AMP-activated protein kinase, stress responses and cardiovascular diseases

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ABSTRACT

AMPK (AMP-activated protein kinase) is one of the key players in maintaining intracellular homoeostasis. AMPK is well known as an energy sensor and can be activated by increased intracellular AMP levels. Generally, the activation of AMPK turns on catabolic pathways that generate ATP, while inhibiting cell proliferation and biosynthetic processes that consume ATP. In recent years, intensive investigations on the regulation and the function of AMPK indicates that AMPK not only functions as an intracellular energy sensor and regulator, but is also a general stress sensor that is important in maintaining intracellular homoeostasis during many kinds of stress challenges. In the present paper, we will review recent literature showing that AMPK functions far beyond its proposed energy sensor and regulator function. AMPK regulates ROS (reactive oxygen species)/redox balance, autophagy, cell proliferation, cell apoptosis, cellular polarity, mitochondrial function and genotoxic response, either directly or indirectly via numerous downstream pathways under physiological and pathological conditions.

INTRODUCTION

All living organisms face the challenge of ever-changing environmental stresses, including nutrient stress [1], oxidative stress [2], DNA damage [3] and hypoxia [4]. Appropriate responses and adaptation to these stresses are essential for eliminating cellular injury, and maintaining or re-establishing intracellular homoeostasis and survival. To pursue these goals, cells rely on the co-ordination of multiple stress response pathways that are controlled at the molecular level by a number of highly conserved signalling molecules and transcriptional regulators, such as the TOR (target of rapamycin) pathway, AMPK (AMP-activated protein kinase) pathways and Sirts.

Key words: AMP-activated protein kinase (AMPK), autophagy, cardiovascular disease, energy sensor, metformin, stress.

Abbreviations: ACC, acetyl-CoA carboxylase; AICAR, 5-amino-4-imidazolecarboxamide riboside; AMPK, AMP-activated protein kinase; AngII, angiotension II; CaMKKβ, Ca2+/calmodulin-dependent protein kinase kinase β; CBM, carbohydrate-binding module; CBS, cystathionine β-synthase; CDK, cyclin-dependent kinase; CDKI, CDK inhibitor; CIDEA, cell-death-inducing DNA fragmentation factor-α-like effector A; CR, caloric restriction; CREB, cAMP-response-element-binding protein; CRTC, CREB-regulated transcriptional co-activator; CVD, cardiovascular disease; 2-DG, 2-deoxy-d-glucose; eNOS, endothelial NO synthase; GLUT4, glucose transporter 4; HF, heart failure; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HIF, hypoxia-inducible factor; HUVEC, human umbilical vein endothelial cell; iκB, inhibitory κB; IKK, iκB kinase; LKB1, liver kinase B1; LV, left ventricular; LVH, LV hypertrophy; MEF, mouse embryonic fibroblast; NF-κB, nuclear factor κB; NPY, neuropeptide Y; O2−, superoxide anion; ONOO−, peroxynitrite; PKC, protein kinase C; PP, protein phosphatase; PPAR, peroxisome-proliferator-activated receptor; PGC1α, PPAR-γ co-activator 1; RBC, red blood cell; RNS, reactive nitrogen species; ROS, reactive oxygen species; siRNA, small interfering RNA; Sirt, sirtuin; SOD, superoxide dismutase; Mn-SOD, manganese SOD; TAK1, transforming growth factor-β-activated kinase 1; TOR, target of rapamycin; mTOR, mammalian TOR; mTORC1, mTOR complex 1; Tdx, thioredoxin; TSC2, tuberous sclerosis complex 2; TZD, thiazolidinedione; UCP, uncoupling protein; ULK1, UNC-51-like kinase 1; WPW, Wolff–Parkinson–White.

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AMPK is one of the key players in maintaining intracellular homoeostasis. AMPK is well known as an energy sensor and can be activated by increased intracellular AMP levels [6]. Generally, the activation of AMPK turns on catabolic pathways that generate ATP, while inhibiting cell proliferation and biosynthetic processes that consume ATP [6,7]. Recent investigations on the regulation and the function of AMPK indicate that AMPK not only functions as an intracellular energy sensor and regulator, but is also a general stress sensor that is important in maintaining intracellular homoeostasis during many kinds of stress challenges.

**AMPK Structure and Regulation**

AMPK is an enzyme that is highly conserved in eukaryotic organisms from yeast (snf1 kinase, a homologue of AMPK) to plants (SnRK1, a homologue of AMPK) to humans [8]. AMPK activity is stimulated by intracellular AMP and inhibited by high levels of ATP. AMPK plays an important role in the regulation of whole-body energy homoeostasis by co-ordinating multiple metabolic pathways. Mammalian AMPK is a heterotrimeric complex comprised of a catalytic subunit (α-subunit) and two regulatory subunits (β- and γ-subunits) [7] (Figure 1). Two α-subunit isoforms (α1 and α2), two β-subunit isoforms (β1 and β2) and three γ-subunit isoforms (γ1, γ2, and γ3) are all encoded by distinct genes (PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2 and PRKAG3 respectively) that are distributed across five chromosomes (α1, 5p12; α2, 1q31; β1, 12q24.1; β2, 1q21.1; γ1, 12q12-14; γ2, 7q35-36; γ3, 2q35) [9].

Figure 1  Structure of AMPK

Schematic representation of the components of the AMPK subunits. The catalytic α-subunit can be phosphorylated at Thr172 by upstream kinases including, LKB1, CaMKKβ and TAK1, leading to enzyme activation. The β-subunit contains a glycogen-binding domain (GBD). The γ-subunits contain four nucleotide-binding modules (CBS domains) capable of co-operatively binding to AMP, ADP and ATP.

**Subunits of AMPK**

The α-subunit contains the catalytic domain of the serine/threonine protein kinase in the N-terminus, as well as the residue Thr172, whose phosphorylation is critical for AMPK activation [10]. The C-terminal domain of the α-subunit is required for interaction with the β- and γ-subunits. An autoinhibitory domain has been mapped to amino acids 312–335 of the α-subunit [11], and the α1 (1–312) truncation is found to be constitutively active without the β- and γ-subunits [12]. It was originally proposed that AMP promoted AMPK activation by facilitating the phosphorylation of AMPK at Thr172 by upstream kinases, whereas more recent work has suggested that AMP binding also inhibits the dephosphorylation of Thr172 [13]. Interestingly, an inhibitory phosphorylation site, Ser485, was identified in the α-subunit [14], but the detailed mechanism by which this phosphorylation site inhibits AMPK activation and how it is regulated has not been demonstrated.

Even though there is 90% amino acid identity in the kinase domains of AMPKα1 and α2 isoforms, the AMPK α-subunits have differential tissue-specific expression and activation. AMPKα1 is relatively evenly distributed in the heart, liver, kidney, spleen, lung and brain, whereas AMPKα2 is highly abundant in skeletal muscle and heart, followed by the liver and kidney [15]. AMPKα1 has been reported to be the predominant isoform of AMPK in endothelial cells [16]. Our group has reported that AMPKα1 is the predominant isoform expressed in RBCs (red blood cells) and the mouse vascular wall [17–19]. Salt
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Figure 2  Regulation of AMPK

AMPK is a heterotrimeric protein complex which consists of a catalytic α-subunit and regulatory β- and γ-subunits. AMPK is activated by increased intracellular AMP levels, which prevents the catalytic subunit from being dephosphorylated. LKB1 and CaMKK are two major upstream regulators of AMPK that exert their function by phosphorylating the AMPK α-subunit at Thr172. Another reported activator of AMPK is TAK1. Activation of AMPK switches off ATP-consuming processes and promotes ATP-generating processes. TGF, transforming growth factor.

et al. [20] have reported that the AMPKα2 isoform is more dependent on AMP than is the AMPKα1 isoform, and that AMPKα2 is largely localized to the nucleus. The β-subunit contributes to the interaction between glycogen and AMPK. The N-terminal region of the β-subunit contains a CBM (carbohydrate-binding module) that is believed to target AMPK to glycogen [21]. In addition, the β-subunit appears to stabilize the interaction between the α- and γ-subunits through a binding domain in its C-terminus (amino acids 186–270) [22,23]. The γ-subunit contains four CBS (cystathionine β-synthase) domains (Figure 1). AMP, ADP and ATP can bind the AMPK γ-subunit to regulate AMPK activation through allosteric structural changes in the catalytic α-subunits, modulating phosphorylation by upstream kinases [24] and regulating the dephosphorylation of Thr172 [13,25].

Regulation of AMPK by AMP/ATP ratios

AMPK plays a key role in regulating cellular energy status. AMPK is activated when the intracellular AMP level increases. The binding of AMP to the AMPK γ-subunit leads to an allosteric structural change in AMPK, resulting in a 2–5-fold increase in AMPK activity [26]. The extent of allosteric activation of AMPK by AMP depends on the specific isoforms of both the α- and γ-subunits present in the enzyme [26]. The greatest activation by AMP occurs in complexes containing the α2 and γ2 isoforms; however, only weak activation occurs in complexes with the γ3 isoforms [26]. The most recent crystal structure for mammalian AMPK indicates that ADP protects AMPK from dephosphorylation after binding to one of the two exchangeable AXP (AMP/ADP/ATP)-binding sites in the γ-subunit [27]. More importantly, the binding of AMP to the γ-subunit of AMPK enhances the phosphorylation of Thr172 by upstream kinases [24] and inhibits the dephosphorylation of Thr172 by PP (protein phosphatase) 2Cα [13] or PP2A [28].

Regulation of AMPK by upstream kinases

In addition to allosteric regulation, AMPK is significantly regulated by upstream kinases (Figure 2). LKB1 (liver kinase B1) and CaMKKβ (Ca2+/calmodulin-dependent protein kinase kinase β) are two well-defined upstream kinases of AMPK. LKB1 was originally discovered as a tumour suppressor in Peutz–Jeghers syndrome patients, who are susceptible to benign intestinal hamartomatous polyps and malignant tumours mainly in the gastrointestinal tract [29]. In vitro, the purified recombinant LKB1 complex directly phosphorylates AMPK on Thr172 [30–32]. LKB1 is not activated by phosphorylation, like most kinases, but is instead regulated by relocation from the nucleus to the cytoplasm upon binding to STRAD (Ste20-related adaptor) and MO25 (mouse protein 25) [33,34]. Research in LKB1-deficient cells revealed CaMKKβ to be another upstream kinase of AMPK [35,36]. Furthermore, CaMKKβ isolated from rat brain or expressed in Escherichia coli phosphorylates and activates AMPK in vitro [37]. The activation of AMPK by CaMKKβ occurs in the absence of a detectable increase in AMP, but depends on an increase in intracellular Ca2+ content [35,36]. More recently, it was reported that TAK1 (transforming growth factor-β-activated kinase 1) can activate AMPK in yeast [38], but the physiological relevance of AMPK activation by TAK1 needs further study.
Other pathways in AMPK activity regulation

The phosphorylation of AMPK by upstream kinases is a reversible procedure modulated by protein phosphatases. Both PP2A and PP2C have been reported to be able to dephosphorylate AMPK, thus inhibiting AMPK activation [39,40]. New mechanisms for regulating AMPK activity have been proposed, indicating that AMPK protein can be modulated by ubiquitin-independent proteasome degradation mediated by CIDEA (cell-death-inducing DNA fragmentation factor-α-like effector A), and a CIDEA-binding site has been identified on the AMPK β-subunit [41]. Oakhill et al. [24] have provided evidence to show that AMPK β-myristoylation plays a key role in the full phosphorylation and activation of AMPK by upstream kinases in response to metabolic stress signals. In a recent paper from Hardie’s group [42], the authors distinguished six pathways that can activate AMPK using isogenic cell lines stably expressing either wild-type AMPK complexes or AMP-insensitive (R531G) γ2 mutants.

AMPK ACTIVATORS AND INHIBITORS

The potential beneficial effects of AMPK activation in disease prevention and treatment have stimulated the interest of researchers in identifying AMPK activators. Thus far, numerous AMPK activators have been reported; some of the direct and indirect activators of AMPK, as well as some AMPK inhibitors, are listed below.

Activation of AMPK in physiological and pathological conditions

AMPK has been implicated in several physiological and pathological conditions, such as hypoxia, CR (caloric restriction) and physiological exercise. In these conditions, activated AMPK modulates numerous downstream targets, allowing the body to adapt to these challenges.

AMPK is activated by hypoxia

Hypoxia is a situation in which the whole body or a region of the body is deprived of adequate oxygen supply. This could happen under physiological conditions, such as during strenuous physical exercise or at high altitudes, and in pathological conditions, such as anaemia, hypoventilation and pulmonary fibrosis. Complete oxygen depletion results in cell death, but cells can survive in reduced oxygen supply conditions by activating broad adaptive processes via HIF (hypoxia-inducible factor)-1, which plays a central role in these adaptations [43]. AMPK is involved in the response to hypoxia in the goldfish, a hypoxia-tolerant species [44], and is activated in hypoxic conditions in rat heart tissue and cultured endothelial cells [45,46]. The mechanisms for the activation of AMPK under hypoxia appear complicated and are dependent on the extent of hypoxia. One of the mechanisms leading to AMPK activation is reduced ATP production due to the inhibition of β-oxidation under hypoxic conditions [47]. On the other hand, ROS (reactive oxygen species) and RNS (reactive nitrogen species) are important in AMPK activation in conditions of non-severe hypoxia. ONOO− (peroxynitrite), generated from NO and O2− (superoxide anion), has been reported to rapidly activate AMPK in endothelial cells [48,49], whereas mitochondrial RNS are reported to be required for the activation of AMPK by metformin [50]. Under these conditions, intracellular AMP/ATP levels remain unchanged, whereas ROS production by the electron transport chain at complexes I, II and III in mitochondria is significantly increased [51,52].

ROS/RNS might activate AMPK via different upstream kinases. ONOO−-activated AMPK in endothelial cells is reported to be a c-Src-mediated PI3K (phosphoinositide 3-kinase)-dependent [53]. Further investigations have demonstrated that PKC (protein kinase C)-ζ-mediated phosphorylation of LKB1 is essential in ONOO−-mediated AMPK activation [54]. On the other hand, ROS, such as H2O2, can trigger AMPK activation in the apparent absence of increased AMP concentration through ROS-dependent CRAC (Ca2+ release-activated Ca2+ channel) activation, leading to increases in cytosolic Ca2+ that activate the AMPK upstream kinase CaM KKβ [55]. Interestingly, AMPK has been reported to be required for HIF induction in the response to hypoxia [56], and an AMPK/HIF1 axis has been suggested to be important for cell survival and growth under conditions of hypoxia [52]. However, the detailed mechanism by which AMPK regulates HIF is not completely clear. In addition, AMPK can also regulate cellular survival in conditions of hypoxic stress through autophagic regulation, which is independent of the HIF-1 pathway [57].

AMPK is activated by physical exercise

Regular physical exercise has numerous benefits in the prevention and treatment of diseases [58,59], and AMPK activation is increased during physical exercise [60]. Notably, rodents that are selectively AMPK-deficient in skeletal muscle showed impaired recovery from fatigue after exercise [61]. The exact mechanism of the beneficial effect of AMPK activation by exercise is not clear. It has been reported that activation of AMPK increases fatty acid oxidation and glucose uptake in rat muscles [62]. Similar results were reported in insulin-resistant high-fat-fed rats [63], suggesting a potential beneficial effect of AMPK activation for insulin-resistant patients and those with Type 2 diabetes. Recently, Evans’s group [64] reported that activation of AMPK by AICAR (5-amino-4-imidazolecarboxamide riboside) mimics the
effect of training and improves exercise performance in mice. Similar effects are observed in mice treated with GW1516, a PPAR (peroxisome-proliferator-activated receptor)-δ activator. On the basis of these findings, the AMPK/PPAR-δ pathway has been proposed to explain the beneficial effects of AMPK activation in exercise, and it has been suggested that AMPK agonists are exercise mimetics [64]. AMPK has also been implicated in the regulation of muscle fibre type change. A previous study has reported that rats fed with AICAR for 14 days have a significantly increased number of type IIX fibres in their extensor digitorum longus [65]. Similarly, in a transgenic mouse model, Goodyear’s group [66] have demonstrated that AMPK is essential for a fibre type IIb to IIX transformation during physical exercise training. Most recently, using skeletal-muscle-specific AMPKβ1/AMPKβ2-knockout mice, O’Neill et al. [68] have demonstrated that AMPK is required in maintaining mitochondrial content and glucose uptake during exercise. AMPK-mediated fibre type change during exercise might result in a significant metabolic switch in muscles, since type IIX fibres are more likely to depend on glycolysis, whereas type IIb fibres rely more on mitochondrial oxidation [69]. Thus increased type IIX fibres during exercise training would promote glucose consumption. Corresponding to this energy-demanding change, there was an increase in glucose uptake capacity supported by an increase in hexokinase expression [66] and increased GLUT4 expression [70] in skeletal muscles of mice after 6 weeks of exercise training. These studies provide molecular mechanisms for the metabolic beneficial effect of AMPK activation during exercise training for obese and diabetic patients.

Natural activators of AMPK

Several physiological hormones and natural plant compounds can activate AMPK. Leptin and adiponectin are hormones secreted by adipocytes. Acute and chronic leptin treatments have been reported to increase AMPK expression and/or phosphorylation in rodent skeletal muscles; this could be an important mechanism by which leptin regulates energy stores [71,72]. Adiponectin has cardioprotective effects against ischaemia/reperfusion injury by a mechanism believed to be due to AMPK activation [73,74]. Resveratrol and berberine are natural compounds found in red grapes and Berberis respectively. Resveratrol exerts an effect similar to CR to extend lifespan, and AMPK is reported to be the key enzyme that mediates this beneficial effect of resveratrol in neurons [75]. Berberine can increase AMPK activity [76] and improve glucose transport in cell lines and animals; this is believed to be the important mechanism by which berberine exerts its beneficial effects in diabetic and insulin-resistant states [77]. Despite existing evidence for the role of AMPK activation by these compounds, the importance of AMPK activation in contributing to the beneficial effects of these chemicals, and the detailed mechanisms by which these compounds activate AMPK, require further investigation.

Chemical activators of AMPK

The most widely used chemical activator of AMPK in research is AICAR. AICAR is a cell-permeant chemical that is converted within the cell into ZMP (monophosphate AICAR), which mimics the effect of AMP in activating AMPK [78]. The glucose analogue 2-DG (2-deoxy-D-glucose) is another strong AMPK activator that is used in experimental research as it can competitively block glucose utilization to mimic the effect of CR [79,80]. Clinically, metformin is the most widely used AMPK activator for the treatment of Type 2 diabetes [81]. TZDs (thiazolidinediones) are PPAR-γ activators used as insulin sensitizers and can rapidly activate AMPK in cell lines and animal tissues [82]. A-769662 is a direct activator of AMPK, identified as the result of screening a chemical library of over 700 000 compounds [83–85]. It is noteworthy that all of these chemicals possess beneficial effects that may be unrelated to AMPK activation. For example, AICAR and metformin have been reported to modulate hepatic glucose metabolism by regulating glucokinase translocation, which is AMPK-independent [86]. Similarly, Viollet’s group [87] have reported that metformin inhibited hepatic glucogenesis in an AMPK-independent manner.

AMPK inhibitors

AMPK is inhibited by high glucose and glycogen

Obesity and diabetes are the fastest growing public health problems in developed and developing countries. Over-nutrition and decreased physical exercise are believed to be the major reasons for these problems. Emerging evidence has suggested that activated AMPK might be beneficial for whole-body energy homoeostasis [88]. On the other hand, AMPK activation is inhibited under high glucose conditions. In animal models, glucose infusion induces acute hyperglycaemia and a reduction in AMPK activation in rat muscle and liver [89]. Similar results are also observed in cultured HepG2 hepatocytes incubated with high glucose [90]. However, the detailed mechanism of AMPK inhibition by high glucose is not completely clear and requires further investigation. High glycogen content represses AMPK activation in rat skeletal muscle without significant changes in adenine nucleotide concentrations [91]. The proposed mechanism suggested McBride and co-workers [92] indicates that glycogen leads to allosteric inhibition of AMPK by binding to the AMPK glycogen-binding domain in the β-subunit.
AMPK is inhibited by lipid overload
High-fat-diet-fed mice have impaired AMPK phosphorylation and protein expression in multiple tissues and organs, including skeletal muscle, heart, liver and hypothalamus [93]. Furthermore, mice fed on a palmitate-enriched diet have a significant inhibition of AMPK activation through the enhancement of PP2A-mediated dephosphorylation of AMPK [28].

AMPK is inhibited by amino acids
A high-protein diet decreases AMPK activity in the hypothalamus, leading to inhibition of NPY (neuropeptide Y) and a reduction in food uptake in mice [94]. Similarly, treatment of C2C12 cells with leucine reduces AMPK phosphorylation with enhanced mTOR (mammalian TOR) activation. Findings further suggest the involvement of an AMPK/mTOR pathway in coordinating amino acid metabolism [95].

Pharmacological AMPK inhibitor
Compound C is a cell-permeant pyrazolopyrimidine compound that is widely used as an AMPK inhibitor [81], but the non-AMPK-targeted effect need to be considered when using this chemical. Bain et al. [96] have reported that Compound C inhibited AMPK with an IC50 value of 0.1–0.2 μM, whereas it also inhibited other protein kinase, such as ERK8 (extracellular-signal-regulated kinase 1), MNK1 (mitogen-activated protein kinase-interacting kinase 1), PHK (phosphorylase kinase), MELK (maternal embryonic leucine-zipper kinase), DYRK (dual-specificity tyrosine-phosphorylated and -regulated kinase) isoforms, HIPK2 (homeodomain-interacting protein kinase 2), Src, Lck and Yes, FGF-R1 (fibroblast-growth-factor receptor 1) and Eph-A2 (Ephrin A2 receptor) with similar IC50 values, as well as the BMP (bone morphogenetic protein) pathway [97]. Thus some other approaches, such as siRNA (small interfering RNA) or transgenic mice are required to confirm whether these specific effects are AMPK-dependent.

AMPK IS AN ENERGY STRESS SENSOR AND MODULATOR
Limited nutrition, as well as an excessive supply of nutrients for cellular requirements, can both cause cellular stress. Nutrient supply status is sensed at multiple levels by the cell and whole body. AMPK is strongly activated by energy depletion and is inhibited in conditions of over-nutrition, such as in Type 2 diabetic patients. This marks AMPK as one of the master sensors and regulators of nutrient stress in maintaining intracellular energy homeostasis, which is essential for normal cell function and survival under physiological and pathological conditions (Figure 3).

AMPK activation by nutrient depletion
Nutrient depletion could happen in both physiological and pathological conditions, such as fasting, exercise and ischaemia, and leads to an increase in intracellular AMP levels, which is a direct activator of AMPK. Exercise and CR, which involves a 20–40 % reduction in caloric intake without compromising daily nutritional needs, are believed to benefit human health. CR is the most consistent way to extend the lifespan of yeast, worms, flies and rodents [98] and to lower the incidence of age-related disease [99]. Interestingly, multiple genetic models have confirmed the role of AMPK as a mediator of the beneficial effects of CR on longevity in yeast [100] and Caenorhabditis elegans [101]. In addition, there is an aging-associated reduction in AMPK activity and mitochondrial biogenesis in rodents [102]. More importantly, CR leads to strong activation of AMPK [60]. The mechanisms by which CR activates AMPK and promotes longevity are not completely understood; however, several mechanisms of AMPK activation in CR conditions have been proposed. For example, CR and muscle contraction during exercise are reported to result in an increase in the cellular AMP/ATP ratio. AMPK is therefore activated in mouse skeletal muscle during exercise and increases glucose transport, an effect that is not seen in muscle from LKB1-deficient mice, indicating that LKB1 is important in regulating AMPK activity in response to muscle contraction [103]. Furthermore, it is believed that CR modulates AMPK through ROS-mediated AMPK activation. This hypothesis is supported by results from 2-DG-induced CR conditions in endothelial cells, which

Figure 3  AMPK is a master regulator of metabolism
AMPK regulates lipid, protein and glucose metabolism by modulating numerous downstream targets. Activation of AMPK promotes fatty acid oxidation and glucose utilization, but inhibits protein, fatty acid and glycogen synthesis. G5, glycogen synthase; PFK2, phosphofructokinase 2; HMG, HMG-CoA reductase; HSL, hormone-sensitive lipase.
trigger autophagy and increased cell survival in response to energy stress. These effects are dependent on AMPK activation [80]. In addition, activated AMPK in CR can modulate mitochondrial function through the regulation of mitochondrial biogenesis via the AMPK/Sirt1/PGC1α (PPAR-γ co-activator 1) pathway and mitochondrial elimination by the AMPK/mTOR/ULK1 (UNC-51-like kinase 1) autophagy pathway [104–106]. Consistent with this, a natural compound, resveratrol, found in red wine and red grapes, can mimic the beneficial effects of CR, and this beneficial effect is dependent on the activation of AMPK and Sirt1 [107,108].

AMPK is a master regulator of energy homeostasis

AMPK modulates multiple downstream pathways in metabolic regulation

Activation of AMPK has been reported to regulate energy balance at multiple organs by targeting several key substrates. AMPK inactivates ACC (acetyl-CoA carboxylase), leading to the inhibition of fatty acid synthesis and the promotion of mitochondrial β-oxidation. Activated AMPK can inhibit HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase to reduce cholesterol synthesis. AMPK increases cellular NAD+ levels and enhances Sirt1 activity, causing the deacetylation and activation of PGC1α and resulting in an increase in mitochondrial gene expression [109]. In parallel, the activation of AMPK leads to increased food intake [110], and increases GLUT4 (glucose transporter 4) expression and glucose transport in skeletal muscle [111]. Most recently, AMPK has been reported to promote fat absorption in mice [112]. AMPK promotes the initiation of autophagy, a major protective mechanism that allows cells to survive in response to multiple stress conditions, especially nutrient depletion, by inhibiting the mTORC1 (mTOR complex 1) pathway. Under low-nutrient conditions, AMPK is activated and phosphorylates TSC2 (tuberous sclerosis complex 2), leading to the inhibition of mTORC1 [113]. Gwinn et al. [114] have reported that AMPK regulates the mTOR pathway by direct phosphorylation of Raptor (regulatory associated protein of mTOR) at Ser722 and Ser792 residues. Recent research further indicates that AMPK could directly act on initiation of autophagy by the direct phosphorylation of ULK1 at Ser550 and Ser777 [115], or at Ser317 and Ser777 [3], in MEFs (mouse embryonic fibroblasts) under conditions of glucose depletion. A study on C. elegans showed that glucose restriction can significantly extend the lifespan of this organism, and that this requires aak-2, a homologue of AMPK [116]. A recent study indicates further that the effects of AMPK activation on longevity are mediated by CREB (cAMP-response-element-binding protein) and CRTCs (CREB-regulated transcriptional co-activators), especially CRTC-1 [117]. Overall, AMPK will be quickly activated by energy depletion, and activated AMPK can regulate multiple pathways involved in maintaining intracellular energy homoeostasis, which is critical for normal cellular function and survival.

AMPK integrates whole-body energy balance

AMPK acts as a metabolic master sensor and regulator by inducing a cascade of events in multiple organs in response to energy challenge in physiological and pathological conditions. Skeletal muscle is one of the major target organs for insulin in stimulating glucose disposal, and insulin resistance is the major characteristic of the development of Type 2 diabetes. In skeletal muscle, AMPK activity is increased by stimuli such as osmotic stress, hypoxia, ischaemia and exercise [118]. Activation of AMPK in muscle recruits GLUT4 to the plasma membrane and enhances glucose transport, independent of insulin stimulation [119]. AMPK also regulates skeletal muscle energy production by regulating mitochondrial biogenesis via the AMPK/Sirt1/PGC1α pathway and mitochondrial homoeostasis, which is critical for normal cellular function and survival.

In adipose tissue, AMPK has been reported to be activated by adipocyte hormones, including leptin [122] and adiponectin [123]. In the liver, activation of AMPK leads to the rapid regulation of hepatocyte metabolism by regulating specific enzymes, such as ACC, thereby resulting in increased fatty acid oxidation and inhibited hepatic lipogenesis, cholesterol synthesis and glucose production [124]. Glucose intolerance and hyperglycaemia has been observed in liver-specific AMPKα2−/− mice in a fasted state [125]. Liver AMPK regulates glucose homoeostasis by regulating multiple glucogenesis genes, such as PEPCk (phosphoenolpyruvate carboxykinase), G6Pase (glucose-6-phosphatase) [126,127], and transcription factors, including HNF (hepatocyte nuclear factor)-4α and FoxO1 (forkhead box O1) [128]. In adipose tissue, activated AMPK improves insulin sensitivity by decreasing lipogenesis and increasing fatty acid oxidation, which results in decreased non-esterified fatty acids (free fatty acids) in adipose tissue, and AMPK activation leads to the inhibition of pre-adipocyte differentiation and triacylglycerol (triglyceride) synthesis in adipocytes (for detailed information, see [129]). Low glucose levels activate AMPK [130], whereas high glucose inhibits AMPK activation in β-cells [131]. Pre-activation of AMPK by AICAR blocked the glucose-induced upregulation of gene expression for insulin secretion in islet β-cells [131]. During food intake regulation, AMPK activation in the hypothalamus is reported to be an important mechanism for anorexigenic and orexigenic signals in integrating whole-body energy homoeostasis [132]. Interestingly, a recent study has highlighted the role of the AMPK/SRC-2 (steroid receptor co-activator-2)
AMPK and the oxidative stress response

Oxidative stress is defined as an imbalance between the levels of pro-oxidants and antioxidants, resulting in macromolecular damage and disruption of redox signalling and controls [133]. Macromolecular damage usually occurs via oxidative mechanisms linked to free radicals, including O₂^−, NO and OH^− (hydroxyl ions). Free radicals are small diffusible molecules that differ from most biological molecules in that they are highly reactive due to an unpaired electron. Free radicals can participate in chain reactions in which a single free radical initiation event can be propagated to damage multiple molecules, including DNA, damage to membrane ion transport systems, enzymes and lipid peroxidation [134]. Recently, studies including those from our group have shown that AMPK may be an oxidative stress sensor and redox regulator in addition to its traditional role as an energy sensor and regulator.

AMPK is activated by intracellular oxidative species

ROS-induced activation of AMPK is believed to be important for the beneficial effect of many medicinal drugs. For example, metformin has been reported to activate AMPK through mitochondrial-derived RNS [50]. Furthermore, ischaemia/reperfusion experiments with sevoflurane protected rat hearts against ischaemic injury in a manner dependent on the activation of AMPK by intracellular ROS. Application of a ROS scavenger resulted in decreased AMPK activation and diminished the cardioprotective effect of sevoflurane, thereby further substantiating the role of ROS in the activation of AMPK [135]. In mouse skeletal muscle, AMPK is activated by oxidative stress and enhances glucose transport, independent of changes in AMP or the AMP/ATP ratio [136]. Similarly, in cell culture conditions, hypoxia-induced AMPK activation is reported to be dependent on mitochondrial ROS without significant changes in the AMP/ATP ratio [137]. H₂O₂ has been observed to be able to strongly induce the activation of AMPK [138]. Recent studies have shown that the underlying mechanism of H₂O₂ activation of AMPK involves the induction of cysteine oxidation [139]. In vascular endothelial cells, our group has reported, in multiple species, that ONOO^− activates AMPK by a PKCζ/LKB1-dependent mechanism [140].

AMPK modulates ROS production

As described above, AMPK is functional as a redox sensor that can be quickly activated by increased intracellular ROS/RNS. Activated AMPK appears to be essential in maintaining intracellular redox status by inhibiting oxidant production by NADPH oxidases, mitochondria, etc. or by increasing the expression of antioxidant enzymes such as SOD (superoxide dismutase)-2 and UCP (uncoupling protein)-2.

AMPK regulates NADPH oxidase

NADPH oxidase (Nox) is a membrane-bound complex made of six subunits: a GTPase (usually Rac1 or Rac2) and five phox units: gp91^phox, p22^phox, p40^phox, p47^phox and p67^phox. NADPH oxidase has been well recognized as an important source of ROS in the vasculature and plays an essential role in normal vascular function and in vascular diseases, such as hypertension, atherosclerosis and diabetes [141]. Overproduction of ROS has been postulated to contribute to a loss of insulin responsiveness. A study that analysed saphenous veins and internal mammary arteries from diabetic patients showed significantly increased NADPH oxidase activity and unregulated expression levels of the p22^phox, p67^phox and p47^phox subunits combined with unpaired endothelial NO synthesis [142] (for reviews, see [143,144]). Activation of AMPK by AICAR has been reported to suppress O₂^− stimulated by phorbol esters or fMLP (N-formylmethionyl-leucylphenylalanine) by reducing the translocation and phosphorylation of p47^phox in human neutrophils [145]. Similarly, rosiglitazone reduced high-glucose-induced NADPH-oxidase-mediated oxidative stress in a manner dependent on AMPK activation in HUVECs (human umbilical vein endothelial cells) [146]. Moreover, metformin suppresses high-glucose-induced oxidative stress in cultured podocytes by an AMPK-mediated reduction of NADPH oxidase activity [147]. In AMPKα2^-/- mice, our group has found a significantly increased expression and activation of NADPH oxidase due to enhanced NF-κB (nuclear factor κB) activation [148]. These findings reveal a novel mechanism for the regulation of NADPH oxidase expression by AMPK, and demonstrate that AMPK is a physiological suppressor of NADPH oxidase and ROS production in endothelial cells. Most recently, a similar observation was reported in AMPKα1-deficient mice treated with chronic AngII (angiotensin II), which showed mild endothelial dysfunction associated with increased NADPH oxidase activation and Nox2 up-regulation [149].

AMPK regulates mitochondrial ROS generation

Mitochondria, especially damaged mitochondria, are the major sources of ROS in cells and are related to many chronic human diseases, such as diabetes mellitus, neurodegeneration and cancer [150]. AMPK can regulate mitochondrial ROS generation through several mechanisms.
UCPs are members of the larger family of mitochondrial anion carrier proteins, and UCPs facilitate the transfer of anions across mitochondrial membranes. UCP2 plays an important role in controlling mitochondrial ROS production [151,152] and is believed to be a potential target in the treatment of obesity, diabetes and aging [153]. Studies have shown that one of the mechanisms of AMPK action in redox regulation is to up-regulate the expression of mitochondrial UCP2. For example, activation of AMPK by AICAR, or overexpression of constitutively activated AMPK, inhibited O$_2^•−$ production and reduced tyrosine nitration of prostacyclin synthase under high-glucose treatment in HUVECs [154]. Furthermore, AMPK is involved in the action of ghrelin on NPY/Agouti-related peptide. The latter seems to be due to the up-regulation of UCP2 expression, resulting in the regulation of mitochondrial ROS production in neurons [155] and cultured MIN6 insulinoma cells [156]. Furthermore, in an animal model, it has been found that endurance training activated AMPK, resulting in an increase in UCP2 expression in rat pancreatic islets [157], which is similar to findings in mouse β-cells deficient in AMPKα2 expression [158]. Despite these findings, the detailed mechanisms by which AMPK regulates UCP2 expression and function are still not completely understood.

Emerging evidence suggests that AMPK might modulate autophagy thereby regulating mitochondrial ROS generation. It is well known that damaged proteins and DNA or dysfunctional mitochondria cause enhanced production of ROS from mitochondria [159]. Accordingly, cells have devised specific mechanisms to control these damaged organelles. One such mechanism is autophagy, which is critical in eliminating dysfunctional mitochondria from cells [160]. Autophagy deficiency leads to elevated ROS production; this is supported by numerous experimental results from autophagy-dysfunctional cell lines or animals. For example, skeletal muscle cells from Agt7-deficient mouse exhibit increased defects in mitochondrial respiration and increased ROS levels [161]. Similar observations have been reported in Atg5−/− cells and beclin1+/− iBMK cells, which show elevated ROS production with stress [162]. More importantly, mitochondrial removal is critical for RBC maturation and survival; accordingly, autophagy-defective RBCs from Nix−/− mice have more ROS production and undergo apoptosis [163]. Furthermore, several other autophagy-deficient gene knockout mice (Agt5−/−, Agt7−/− and Ulk1−/−) showed similar anaemic phenotypes due to a shortened RBC lifespan [164]. It has been well established that AMPK regulates autophagy through inactivation of the mTORC1 pathway by phosphorylation and activation of the TSC2 exchange factor [113]. Most recently, a new mechanism involving AMPK in the control of autophagy has been reported by several research groups, involving the direct phosphorylation of Ulk1 by AMPK to regulate autophagy [165], indicating that AMPK can regulate ROS through the regulation of autophagy. Indeed, AMPK inactivation by palmitate leads to defective autophagy and increased generation of mitochondrial ROS in bone-marrow-derived macrophages [166].

AMPK activation increases the antioxidant potential

Oxidative stress is a result of an imbalance of oxidants and antioxidants. Emerging evidence suggests that AMPK also plays an important role in the regulation of cellular antioxidant defence. For instance, activation of AMPK by AICAR is reported to inhibit palmitate-induced ROS levels by increasing the expression of Trx (thioredoxin) in human aortic endothelial cells [167]. Similarly, AMPK activation in endothelial cells increases the expression of Mn-SOD (manganese SOD) by up-regulating the expression of PGC-1α [168]. Consistently, inhibition of AMPKα1 in cultured HUVECs is reported to decrease the expression of some antioxidant genes, including γ-GS (γ-glutamylcysteine synthase), Mn-SOD, catalase and Trx, resulting in the accumulation of ROS and increased apoptosis [169]. Using transgenic mice, our group has reported that AMPKα1−/− mice exhibit an anaemic phenotype because of the imbalance of antioxidants/oxidants in RBCs, leading to a significantly shortened RBC lifespan [19].

ROLE OF AMPK IN CELL PROLIFERATION AND CELL DEATH

AMPK in cell proliferation

Mammalian cell proliferation is controlled by the cell-cycle machinery that is regulated by CDKs (cyclin-dependent kinases), such as Rb (retinoblastoma protein) and CDKIs (CDK inhibitors), including as p21cip and p27kip[170]. AICAR, an AMPK activator, has been reported to induce cell-cycle arrest in multiple types of cells, including carcinoma and non-carcinoma cells [171,172]. AMPK regulates cell proliferation by controlling several key factors in metabolic pathways that are important for cell growth and division (Figure 4). Activated AMPK has been reported to suppress mTOR signalling by growth factors and amino acids to control protein synthesis, which is required for cell growth and division [173]. Furthermore, activation of AMPK by AICAR or rosiglitazone leads to the suppression of prostate cancer cell proliferation by reducing Fas expression [174], which controls fatty acid synthesis. Furthermore, HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, is a direct substrate of AMPK [175]. Importantly, studies suggest that AMPK has a more direct effect on cell growth control by controlling CDKIs. For instance, Jones et al. [176] have reported that AMPK directly regulates p53 function.
by modifying the Ser\(^{15}\) phosphorylation site, which controls p21\(^{\text{CIP}}\) expression in MEFs. Our most recent findings have shown that AMPK, especially AMPK\(\alpha_2\), controls mVSMC (mouse vascular smooth muscle cell) proliferation by modulating p27\(^{\text{KIP}}\) expression through p52 NF-\(\kappa\)B-2-dependent Skp2 (S-phase kinase-associated protein 2) regulation [17].

### AMPK in cellular survival and apoptosis

The function of AMPK in cellular apoptosis is complicated and sometimes controversial. For example, AICAR has been reported to induce apoptosis in adipocytes through AMPK-dependent eIF2\(\alpha\) (eukaryotic initiation factor 2\(\alpha\)) regulation [177], whereas another study has reported that AICAR-induced apoptosis is independent of AMPK activation in leukaemia cell lines [178]. Similarly, resveratrol has been reported to induce apoptosis in chemoresistant cancer cells through AMPK modulation [179], but the same group has also reported that resveratrol protects ROS-induced cell death in H9c2 cardiac muscle cells by activating AMPK [180]. Therefore the role of AMPK in cellular fate decision seems to be cell-type- and treatment-specific, and dependent on the duration and tension of the stress. One explanation for these observations is the multiple functions of downstream substrates of AMPK, such as p53, which might play an anti-apoptotic role by inducing cell growth arrest and promoting the expression of genes involved in DNA repair, including GADD45 (growth-arrest and DNA-damage-inducible protein 45) and PCNA (proliferating-cell nuclear antigen), which are important for stem cell survival [181]. At the same time, p53 has a pro-apoptotic effect by up-regulating the expression of several apoptosis genes including Bax [182] or by directly attacking mitochondria [183]. Another example is the function of AMPK in autophagy regulation. Autophagy is generally believed to be a cytoprotective mechanism that enables cells to survive in conditions of unfavourable stress, especially metabolic stress [184]. However, autophagy is also reported to contribute to cell death in some types of damage; for example, damage-regulated modulator-mediated autophagy is reported to play a critical role in p53-modulated apoptosis [185]. In cancer cells, autophagy can increase apoptosis in a caspase-independent manner [186,187]. As discussed above, AMPK can regulate autophagy through the mTOR and ULK1 pathways, and thereby play dual roles in cellular apoptosis. Current evidence supports the theory that AMPK plays important, but complicated, roles in cell fate decisions, which could be cell-type-, stress-tension- and sustention-dependent. It is obvious that the role of AMPK in cell apoptosis or cell fate decisions, particularly the relationship among AMPK, p53 and autophagy, requires further investigation.

### ROLE OF AMPK IN CVD (CARDIOVASCULAR DISEASE)

A healthy heart requires a sustained energy supply for its contraction. Many energy substrates, including fatty acids, glucose, ketones and amino acids, can be used by the heart, but fatty acid \(\beta\)-oxidation in mitochondria is its primary source of ATP. AMPK works to restore ATP supply and plays an important role in the heart in both physiological states and stress conditions, such as excessive load and ischaemia/reperfusion. Recent evidence suggests that AMPK might also regulate the oxidative stress response and that dysfunctional AMPK could be the basis of the pathogenesis of several CVDs [188].

### Alterations in AMPK cause cardiomyopathy in humans

WPW (Wolff–Parkinson–White) syndrome is a hereditary disease that is characterized by ventricular pre-excitation [189]. WPW syndrome is associated with unexplained LVH [LV (left ventricular) hypertrophy] and is diagnosed as FHC (familial hypertrophic cardiomyopathy) [190]. AMPK \(\gamma\)-subunit mutations have been identified as the molecular basis for the pathogenesis of WPW syndrome and LVH [191,192]. Subsequently, multiple mutations have been reported and distinct clinical features associated with AMPK\(\gamma\) subunit mutations (\(PRKAG2\) cardiac syndrome) [193]. The exact mechanism by which AMPK\(\gamma\)-subunit mutations cause cardiac dysfunction is not clear, but findings suggest that a glycogen storage disorder might be the common reason...
Mice overexpressing human PRKAG2 mutant genes, R531G [195], N488I [196] and R302Q [197], closely mimic clinical WPW syndrome, with cardiomyopathy and glycogen accumulation in cardiomyocytes. A glycogen storage problem in PRKAG2 cardiomyopathy patients is also believed to be one of the mechanisms for the cardiac pre-excitation conduction syndrome seen in these patients [198]; notably, other glycogen storage disorders, such as Danon disease, also lead to pre-excitation [199]. Modulating AMPK activation in epithelial cells affects their transmembrane conductance [200,201], potentially indicating a more direct role of AMPK in regulating cardiac electrophysiology; however, this hypothesis needs further investigation.

**Protective effect of AMPK activation on cardiomyocytes**

In the clinical situation, doxorubicin is a widely used and highly effective medicine in the treatment of cancer, but the significant side effects to non-carcinoma cells, especially cardiomyocytes, limit its utilization [202]. The underlying mechanism of doxorubicin-induced cardiomyocyte apoptosis is not fully known, but findings indicate that AMPK dysfunction might be involved in the development of doxorubicin-induced cardiotoxicity. In isolated perfused hearts, doxorubicin causes a rapid reduction in AMPK activity and protein expression [203]. In line with this observation, activation of AMPK with 2-DG antagonizes doxorubicin-induced cardiomyocyte death in neonatal rat cardiomyocytes, and blocking AMPK activation with Compound C or siRNA attenuates the protective effects of 2-DG, indicating the protective role of AMPK activation against doxorubicin-induced cardiotoxicity [79]. Consistent with this, activation of AMPK with metformin protects adult mouse cardiomyocytes (HL-1 cells) from doxorubicin-induced apoptosis [204]. Furthermore, adiponectin treatment protects mice against doxorubicin-induced cardiomyopathy, and this beneficial effect is dependent on AMPK activation [205]. Mice expressing KD-AMPK (kinase-dead AMPK), which has a non-functional mutant kinase domain, do not exhibit dramatic cardiac dysfunction, although their hearts show impaired recovery of LV contractile function with increased caspase 3 activity and TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick-end labelling) staining after ischaemia and reperfusion. The results indicate a protective role of AMPK in limiting damage to cardiomyocytes from ischaemic injury [206]. Most recently, our group has reported in OVE26 mice that AMPK activation is reduced along with cardiac dysfunction, and that metformin can preserve cardiac function [207]. More importantly, genetic AMPK inhibition attenuated the protective effect of metformin on cardiomyocytes in a mouse diabetic model [208]. Despite all of these exciting lines of evidence for a beneficial role of AMPK activation in cardiomyocytes under multiple stress conditions, the exact mechanisms by which AMPK protects cardiomyocytes against injury require further investigation.

**Anti-inflammatory function of AMPK in CVD**

Inflammation has been well known to play an important role in CVD development and progress [209], and inflammation might be a link between the metabolic syndrome and CVD [210]. Extensive evidence suggests that AMPK might be an inflammatory repressor. For example, activation of AMPK by AICAR or metformin have been reported to inhibit inflammation [211,212], whereas inhibition of AMPK leads to increased inflammation in obese humans [213] and in an AMPKα1 genetic knockdown cell culture model [214]. AMPK activation might inhibit inflammation through multiple pathways and recent evidence suggests that inhibition of the NF-κB pathway might play a central role for this inhibitory effect. For example, overexpression of AMPK has been demonstrated to inhibit the NF-κB pathway in endothelial cells [215]. In support of this, NF-κB activity was increased in endothelial cells isolated from AMPKα2-knockout mice [148]. Furthermore, adiponectin application inhibited AngII-induced cardiac hypertrophy in rats by decreasing NF-κB activity and this beneficial effect was blocked by AMPK inhibition [217]. In cultured HUVECs, metformin has been shown to inhibit TNF-α-induced IKK [IkB (inhibitory κB) kinaseα/β] phosphorylation, IkBα degradation and IL (interleukin)-6 production in an AMPK-dependent manner. Most recently, Bess et al. [218] have reported that AMPK could contribute to the anti-inflammatory effect of NO in endothelial cells by directly phosphorylating IKK at Ser177 and Ser181. Despite all of these findings, whether there is a direct link between the AMPK/NF-κB pathway and CVD requires further investigation.

**Cardioprotective effects of AMPK activators**

Metformin lowers the risk of cardiovascular incidents in patients with Type 2 diabetes

Patients with diabetes are at increased risk of CVDs, such as atherosclerotic CHD (coronary heart disease), diabetic cardiomyopathy and stroke (for detailed information, see [219,220]). Metformin is one of the most widely prescribed antidiabetic medicines in the world. Interestingly, a large clinical study showed that patients with Type 2 diabetes treated with metformin have a 36 % lower risk of mortality and 39 % lower risk of myocardial infarction than those exposed to conventional treatment [221]. Similar results were reported later in a clinical
study that focused on older patients (over 65 years of age) with diabetes and HF (heart failure) [222]. These results are supported further by a comprehensive study which suggests that metformin is the only antidiabetic medicine that is not associated with deleterious effects, but is instead associated with reduced all-cause mortality in patients with HF and diabetes [223]. The exact mechanisms of the beneficial effects of metformin are not fully known; however, results, including reports from our group, have suggested that AMPK activation is required for the cardiovascular protective effects of metformin [50,81,208]. In vivo administration of metformin increases AMPK phosphorylation in the mouse aorta, resulting in increased NO synthesis and bioavailability [50]. A further study has demonstrated that AMPK activation by metformin increases the association between Hsp90 (heat-shock protein 90) and eNOS (endothelial NO synthase), which reduces eNOS-derived $\text{O}_2^{-}$ [224]. Although there is still considerable work to be done, current evidence suggests that patients with Type 2 diabetes with CVD will benefit from the activation of AMPK by metformin.

Protective role of statins in CVD

Statins are HMG-CoA reductase inhibitors used to lower cholesterol levels in patients. A clinical study has demonstrated that statin treatment reduced total stroke rates, including a substantial reduction in coronary event rates, in patients ranging widely in age [225]. In addition, a nationwide study of older patients with HF indicated that statin therapy significantly lowered mortality and improved outcomes in these patients [226]. The most recent clinical trial results further indicate that statins safely reduce the incidence of major atherosclerotic events in patients with advanced chronic kidney disease. Overall, the beneficial effect of statins in patients with CVD is unquestionable. Mechanistically, the beneficial effect of statins in the cardiovascular system derives from their cholesterol-lowering effect. Recent findings suggest that the direct beneficial effect of statins in the prevention of CVD might be mediated at the molecular level by AMPK. For instance, statins have been shown to increase AMPK activation and NO production in HUVECs and in mouse aortas and myocardium [227]. Similar results have been observed in bovine aortic endothelial cells [228]. Interestingly, the activation of AMPK by statins has been reported to promote the differentiation of EPCs (endothelial progenitor cells) by the activation of eNOS; these effects were blocked by the AMPK antagonist Compound C [229]. Thus activation of AMPK by statins might be an important mechanism involved in the beneficial effects of statins against CVDs. Despite exciting evidence on the role of statins in CVD, more work is required to define the mechanism of action of these compounds and the role of AMPK in its effects.

Other AMPK activators in CVD

As described above, several natural compounds are AMPK activators, including resveratrol and berberine. Studies in humans provide promising evidence of multiple beneficial effects of these two chemicals in both physiological and pathological conditions. For example, resveratrol can modulate cerebral blood flow and cognitive performance in healthy humans [230]. Furthermore, resveratrol supplementation has been reported to improve flow-mediated dilation of the brachial artery in 19 obese volunteers [231]. Consistent with these observations, a study has demonstrated the significant cardiovascular-protective effects of resveratrol in vivo and in vitro [232]. Similarly, berberine supplement has been reported to have beneficial cardiovascular effects in a study including 156 patients with chronic CHF (congestive HF) [233]. Importantly, a clinical study has reported that berberine might be used as a hypolipidaemic drug, as oral administration of berberine for 3 months reduced serum cholesterol by 29%, triacylglycerols by 35% and LDL (low-density lipoprotein)-cholesterol by 25% in 32 hypercholesterolaemic patients [234]. Whether those clinical beneficial effects are AMPK-mediated, and the exact mechanisms by which these compounds activate AMPK, deserve more investigation.

CONCLUSIONS

Taken together, the information described above clearly indicates that AMPK functions far beyond its proposed energy sensor and regulator function. AMPK regulates ROS/redox balance, autophagy, cell proliferation, cell apoptosis, cellular polarity, mitochondria function and genotoxic response, either directly or indirectly via numerous downstream pathways. Further investigations are required to clarify the exact molecular targets of AMPK in physiological and pathological conditions and to develop potential strategies to modulate AMPK activation in diabetes, cancer, and cardiovascular dysfunction.

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