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The dark face of AMPK as an essential tumor promoter

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Numerous studies have shown that supraphysiological activation of AMPK could inhibit tumor growth. On the other hand, accumulating data also suggest that AMPK activity is required for tumor growth and migration. These findings suggest that physiological activation of AMPK is critical for tumor growth/migration, possibly through maintenance of ATP levels. Our recent study provides the first evidence that the maintenance of cellular NADPH homeostasis is the predominant mechanism by which AMPK promotes tumor cell survival and solid tumor formation. We showed that AMPK activation is required to maintain intracellular NADPH levels through the activation of fatty acid oxidation (FAO) or the inhibition of fatty acid synthesis (FAS) during glucose deprivation or matrix detachment respectively. Through these processes AMPK activation inhibits the rise in reactive oxygen species (ROS) levels and promotes metabolic adaptation in response to metabolic stress. This finding also provides a new therapeutic opportunity through targeting metabolic adaptation of cancer cells, either alone or in combination with conventional anti-cancer drugs that cause metabolic stress.

Keywords: AMPK, LKB1, CaMKK2, ACC, NADPH, ROS, fatty acids, metabolic stress, cancer

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Introduction

AMP-activated protein kinase (AMPK) plays a pivotal role in the regulation of energy homeostasis at both the cellular and organismal levels. Diverse stress conditions, such as nutrient starvation or hypoxia, that increase AMP, ADP, Ca^{2+} and reactive oxygen species (ROS) levels have been shown to activate AMPK (**Fig. 1**, and reviewed in ref. 1). AMPK exists as a heterotrimer consisting of an α-catalytic subunit and β, γ-regulatory subunits, and the phosphorylation of T172 in the α-catalytic subunit is a critical event for the full activation of AMPK under these conditions. Two major upstream kinases are responsible for the phosphorylation of the catalytic subunit: the liver kinase B1 (LKB1) and Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2)[2-6] (**Fig. 1**). LKB1 is responsible for the activation of AMPK during energy stress when intracellular ATP levels are reduced and ADP and AMP levels are elevated. Thus, the LKB1-AMPK pathway has a major role in the regulation of metabolic adaptation during energy stress conditions. CaMKK2 activity is usually elevated when intracellular Ca^{2+} levels are elevated, and thus can activate AMPK regardless of the energy status of the cells. However, the physiological conditions in which the CaMKK2-AMPK axis plays a major role remain to be elucidated. In addition to AMP, ADP and Ca^{2+}, recent studies also have identified reactive oxygen species (ROS) as another upstream activators of AMPK.[7-11] Although ROS can increase the phosphorylation of T172, it was not yet determined which upstream kinases (or phosphatases) are responsible for this increase. Moreover, there is a debate whether ROS can activate AMPK directly through the oxidation of cysteine residues in AMPK, or indirectly through the increase of AMP, ADP or Ca^{2+} levels.[7,8,10-12]

Once activated, AMPK maintains energy balance through the activation of catabolism to increase ATP production and through the inhibition of anabolism

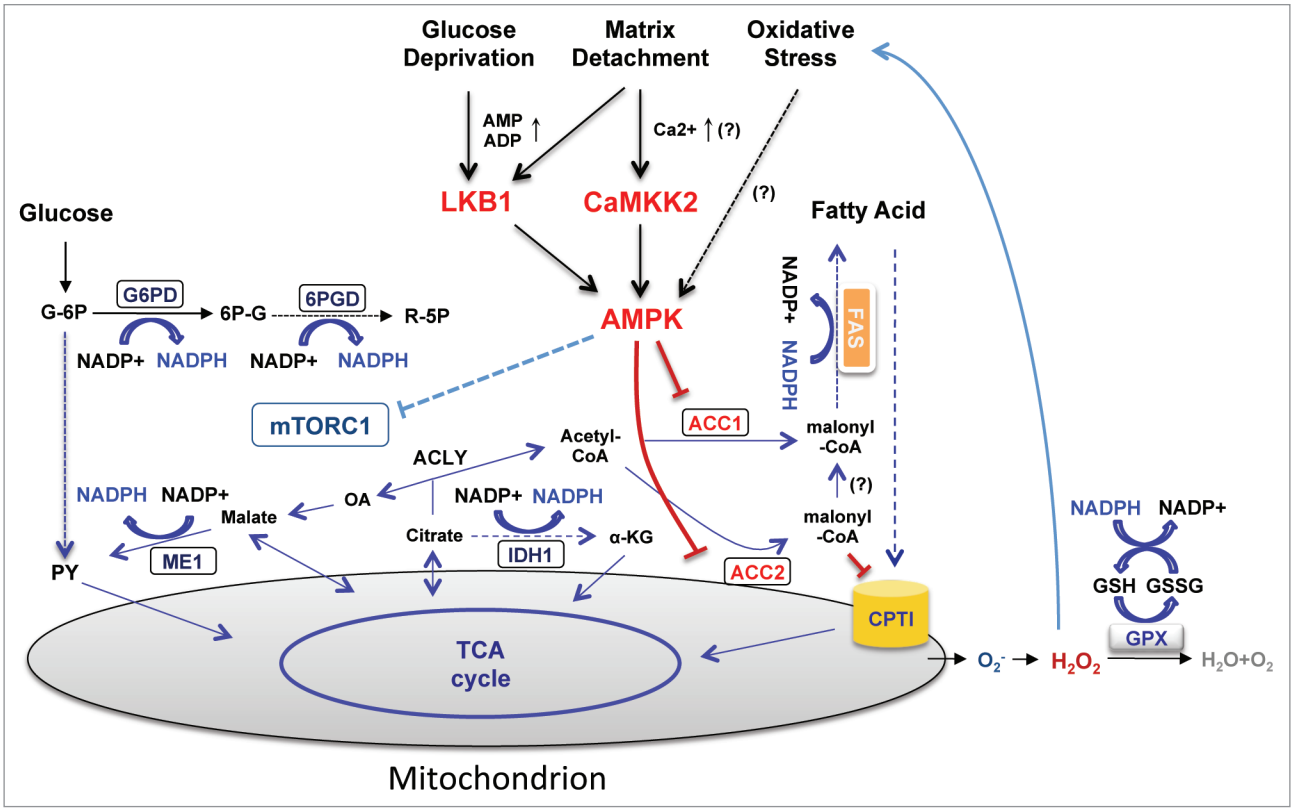

Figure 1. The mechanisms by which AMPK regulate NADPH homeostasis during energy stress. When glucose is available, NADPH is generated by the PPP and mitochondrial metabolism. NADPH is consumed in FAS and in regeneration of GSH to detoxify ROS. Energy stress conditions, such as glucose deprivation or matrix detachment, which decrease glucose metabolism, impair NADPH production by the PPP. Under these conditions, AMP/ATP and ADP/ATP ratios are increased and the LKB1-AMPK pathway is activated. Matrix detachment also activates the CaMKK2-AMPK pathway. Oxidative stress is also known to activate AMPK through poorly understood mechanisms. Activated AMPK could inhibit cell proliferation through the inhibition of mTORC1. However, AMPK also phosphorylates and inactivates ACC1 and ACC2, which result in the inhibition of FAS and activation of FAO respectively. Inhibition of FAS reserves intracellular NADPH levels by blocking NADPH consumption during FAS. The activation of FAO increases NADPH production by increasing TCA cycle metabolites and substrates for ME1 and IDH1 that generate NADPH from malate and isocitrate respectively (for details see text). Abbreviations: PY, pyruvate; OA, oxaloacetate; α-KG, α-ketoglutarate.

to reduce ATP consumption through the phosphorylation of diverse substrates. Thus AMPK could restrain cell growth by inhibiting protein and fatty acid synthesis, while promoting cell survival mechanism through the elevation of mitochondrial metabolism and autophagy.[13-21] Because of these versatile and conflicting functions of AMPK in cell growth and survival, its role in cancer has been hotly debated. In this addendum, we briefly summarize recent literatures supporting the pro-tumorigenic role of AMPK and highlight our recent findings connecting AMPK and NADPH homeostasis as the potential mechanisms to explain those previous observations. Further, we discuss possible scenarios that can resolve the LKB1-AMPK paradox in cancer and potential applications of this finding to cancer therapy.

The Emerging Evidences for the Pro-Tumorigenic Role of AMPK

Despite the concept that AMPK hyperactivation is anti-tumorigenic, there are multiple observations that indicate that physiological AMPK activation is pro-tumorigenic (summarized in **Table 1**). Using an established model of glioblastoma, it was shown that AMPK is strongly activated during the early stages of solid tumor formation.[22] This finding supports the notion that physiological activation of AMPK could be important for tumorigenesis. Consistently, it has been shown that LKB1-null mouse embryo fibroblasts (MEFs) are resistant to oncogene-induced anchorage-independent growth and that oncogene-transformed AMPKα1/α2-null (AMPKα-KO) MEFs are sensitive to anoikis and are severely impaired in their ability to form tumors in vivo.[23-25]

The silencing of AMPK in pancreatic cancer cells impairs both their ability to grow in an anchorage-independent manner and to form tumors in vivo.[26] Moreover, it has been shown that the LKB1-AMPK signaling pathway is required for glioma cell survival and spheroid migration in low-glucose conditions.[27] The importance of AMPK activation for tumor cell growth, survival and migration has also been shown in prostate cancer.[28-31] Unbiased RNAi screening identified that AMPK β subunit is essential for prostate cancer cell survival.[30] The same study found that the expression of AMPK β subunit is highly elevated in metastatic prostate cancers when compared with primary prostate cancers. Interestingly, a critical role of the CaMKK2-AMPK axis

Table 1. Summary of recent findings supporting the pro-tumorigenic role of AMPK

Cell lines/animal models	Stress conditions (assays)	Major findings supporting pro-tumorigenic role of AMPK	References
Established model of glioblastoma development in the offspring of rats exposed prenatally to the mutagen N-ethyl-N-nitrosourea (ENU)	Solid tumor formation in vivo (brain tumor model induced by carcinogen)	AMPK is strongly activated during early stage of tumorigenesis in vivo.	22
LKB1-null MEFs transformed by oncogene	Anchorage-independent growth (soft agar)	LKB1-null MEFs are resistant to oncogene-induced transformation.	23
AMPKα1α2-double knockout MEFs transformed by H-Rasv^{12}	Solid tumor formation in vivo (xenograft)	AMPKα1α2-double knockout MEFs are severely impaired in their ability to form tumors in vivo.	24
AMPKα1α2 double knockout MEFs transformed by H-Rasv^{12}	Matrix detachment (cell viability)	AMPK confers anoikis resistance in transformed cells	25
Pancreatic cancer cell lines	Glucose deprivation (cell viability), anchorage-independent growth (soft agar) and solid tumor formation (xenograft)	AMPK knockdown using siRNA sensitizes cell death during glucose deprivation and also impairs anchorage-independent growth and solid tumor formation.	26
Glioblastoma cell lines	Glucose deprivation (cell viability and spheroid migration)	AMPK signaling promotes cell survival and migration during glucose deprivation	27
Prostate cancer cell lines	Non-stress conditions (migration?)	CaMKK2 is increased by androgen and mediates androgen dependent regulation of cell migration through AMPK.	28
Prostate cancer cell lines	Non-stress conditions (cell proliferation and apoptosis)	Inhibition of AMPK by RNAi or compound C decreases cell proliferation and induces apoptosis.	29
Prostate cancer cell lines	Lipid deprivation (caspase activity and cell mass) and solid tumor formation (xenograft)	siRNA screening in prostate cancer cell lines under lipid deprivation identified AMPKβ1 subunit as an essential gene for survival.	30
Prostate cancer cell lines	Glucose deprivation (cell viability)	AMPK promotes prostate cancer cell survival during glucose deprivation.	31
Hepatocarcinoma cells isolated from mouse primary tumor induced by myc, Akt, p53$^{-/-}$	Solid tumor formation (orthotopic transplantation into liver capsule)	Myc-driven tumorigenesis requires AMPK activity that promotes mitochondrial metabolism.	32
Pancreatic cancer cell line and glioblastoma cell line	Matrix detachment (cell viability) and anchorage-independent growth (soft agar)	KSR2 promotes metabolic activity, anoikis resistance and anchorage-independent growth via AMPK.	33

also has been shown in prostate cancer.[28] It was found that CaMKK2 expression is highly elevated in prostate cancers and the activation of the CaMKK2-AMPK pathway is a critical downstream effector of androgen receptor (AR)-dependent migration and invasion, suggesting that the CaMKK2-AMPK axis is a promising therapeutic target in prostate cancer. Finally, it was recently shown that AMPK is essential for Myc-driven tumorigenesis using orthotopic transplantation of mouse hepatocellular carcinoma (HCC) cells[32] and for the Kinase Suppressor of Ras 2 (KSR2)-induced anoikis resistance and anchorage-independent growth of cancer cells.[33]

The Mechanisms by Which AMPK Activation Promotes Tumorigenesis: The Redox Connections

Previous studies suggested that the LKB1-AMPK pathway promotes cell survival during glucose deprivation, in part, through inhibition of mammalian target of rapamycin complex 1 (mTORC1) or activation of p53.[13,34] These previous studies suggested that AMPK promotes cell survival by conserving ATP levels during metabolic stress through the inhibition of ATP consuming processes or the activation of ATP producing processes, although there is no direct experimental evidence to support this view.

Our recent study unraveled a new function of AMPK in NADPH homeostasis, which is required to prevent

oxidative stress and promote cancer cell survival under metabolic stress conditions[35] (**Fig. 1**). NADPH is consumed and required for FAS, and for the maintenance of reduced glutathione, which is required to detoxify H_2O_2 (**Fig. 1**). We showed that AMPK maintains NADPH homeostasis through the regulation of fatty acid metabolism by phosphorylating and inactivativating acetyl-CoA carboxylase 1 (ACC1) and ACC2.[35] ACC1 and ACC2, which are the major targets of AMPK in the regulation of FAS and FAO, convert acetyl-CoA to malonyl-CoA[36] (**Fig. 1**). Malonyl-CoA is not only a precursor for FAS, but is also an allosteric inhibitor of carnitine palmitoyl transferase 1 (CPT1). CPT1 is associated with the outer mitochondrial membrane and transfers long-chain fatty acids from the cytosol into the mitochondria for FAO. ACC1 and ACC2 might have overlapping function. However, malonyl-CoA generated by ACC1, which is localized to the cytosol is largely channeled to FAS.[37] On the other hand, malonyl-CoA generated by ACC2, which is localized to the outer mitochondrial membrane and in close proximity to CPT1, inhibits CPT1 and consequently FAO[38] (**Fig. 1**). Since FAO fuels mitochondrial metabolism and increases TCA cycle metabolites, FAO could increase NADPH production through malic enzyme 1 (ME1) and isocitrate dehydrogenase 1 (IDH1) by converting malate and isocitrate to pyruvate (PY) and α-ketoglutarate (α-KG) respectively (**Fig. 1**).

In our study, we used two different conditions for metabolic stress, glucose deprivation and matrix detachment, which are both associated with solid tumor microenvironment.[35,39] When glucose uptake is decreased, both glycolysis and the pentose phosphate pathway (PPP) can be inhibited (**Fig. 1**). The PPP is the major pathway that generates NADPH via glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD). As a consequence of metabolic stress conditions when glucose availability is decreased in LKB1 deficient cancer cells, we showed that NADPH production is decreased from PPP and consequently H_2O_2 levels are elevated, which is the main cause of cell death.[35] However, the concurrent activation of LKB1-AMPK pathway under these conditions increases NADPH production and H_2O_2 detoxification via inhibition of ACC2 and the increase of FAO.[35] Only ACC2 inhibition and the induction of FAO—not ACC1 and FAS inhibition—is required for cell survival under glucose deprivation because FAS inhibition is occurring regardless of ACC1 or ACC2 status in response to glucose deprivation.[35] This is consistent with the previous finding showing that in the absence of glucose, the activation of AMPK and FAO could promote the survival of cancer cells displaying hyperactive Akt.[20] This is also consistent with the recent study showing that AMPK is essential for Myc-driven tumorigenesis through promoting mitochondrial respiration,[32] further underscoring the importance of the AMPK-induced mitochondrial metabolism in tumorigenesis.

On the other hand, during matrix detachment, we found that the inhibition of FAS and therefore the inhibition of NADPH consumption by ACC1 inhibition is the predominant mechanism by which AMPK sustains NADPH levels and prevents oxidative stress[35] (**Fig. 1**). Importantly, we found that even in the absence of LKB1, AMPK can be significantly activated during matrix detachment in a CAMKK2-dependent manner[35] (**Fig. 1**). The mechanism by which the CAMKK2-AMPK axis is activated during matrix detachment is not known, but this activation of AMPK is not sufficient to fully protect the cells from oxidative stress induced by matrix detachment. The importance of AMPK activation in reducing oxidative stress during tumorigenesis was underscored by the observations that antioxidant treatment in LKB1- or AMPK-deficient cells could restore anchorage-independent growth of these cells. Consistently the inhibition of ACC1 or ACC2 could restore anchorage-independent growth and solid tumor formation of AMPK-deficient cells in vivo.[35]

Collectively, our study demonstrates that NADPH and ROS maintenance by ACC inhibition is a major mechanism by which AMPK promotes cancer cell survival during energy stress, anchorage-independent growth and solid tumor formation. Further, the study established that in addition to ATP, NADPH maintenance is an important function of AMPK activation. It appears that in cancer cells, which are exposed to energy stress, the maintenance of NADPH is the predominant mechanism by which AMPK activation promotes cell survival.

Understanding the Previous Findings in the Context of AMPK-NADPH Paradigm

Our findings could explain several aspects regarding the role of the LKB1-AMPK pathway in cancer raised by previous studies, and which are not fully understood. For example, our results may explain why patients with Peutz-Jeghers syndrome, who have an inherited deficiency of LKB1, and most mouse models of LKB1 deficiency, develop only benign tumors.[23] Although cells with LKB1 deficiency have an advantage in cell growth and proliferation as a result of mTORC1 activation, they cannot become fully malignant due to a disadvantage in cell survival during solid tumor formation, which involves matrix detachment and limited glucose supply. It would be, therefore, interesting to assess this possibility by crossing LKB1 deficient mice with the mice overexpressing certain antioxidant genes such as catalase or the transcription factor NRF2, a master regulator of antioxidant enzymes and analyzing tumor development.[40] In addition, our results could potentially explain why LKB1- or AMPK-deficient MEFs are resistant to both oncogene-induced anchorage-independent growth and tumor formation in vivo, and why AMPK is required for KSR2-mediated anoikis resistance and anchorage-independent growth.[23-25,33] Interestingly, a recent study by Sorensen and colleagues also found that AMPK inhibits anoikis partially through the inhibition of mTORC1-mediated translation.[25] This study adds additional mechanism for the protective role of AMPK during matrix detachment, although it is still possible that mTORC1 can regulate FAS and NADPH levels through regulation of sterol-regulatory-element-binding proteins 1 (SREBP1) that induces the expression of lipogenic enzymes.[41,42] Likewise, this may also explain why Tsc2-null cells are hypersensitized to glucose deprivation,[15,43] as mTORC1 hyperactivation accelerates FAS

through its effect on SREBP1, which further increases NADPH consumption. Our results may also explain why Akt activation sensitizes cells to glucose deprivation and why FAO activation reverses this sensitization.[20] Akt activation may sensitize cells to glucose deprivation because it inhibits AMPK[44] or activates ATP-citrate lyase (ACLY),[45] which in turn activates FAS and inhibits FAO, leading to NADPH depletion. Thus, FAO activation could promote cell survival under these conditions by increasing NADPH production. Similarly, this can also potentially explain why Myc-driven tumorigenesis requires AMPK-induced mitochondrial gene expression and mitochondrial metabolism.[32] Enhancing mitochondrial metabolism by AMPK could increase NADPH production and provide potential benefits during Myc-driven tumorigenesis which is known to increase ROS levels.[46] Finally, based on our findings showing that the CaMKK2-AMPK-NADPH axis plays a major role in cancer cell survival during matrix detachment, our study may explain why CaMKK2 is overexpressed in certain cancer and also may provide new mechanistic insights regarding the pro-tumorigenic role of the CaMKK2-AMPK pathway in prostate cancer migration and invasion.[28]

The LKB1-AMPK Paradox: Unresolved Questions

Although somatic mutations in LKB1 are rare in most tumors, the mutations are prevalent in certain cancers, such as non-small cell lung carcinoma and cervical carcinoma.[47] In mouse models, the deficiency in LKB1 accelerates Ras-induced lung tumorigenesis.[48] Thus, one critical question is how the loss of function mutations in LKB1 occurs in these cancer cells, despite the disadvantages in metabolic adaptation. The simplest explanation would be that LKB1-deficiency could be pro-tumorigenic through the activation of mTORC1 or inactivation of other kinases downstream of LKB1[48] in a microenvironment where metabolic adaptation does not play a critical role. Indeed, it was recently shown that lung tumors are less hypoxic as compared with other solid tumors,[49] suggesting that the LKB1-AMPK-mediated metabolic adaptation may not be critical for lung tumorigenesis. It is also possible that in these cases certain microenvironmental factors enable activation of AMPK through other upstream activators, such as CaMKK2, in the absence of LKB1. A more intriguing possibility is that LKB1 mutations could lead to cancer only if other occurring mutations could compensate for the loss of metabolic adaptation mediated by the LKB1-AMPK axis. Thus, it would be interesting to analyze genetic or epigenetic changes in these cancers to verify whether there are additional mutations that co-exist with LKB1 mutation, and that elicit protection from oxidative stress through alternative mechanisms. Finally, tracing metabolic fluxes redirected by genetic or epigenetic changes to compensate for the loss of LKB1 would be also important to resolve this issue.

Therapeutic Opportunity

Forced AMPK activation is being considered for cancer therapy as it was shown that it induces a cytostatic effect primarily through the inhibition of mTORC1. However, our results suggest that physiological activation of AMPK in cancer cells could promote their survival during two critical processes at the early stages of solid tumor formation. The metabolic adaptation mediated by AMPK activation could occur when cancer cells are exposed to an environment with limited nutrients and glucose and/or detachment from the matrix inside of a solid tumor, and when they migrate from the primary tumor and colonize in a secondary site during metastasis.

Our results suggest that the combination of AMPK activators and potential ACC activators could eliminate the pro-survival effect of AMPK activation and would elicit cancer cell death during the two critical stages in cancer development. In addition, targeting metabolic adaptation could be a promising strategy if combined with conventional anti-cancer drugs that cause metabolic stress. Recently, it has been shown that anti-VEGF therapy in ovarian cancers induces oxygen and glucose deprivation and increases AMPK activity.[50] Intriguingly, the AMPK activity as measured by p-ACC level is well correlated with the resistance to anti-VEGF therapy, while silencing AMPKα2 in ovarian cancer enhanced the therapeutic effect of anti-VEGF. Thus, it would be of importance to further assess the potential clinical benefits of this combination therapy. Further, as described above, analyzing specific compensatory genetic or epigenetic changes in LKB1-mutated cancers will help design new therapeutic strategies to treat those cancers.

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