Cell Metabolism
Review

AMPK: An Emerging Drug Target for Diabetes and the Metabolic Syndrome

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Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) is a key player in regulating energy metabolism, placing it at the center stage in studies of diabetes and related metabolic diseases. Expressed in key metabolically relevant organs, AMPK is activated in response to a variety of stimuli, including cellular stress, exercise, and a wide range of hormones and agents that exert impacts on cellular metabolism. Genetic and pharmacological studies demonstrate that AMPK is required for maintaining glucose homeostasis. Activation of AMPK by pharmacological agents presents a unique challenge, given the complexity of the biology, but holds a considerable potential to reverse the metabolic abnormalities associated with type 2 diabetes.

Introduction

According to National Diabetes Statistics, the number of people with diagnosed and undiagnosed diabetes in the United States reached 23.6 million, which is 7.8% of the general population, in 2007. The total number of people worldwide with diabetes is projected to rise to 366 million in 2030. A number of therapeutic agents exist for the treatment of type 2 diabetes mellitus (T2DM), including metformin, sulfonylureas, DPP-4 inhibitors, PPARγ agonists, α-glucosidase inhibitors, insulin, and GLP-1 analogs. However, in addition to inadequate efficacy and durability, some of these agents suffer from liabilities, including hypoglycemia, weight gain, edema, fractures, lactic acidosis, and gastrointestinal intolerance (Nathan et al., 2009). In aggregate, there is a pressing need to develop novel modalities for the treatment of diabetes to stem the spread of this global epidemic. AMP-activated protein kinase (AMPK) is a potential target for novel agents that may meet this challenge.

AMPK is a major cellular energy sensor and a master regulator of metabolic homeostasis. AMPK is a heterotrimeric enzyme comprised of a catalytic (α1 or α2) subunit and two regulatory (β1 or β2 and γ1, γ2, or γ3) subunits, all of which are encoded by separate genes, making it possible to form a total of 12 complexes (Hardie, 2008) (Figure 1). AMPKs are activated by two distinct signals: a Ca^{2+}-dependent pathway mediated by CaMKKβ and an AMP-dependent pathway mediated by LKB1 (Sanders et al., 2007b). These and other upstream kinases, including TGF-β-activated kinase-1 (Tak1), phosphorylate Thr172 on the α subunit. Binding of AMP to the γ subunit leads to allosteric activation of AMPK as well as protection of Thr172 from dephosphorylation, thereby maintaining the enzyme in the activated state. AMPK thus serves as a unique metabolic control node as it senses cellular energy status through modulation of its activities via phosphorylation and allosteric activation by AMP. Activation of AMPK by Tak1 leads to cytoprotective autophagy in untransformed cells against TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (Herrero-Martin et al., 2009).

A number of physiological processes have been shown to stimulate AMPK, including conditions that lead to alterations of the intracellular AMP/ATP ratio (e.g., hypoxia, glucose deprivation) and calcium concentration, as well as the action of various hormones, cytokines, and adipokines. The activated form of the enzyme is responsible for metabolic changes via phosphorylation of various downstream substrates. The net effect is a change in local and whole-body energy utilization from an energy-consuming state to an energy-producing state in order to restore energy balance. These findings, coupled with reports that AMPK in muscle is activated in response to exercise (Long and Zierath, 2006; Reznick and Shulman, 2006), have led to an intense interest in developing AMPK activators as potential therapies for T2DM and obesity (Hardie, 2008).

The effects of AMPK activation are pleiotropic in key metabolically relevant tissues, such as liver, skeletal muscle, adipose, and hypothalamus. In this review, we will focus the discussion on the beneficial effects of AMPK activation in liver and muscle on modulation of insulin sensitivity and energy homeostasis. We will explore key issues and challenges that need to be addressed in the pursuit of AMPK activators for the treatment of diabetes, obesity, and related metabolic diseases.

Role of AMPK Activation in Countering Liver and Muscle Insulin Resistance

In T2DM, the major insulin-resistant organs include liver, muscle, and adipose tissue. In a state of insulin resistance, glucose uptake and utilization are dramatically decreased, and skeletal muscle becomes metabolically inflexible, unable to switch between glucose and fatty acid use (Kelley et al., 1999). More recently, defects in fatty acid oxidation have been considered the primary causal factors in the development of muscle insulin resistance (Petersen et al., 2004). While deficiencies in skeletal muscle mitochondrial capacity and fatty acid metabolism may be responsible for intramyocellular lipid accumulation leading to an insulin-resistant state (Petersen et al., 2004), it is important to point out that incomplete fatty oxidation and mitochondrial overload also contribute to skeletal muscle insulin resistance (Koves et al., 2008), considering that the capacity of muscle to oxidize substrate is far in excess of what is needed to supply energy to resting muscle (Holloszy, 2009).

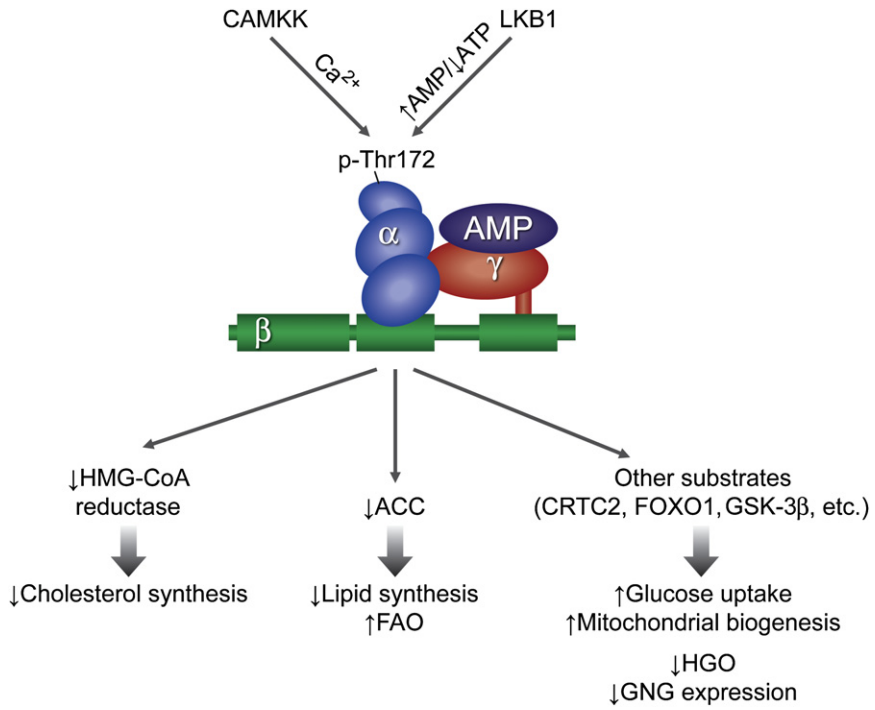

Figure 1. Activation of AMPK by Upstream Kinases and the Potential Beneficial Consequences

LKB1 is activated by an increase of the AMP/ATP ratio, while CAMKK is activated by an increase in intracellular Ca^{2+}. Both LKB1 and CAMKK phosphorylate AMPK on Thr172. The key substrates of AMPK as well as the consequences of AMPK activation are indicated. FAO, fatty acid oxidation; HGO, hepatic glucose output; GNG, gluconeogenic genes.

AMPK Action in Liver

Liver AMPK controls glucose homeostasis mainly through the inhibition of gluconeogenic gene expression and hepatic glucose production. In primary hepatocytes, AMPK activation mediated by both AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside [see below]) and metformin has been demonstrated to downregulate PEPCK and G6Pase (Lochhead et al., 2000; Zhou et al., 2001). Expression of PEPCK and G6Pase are induced by transcription factor binding to the cAMP-responsive element (CRE) of these genes involving CRTC2 (CRE-binding protein [CREB]-regulated transcription coactivator 2) and FOXO1 (forkhead box O1), both of which are suppressed by AMPK (Liu et al., 2008). CRTC2 also drives the expression of PPARγ coactivator 1α (PGC-1α), which in turn increases gluconeogenesis (Shaw et al., 2005). In addition, AMPK activation increases phosphorylation of GSK-3β (glycogen synthase kinase 3β) and thereby reduces CRE transcriptional activity and PEPCK-C gene expression in the liver, reducing gluconeogenesis (Horike et al., 2008).

Glucose production in primary cultured hepatocytes was suppressed by both metformin (Zhou et al., 2001) and an adipokine, adiponectin (Yamauchi et al., 2002), as well as by adenovirus expressing a constitutively active form of AMPKα2 (Foretz et al., 2005). In animal models, infusion of AICAR suppressed endogenous hepatic glucose production and lowered plasma glucose (Bergeron et al., 2001). In diabetic patients, the glucose-lowering effect of metformin is at least partly attributed to its ability to suppress gluconeogenesis (Hundal et al., 2000). The effect of metformin on AMPK activation and glucose lowering was abolished in *LKB1*$^{-/-}$ mice (Shaw et al., 2005), demonstrating the importance of LKB1, the major kinase of AMPK in liver, in glucose control by this drug.

Liver AMPK also decreases hepatic lipogenesis. AMPK phosphorylates and inactivates acetyl-CoA carboxylase 1 (ACC1) and 3-hydroxy-3-methylglutaryl-CoA reductase, leading to inhibition of de novo fatty acid and cholesterol synthesis. Phosphorylation of ACC2 by AMPK, on the other hand, causes increases of fatty acid oxidation (Viollet et al., 2009). ACC is a rate-determining enzyme for the synthesis of malonyl-CoA, both a critical substrate for fatty acid biosynthesis and a potent inhibitor of fatty acid oxidation (Figure 2). AMPK can also increase the activity of malonyl-CoA decarboxylase (MCD) (Assifi et al., 2005) to further decrease hepatic malonyl-CoA levels. Hypertriglyceridemia has been documented in both whole-body *AMPKα2*$^{-/-}$ and liver-specific *AMPKα2*$^{-/-}$ mice (Andreelli et al., 2006). In addition, AICAR infusion has been shown to decrease plasma TG (Bergeron et al., 2001). AMPK also mediates suppression of lipogenic gene expression (fatty acid synthase [FAS] and ACC) via decreasing the actions of transcriptional factors SREBP-1c (Foretz et al., 2005; Zhou et al., 2001) and ChREBP (Kawaguchi et al., 2002). AMPK inactivates mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) (Muoio et al., 1999), providing a means to reciprocally regulate acyl-CoA channeling toward β-oxidation and away from glycerolipid biosynthesis.

AMPK Action in Muscle

Muscle AMPK is activated during contraction and may mediate multiple beneficial effects of exercise. As in the liver, acute muscle AMPK activation increases fatty acid oxidation by decreasing malonyl-CoA concentrations though inhibiting ACC and activating MCD. Increased mitochondrial fatty acid oxidation results in decreased intramyocyte lipid accumulation and increased muscle insulin sensitivity (Ruderman et al., 2003). Chronic muscle AMPK activation, by exercise training and by AICAR treatment, can further enhance muscle lipid breakdown and the capacity for ATP generation by transcriptional activation of mitochondrial fatty acid β-oxidation enzymes via PGC-1α (Suwa et al., 2003).

Mediation of glucose uptake by both muscle contraction and AMPK activation is insulin independent and additive with the effects of insulin. Studies have shown that the capacity for AMPK-mediated glucose uptake is intact in muscle cells from patients with T2DM, while insulin-induced glucose uptake is impaired (Koistinen et al., 2003). Similar to a single bout of exercise, the insulin sensitivity of muscle, liver, and whole body was increased 24 hr after a single dose of AICAR in a high-fat-fed

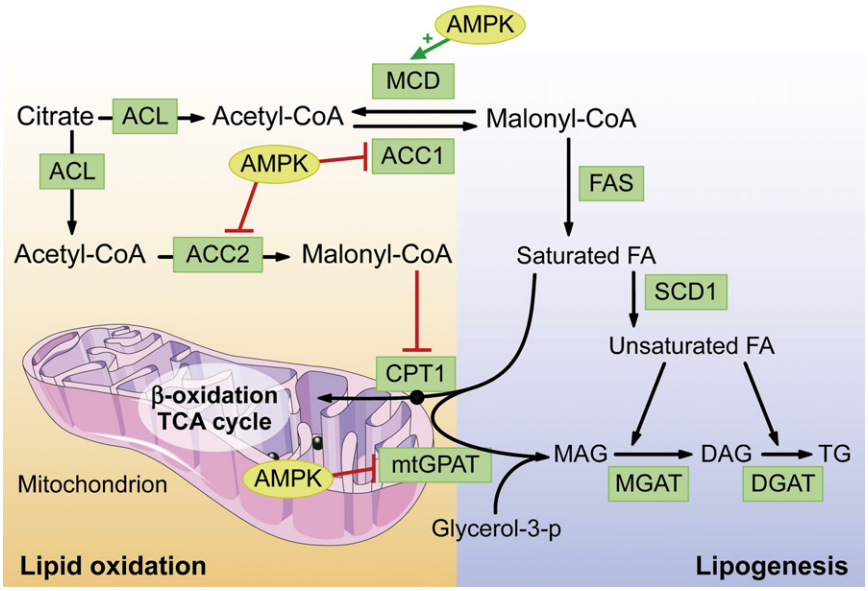

Figure 2. Schematic Diagram of Lipid Metabolism and the Regulation by AMPK
AMPK phosphorylates ACC1/2 and mtGPAT, inhibiting lipogenesis; AMPK activates MCD, reducing the levels of malonyl-CoA, thus inhibiting the substrate supply of lipogenesis. Reduction of malonyl-CoA also relieves CPT1 inhibition, increasing lipid oxidation.

insulin-resistant rat model (Iglesias et al., 2002). Contraction stimulates phospho-Akt substrate (PAS)-TBC1D1 and glucose transport but not PAS-TBC1D4 (also referred to as Akt substrate of 160 kDa [AS160]) in an AMPK-dependent manner (Funai and Cartee, 2009). Chronic AMPK activation has been shown to increase muscle Glut4, hexokinase, and glycogen content in skeletal muscle, similar to the effects of endurance training (Holmes et al., 1999).

AMPK activation has been shown to increase mitochondrial genes in skeletal muscle via PGC-1α induction (Jager et al., 2007). In parallel, AMPK increases cellular NAD(+) levels and enhances sirtuin 1 (SIRT1) activity, resulting in the deacetylation and activation of PGC-1α (Canto et al., 2009). Both insulin resistance and metabolic inflexibility are associated with muscle mitochondrial dysfunction in type 2 diabetic and obese subjects (Kelley et al., 2002), suggesting that AMPK-mediated mitochondrial improvement may overcome the metabolic inflexibility as well as insulin resistance. Indeed, 4 weeks of AICAR treatment was sufficient to induce genes involved in muscle metabolism and to enhance running endurance in sedentary mice (Narkar et al., 2008). In muscle-specific transgenic mice expressing an inactive form of the AMPKα2 catalytic subunit, the lack of skeletal muscle AMPKα2 activity exacerbated the development of glucose intolerance and insulin resistance caused by high-fat feeding (Fujii et al., 2008), suggesting a key role of AMPKα2 in glucose homeostasis.

Taken together, activation of AMPK in the liver and muscle is expected to elicit a spectrum of beneficial metabolic effects with the potential to ameliorate the defects associated with T2DM and the metabolic syndrome.

Other AMPK Substrates

In addition to the well-known AMPK substrates ACC and HMG-CoA reductase, many other substrates of AMPK are also documented. One example is histone deacetylase (HDAC) 5. Phosphorylation of HDAC5, a transcriptional repressor, causes HDAC5 to associate with 14-3-3. HDAC5 is then acetylated and falls off from GLUT4 promoter and exits the nucleus. The net result of AMPK activation is increased Glut4 transcription (McGee et al., 2008). This and other yet to be discovered actions of AMPK will further broaden our understanding of the biological consequences of AMPK activation.

Activation of AMPK by Indirect Activators

A number of hormones and pharmacological agents have been reported to activate AMPK in vivo or upon treatment of cells and/or tissues (Figure 3). While the detailed modes of action by which these agents activate AMPK are not fully delineated, they act in an indirect manner. Changes in mitochondrial coupling and cellular energy state could account for, at least in part, the mechanisms leading to cellular AMPK activation. Studies using these agents have aided our understanding of the role of AMPK in the regulation of cellular processes and whole-body energy homeostasis.

Metformin

Metformin is an antihyperglycemic agent with effects on suppressing hepatic glucose output. In 2001, it was reported that metformin activated AMPK in hepatocytes, reducing ACC activity and inducing fatty acid oxidation (Zhou et al., 2001), thus linking metformin action to AMPK activation. SREBP-1, a key lipogenic transcription factor, was reduced at both mRNA and protein levels in cells following metformin treatment. Metformin requires LKB1, which phosphorylates and activates AMPK in the liver to lower blood glucose levels (Shaw et al., 2005). These studies strongly suggest that activation of AMPK provides a unified explanation for the pleiotropic beneficial effects of metformin. Correlative results were also observed with respect to metformin increasing AMPK activity in skeletal muscle of subjects with type 2 diabetes (Musi et al., 2002).

Thiazolidinediones (TZDs)

TZDs are activators of PPARγ and function as insulin sensitizers. Incubation of isolated rat EDL muscles in culture medium containing 5 μM troglitazone for 15 min (a time course too brief to be attributable to transcriptional effects) significantly increased phosphorylation of AMPK and ACC, paralleled by a transient increase in the AMP/ATP ratio (LeBrasseur et al., 2006). In clamp studies, rosiglitazone treatment significantly enhanced AICAR-stimulated whole-body glucose disposal (Ye et al., 2006), suggesting that TZDs can activate AMPK by a mechanism that is likely independent of PPARγ-regulated gene transcription. However, the major effect of TZDs is likely to be on the release of adiponectin by adipocytes, leading to activation of AMPK in liver to reduce glucose production (Kubota et al., 2006).

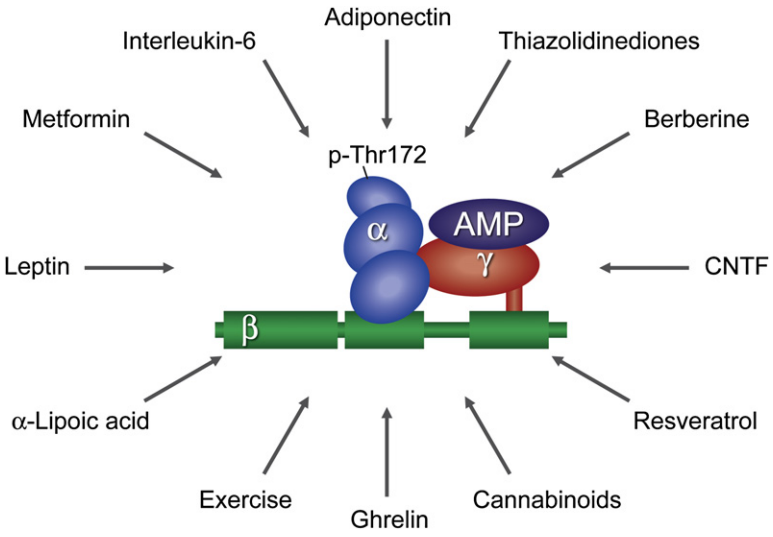

Figure 3. Modulators of AMPK Activation
Exercise and the agents listed have been shown to modulate the activity of AMPK. See text for more details.

Adiponectin

Full-length adiponectin induces phosphorylation and activation of AMPK in skeletal muscle and liver, increasing phosphorylation of ACC and fatty acid oxidation, enhancing glucose uptake and lactate production in myocytes, and reducing glucose levels in vivo (Yamauchi et al., 2002). The requirement of AMPK activation in mediating the adiponectin effects was confirmed by blocking AMPK activation with dominant-negative mutants of AMPK, which inhibited the stimulation of glucose utilization and fatty acid oxidation by adiponectin. Insulin but not adiponectin totally inhibited endogenous glucose production in liver-specific *AMPKα2*$^{-/-}$ mice, further demonstrating the role of AMPK in adiponectin action (Andreelli et al., 2006).

Leptin

Leptin selectively stimulates phosphorylation and activation of the α2 catalytic subunit of AMPK in skeletal muscle. It was reported that early activation of AMPK occurred by leptin acting directly on muscle, whereas the later-phase activation depended on leptin functioning through the hypothalamic-sympathetic nervous system axis (Minokoshi et al., 2002). In addition to AMPK phosphorylation, leptin also induced PPARα gene transcription in C2C12 cells. In these cells, the α2β1-containing AMPK complexes resided in the cytoplasm and phosphorylated ACC, leading to increases in fatty acid oxidation. In contrast, α2β2-containing AMPK complexes underwent translocation to the nucleus, where they induced PPARα gene transcription. A nuclear localization signal and Thr172 phosphorylation of α2 were found to be essential for nuclear translocation of AMPKα2, whereas the myristoylation of β1 anchored AMPKα2 complex in the cytoplasm (Suzuki et al., 2007).

Ciliary Neurotrophic Factor (CNTF)

CNTF signals through the CNTFRα-IL-6R-gp130β receptor complex to increase fatty acid oxidation and improve insulin sensitivity in skeletal muscle by activating AMPK, independent of signaling through the brain (Watt et al., 2006). Intraperitoneal injection of a second-generation CNTF analog, CNTF(Ax15), reduced hypothalamic AMPKα2 activity, AMPKα2 Thr172 phosphorylation, and ACC phosphorylation in both lean mice as well as diet-induced-obesity mice. Intracerebroventricular injection of CNTF(Ax15) reduced AMPK signaling and food intake, an effect possibly mediated by the arcuate nucleus. These studies suggest that CNTF(Ax15) bypasses diet-induced leptin resistance to reduce hypothalamic AMPK activity (Steinberg et al., 2006).

Ghrelin/Cannabinoids

Both endocannabinoids and ghrelin are potent appetite stimulators that increase AMPK activity in the hypothalamus and the heart while inhibiting AMPK in liver and adipose tissue, which may contribute to the well known effects of endocannabinoids and ghrelin on the metabolism of peripheral tissues, including the reduction in infarct size in the myocardium, the enlargement in adipose tissue, and changes in carbohydrate and lipid metabolism (Kola et al., 2005). Pharmacological and genetic approaches reveal that the physiological orexigenic response to ghrelin involved activation of AMPK in the CNS, leading to inhibition of fatty acid biosynthesis via ACC inhibition, decreases in hypothalamic levels of malonyl-CoA, and the resulting increases in carnitine palmitoyltransferase 1 (CPT1) activity (Lopez et al., 2008). Ghrelin increased the endocannabinoid content of hypothalamus. Since ghrelin did not induce an orexigenic effect in CB1 knockout mice, an intact cannabinoid signaling pathway is necessary for the stimulatory effects of ghrelin on AMPK activity and food intake (Kola et al., 2008).

Interleukin-6

IL-6 is produced and released by skeletal myocytes in response to contraction. Basal IL-6 release from soleus was increased in AMPKα2 knockdown and AMPKα1 knockout mice, suggesting AMPK participates in the regulation of IL-6 release from oxidative muscle. Conversely, IL-6 release from soleus was reduced 45% when AMPK was activated by A-769662 (a direct activator of AMPK, see below). These observations suggest a role of AMPK in the regulation of IL-6 release from oxidative skeletal muscle (Glund et al., 2008). IL-6 also rapidly and markedly increased AMPK activity in myotubes, enhancing fatty acid oxidation as well as basal and insulin-stimulated glucose uptake (Carey et al., 2006). Correspondingly, IL-6 deficiency is associated with a decrease in AMPK activity (Kelly et al., 2004).

Natural Products

A number of natural products, including alkaloids, bitter melon extracts, etc., have been found to have effects on AMPK activation. Epigallocatechin-3-gallate, a main catechin of green tea, suppressed hepatic gluconeogenesis by AMPK activation, mediated by CaMKK (Collins et al., 2007).

Berberine, an alkaloid isolated from *Rhizoma coptidis,* is an oral antihyperglycemic agent. Berberine caused inhibition of mitochondrial function that increases the AMP/ATP ratio, which could explain the activation of the AMPK pathway by berberine as well as its beneficial effects in the treatment of diabetes and obesity in animal models (Yin et al., 2008), a hypothesis consistent with and confirmed by additional in vivo studies (Turner et al., 2008).

α-Lipoic Acid

α-Lipoic acid (α-LA) is a cofactor of mitochondrial enzymes that play key roles in catalyzing the decarboxylation of α-keto acids. α-LA decreased hypothalamic AMPK activity and caused profound weight loss in rodents by reducing food intake and enhancing energy expenditure (Kim et al., 2004). In peripheral tissues, α-LA activated AMPK in skeletal muscle as well as endothelial cells. α-LA also increased insulin-stimulated whole-body glucose uptake and fatty acid oxidation in obese rats (Lee et al., 2005). However, α-LA-stimulated glucose oxidation in isolated working rat hearts was independent of AMPK activation (Onay-Besikci et al., 2007).

Resveratrol/SIRT1

SIRT1 is a member of a highly conserved gene family (sirtuins) encoding NAD(+)-dependent deacetylases. LKB1 was shown to be acetylated in HEK293T cells, mouse white adipose tissue, and rat liver. In vivo, total LKB1 lysine-acetylation was decreased by 60% in the liver of 48 hr starved rats compared to starved-refed rats, and this was associated with modest but significant increases in both LKB1 and AMPK activities, establishing a mechanistic link between LKB1 deacetylation by SIRT1 and LKB/AMPK activation (Lan et al., 2008). In parallel, SIRT1 activation by resveratrol and the synthetic polyphenol S17834, while increasing SIRT1 deacetylase activity, also increased LKB1 phosphorylation at Ser428 and AMPK activity (Hou et al., 2008). Resveratrol analogs, such as combretastatin A-4 (CA-4), can function similarly as resveratrol and activate AMPK (Zhang et al., 2008).

Activation of AMPK by Direct Activators

As can be inferred from the discussions above, direct activators of AMPK that act without increasing cellular AMP/ATP ratios are being pursued as novel therapeutics. By definition, a direct activator should bind directly to the three subunits (α, β, or γ) of the AMPK holo enzyme. Summarized below are two examples of direct AMPK activators (Cool et al., 2006; Pang et al., 2008).

A-769662

A-769662 is a recently identified thienopyridine family of AMPK activators (Cool et al., 2006). It acts by directly stimulating partially purified rat liver AMPK (EC_{50} = 0.8 μM) and inhibiting fatty acid synthesis in primary rat hepatocytes (IC_{50} = 3.2 μM). Short-term treatment of normal Sprague-Dawley rats with A-769662 decreased liver malonyl-CoA levels and the respiratory exchange ratio, VCO_2/VO_2, indicating an increased rate of whole-body fatty acid oxidation. Treatment of *ob/ob* mice with A-769662 decreased hepatic expression of PEPCK, G6Pase, and FAS; lowered plasma glucose by 40%; reduced bodyweight gain; and significantly decreased both plasma and liver triglyceride levels.

How does A-769662 activate AMPK? When AMPK is in the inactive conformation, ATP is bound to the γ subunit, and Thr172 within the catalytic α subunit is freely accessible to protein phosphatases and is maintained predominantly in the unphosphorylated form. Similar to AMP, A-769662 activates AMPK both allosterically and by inhibiting dephosphorylation of AMPK on Thr172. In contrast, mutation of Ser108 in the carbohydrate-binding module (CBM) of the β subunit to alanine reduces activation of AMPK by A-769662 but not by AMP. However, AMP binding of the γ subunit and the subsequent autophosphorylation of Ser108 is not sufficient to maintain the conformation of AMPK in the active form (Goransson et al., 2007; Sanders et al., 2007a). Using recombinant enzyme preparations, it has been reported that A-769662 exclusively activates AMPK complexes containing the β1 subunit (Scott et al., 2008), and activation of AMPK by A-769662 involves an interaction between the β subunit CBM and residues from the γ subunit that are not involved in AMP binding. Intriguingly, the CBM also binds glycogen, which then interferes with AMPK activation by LKB1 or CamKKβ as well as allosterically inhibits the activity of AMPK (McBride et al., 2009). It remains to be determined if A-769662 also alters the interaction of CBM with glycogen.

Compared to ZMP and AMP, A-769662 has improved specificity for AMPK over other enzymes. On the other hand, a recent paper highlighted an off-target activity for the compound being inhibition of the 26S proteasome (IC_{50} = 62 μM) (Moreno et al., 2008). Using hepatocytes generated from AMPKβ1 null mice, it was observed that A-769662 retained its glucose-lowering effect independent of AMPK activation, suggesting additional off-target activity of A-769662 (Scott et al., 2008).

PT1

Based on a previous report that inactive mammalian AMPKα1 catalytic subunit contains an autoinhibitory domain, screening of a chemical library with inactive human α1 (residues 1–394) led to the identification of a novel small molecule activator, PT1, which allosterically activated AMPKα1 (1–394), α1 (1–335), α2 (1–398), and α1β1γ1 in vitro and promoted phosphorylation of AMPK and ACC in L6 myotubes and HeLa cells without an increase in cellular AMP/ATP ratio (Pang et al., 2008). Modeling studies revealed that PT1 may interact with AMPKα1 near the autoinhibitory domain and directly relieve autoinhibition (Pang et al., 2008). The in vivo activity of PT1 as well as the potential of this lead structure to be further developed into a candidate for testing in humans remain unknown at present.

Insights of AMPK Activation by Small Molecule Direct Activators from Structural Studies

Prior crystallographic studies with fission yeast AMPK revealed a single site in the γ subunit that binds either ATP or AMP within its Bateman domain B (Townley and Shapiro, 2007). However, crystal structures of a heterotrimeric regulatory core fragment from *Schizosaccharomyces pombe* AMPK in complex with ADP, ADP/AMP, ADP/ATP, and ZMP provided new insight that the structure of *Schizosaccharomyces pombe* AMPK embraces two moles of ADP by its γ subunit, suggesting a possible role for ADP in regulating AMPK response to changes in cellular energy status (Jin et al., 2007).

The activation of AMPK by A-769662, PT1, and AMP is schematically shown in Figure 4. Structural studies should lead to a more informed strategy in the screening and design of more potent AMPK activators.

Challenges of Development of AMPK Activators as a Therapy

Given the aforementioned critical actions of AMPK on glucose and lipid metabolism and the encouraging data from indirect as well as direct activators of AMPK in preclinical setting, targeting AMPK activation appears to be an attractive therapeutic strategy for the treatment of T2DM and related metabolic disorders. However, the following critical issues and challenges will

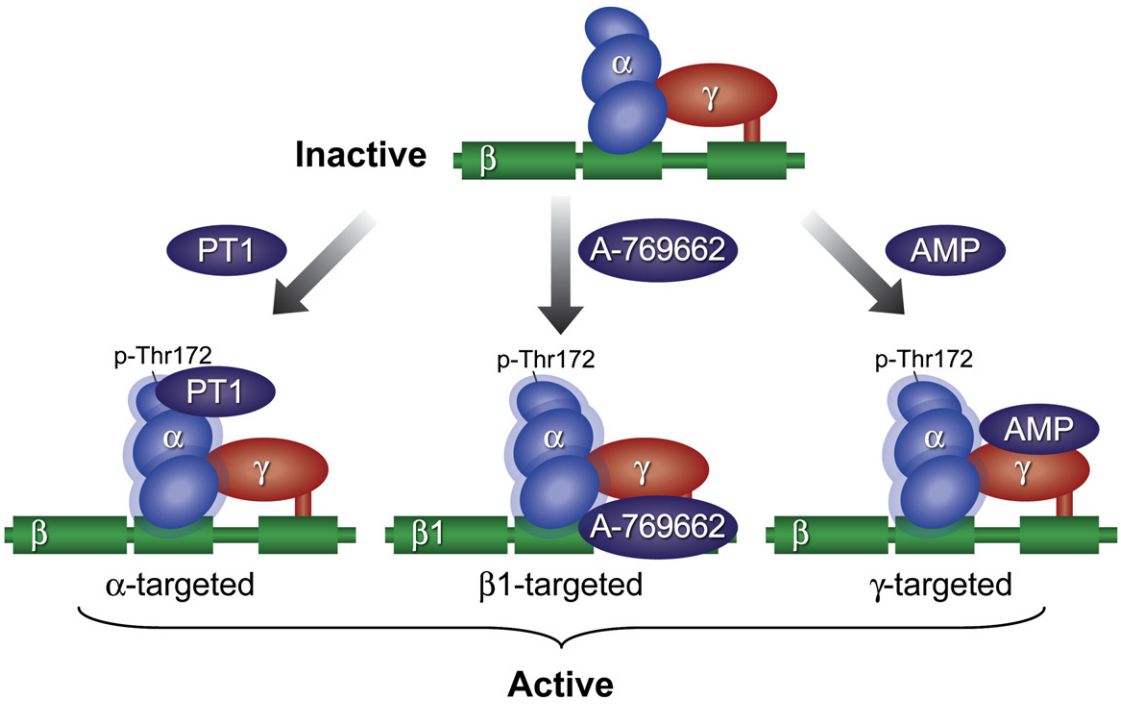

Figure 4. Mechanisms of AMPK Activation by Direct Small Molecule Activators
Shown are examples of AMPK activation by direct small molecule activators that act on the α (*PT1*), β (*A-769662*), or γ (*AMP*) subunits, illustrating the possibility of targeting a subset of or all AMPK complexes with activators.

need to be fully considered in order to realize the potential of this target.

What Are the AMPK Isoforms that Need to Be Targeted?

One major challenge in developing an AMPK activator is the issue of isoform specificity. Given the possibility that 12 AMPK complexes can form in vivo and that not a single subunit is shared among all 12 complexes, it is a tremendous task to develop a pan-activator, which might be required for efficacy in preclinical species and in humans. The observation that PT1 can activate both α1 and α2 (Pang et al., 2008) suggests that it is feasible to activate all 12 AMPK complexes by a single compound. However, it is not clear whether all 12 AMPK complexes could be or need to be activated to the same degree in order to realize the maximum benefits of AMPK activation in vivo. Furthermore, it is conceivable that distinct subunit distributions exist in different tissues of a given species. In addition, AMPK complex composition in a given tissue (e.g., liver) across different species could be different. This complexity makes it highly challenging to translate the in vivo efficacy of an AMPK activator in preclinical models to humans.

Are There Liabilities of Systemic AMPK Activation?

Agents that decrease the activity of AMPK in hypothalamus reduce food intake (Minokoshi et al., 2008), while agents that increase the activity of AMPK in hypothalamus may increase food intake as well as hepatic glucose output in rodents (Kubota et al., 2007; McCrimmon et al., 2008). These observations raise the question of whether a brain penetrant direct AMPK activator may cause undesired consequences. However, genetic studies led to a more complex picture of the effect of AMPK in the brain. Mice lacking AMPKα2 in different brain regions developed opposite phenotypes: knockout of AMPKα2 in pro-opiomelanocortin neurons caused obesity while mice with knockout of the same gene in agouti-related protein neurons developed an age-dependent lean phenotype. Interestingly, neurons from both lines of knockout mice exhibited normal leptin or insulin action but lacked the responses to alterations in extracellular glucose levels. These findings suggest that while AMPK plays a key role in hypothalamic function, it does not act as a general sensor and integrator of energy homeostasis in this region of the hypothalamus (Claret et al., 2007). Nonetheless, these data caution against developing AMPK activators that cross the blood-brain barrier.

Wolff-Parkinson-White (WPW) syndrome is manifested mainly as a cardiac conduction system defect. While the genetic basis for the majority of patients afflicted with WPW syndrome has not been delineated, mutations in the AMPKγ2 subunit have been identified in a small percentage of patients with the syndrome (Arad et al., 2007). These mutations cause disparate effects on AMPK activity, and it is uncertain whether the effects of mutations on cardiac AMPK activity are the cause of WPW syndrome (Young, 2008). A recent report showed that macrophage migration inhibitory factor, an upstream regulator of inflammation, was released in the ischemic heart, where it stimulated AMPK through CD74, promoted glucose uptake, and protected the heart during ischemia-reperfusion injury (Miller et al., 2008). While unsettled (Akman et al., 2007; Luptak et al., 2007), the effect of AMPK activation on cardiac function will need to be closely monitored.

The effects of AMPK activation in pancreatic islets and adipose tissue are less well characterized. Expression of constitutively active AMPK induced apoptosis in clonal MIN6 β cells

Figure 5. Multiple Effects of AMPK Activation in Liver and Muscle Leading to Improved Metabolic Control
These effects include modulation of lipid metabolism, mitochondrial biogenesis, mimicking the beneficial effects of exercise, improvement in insulin sensitivity, and reduction in blood glucose. The stick figure in the center of the illustration denotes the AMPK complex containing the α, β, and γ subunits. The complex is activated by phosphorylation and binding to AMP.

and CD1 mouse pancreatic islets (Riboulet-Chavey et al., 2008). In adipose tissue, AICAR-induced AMPK activation suppressed the activity of HSL but promoted a 4-fold increase in ATGL content in adipocytes (Gaidhu et al., 2008). The effect of activation of AMPK by specific activators in adipose tissue and in islet will warrant further investigation.

Should a Strategy of Tissue-Selective AMPK Activation Be Employed?

Given the potential adverse effects that may be associated with sustained systemic pharmacological activation of AMPK, an intriguing concept is to target AMPK activation in specific tissues. As discussed above, liver and skeletal muscle are the two main tissues in which activation of AMPK would be expected to lead to improved insulin sensitivity and whole-body energy homeostasis.

There are a number of factors that can be exploited to attempt to enrich liver exposure of AMPK activators relative to extrahepatic tissues. In particular, the liver is rich in metabolic enzymes and uptake transporters, and it is the first organ exposed to compounds after adsorption in the gastrointestinal tract. A prerequisite for any successful liver targeting strategy is that passive diffusion out of the liver be minimized, since this opposes the desired gradient and is likely to mirror passive diffusion into other tissues. One particularly attractive strategy is the development of cell-permeable prodrugs that are cleaved or oxidized by metabolic enzymes (CYP isozymes, esterases, amidases, etc.) to generate less cell-permeable active species. Another approach is exemplified by metformin, where organic cation transporter 1 is responsible for its hepatic uptake as well as playing a role in its intestinal uptake (Wang et al., 2002). Liver-targeted AMPK activators may provide an effective means to avoid exposure to other tissues, while they will limit the potential to harness the benefit of AMPK activation in skeletal muscle.

Approaches relying on drug distribution to skeletal muscle seem unlikely to be successful, given the high level of cardiac perfusion and the lack of known, suitable, skeletal muscle-specific

uptake transporters. The most viable strategy would appear to be targeting specific AMPK complexes that are enriched in skeletal muscle. In this regard, the AMPK$\alpha 2\beta 2\gamma 3$ is particularly interesting, since this $\gamma 3$-containing complex is expressed only in skeletal muscle, and it is the only complex that is predominately activated during high-intensity exercise in human subjects (Birk and Wojtaszewski, 2006). The need to develop complex-specific AMPK activators requires expression of all 12 possible complexes in order to identify and optimize selective compounds.

Will AMPK Activation Result in Glucose Lowering in Humans?

The test of activating AMPK as a therapeutic approach requires a proof of concept study for glucose-lowering efficacy in the clinic. It is not clear how much activation relative to that achieved by AMP is needed for efficacy. Furthermore, it needs to be established whether continuous activation is desirable or whether intermittent activations of AMPK mimicking bouts of exercise will yield greater efficacy in the control of glucose homeostasis. Much of the preclinical validation guiding the development of AMPK activators is from rodent genetic models as well as the use of nonselective activators such as AICAR that also affect many AMP-modulated metabolic enzymes, including fructose 1,6-bisphosphatase and glycogen phosphorylase. In addition, because animal models deficient in all AMPK complexes, such as double knockout of $\alpha 1$ and $\alpha 2$ subunits of AMPK, are not viable, it is not feasible to test direct systemic AMPK activators in these models to definitively establish that the beneficial effects are on target. In this regard, mice with liver-specific double knockout of $\alpha 1$ and $\alpha 2$ are viable (Guigas et al., 2006), providing a possible means to test the on-target activity of liver-targeted AMPK activators. As a novel therapy, the net efficacy of an AMPK activator relative to other glucose lowering agents will also need to be defined. Finally, since metformin is a first-line therapy in the treatment of diabetes, it will be advantageous if an AMPK activator is combinable with or has advantage over metformin. While these questions pose significant challenges in developing a direct AMPK activator for the treatment of T2DM and the metabolic syndrome, the potential benefits of AMPK activation will make this task ultimately a rewarding journey, as depicted in Figure 5.

Future Prospects

Intense research in recent years has revealed the critical roles that AMPK plays in modulating an ever-expanding array of biological pathways. The broad spectrum of activities of AMPK in lipid and glucose metabolism makes it a very attractive target for drug discovery. The feasibility of developing potent and selective activators has begun to emerge, and the recent preliminary proof of concept in preclinical models suggests that there is great hope for therapeutic use of AMPK activators in humans afflicted with T2DM and related metabolic disorders. With the challenges faced by targeting this complex enzyme, the question is not whether but when this possibility will be realized in the clinic.

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