

Critical Review

The Multifaceted Activities of AMPK in Tumor Progression—Why the “One Size Fits All” Definition Does Not Fit at All?

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Abstract

AMP-activated kinase (AMPK) is a central cellular energetic biosensor and regulator of a broad array of cellular metabolic routes activated by nutrient deprivation, mitochondrial dysfunction, oxidative stress, and cytokines. The activation of AMPK maintains ATP levels in response to hypoxia, mitochondrial dysfunction, and shortage of essential metabolic fuels. Activated AMPK turns on energy sparing pathways and promotes antiapoptotic functions thereby permitting cells to survive extremely hostile conditions for prolonged periods of time. Cancer cells in solid tumors are generally subjected to such harsh conditions; however, they manage to efficiently survive and proliferate. This is likely due, in great part, to a peculiar form of metabolism that is heavily reliant on glycolysis and which promotes cancer cell adaptation and tumor progression. AMPK controls the influx and utilization of glucose by cancer cells and therefore has emerged as an attractive target to treat cancer. Investigations exploring this possibility demonstrated that activators or inhibitors of AMPK impact cancer cell viability and possibly cancer

progression. For example, the AMPK activator metformin induces apoptosis in a variety of cancer cell lines and models. A major problem with many of the studies on metformin is that little effort has been invested in unraveling how metformin activates AMPK in the many contexts it has been tested. This is significant because many AMPK-independent effects of metformin have been documented. The notion that AMPK acts solely as a tumor suppressor also conflicts with findings that it confers resistance to nutrient deprivation, sustains NADPH levels in cancer cells, facilitates stress-induced gene transcription, promotes cell survival via antiapoptotic function upregulation, intermediates epithelial-to-mesenchymal transition, and increases malignant transformation. These are all recognized steps necessary for the successful evolution of tumors. This review highlights some of these findings and proposes that the role of AMPK in cancer should be reconsidered in light of the complex roles of AMPK under different metabolic conditions. © 2013 IUBMB Life, 65(11):889–896, 2013

Keywords: signaling; stress-activated signaling; complex diseases; AMPK; nutrient deprivation; metformin

Introduction

AMP-activated kinase (AMPK) is a major energetic biosensor and metabolic switch that controls a broad array of biosynthetic and

catabolic pathways in the cell. Its activation maintains cellular energetic viability under conditions of reduced mitochondrial ATP biosynthetic capacity by promoting the recycling of cellular components through autophagy, optimizing oxygen utilization, activating alternative mechanisms of ATP synthesis independent of mitochondria, and increasing glucose influx through glucose transporter localization to the plasma membrane. The mammalian AMPK enzyme is composed by a number of catalytic ($\alpha 1$ and $\alpha 2$), regulatory ($\beta 1$ and $\beta 2$) and adenine nucleotide binding ($\gamma 1$, $\gamma 2$, and $\gamma 3$) subunits. It is regulated by allosteric interactions, acetylation, and phosphorylation that is mediated largely by upstream kinases such as LKB1 and CAMKK β . The basic biochemistry of AMPK has been thoroughly reviewed elsewhere (1).

Eil and Wool first isolated AMPK in 1971. They found that several ribosomal component proteins were phosphorylated in

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response to AMP stimulation by two different kinases (2). It would be 20 years before AMPK function in mammalian cells would be better appreciated spurred by findings that the enzyme controlled carbon metabolism similarly to the yeast and plant serine/threonine kinase SNF1 (3–5). From there, evidence rapidly accumulated in support of AMPK inhibiting lipid biosynthesis (6–8), increasing fatty acid oxidation (9), increasing glucose uptake into the cell (10–13), glucose utilization (14,15), and insulin secretion (16). Later, AMPK was also demonstrated to inhibit cellular protein biosynthetic pathways (17,18) and increase autophagy (19,20) by suppressing the mammalian target of rapamycin (mTOR). Findings in support of AMPK being a potent regulator of glucose, lipid, and ketogenic metabolism made it apparent that manipulating the activity of the enzyme could affect diabetes, a condition in which glucose utilization by cells is heavily impaired forcing them to operate under a persistent state of fasting despite an abundant extracellular supply of glucose. The search for pharmacologic activators of AMPK that could be useful in the management of metabolic syndrome and type 2 diabetes led to the discovery that thiazolidones and biguanidines, in particular metformin, activate AMPK and to some extent normalize various abnormalities that characterize glucose handling in these conditions (21).

These AMPK activators are not without some problems, however. In cancer cell lines, metformin was found to induce apoptosis and while this effect was largely attributed to AMPK activation, it is important to note that metformin functions as an inhibitor of complex I of the electron transport chain (22,23). This important effect of metformin is more generally overlooked in typical studies addressing metformin-induced apoptosis. Importantly, mitochondrial depolarization is capable of simultaneously activating apoptosis and AMPK by different mechanisms. Similarly, AICAR, another pharmacologic activator of AMPK, was shown to mimic the pro-apoptotic effects of metformin in cancer cell culture models (24) but not surprisingly was unequivocally demonstrated to exert AMPK-independent effects on cellular metabolism similar to those of metformin and oligomycin in AMPK deficient cells (25), suggesting that the metabolic effects may be more important than previously appreciated. AICAR has subsequently been shown to have variable effects on different cancer cell types, and in many cases, these effects are linked to the stimulation of mitochondria-dependent apoptosis (26,27). The AMPK inhibitor, compound C, has similarly been shown to affect cell survival by both AMPK-dependent and AMPK-independent mechanisms (28).

The controversy that arises from these various pharmacologic approaches demands more reliable assessments of the functions of AMPK in cancer. To circumvent the off target effects of drug studies, genetically manipulated systems with altered AMPK activity could be used. Consistent with the AMPK-independent effects of metformin and AICAR in cancer cell death, AMPK was shown to be critical for cells to resist metabolic stress and evade apoptosis under conditions of

reduced mitochondrial function or nutrient deprivation (29–31). Because the roles of AMPK in controlling various aspects of metabolism and signaling have been amply discussed, this review will focus on some of the findings that indicate that AMPK is involved in several critical steps required for malignant transformation and cancer progression. Specifically we will discuss evasion of apoptosis, epithelial to mesenchymal transition (EMT) and reprogramming. We will focus, where possible, on separating AMPK-dependent and -independent effects of pharmacological data to highlight the need for a broader understanding of the complexity of AMPK signaling in cancer.

Antiapoptotic Functions of AMPK in Healthy and Cancerous Cells

AMPK activation following stress has been associated with increased resistance to death in many cellular systems. For instance, transfection of myocytes with a dominant negative, inactive form of AMPK (AMPK-DN) was shown to attenuate survival on glucose deprivation, which induces the activation of AMPK, and subsequent inhibition of mTOR (32). This work concurs with previous observations that have supported a critical role for AMPK in adaptive responses of the heart to reduced blood flow (33) as well as the finding that ablation of AMPK activity in mouse embryonic fibroblasts and cardiomyocytes strongly affected the accumulation of p53 and increased apoptosis (34). Interestingly, Zou and coworkers observed that AMPK α 1 activation in endothelial cells results in the upregulation of the antiapoptotic genes, Bcl-2 and survivin (35). In our own laboratory, we recently demonstrated that this occurs in breast cancer epithelial cell lines derived from MCF-7 cells as well (Ansenberger-Fricano et al., submitted). Indeed, we observed that in advanced breast cancer biopsies AMPK is strongly activated in parallel with the expression of antiapoptotic proteins. It is well documented that Bcl-2 and survivin upregulation correlate with poor outcomes in cancer (36–39). This linkage between AMPK activation and cancer cell survival is seen in a variety of cancer cell lines, particularly under nutritional stress and hypoxia *in vitro* and *in vivo*. For instance, it has been demonstrated that AMPK sustains pancreatic cancer cell (PANC-1) survival in the face of glucose withdrawal and supports the growth of tumor xenograft from these cells implanted in immunodeficient mice (40). AMPK was also shown to inhibit detachment-induced apoptosis (anoikis) in K-Ras transformed fibroblasts. In these cells, the genetic ablation of AMPK led to a significant reduction in steady state levels of ATP in cells in suspension paralleled to a marked increase in the activity of caspase-3 (41). In the same study, it was demonstrated that AMPK critically contributes to anoikis resistance in a number of breast cancer cell lines. This aspect of AMPK cellular biology is critically important for cancer progression, since metastasis requires that cells detach from the matrix and survive in suspension until engrafting at a distant

site. More recently, our laboratory has found that AMPK maintains the breast cancer cell's energetic viability by sustaining ATP production despite progressive mitochondrial failure induced by MnSOD upregulation (Ansenberger-Fricano et al., submitted). So, it is becoming increasingly clear that AMPK plays a critical role in suppressing apoptosis by sustaining cellular energetic viability and fostering the expression of antiapoptotic factors, both of which are important for the progression of cancer.

Are The Effects of Metformin on Cancerous Cells Always AMPK-Dependent?

Metformin exerts effects on a great variety of signaling pathways (Fig. 1). In particular, metformin inhibits NADH dehydrogenase (complex I) of the mitochondrial electron transport chain (42,43). Zakikhani et al. found that metformin dampens steady state levels of ATP to values comparable to those measured when MCF-7 cells were treated with rotenone, a powerful inhibitor of complex I (42). Inhibition of complex I has been linked to increased production of mitochondrial reactive oxygen species (mtROS) and a drastic reduction in ATP synthesis. Both reactive oxygen species and a reduction in ATP are known to activate AMPK by different mechanisms. Loss of mitochondrial potential and oxidative stress are known to elicit apoptosis via release of cytochrome c. In light of this, it is highly unlikely that effects of metformin on cell cycle arrest and apoptosis are strictly the result of AMPK activation, but rather, represent a hybrid of AMPK-dependent and independent effects on mitochondrial function. Given this, we present a radically different view of AMPK activation in cancer, not as a cause of apoptosis, but rather as an enabler. This idea is built on the premise that critically compromised cells with failing mitochondria need to activate alternative pathways to maintain minimal steady state concentrations of ATP that are required to direct and complete apoptosis. AMPK is an obvious

candidate for this sort of metabolic function, which in non-transformed cells could avoid necrosis in view of irreversible damage, allowing for orderly elimination by apoptosis rather than inflammation caused by necrosis. The potential for such a pathway in cancer cells is intriguing.

Numerous studies have shown that many of the downstream effects of AMPK can be emulated by biguanidines acting on alternative effectors, even the well-described inhibition of mTOR. Sahra et al. observed that in prostate cancer, REDD1 is directly activated by metformin and mediates the inhibition of mTOR without the need for AMPK (44). Buler et al. observed that metformin rapidly depletes the mitochondrial deacetylase SIRT3 (45), which in turn, regulates manganese superoxide dismutase (MnSOD) activity (46). Thus, by reducing SIRT3 activity metformin inhibits MnSOD increasing mtROS, which activate AMPK. Another indirect mechanism of AMPK activation by metformin was identified by Ouyang et al. who observed that the drug impedes the degradation of AMP by inhibiting the enzyme AMP deaminase. Accumulation of AMP activates AMPK (47). Importantly, it was recently demonstrated that AMPK is critical for the maintenance of NADPH levels in epithelial lung cancer cells (48). The authors demonstrated that in p53-competent as well as in p53-null cells, LKB1 reconstitution parallel to AMPK activation inhibited glucose-deprivation induced cell death.

Taken together, the majority of the published observations indicate that while metformin has obvious and consistent anticancer effects, AMPK's involvement in producing such effects depends on the context. It is clear that AMPK should not simplistically be assumed to be a tumor suppressor under all circumstances, but rather must be understood as a central effector of outcomes that depend on dynamic interconnected signaling events that can themselves be modulated intrinsically and extrinsically by the microenvironment, genetic and epigenetic variants, energetic, and functional states of the cell leading to survival, apoptosis, or adaptation.

AMPK in Cancer—A Tumor Suppressor?

Current evidence supports a role for AMPK as either a tumor promoter or a tumor suppressor, depending on the context (1). Many recent studies have found that the cellular metabolic state alone, which is critically determined by AMPK, can dramatically affect multiple cellular processes relevant to carcinogenesis and cancer progression (48–50); Fig. 2). While it is true that AMPK activation has been found to inhibit cell growth (reviewed in) (51), it remains poorly defined whether and how AMPK affects other critically important aspects of tumorigenesis such as EMT, the reverse process of mesenchymal to epithelial transition (MET), reprogramming, transition between quiescence and proliferative states, mitosis, etc. AMPK can be activated by both liver kinase B1 (LKB1) and calmodulin kinase kinase beta (CAMKK β). Interestingly, LKB1

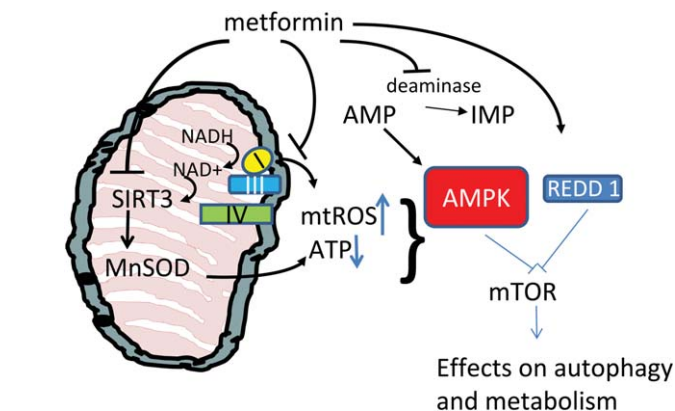
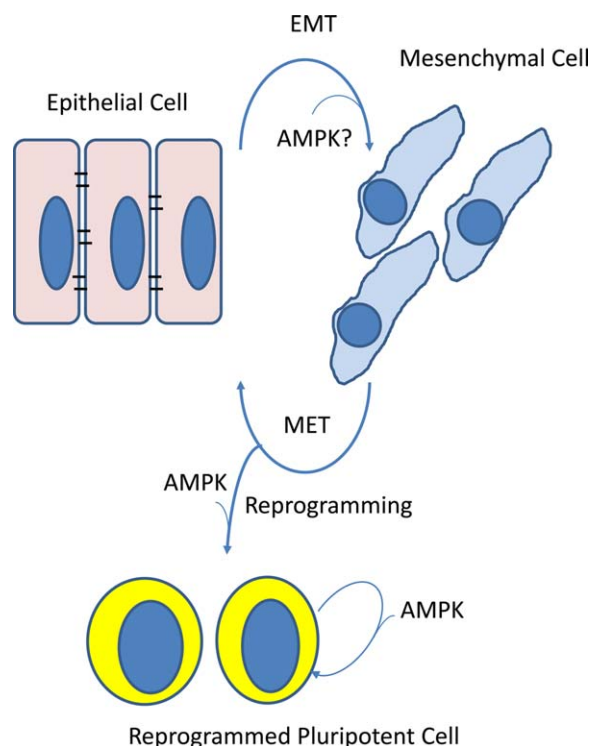


Fig 1

Examples of pathways that are affected by metformin and overlap with those controlled by AMPK.


Fig 2

Schematic representation of the many pathways related to cancer progression and metastasis possibly regulated (directly or indirectly) by AMPK. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

appears to be a powerful tumor suppressor and mice deficient in this enzyme are prone to cancer development (52). Importantly, these studies have implicated AMPK in the downstream tumor suppressive effects of LKB1 (29; reviewed in 1). On the contrary, blockade of the CAMKK β /AMPK pathway has been shown to result in dampened migration, invasion, and growth of prostate cancer cells (53,54). These observations indicate that the route to AMPK activation likely affects downstream outcomes. In a recently published study, Cuezva and coworkers (55) reported that AMPK is not merely responding to the dampening of mitochondrial bioenergetic activity in cancer but might be actively promoting it. Cooperatively with the stress activated kinase GCN2, AMPK is thought to repress the transcription of mitochondrial genes. This concept challenges the simplistic bioenergetic sensor model and promotes AMPK to the level of an active regulator of mitochondrial function in tumorigenesis.

AMPK Involvement in the Activation of AKT

A well-appreciated aspect of tumorigenesis is the modification of the serine threonine kinase, Akt, and its associated signaling pathways (56). The roles that AMPK may play in the process of Akt regulation are not clear. Reports exist demonstrating that

AMPK can both inhibit and activate Akt, but these have used tools like AICAR and metformin whose problems were discussed above (57–59). The activation of Akt is complex and involves the activation of PI3Kinases or suppression of lipid phosphatases such as phosphatase and tensin homolog (PTEN) to promote the accumulation of phosphoinositides. These in turn recruit kinases such as phosphoinositide dependent kinase (PDK-1) and the mTORC2 complex to Akt to promote its activation via phosphorylation on Thr308 and Ser473, respectively. Optimal activation of Akt requires both sites to be phosphorylated (60). mTORC2 is indirectly inhibited by the activity of mTORC1, in a pathway involving insulin receptor substrate-1 and, in fact, many groups have shown that rapamycin, a drug that inhibits the activity of the mTORC1 complex but not mTORC2, increases Akt phosphorylation in a number of cellular systems (61–63). Since AMPK has a well-described role in the suppression of mTORC1, it remains at least theoretically possible that AMPK could be playing a role in supporting the activation of Akt during tumorigenesis.

AMPK in the Process of Malignant Transformation

Malignant transformation radically reformats the cellular metabolism; this generally accompanies changes in membrane proteins and channels, acquisition of the capacities to migrate, invade healthy tissue, and undergo unrestricted proliferation. All of these processes demand considerable amounts of energy. In cancer, the energetic function of mitochondria appears to fade favoring the redirection of mitochondria toward an increased biosynthetic capacity gradually (64). Despite the diversion of the mitochondrial machinery to biosynthesis, ATP is still required to support the metabolic, proliferative, and migratory capacities of the cancer cell. A model wherein AMPK and related enzymes serve to “balance the budget” for cellular energy by promoting efficient processes and restraining energy consuming ones seems to be adequate to maintain the cellular energetic stability during transformation. Utilizing immortalized mammary epithelial cells that express v-Src on tamoxifen stimulation, we demonstrated that AMPK is activated during transformation (Ansenberger-Fricano K., et al., submitted). In its role of governing the cellular energetic expenditure, AMPK occupies a central role in supporting cancer cell survival, resistance to apoptosis, EMTs, and reprogramming. Below we will attempt to critically review some of the recent work that has supported or refuted this concept.

AMPK Involvement in EMT

EMT and MET are processes whereby mature cells acquire features of alternate cell fates. EMT and MET are generally viewed as fundamental to the progression of cancer. EMT promotes cancer progression by fostering the formation of cells

capable of migration and engraftment at distal locations (metastasis), and as such, serves as an important prognostic clinical indicator. Further, cycles of EMT-MET may promote more radical reprogramming events, such as dedifferentiation. It remains controversial whether somatic cell dedifferentiation contributes to the formation of cancer stem cells, which are thought to be more menacing due to their increased drug resistance and capacity to repopulate tumors. Some recent work has indicated that these cancer stem cells may be driving cancer progression in a number of different contexts (reviewed in) (65,66). The demonstration that differentiated cells can be reprogrammed to acquire stemness through expression of well-defined factors (67) has raised the possibility that somatic cells harbor the potential to produce pluripotent and self-renewing cells *in vivo*. Intriguingly, Pei and coworkers (68) have recently demonstrated that MET is a required first step in reprogramming of induced pluripotent stem cell (iPSC) formation. While inhibition of MET prevents dedifferentiation, enhancing MET increases the efficiency of reprogramming. Is AMPK involved in EMT or MET, thereby promoting the formation of especially difficult to eliminate cancer stem cells? Two different groups have provided intriguing, if somewhat dissonant, answers. In a study by Song and coworkers, EMT induced by transforming growth factor beta (TGF β) was strongly attenuated by AMPK inhibition with compound C or by the expression of dominant negative AMPK in hepatocytes and AML cells (69). Interestingly, this study also showed that AMPK inhibition increases TGF β induced apoptosis. These two observations indicate that AMPK activation leads to apoptosis of a subpopulation of cells while enriching surviving populations with cells possessing increased plasticity. Conversely, Menendez and coworkers (70) found that metformin impedes the reprogramming of mouse embryonic fibroblasts to stemness induced by Oct-4/Sox-2/KLF-4 ectopic coexpression. Their conclusion is that AMPK activation imposes a metabolic barrier to reprogramming. At the very least, the comparison between these studies provides a solid basis to believe, as discussed above, that the effect of AMPK activity is likely to be highly complex and must be studied using an array of approaches to draw appropriate conclusions about its function in any particular context.

AMPK and Cancer Stem Cells—Is AMPK Involved in Reprogramming?

The precise role of AMPK in the dedifferentiation of cancerous somatic cells to stemness remains unresolved. However, recent work indicates that mitochondria are actively reprogrammed in cancer both morphologically (71) and functionally (64), in concert with nuclear-directed transitions (72–74), leading to the formation of cells with increased capacities to grow in suspension, migrate, proliferate, and initiate tumors. These are characteristics shared by primitive undifferentiated cells such as tumor initiating cells, iPSC, and embryonic stem cells (ESC).

Further, a study comparing the metabolism of iPSC, ESC, and cancer cells has found evidence in support of the idea that these cell types share striking metabolic commonalities (75). Both in cancer and in the reprogramming of somatic cells using defined factors, mitochondria progressively shut down ATP synthesis while the cell increases glycolytic activity. Taken together these data suggest that metabolic transition is a requirement for dedifferentiation, enabling reprogrammed cell survival, and the maintenance of stemness.

AMPK, a critical regulator of metabolic transitions is highly likely to play a critical role. In support of this, AMPK was found to be required to maintain cellular proliferation in astrocytic tumors (76), facilitate stem cell self-renewal by fomenting the glycolytic metabolism of pluripotent cells (77), and has been shown to be crucial for the maintenance of the metabolic viability of myc-activated cells (78,79). AMPK plays essential roles in activating glycolysis as reviewed above and inhibits mTOR whose activation favors senescence over pluripotency as reviewed elsewhere (80). Thus, it is likely that AMPK activation critically impacts reprogramming, and may directly regulate it. In support of this concept, an array of recent studies has placed AMPK as a central controller of many critically important regulators of reprogramming such as Notch (81) and Wnt pathways (82).

Conclusions

Why is the “tumor suppressor” model just not enough to understand the role of AMPK in cancer? First, many of the findings that led to the belief that AMPK is a tumor suppressor were actually focused on readouts of LKB1 deficiency and treatment with metformin or related drugs. LKB1 has many downstream targets including AMPK and as reviewed above, cancer cells may have evolved to distinguish signaling mediated by AMPK that is initiated by LKB1 or CAMKK β , minimizing the first and amplifying the latter. Metformin and related compounds most likely activate AMPK as a consequence of their mitochondrial effects. Therefore, such studies likely include the additive roles of many variables in addition to AMPK activation and must be interpreted with caution. Second, different cancers are exposed to different niches. The cells will have unique epigenetic signatures and active metabolic networks, and the effects of AMPK will depend heavily on this context. Modulating AMPK will lead to different outcomes as a result. Expanding on this point, as the tumor progresses, it provides a variety of microenvironments itself. It is likely that the role of AMPK and its impact on each of the cells within a tumor will be unique depending on availability of oxygen, nutrients, exposure to cytokines, or cell-cell interactions. Compounding this, within the tumor, degrees of differentiation exist. Some cells are terminally differentiated, some are plastic, some are primitive and a few are progenitors. AMPK will have a different role in the survival and maintenance of each of these related cell subtypes, and may be involved in preventing or directing differentiation of some cells (83,84), promoting

self-renewal and survival of stem-like cells (77), and potentially facilitating or restricting EMT, MET, and the reprogramming of others. Thus understanding the context-dependent effects of AMPK will be critical to define when and how AMPK manipulation can be therapeutically beneficial.

In summary, the waters remain uncharted when it comes to understanding the specific roles of AMPK in the many processes that critically impact tumor progression. In view of the variety and breadth of AMPK cellular actions it is not unexpected that controversy arises as a result of the incomplete knowledge about AMPK biochemistry and the sequence of events that promote each significant step in the complex process of malignant transformation. We urge caution in interpreting studies which rely solely on pharmacologic manipulation of AMPK. With the introduction of genetic models to manipulate AMPK in cell cultures and mouse models in which AMPK catalytic subunits $\alpha 1$ and $\alpha 2$ have been floxed new data will likely clarify many of the pending questions about the role of AMPK in cancer and in many other conditions. These will undoubtedly provide a revolution in our understanding of AMPK biology and signaling in the years to come and identify new therapeutic opportunities as a consequence.

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