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Review Article

Protective benefits of AMP-activated protein kinase in hepatic ischemia-reperfusion injury

Min Zhang[1*], Dan Yang[1*], Xianqiong Gong[2*], Pu Ge[1], Jie Dai[3], Ling Lin[1], Li Zhang[1,4]

[1]Department of Pathophysiology, Chongqing Medical University, Chongqing, China; [2]Hepatology Center, Xiamen Hospital of Traditional Chinese Medicine, Xiamen, Fujian Province, China; [3]Hospital of Chongqing University of Arts and Sciences, Chongqing, China; [4]Laboratory of Stem Cell and Tissue Engineering, Chongqing Medical University, Chongqing, China. [*]Equal contributors.

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Abstract: Hepatic ischemia-reperfusion injury (HIRI) is a major cause of hepatic failure and death after liver trauma, haemorrhagic shock, resection surgery and liver transplantation. AMP-activated protein kinase (AMPK) is an energy sensitive kinase that plays crucial roles in the regulation of metabolic homeostasis. In HIRI, ischemia induces the decline of ATP and the increased ratio of AMP/ATP, which promotes the phosphorylation and activation of AMPK. Three AMPK kinases, liver kinase B1 (LKB1), Ca^{2+}/calmodulin-depedent protein kinase kinase β (CaMKKβ) and TGF-β-activated kinase-1 (TAK1), are main upstream kinases for the phosphorylation of AMPK. In addition to the changed AMP/ATP ratio, the activated CaMKKβ by increased intracelluar Ca^{2+} and the overproduction of reactive oxygen species (ROS) are also involved in the activation of AMPK during HIRI. The activated AMPK might provide protective benefits in HIRI via prevention of energy decline, inhibition of inflammatory response, suppression of hepatocyte apoptosis and attenuation of oxidative stress. Thus, AMPK might become a novel target for the pharmacological intervention of HIRI.

Keywords: AMP-activated protein kinase, hepatic ischemia-reperfusion injury, oxidative stress, inflammation, apoptosis

Hepatic ischemia-reperfusion injury (HIRI) happens when the blood supply to liver is interrupted and subsequently returned, resulting in robust oxidative stress and inflammatory response in liver [1]. HIRI is a major cause of hepatic failure and death after liver trauma, haemorrhagic shock, resection surgery and liver transplantation [2, 3]. In HIRI, ischemia and hypoxia induce decline of ATP, a status of shortage of energy, thus directly or indirectly lead to hepatic damage [4]. Meanwhile, the fallen energy status activates several energy sensors such as AMP-activated protein kinase (AMPK) [5]. AMPK plays important roles in the maintenance of energy homeostasis via regulating energy metabolism [6]. In addition, there are increasing evidence indicated that AMPK also participated in the regulation of oxidative stress, inflammatory response and cellular apoptosis [7, 8]. Recent studies have revealed that AMPK could provide beneficial effects in HIRI and AMPK is emerging as a novel target for pharmacological intervention of HIRI [9].

The structure of AMPK

AMPK is a heterotrimeric complexes of catalytic α subunit, regulatory β/γ subunits in all eukaryotic cells [10]. In mammals, there are several subunit isoforms including α1, α2, β1, β2, γ1, γ2 and γ3 [11]. For α subunit, there is a binding segment for β/γ subunit in the C-terminal domain, an auto-inhibitory domain (AID) in the middle position and an activation of kinase domain which contains the Thr^{172} residue in the N-terminal domain [12]. The C-terminal domain of β isoform is crucial for its interaction with α and γ subunits and a glycogen/carbohydrate-binding domain/motif (GBD/CBM) is located in the middle region of β isoform [13]. α1/α2 and β1/β2 isoforms are very similar in mammalian. AMPKγ subunits whose sizes vary from the length of N terminal domain, contain four

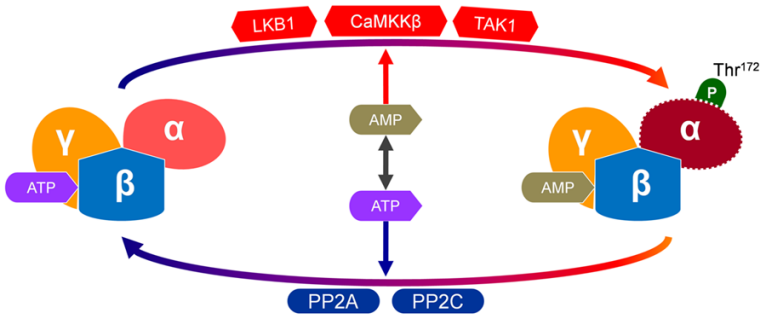

Figure 1. Activation of AMPK. AMPK is sensitive to AMP/ATP ratio, it can be activated when intracellular AMP increases. Binding AMP to AMPKγ subunit can change AMPK into a better substrate for its upstream kinases to phosphorylate and activate AMPK. The phosphorylation of AMPKα catalytic subunit at Thr^{172} is a hallmark of AMPK activation. Three AMPK kinases, liver kinase B1 (LKB1), Ca^{2+}/calmodulin-depedent protein kinase kinase β (CaMKKβ) and TGF-β-activated kinase-1 (TAK1), are main upstream kinases for the phosphorylation of Thr^{172} in AMPKα. On the contrary, AMPK can be deactivated by protein phosphatase-2A (PP2A) and protein phosphatase-2C (PP2C).

cystathionine-β-synthase (CBS) which make up two Bateman domains in a series of tandem repeats (CBS1 and CBS2 in Bateman domains 1, CBS3 and CBS4 in Bateman domains 2) [14]. Binding AMP or ADP to the AMPKγ subunits is crucial for the sensing of lower energy status and the activation of AMPK [15].

Activation of AMPK in HIRI

The phosphorylation of AMPKα catalytic subunit at Thr^{172} is a hallmark of AMPK activation [13]. AMPK is activated by metabolic stress when the intracellular AMP/ATP ratio and/or ADP/ATP ratio increases [5]. Binding AMP or ADP to AMPKγ subunit can change AMPK into a better substrate for its upstream kinases [7]. Three AMPK kinases, liver kinase B1 (LKB1), Ca^{2+}/calmodulin-depedent protein kinase kinase β (CaMKKβ) and TGF-β-activated kinase-1 (TAK1, a member of the mitogen-activated protein kinase family) have been identified as the main upstream kinases to mediate the phosphorylation of AMPKα at Thr^{172} [16]. The phosphorylation of AMPK is reversed mainly by protein phosphatase-2A (PP2A) and protein phosphatase-2C (PP2C) [17]. In addition to promoting AMPK phosphorylation, AMP can also prevent AMPK against dephosphorylation and the subsequent deactivation (**Figure 1**) [18]. In addition, the endogenous hormones including ghrelin, cannabinoids, glucocorticoids, resistin, adiponectin also play pivotal regulatory roles in the activation of AMPK [19-23].

There is evidence suggested that AMPK was activated in HIRI [24], but the underlying mechanisms largely remains unknown. Here, we will present several possible pathways (**Figure 2**). Firstly, AMPK might be activated in response to the changed ratio of AMP/ATP in liver during HIRI [9]. Secondly, the increase of intracelluar Ca^{2+} during HIRI could act as a second messenger and induce the activation of CaMKKβ [25], which is reported to be an upstream kinase of AMPK and be involved in the phosphorylation of AMPK [26]. Thirdly, increased reactive oxygen species (ROS), such as H_2O_2, were reported to be able to activate AMPK during HIRI because H_2O_2 could induce the oxidation of cysteine residues of the subunits of AMPK and then assist the phosphorylation of AMPK by increased AMP [27, 28].

The beneficial actions of AMPK in HIRI

The beneficial effects of AMPK in ischemia-reperfusion have been observed in heart and kidney [29, 30]. In rats with HIRI, administration of AMPK activator AICAR preserved ATP content, decreased lactate accumulation, suppressed hepatocyte apoptosis and alleviated hepatic injury [9]. Adiponectin is an important adipocytokine that involved in energy metabolism and other important physiological or pathological processes [23, 31]. There is increasing evidence suggests that the biological activities of adiponectin largely depend on AMPK [32-34]. Recent research found that treatment with adiponectin suppressed the elevation of aminotransferase and the degree of histological abnormalities, these beneficial effects were associated with enhanced activation of AMPK while inhibition of AMPK abolished the protective effects of adiponectin [35]. These data also support the protective actions of AMPK in HIRI.

The potential mechanism underlying the benefits of AMPK

Although the beneficial effects of AMPK in HIRI and ischemia-reperfusion injury in other organs

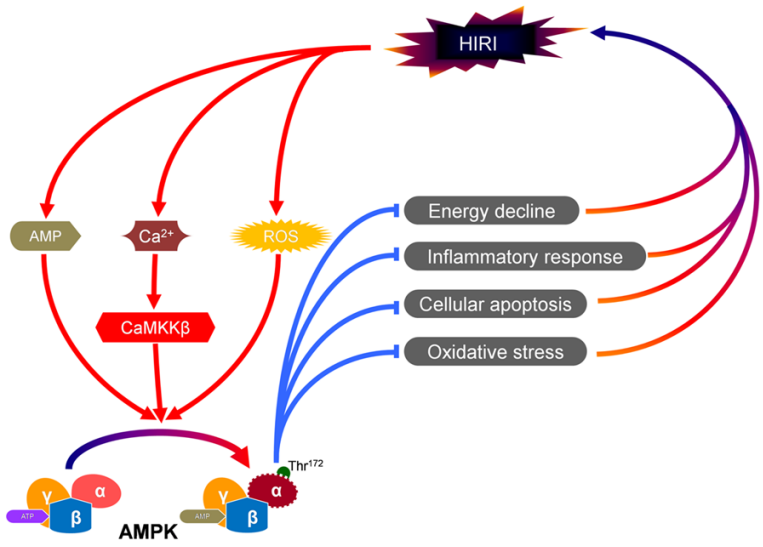

Figure 2. Pathophysiological significance of AMPK in HIRI. AMPK is activated in HIRI via several possible pathways. Firstly, AMPK might be activated in response to increased ratio of AMP/ATP. Secondly, the increased intracelluar Ca^{2+} during HIRI could induce the activation of CaMKKβ, an upstream kinase of AMPK. Thirdly, increased reactive oxygen species (ROS) might also be involved in the activation of AMPK. The activated AMPK might provide protective benefits via prevention of energy decline, inhibition of inflammatory response, suppression of hepatocyte apoptosis and attenuation of oxidative stress.

have been confirmed by various researchers, the underlying mechanisms largely remains unknown. It is well-established that the primary role of AMPK is maintenance the balance of energy metabolism [36], therefore, preventing the decline of ATP might be the basic mechanism contributes to the protective benefits of AMPK in HIRI [9]. In addition, AMPK also have pivotal regulatory roles in inflammatory response, oxidative stress and cellular apoptosis [37-39], these actions of AMPK could also provide beneficial effects in HIRI (**Figure 2**).

Maintenance of energy homeostasis

It was reported that ischemia-reperfusion could induce marked reduction in hepatic ATP level [9]. Preconditioning, a well documented approach against ischemia injury [40], stimulated the activation of hepatic AMPK, suppressed ATP decline and attenuated HIRI, treatment with AMPK activator could also maintain ATP level and provide beneficial effects [9]. These data suggested that preservation of ATP level might be closely associated with the protective effects of AMPK in HIRI [41]. The central roles of AMPK in maintenance of energy balance have been widely recognized. AMPK preserve ATP level via switching on catabolic pathways to produce ATP and shutting off anabolic pathways to prevent ATP consumption [42].

Suppression of inflammation

In addition to metabolic regulation, AMPK is also involved in several energy-intensive physiological and pathological processes such as inflammation [43, 44]. It was reported that transfection with constitutively active AMPKα significantly suppressed LPS-induced production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) in macrophages, whereas inhibition of AMPK by RNA interference dramatically enhanced the expression of TNF-α and IL-6 in LPS-stimulated macrophages [45, 46]. Additionally, the anti-inflammatory actions of AMPK activators have extensively confirmed in vitro and in vivo [47, 48].

Ischemia-reperfusion injury is usually accompanied with severe activation and infiltration of leukocytes. In HIRI, the activation of liver kupffer cell, neutrophils, T-lymphocytic and monocytes results in the generation of pro-inflammatory cytokines and matrix metalloproteinases (MMPs), these inflammatory mediators would greatly aggravate hepatic injury in HIRI [49]. It has been suggested that activation of AMPK in HIRI could suppress the expression of adhesion molecules, reduce the infiltration of leukocytes and decrease the level of pro-inflammatory cytokines [35]. Thus, suppression of inflammatory response might contribute to the protective benefits of AMPK in HIRI.

Modulation of apoptosis

AMPK is also an important regulator involved in determining the fate of cells. It was recently reported that activation of AMPK suppressed glucose deprivation-induced apoptosis in neu-

rons, hyperglycemia-induced apoptosis in endothelial cells and free fatty acids-induce apoptosis in retinal pericytes [50-52]. In rats with HIRI, administration of AMPK activator AICAR significantly suppressed the apoptosis of hepatocytes [9]. In addition, the suppressive effects of adiponectin on the cleavage of caspase-3 and the percentage of TUNEL-positive cells in rats with HIRI could be reversed by AMPK inhibitor [35]. The above data suggests that the protective benefits of AMPK in HIRI might also attribute to its anti-apoptotic activities.

Regulation of oxidative stress

Severe oxidative stress induced by ischemia-reperfusion is another crucial tache in the development of HIRI [53]. Several studies have also revealed the important roles of AMPK in antioxidant defenses. It was reported that activation of AMPK induced the expression heme oxygenase-1 (HO-1), a representative anti-oxidative enzyme, via E2-related factor 2 (Nrf2)-dependent manner [54, 55]. AMPK could also increase the expression of manganese superoxide dismutase and catalase via phosphorylation and activation of forkhead box O1 (FoxO1) [56, 57]. In addition to the enhanced antioxidant capacity, AMPK also suppressing ROS generation via inhibiting the NAD(P)H oxidase [58, 59]. These anti-oxidative activities of AMPK might also result in beneficial effects in HIRI.

Conclusions and prospects

AMPK is a critical enzyme involved in metabolic regulation and other energy-associated processes. Ischemia-reperfusion is a typical situation with severe disturbance of energy metabolism. There is increasing evidence suggests that AMPK is activated during ischemia-reperfusion and activated AMPK plays crucial roles against ischemia-reperfusion injury [39, 60]. The protective benefits of AMPK activator, such as AICAR, have been confirmed in HIRI and ischemia-reperfusion injury in other organs [9, 30]. In addition, the widely used first-line anti-diabetic drug metformin is an indirect activator of AMPK and most of the hypoglycemic actions of metformin depend on AMPK [61, 62]. Interestingly, administration of metformin also prevented ischemia-reperfusion injury, including HIRI [63-67]. Therefore, AMPK might become a novel target for the pharmacological intervention of HIRI and ischemia-reperfusion injury in other organs.

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Disclosure of conflict of interest

None.

Address correspondence to: Li Zhang, Department of Pathophysiology, Chongqing Medical University, 1 Yixueyuan Road, Chongqing 400016, China. E-mail: zhangli@cqmu.edu.cn

References

[1] Saidi RF and Kenari SK. Liver ischemia/reperfusion injury: an overview. J Invest Surg 2014; 27: 366-379.

[2] Karatzas T, Neri AA, Baibaki ME and Dontas IA. Rodent models of hepatic ischemia-reperfusion injury: time and percentage-related pathophysiological mechanisms. J Surg Res 2014; 191: 399-412.

[3] Nastos C, Kalimeris K, Papoutsidakis N, Tasoulis MK, Lykoudis PM, Theodoraki K, Nastou D, Smyrniotis V and Arkadopoulos N. Global consequences of liver ischemia/reperfusion injury. Oxid Med Cell Longev 2014; 2014: 906965.

[4] Evans ZP, Mandavilli BS, Ellett JD, Rodwell D, Fariss MW, Fiorini RN, Schnellmann RG, Schmidt MG and Chavin K. Vitamin E succinate enhances steatotic liver energy status and prevents oxidative damage following ischemia/reperfusion. Transplant Proc 2009; 41: 4094-4098.

[5] Hardie DG, Schaffer BE and Brunet A. AMPK: an energy-sensing pathway with multiple inputs and outputs. Trends Cell Biol 2016; 26: 190-201.

[6] Bertoldo MJ, Faure M, Dupont J and Froment P. AMPK: a master energy regulator for gonadal function. Front Neurosci 2015; 9: 235.

[7] Antonioli L, Colucci R, Pellegrini C, Giustarini G, Sacco D, Tirotta E, Caputi V, Marsilio I, Giron MC, Nemeth ZH, Blandizzi C and Fornai M. The AMPK enzyme-complex: from the regulation of cellular energy homeostasis to a possible new molecular target in the management of chron-

ic inflammatory disorders. Expert Opin Ther Targets 2016; 20: 179-191.
[8] Steinberg GR and Schertzer JD. AMPK promotes macrophage fatty acid oxidative metabolism to mitigate inflammation: implications for diabetes and cardiovascular disease. Immunol Cell Biol 2014; 92: 340-345.
[9] Peralta C, Bartrons R, Serafin A, Blazquez C, Guzman M, Prats N, Xaus C, Cutillas B, Gelpi E and Rosello-Catafau J. Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat. Hepatology 2001; 34: 1164-1173.
[10] Liu WY and Jiang RS. Advances in the research of AMPK and its subunit genes. Pak J Biol Sci 2013; 16: 1459-1468.
[11] Bright NJ, Thornton C and Carling D. The regulation and function of mammalian AMPK-related kinases. Acta Physiol (Oxf) 2009; 196: 15-26.
[12] Oakhill JS, Scott JW and Kemp BE. Structure and function of AMP-activated protein kinase. Acta Physiol (Oxf) 2009; 196: 3-14.
[13] Sanz P. AMP-activated protein kinase: structure and regulation. Curr Protein Pept Sci 2008; 9: 478-492.
[14] Novikova DS, Garabadzhiu AV, Melino G, Barlev NA and Tribulovich VG. AMP-activated protein kinase: structure, function, and role in pathological processes. Biochemistry (Mosc) 2015; 80: 127-144.
[15] Kim J, Yang G, Kim Y, Kim J and Ha J. AMPK activators: mechanisms of action and physiological activities. Exp Mol Med 2016; 48: e224.
[16] Russo GL, Russo M and Ungaro P. AMP-activated protein kinase: a target for old drugs against diabetes and cancer. Biochem Pharmacol 2013; 86: 339-350.
[17] Salminen A, Kaarniranta K and Kauppinen A. Age-related changes in AMPK activation: role for AMPK phosphatases and inhibitory phosphorylation by upstream signaling pathways. Ageing Res Rev 2016; 28: 15-26.
[18] Suter M, Riek U, Tuerk R, Schlattner U, Wallimann T and Neumann D. Dissecting the role of 5'-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. J Biol Chem 2006; 281: 32207-32216.
[19] Wang Y, Nishi M, Doi A, Shono T, Furukawa Y, Shimada T, Furuta H, Sasaki H and Nanjo K. Ghrelin inhibits insulin secretion through the AMPK-UCP2 pathway in beta cells. FEBS Lett 2010; 584: 1503-1508.
[20] Vara D, Salazar M, Olea-Herrero N, Guzman M, Velasco G and Diaz-Laviada I. Anti-tumoral action of cannabinoids on hepatocellular carcinoma: role of AMPK-dependent activation of autophagy. Cell Death Differ 2011; 18: 1099-1111.
[21] Nader N, Ng SS, Lambrou GI, Pervanidou P, Wang Y, Chrousos GP and Kino T. AMPK regulates metabolic actions of glucocorticoids by phosphorylating the glucocorticoid receptor through p38 MAPK. Mol Endocrinol 2010; 24: 1748-1764.
[22] Tsai CH, Tsai HC, Huang HN, Hung CH, Hsu CJ, Fong YC, Hsu HC, Huang YL and Tang CH. Resistin promotes tumor metastasis by down-regulation of miR-519d through the AMPK/p38 signaling pathway in human chondrosarcoma cells. Oncotarget 2015; 6: 258-270.
[23] Luo Y and Liu M. Adiponectin: a versatile player of innate immunity. J Mol Cell Biol 2016; 8: 120-128.
[24] Qin Y, Li Z, Wang Z, Li Y, Zhao J, Mulholland M and Zhang W. Ghrelin contributes to protection of hepatocellular injury induced by ischaemia/reperfusion. Liver Int 2014; 34: 567-575.
[25] Evankovich J, Zhang R, Cardinal JS, Zhang L, Chen J, Huang H, Beer-Stolz D, Billiar TR, Rosengart MR and Tsung A. Calcium/calmodulin-dependent protein kinase IV limits organ damage in hepatic ischemia-reperfusion injury through induction of autophagy. Am J Physiol Gastrointest Liver Physiol 2012; 303: G189-198.
[26] Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR, Carlson M and Carling D. Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metab 2005; 2: 21-33.
[27] Wang G, Yao J, Li Z, Zu G, Feng D, Shan W, Li Y, Hu Y, Zhao Y and Tian X. miR-34a-5p inhibition alleviates intestinal ischemia/reperfusion-induced reactive oxygen species accumulation and apoptosis via activation of SIRT1 signaling. Antioxid Redox Signal 2016; 24: 961-973.
[28] Emerling BM, Weinberg F, Snyder C, Burgess Z, Mutlu GM, Viollet B, Budinger GR and Chandel NS. Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. Free Radic Biol Med 2009; 46: 1386-1391.
[29] Paiva MA, Goncalves LM, Providencia LA, Davidson SM, Yellon DM and Mocanu MM. Transitory activation of AMPK at reperfusion protects the ischaemic-reperfused rat myocardium against infarction. Cardiovasc Drugs Ther 2010; 24: 25-32.
[30] Decleves AE, Sharma K and Satriano J. Beneficial effects of AMP-activated protein kinase agonists in kidney ischemia-reperfusion: autophagy and cellular stress markers. Nephron Exp Nephrol 2014; [Epub ahead of print].

[31] Ruan H and Dong LQ. Adiponectin signaling and function in insulin target tissues. J Mol Cell Biol 2016; 8: 101-109.
[32] Ding W, Zhang Q, Dong Y, Ding N, Huang H, Zhu X, Hutchinson S, Gao X and Zhang X. Adiponectin protects the rats liver against chronic intermittent hypoxia induced injury through AMP-activated protein kinase pathway. Sci Rep 2016; 6: 34151.
[33] de Boer MP, Meijer RI, Richter EA, van Nieuw Amerongen GP, Sipkema P, van Poelgeest EM, Aman J, Kokhuis TJ, Koolwijk P, van Hinsbergh VW, Smulders YM, Serne EH and Eringa EC. Globular adiponectin controls insulin-mediated vasoreactivity in muscle through AMPKalpha2. Vascul Pharmacol 2016; 78: 24-35.
[34] Zhao L, Fu Z, Wu J, Aylor KW, Barrett EJ, Cao W and Liu Z. Globular adiponectin ameliorates metabolic insulin resistance via AMPK-mediated restoration of microvascular insulin responses. J Physiol 2015; 593: 4067-4079.
[35] Zhang C, Liao Y, Li Q, Chen M, Zhao Q, Deng R, Wu C, Yang A, Guo Z, Wang D and He X. Recombinant adiponectin ameliorates liver ischemia reperfusion injury via activating the AMPK/eNOS pathway. PLoS One 2013; 8: e66382.
[36] Grahame Hardie D. AMP-activated protein kinase: a key regulator of energy balance with many roles in human disease. J Intern Med 2014; 276: 543-559.
[37] O'Neill LA and Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. Nature 2013; 493: 346-355.
[38] Wu SB, Wu YT, Wu TP and Wei YH. Role of AMPK-mediated adaptive responses in human cells with mitochondrial dysfunction to oxidative stress. Biochim Biophys Acta 2014; 1840: 1331-1344.
[39] Qi D and Young LH. AMPK: energy sensor and survival mechanism in the ischemic heart. Trends Endocrinol Metab 2015; 26: 422-429.
[40] Suyavaran A and Thirunavukkarasu C. Preconditioning methods in the management of hepatic ischemia reperfusion-induced injury: update on molecular and future perspectives. Hepatol Res 2017; 47: 31-48.
[41] Huynh MK, Kinyua AW, Yang DJ and Kim KW. Hypothalamic AMPK as a regulator of energy homeostasis. Neural Plast 2016; 2016: 2754078.
[42] Ross FA, MacKintosh C and Hardie DG. AMP-activated protein kinase: a cellular energy sensor that comes in 12 flavours. FEBS J 2016; 283: 2987-3001.
[43] Yang CH, Shen YJ, Lai CJ and Kou YR. Inflammatory role of ROS-sensitive AMP-activated protein kinase in the hypersensitivity of lung vagal C fibers induced by intermittent hypoxia in rats. Front Physiol 2016; 7: 263.
[44] Ren J, Xu X, Wang Q, Ren SY, Dong M and Zhang Y. Permissive role of AMPK and autophagy in adiponectin deficiency-accentuated myocardial injury and inflammation in endotoxemia. J Mol Cell Cardiol 2016; 93: 18-31.
[45] Sag D, Carling D, Stout RD and Suttles J. Adenosine 5'-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. J Immunol 2008; 181: 8633-8641.
[46] Carroll KC, Viollet B and Suttles J. AMPKalpha1 deficiency amplifies proinflammatory myeloid APC activity and CD40 signaling. J Leukoc Biol 2013; 94: 1113-1121.
[47] Jhun BS, Jin Q, Oh YT, Kim SS, Kong Y, Cho YH, Ha J, Baik HH and Kang I. 5-Aminoimidazole-4-carboxamide riboside suppresses lipopolysaccharide-induced TNF-alpha production through inhibition of phosphatidylinositol 3-kinase/Akt activation in RAW 264.7 murine macrophages. Biochem Biophys Res Commun 2004; 318: 372-380.
[48] Zhao X, Zmijewski JW, Lorne E, Liu G, Park YJ, Tsuruta Y and Abraham E. Activation of AMPK attenuates neutrophil proinflammatory activity and decreases the severity of acute lung injury. Am J Physiol Lung Cell Mol Physiol 2008; 295: L497-504.
[49] van Golen RF, Reiniers MJ, Olthof PB, van Gulik TM and Heger M. Sterile inflammation in hepatic ischemia/reperfusion injury: present concepts and potential therapeutics. J Gastroenterol Hepatol 2013; 28: 394-400.
[50] Culmsee C, Monnig J, Kemp BE and Mattson MP. AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. J Mol Neurosci 2001; 17: 45-58.
[51] Ido Y, Carling D and Ruderman N. Hyperglycemia-induced apoptosis in human umbilical vein endothelial cells: inhibition by the AMP-activated protein kinase activation. Diabetes 2002; 51: 159-167.
[52] Cacicedo JM, Benjachareonwong S, Chou E, Yagihashi N, Ruderman NB and Ido Y. Activation of AMP-activated protein kinase prevents lipotoxicity in retinal pericytes. Invest Ophthalmol Vis Sci 2011; 52: 3630-3639.
[53] Elias-Miro M, Jimenez-Castro MB, Rodes J and Peralta C. Current knowledge on oxidative stress in hepatic ischemia/reperfusion. Free Radic Res 2013; 47: 555-568.
[54] Liu XM, Peyton KJ, Shebib AR, Wang H, Korthuis RJ and Durante W. Activation of AMPK stimulates heme oxygenase-1 gene expression and human endothelial cell survival. Am J Physiol Heart Circ Physiol 2011; 300: H84-93.

[55] Zimmermann K, Baldinger J, Mayerhofer B, Atanasov AG, Dirsch VM and Heiss EH. Activated AMPK boosts the Nrf2/HO-1 signaling axis--A role for the unfolded protein response. Free Radic Biol Med 2015; 88: 417-426.

[56] Awad H, Nolette N, Hinton M and Dakshinamurti S. AMPK and FoxO1 regulate catalase expression in hypoxic pulmonary arterial smooth muscle. Pediatr Pulmonol 2014; 49: 885-897.

[57] Yun H, Park S, Kim MJ, Yang WK, Im DU, Yang KR, Hong J, Choe W, Kang I, Kim SS and Ha J. AMP-activated protein kinase mediates the antioxidant effects of resveratrol through regulation of the transcription factor FoxO1. FEBS J 2014; 281: 4421-4438.

[58] Alba G, El Bekay R, Alvarez-Maqueda M, Chacon P, Vega A, Monteseirin J, Santa Maria C, Pintado E, Bedoya FJ, Bartrons R and Sobrino F. Stimulators of AMP-activated protein kinase inhibit the respiratory burst in human neutrophils. FEBS Lett 2004; 573: 219-225.

[59] Song P and Zou MH. Regulation of NAD(P)H oxidases by AMPK in cardiovascular systems. Free Radic Biol Med 2012; 52: 1607-1619.

[60] Ahn YJ, Kim H, Lim H, Lee M, Kang Y, Moon S, Kim HS and Kim HH. AMP-activated protein kinase: implications on ischemic diseases. BMB Rep 2012; 45: 489-495.

[61] Sliwinska A and Drzewoski J. Molecular action of metformin in hepatocytes: an updated insight. Curr Diabetes Rev 2015; 11: 175-181.

[62] Foretz M, Guigas B, Bertrand L, Pollak M and Viollet B. Metformin: from mechanisms of action to therapies. Cell Metab 2014; 20: 953-966.

[63] Asghari A, Akbari G, Meghdadi A and Mortazavi P. Protective effect of metformin on testicular ischemia/reperfusion injury in rats. Acta Cir Bras 2016; 31: 411-416.

[64] Wang M, Weng X, Guo J, Chen Z, Jiang G and Liu X. Metformin alleviated EMT and fibrosis after renal ischemia-reperfusion injury in rats. Ren Fail 2016; 38: 614-621.

[65] Cahova M, Palenickova E, Dankova H, Sticova E, Burian M, Drahota Z, Cervinkova Z, Kucera O, Gladkova C, Stopka P, Krizova J, Papackova Z, Oliyarnyk O and Kazdova L. Metformin prevents ischemia reperfusion-induced oxidative stress in the fatty liver by attenuation of reactive oxygen species formation. Am J Physiol Gastrointest Liver Physiol 2015; 309: G100-111.

[66] Ashabi G, Khalaj L, Khodagholi F, Goudarzvand M and Sarkaki A. Pre-treatment with metformin activates Nrf2 antioxidant pathways and inhibits inflammatory responses through induction of AMPK after transient global cerebral ischemia. Metab Brain Dis 2015; 30: 747-754.

[67] Barreto-Torres G, Parodi-Rullan R and Javadov S. The role of PPARalpha in metformin-induced attenuation of mitochondrial dysfunction in acute cardiac ischemia/reperfusion in rats. Int J Mol Sci 2012; 13: 7694-7709.