BMB
Reports

Invited Mini Review

AMP-activated protein kinase: implications on ischemic diseases

Yong-Joo Ahn[1,#], Hwewon Kim[1,#], Heejin Lim[2], Max Lee[1], Yuhyun Kang[1], SangJun Moon[2], Hyeon Soo Kim[3] & Hyung-Hwan Kim[1,]*

[1]Vascular Medicine Research Unit, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Cambridge, Massachusetts 02139, USA, [2]Department of Robotics Engineering, DGIST, Daegu 700-742, [3]Department of Anatomy, Korea University College of Medicine, Seoul 136-705, Korea

Ischemia is a blockage of blood supply due to an embolism or a hemorrhage in a blood vessel. When an organ cannot receive oxygenated blood and can therefore no longer replenish its blood supply due to ischemia, stresses, such as the disruption of blood glucose homeostasis, hypoglycemia and hypoxia, activate the AMPK complex. LKB1 and CaMKKβ are essential activators of the AMPK signaling pathway. AMPK triggers proangiogenic effects through the eNOS protein in tissues with ischemic conditions, where cells are vulnerable to apoptosis, autophagy and necrosis. The AMPK complex acts to restore blood glucose levels and ATP levels back to homeostasis. This review will discuss AMPK, as well as its key activators (LKB1 and CaMKKβ), as a central energy regulator and evaluate the upstream and downstream regulating pathways of AMPK. We will also discuss how we can control this important enzyme in ischemic conditions to prevent harmful effects in patients with vascular damage. [BMB Reports 2012; 45(9): 489-495]

INTRODUCTION

Ischemia is a condition in which blood flow to a certain organ, such as the brain or the heart, cannot be maintained or is stopped entirely (1). People who have atherosclerosis, hypertension and cerebral vascular diseases are at a higher risk of developing an ischemic disease (2). Ischemic disease is characterized by inflammatory responses, metabolic disorganization and eventual cell death (3, 4). Mammalian 5-adenosine monophosphate [AMP]-activated protein kinase (AMPK) is an enzyme involved in cellular energy homeostasis. AMPK is the master metabolic regulator, and its activation leads to the down-regulation of several energy-demanding, anabolic processes, such as fatty acid oxidation, glucose uptake, and glycolysis (5, 6). It is the intracellular low fuel warning system in the body, which is active during exercise and high AMP/ATP state (6). This pathway can be activated by many factors including LKB1 and CaMKKβ (7, 8). AMPK propagates a cascade of events within cells in response to stress signals in the body that can be caused by exercise, calorie restriction, low carbohydrate diet and ischemic damage to the body (9). When blood gas and blood glucose homeostasis is disrupted and the AMP/ATP level is high, the body activates the AMPK pathway to generate ATP and restore blood gas and blood glucose back to a state of homeostatic balance (6). AMPK is activated by two complexes. The LKB1 complex activates AMPK during the normal resting state, and CaMKKβ activates AMPK during exercise and physical stress, when calcium levels are high in the body (5, 7). LKB1 is a tumor suppressor serine/threonine kinase that is encoded by the STK11 gene and is the key upstream regulator of the AMPK pathway (10). LKB1 requires two binding partners, STRAD and MO25, to activate AMPK (8). CaMKKβ, calcium dependent protein kinase kinase beta, is an upstream activator of the AMPK pathway (7). Under stress or following injury, our bodies naturally activate the AMPK pathway to turn on key metabolic pathways, such as ribosome and protein biogenesis, cell growth and proliferation, cellular autophagy, cell apoptosis, lipolysis, fatty acid synthesis, steroid synthesis, gluconeogenesis, glycogen synthesis and ultimately, cardiovascular homeostasis via the eNOS complex which is a major regulator of vascular tone. In this review, we summarize the current understanding of the associations between the AMPK signaling pathway and ischemic disease and discuss therapeutic strategies. We also speculate on the importance of the AMPK signaling pathway and its many activators in ischemic conditions to help uncover potentially effective therapeutic methods and reverse the damaging effects made by ischemic injury.

AMPK COMPLEX AND ITS MOLECULAR COMPOSITION

AMPK exists as a heterotrimeric protein complex and is expressed in virtually all eukaryotic cells, including plants, fungi

*Corresponding author. Tel: +1-617-768-8415; Fax: +1-617-768-8444; E-mail: hkim@rics.bwh.harvard.edu
[#]Denote equal contribution by the authors.
http://dx.doi.org/10.5483/BMBRep.2012.45.9.169

Received 19 July 2012

Keywords: AMPK, CaMKKβ, Ischemia, LKB1

and animals (11). AMPK is composed of a catalytic (AMPK α) subunit and two regulatory (AMPK β and γ) subunits (12). Two isoforms of AMPK α (PRKAA1 and PRKAA2), two isoforms of AMPK β (PRKAB1 and PRKAB2) and three isoforms of AMPK γ (PRKAG1, PRKAG2 and PRKAG3) exist (Fig. 1). Both catalytic PRKAA1 and PRKAA2 subunits of the AMPK complex contain the protein kinase domain Thr^{172}. The β regulatory subunits, PRKAB1 and PRKAB2, possess a glycogen-binding region (GBR) and also modulate AMPK activity (Fig. 2) (13). The γ regulatory subunits, PRKAG1, PRKAG2 and PRKAG3, each contain four repeating cystathionine-β-synthase (CBS) units; two CBS units constitute one Bateman domain, which serves as a cooperative regulatory AMP- and ATP-binding site within the AMPK complex (Fig. 1) (5, 14, 15).

AMPK ACTIVATION AND INHIBITION

A wide variety of agents activate AMPK (Table 1), and AMP activation occurs mainly via the 5-amino-4-imidazolecarboxamide riboside-1-β-D-ribofuranoside (AICAR) analogue, 5-amino-4-imidazolecarboxamide ribotide (ZMP) (9). ZMP phosphorylates and activates a complex consisting of STK11 (LKB1), mouse protein 25 (MO25) and the pseudokinase STE-related adaptor protein (STRAD) (8). This LKB1/MO25/STRAD complex phosphorylates AMPK on its active site, Thr^{172}; this phosphorylation is required for AMPK to become active (8, 17). LKB1 is a tumor suppressor and is mutated in the Peutz-Jeghers syndrome (8). LKB1 is the primary AMPK activator in skeletal muscle, and ablation of LKB1 eliminates the activation of AMPK (12). It requires two binding partners STRAD and MO25 (17). LKB1 phosphorylates serine-threonine kinase (Thr^{172}) in AMPK. AMPK can also be phosphorylated by the calcium-dependent protein kinase kinase beta (CaMKKβ) protein. Inhibition of CaMKK also leads to an inhibition of AMPK phosphorylation in muscles after a few minutes of contraction. Decrease in inhibition resulted in a low contraction because exercise is activating (18). CaMKK inhibitors had no effect on AMPK activation via AICAR phosphorylation (9). Muscle contraction activates the AMPK complex via an adenylate kinase reaction that results in high AMP/ATP levels (1, 9, 19). Adenylate kinase converts two ADP molecules into one ATP molecule and one AMP molecule (Fig. 3) (19, 20). The newly synthesized AMP molecules bind to the active site Thr^{172} and phosphorylate and activate the AMPK complex. C75, fatty acid synthase inhibitor, inhibits AMPK in hypothalamus (53). Compound C and adenosine 9-β-$_D$-arabinofurano-

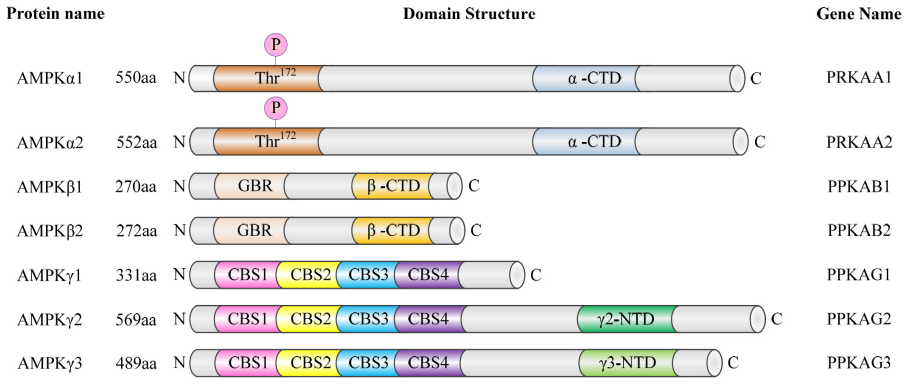

Fig. 1. AMPK is composed of a catalytic (α) subunit and two regulatory (β and γ) subunits. The kinase domain (Thr^{172}) with attached phosphate (P) and the α carboxyl terminal domain (CTD) are located on α subunits are represented in red and blue respectively. The glycogen-binding region (GBR) and the CTD located on the β subunits are represented in pink and gold, respectively. The cystathionine β-synthase (CBS) regions 1-4 and the γ N-terminal domain (NTD) on the γ subunits are represented in pink, yellow, blue, purple and green respectively. Two CBS units constitute one Bateman domain.

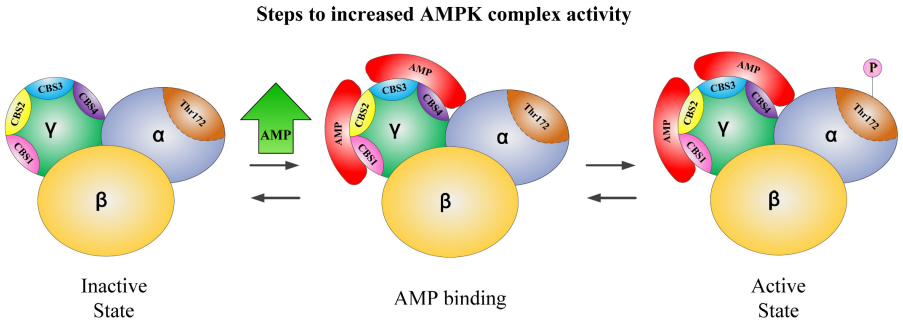

Fig. 2. For optimal activity, three conditions must be met to produce a functional AMPK complex. First, high AMP/ATP levels must be present. Second, the γ subunit must undergo a conformational change to expose the active site, Thr^{172}, on the α subunit. This conformational change is due to high AMP/ATP levels; two AMP molecules bind to the two Bateman domains on the γ subunit. Finally, AMPK kinase must be phosphorylated and thereby activate AMPK on the active site Thr^{172}. Note that the AMPK pathway is allosterically regulated (i.e., 3PG/AMPK pathway) (16).

Table 1. Activators and Inhibitors of AMPK

Anabolic processes	Hepatic Fatty Acid Oxidation (17)
	Ketogenesis (17)
	Lipogenesis (35)
	Mitochondrial Biogenesis (36)
	Triglyceride Synthesis (36)
	Inhibition of Adipocyte (37) Lipolysis (37)
	Inhibition of Adipocyte (35) Lipogenesis (35)
	Decrease Malonyl-CoA (36)
	Increase Co-activator-1 (PGC-1) (36) and associated proteins (36)
AMPK activators (supplemental and drug)	AICAR (9)
	CaMMKβ (7)
	LKB1/MO25/STRAD (8)
	Metformin (38)
	Phenformin (39)
	Resveratrol (40)
	Ghrelin (41)
	Lipoic Acid (35)
	Leptin (42)
	ADIPONECTIN (42)
	Guanidinopropionic acid (GPA) (36)
	Homocysteine (HCY) (37)
	2-deoxyglucose (39)
	Hydrogen peroxide (39)
	Creatine (20)
	Galegine (39)
	Troglitazone (39)
	Phenobarbital (39)
	Captopril (2)
	Berberine (39)
AMPK activators (natural)	Exercise (9, 40)
	Ischemia (43)
	Decreased Glucose Levels (44)
	Increased CA^{2+} Levels (9)
	Low Calorie Consumption (9, 21)
	Low Carbohydrate Consumption (9, 21)
	Insulin (9)
AMPK inhibitors	Compound C (2)
	C57 53
	9-β-D-arabinofuranoside (araA) 54

side (araA) inhibit AMPK (54).

UPSTREAM AND DOWNSTREAM DELETED OF AMPK SIGNALING PATHWAYS

In mammals, there are several alternative upstream kinases, including the tumor suppressor LKB1, CaMKKα and CaMMKβ (10, 21-24). The AMPK signaling pathway responds to various energy stresses that cause fluctuation in the intricate balance of AMP/ATP ratio. As ATP levels fall, there is an increase in the AMP levels, which triggers the activation of the AMPK pathway (Fig. 3) (3, 6). The upstream regulator of AMPK, LKB1, allows for AMP phosphorylation at a specific site (Thr^{172}) on the α subunit of AMPK (25). Two other subunits, STRAD and MO25, form a complex with LKB1 to enhance this process (16, 19). CaMKK is also able to activate AMPK. (10, 21) Of the CaMKK proteins, CaMKKβ has the strongest regulation of AMPK (10). CaMKKβ regulates AMPK in response to changes

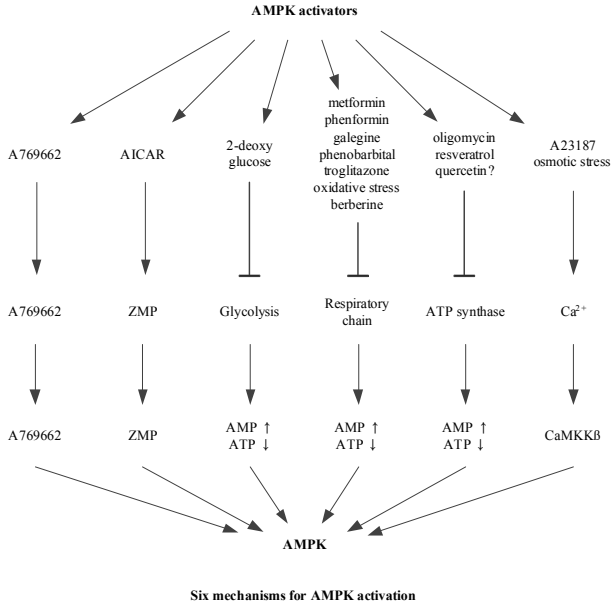

Fig. 3. Diverse mechanisms of AMPK activation (35).

in intracellular calcium concentrations but not to changes in the AMP:ATP ratio (22, 23). Downstream kinases of AMPK include eukaryotic elongation factor-2 kinase (eEF-2), mammalian target of rapamycin (mTOR) and ULK1. AMPK directly phosphorylates eEF-2 at Ser398 and inhibits protein synthesis by inhibiting protein elongation (26). AMPKα2 activation by AICAR regulates cell growth by decreased activation of mTOR on Ser^{2448} (27) and evidence suggests that mTOR inhibition by AMPK activation is mediated by Tsc2 (28). In $Tsc2^{-/-}$ MEFs cells, cell growth is controlled by AMPK activation and results in the direct phosphorylation of Raptor of mTORC1 (29, 30). AMPK directly phosphorylates the ULK1 complex (31-34).

AMPK AND ISCHEMIC DISEASES

Ischemic disease results in a perturbation of blood glucose homeostasis that leads the to activation of AMPK. There are two major pathways to activate AMPK. CaMKKβ can be activated through changes in calcium concentrations, or LKB1 can become active through a currently unknown mechanism (43). The activation of AMPK leads to the phosphorylation of eNOS at residue Ser^{1177}, resulting in the production of nitric oxide. Therefore, it is reasonable to state that the activation of AMPK has a proangiogenic effect because it stimulates the eNOS pathway and allows new blood vessels to form in endothelial cells during ischemic condition (45, 46). Additionally, the Akt pathway is activated by AMPK through a mechanism that remains elusive (21). The activation of Akt is crucial for cell survival. Akt can be activated through PI3K - this is activated by insulin (41). It has been shown that PI3K also plays a crit-

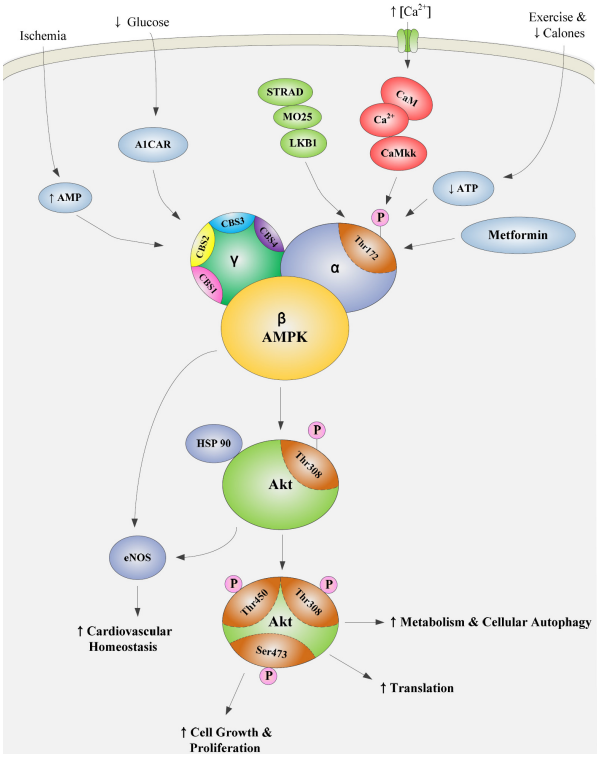

Fig. 4. Representative signaling in the AMPK pathway. Ischemic conditions increase AMP concentrations, leading to AMP binding to the CBS regions of the γ subunits of the AMPK complex and subsequent AMPK complex activation. Two AMP molecules bind to the γ subunit at each Bateman region, which is made up of two CBS regions (5). Additionally, there are three common ways in which the AMPK complex may be activated: 1) direct activation via AICAR (9, 16), 2) phosphorylation at the active site Thr^{172} by the LKB1/MO25/STRAD complex or the Ca^{2+}/calmodulin complex (16, 18), and 3) phosphorylation induced by the misregulation of the ADP/ATP (4, 6). Metformin activates the AMPK complex by decreasing ATP levels, as well phosphorylating the active site Thr^{172} (38).

ical role in cell survival by stimulating cell proliferation and inhibiting apoptosis (38). Upon the activation of receptor tyrosine kinases (RTKs), PI3K becomes activated, PI3K then triggers the conversion of PIP2 to PIP3, which then activates Akt by phosphorylation (43). Furthermore, Akt helps regulate pathways that promote cell cycle, cell proliferation and anti-apoptosis, such as eNOS (Fig. 4) (25). Adiponectin that activates AMPK improves left ventricular function after cardiac I/R injury through the combined phosphorylations of AMPK (Thr^{172}), Akt (Ser^{473}) and eNOS (Ser^{1177}) (47). AMPKα1 knockdown attenuated Akt1 and eNOS phosphorylation by VEGF and S1P, whereas Akt1 knockdown did not affect AMPK activation. This implies that Akt1 is downstream of AMPKα1 (49). A constitutively active mutant of AMPKα1 in HUVECs reduced caspase 3/7 and increased Akt (Ser^{473}) and eNOS (Ser^{1177}) phosphorylation in HUVEC cells in anoxic conditions (50). AMPK activation by metformin protects myocardial I/R mediated by AMPK/eNOS and does not result in activation of Akt (51). The role of Akt in the AMPK-related signaling pathway in ischemic conditions is not well defined. Pharmacological and genetic inhibition of AMPK results in neuroprotection after ischemic stroke (48); however, intraperitoneal injection of metformin, resulted in the activation of AMPK and significantly reduced neuronal damage after experimental stroke model (52, 53). A recent report showed that mice treated with leptin in a photothrombotic stroke had increased leptin receptor and AMPK phosphorylation; which led to an induction of neurogenesis and angiogenesis (55).

CONTROLLING AMPK SIGNALING PATHWAYS AND THERAPEUTIC IMPLICATIONS IN ISCHEMIC DISEASES

Once activated, AMPK induces a signaling cascade that works to boost ATP levels back to homeostatic levels. In this way, the AMPK pathway is the most important energy-regulating pathway in the body (4). AMPK activation also exerts its effect on glucose homeostasis, lipid metabolism, and protein synthesis in the liver, pancreas, skeletal muscle, heart, and brain (44). AMPK is the master regulator of energy, glucose, and fat storage. In diseased conditions, AMPK is less active, and therefore, activators are essential for the proper upkeep and regulation of these anabolic processes and proper body functions (4). While many activators exist for this important pathway, LKB1 and CaMKKβ are the major primary activators of AMPK (8, 16). The actions of LKB1 and CaMKKβ are well understood, and, as previously mentioned, extensive research has been focused on these activators to prevent the inhibition or breakdown of the AMPK pathway in disease conditions, such as ischemic injury. Many AMPK studies have focused on other human diseases, particularly breast cancer, diabetes mellitus, obesity, cardiovascular disease, and neurological disease, such as ischemia and stroke (9, 52). Further study is necessary to understand the potential of targeting AMPK activators for treatment in ischemic disease. Currently, there are two drugs that are agonists of AMPK: AICAR and metformin. AICAR enhances insulin-stimulated glucose uptake; metformin is an oral drug used to treat type II diabetes (9, 52). Metformin suppresses the activation of Complex I, thereby shifting the AMP/ATP ratio which results in the activation of AMPK (38). Compound C has been used to inhibit AMPK (2). Although this compound is commercially available, most studies focus on activating AMPK to trigger its beneficial effects (35). In terms of ischemic diseases, activation of AMPK would be far more beneficial than inhibition of AMPK.

Acknowledgements
This work was supported by AHA NSDG, CHOM, NOMC, ZEMA, and Jungwon grants to Hyung-Hwan Kim. Hyung- Hwan Kim is a principal investigator at the Brigham and Women's

Hospital and Harvard Medical School. No author has a conflict of interest related to the work reported in this manuscript.

REFERENCES

1. Soler, E. P. and Ruiz, V. C. (2010) Epidemiology and risk factors of cerebral ischemia and ischemic heart diseases: Similarities and differences. *Curr. Cardio. Rev.* **6**, 138-149.
2. Jin, J., Mullen, T. D., Hou, Q., Bielawski, J., Bielawska, A., Zhang, X., Obeid, L. M, Hannun, Y. A, and Hsu, Y., T. (2009) AMPK inhibitor Compound C stimulates ceramide production and promotes Bax redistribution and apoptosis in MCF7 breast carcinoma cells. *J. Lipid Res.* **50**, 2389-2397.
3. Zhao, Z. Q., Corvera, J. S., Halkos, M. E., Kerendi, F., Wang, N. P., Guyton, R. A., and Vinten-Johansen, J. (2003) Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am. J. Physiol. Heart Circ. Physiol.* **285**, 579-588.
4. Hardie, D. G. and Sakamoto, K. (2006) AMPK: a key sensor of fuel and energy status in skeletal muscle. *Physiology*. **21**, 48-60.
5. Arad, M., Seidman, C. E. and Seidman, J. (2007) AMP-Activated Protein Kinase in the Heart during health and disease. *Circ. Res.* **100**, 474-488.
6. Carling, D., Mayer, F. V., Sanders, M. J. and Gamblin, S. J. (2011) AMP-activated protein kinase: nature's energy sensor. *Nat. Chem. Biol*. **7**, 512-518.
7. Green, M. F., Anderson, K. A. and Means, A. R. (2011) Characterization of the CaMKK [beta]-AMPK signaling complex. *Cell Signaling* **23**, 2005-2012.
8. Zeqiraj, E., Filippi, B. M., Deak, M., Alessi, D. R. and Van Aalten, D. M. F. (2009) Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation. *Science* **326**, 1707-1711.
9. Iglesias, M. A., Ye, J. M., Frangioudakis, G., Saha, A. K., Tomas, E., Ruderman, N. B., Cooney, G. J. and Kraegen, E. W. (2002) AICAR administration causes an apparent enhancement of muscle and liver insulin action in insulin-resistant high-fat-fed rats. *Diabetes* **51**, 2886-2894.
10. Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. G. D., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M. and Carling, D. (2003) LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr. Biol*. **13**, 2004-2008.
11. Hardie, D. G. (2008) Role of AMP-activated protein kinase in the metabolic syndrome and in heart disease. *FEBS Lett*. **582**, 81-89.
12. Shackelford, D. B. and Shaw, R. J. (2009) The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat. Rev. Cancer* **9**, 563-575
13. McBride, A., Ghilagaber, S., Nikolaev, A. and Hardie, D. G. (2009) The glycogen-binding domain on the AMPK [beta] subunit allows the kinase to act as a glycogen Sensor. *Cell Metab*. **9**, 23-34.
14. Kemp, B. E. (2004) Bateman domains and adenosine derivatives form a binding contract. *J. Clin. Invest*. **113**, 182-184.
15. Scott, J. W., Hawley, S. A., Green, K. A., Anis, M., Stewart, G., Scullion, G. A., Norman, D. G. and Hardie, D. G. (2004) CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J. Clin. Invest*. **113**, 274-284.
16. Hawley, S. A., Selbert, M. A., Goldstein, E. G., Edelman, A. M., Carling, D. and Hardie, D. G. (1995) 5'-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. *J. Biol. Chem*. **270**, 27186-27191.
17. Vàzquez-Chantada, M., Ariz, U., Varela-Rey, M., Embade, N., Martínez-Lopez, N, Fernàndez-Ramos, D., Gómez-Santos, L, Lamas, S., Lu, S. C. and Martínez-Chantar, M. L. (2009) Evidence for LKB1/AMP activated protein kinase/endothelial nitric oxide synthase cascade regulated by hepatocyte growth factor, S adenosylmethionine, and nitric oxide in hepatocyte proliferation. *Hepatology* **49**, 608-617.
18. Park, S., Scheffler, T. and Gerrard, D. (2011) Chronic high cytosolic calcium decreases AICAR-induced AMPK activity via calcium/calmodulin activated protein kinase II signaling cascade. *Cell Calcium* **50**, 73-83.
19. Hawley, S, A., Davison, M., Woods, A., Davies, S. P., Beri, R. K., Carling, D. and Hardie, D. G. (1996) Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *J. Biol. Chem*. **271**, 27879-27887.
20. Darrabie, M. D., Arciniegas, A. J. L., Mishra, R., Bowles, D. E., Jacobs, D. O. and Santacruz, L. (2011) AMPK and substrate availability regulate creatine transport in cultured cardiomyocytes. *Am. J. Physiol. Endocrinol. Metab*. **300**, E870-876
21. Hawley, S., Boudeau, J., Reid, J., Mustard, K., Udd, L., Mäkelä, T., Alessi, D. and Hardie, D. G. (2003) Complexes between the LKB1 tumor suppressor, STRADα/β and MO25α/β are upstream kinases in the AMP-activated protein kinase cascade. *J. Biol*. **2**, 28.
22. Hurley, R. L., Anderson, K. A., Franzone, J. M., Kemp, B. E., Means, A. R. and Witters, L. A. (2005) The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J. Biol. Chem*. **280**, 29060-29066.
23. Woods, A., Dickerson, K., Heath, R., Hong, S. P., Momcilovic, M., Johnstone, S. R., Carlson, M. and Carling, D. (2005) Ca2+/calmodulin-dependent protein kinase kinase-[beta] acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab*. **2**, 21-33.
24. Hawley, S. A., Pan, D. A., Mustard, K. J., Ross, L., Bain, J., Edelman, A. M., Frenguelli, B. G. and Hardie, D. G. (2005) Calmodulin-dependent protein kinase kinase-[beta] is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab*. **2**, 9-19.
25. Thors, B., Halldórsson, H. and Thorgeirsson, G. (2010) eNOS activation mediated by AMPK after stimulation of endothelial cells with histamine or thrombin is dependent on LKB1. *Biochim. Biophys. Acta*. **1813**, 322-331.
26. Browne, G. J, Finn, S. G. and Proud, C. G. (2004) Stimula-

tion of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site, serine 398. *J. Biol. Chem.* **279**, 12220-12231.
27. Bolster, D. R, Crozier, S. J, Kimball, S. R. and Jefferson, L. S. (2002) AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J. Biol. Chem.* **277**, 23977-23980.
28. Inoki, K., Zhu, T. and Guan, K. L. (2003) TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577-590.
29. Gwinn, D. M., Shackelford, D. B., Egan, D. F, Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E. and Shaw, R. J. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* **30**, 214-226.
30. Kalender, A., Selvaraj, A., Kim, S. Y., Gulati, P., Brûlé, S., Viollet, B., Kemp, B. E., Bardeesy, N., Dennis, P. and Schlager, J. J. (2010) Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metab.* **11**, 390-401.
31. Packer, L. and Cadenas, E. (2011) Lipoic acid: energy metabolism and redox regulation of transcription and cell signaling. *J. Clin. Biochem. Nutr.* **48**, 26-32.
32. Horvath, T. L., Erion, D. M., Elsworth, J. D., Roth, R. H., Shulman, G. I. and Andrews, Z. B. (2011) GPA protects the nigrostriatal dopamine system by enhancing mitochondrial function. *Neurobiol. Dis.* **43**, 152-162.
33. Wang, Z., Pini, M., Yao, T., Zhou, Z., Sun, C., Fantuzzi, G. and Song, Z. (2011) Homocysteine suppresses lipolysis in adipocytes by activating the AMPK pathway. *Am. J. Physiol. Endocrinol. Metab.* **301**, E703-E712.
34. Vazquez-Martin, A., Oliveras-Ferraros, C., Del Barco, S., Martin-Castillo, B. and Menendez, J. (2009) The antidiabetic drug metformin: a pharmaceutical AMPK activator to overcome breast cancer resistance to HER2 inhibitors while decreasing risk of cardiomyopathy. *Ann. Oncol.* **20**, 592-595.
35. Hawley, S. A, Ross, F. A, Chevtzoff, C., Green, K. A., Evans, A., Fogarty, S., Towler, M. C., Brown, L. J., Ogunbayo, O. A. and Evans, A. M. (2010) Use of cells expressing [gamma] subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab.* **11**, 554-565.
36. Centeno-Baez, C., Dallaire, P. and Marette, A. (2011) Resveratrol inhibition of inducible nitric oxide synthase in skeletal muscle involves AMPK but not SIRT1. *Am. J. Physiol. Endocrinol. Metab.* **301**, E922-E930.
37. Wang. Y., Nishi, M., Doi, A., Shono, T., Furukawa, Y., Shimada, T., Furuta, H., Sasaki, H. and Nanjo, K. (2010) Ghrelin inhibits insulin secretion through the AMPK-UCP2 pathway in [beta] cells. *FEBS Lett.* **584**, 1503-1508.
38. Pene, F., Claessens, Y. E., Muller, O., Viguie, F., Mayeux, P., Dreyfus, F., Lacombe, C. and Bouscary, D. (2002) Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6- kinase pathways in the proliferation and apoptosis in multiple myeloma. *Oncogene.* **21**, 6587-6597.
39. Altarejos, J. Y., Taniguchi, M., Clanachan, A. S. and Lopaschuk, G. D. (2005) Myocardial ischemia differentially regulates LKB1 and an alternate 5'-AMP-activated protein kinase kinase. *J. Biol. Chem.* **280**, 183-190.
40. Zhang, H., Zhang, G., Gonzalez, F. J., Park, S. and Cai, D. (2011) Hypoxia-inducible factor directs POMC gene to mediate hypothalamic glucose sensing and energy balance regulation. *PLoS Biol.* **9**, e1001112.
41. Nagata, D., Mogi, M. and Walsh, K. (2003) AMP-activated protein kinase (AMPK) signaling in endothelial cells is essential for angiogenesis in response to hypoxic stress. *J. Biol. Chem.* **278**, 31000-31006.
42. Ouchi, N., Kobayashi, H., Kihara, S., Kumada, M., Sato, K., Inoue, T., Funahashi, T. and Walsh, K. (2004) Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. *J. Biol. Chem.* **279**, 1304-1309.
43. Sansal, I. and Sellers, W. R. (2004) The biology and clinical relevance of the PTEN tumor suppressor pathway. *J. Clin. Oncol.* **22**, 2954-2963.
44. Gonon, A. T, Widegren, U., Bulhak, A., Salehzadeh, F., Persson, J., Sjöquist, P. O. and Pernow, J. (2008) Adiponectin protects against myocardial ischaemia-reperfusion injury via AMP-activated protein kinase, Akt, and nitric oxide. *Cardiovasc. Res.* **78**, 116-122.
45. Levine, Y. C., Li, G. K. and Michel, T. (2007) Agonist-modulated regulation of AMP-activated protein kinase (AMPK) in endothelial cells. *J. Biol. Chem.* **282**, 20351-20364
46. Nagata, D., Kiyosue, A., Takahashi, M., Satonaka, H., Tanaka, K., Sata, M., Nagano, T., Nagai, R. and Hirata, Y. (2009) A new constitutively active mutant of AMP-activated protein kinase inhibits anoxia-induced apoptosis of vascular endothelial cell. *Hypertens Res.* **32**, 133-139.
47. Calvert, J. W., Gundewar, S., Jha, S., Greer, J. J. M., Bestermann, W. H., Tian, R. and Lefer, D. J. (2008) Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-Mediated signaling. *Diabetes* **57**, 696-705.
48. McCullough, L. D., Zeng, Z., Li, H., Landree, L. E., McFadden, J. and Ronnett, G. V. (2005) Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. *J. Biol. Chem.* **280**, 20493-20502.
49. Li, J., Zeng. Z., Viollet, B., Ronnett, G. V. and McCullough, L. D. (2007) Neuroprotective effects of adenosine monophosphate-activated protein kinase inhibition and gene deletion in stroke. *Stroke* **38**, 2992-2999.
50. Harada, S., Fujita-Hamabe, W. and Tokuyama, S. (2010) The importance of regulation of blood glucose levels through activation of peripheral 5'-AMP-activated protein kinase on ischemic neuronal damage. *Brain Res.* **1351**, 254-263.
51. Avraham, Y., Davidi, N., Lassri, V., Vorobiev, L., Kabesa, M., Dayan, M., Chernoguz, D., Berry, E. and Leker, R. (2011) Leptin induces neuroprotection neurogenesis and angiogenesis after stroke. *Curr. Neurovasc. Res.* **8**, 313-322.
52. Ellingson, W. J., Chesser, D. and Winder, W. W. (2007) Effects of 3-phosphoglycerate and other metabolites on the activation of AMP-activated protein kinase by LKB1-STRAD-MO25. *Am. J. Physiol. Endocrinol. Metab.* **292**, E400-E407.
53. Kim, E. K, Miller, I., Aja, S., Landree, L, E., Pinn, M., McFadden, J., Kuhajda, F. P., Moran, T. H and Ronnett, G.

V. (2004) C75, a fatty acid synthase inhibitor, reduces food intake via hypothalamicAMP-activated protein kinase. *J. Biol. Chem*. **279**, 19970-19976.
54. Wu, X., Motoshima, H., Mahadev, K., Stalker, T. J., Scalia, R. and Goldstein, B. J. (2003) Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes* **52**, 1355-1363.