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The double-edged sword of AMPK signaling in cancer and its therapeutic implications

Sang-Min Jeon and

College of Pharmacy, Ajou University, Suwon, Gyeonggi-do 443-749, Republic of Korea

Nissim Hay

Department of Biochemistry and Molecular Genetics, College of Medicine, University of Illinois at Chicago, Chicago, IL 60607, USA

Abstract

5'-AMP-activated protein kinase (AMPK) plays a pivotal role in maintaining energy and redox homeostasis under various metabolic stress conditions. Metabolic adaptation, which can be triggered by the activation of AMPK during metabolic stress, is the critical process for cell survival through the maintenance of ATP and NADPH levels. The importance of such regulation of fundamental process poses the AMPK signaling pathway in one of the most attractive therapeutic targets in many pathologies such as diabetes and cancer. In cancer, however, accumulating data suggest that the role of AMPK would not be simply defined as anti- or pro-tumorigenic, but it seems to have two faces like a double-edged sword. Importantly, recent studies showed that the anti-tumorigenic effects of many 'indirect' AMPK activators such as anti-diabetic biguanides are not dependent on AMPK; rather the activation of AMPK induces the resistance to their cytotoxic effects, emphasizing the pro-tumorigenic effect of AMPK. In this review, we summarize and discuss recent findings suggesting the two faces of AMPK in cancer, and discuss how we can exploit this unique feature of AMPK for novel therapeutic intervention.

Keywords

AMPK; LKB1; CAMKK2; mTORC1; ACC; NADPH; ROS; Fatty acids; Metabolic stress; Mitochondria; Biguanides; Cancer

Introduction

In 1987, AMPK has been discovered as a key regulator of fatty acid and cholesterol biosynthesis after the identification of AMPK as an upstream kinase of acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) (Carling et al. 1987). After the discovery of AMPK as a key regulator of lipid metabolism, it has now become one of the most versatile key players involved in the diverse and fundamental metabolic processes that counter metabolic stresses (Hardie and Alessi 2013). Upon activation of AMPK under these conditions, anabolism is shut down to prevent ATP and

NADPH consumption while catabolism is turned on to increase ATP and NADPH production (Hardie et al. 2012; Jeon and Hay 2012). As such, AMPK plays a pivotal role in maintaining energy and redox homeostasis at the cellular and organismal level, and deregulation of this balance can lead to major metabolic diseases such as diabetes and cancer (Steinberg and Kemp 2009; Hardie et al. 2012).

The first link between AMPK and cancer came from the finding that AMPK can mediate the tumor suppressive signaling of liver kinase B1 (LKB1) to the inhibition of mechanistic target of rapamycin complex 1 (mTORC1), which is activated in most cancers (Shackelford and Shaw 2009). Since this discovery, the LKB1-AMPK axis has been considered as a tumor suppressor pathway through the inhibition of mTORC1. Consistent with this hypothesis, it has been shown that metabolic inhibitors such as anti-diabetic biguanides metformin and phenformin, which indirectly activate AMPK inhibits tumor growth through the inhibition of mTORC1 (Shackelford and Shaw 2009). However, despite much pharmacological evidence, there has been little genetic evidence supporting this tumor suppressive role of AMPK.

Recently and in contrast to prior expectation, multiple research groups have shown that AMPK actually plays a pivotal role in tumor survival and growth, both in vitro and in vivo (Jeon and Hay 2012; Faubert et al. 2014b; Liang and Mills 2013; Hardie and Alessi 2013). The accumulated data suggest that the reprogramming of metabolism, which is triggered by the activation of AMPK, is the critical process for cell survival during metabolic stress that can often occur under pathophysiological conditions such as those found in the tumor microenvironment. Thus, it would not be surprising if AMPK could promote survival of not only normal cells but also tumor cells in such cases, and consequently play an essential role as a tumor promoter. In this review, we summarize and discuss the various causes and two opposing consequences of AMPK activation in tumors in the context of the tumor microenvironment, and propose a novel therapeutic opportunity conferred by this unique feature of AMPK and its role in cancer.

AMPK as a converging point of various stress signals arising from the tumor microenvironment

As a key player in metabolic adaptation, AMPK mediates diverse metabolic stress signals arising from nutrient starvation, matrix detachment, oxidative stress and hypoxia (Hardie et al. 2012; Jeon and Hay 2012; Cardaci et al. 2012). Importantly, all of these stresses are well-known factors that are actually present in the tumor microenvironment (Fig. 1). In addition, recent studies have shown that AMPK can be also activated by a variety of oncogenic signaling triggered by the activation of proto-oncogenes or the inactivation of tumor suppressor genes (Rios et al. 2013, Tennakoon et al. 2013; Possik et al. 2014; Yan et al. 2014). The activation of AMPK is mainly mediated by an increase in the phosphorylation of T172 in the α -catalytic subunit of AMPK; a heterotrimer consists of one catalytic α -subunit and two regulatory β - and γ -subunits. In recent decades, major upstream kinases and phosphatases have been identified that are responsible for the phosphorylation and dephosphorylation of this residue: LKB1, calcium/calmodulin-dependent kinase kinase 2

(CAMKK2), and protein phosphatase 2C and 2A (PP2C and PP2A) (Woods et al. 2003; Hawley et al. 2003, 2005; Woods et al. 2005; Davies et al. 1995).

Energetic stress

Under normal circumstances, PP2C and PP2A can freely access the T172 residue and keep it unphosphorylated (Fig. 1a) (Hardie et al. 2012). However, the energetic stress driven increase in the levels of AMP and ADP leads to an increase in their binding with the γ -subunit of AMPK, and this induces conformational changes that prevent the access of the phosphatases to this residue, resulting in an increase in T172 phosphorylation (Fig. 1b) (Hardie et al. 2012). Moreover, conformational changes induced by the binding of AMP but not ADP can induce allosteric activation of AMPK without T172 phosphorylation (Fig. 1b) (Hardie et al. 2012). Since LKB1 is considered to be constitutively active while CAMKK2 can be only activated by Ca^{2+} flux, it has been accepted that LKB1 does play a major role in AMPK phosphorylation and activation during energetic stress. Recently, further mechanisms of AMPK activation by LKB1 under energetic stress have been reported. Hardie and colleagues proposed that AMP, in spite of being at a lower cellular concentration, is a greater physiological regulator of AMPK than ADP; they showed that AMP is ten times more potent than ADP in inhibiting dephosphorylation of T172 and, more importantly, AMP but not ADP can enhance LKB1-induced AMPK phosphorylation (Fig. 1b) (Gowans et al. 2013). In addition, Lin and colleagues proposed the mechanism by which LKB1-induced T172 phosphorylation of AMPK under energetic stress. Interestingly, they showed that conformational change of AMPK induced by the binding of AMP recruits AMPK to the scaffold protein Axin-LKB1 complex, which enables LKB1 to phosphorylate AMPK in close proximity (Zhang et al. 2013). These two independent observations are consistent, in that only AMP and not ADP can enhance LKB1-induced AMPK phosphorylation, while both AMP and ADP can inhibit dephosphorylation of T172 by blocking access by phosphatases.

Matrix detachment

Recently, several groups have shown that matrix detachment that can occur during solid tumor growth and metastasis, can also activate AMPK (Jeon et al. 2012; Fung et al. 2008; Ng et al. 2012; Hindupur et al. 2014). Matrix detachment has been reported to inhibit glucose uptake and glycolysis, thereby inducing energetic stress (Schafer et al. 2009), which suggests that LKB1 may play a major role in AMPK activation in such cases. Recently, we provided the first evidence showing that the activation of AMPK upon matrix detachment is mediated through both LKB1 and CAMKK2 (Fig. 1b) (Jeon et al. 2012). We found that LKB1-null cells, such as A549 cells, could still substantially activate AMPK upon matrix detachment, although this activity is less than in LKB1-reconstituted A549 cells, and so this suggests that LKB1-independent mechanisms are also present. Furthermore, we showed that this phosphorylation is dependent on CAMKK2, since the treatment of A549 cells with the CAMKK2 inhibitor STO609 almost completely inhibited the AMPK activation induced by matrix detachment. Thus, these data suggest that matrix detachment may also stimulate Ca^{2+} flux in these cells, although there is no direct evidence for this and the mechanisms are currently unknown.

Oxidative stress

The first report demonstrating that exogenous H_2O_2 can activate AMPK in cultured cells was published more than 10 years ago (Choi et al. 2001). Although they showed that H_2O_2 can increase the AMP/ATP ratio, further mechanisms have not been extensively studied until recently. Interestingly, a recent study showed that reactive oxygen species (ROS) that are constantly generated in the medium in cell culture by the addition of glucose oxidase can induce the oxidative modification including *S*-glutathionylation of two conserved cysteine residues within the α -catalytic subunit of AMPK, which is responsible for the activation of AMPK by ROS (Zmijewski et al. 2010). They showed that this activation and oxidation of AMPK occurs before any changes in intracellular ADP/ATP ratio and non-oxidative mutation of the cysteine residues within the α -catalytic subunit was sufficient to prevent the activation of AMPK by ROS. Conversely, Hardie and colleagues used AMP/ADP insensitive γ -subunit mutant (RG) expressing cells in order to thoroughly investigate the involvement of AMP/ADP levels in ROS-induced AMPK activation (Auciello et al. 2014; Hawley et al. 2010). They showed that ROS fail to activate AMPK in RG expressing cells suggesting that ROS-induced activation of AMPK requires AMP/ADP binding of the γ -subunit. They also showed that ROS can inhibit mitochondrial oxygen consumption, resulting in the increase of AMP/ADP levels. Furthermore, using the cell lines expressing glucose oxidase, which induces constant and physiological levels of H_2O_2 in the cells, they showed that both exogenous and endogenous ROS can activate AMPK in an AMP/ADP dependent manner (Auciello et al. 2014). Thus, it seems that ROS may activate AMPK by two distinct mechanisms involving direct oxidation of cysteine residues in AMPK as well as energetic stress through the inhibition of mitochondrial metabolism (Fig. 1b).

Hypoxia

Although hypoxia is a well-known feature of tumor microenvironments, the underlying mechanisms of AMPK activation remain elusive. At least two groups have reported mechanisms for the hypoxic activation of AMPK. Using mouse embryonic fibroblasts (MEFs), Chandel and colleagues showed that hypoxia activates AMPK by increasing the levels of mitochondrial ROS (mtROS) but not the AMP/ATP ratio (Emerling et al. 2009). Despite no change in the AMP/ATP ratio, they demonstrated that this activation of AMPK is dependent on LKB1. Interestingly, in contrast to hypoxic ROS, exogenous H_2O_2 (100 μM) under normoxia could significantly activate AMPK even in the absence of LKB1, suggesting that hypoxic ROS and normoxic ROS may have different mechanisms for AMPK activation. Conversely, Schumacker and colleagues proposed different mechanisms for the hypoxic activation of AMPK using MEFs and 143B osteosarcoma cells (Mungai et al. 2011). Although they also found that mtROS but not AMP/ATP ratio are responsible for the hypoxic activation, they showed that the hypoxia-induced ROS activate AMPK through an increase in Ca^{2+} levels and the activation of CAMKK2 but not LKB1. Since these two groups have reported two conflicting mechanisms (Fig. 1c), further studies will be necessary to elucidate the mechanisms underlying the hypoxic activation of AMPK.

Oncogenic signaling

Although AMPK signaling was originally suggested as a tumor suppressive pathway, as mentioned above, recent studies have shown that several oncogenic signaling molecules can activate AMPK. Oncogene SRC, which is overexpressed and activated in many cancers, has been shown to activate AMPK through the activation of the PKC α -LKB1 pathway in certain cancer cell lines (Mizrachy-Schwartz et al. 2011). Similarly, the expression of MYC and H-Ras^{V12} in osteosarcoma cell lines and astrocytic tumors, respectively, also activate AMPK, although the mechanisms are not clear (Liu et al. 2012; Rios et al. 2013). Moreover, androgen receptor (AR) signaling also activates AMPK through the transactivation of CAMKK2 in prostate cancer (Massie et al. 2011, Tennakoon et al. 2013; Frigo et al. 2011). In addition to the activation of oncogenes, the loss of the tumor suppressor gene folliculin (*FLCN*), which is associated with Birt-Hogg-Dubé syndrome (BHD), has been shown to activate AMPK (Possik et al. 2014; Yan et al. 2014); this is discussed later.

AMPK as a pro-tumorigenic regulator: changing the old paradigm

As described above, many factors arising in the tumor microenvironment, as well as some oncogenic signaling, are bona fide AMPK activators in cancer. Indeed, AMPK has been shown to be strongly activated in vivo in both rodent and human glioblastomas (Rios et al. 2013; Jang et al. 2011). Thus, the main question arising from this is what are the consequences of AMPK activation in tumors; are they pro-tumorigenic or anti-tumorigenic? This has been debated in recent years, but it is now becoming clear that the activation of AMPK under these conditions can be a pro-tumorigenic signal in cancer.

The initial observations that LKB1 and AMPK have pro-tumorigenic roles came from the setting of oncogene-induced transformation experiments using MEFs. Two groups independently showed that both LKB1-null MEFs and AMPK α 1/ α 2-null (AMPK α -KO) MEFs are resistant to oncogenes such as H-Ras^{V12}/SV40T-induced anchorage-independent growth as well as solid tumor growth in vivo (Bardeesy et al. 2002; Laderoute et al. 2006). Remarkably, three recent studies have shown that AMPK is also essential for other oncogene-induced tumorigenesis, such as the MYC-induced development of hepatocellular carcinoma (HCC), H-Ras^{V12} $\pm$ Pten^{-/-} induced astrocytic tumor cell proliferation, and kinase suppressor of ras 2 (KSR2) induced anoikis resistance of MEFs and some cancer cell lines (Liu et al. 2012; Rios et al. 2013; Fernandez et al. 2012). In addition, multiple groups have recently reported that AMPK has an essential role in a variety of cancer types. The silencing of AMPK in human breast and pancreatic cancer cells impairs their ability to grow in an anchorage-independent manner and to form tumors in vivo (Laderoute et al. 2014; Hindupur et al. 2014; Kato et al. 2002). In addition, the LKB1-AMPK signaling pathway is also required for glioma cell survival and spheroid migration in low-glucose conditions (Godlewski et al. 2010; Rios et al. 2013). Interestingly, two recent unbiased approaches have identified *AMPK α 1* and *AMPK β 1* subunits as essential pro-tumorigenic genes in melanomas and prostate cancers, respectively. By chemical proteomic screening of melanoma cells, AMPK α 1 was identified as playing a key role in maintaining cell viability through the regulation of microphthalmia-associated transcription factor (MITF), a melanoma oncoprotein (Borgdorff et al. 2014). Conversely, the expression of the AMPK β 1

subunit in prostate cancer was found to be essential for cancer cell survival and was highly elevated in metastatic prostate cancers when compared with primary prostate cancers (Ros et al. 2012). Importantly, the AR-CAMKK2-AMPK axis was recently reported to have critical roles in prostate cancer. An interdisciplinary approach integrating genomic studies and metabolomic profiling found that CAMKK2, which is overexpressed in prostate cancers, is the critical transcriptional target of AR, and that the CAMKK2-AMPK axis is critical for AR-induced prostate cancer cell growth and survival (Massie et al. 2011). Consistently, other groups have also shown that the CAMKK2-AMPK pathway is a critical downstream effector of AR-dependent prostate cancer cell growth, migration and invasion, suggesting that the CAMKK2-AMPK axis is a promising therapeutic target in AR-dependent prostate cancer (Tennakoon et al. 2013; Frigo et al. 2011).

In general, the mechanisms underlying the pro-tumorigenic role of AMPK have been attributed to the maintenance of ATP levels by reprogramming energy metabolism, since this is the best-known function of AMPK. Supporting this view, it was shown that the activation of AMPK by MYC maintains the expression of mitochondrial respiratory chain complexes and respiratory capacity, which would be required for efficient glutamine metabolism for energy production (Liu et al. 2012). Similarly, the activation of AMPK by loss of tumor suppressor FLCN increases mitochondrial biogenesis by inducing PGC1 α expression and consequently resulting in ROS production (Yan et al. 2014). They also showed that high levels of ROS activate HIF1 α transcriptional activity resulting in an increase in the expression of glycolytic enzymes, which drive ATP production. Other studies of prostate cancer also proposed that the AR-CAMKK2-AMPK axis increases aerobic glycolysis and anabolic metabolism in part through PGC1 α -mediated mitochondrial biogenesis, which can increase the supply of both the energy and the building blocks required for rapid cell growth (Massie et al. 2011; Tennakoon et al. 2013).

Recently, different mechanisms have been proposed, other than those involving the provision of energy or building blocks; these mechanisms are associated with the metabolic functions of AMPK. We showed that, in the absence of AMPK activity, oxidative stress is the major cause of cancer cell death during metabolic stress, such as glucose deprivation and matrix detachment (Jeon et al. 2012). Under these conditions, it is the maintenance of NADPH levels, rather than of ATP levels through phosphorylation and inhibition of acetyl-CoA carboxylases (ACCs), that are critical mechanisms of AMPK-induced tumor cell survival, anchorage-independent growth and solid tumor formation in vivo. The inhibition of ACC1 and ACC2, which reduce NADPH consumption by inhibition of fatty acid synthesis and increase NADPH production by activation of fatty acid oxidation, respectively, is required for maintaining the GSH/GSSG ratio and for reducing hydrogen peroxide levels, thereby protecting against oxidative stress induced cell death. Concordantly, recent studies have shown that AMPK protects against H₂O₂ induced cell death through NADPH maintenance in osteosarcoma and loss of folliculin was shown to confer an AMPK-dependent resistance to oxidative stress induced cell death (She et al. 2014; Possik et al. 2014).

Conversely, recent studies have also proposed different mechanisms that are not associated with the metabolic functions of AMPK. As mentioned above, AMPK has been found to inhibit the degradation of the oncoprotein MITEF, which is required to maintain cell viability

in melanoma (Borgdorff et al. 2014). MITF has been shown to be a melanoma-specific oncogene as it is amplified or mutated in a significant proportion of melanomas. However, the mechanisms by which the AMPK-MITF axis regulates cell viability in melanoma are not well understood. In breast cancer cells, AMPK has been shown to phosphorylate phosphoprotein enriched in astrocytes 15 (PEA15), a protein that is critical for mammosphere formation and solid tumor growth in vivo (Hindupur et al. 2014). Although they suggested that the AMPK-PEA15 axis can inhibit apoptosis, at least in part, through the inhibition of FADD-mediated apoptosis signaling, further studies would be needed to elucidate the significance of this mechanism.

AMPK as an anti-tumorigenic regulator: re-evaluation is needed

Although many recent studies have suggested the pro-tumorigenic role of AMPK, it was first suggested as having anti-tumorigenic activity after the discovery of LKB1 and mTORC1 as an upstream kinase and a downstream effector of AMPK, respectively (Hawley et al. 2003; Woods et al. 2003; Corradetti et al. 2004; Inoki et al. 2003). *LKB1* was originally identified as a tumor suppressor gene since its germline mutations were found in Peutz-Jeghers Syndrome (PJS), which is characterized by intestinal hamartomas and increased cancer predisposition in certain tissues (Hemminki et al. 1998). In addition, somatic mutations in *LKB1* were also found in certain cancers including lung and cervical cancers underscoring the tumor suppressive function of LKB1 (Shackelford and Shaw 2009). mTORC1, which is inactivated by the LKB1-AMPK axis, is a central integrator of growth factor and nutrient signals that regulate protein synthesis or cell growth, and are hyperactivated in most human cancers (Shackelford and Shaw 2009). Thus, the findings that LKB1 activates AMPK, and that AMPK inhibits mTORC1 have made it reasonable to classify AMPK under the tumor suppressive signaling pathway.

Indeed, recent studies using genetically modified mouse models supported the tumor suppressive role of LKB1. Although *LKB1* deletion alone is not sufficient to develop lung tumors, it enhances K-Ras activation- or Pten deletion-induced lung tumorigenesis with elevated mTORC1 and SRC activity (Ji et al. 2007; Carretero et al. 2010; Xu et al. 2014). Similarly, LKB1 deletion has been shown to accelerate ERBB2-induced breast tumorigenesis with elevated mTORC1 activity (Dupuy et al. 2013). However, LKB1 deficiency in breast tumors reduces the lung metastatic burden, possibly due to the impaired metabolic flexibility during metastasis. Notably and in contrast to lung and breast, *LKB1* deletion alone in the endometrium is sufficient to develop invasive endometrial carcinoma, which is highly responsive to rapamycin, an mTORC1 inhibitor, suggesting that mTORC1 activation could be the major downstream effector of *LKB1* inhibition (Cheng et al. 2014; Contreras et al. 2010, 2008). Interestingly, recent studies have also suggested that LKB1 has an AMPK-mTORC1-independent tumor suppressive role, which would be dependent on MARK-mediated regulation of Snail or Hippo signaling (Mohseni et al. 2014; Goodwin et al. 2014).

In contrast with the several lines of evidence supporting the anti-tumorigenic role of LKB1, only one study to date has provided the genetic evidence supporting the anti-tumorigenic role of AMPK in vivo. A recent study by Jones and colleagues showed that deletion of

AMPK α 1, which is the sole catalytic subunit expressed in B lymphocytes, accelerates MYC-induced lymphomagenesis (Faubert et al. 2013), which seems to oppose the findings of the previous study that showed that AMPK has an essential role in MYC-induced hepatocellular carcinoma (HCC) development (Liu et al. 2012). Faubert et al. also provided evidence that the deletion of either *LKB1* or *AMPK α 1* induces mTORC1-HIF1 α -dependent metabolic reprogramming that supports the energetic and anabolic demands of cancer cells (Faubert et al. 2013, 2014a). This also conflicts with another study mentioned above (Yan et al. 2014), which suggested that AMPK activates aerobic glycolysis through the PGC1 α -ROS-HIF1 α axis in the absence of FLCN. Although this discrepancy could be explained by the tissue-specific and context-dependent functions of AMPK, further work would be needed to better understand this complexity.

In addition to the genetic evidence, much conflicting pharmacological evidence regarding the role of AMPK in cancer has been reported. Retrospective epidemiological analysis proposed that long-term administration of the anti-diabetic drug metformin, which is known to indirectly activate AMPK through the inhibition of mitochondrial metabolism, has reduced the incidence and/or mortality of breast cancer among diabetic patients (Evans et al. 2005; Decensi et al. 2010). Moreover, numerous studies have shown that AMPK activators, such as AICAR and the anti-diabetic drugs metformin and phenformin, can inhibit cancer cell growth and proliferation (Rosilio et al. 2013; El-Masry et al. 2012; Buzzai et al. 2007; Appleyard et al. 2012). However, three recent studies have challenged these conclusions, which were drawn by the use of indirect or non-specific AMPK activators. Dasgupta and colleagues showed that AICAR and metformin, while non-specific or indirect activators of AMPK, can reduce glioblastoma proliferation and viability (Liu et al. 2014). Furthermore, a direct AMPK activator A769662 has no effect on proliferation, suggesting that the anti-tumorigenic effects of AICAR and metformin are AMPK-independent (Liu et al. 2014). Shaw and colleagues also showed that phenformin preferentially kills LKB1-deficient lung tumor cells in vivo, suggesting that the anti-tumor effect of phenformin is not dependent on the activation of AMPK, rather activation of AMPK in the presence of LKB1 actually compromises the cytotoxic effect of phenformin (Shackelford et al. 2013). Importantly, Jones and colleagues recently showed that all these nonspecific and indirect AMPK activators strongly inhibit cell proliferation and viability in the absence of AMPK, while these cytotoxic effects are reduced in the presence of AMPK; this suggests that the activation of AMPK actually antagonizes the cytotoxic effect of these indirect AMPK activators (Vincent et al. 2014). Furthermore, Jones and colleagues also showed that the direct AMPK agonist A769662 actually enhances cell proliferation under metabolic stress conditions, which supports the suggestion that AMPK has a pro-tumorigenic role in the tumor microenvironment. Thus, in contrast to the relatively solid experimental and genetic evidence supporting the anti-tumorigenic role of LKB1 in cancer, it seems that the role of AMPK in cancer need to be re-evaluated as an essential tumor promoter.

Resolving the LKB1/CAMKK2-AMPK paradox in cancer

While the original view suggested a tumor suppressive role for the LKB1-AMPK pathway, due to the finding of a loss of function of LKB1 in certain cancers and the inhibition of mTORC1 by the LKB1-AMPK pathway, the accumulated data also suggest that LKB1

might be a pro-tumorigenic regulator in other contexts. Thus, the key question arising is how can LKB1 be lost in certain cancers despite the disadvantages to metabolic adaptation and survival in the tumor microenvironment since the LKB1-AMPK pathway is essential for metabolic adaptation through the maintenance of energy and redox balance?

One possibility is that the levels of AMPK activity determine the different consequences of the AMPK activation. A recent study suggested that although AMPK α 1-KO MEFs (α 1 is the major isoform of AMPK in MEFs) and AMPK α 1 α 2-DKO MEFs are resistant to oncogene-induced transformation, AMPK α 2-KO MEFs (KO of a minor isoform) are sensitive to oncogene-induced transformation (Phoenix et al. 2012). These data raise the interesting possibility that in contrast to severe depletion of AMPK, slight or partial reduction of AMPK activity may be sufficient to trigger metabolic adaptation, while insufficient to inhibit mTORC1 activity (Fig. 2a). However, we cannot exclude the possibility that AMPK α 1 and AMPK α 2 may have different substrates and functions. Based on this observation, it may be plausible to speculate that in the absence of LKB1, moderate activation of AMPK by another upstream kinase such as CAMKK2 would be sufficient to trigger metabolic adaptation in tumor microenvironment, while insufficient to inhibit mTORC1 signaling (Fig. 2a). Indeed, it has been shown that matrix detachment of LKB1-deficient cancer cells can moderately activate AMPK in a CAMKK2-dependent manner, which is critical for anchorage-independent growth (Jeon et al. 2012). However, this hypothesis cannot fully explain the paradox since, unlike matrix detachment, nutrient deprivation-induced AMPK activation is mostly dependent on LKB1 (Hardie et al. 2012). This suggests that additional mechanisms that could compensate for the loss of LKB1-induced metabolic adaptation may be operating in LKB1-null cancer cells (Fig. 2b). Since previous studies have shown that the combination of K-Ras activation and LKB1 deficiency developed lung cancer (Ji et al. 2007), it would be interesting to investigate how K-Ras activation can compensate for the loss of LKB1-induced metabolic adaptation in *K-Ras/LKB1* mutated lung cancers. Moreover, analysis of human cancer genome sequencing data should be very informative for finding the co-existing mutations in LKB1 mutated cancers, which synergize tumorigenesis by complementing the loss of the LKB1-AMPK pro-tumorigenic signaling (Fig. 2b).

In addition, although the LKB1-AMPK pathway in cancer has conflicting roles in cancer, the CAMKK2-AMPK pathway has been mostly reported as a pro-tumorigenic regulator, at least in prostate cancer (Frigo et al. 2011; Massie et al. 2011; Tennakoon et al. 2013). Thus, another interesting issue is to determine whether the activation of AMPK by LKB1 versus CAMKK2 results in different outcome or not. For example, it would be interesting to speculate that, unlike the LKB1-AMPK pathway, the CAMKK2-AMPK pathway may only activate metabolic adaptation pathways without inhibiting mTORC1 signaling, and thus can promote tumor growth (Fig. 2c). Supporting this possibility, a recent study showed that the inhibition of CAMKK2 cannot activate mTORC1 in prostate cancer (Massie et al. 2011). Therefore, further investigation of the differential consequences of AMPK activation by LKB1 and CAMKK2 may reveal novel mechanisms that determine the roles of AMPK in cancer.

Therapeutic implications in cancer: re-connection to biguanides

Since the discovery of the LKB1-AMPK-mTORC1 signaling axis, the activation of AMPK has been considered a reasonable anti-cancer strategy through its inhibition of mTORC1 (Kim and He 2013; Hadad et al. 2008). However, as discussed above, the roles of AMPK in cancer have turned out to be more complicated, suggesting that there are ‘two faces’ to the activity of AMPK in cancer (Fig. 3a). Importantly, the finding that the cytotoxic effects of metabolic inhibitors, which can indirectly activate AMPK, are enhanced in the absence of LKB1 or AMPK. This strongly suggests that AMPK activation could have more pro-survival effects in a therapeutic setting (Vincent et al. 2014). Thus, the combination of AMPK inhibitors with various metabolic inhibitors may be a reasonable therapeutic strategy for cancer.

Currently, only one molecule, compound C, has been identified as a selective AMPK inhibitor (Zhou et al. 2001). Compound C was identified in a high-throughput kinase assay and is known to bind to the AMPK α subunit. However, many studies have shown that compound C can actually inhibit many other kinases as well as bone morphogenetic protein (BMP) receptor, indicating its promiscuity (Sinnott and Brenman 2014). Interestingly, a recent study showed that sunitinib, a multiple tyrosine kinase inhibitor that is used clinically against advanced renal cell carcinoma (RCC) and gastrointestinal stromal tumor (GIST), can directly inhibit AMPK activity by binding to the AMPK α subunit (Laderoute et al. 2010). Laderoute et al. also demonstrated that sunitinib is a more potent inhibitor of AMPK than compound C, both in vitro and in vivo. Congruently, another study showed that AMPK α 1 was pulled-down with sunitinib and midostaurin when they were treated in melanoma cell lines (Borgdorff et al. 2014). They demonstrated that these two inhibitors can inhibit AMPK resulting in the degradation of MITF, and consequently induce cell death in melanoma cell lines; this was discussed earlier. Therefore, it is possible that the cytotoxic effects of sunitinib and midostaurin can be attributed, at least in part, to their inhibition of AMPK (Fig. 3a, b).

However, one concern and drawback of the inhibition of AMPK would be the hyperactivation of mTORC1, which can compromise the anti-cancer effect of the AMPK inhibition (Fig. 3b). Among the various metabolic inhibitors, the anti-diabetic biguanides phenformin and metformin, which inhibit mitochondrial metabolism, have been shown to inhibit mTORC1 in both AMPK-dependent and independent mechanisms (Fig. 3c) (Nair et al. 2014; Liu et al. 2014; Ben Sahra et al. 2011; Vincent et al. 2014). Moreover, given that the pro-survival effects of AMPK activation are mediated, at least in part, through the activation of mitochondrial metabolism, the use of biguanides should be an attractive strategy for bypassing part of their pro-survival effects since they are mitochondrial inhibitors (Fig. 3c). Furthermore, recent studies have shown that cancer stem cells are dependent on mitochondrial metabolism, and metformin and phenformin preferentially kill various cancer stem cells (Janzer et al. 2014; Honjo et al. 2014; Lonardo et al. 2013; Shank et al. 2012; Song et al. 2012; Vazquez-Martin et al. 2011; Hirsch et al. 2009; Viale et al. 2014). Indeed, a number of clinical trials of metformin are ongoing as therapy for a variety of cancers (Pollak 2013). Thus, we propose that the combination of biguanides with AMPK inhibitors would be the ‘best bet’ as this strategy would strongly enhance metabolic stress

with preventing pro-tumorigenic signals such as the activation of mTORC1 and mitochondrial metabolism (Fig. 3c). For proof of concept, although no specific AMPK inhibitors are currently available, it would be important to examine if sunitinib or midostaurin in combination with phenformin or metformin do indeed have synergistic anti-cancer effects.

Concluding remarks

Although AMPK was originally suggested as suppressing tumors by mediating LKB1 signaling to mTORC1 inhibition, now the accumulated data suggest that AMPK could be a tumor promoter by protecting cancer cells from death in harsh tumor microenvironments. Importantly, it turns out that the secondary activation of AMPK by many metabolic inhibitors actually induces resistance to cell death. This suggests that, rather than activation, inhibition of AMPK with concurrent induction of metabolic stress should be a more reasonable strategy for treating cancers and this in combination with biguanides should be the best combination strategy. Thus, this would be reasonable approach for investigating the synergistic anti-cancer effects of the combination of clinically relevant AMPK inhibitors with anti-diabetic biguanides, which will change the paradigm of AMPK inhibitors as a novel and promising anti-cancer strategy.

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Fig. 1.

The mechanisms of AMPK activation by various factors present in the tumor microenvironment. **a** The regulation of AMPK activity under normal conditions. **b** The mechanisms of AMPK activation by energy stress, matrix detachment and oxidative stress. **c** The mechanisms of AMPK activation by hypoxia. See text for details. *Arrows* denote activation, whereas *horizontal bars* indicate inhibition. *PS* protein synthesis, *FAS* fatty acid synthesis, *FAO* fatty acid oxidation, *mtROS* mitochondrial reactive oxygen species

Fig. 2.

The hypotheses to explain the paradox of the LKB1-AMPK pathway and the CAMKK2-AMPK pathway in cancer. **a** The LKB1-AMPK pathway. Partial activity of AMPK in the absence of LKB1 may be sufficient to trigger metabolic adaptation, while insufficient to inhibit mTORC1 in the tumor microenvironment. **b** The LKB1-AMPK pathway. Additional mutations that could compensate for the loss of LKB1-induced metabolic adaptation may exist in LKB1-deficient cancer. **c** The CAMKK2-AMPK pathway. Unlike to LKB1 induced activation of AMPK, AR-CAMKK2 induced activation of AMPK can only activate metabolic adaptation signaling but not growth inhibitory signaling. See text for details. *Arrows* denote activation, whereas *horizontal bars* indicate inhibition

Fig. 3.

Novel therapeutic strategies targeting AMPK and mitochondrial metabolism. **a** The dual effects of AMPK activation on tumorigenesis. **b** The effects of AMPK inhibitors such as sunitinib or midostaurine on anti- and pro-tumorigenic signaling of AMPK. **c** The effects of biguanides in combination with AMPK inhibitors on anti- and pro- tumorigenic signaling of AMPK. See text for details. *Arrows* denote activation, whereas *horizontal bars* indicate inhibition. *Gray text* indicates inactivation. *REDD1* regulated in development and DNA damage responses 1, *PRAS40* proline-rich Akt substrate of 40 kDa