Commentary

Role of AMPK activation in oxidative cell damage: Implications for alcohol-induced liver disease

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A B S T R A C T

Chronic alcohol consumption is a well-known risk factor for liver disease. Progression of alcohol-induced liver disease (ALD) is a multifactorial process that involves a number of genetic, nutritional and environmental factors. Experimental and clinical studies increasingly show that oxidative damage induced by ethanol contributes in many ways to the pathogenesis of alcohol hepatotoxicity. Oxidative stress appears to activate AMP-activated protein kinase (AMPK) signaling system, which has emerged in recent years as a kinase that controls the redox-state and mitochondrial function. This review focuses on the most recent insights concerning the activation of AMPK by reactive oxygen species (ROS), and describes recent evidences supporting the hypothesis that AMPK signaling pathways play an important role in promoting cell viability under conditions of oxidative stress, such as during alcohol exposure. We suggest that AMPK activation by ROS can promote cell survival by inducing autophagy, mitochondrial biogenesis and expression of genes involved in antioxidant defense. Hence, increased intracellular concentrations of ROS may represent a general mechanism for enhancement of AMPK-mediated cellular adaptation, including maintenance of redox homeostasis. On the other hand, AMPK inhibition in the liver by ethanol appears to play a key role in the development of steatosis induced by chronic alcohol consumption. Although more studies are needed to assess the functions of AMPK during oxidative stress, AMPK may be a possible therapeutic target in the particular case of alcohol-induced liver disease.

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1. Introduction

Chronic alcohol consumption is a well-known risk factor for liver disease, which represents a major cause of morbidity and mortality worldwide [1]. Progression of alcohol-induced liver disease (ALD) is a multifactorial process that involves a number of genetic, nutritional and environmental factors [2]. Among the mechanisms implicated in the pathogenesis of ALD, oxidative stress has received growing interest [3]. Oxidative stress exists when there is an imbalance between oxidants and antioxidant defenses in favor of the oxidants in the cell. Reactive oxygen species (ROS) are produced by normal cellular metabolism with beneficial effects such as cytotoxicity against bacteria and other pathogens. However, these reactive species also may affect cells of the host organism, by leading to the oxidation of cellular macromolecules, such as lipids, protein or DNA, inhibiting normal function [4]. For instance, peroxidation of lipids can result in destruction of biological membranes [5], while alterations induced by ROS in different signaling pathways may modulate gene expression, cell metabolism, cell cycle and cell death [5,6].

Oxidative stress appears to activate the AMP-activated protein kinase (AMPK) signaling system in various cell types, including neuronal, heart, skeletal and vascular smooth muscle, pancreatic and liver cells [7]. AMPK plays a key role in cellular and organism survival during metabolic stress by its ability to maintain metabolic homeostasis. However, it also controls the redox-state and mitochondrial function. Interestingly, AMPK-associated pathways may suppress the cell death induced by oxidative stress [8]. For example, AMPK seems to be required for ROS-triggered autophagy, which promotes cell survival in response to cellular stresses, such as nutrient starvation, hypoxia or ischemia [9].

The first part of this review focuses on the most recent insights concerning the activation of AMPK by oxidative stress. We then describe recent evidence supporting the hypothesis that AMPK

Abbreviations: AMPKK, AMP-activated protein kinase kinase; PKA/PKB, Protein Kinase A/B; VSMCs, Vascular Smooth Muscle Cells; BrDU, 5-bromo-2′-deoxyuridine; PI3K, Phosphatidylinositol 3-kinase; VDAC, Voltage-dependent anion channels; ANT, Adenine Nucleotide Translocase; G6PD, Glucose-6-phosphate dehydrogenase; 6-ANAD, 6-aminonicotinic acid amide-analog of NAD; DHEA, Dehydroepiandrosterone.

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signaling pathways play an important role in promoting cell viability under conditions of oxidative stress, such as during alcohol exposure.

2. AMPK activation and oxidative stress

2.1. Involvement of oxidative stress and AMPK in ALD

Steatosis induced by chronic alcohol consumption can be directly linked to a critical signaling pathway that increases lipogenesis in the liver, i.e., AMPK inhibition [10]. Indeed, AMPK activates fatty acid oxidation and inhibits lipogenesis in rat hepatocytes and in the livers of ethanol-fed mice [11]. The inhibition of AMPK leads to activation of acetyl-CoA carboxylase (ACC), enhancing malonyl CoA levels, which inhibit fatty acid uptake and β-oxidation in mitochondria. However, chronic ethanol exposure has been shown to inhibit AMPK activity in cultured rat hepatocytes through the inhibition of PK-ζ and LKB1 phosphorylation [11] and impaired AMPK activity was shown in hepatocytes isolated from rats fed with ethanol [12]. Moreover, pharmacological AMPK activation abrogated ethanol-induced induction of lipogenesis and reduction of fatty acid oxidation [12,13]. Thus, alcohol-associated inhibition of AMPK contributes to fat accumulation via stimulation of lipogenesis and inhibition of fat oxidation. Additionally, it has been demonstrated that activation of AMPK by adiponectin and other agents, such as rosiglitazone, improves alcohol-induced fatty liver disease in rodent models [14,15], demonstrating the key role played by AMPK inhibition in the development of steatosis.

However, this AMPK inhibition by alcohol is a paradox, because ethanol metabolism produces ROS. ROS is a collective term used to designate oxygen free radicals, such as superoxide anion (O2•−) and hydroxyl radical (HO•), and also oxygen derivatives that do not contain unpaired electrons, such as hydrogen peroxide (H2O2), singlet oxygen (¹O2) and hypochlorous acid (HOCI). Under chronic ethanol exposure, it is well established that ROS production is enhanced [3]. ROS are critical controlling factors in the activation of AMPK. Indeed, ROS-mediated AMPK activation is induced by a wide range of stimuli, including hyperglycemia and treatment with metformin [7,16]. These results suggest that inhibition of AMPK by alcohol and activation of AMPK by ROS occur by different mechanisms and likely at different signaling pathways that regulate AMPK. Recently, Everitt et al. [17] demonstrated that hepatic AMPK signaling was severely impaired in the livers of chronically ethanol-fed ob/ob mice by ROS-independent mechanisms. They suggest that ethanol may disrupt AMPK signaling through an augmented shift in the ratio of hepatic NAD⁺ to NADH concentration. Another study showed that the inhibitory effect of ethanol on AMPK activity appears to be mediated in part through increased cellular levels of hepatic ceramide and activation of protein phosphatase 2A [18].

On the other hand, although much of the studies show that alcohol inhibits AMPK, there are some reports which do not observe these inhibitory effects [15,19]. For example, dietary fat plays an important role in regulation of AMPK by ethanol. Indeed, a recent study shows a significantly decreased hepatic AMPK activation in mice fed with moderate dietary fat combined with ethanol, whereas high dietary fat plus ethanol led to an increase in AMPK activation [20]. Thus, effects of alcohol on AMPK pathway vary depending on the animal model of ethanol exposure utilized.

2.2. AMPK, the metabolic master switch

AMPK is an evolutionarily conserved serine/threonine kinase that is ubiquitously expressed. It is a heterotrimer consisting of a catalytic α-subunit and two regulatory subunits, β and γ. Multiple isoforms exist, giving twelve possible combinations of holoenzymes with different tissue distributions and subcellular localization. AMPK was initially characterized as a “fuel gauge”, modulating cellular energy flux in eukaryotic cells in response to changes in the AMP/ATP ratio [21].

AMPK is activated by conditions that increase intracellular AMP such as hypoglycemia, hypoxia and exercise, as well as certain cytokines and drugs, such as adiponectin, leptin, metformin, statin, rosiglitazone and 5-aminoimidazole-4-carboxamide riboside (AICAR). The Pellez-Jeghers protein, or liver kinase B1 (LKB1), Ca2+/calmodulin-dependent protein kinase kinase-β (CaMKKβ) and transforming growth factor-β-activated kinase 1 (TAK1) are upstream kinases that activate AMPK by phosphorylating Thr172 in the activation loop of the catalytic α-subunits (Fig. 1) [22].

Protein phosphatase 2A (PP2A) and PP2C dephosphorylate AMPK at Thr172. Moreover, α1- and α2-containing AMPK complexes are both inactivated by PP2C, and the α1-isoform is more resistant than α2 to inactivation by PP2A. Both PKB and PKA have been reported to phosphorylate AMPKα1/α2 at Ser485/491, which inhibits AMPK phosphorylation at Thr172 [23]. More recent evidence in primary mouse adipocytes has shown that AMPKα1 phosphorylation at Thr172 can be impeded by AMPKα1 phosphorylation at Ser173 mediated by PKA [24].

An increase in AMP allosterically stimulates AMPK activity by binding to the γ-subunits and also prevents dephosphorylation of Thr172. Once activated, AMPK switches on catabolic pathways that generate ATP (i.e., glucose uptake, glycolysis, fatty acid oxidation, mitochondrial biogenesis, and autophagy), while switching off ATP-consuming processes (i.e., protein synthesis and cell division). The AMPK signaling system plays a key role in cellular and organism survival during metabolic stress by its ability to maintain metabolic homeostasis [25].

Interestingly, it is now becoming clear that metabolic stress is not the only pathological or physiological condition that activates AMPK and that the function of this enzyme extends beyond metabolic control and energy homeostasis, to include, for example, the control of cell architecture, cell polarity and ion transport [26]. For example, osmotic stress is able to regulate the AMPK signaling system. Another example is provided by hypertonic shock, which leads to Ca2+-dependent AMPK activation via CaMKKKβ in human erythrocytes and in epithelial cells [27,28]. Moreover, hyperosmolarity-induced AMPK activation leads to actin cytoskeleton reorganization in epithelial cells [28].

2.3. AMPK activation by oxidative stress

The first studies on the effects of oxidative stress on AMPK activation started ten years ago when Choi et al. [29] demonstrated that AMPK cascades were highly sensitive to ROS. These authors showed a transient and concentration-dependent activation of AMPK by H2O2 in NIH-3T3 cells. Consistent with many previous reports showing that H2O2 induces intracellular ATP depletion [30], they observed that the profile of AMPK activation caused by H2O2 was tightly associated with a rapid increase in the AMP:ATP ratio. Moreover, DMSO, a potent hydroxyl radical scavenger, significantly blocked AMPK activation as well as ATP depletion caused by H2O2, suggesting that the change in the intracellular AMP:ATP ratio caused by H2O2 is a major signal for AMPK regulation under oxidative stress. Other research groups then demonstrated that addition of H2O2 to neuronal cells resulted in activation of AMPK [31]. Because this activation was blocked by the antioxidant, N-acetyl-cysteine (NAC), these authors concluded that oxidative stress activates AMPK.

Recent studies have demonstrated that ROS stimulate AMPK activity via an AMP-independent mechanism. Indeed, because the effects of hypoxia on AMPK activation in the embryos of pregnant
mice were prevented by the antioxidants, GSH-ethyl ester or vitamin E, it was concluded that the effects of hyperglycemia-induced hypoxia in stimulating AMPK are solely due to the production of ROS [32]. Finally, Quintero et al. [33] showed that mitochondrial-derived ROS activate AMPK by a mechanism independent of nucleotide concentration in vascular endothelium cells.

Glucose deprivation induces depletion in intracellular ATP [34], which consequently elevates the AMP/ATP ratio and activates AMPK [35]. In addition, glucose deprivation increases O$_2^*$ and H$_2$O$_2$ generation in human colon and breast cancer cells [36] and activates AMPK in pancreatic β cells via ROS production [37]. A recent study has further demonstrated the involvement of H$_2$O$_2$ in AMPK activation in endothelial cells under glucose deprivation [38].

2.3.1. AMPK activation via LKB1

The correlation between AMPK activity and the phosphorylation level of AMPK at Thr172 indicates that phosphorylation of AMPK by an upstream AMPKK is a crucial step for AMPK activation in intact cells in response to H$_2$O$_2$-induced oxidative stress (Fig. 1). Wood et al. [39] showed that blocking LKB1 activity resulted in an
inability of H2O2 to activate AMPK, but H2O2 did not directly activate LKB1. Another group demonstrated that thromboxane receptors activate AMPK in VSMCs via LKB1 in an H2O2-dependent manner [40]. These authors suggested that this effect was mediated by protein kinase C (PKC)-ζ, which phosphorylated LKB1 at Ser428 [41]. This phosphorylation of LKB1 would promote its export from the nucleus, resulting in activation of AMPK [42]. LKB1 has been shown to be a target for the phosphorylation of the cellular damage sensor, Ataxia-telangiectasia mutated (ATM), at Thr366 in response to DNA damage [43]. ATM is a protein kinase involved in metabolic regulation, and ATM deficiency is associated with elevated ROS [44]. Recently, it has been shown that following treatment with H2O2, ATM-dependent phosphorylation of LKB1 at Thr366 mediated activation of AMPK [45].

Finally, drugs such as metformin and berberine, which are able to inhibit complex I of the respiratory chain to generate mitochondrial superoxide anion, O2•-, and then peroxynitrite, ONOO-, lead to AMPK activation via a c-Src and PI3K-dependent pathway [7,46]. It should be noted that phosphorylation of LKB1 at Ser307 is involved in LKB1-mediated AMPK activation induced by berberine [47].

2.3.2. AMPK activation via CaMKKβ

Several studies have shown that AMPK can also be activated by CaMKKβ [48]. Liangpunsakul et al. [49] showed that the level of AMPK phosphorylation in HeLa cells, which lack LKB1 [50], was significantly increased after H2O2 treatment. Moreover, the CaMKKβ inhibitor, STO-609, inhibited AMPK phosphorylation by 75% and prevented AMPK activation by H2O2 in these cells. These data suggest that phosphorylation of AMPK-α on Thr-172 by H2O2 can result from activation of either PKC-ζ/LKB1 or CaMKKβ depending on the cell type (Fig. 1).

2.3.3. AMPK activation by S-glutathionylation

More recently [51], it has been demonstrated that H2O2 directly causes the oxidative modification of the AMPKα subunit, including S-glutathionylation of cysteine residues (C299/304), without depletion of cellular ATP (Fig. 2). Moreover, the catalytic AMPK-α1 subunit is the only one present in endothelial cells [33]. This distribution is different to liver, cardiomyocytes, and skeletal muscle [52], in which both catalytic subunits are expressed. Although activation of the α2-subunit of AMPK is dependent on bioenergetic crisis [53], the α1-subunit is known to be far less responsive to changes in AMP. Oxidative stress, therefore, only activates α1-subunit-containing AMPK, and the sensitivity of the AMPK cascade to oxidative stress will, thus, depend on the tissue model or cell type.

Taken together, these data demonstrate that increased intracellular concentrations of ROS may be a general mechanism for AMPK activation under certain physiological or pathological conditions, including hyperglycemia, hypoxia, glucose deprivation, and enhancement of AMPK-mediated cellular adaptation, including maintenance of redox homeostasis [54], such that AMPK might function as an “early warning system” in response to oxidants to attenuate oxidative injury [55].

![Fig. 2. AMPK activation by oxidative stress. Glucose deprivation and drugs like metformin and berberine, which are AMPK activators, are able to inhibit complex I of the respiratory chain to generate mitochondrial superoxide anion O2•-. ROS can activate AMPK by three pathways: activation of both AMPKK LKB1 and CaMKKβ, and by direct oxidative modification of the AMPKα catalytic subunit. ROS have been shown to activate LKB1 via protein kinase C-ζ, which phosphorylates LKB1 at Ser428. Moreover, LKB1 is a target for the cellular damage sensor ATM phosphorylation at Thr366 in response to H2O2 treatment. At last, ROS lead to phosphorylation of LKB1 at Ser307 via a c-Src and PI3K-dependent pathway. Alternatively, AMPK activation by H2O2 treatment can also result from CaMKKβ activation. Finally, ROS directly cause the S-glutathionylation of cysteine residues (C299/304).](attachment:image.png)
3. AMPK-associated signaling pathways mediate cell survival under oxidative stress

3.1. AMPK regulates cell death induced by ROS

A decade ago, many studies have shown that AMPK played an important role in the decision between cell survival and death [56]. Early reports showed that pharmacological activation of AMPK protected fibroblast from apoptosis induced by serum withdrawal [57]. AMPK was also shown to play a critical role in protecting the liver from ischemia-reperfusion injury [58]. Based on experiments with AMPK activators and inhibitors, Saberi et al. [59] showed that the upregulation of AMPK promoted hepatocyte survival and significantly reduced H$_2$O$_2$-induced necrosis. However, recent evidence demonstrates that AMPK can have different roles. For example, AMPK activation during transitory bioenergetic collapse by excitotoxic stress in neurons leads to the induction of apoptosis [60,61]. Conversely, activation of AMPK inhibits lipid-mediated endoplasmic reticulum stress and apoptosis in hepatocytes [62]. Thus, the role of AMPK in cell death induced by ROS seems to depend on the cell environmental context, and notably on the exposure time of the cell to the oxidative stress (Fig. 3).

3.1.1. Pro-survival signaling pathways

AMPK has been identified as a major regulator of mitochondrial biogenesis in response to energy depletion [63]. Pharmacologically activated AMPK conveys its signal to induce mitochondrial biogenesis via the PGC-1α/NRF pathway [63,64]. Another study, using endothelial cells in which the α1-subunit of AMPK had been silenced, showed that this kinase was able to induce the expression of genes involved in antioxidant defense, such as MnSOD, catalase, γ-glutamylcysteine synthase and thioredoxin [54]. The authors observed that Foxo3a was significantly reduced in these AMPKα1-silenced cells both at the protein and mRNA levels, suggesting its involvement in the regulation of the antioxidant defense genes by AMPK (Fig. 3).

Moreover, silencing of the α1-subunit of AMPK leads to a decreased mitochondrial content and increased accumulation of ROS, suggesting that AMPKα1 regulates both mitochondrial content and antioxidant defenses in endothelial cells. Furthermore, these authors observed that AMPKα1 silencing increased the sensitivity of endothelial cells to oxidative stress. It has also been reported that H$_2$O$_2$ increases mitochondrial biogenesis in human osteosarcoma 143B cells [65]. These data suggest that ROS may increase mitochondrial biogenesis through the AMPK pathway and, therefore, AMPK activation by ROS may promote cell survival by inducing mitochondrial biogenesis.

Resveratrol, a natural polyphenolic compound, has been shown to inhibit both mitochondrial ROS generation and mitochondrial permeability transition, thereby protecting the mitochondria against the oxidative stress [66]. This cytoprotective effect is dependent on AMPK activation. Shin et al. [66] also showed that resveratrol activated AMPK, which phosphorylates the glycogen synthase kinase-3β (GSK3β) at the Ser9 residue. This phosphorylation leads to the inhibition of the activity of GSK3β. However, activated GSK3β in mitochondria binds to and phosphorylates the components of the mitochondrial membrane pore, such as VDAC...
and ANT, and thereby induces MMP transition [67]. This demonstrates that the AMPK-dependent mitochondrial protection of resveratrol against oxidative stress may be associated with the downstream inhibitory phosphorylation of GSK3β.

Finally, an AMPK-mediated increase in glycolysis in skin fibroblasts was demonstrated to be essential for the survival of cells under oxidative stress [68]. These findings are in line with previous reports that AMPK-mediated activation of glycolysis was required for the protection of astrocytes against oxidative stress [69]. It is well known that intracellular NADPH production is controlled by G6PD [70]. The expression of G6PD is regulated by oxidative stress because of the presence of an antioxidant response element (ARE) in the promoter region of the G6PD gene [71]. Nevertheless, up-regulation of G6PD protein expression by H2O2 was observed in shAMPK–1α-transfected cells, suggesting that the expression of G6PD was not regulated by AMPK [68]. A recent report showing that G6PD activity can be regulated by reversible tyrosine phosphorylation [72], raised the question of whether AMPK can activate G6PD by post-translational modification. Although glycolysis and the pentose phosphate pathway (PPP) are parallel pathways in glucose metabolism, the redistribution of glycolytic flux can regulate PPP activity for the generation of NADPH [73]. The findings of this study further suggest that the increase in glycolytic flux exerted by AMPK activation can regulate intracellular NADPH production. However, we recently observed that inhibition of PPP by 6-ANAD or DHEA did not abolish the AMPK-induced increased in GSH, or the decrease in ROS content in HepG2 hepatocarcinoma cells [unpublished data].

The AMPK signaling system is, therefore, essential for the survival of cells under oxidative stress. However, the mechanism of action of AMPK in cells under oxidative stress remains unclear. Indeed, the involvement of AMPK in the induction of cell death has also been demonstrated, mostly in conditions of sustained activation [61].

3.1.2. Pro–death signaling pathways

Multiple studies have demonstrated that prolonged AMPK activation with AICAR can be toxic to cells [74]. Prolonged AMPK activation has been shown to trigger apoptosis [37,60,74] and potentiate apoptosis induced by high glucose concentrations [16] in pancreatic β cells. These effects seem to be the result of enhanced production of mitochondria-derived ROS and the onset of the intrinsic mitochondrial apoptosis pathway, followed by caspase activation and Bcl-2 cleavage which may amplify the death signal. It has been demonstrated that AMPK negatively regulates cell proliferation and induces cell cycle arrest by promoting phosphorylation of p53 or p27 in various tumor cells [75]. Moreover, AMPK and mTOR pathways seem to configure a network connecting nutritional status with the apoptotic machinery in diverse cancer cells. In fact, various pro-apoptotic BH3-only and anti-apoptotic Bcl-2 proteins can be regulated by each of these kinases: prolonged AMPK activation is directly associated with the transcriptional induction of the BH3-only protein, Bim, probably via FOXO3A or JNK pathways, and activation of the Bcl-2-regulated apoptotic pathway [60]; Puma is regulated indirectly by p53 downstream AMPK signaling [76]; and Noxa can be up-regulated in the same way [77,78]. In turn, since Bcl-2 is both induced and protected by phosphorylation by mTOR signaling [79], inhibition of this latter by AMPK may favor the onset of apoptosis.

AMPK activation appears to drive either cell death or survival, but some of the AMPK targets that are required to propagate this signal through phosphorylation cascades are also involved in both cell death and survival processes. This suggests that other intermediate modulators or interactors, which determine whether cell death or survival pathways predominate under oxidative stress, remain to be identified [80]. However, we can hypothesize that the roles played by AMPK may be dictated by the degree of intracellular ROS content and by the duration of the oxidative stress conditions. Indeed, it has been demonstrated that AMPK activation can lead to the induction of apoptosis in liver cells and that AMPK activation had to be sustained (>10 h) to trigger apoptosis. The delay between AMPK activation and the onset of apoptosis may correspond to the time required to accumulate pro-apoptotic proteins in excess of anti-apoptotic proteins [74]. Thus, AMPK activation by short exposure to oxidative stress would promote a cell survival pathway preferentially, whereas AMPK activation by prolonged exposure to ROS would trigger cell death (Fig. 3).

3.2. AMPK induces autophagy under oxidative stress conditions

Among the mechanisms involved in the regulation of cell survival and death, autophagy is emerging as an important mediator of pathological responses and is engaged in cross-talk with ROS in cell signaling and removal of damaged proteins [81]. Autophagy, primarily described as a survival mechanism, can also lead to cell death. However, in most liver diseases, it seems clear that one of its major functions is to fight to keep cells alive under stressful “life-threatening” conditions [82]. It is now widely accepted that ROS also induce autophagy [83], and that autophagy, in turn, serves to reduce oxidative damage [84,85].

3.2.1. Autophagy activation by oxidative stress

Macroautophagy, referred to herein as autophagy, is an intracellular bulk degradation process involved in the degradation of many long-lived cytosolic proteins and organelles, which affects various physiological and pathological processes [86]. Autophagy starts with the formation of isolation membranes called phagophores, which elongate and engulf a portion of the cytoplasm to form mature autophagosomes. These autophagosomes then fuse with lysosomes to form autolysosomes, in which acidic lysosomal hydrolases digest the engulfed contents [87].

Autophagosome formation involves autophagy-related proteins (Atg), which are tightly regulated, most notably downstream of mTOR Ser/Thr kinase [88]. Atg4, an essential protease in the autophagic pathway, has been identified as a direct target for oxidation by H2O2. This cysteine protease cleaves Atg8 at the C-terminus, priming Atg8 for subsequent conjugation to phosphatidylethanolamine (PE) [89]. Atg8–PE will be incorporated into the autophagosome membrane, which is necessary for autophagosome maturation [90]. However, Atg4 can also deconjugate Atg8 from PE, resulting in autophagosome disassembly. Thus, inactivation of Atg4 following the initial cleavage of Atg8 is needed to ensure the structural integrity of the mature autophagosome. During cellular starvation, there is an increased production of mitochondrial-derived ROS through a P38 kinase-dependent pathway [91]. This results in oxidation and inhibition of Atg4, increasing Atg8–PE conjugation and promoting autophagy. ROS can also induce autophagy through a beclin-1-dependent mechanism [92]. Under basal conditions, beclin-1 is negatively regulated by its interaction with Bcl-2 [93]. However, increased ROS concentrations activate the ubiquitin-dependent–proteasome system, which degrades Bcl-2 allowing for beclin-1 activation and subsequent autophagy [94].

3.2.2. Role of AMPK–mTOR pathway in ROS-induced autophagy

mTOR is one of the key regulators of autophagy. This phenomenon occurs at low basal levels in virtually all cells to perform homeostatic functions and is rapidly upregulated through inhibition of mTOR when cells need to generate intracellular nutrients and energy [95]. Thus, depletion of ATP and subsequent...
AMPK activation will hamper autophagy. A study employing an AMPK inhibitor, compound C, or a dominant-negative AMPK mutant, indicated that AMPK was required for autophagy by amino acid deprivation [96]. This finding, that AMPK is a positive regulator of autophagy, is supported by other studies in yeast [97] and in mammalian cells [98] under various conditions, including ischemia.

Interestingly, ROS inhibit mTOR through activation of AMPK and inhibition of AKT, leading to apoptosis [99] and autophagy-dependent cell death [100]. Notably, this pathway may be mediated via cytoplasmic ATM. Indeed, in response to H2O2, ATM activates the Tuberous sclerosis complex 2 (TSC2) in the cytoplasm via AMPK. This activation results in inhibition of mTOR and induces autophagy [34].

mTOR is also known to phosphorylate and actively sequester the mammalian homolog of Atg1, ULK1, in a complex with Atg13 and FIP200 in an inactive state [101]. The activation of AMPK during nutrient deprivation can inhibit mTOR activity [102], consequently reducing ULK1 phosphorylation and promoting its release from mTORC1 [103]. In addition, two recent studies suggest that AMPK directly regulates autophagy by phosphorylating and activating ULK1 [104,105]. AMPK-mediated phosphorylation of ULK1 at a different residue supports its localization in the region of autophagosome formation and the initiation of autophagy [105]. AMPK can also activate autophagy by inhibiting mTOR activity via direct phosphorylation of Raptor [106]. Taken together, these data indicate that AMPK regulates ROS-induced cellular damage through the regulation of autophagy (Fig. 4).

4. Involvement of AMPK–autophagy axis in ALD

Several direct and indirect arguments suggest that alcohol consumption suppresses liver cell autophagy. First, rats chronically fed with ethanol have a reduced number of autophagic vacuoles in liver cells [107]. Second, chronic ethanol consumption slows down the catabolism of long-lived proteins in the rat liver [108]. Moreover, hepatocytes from patients with alcoholic steatohepatitis contain protein aggregates called Mallory-Denk bodies (MDBs). These Mallory-Denk bodies, the major constituents of which are keratins 8 and 18 (K8/18), ubiquitin and p62, may witness a decrease in autophagy level. Indeed, autophagy participates in the elimination of components of MDBs [109]. Finally, loss of autophagy in transgenic mice induces the formation of protein aggregates in hepatocytes, resembling MDBs [110].

Recently, Noh et al. [111] showed that ethanol incubation reduced autophagy significantly as assessed by microtubule-associated protein1 light chain 3 (LC3) expression using immunohistochemistry and immunoblot analysis, and that the reduced expression of LC3 was restored by AMPK activation in ethanol-treated hepatocytes. The mechanisms responsible for the decrease in autophagy are not clear, but one explanation can be proposed [112]: ethanol consumption has been shown to significantly reduce AMPK activity in the liver [10–13,17,18,15,19,20]. Moreover, ethanol inhibited H2O2-induced AMPK activation in hepatoma cells by inhibiting PKC-ζ/LKB1 and activating PP2A, and this effect requires its metabolism by ADH [38]. However, AMPK is described as an activator of autophagy notably via the mTOR pathway. AMPK suppression by ethanol could thus reduce autophagy and therefore promote oxidative liver damage. Indeed, ROS has been described to induce autophagy in part through activation of AMPK. By inhibiting AMPK signaling, ethanol could abolish the effect of ROS production on the induction of autophagy. Nevertheless, it is clear that further investigations will be necessary to confirm this hypothesis.

5. Conclusion and future perspectives

Although AMPK has been studied for more than three decades, many questions remain regarding its function, regulation and downstream targets. Depending on the model used, the type of stress applied and the experimental conditions, AMPK may have different roles.

AMPK activation by ROS can promote cell survival by inducing autophagy, mitochondrial biogenesis and expression of genes involved in antioxidant defense. Hence, increased intracellular concentrations of ROS may represent a general mechanism for enhancement of AMPK-mediated cellular adaptation, including maintenance of redox homeostasis, such that AMPK might function as an "early warning system" in response to oxidants to attenuate oxidative injury. However, the mechanism of action of AMPK in cells under oxidative stress remains unclear. Indeed, AMPK appears to be activated to drive either cell death or survival, but some of the required AMPK targets that propagate this signal through phosphorylation cascades have been implicated in both processes. This finding suggests that other intermediate modulators, which are able to determine whether cell death or survival
pathways predominate under oxidative stress, remain to be identified. Nevertheless, we can speculate that the role that AMPK plays may be dictated, in part, by the duration of its activation: thus, short and transitory AMPK activation would promote cell survival pathways preferentially, whereas sustained AMPK activation would trigger cell death.

Experimental and clinical studies increasingly show that oxidative damage induced by ethanol contributes in many ways to the pathogenesis of alcoholic hepatotoxicity. In the liver, AMPK inhibition by ethanol appears to be a paradox, because ethanol metabolism produces ROS, which are critical controlling factors in the activation of AMPK. These observations suggest that inhibition of AMPK by alcohol and activation of AMPK by ROS occur by different mechanisms and likely at different sites of signaling pathways that regulate AMPK. Moreover, effects of alcohol on AMPK pathway vary depending on the animal model of ethanol exposure utilized.

It is clear that more in vivo studies are needed to assess the functions of AMPK in the liver under oxidative stress conditions. A complete understanding of the different mechanisms involved in AMPK regulation by ethanol, as well as in ROS-induced AMPK signaling are critical for the development of new effective therapies of alcohol-induced liver disease. However, extrapolation of findings from rodents to humans will not be easy because reodox homeostasis in humans may be affected by nutritional, environmental and drug influences. Nevertheless, these studies could possibly lead to the evaluation of AMPK as a possible therapeutic target in alcohol-induced liver disease. Indeed, identifying potential new therapeutic target, whose signaling pathway is specifically involved in steatosis, such as AMPK, could help ameliorate the complications of liver diseases and improve the quality of life of many patients worldwide.

AMPK has become a well-known therapeutic target in type 2 diabetes and obesity. The intravenous administration of AMPK activator AICAR reduces hepatic glucose output, thereby lowering blood glucose concentrations in vivo in type 2 diabetic patients. Furthermore, AICAR administration stimulates hepatic fatty acid oxidation and/or inhibits whole body lipolysis [113]. Metformin and thiazolidinedione (TZD) are also used for therapeutic intervention in type 2 diabetes and lead to the activation of AMPK. In order to modulate AMPK activity in a more localized way as compared to the currently used drugs, the ability to regulate AMPK in different organs or tissues should be further investigated. For instance, oral administration of metformin is believed to preferentially alter liver metabolism, whereas TZD affects adipose tissue and skeletal muscle. Since tissue-specific differences in AMPK subunits have been established, developing compounds that preferentially interact with individual subunits or specific subunit combinations of heterotrimers could provide a means to increase the specificity of AMPK action [114]. For example, the α1 subunit predominates in the liver and could therefore serve as a target to alter AMPK in this organ. Moreover, AMPKα1 and α2-containing complexes account each for about half of total AMPK activity in liver. However, the AMPKα2 subunit, rather than α1, seems to play a critical role in the control of the balance between hepatic lipogenesis and β-oxidation [115]. Thus, a comprehensive and comparative analysis of the various AMPK isoforms expressed in animals and humans could help to improve an AMPK-mediated therapeutic approach.

Future investigations should focus on newly discovered direct and indirect AMPK-activators, as well as novel synthesized AMPK-activating compounds, which will highlight the potential for further exploiting AMPK in a therapeutic context for alcoholic-liver diseases.

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