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Targeting AMPK for Cardiac Protection: Opportunities and Challenges

Maengjo Kim and Rong Tian

Mitochondria and Metabolism Center, Department of Anesthesiology and Pain Medicine, University of Washington, Seattle WA

Abstract

AMP-activated protein kinase (AMPK) regulates cellular energy homeostasis and multiple biological processes in cell growth and survival, hence an attractive drug target. AMPK is a heterotrimeric protein consisting of α catalytic, β and γ regulatory subunits; two isoforms of each subunit are present in the heart. Studies using both genetic and pharmacological approaches have demonstrated important roles of AMPK in protecting the heart during ischemia/reperfusion injury as well as in pathological hypertrophy and failure. There is also emerging evidence suggesting isoform-specific function of AMPK, e.g. mutations of the $\gamma 2$ subunit cause human cardiomyopathy. Thus, strategies avoiding the undesirable effects of altering $\gamma 2$ -AMPK activity, such as isoform selective activation of AMPK may lead to cardioprotective therapies with greater efficacy and safety.

Keywords

AMPK; energy homeostasis; drug target; isoform; cardiomyopathy

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase and is phylogenetically conserved from yeast to mammals. AMPK is sensitive to a broad spectrum of stresses, especially those that cause changes in cellular energy status. Activation of AMPK stimulates ATP-generating pathways, such as glucose uptake, glycolysis and fatty acid oxidation, and inhibits ATP-consuming pathways, such as fatty acid and cholesterol synthesis [1,2]. Therefore, the kinase is originally considered a master metabolic switcher that controls the cellular and whole body energy homeostasis. More recent work has also revealed an important role of AMPK in cell growth and survival through its interactions with mitochondrial biogenesis, protein synthesis and degradation pathways [3-5]. Thus, there has been intense interest in targeting AMPK for the treatment of multiple prevalent diseases, such as diabetes and obesity, cancer and cardiovascular diseases.

AMPK structure and activity

AMPK is a heterotrimeric complex composed of a catalytic α - subunit and two regulatory β - and γ - subunits. Each subunit exists in multiple isoforms encoded by separate genes ($\alpha 1$, $\alpha 2$,

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Correspondence: Rong Tian, MD, PhD, Mitochondria and Metabolism Center, University of Washington School of Medicine, 815 Mercer St., Seattle, WA 98109, Phone: 206-543-8982, Fax: 206-616-4819, rongtian@u.washington.edu .

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$\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, and $\gamma 3$), and their combination give rise to a variety of AMPK holoenzymes (Figure 1). Phosphorylation of Threonine 172 in the catalytic domain of the α subunit is required for AMPK activation. The C-terminal domain is required for binding with the β and γ subunits. The β subunit has scaffold/docking properties and contains two domains, glycogen binding domain and the C-terminal domain for binding with the α and γ subunits [6]. The β subunit also contains several sites for post-translational modifications such as myristoylation and phosphorylation which may regulate the subcellular localization of the heterotrimer [7]. The γ subunit contains four cystathionine- β -synthase (CBS) motifs in the C-terminus. Each pair of the CBS motifs tightly associate in a pseudo dimeric arrangement through their β -sheets forming a so-called Bateman domain. Nucleotide binding assays suggested that each Bateman domain bound one molecule of AMP or ATP in an exclusive manner [8]. This observation was supported by a recent crystal structure study which in addition revealed a third site containing a non-exchangeable AMP [9]. The binding of AMP activates while the binding of ATP inactivates the kinase, thus, the two exchangeable sites on the γ subunit are responsible for the sensitivity of AMPK to the AMP/ATP ratio in the environment.

The AMP/ATP ratio regulates AMPK activity by several mechanisms (Figure 1). First, the binding of AMP in the γ subunit allosterically activates the kinase. Increased AMP binding also facilitates the phosphorylation of and protects against the dephosphorylation of Thr 172, thus promotes and sustains the kinase activity [10]. The combined effects of the allosteric activation and Thr 172 phosphorylation increase AMPK activity by 1000-fold [11]. Since the [ATP] in the cell is several orders of magnitude higher than [AMP], AMPK activity is very low in unstressed conditions. However, because of the large difference in free concentrations of the nucleotides, a small decrease in [ATP] can lead to a large increase of [AMP] ($\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP}$) resulting in a great change of AMP/ATP. In this way, AMPK functions as an ultra-sensitive gauge of cellular energetic status [12].

Multiple upstream kinases of AMPK (AMPKK) have been reported, including LKB1, Ca^{2+} /calmodulin-dependent protein kinase kinase β (CaMKK β), and transforming growth factor- β activated kinase 1 (TAK1) (Figure 1). LKB1, encoded by the Peutz-Jeghers syndrome tumor suppressor gene, exists in a heterotrimeric complex associated with two accessory proteins, mouse protein 25 (MO25 α/β) and Ste20-related adaptor protein (STRAD α/β). LKB1 complex functions as a constitutively active kinase that phosphorylates AMPK in the presence of AMP [13]. LKB1-AMPK pathway exists in majority of cell and tissue types except in HeLa cells where LKB1 is absent [14]. CaMKK β has considerable sequence and structural homology with LKB1 but its activity is dependent on intracellular Ca^{2+} levels. Recent studies suggest that CaMKK β regulates AMPK in a Ca^{2+} /calmodulin-dependent and AMP-independent manner [11]. The expression of CaMKK β in the heart is very low but the CaMKK β -AMPK cascade likely has an important role in the neurological tissues [15,16]. TAK1 was first shown to activate the yeast AMPK homologue Snf1 and purified mammalian AMPK and more recently in human cell culture [17,18]. Deletion of TAK1 results in decreased phosphorylation of AMPK in mice [19].

Recent studies have also proposed that the AMPKKs may be constitutively active and the regulation of AMPK phosphorylation and activity is, instead, achieved by the dephosphorylation process. Increased AMP has been shown to prevent the dephosphorylation and inactivation of AMPK by protein phosphatase [10] but the specific phosphatase for the AMPK *in vivo* as well as its regulation remains to be defined.

Several endocrine factors/hormones have been reported to regulate AMPK activity, such as adiponectin, leptin, ciliary neurotrophic factor and ghrelin [20-23]. The mechanisms of action for these factors/hormones are complex involving both central nervous system and

direct effects on the peripheral tissue. It is though very unlikely these factors/hormones interact directly with the AMPK complex.

Tissue Distribution of AMPK

Although AMPK is widely distributed, tissue-specific expressions of selective isoforms have been reported [24-26]. Among the seven isoforms, the $\alpha 1$, $\beta 1$ and $\gamma 1$ isoforms are ubiquitously expressed. The $\alpha 2$ and $\beta 2$ isoforms are highly expressed in heart and skeletal muscle. The $\gamma 2$ isoform is expressed in several tissues including the heart, whereas the $\gamma 3$ isoform is exclusively expressed in skeletal muscle. The differential expression pattern of the isoforms enables a tighter regulation of the AMPK activity in a tissue dependent manner. It also provides a unique opportunity to modulate AMPK activity in selective tissues or to target isoform-specific AMPK function(s) using pharmacological approaches.

Summarized in Table 1 are reported distributions of each subunit isoform in mammalian tissues. In cardiac and skeletal muscle, $\alpha 2$ AMPK complexes accounted for 70-80% of total AMPK activity while $\alpha 1$ complexes accounted for the remaining 20-30% [26]. In contrast, $\alpha 1$ complexes accounted for 60-90% of total AMPK activity with $\alpha 2$ complexes for the remaining 10-40% in lung, kidney, testis and brain. Liver had the same contribution of $\alpha 1$ and $\alpha 2$ AMPK to the total activity. Yang et al. have demonstrated that $\alpha 1$ subunit isoform was more abundantly expressed in adipose tissue, peritoneal macrophages, and spleen compared to muscle and might account for AMPK activation in those tissues [27]. The distribution of γ -subunit isoform was different. The $\gamma 1$ complexes accounted for the major part (80-90%) of total AMPK activity in liver, lung, kidney, pancreas, cardiac and skeletal muscles in rodents. The $\gamma 2$ complexes accounted for 10-20% of the total activity in these tissues [26]. Even though $\gamma 3$ mRNA level was readily detected in the skeletal muscle, the activity of $\gamma 3$ complexes was elusive [26]. A recent study showed that ~20% of $\alpha 2$ complexes and none of the $\alpha 1$ complexes in human skeletal muscle contained $\gamma 3$ -subunit [28-30].

The functional significance of the isoform composition of AMPK was not recognized until recently when studies of human skeletal muscle have shown that only 3 of the 12 possible AMPK complexes were present ($\alpha 1\beta 2\gamma 1$, $\alpha 2\beta 2\gamma 1$, and $\alpha 2\beta 2\gamma 3$), and their expressions were differentially regulated during exercise depending on strength and duration [28-30]. Furthermore, exercise caused nuclear translocation of $\alpha 2$ -AMPK, which likely mediated the transcriptional regulation of glucose metabolism in human skeletal muscle [31,32]. In mouse C2C12 myoblasts, nuclear translocation of $\alpha 2$ -AMPK by leptin occurred only in complexes containing $\beta 2$ subunit while $\alpha 2$ -AMPK containing the $\beta 1$ subunit was retained in the cytoplasm [33]. The subcellular redistribution of AMPK complexes in the heart has not been explored. In addition, mechanisms responsible for the isoform-specific responses to various physiological or pathological stimuli are largely unclear at present. A thorough understanding of these questions holds a great promise to achieve isoform-selective targeting of AMPK for therapeutic purposes, and is thus highly warranted.

AMPK function in the heart

AMPK activity in the heart is very low under normal conditions; its function in the unstressed heart is not clearly defined. However, activation of AMPK is rapid and robust in response to a variety of stresses such as ischemia, exercise and chronic pressure overload [34-36]. A number of studies using pharmacological activators of AMPK or transgenic mice expressing inactive catalytic subunit of AMPK have been carried out to understand the functional role of AMP during stresses.

Gain of Function Studies

Most of the studies in the literature used AICAR or metformin to activate AMPK. AICAR is a nucleoside 5-aminoimidazole-4-carboxamide riboside that is taken up by the cells and then converted into the AMP analog, ZMP. The ZMP activates AMPK by binding to the γ subunit as an AMP mimetic. Metformin is a biguanide derivative that has been used for the treatment of hyperglycemia in diabetes with unknown mechanisms. Recent studies have shown that metformin indirectly activates AMPK by increasing AMP/ATP in hepatocytes through inhibition of complex I in the mitochondrial respiratory chain [37].

Activation of AMPK in the heart by ischemia or AICAR stimulates glucose uptake and glycolysis by translocating the glucose transporters to the cell membrane and activating phosphofructokinase-2 via insulin-independent mechanism(s) [38,39]. These effects of AMPK have been shown to maintain energy homeostasis and protect against ischemic injury in the heart [40,41]. AMPK also stimulates fatty acid oxidation (FAO) via phosphorylation and inactivation of acetyl-CoA carboxylase 2 (ACC2) [42]. Although increased FAO promotes ATP production, previous studies have shown that a high level of FAO contributes to reperfusion injury, thus, suggesting that increased AMPK activity during reperfusion is not desirable [43,44]. However, failure to activate AMPK *in vivo* results in more cell death and larger infarct size, suggesting that the biological effects of AMPK during the ischemia/reperfusion insult may have extended beyond the acute regulation of cell metabolism [40]. Recent evidence suggest that AMPK modulates mTOR signaling and autophagy, both are closely linked to cell survival during stress [16,45].

Studies in the last decade have also suggested a cardioprotective role of AMPK during chronic stresses that lead to pathological cardiac hypertrophy. Increased AMPK activity was first found in hypertrophied hearts with impaired energetics leading to the hypothesis that activation of AMPK is a compensatory response to restore energy balance [36]. Activation of AMPK by pharmacological compounds such as, AICAR or metformin, has been shown to inhibit cardiac hypertrophy, blunt cardiac remodeling and delay the development of heart failure [46,47]. Several signaling mechanisms downstream of the AMPK cascade have been implicated in cardiac hypertrophy, including metabolic pathways, protein synthesis and degradation mechanisms, mitochondrial biogenesis, and NO signaling [47-49]. It has been shown that activation of AMPK in neonatal rat cardiac myocytes by AICAR or metformin leads to phosphorylation and inactivation of eEF-2 kinase thus blunting the hypertrophic response to phenylephrine [47]. Metformin treatment in mouse models of myocardial infarction improved mitochondrial function and reduced LV remodeling in an AMPK and eNOS dependent mode [50]. Even though AMPK is activated during cardiac stress, pharmacological activation that induces an early and sustained increase of AMPK activity likely provides additional beneficial effects.

It should be noted that although effective and widely used as AMPK activators, pharmacological compounds such as metformin or AICAR have significant off-target effects [51-54]. For example, recent studies have shown that metformin affects hepatic gluconeogenesis and mTOR signaling via AMPK-independent mechanisms [55,56]. Thus, results from these studies need to be interpreted with caution and best to be confirmed with genetic approaches.

Activation of AMPK by genetic approach appeared to be more complicated than expected. There has been no report of mouse models of increased AMPK activity by simply overexpressing the catalytic subunits likely due to the fact that the kinase is tightly regulated and a heterotrimer is required for the activity [57,58]. Recently, mutations of the γ regulatory subunit have been found to abolish the sensor function of the Bateman domains thus altering the kinase activity [8,59]. Mutations of the $\gamma 3$ subunit (encoded by PRKAG3)

caused gain of function changes of AMPK resulting in glycogen storage in the skeletal muscle of pigs and mice [60-62]. Similar mutations of the $\gamma 2$ subunit (encoded by PRKAG2) cause human cardiomyopathy with glycogen storage, severe cardiac hypertrophy and arrhythmias [63-65]. The PRKAG2 cardiomyopathy has been attributed to the aberrant activation of AMPK that causes metabolic derangement and glycogen storage [63,66,67]. However, decreased AMPK activity has also been observed in mouse hearts expressing some PRKAG2 mutants, possibly due to feedback inhibition of the kinase activity [68,69]. Interestingly, the PRKAG2 mutations present primarily a cardiac phenotype suggesting a cardiac-specific function of this particular isoform.

The heart is made up of cardiomyocytes and non-myocyte cell types. AMPK is expressed in the cardiomyocytes as well as fibroblasts, endothelial cells and smooth muscle cells in the heart. The functional role of AMPK in non-cardiomyocytes is just being understood. AMPK in endothelial cells has been shown to be involved in maintaining endothelial function through the anti-inflammatory and anti-atherogenic properties [70-72]. Previous studies showed that AMPK activated eNOS (endothelial NO synthase) in endothelial cells in response to shear stress and myocardial ischemia contributing to the regulation of vascular tone under these conditions [73,74]. AMPK activation inhibited the vascular smooth muscle cell hypertrophy induced by angiotensin II [75], and activation of AMPK in cardiac fibroblasts was also reported to be cardioprotective [76].

Loss of Function Studies

There are a number of genetic models for reduced AMPK activity including deletions of $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$ or $\gamma 3$ subunit of AMPK in mice [60,77-79]. Mice expressing dominant negative or kinase inactive catalytic subunit of AMPK in the heart or in the heart and skeletal muscles are also available [40,41]. There is, however, no report of genetic models with complete loss of AMPK activity in the heart at the present time. Many studies with genetic null mice for AMPK have shown that the reduced AMPK activity did not cause any cardiac dysfunction in normal conditions but did affect on cardiac function during stress. Mice deficient of $\alpha 2$ catalytic subunit of AMPK exhibited exacerbated left ventricular hypertrophy and dysfunction during chronic pressure-overload [80]. In isolated perfused mouse hearts with the kinase inactive mutant of $\alpha 2$ subunit or complete deletion of $\alpha 2$ subunit, the reduced AMPK activation developed a more rapid and severe contracture of the ischemic hearts and was detrimental for the heart during ischemia [40,41,81]. But, the response to ischemia/reperfusion was somewhat dependent on the perfusion condition [40,82].

Several AMPK inhibitors have been used in a number of in vitro studies. The most widely used AMPK inhibitor, Compound C, is a cell permeable pyrazolopyrimidine compound and acts as ATP-competitive inhibitor of AMPK [37]. Like many other kinase inhibitors, this inhibitor is not strictly specific to AMPK. Several studies have reported that Compound C can inhibit a number of other protein kinases and hence inhibits various biological events independently of AMPK activity [83,84]. Furthermore, the very short half-life of the Compound C significantly limits its use for studies *in vivo*. Thus, specific AMPK inhibitors and/or additional loss of function models are necessary for our further understanding of the kinase function in vivo.

AMPK as a potential therapeutic target

AMPK is a key enzyme that regulates cardiac energy homeostasis and multiple biological processes in cell growth and survival, hence an attractive drug target. A number of pharmacological compounds currently on the market have been shown to activate AMPK, including the anti-diabetic drugs metformin, thiazolidinediones (TZD) and the cholesterol lowering drug statins [70]. It is likely that these compounds activate AMPK through an

indirect effect of increasing AMP/ATP, and in the case of metformin via inhibition of mitochondrial respiration [85]. To achieve potent and specific activation of AMPK, “direct” activators would be most desirable. By definition, a direct activator should directly interact with the kinase to increase its activity. Several compounds in this category have been reported in cell culture or animal studies. A-769662, a compound developed by Abbot Laboratory, is a nonnucleoside thienopyridone derivative that directly and exclusively activates AMPK complexes containing the $\beta 1$ subunit [86]. Although it may not achieve maximal activation of AMPK in tissues expressing both isoforms of β subunit, it presents the exciting possibility of implementing AMPK-isoform selective therapeutics. A-769622 was well tolerated in small animals but a recent study showed that it caused inhibition of 26S proteasome function and arrest of cell cycle progression via an AMPK independent mechanism [87]. Another small molecule compound, PT1, is shown to activate both $\alpha 1$ and $\alpha 2$ isoforms through the relief of autoinhibition in the catalytic α subunits [88]. It is thus possible that PT1 can activate all 12 AMPK complexes although the in vivo studies of PT1 are still pending.

There are several concerns of activating AMPK systemically as a therapeutic strategy. AMPK activity in hypothalamus is positively correlated with food intake [23,89]. The potential effectiveness of perturbing energy homeostasis via AMPK activation was also challenged by recent studies demonstrating that direct targeting of ACC2, the primary downstream target of AMPK, minimally affects whole body energy balance [90,91]. This, however, does not exclude the possibility of achieving metabolic modulation via other AMPK downstream targets. Furthermore, these concerns do not detract from the potential benefit of AMPK activation for cardioprotection. The conceptual obstacle in developing cardiovascular selective AMPK activators is the observation that the gain of function mutation in the γ subunit of AMPK causes glycogen storage in the skeletal muscle ($\gamma 3$ subunit) and the heart ($\gamma 2$ subunit) in both animals and human [61,62,64-66]. The glycogen storage cardiomyopathy is associated with severe cardiac hypertrophy and arrhythmias suggesting that activation of $\gamma 2$ -containing AMPK in the heart may not be desirable. In the regard, it becomes critical to understand the isoform-specific function of the γ -subunit. If the PRKAG2 cardiomyopathy represents a unique phenotype of altered $\gamma 2$ -AMPK function, selective activation of $\gamma 1$ -AMPK will be a viable therapeutic strategy. These considerations further emphasize the significance of developing tissue selective and isoform specific activators of AMPK. Therefore, understanding of the isoform specific functions of the AMPK complexes is an important focus of future research in the field.

Conclusions

AMPK is a master protein kinase that mediates energy homeostasis and has been considered as a drug target for obesity and metabolic syndrome. The accumulating evidence indicates that AMPK is also critically involved in cardiovascular diseases such as cardiac hypertrophy and myocardial ischemia. Future research should be directed to further understanding of cell type-specific and isoform-specific AMPK activation in the pathogenesis as well as the treatment of cardiovascular diseases. Advances in the development of isoform-selective and direct activators of AMPK will likely deliver the therapy with greater efficacy and safety.

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Figure 1.

Regulation of the AMPK signaling cascade. The intracellular AMP/ATP ratio is a primary regulator of the system. Increased AMP/ATP activates AMPK via three mechanisms 1) allosteric activation; 2) promotes the Thr 172 phosphorylation of the α -AMPK by its upstream kinase (AMPKK) such as LKB1, and 3) prevents protein phosphatase from dephosphorylating AMPK. Stress can also directly activate the CAMKK β via increased intracellular calcium levels. It is proposed that certain stresses may directly affect the dephosphorylation process by yet undetermined mechanisms. AMPK, AMP-activated protein kinase; AMPKK, AMPK-kinase; CaMKK β , Ca²⁺/calmodulin-dependent protein kinase kinase β ; TAK1, transforming growth factor- β activated kinase 1; CD, catalytic domain; GBD, glycogen binding domain; N, NH₂-terminus of subunits; CBS, cystathionine- β -synthase motif.

Table 1

Tissue distribution of AMPK subunit isoforms

Tissue	α -subunit	β -subunit	γ -subunit
Adipose tissue	$\alpha 1$: 90% ^a $\alpha 2$: 10% ^a	ND	ND
Brain	$\alpha 1$: 75% ^b $\alpha 2$: 25% ^b	ND	$\gamma 1$: 36% ^b $\gamma 2$: 36% ^b
Bone Osteoblast Osteoclast	$\alpha 1$: 100% ^c $\alpha 1$: 100% ^d	ND $\beta 1$: 100% ^d	$\gamma 1$: 100% ^d $\gamma 1$: 100% ^d
Heart Endothelial cell Smooth muscle	$\alpha 1$: 30% ^b $\alpha 2$: 70% ^b $\alpha 1 > \alpha 2$ ^f ND	$\beta 1 < \beta 2$ ^e $\beta 1 > \beta 2$ ^f ND	$\gamma 1$: 85% ^b $\gamma 2$: 15% ^b $\gamma 1 < \gamma 2$ ^f ND
Kidney	$\alpha 1$: 80% ^b $\alpha 2$: 20% ^b	$\beta 1 < \beta 2$ ^e	$\gamma 1$: 80% ^b $\gamma 2$: 20% ^b
Liver	$\alpha 1$: 45% ^b $\alpha 2$: 55% ^b	$\beta 1 > \beta 2$ ^e	$\gamma 1$: 90% ^b $\gamma 2$: 10% ^b
Lung	$\alpha 1$: 90% ^b $\alpha 2$: 10% ^b	$\beta 1 > \beta 2$ ^e	$\gamma 1$: 80% ^b $\gamma 2$: 20% ^b
Skeletal muscle	$\alpha 1$: 20% ^b $\alpha 2$: 80% ^b	$\beta 1 < \beta 2$ ^e	$\gamma 1$: 90% ^b $\gamma 2$: 10% ^b $\gamma 3$: ND

ND: the isoforms are present but the relative abundance is not determined.

^a determined by activity assay in rodent [26,89]^b determined by activity assay in rodent [25]^c determined by RT-PCR in rodent [90]^d determined by Western blot in rodent [71]^e determined by Northern and Western blots in human and rodent [24]^f determined by RT-PCR and Western blot in rodent [91]