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Critical role of AMPK in redox regulation under glucose starvation

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ABSTRACT

Glucose starvation is one of the major forms of metabolic stress in cancer cells. Deprivation of glucose impairs glycolysis and the pentose phosphate pathway, which elicits oxidative stress due to enhanced production of reactive oxygen species (ROS) and impaired antioxidant system, leading to redox imbalance and cell death. Under glucose starvation, the 5′ AMP-activated protein kinase (AMPK) plays a critical role in maintaining redox homeostasis and cell survival via multiple pathways, such as regulation of fatty acid metabolism and antioxidant response. Convergence of ROS and the glucose metabolic pathway reveals novel molecular targets for the development of effective cancer therapeutic strategies. Interestingly, AMPK, along with its upstream kinase liver kinase B1 (LKB1), has been regarded to play a tumor suppressor role. However, emerging studies have provided novel insights into the pro-tumor survival function of the LKB1-AMPK pathway. Therefore, targeting metabolic and oxidative stress in cancer cells, with manipulation of AMPK activity, is a promising strategy in developing novel cancer therapeutic agents.

1. Introduction

Cancer is one of the most deathful diseases worldwide, leading to estimated 9.6 million deaths in 2018 [1]. Despite extensive research and considerable efforts devoted to developing effective targeted therapies, many cancers remain poorly prognosed and incurable. Thus, novel therapies that improve the treatment outcomes of aggressive and malignant cancers are urgently needed. Cancer cells frequently undergo metabolic stress due to shortages in supply of oxygen, nutrients and growth factors, as a consequence of uncontrolled cell growth and proliferation, as well as insufficient angiogenesis and blood supply [2–4]. Glucose starvation is one of the major forms of metabolic stress in cancer cells because of a combination of reduced tumor vascularization and high rates of glucose consumption by cancer cells. It is estimated that glucose concentrations in tumors can be 3–10 folds lower than that in non-transformed tissues [5,6]. Glucose starvation can lead to

Abbreviations: 2DG, 2-deoxyglucose; 2DG-6P, 2-deoxyglucose 6-phosphate; 3PG, 3-phosphoglyceric acid; 5TG, 5-thioglucose; 6P-2DG, 6-phospho 2-deoxygluconate; 6P-G, 6-phosphogluconate; 6PGD, 6-phosphogluconate dehydrogenase; α-KG, α-ketoglutaric acid; ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMPK, 5′ AMP-activated protein kinase; ARE, antioxidant response; BRAF, B-Raf proto-oncogene; CaMKKβ, calcium/calmodulin-dependent protein kinase kinase β; CAT, catalase; COX-2, cyclooxygenase-2; CPT1, carnitine palmitoyltransferase 1; CRC, colorectal cancer; CRM, calorie restriction mimetic; DHA, dehydroascorbic acid; ECM, extracellular matrix; ERK2, extracellular signal-regulated kinase 2; ETC, electron transport chain; $FADH_2$, the quinone form flavin adenine dinucleotide; FAS, fatty acid synthesis; FASN, fatty acid synthase; FAO, fatty acid oxidation; G-6P, glucose 6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Glc, glucose; GLUT1, glucose transporter 1; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; HIF-1α, hypoxia-inducible factor 1-α; HK, hexokinase; HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase 1; IDH, isocitrate dehydrogenase; LKB1, liver kinase B1; KEAP1, Kelch-like ECH-associated protein 1; KO, knockout; KRAS, Kirsten rat sarcoma; MAGE A3/6, melanoma-associated antigen 3/6; MAPK, mitogen-activated protein kinase; ME, malic enzyme; MEF, mouse embryonic fibroblasts; MEK, MAPK Kinase; mTOR, the mechanistic target of rapamycin; mTORC1, mTOR Complex 1; MTP, mitochondrial trifunctional protein; NAC, N-acetyl cysteine; NADH, the reduced nicotinamide adenine dinucleotide; NADP+, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NQO1, NADPH dehydrogenase (quinone 1); NOX, NADPH oxidases; NRF2, nuclear factor (erythroid-derived 2)-like 2; NSCLC, non-small cell lung cancer; OXPHOS, oxidative phosphorylation; PDAC, pancreatic ductal adenocarcinoma; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1-α; PI3K, phosphoinositide-3-kinase; PPAR, peroxisome proliferator-activated receptor; PPP, the pentose phosphate pathway; PTEN, phosphatase and tensin homolog; R-5P, ribose 5-phosphate; RAPTOR, regulatory-associated protein of mammalian target of rapamycin; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase-1; SGLT2, sodium/glucose cotransporter 2; SOD, superoxide dismutase; SREBP, sterol regulatory element binding protein; TCA, tricarboxylic acid cycle; TP, trifunctional protein; TRIM28, tripartite motif-containing 28; Trx, thioredoxin; TSC2, tuberous sclerosis complex 2; UBE2O, (E3-independent) E2 ubiquitin-conjugating enzyme; UCP2, mitochondrial uncoupling protein 2; ULK1, UNC-51-like kinase 1

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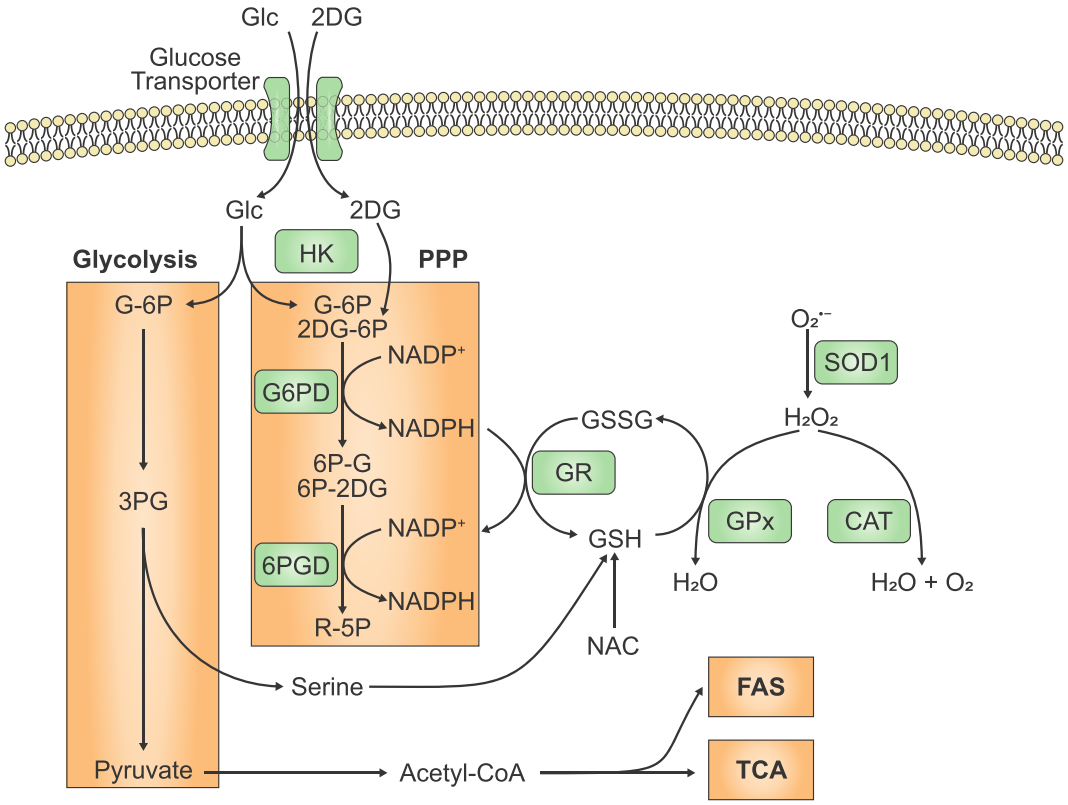

Fig. 1. Redox Regulation in Glucose Metabolism. A schematic diagram showing the redox regulation in glucose metabolism, especially glycolysis and the pentose phosphate pathway. Major metabolic pathways are shown in the yellow boxes. Enzymes that promote antioxidant effects are labeled green. Black arrows show the direction of the biological reaction(s). Abbreviations for proteins and metabolites are used as following: 2DG, 2-deoxyglucose; 2DG-6P, 2-deoxyglucose 6-phosphate; 3PG, 3-phosphoglyceric acid; 6P-2DG, 6-phospho 2-deoxygluconate; 6P-G, 6-phosphogluconate; 6PGD, 6-phosphogluconate dehydrogenase; CAT, catalase; FAS, fatty acid synthesis; G-6P, glucose 6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; Glc, glucose; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; HK, hexokinase; NAC, N-acetyl cysteine; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PPP, the pentose phosphate pathway; R-5P, ribose 5-phosphate; SOD, superoxide dismutase; TCA, tricarboxylic acid cycle.

decreased ATP production and increased reactive oxygen species (ROS) generation, leading to the disruption of redox status in cells, and rendering cancer cells more susceptible to cell death [7]. Meanwhile, glucose starvation is a canonical activator of the 5′ AMP-activated protein kinase (AMPK), a key regulator in energy homeostasis. At present, there is emerging evidence demonstrating the importance of ROS and oxidative stress in the regulation of AMPK activity under glucose starvation. In this review, we will discuss the recent findings on the crosstalk between redox regulation and AMPK under glucose starvation, and the possibility of developing novel targeted therapies based on the vulnerability of cancer cells to metabolic and oxidative stresses.

2. ROS and oxidative stress

ROS are the byproducts of aerobic metabolism, including the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$). Superoxide is mainly generated from the mitochondrial electron transport chain (ETC), predominantly complexes I and III, through univalent reduction of molecular oxygen (O_2) by leaked electrons, and from nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) at plasma membrane which produce $O_2^{\bullet -}$ using NADPH as a reductant [8,9]. Superoxide has been shown to inactivate iron-sulfur proteins, in particular, aconitase, the reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase (complex I), and succinate dehydrogenase (complex II), by oxidizing [4Fe-4S] to [3Fe-4S] clusters and releasing iron, therefore either promoting adaptation to increased oxidative stress or initiating cell death [10]. Elimination of superoxide is achieved by superoxide dismutase 1 (SOD1) and SOD2, which rapidly convert it into less reactive H_2O_2 [11]. Hydrogen peroxide can mediate the oxidation of cysteine residues of proteins. Under physiological conditions, cysteine residues exist as thiolate anions ($Cys\text{-}S^-$), which can be oxidized by H_2O_2 to the sulfenic form (Cys-SOH). Such oxidation causes conformational changes of proteins and regulates their functions. However, higher levels of H_2O_2 further oxidize thiolate anions to sulfinic (SO_2H) or sulfonic (SO_3H) forms. The sulfenic oxidation can be reduced by disulfide reductases thioredoxin (Trx) and glutaredoxin (Grx), thus recovering the protein functions to their original status. In contrast, sulfinic and sulfonic modifications are irreversible and cause permanent damages to the proteins [12,13]. Hydrogen peroxide can be reduced to H_2O by professional antioxidant proteins, including catalase, peroxiredoxins and glutathione peroxidase [14]. Alternatively, in the presence of ferrous ions, $^{\bullet}OH$ is generated from H_2O_2 through the Fenton reaction, which is highly reactive and toxic, and causes irreversible damage to lipids, proteins, and DNA [15].

3. ROS and glucose metabolism

It has been long proposed that cancer cells exhibit persistently high ROS levels due to defective mitochondrial functions [16,17], however, other studies have also suggested that many cancer cells possess functional mitochondria and utilize oxidative phosphorylation (OXPHOS) to produce ATP [18,19]. Nevertheless, one key feature of cancer cells in glucose metabolism is the shift from OXPHOS to aerobic glycolysis, a phenomenon called the Warburg effect [2]. Although glycolysis generates much less ATP per glucose molecule compared to complete oxidation, the rate of glucose metabolism through aerobic glycolysis is

higher, which may provide cancer cells with a selective advantage when competing with other cells such as stromal cells for the limited nutrients [20–23]. Meanwhile, glycolytic intermediates can be used in other metabolic pathways, such as the pentose phosphate pathway (PPP), fatty acid synthesis (FAS) and nucleotide synthesis, which favor the fast growth of cancer cells by providing the building blocks. More importantly, this metabolic shift enables cancer cells to reduce the generation of ROS from mitochondria and to enhance the generation of NADPH from the PPP [24,25]. NADPH converts oxidized glutathione (GSSG) to reduced glutathione (GSH) through glutathione reductase (GR), therefore regenerating this antioxidant to maintain the low level of ROS [24] (Fig. 1).

4. Redox regulation by AMPK under glucose starvation

AMPK is an evolutionarily conserved serine/threonine protein kinase complex which is ubiquitously expressed in almost all eukaryotes except in some intracellular parasites [26,27]. It is activated when cells are under stress conditions where intracellular ATP level reduces, while ADP and AMP levels increase. The activated AMPK exhibits dual functions on cell metabolism, *i.e.*, inhibiting anabolic pathways to minimize ATP consumption, including lipid and sterol syntheses, glycogen synthesis, RNA and protein syntheses, and cell cycle progression; and promoting catabolic pathways to replenish ATP and building blocks, including autophagy, glucose uptake and utilization, mitochondrial biogenesis, as well as lipid utilization [28]. So far, numerous studies have provided strong evidence showing that liver kinase B1 (LKB1) is responsible for almost all of the AMPK activation under energy stress conditions, through AMPKα Thr172 phosphorylation [29–32].

It was first reported that the LKB1-AMPK axis is critical to maintaining cell survival under glucose starvation [33–35]. The underlying mechanism of cell death and survival has been shown recently to involve ROS [36]. Glucose starvation rapidly depletes NADPH/GSH and elevates H_2O_2 level as a consequence of the impaired PPP, supported by the interesting findings that the non-metabolizable glucose analog 2-deoxyglucose (2DG), which is often used to mimic glucose starvation by inhibiting glycolysis, totally blocks glucose starvation-induced cell death [36]. The protective effect cannot be achieved by 5-thioglucose (5TG), another non-metabolizable glucose analog. The reason is that despite inhibiting glycolysis after phosphorylation by hexokinase, 2DG but not 5TG produces 2-deoxyglucose 6-phosphate (2DG-6P), which is still able to enter the PPP and generate NADPH through glucose-6-phosphate dehydrogenase (G6PD) to suppress ROS and subsequent cell death [36], as shown in Fig. 1. Consistently, treatment of the antioxidant N-acetyl cysteine (NAC) or GSH reduces ROS levels and protects cells from glucose starvation-induced cell death, indicating that oxidative stress is the cause of cell death. Moreover, LKB1-dependent AMPK activation by glucose starvation maintains NADPH levels through the phosphorylation and inhibition of acetyl-CoA carboxylase 1 (ACC1) and ACC2, which in turn inhibits the conversion from acetyl-CoA to malonyl-CoA [36], as illustrated in Fig. 2. Malonyl-CoA is the precursor of FAS and a potent inhibitor of carnitine palmitoyltransferase 1 (CPT1), the essential and rate-limiting enzyme in fatty acid oxidation (FAO), which transports long chain fatty acids such as palmitoyl-CoA to the mitochondrial matrix. FAO breaks down long-chain fatty acids to generate acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle [37]. The increased acetyl-CoA in the TCA cycle promotes the generation of malate and isocitrate. The increased malate can be converted to oxaloacetate or a step further to pyruvate by $NADP^+$-dependent malic enzyme1 (ME1) in cytosol or ME3 in mitochondria [38–40]. Isocitrate can be converted to α-ketoglutaric acid (α-KG) by $NADP^+$-dependent isocitrate dehydrogenase (IDH) [41–43]. These two processes generate NADPH, therefore providing cells with antioxidants [44] (Fig. 2). The importance of FAO and ME in mediating NADPH homeostasis is further corroborated by two recent studies. In pancreatic ductal adenocarcinoma (PDAC) and gastric cancer, the mitochondrial NAD^+-dependent ME2 is frequently co-deleted with the tumor suppressor SMAD4, whereas ME2 paralogue ME1 or ME3 catalyzes the conversion of malate to pyruvate, which is essential for NADPH regeneration, redox homeostasis and cell survival under glucose starvation and anchorage-independent growth [45,46]. Moreover, active AMPK phosphorylates and inhibits sterol regulatory element binding protein 1c (SREBP1c) and SREBP2, therefore decreasing the expression of lipogenic proteins such as ACC1, fatty acid synthase (FASN) and stearoyl-CoA desaturase-1 (SCD1) [47]. Similarly, extracellular matrix (ECM) detachment, which inhibits glucose uptake, elevates ROS levels and induces cell death [36,48]. Indeed, during metastasis, when cancer cells migrate from the original lesion, ROS levels increase in circulating melanoma cells [49]. Although the reason behind it was not clearly elucidated, it may be explained by the mechanisms described above. These studies thus suggest that glucose starvation or ECM detachment elicits oxidative stress through impaired PPP, and that AMPK activation is necessary to maintain NADPH homeostasis through FAS inhibition and FAO activation, as highlighted in Fig. 3.

Another signaling pathway of redox regulation by FAO in melanoma cells under glucose starvation or ECM detachment has also been described [50]. Glucose starvation elevates ROS levels, which activates extracellular signal-regulated kinase 2 (ERK2). ERK2 phosphorylates the orphan receptor Nur77 and induces its translocation to mitochondria, where Nur77 binds to mitochondrial trifunctional protein (MTP) β subunit (TPβ), the last enzyme in FAO that generates two-carbon shortened fatty acyl-CoA and acetyl-CoA. The interaction between Nur77 and TPβ protects TPβ from oxidation in a Nur77 self-sacrifice manner, thus maintaining FAO and NADPH homeostasis [50] (Fig. 2). However, whether AMPK is involved in this signaling pathway remains elusive. Firstly, it has been reported that administration of cytosporone B, an agonist for Nur77, resulted in the elevation of blood glucose in fasting C57BL/6 J mice, through the induction of a group of genes involved in gluconeogenesis [51]. Secondly, the same group proposed a novel signaling pathway of Nur77 in regulating AMPK activity in which Nur77 binds and sequesters LKB1 in the nucleus, thereby attenuating AMPK phosphorylation by LKB1 under glucose starvation or metformin treatment [52]. Taken together, these lines of evidence suggest that Nur77 may exhibit opposite effects on FAO through two distinct signaling pathways, possibly depending on the subcellular localization of Nur77. More studies focusing on the spatiotemporal regulation of FAO and LKB1-AMPK by Nur77, especially under glucose starvation, are needed to further address the question above. Nevertheless, these studies shed more lights on the FAO-NADPH-ROS pathway and further emphasize the importance of redox regulation in cancer cells under metabolic stress during which AMPK is a key player.

In addition to FAS and FAO regulation, AMPK also regulates gene transcription in response to glucose starvation and oxidative stress, via several distinctive pathways, as shown in Fig. 3. Firstly, in mouse embryonic fibroblasts (MEFs), glucose starvation enhances mitochondrial ROS generation and activates AMPK, which then induces the expression of several antioxidant genes, including *Catalase*, *Sod2*, and *Ucp2*, in a peroxisome proliferator-activated receptor (PPAR) γ coactivator 1-α (PGC-1α)-dependent manner [53]. Secondly, in cancer cells, AMPK is also required to upregulate PGC-1α and transcription factor A, mitochondrial (TFAM) through p38 activation, which promotes mitochondria biogenesis [54]. Thirdly, activated LKB1-AMPK signaling pathway by glucose starvation-induced ROS facilitates autophagy, which in turn enhances the autophagic degradation of Kelch-like ECH-associated protein 1 (KEAP1) [55]. KEAP1 is an adapter protein between the Cullin-3 E3 ubiquitin ligase complex and the nuclear factor (erythroid-derived 2)-like 2 (NRF2), leading to NRF2 ubiquitination and degradation by the proteasome [56,57]. Degradation of KEAP1 thus stabilizes NRF2, which regulates the transactivation of multiple antioxidant genes through binding to the antioxidant response element

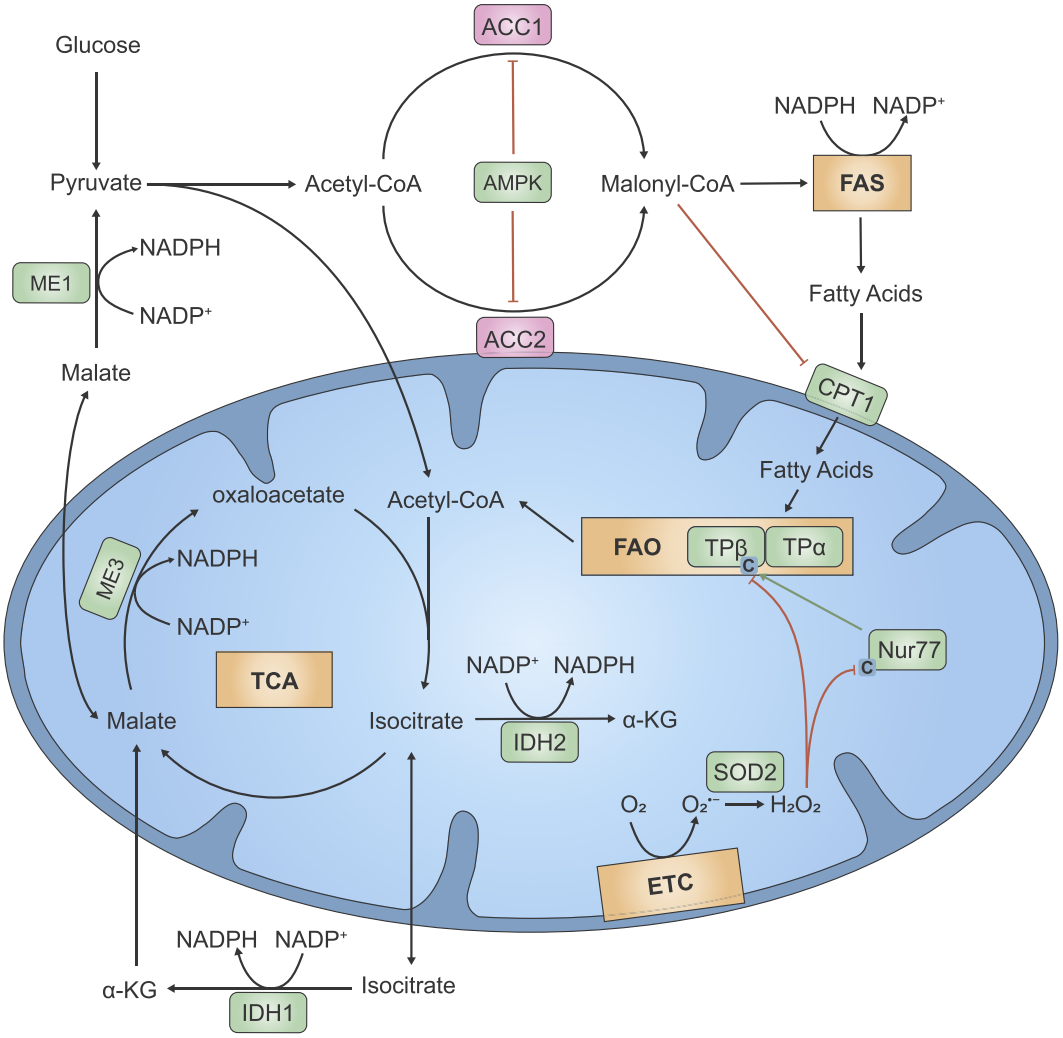

Fig. 2. Redox Regulation in Mitochondria and Fatty Acid Metabolism related to AMPK. A schematic diagram showing the redox regulation in mitochondria and fatty acid metabolism related to AMPK. Major metabolic pathways are shown in the yellow boxes. Enzymes that promote antioxidant effects are labeled green, whereas enzymes that promote ROS generation are labeled red. Green arrows indicate the positive regulation. Red blunt ends indicate the negative regulation. Black arrows show the direction of the biological reaction(s). Abbreviations for proteins and metabolites are used as following: C, cysteine oxidation; α-KG, α-ketoglutaric acid; ACC, acetyl-CoA carboxylase; AMPK, 5′ AMP-activated protein kinase; CPT1, carnitine palmitoyltransferase 1; ETC, electron transport chain; FAS, fatty acid synthesis; FAO, fatty acid oxidation; IDH, isocitrate dehydrogenase; ME, malic enzyme; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; SOD, superoxide dismutase; TCA, tricarboxylic acid cycle; TP, mitochondrial trifunctional protein.

(ARE) in a number of target genes, including *NADPH dehydrogenase (quinone 1)* (*NQO1*) and *heme oxygenase 1* (*HO-1*) [55].

5. AMPK regulation by ROS

In addition to the regulatory role of AMPK in ROS and oxidative stress as described in detail above, in turn, AMPK is also subject to regulation by ROS.

On one hand, ROS are primarily regarded as a positive regulator of AMPK. In NIH-3T3 cells, exogenous H_2O_2 transiently activates AMPK in a dose-dependent manner, associated with an increased AMP/ATP ratio [58]. However, it is unclear which kinase(s) activates AMPK upon H_2O_2 treatment. It has been reported that LKB1 is required for AMPK activation by H_2O_2 [35]. In contrast, in LKB1-deficient MEFs or HeLa cells, AMPK is still activated by H_2O_2, which is mediated by calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) [59]. Recently, using AMPKγ2 R531G (RG) expressing cells, which generates an AMP-insensitive AMPK complex, researchers were able to show that without the AMP sensing mechanism, AMPK cannot be activated by H_2O_2. This finding suggests that the target for exogenous H_2O_2 may not be AMPK itself but the component(s) of the respiratory chain, leading to a secondary effect on AMPK through increased AMP/ATP ratio [60]. Notably, exogenous or glucose oxidase (GO)-generated H_2O_2 induces direct *S*-glutathionylation of cysteine residues Cys299 and Cys304 on AMPKα subunit, which has been shown to activate AMPK [61]. However, this point of view has been challenged recently with the following evidence: 1) AMPK activation correlates with nucleotides change when H_2O_2 is generated by GO; 2) AMPKγ2 RG cells are insensitive to endogenous H_2O_2 induced by GO; 3) MitoParaquat (MitoPQ), a mitochondria-targeted redox cycler that generates physiological ROS levels within mitochondria at complex I without disrupting oxidative phosphorylation, induces mitochondrial ROS but not activates AMPK; and 4) H_2O_2-induced AMPK activity was unaffected by replacing the putative redox-sensitive cysteine residues with redox-insensitive alanine residues [62,63]. The reasons for the discrepancy between these different studies are most likely to be context-dependent, due to different cell models, methodological or biological differences, and different methodologies in measuring nucleotides or ROS level. Thus, the exact mechanisms of how H_2O_2 activates AMPK are to be further investigated.

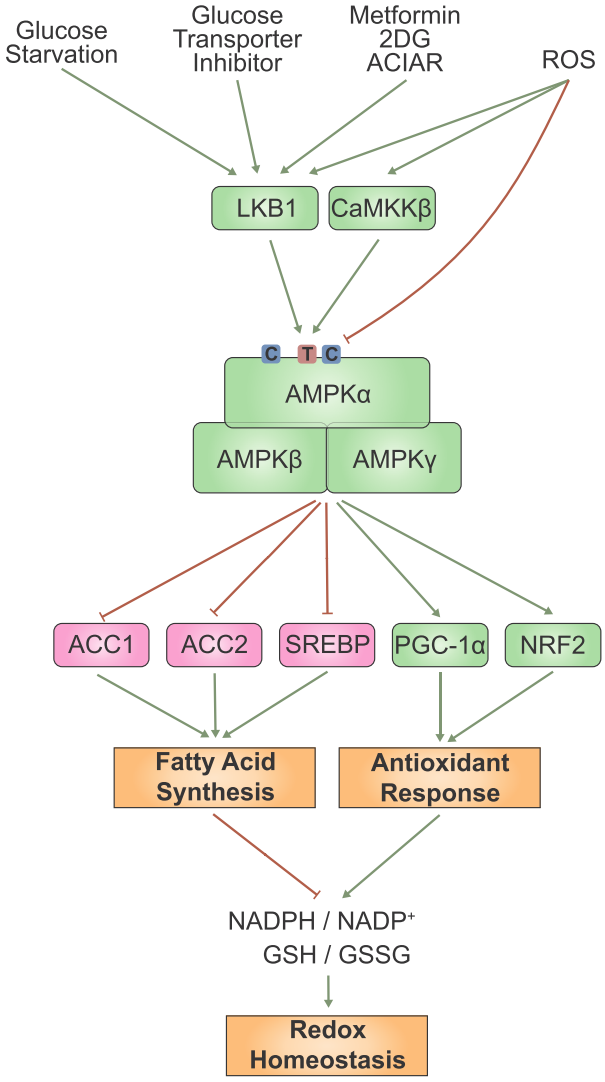

Fig. 3. Redox Regulation by AMPK. A schematic diagram showing the redox regulation by AMPK signaling pathway. Major biological pathways are shown in the yellow boxes. Enzymes that promote antioxidant effects are labeled green, whereas enzymes that promote fatty acid synthesis are labeled red. Green arrows indicate the positive regulation. Red blunt ends indicate the negative regulation. Abbreviations used in the figure: C, cysteine oxidation; T, threonine phosphorylation; 2DG, 2-deoxyglucose; ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMPK, 5′ AMP-activated protein kinase; CaMKKβ, calcium/calmodulin-dependent protein kinase kinase β; GSH, reduced glutathione; GSSG, oxidized glutathione; LKB1, liver kinase B1; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NRF2, nuclear factor (erythroid-derived 2)-like 2; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1-α; ROS, reactive oxygen species; SREBP, sterol regulatory element binding protein.

Nevertheless, the studies mentioned above mainly used exogenous H_2O_2 as the source of ROS, thus, more physiologically or pathologically relevant conditions with endogenous ROS and AMPK activation should be considered. Hypoxia (1.5% O_2), but not anoxia (0% O_2) has been reported to activate AMPK without changing AMP/ATP levels, but through induction of ROS [64]. Interestingly, AMPK activation requires mitochondria ROS, as ρ^0 143B cells that lack functional ETC did not respond to hypoxia in AMPK activation [64]. Another study has also reported that hypoxia activates AMPK through increased ROS and Ca^{2+}, but not increased AMP/ATP ratio [65]. Interestingly, it is controversial whether LKB1 is involved in hypoxia-induced AMPK activation. On one hand, $LKB1^{-/-}$ MEFs under hypoxia for 30 min cannot increase ACC phosphorylation, indicating that LKB1 is required for AMPK activation [64]. On the other hand, $LKB1^{-/-}$ MEFs under hypoxia for 2 h activates AMPK through CaMKKβ [65]. It remains unknown whether the disparity of treatment time between two studies affect the results. Thus, it would be of great interest to sophisticatedly study the temporal regulation of AMPK by hypoxia and ROS.

On the other hand, several lines of evidence have also suggested that ROS or other reactive molecules may inhibit the LKB-AMPK axis [66,67]. For instance, chemically reactive lipids, such as cyclopentenone prostaglandins, either exogenous or generated from cyclooxygenase-2 (COX-2), are able to form a covalent adduct with LKB1 Cys210 in its activation loop, therefore inhibiting the kinase activity of LKB1 [66]. Similarly, in the spontaneously hypertensive rat model, the development of left ventricular hypertrophy is associated with an increase in the oxidative stress–derived lipid peroxidation byproduct 4-hydroxy-2-nonenal (HNE), which results in the formation of HNE-LKB1 adducts that inhibit LKB1 and subsequent AMPK activity [67]. However, these two above-mentioned studies did not describe any direct post-translational modification of AMPK by ROS. Recently, it has been reported that under glucose starvation or exogenous H_2O_2 treatment, AMPK is negatively regulated by oxidation of cysteine residues Cys130 and Cys174 on its α subunit, which causes oxidative aggregation and interferes the interaction between LKB1 and AMPK [68]. The disulfide reductase Trx1 is required to cleave the disulfides in the AMPKα and prevent AMPK oxidation, thus serving as an essential cofactor for AMPK activation in response to oxidative stress induced by glucose starvation or ischemia [68]. Interestingly, AMPK is also capable of upregulating Trx1, thus forming a feedforward loop to maintain redox homeostasis [68], and further emphasizing the critical role of AMPK in redox regulation.

Taken together, AMPK activation is intricately correlated with oxidative stress and ROS, a process that is context-dependent. It is likely that both the antioxidant level and the dose and/or the nature of the oxidative stress affect the overall effect of ROS on AMPK activity. Indeed, the same dosage of H_2O_2 induces AMPK oxidation in cardiomyocytes but not in HEK293 cells [68]. Compared to primary cardiomyocytes, transformed cell lines or cancer cell lines may have elevated levels of antioxidants such as Trx1 that prevent AMPK oxidation and aggregation [68]. In addition, AMPK activity may also be regulated differently by distinct mechanisms under energy-sufficient and energy-deficient conditions, and by different ROS stimuli. Moreover, the methodological difference in measuring AMPK activity, ROS level, nucleotides level, and Ca^{2+} level may lead to discrepancies observed in the literature. To further depict the regulatory network of AMPK by ROS, more systematic and comprehensive studies are needed.

6. Relevance of ROS-AMPK signaling in cell death under glucose starvation

Based on the critical role of AMPK in regulating redox homeostasis in cells under glucose starvation, it is reasonable to speculate that impaired LKB1-AMPK pathway renders cancer cells sensitive to metabolic and oxidative stresses. Indeed, not only glucose starvation and ECM detachment, other mitochondria inhibitors such as phenformin and berberine are able to increase ROS and activate AMPK [69,70]. Moreover, LKB1 deficiency, which is frequently detected in non-small cell lung cancer (NSCLC), dictates the susceptibility to glucose starvation and phenformin [36,70,71].

However, in the cell culture model, glucose starvation is easily achieved through depleting glucose from the culture media, whereas mimicking glucose starvation *in vivo* remains to be a challenge. Several studies have suggested that targeting glucose transporters that are overexpressed in multiple cancer types, *e.g.*, glucose transporter 1 (GLUT1) by small molecule inhibitors, selectively kills cancer cells *in vitro* [72,73]. It has been further reported that GLUTs inhibitor WZB117

decreases glucose level and increases ROS levels in parasites-infected red blood cells [72]. However, the ubiquitous expression of GLUT1 in normal mammalian cells and the importance of GLUT1 in glucose uptake into the brain may preclude the clinical use of GLUT1 inhibitors as cancer therapeutics [74]. Notably, GLUT1 not only transports glucose, but it is also a transporter of dehydroascorbic acid (DHA), the oxidized form of vitamin C [75]. DHA is reduced to vitamin C, thus consuming NADPH and GSH. It was recently reported that high doses of DHA increase ROS levels in colorectal cancer (CRC) cells that carry activating *Kirsten rat sarcoma (KRAS)* and *B-Raf proto-oncogene (BRAF)* mutations and express high levels of GLUT1 [76]. The accumulated ROS, in turn, oxidizes and inactivates glyceraldehyde-3-phosphate dehydrogenase (GAPDH), leading to energetic crisis and cell death [76]. It is of note that AMPK is activated by vitamin C and NAC diminishes AMPK activation and blocks cell death, thus it is important to take LKB1 mutation status into consideration when exploring the possibility of developing DHA as a selective treatment for cancer cells with high GLUT1 expression.

The development of calorie restriction mimetics (CRMs) as anti-diabetic drugs provides the possibility of achieving glucose starvation *in vivo*. These CRMs such as insulin sensitizer metformin and the sodium/glucose cotransporter 2 (SGLT2) inhibitors are capable of lowering blood glucose level and activating AMPK [77–79]. Metformin is the most well studied anti-diabetic CRM, which functions by inhibiting mitochondrial complex I and activating AMPK. Numerous studies have suggested that metformin suppress cell proliferation of various types of cancer [80,81]. Similarly, SGLT2 inhibitors block the reabsorption of glucose in the kidney and lower blood glucose level. Recently, it has been reported that SGLT2 inhibitors activate AMPK and exhibit anti-cancer effects through dual functions, i.e., inhibiting glucose uptake and blocking mitochondrial complex I [79,82,83]. It remains to be further tested whether such anti-diabetic agents could be developed as novel cancer therapeutics by targeting AMPK.

7. The positive and negative effects of AMPK on cancer cell survival and cancer progression

LKB1 mutations have been discovered in multiple types of cancer, *e.g.*, NSCLC and cervical cancer [84–86]. It is estimated that loss-of-function mutations of LKB1 occur in 15–30% of NSCLC and 20% of cervical cancer, however, the real mutation frequency might be higher because the loss of heterozygosity of LKB1 gene locus commonly occurs [84,86–88]. In contrast, AMPK mutation is rarely observed in cancer. Since the tumor suppressor role of LKB1 has been well established, AMPK was primarily considered as a component of the LKB1-mediated tumor suppressor. It is well known that AMPK is able to inhibit the mechanistic target of rapamycin (mTOR), a well-established tumor promoter, through multiple ways, including phosphorylation of tuberous sclerosis complex 2 (TSC2) and regulatory-associated protein of mammalian target of rapamycin (RAPTOR), a negative and positive regulator of mTOR complex 1 (mTORC1), respectively [89,90]. Moreover, knockout (KO) of AMPKα1, the only catalytic α subunit in B cells, accelerates the development of lymphomas in transgenic mice overexpressing c-Myc, suggesting that AMPK loss can cooperate with oncogenic drivers to promote tumorigenesis in a tissue-specific manner [91]. The tumor suppressive function of AMPK is believed to be through downregulating hypoxia-inducible factor-1α (HIF-1α) and its downstream glycolytic genes [91]. In addition, activation of AMPK has been found to prevent cancer cells from completing mitosis, arresting them at G2/M checkpoint by inhibiting ACC [92]. Further, it has been recently described that AMPKα can be targeted for ubiquitination and degradation by the cancer-specific melanoma-associated antigen 3/6 (MAGE-A3/6)-tripartite motif-containing 28 (TRIM28) ubiquitin ligase (AMPKα1) or (E3-independent) E2 ubiquitin-conjugating enzyme (UBE2O) (AMPKα2), therefore promoting the downstream mTOR signaling pathway and cancer progression [93,94]. Finally, AMPK is able to inhibit other key oncogenic signaling pathways. For instance, AMPK phosphorylates the oncogenic protein BRAF, leading to attenuation of the mitogen-activated protein kinase (MAPK) kinase (MEK)-ERK pathway [95]. AMPKα itself can be inhibited by AKT through phosphorylation at the C-terminal serine/threonine-rich loop, which might occur in tumors where AKT is hyperactivated due to phosphatase and tensin homolog (PTEN) loss-of-function mutations, or activating mutations in phosphoinositide-3-kinase (PI3K) [96]. Collectively, these studies suggest that AMPK can exert a tumor suppressor role in specific genetic, metabolic, and signaling contexts.

On the contrary, emerging evidence has proposed that AMPK can protect cancer cells from metabolic or oxidative stress, thus possessing a tumor-promoting property. The overall function of LKB1-AMPK is to maintain cellular homeostasis and support cell survival when cells are under stress conditions, therefore cells lacking LKB1 are more vulnerable to energy crisis due to failed AMPK activation. Indeed, several studies have shown that LKB1 deficiency renders cells susceptible to various stress conditions, such as oxidative stress induced by H_2O_2, glucose starvation mimicked by 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and 2DG, as well as ER stress induced by tunicamycin and Brefeldin A [35,97,98]. Moreover, in LKB1-deficient NSCLC cells, overexpression of LKB1 can protect cells against cell death induced by glucose starvation and phenformin, through maintaining mitophagy and reducing ROS levels [36,70]. Part of the tumor-promoting function of AMPK is associated with its regulatory role in autophagy via multiple pathways [89,90,99,100]. One example is that AMPK phosphorylates UNC-51-like kinase 1 (ULK1) to initiate mitophagy and to maintain mitochondrial homeostasis and cell survival during starvation [101]. Furthermore, AMPK positively regulates CPT1C in cancer cells to sustain FAO and ATP production [102]. Taken together, AMPK is able to promote tumor growth or maintain cell survival in specific tumor types and genetic contexts. Therefore, it is possible that AMPK inhibitors have synergetic effects with conventional chemotherapy in the treatment of cancer, offering a new therapeutic strategy in those cancers with constitutive AMPK activation.

8. Conclusion and future directions

As discussed above, the critical role of AMPK in redox regulation has been extensively studied. Findings from those investigations have provided strong evidence that glucose starvation promotes ROS production and elicits oxidative stress, in which AMPK plays a key role in maintaining the redox homeostasis and cell survival via regulation of fatty acid metabolism and antioxidant response, as summarized in Fig. 3. Accordingly, AMPK and the glucose metabolic pathway have emerged as novel molecular targets for the development of effective therapeutic strategies. It is of great interest to develop mitochondria inhibitors, glucose metabolism inhibitors and other applicable drugs that can elicit metabolic and oxidative stresses in cancer cells as targeted therapies, especially for cancers with impaired LKB1-AMPK function. Alternatively, if potent and specific AMPK inhibitors can be developed into clinical use, the combination of AMPK activators or AMPK inhibitors with other chemotherapeutics might benefit more cancer patients regardless of their genetic backgrounds.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2019.101154.

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