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 Thr172 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...
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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 the 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), 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).

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 intracellular 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_{2}O_{2}, were reported to be able to activate AMPK during HIRI because H_{2}O_{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 amiotransferase 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 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).

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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-
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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 (Nr2f2)-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.

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