Review

Molecular Cancer Research

Dissecting the Dual Role of AMPK in Cancer: From Experimental to Human Studies

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Abstract

The precise role of 5′AMP-activated kinase (AMPK) in cancer and its potential as a therapeutic target is controversial. Although it is well established that activation of this energy sensor inhibits the main anabolic processes that sustain cancer cell proliferation and growth, AMPK activation can confer on cancer cells the plasticity to survive under metabolic stress such as hypoxia and glucose deprivation, which are commonly observed in fast growing tumors. Thus, AMPK is referred to as both a "conditional" tumor suppressor and "contextual" oncogene. To add a further layer of complexity, AMPK activation in human cancer tissues and its correlation with tumor aggressiveness and progression appears to vary in different contexts. The current review discusses the different faces of this metabolic regulator, the therapeutic implications of its modulation, and provides an overview of the most relevant data available on AMPK activation and AMPK-activating drugs in human studies. *Mol Cancer Res; 13(7); 1059–72. ©2015 AACR.*

Introduction

5′ AMP-activated kinase (AMPK) is a central metabolic sensor that stands at the crossroad between metabolic and signaling networks. In 2003, the discovery of the tumor suppressor liver kinase B1 (LKB1) as the major upstream kinase of AMPK established a link between an energy regulator and cancer pathogenesis, suggesting that the tumor suppressor functions of LKB1 could be mediated by AMPK (1–3). Since then, *in vitro* and *in vivo* studies have been conducted to dissect the role of AMPK in cancer initiation and progression, using AMPK-modulating drugs. The functional consequences of AMPK activation in cancer appear to be much more complex than initially thought, and AMPK can behave as both cancer "friend" or "foe" in a context-specific manner.

Drug-induced supraphysiological activation of AMPK reduces tumor growth *in vitro* and in preclinical models through the suppression of key biosynthetic pathways (reviewed in refs. 4, 5). However, physiological activation of AMPK in response to a broad range of stresses (e.g., hypoxia, glucose deprivation, and matrix detachment) provides cancer cells with the flexibility to adapt and survive metabolic stress (metabolic adaptation; reviewed in ref. 6). IHC evaluation of AMPK status in human tissues has revealed that the levels of AMPK activation are heterogeneous in different tumor types, while discordant data have been reported on the correlation between AMPK activation and tumor prognosis.

Here, we discuss the "two faces" of AMPK, the therapeutic benefit of AMPK modulators and we review the current data available on AMPK activation and AMPK-activating drugs in human studies. Throughout the review, we associate AMPK with both the terms "tumor promoter" and "tumor suppressor." However, we do not intend to define AMPK as a classic *bona fide* tumor suppressor gene such as LKB1, which is mutated or deleted in several cancers, rather to emphasize the fact that AMPK activation may result in tumor growth inhibition, cell-cycle arrest, and apoptosis of cancer cells in some tumor types/contexts. Interrogating the cBioPortal data, the frequency of mutation/deletion in the genes codifying for AMPK catalytic subunits α1 (*PRKAA1*) and α2 (*PRKAA2*) ranges from 0.2% to 3.4% and from 0.2% to 10.3%, respectively (7).

AMPK: A Unique Metabolic "Guardian" with Pleiotropic Downstream Targets

AMPK is a heterotrimeric Ser/Thr kinase complex characterized by a catalytic α subunit and two regulatory subunits (β, γ), which exist in different isoforms, making up to 12 different heterotrimers. The different subunits show tissue specificity and may contribute to tumor cell growth and proliferation independently 10–8)). The γ subunit contains four-tandem sequence repeats known as CBS repeats, which functions as four adenine nucleotide-binding domains. Site 2 is always unoccupied, site 4 is permanently bound by AMP, whereas sites 1 and 3 can be competitively bound by either AMP, or ADP, or ATP (11, 12).

AMPK functions as an energy sensor to restore energy homeostasis at cell and organismal levels in conditions of metabolic stress that reduce ATP levels either by inhibiting its production (e.g., hypoxia, glucose deprivation, and treatment with biguanides drugs or xenobiotics) or by accelerating its consumption (e.g., muscle contraction), resulting in increased ADP and AMP levels. For a detailed description of AMPK regulation, we refer

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readers to other excellent reviews (13, 14). However, a brief description of the biochemical circuits regulating AMPK follows. The binding of ADP and/or AMP to the γ subunit both promotes phosphorylation by upstream kinases and inhibits dephosphorylation of the residue Thr172 within the activation loop of the catalytic domain, which is required for the full activity of the kinase. Furthermore, the binding of AMP (but not ADP) causes a further allosteric activation of the phosphorylated kinase. The two major upstream kinases responsible for AMPK activation are the tumor suppressor LKB1 and $Ca2^{+}$/calmodulin-dependent protein kinase kinase 2 (CaMKK2). An activating role, still not well characterized, for the transforming growth factor β-activated kinase 1 (TAK1) has also been described. LKB1 activates AMPK during energy stress, whereas CaMKK2 activity is induced by increased intracellular $Ca2^{+}$ levels, regardless of the energy status of the cells (reviewed in ref. 13). However, CaMKK2 can compensate for the absence of LKB1 in mediating AMPK phosphorylation (15). In addition to AMP, ADP, and $Ca2^{+}$, recent studies have also identified reactive oxygen species (ROS) as additional upstream activators of AMPK, acting in an LKB1-independent manner (Fig. 1; ref. 16). Once activated, AMPK maintains energy balance by switching off anabolic pathways that consume ATP and NADPH, while switching on catabolic pathways that generate ATP both by direct phosphorylation of metabolic enzymes, and through longer-term effects mediated by phosphorylation of transcription factors and coactivators (14). Thus, AMPK can restrain cell growth by (i) inhibiting protein synthesis [through direct phosphorylation of mammalian target of rapamycin complex 1 (mTORC1) signaling members tuberous sclerosis complex 2 (TSC2) and Raptor], (ii) blocking fatty acid (FA) and cholesterol biosynthesis [through direct phosphorylation of the enzymes acetyl-CoA carboxylase 1 (ACC1) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and inhibition of the lipogenic transcription factors sterol regulatory element-binding proteins (SREBP) and carbohydrate-responsive element-binding protein (ChREBP)], required for new membrane formation in proliferating cells, (iii) inducing cell-cycle arrest and apoptosis [through several mechanisms, including stabilization of p53, regulation of the cyclin-dependent kinase inhibitors $p21^{Waf1}$ and $p27^{Cip1}$, phosphorylation of the hippo signaling member angiomotin-like 1 (AMOTL1), an upstream inhibitor of Yes-associated protein (YAP); refs. 13, 17, 18], while promoting cell survival mechanisms during metabolic stress (19), as discussed below (Fig. 2).

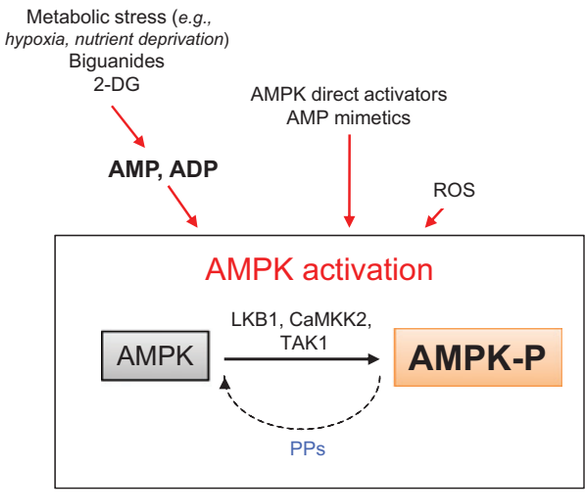

Figure 1.
Mechanisms of AMPK activation. AMPK functions as a metabolic sensor that is activated by metabolic stress induced by hypoxia, nutrient deprivation, and drugs/compounds [e.g., biguanides, 2-deoxyglucose (2-DG)], AMP mimetic, direct AMPK activators, or ROS. For the full activity of the kinase, a phosphorylation at the residue Thr172 in the catalytic loop is required. The main upstream kinases are LKB1, CaMKK2, and TAK1. Uncharacterized protein phosphates (PPs) can reverse this phosphorylation.

Role of AMPK in Cancer: Preclinical Studies

AMPK as a tumor suppressor

Because the role of LKB1 as tumor suppressor was well established, AMPK was primarily considered as a component of the LKB1-mediated tumor suppressor cascade and much less was known regarding its own independent role in cancer. This was due to the fact that most of the data were generated utilizing the AMPK activators AICAR and metformin, which also display AMPK-independent mechanisms or by experimental evidence in models of LKB1 inactivation, which affect an additional 12 AMPK-related downstream kinases, beyond AMPK. The role of the AMPK-related kinases is still not very well characterized, although they might themselves contribute to the tumor suppressive functions of LKB1, as well as have independent functions (20). Experiments of genetic ablation of AMPK, the use of direct

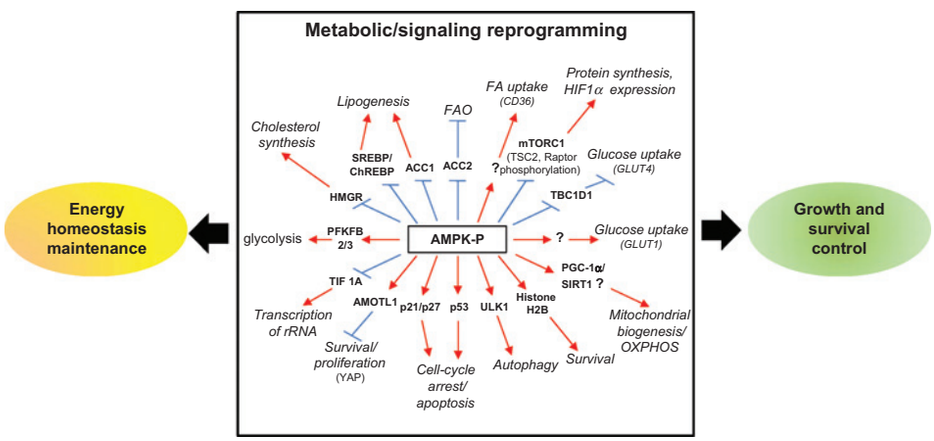

Figure 2.
AMPK-mediated metabolic and signaling reprogramming. Once activated, AMPK switches off anabolic pathways while turning on catabolic pathways to restore energy homeostasis. Thus, AMPK controls pathways involved in metabolism, cell growth, and survival. Red lines indicate direct activation, whereas inhibition is depicted in blue. A question mark indicates that it is not yet certain that the protein is directly phosphorylated. GLUT1/4, glucose transporter 1, 4; PFKFB2/3,6-phosphofructo-2-kinase/ fructose-2, 6-bisphosphatases 2 and 3; TBC1D1, TBC1 domain protein-1; SIRT1, sirtuin 1; PGC-1α, PPARγ-coactivator-1α.

AMPK activators, and detailed phosphorylation studies in different cancer models have recently helped to address this issue. Faubert and colleagues have reported that the ubiquitous knockout (KO) of AMPKα1, the only catalytic subunit expressed 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. The underpinning mechanism for AMPK tumor suppressor activity is the ability of the kinase to exert an "anti-Warburg" effect by downregulating hypoxia-inducible factor 1-alpha (HIF1α) and its downstream glycolytic genes, which, conversely, are upregulated in AMPKα1 KO mice (21).

Aside from antagonizing the Warburg effect, AMPK has also been shown to exert its "metabolic" tumor-suppressor role by inhibiting unchecked mTORC1 activity and *de novo* lipogenesis, required both during G_1–S and G_2–M phases. We have recently observed increased *de novo* FA synthesis concomitant to reduced AMPK activation and phosphorylation of its major target ACC1 (the rate-limiting enzyme for FA synthesis), prior to cytokinesis initiation. In this view, by inhibiting *de novo* FA synthesis and FA incorporation into membranes, activation of AMPK would prevent cells from completing mitosis, arresting them at a "lipogenic" G_2–M checkpoint. This was indeed observed under direct supraphysiological activation of AMPK (22). Cell-cycle arrest (via decreased fraction of cells in the S phase) and/or apoptosis was previously confirmed using ACC1 and fatty acid synthase (FASN) siRNA to directly inhibit FA synthesis (23, 24).

AMPK also plays a direct metabolic-independent role in cell-cycle regulation (25–27). A fine-tuned biphasic activation of AMPK has been shown to be required for proper mitotic progression (28). However, alteration of the dynamic spatial and temporal regulation of AMPK by either its sustained activation or depletion can result in microtubule misalignment, spindle misorientation, abnormal chromosome segregation followed by mitotic catastrophe and polyploidy (e.g., observed under metformin treatment) or mitotic delay (e.g., observed in AMPK-silenced cells; refs. 27, 29). Thus, cell-cycle arrest induced by persistent supraphysiological activation of AMPK could be ascribed to both the inhibition of *de novo* FA synthesis (metabolic role) as well as mitotic spindle assembly/chromosome segregation abnormalities (nonmetabolic role). Recently, a role for the subunit AMPK α1 in the direct regulation of cell cycle, independently of energy balance, has also emerged (30).

A third mechanism in favor of AMPK's behavior as a "tumor suppressor" has been described by Shen and colleagues, showing AMPK-dependent phosphorylation of the oncogene BRAF at Ser729. This phosphorylation prevents BRAF interaction with the scaffolding protein kinase suppressor of Ras 1 (KSR1), leading to the suppression of the oncogenic MEK–ERK signaling and consequent impairment of cell proliferation and cell-cycle progression (31).

Furthermore, additional mechanisms of action to suppress tumor growth have been proposed. Chou and colleagues showed that AMPK knockdown promotes epithelial-to-mesenchymal transition (EMT) in breast and prostate cancer cell lines by reducing the expression of forkhead box O3 (Foxo3a) and E-cadherin in conjunction with increased expression of vimentin, Y-box-binding protein-1 (YB-1), Snail, and the formation of F-actin stress fibers (32). These results suggested that AMPK activation counteracts EMT, the process through which epithelial cells are thought to acquire cancer stem cell–like properties and gain the ability to breach basement membranes and metastasize to distant sites. DeRan and colleagues showed that AMPK activation induces phosphorylation of the hippo signaling component AMOTL1, which results in the cytoplasmic sequestration and inhibition of YAP and its targeted genes, involved in proliferation and survival. This mechanism was abolished when AMPK expression was silenced, suggesting that loss of AMPK activity may contribute to tumorigenesis through AMOTL1 destabilization, leading to hyperactivation of YAP (18). Finally, AMPK may be inactivated by its ubiquitination and degradation by the cancer-specific MAGE-A3/6-TRIM28 ubiquitin ligase. MAGE-A3 and MAGE-A6 proteins, normally expressed only in the male germline, are frequently reactivated in human cancers, they are necessary for cancer cell viability, and sufficient to induce cell transformation. Screening for targets of the MAGE-A3/6–TRIM28 complex revealed that it ubiquitinates and degrades AMPKα1, leading to inhibition of autophagy, activation of mTORC1 signaling, and hypersensitization to AMPK agonists, such as metformin. These findings elucidated a germline mechanism commonly hijacked in cancer to suppress AMPK (33).

Further evidence also supports the tumor suppressor role of AMPK in some tumor types and genetic contexts. First, protein kinase B (Akt) has been reported to induce AMPK phosphorylation at Ser485, reducing its activation by LKB1 (34). This might occur in tumors in which Akt is hyperactivated due to phosphatase and tensin homolog (PTEN) loss-of-function mutations, or activating mutations in phosphoinositide-3-kinase (PI3K). Second, AMPK activation is suppressed in melanoma cells carrying the most common BRAF mutation V600E, which induces a constitutively active downstream ERK. The lack of AMPK activity is due to ERK and ribosomal S6 kinase (RSK)-mediated phosphorylation of LKB1, which prevents its binding/activation of AMPK. These data suggested that suppression of the LKB1/AMPK pathway might play an important role in $BRAF^{V600E}$-driven tumorigenesis (35). Third, inhibition of AMPK has been observed in a PTEN-deficient model of thyroid cancer and in non–small cell lung cancer (NSCLC) cells expressing the mitochondrial heat-shock protein 90 chaperone TRAP-1 (36). Fourth, in fumarate hydratase-deficient kidney tumors and cell lines from patients with hereditary leiomyomatosis renal cell cancer (HLRCC), which are characterized by a metabolic shift to aerobic glycolysis, AMPK levels are decreased. AMPK reduction leads to diminished expression of the DMT1 iron transporter, cytosolic iron deficiency, and activation of the iron regulatory proteins IRP1 and IRP2, resulting in increased expression of HIF1α. Silencing of HIF1α or activation of AMPK diminishes invasive activities of the HLRCC cell line UOK262, indicating that overexpression of HIF1α and downregulation of AMPK contribute to the oncogenic growth of fumarate hydratase-deficient cells (37). Recently, a study from Rodriguez and colleagues showed that cytochrome P450-1A1, constitutively expressed in the majority of breast cancer tumors, promotes breast cancer proliferation and survival, at least in part, through suppression of AMPK signaling (38). Finally, reduced expression of the catalytic α2 subunit has been reported in some cases of hepatocellular carcinomas, and it is associated with enhanced tumor cell growth in mouse xenografts (10).

Taken together, these results suggest that in specific genetic, metabolic, and signaling contexts, AMPK can exert a tumor suppressor role (Fig. 3).

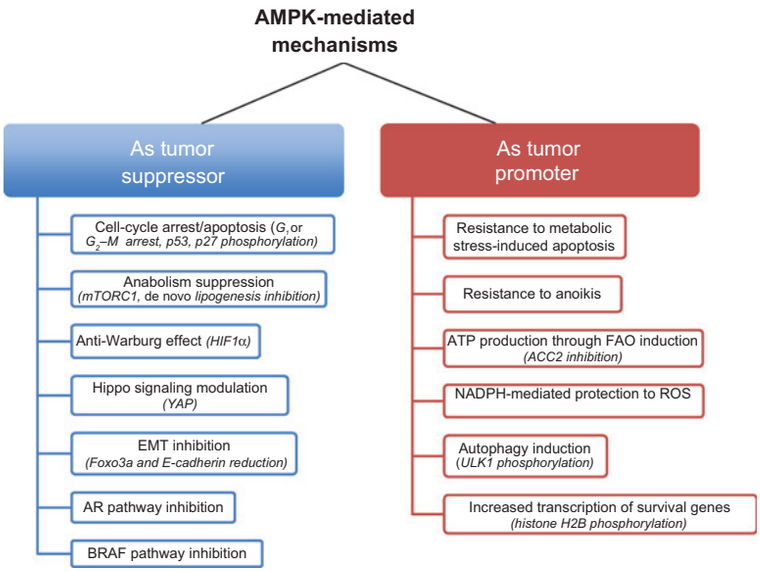

Figure 3.
Main mechanisms through which AMPK can exert its double-faced role in cancer. AMPK activation triggers cellular processes that can both suppress and promote tumor development/progression by activating different downstream pathways in a context-specific manner. NADPH, reduced form of nicotinamide adenine dinucleotide phosphate.

AMPK as contextual tumor promoter

The ability to survive in conditions of metabolic stress, such as hypoxia/nutrient deprivation, or matrix detachment is fundamental to cancer cells. Several mechanisms by which the AMPK pathway supports this plasticity have been described. These include: (i) the induction of autophagy by AMPK-dependent phosphorylation of the unc-51-like kinases (ULK; ref. 39), (ii) the promotion of FA oxidation (FAO) to generate ATP (40, 41), (iii) transcriptional changes induced by phosphorylation of the core histone H2B (42), and (iv) the increase of intracellular NADPH levels through the activation of FAO/inhibition of FA synthesis to neutralize cytotoxic ROS (Fig. 3; 43). Intriguingly, while in nutrient-replete conditions, the AMPK energy-sensing pathway and the PI3K/Akt cascade converge on mTOR with opposing regulatory effects, under glucose depletion, both AMPK and Akt are activated and coordinately support cell survival (44). Thus, whereas the LKB1/AMPK pathway can act as a tumor suppressor through its ability to restrain tumor growth, it can also behave as "tumor promoter," allowing tumor cells to be more resistant to metabolic stress, such as when tumor growth exceeds the capacity of its blood supply to deliver oxygen and nutrients (Fig. 4). Recent experimental evidence *in vitro*, using the direct AMPK activator A-769662, indeed supports this notion (45). AMPK activation can also promote tumor growth in specific tumor types and genetic contexts, even in nutrient-replete conditions.

Recent evidence showed the key role of AMPK in supporting tumor growth in aggressive breast and astrocytic tumors (46–49). Moreover, in contrast to the results obtained by Faubert and colleagues in a lymphoma model (21), MYC has been shown to establish a dependence on AMPK-related kinase 5 (ARK5) to maintain metabolic homeostasis and cell survival. Depletion of ARK5 prolongs survival in MYC-driven mouse models of hepatocellular carcinoma, suggesting that targeting cellular energy homeostasis is a valid therapeutic strategy to eliminate tumor cells that express deregulated MYC (50).

The therapeutic benefit of AMPK modulators: the metformin paradox

The better understanding of the dichotomous role of AMPK in cancer has also brought about the careful reevaluation of the use of AMPK modulators in cancer therapy. In this regard, the case of metformin is emblematic.

The interest in using AMPK activators began as evidence was accumulating for the antitumorigenic role of the LKB1/AMPK axis. The antiproliferative and growth-suppressing effects of supraphysiological activation of AMPK have been shown *in vitro* and in preclinical models. Activation was achieved with natural compounds, the AMP mimetic drug AICAR as well as the biguanides metformin and phenformin, which inhibit complex I of the mitochondrial electron transport chain, leading to increased

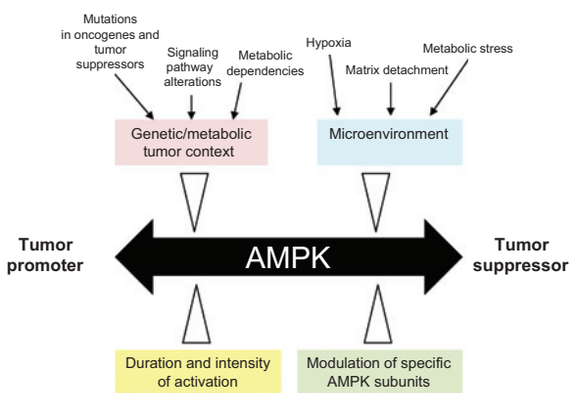

Figure 4.
AMPK functions as "conditional" tumor suppressor and "contextual" tumor promoter. The outcome of AMPK activation in cancer is affected by the genetic context, metabolic dependency of cancer cells, and the surrounding microenvironment. Differences in the intensity/duration of AMPK activation (e.g., physiological activation vs. drug-induced supraphysiological activation) as well as in the expression/activation of specific subunits of the heterotrimer contribute to the antitumorigenic vs. protumorigenic role of AMPK in different cancer types.

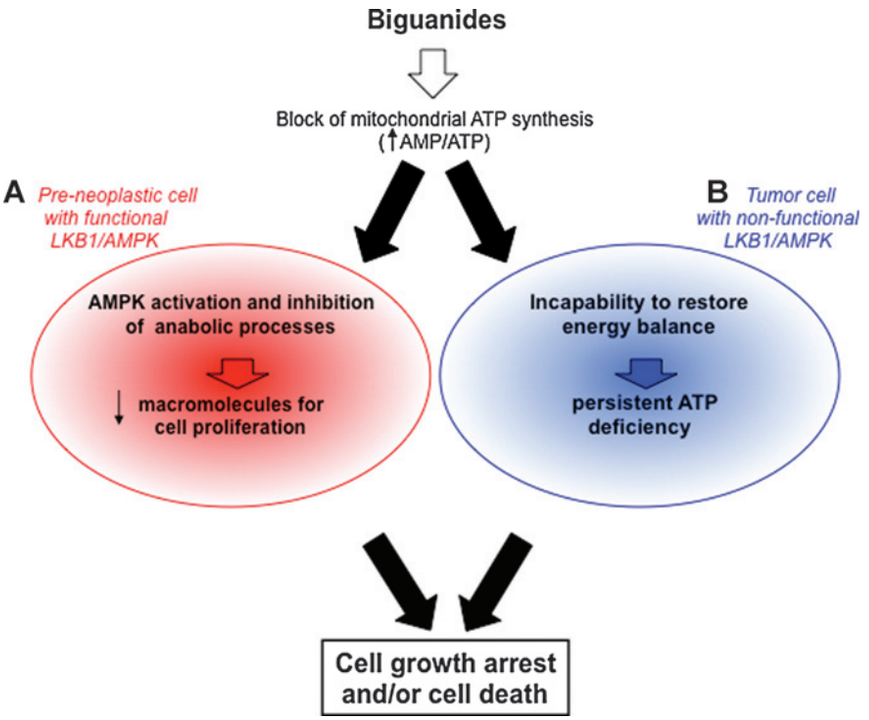

Figure 5.
Mechanisms by which biguanides are therapeutically beneficial in LKB1-positive and -negative tumors. A, metformin or phenformin activates AMPK in preneoplastic cells with the functional LKB1/AMPK pathway, restraining their growth and proliferation and thus delaying the onset of tumorigenesis; B, cancer cells, in which the LKB1–AMPK pathway is not functional, cannot restore biguanides-induced energy stress and they are more sensitive to cell death (biguanide paradox).

levels of intracellular ADP, AMP, and energy stress (reviewed in refs. 4, 14, 51). Metformin has received particular attention, because it is a safe medication, used as first choice in the treatment of type II diabetes, and has been associated with reduced cancer incidence in diabetic patients (52). Thus, it is currently being tested for cancer treatment/prevention in several clinical trials, as discussed below. However, ascribing metformin's antitumor properties *in vivo* to AMPK activation has been criticized because the major effect of the drug is the inhibition of hepatic gluconeogenesis, resulting in reduced circulating levels of glucose and insulin, two well-known promoters of tumor cell proliferation. This is also valid for metformin's antitumor effects *in vitro*, where several AMPK-independent mechanisms have been described (45, 53–56). Moreover, the discovery of the so-called "biguanide paradox" has recently suggested that, in specific contexts, metformin-mediated suppression of tumor growth does not depend on AMPK activation but, rather, on its downregulation. Because cells with a defective LKB1/AMPK pathway are less able to restore ATP levels in response to metabolic stress induced by metformin treatment, LKB1/AMPK-deficient cancer cells are more susceptible to cell death than their counterparts with a functional LKB1/AMPK axis (Fig. 5). Several *in vitro* and *in vivo* studies using metformin, phenformin, or other compounds that cause metabolic stress (AICAR, salicylate, and 2-deoxyglucose) have supported this mechanism (discussed in ref. 57, 58). In light of this, the use of biguanides may be most effective in combination with agents that inhibit, rather than activate, AMPK and, overall, these data suggest that the use of AMPK inhibitors rather than activators would preferentially trigger cancer cell death in the context of metabolic stress. Interestingly, the chemotherapeutic agent sunitinib has been shown to inhibit AMPK, suggesting that combinatorial treatment of sunitinib and metformin could be clinically relevant (59).

Novel direct AMPK activators have been developed to overcome the off-target effects of metformin and AICAR treatment. The direct activator A-769662 (which binds the β1 subunit) delays tumor formation in PTEN-null/LKB1 hypomorphic mice (60). The same compound has been shown to suppress the proliferation of breast, colon, and prostate cancer cells (61–63). A-769662 was, however, ineffective in models of glioma (56). OSU-53, a direct activator that binds the autoinhibitory domain of AMPK, displays tumor growth inhibition *in vitro* and *in vivo* in triple-negative breast cancer models (64). The same group reported that AMPK activation by OSU-53 blocks "EMT" in breast and prostate cancer cells by activating Foxo3a, which results in the inhibition of invasive phenotypes *in vitro* and metastatic properties *in vivo* (32). Direct supraphysiological activation of AMPK in nutrient-replete conditions has also been shown to suppress prostate cancer cells growth, in association with mitotic arrest and apoptosis, and to potentiate the effect of antiandrogens *in vitro* (65). The inhibitory effect of AMPK activation on the androgen receptor (AR) axis at both transcriptional and posttranslational levels was previously observed when a supraphysiological activation of AMPK was achieved by treatment with metformin or AICAR (66, 67). Finally, Compound 1, a novel AMPK activator, induces a significant antitumor activity *in vitro* and tumor growth delay in a mouse xenograft model of colorectal cancer (68). The mechanism through which Compound 1 activates AMPK is, however, still uncharacterized.

Taken together, the induction of a persistent, supraphysiological activation of AMPK results in tumor suppression in some cancer types (Fig. 3).

Salicylate, the active metabolite of aspirin following absorption from the gut, was recently identified as a direct AMPK activator, which binds to the same site on the β1 subunit as A-769662 (69). This suggests that AMPK activation might be involved in mediating aspirin's protective effects against cancer. Future preclinical studies in genetically engineered AMPK models are however required to validate this hypothesis.

Overall, these apparently conflicting data suggest that both AMPK activators and inhibitors can provide therapeutic benefit in different tumor types, different genetic/metabolic contexts, and different microenvironment conditions. Thus, the choice of AMPK modulators may be different at various phases of tumorigenesis/tumor progression.

AMPK Role in Cancer: Human Studies

AMPK activation in human cancers

Evaluation of AMPK activation in human tissues is not trivial. Early studies have demonstrated that when tissues and organs are removed by dissection at ambient temperature rather than by freeze clamping, ACC phosphorylation occurred as a postmortem artifact. Dissection at ambient temperature leads to elevation of AMP and depletion of ATP, presumably due to hypoxia following interruption of the blood supply, resulting in AMPK activation. Moreover, ACC phosphorylation in tissues such as liver has also been shown to follow a diurnal rhythm and to be influenced by dietary behavior (70). Therefore, analysis of AMPK activity and ACC phosphorylation in human tissues should be interpreted with caution.

AMPK activation has been investigated in fresh frozen and archival tumor tissue from numerous cancer sites, including prostate (63, 71, 72), breast (73, 74), head and neck (75), colorectal (76, 77), gastric (78, 79), liver (80), lung (81–83), ovary (84), and kidney (85, 86). Table 1 summarizes the population-based studies of AMPK activation, measured by protein expression of phosphorylated AMPKα1 (p-AMPKα1, $n = 16$ studies) or its phosphorylated substrate ACC (p-ACC, $n = 6$ studies), with cancer prognosis and clinicopathologic features. Of the 13 studies reporting on p-AMPKα1 at Thr172 and overall, cancer-specific, or progression-free survival (PFS), 8 studies found that AMPK activation was associated with improved prognosis among head and neck (75), colorectal (76, 77), gastric (79), liver (80), lung (81), and kidney (85, 86) cancer patients either within the entire study population or within subgroups. Consistent with the findings for p-AMPKα1 at Thr172, one additional study of lung cancer found that higher expression of p-AMPKα1 at Ser485, which inhibits AMPK signaling (14), was associated with shorter survival (82). Conversely, two studies in gastric cancer (78) and in prostate cancer (72) reported associations between higher p-AMPKα1 and disease recurrence; however, the gastric cancer study population was substantially smaller than that of Kim and colleagues (79). Three additional studies in lung (83) and breast cancer patients (73, 74) found no association between p-AMPKα1 expression and overall survival (OS). In cross-sectional analyses, higher p-AMPKα1 expression was associated with lower tumor grade and/or stage in breast (73), head and neck (75), colorectal (76), gastric (79), liver (80), and ovarian (84) cancer, while 4 additional studies in prostate (72), breast (74), gastric (78), and lung (81) cancer found no associations with clinicopathologic features. In contrast, Choudhury and colleagues found increasing p-AMPKα1 expression with higher tumor grade in prostate cancer specimens (63). Overall, these human studies support the hypothesis that AMPK activation may delay disease progression in several cancer types.

Of the 6 studies that used protein expression of p-ACC at Ser79 to characterize AMPK activation, higher p-ACC was associated with worse OS (82) and disease recurrence (83) among lung cancer patients, and with worse OS among head and neck cancer (75) and kidney cancer (86) patients. In contrast, higher p-ACC was associated with improved OS and PFS in colorectal cancer patients (77). Lastly, no correlation was observed between p-ACC expression and Gleason grade in prostate tumors (71). A better understanding of the effects of ACC inactivation and its downstream targets in different tumor tissues will help elucidate the complex role of AMPK activation in carcinogenesis.

Tumor expression of specific AMPK α, β, and γ subunits in relation to cancer outcomes has been explored in patients with melanoma (87), kidney cancer (85, 86), breast cancer (74), cervical cancer (88), lymphoma (89), ovarian cancer (84, 90, 91), lung cancer (82), and colorectal cancer (92). Total AMPKα1 protein expression, which captures both phosphorylated and nonphosphorylated AMPKα1, was associated with improved overall and disease-specific survival among 128 melanoma patients (87). Total AMPKα1/α2 protein expression was associated with improved PFS ($P = 0.04$) and borderline associated with OS ($P = 0.06$) in 37 renal cell carcinoma patients (85). Using publicly available data from The Cancer Genome Atlas (TCGA), overexpression of the genes encoding for AMPKα1, α2, β1, β2, and γ1 subunits was also associated with improved OS ($P \leq 0.05$) in 417 clear-cell renal cell carcinoma patients (86). In a discovery ($n = 166$) and validation ($n = 609$) cohort of breast cancer patients, total AMPKα expression was associated with longer relapse-free ($P = 0.016$ and $P = 0.06$, respectively) and breast cancer-specific ($P < 0.001$ and $P = 0.005$, respectively) survival (74). Using fluorescence *in situ* hybridization, amplification of the gene encoding AMPKα1 was not significantly associated with lymph node positivity ($P = 0.085$) in pretreatment cervical biopsies among 31 cervical cancer patients (88). Using the Oncomine database, Hoffman and colleagues reported an association between higher expression of the genes encoding the regulatory AMPKβ1 and β2 subunits and increased 5-year survival ($P = 0.001$ and 0.021, respectively) among diffuse large B-cell lymphoma patients; marginal associations were found for higher expression of the gene encoding AMPKα1 and improved survival ($P = 0.0751$), and higher expression of the gene encoding AMPKγ3 and worse survival ($P = 0.0646$; ref. 89). Similarly, in a series of 70 ovarian cancer patients, higher protein expression of p-AMPKβ1 at Ser182 was associated with lower tumor grade ($n = 70$, $P = 0.009$) and improved OS in the subgroup of patients with serous subtype ($n = 46$, $P = 0.037$) and advanced-stage disease ($n = 54$, $P = 0.0016$; ref. 90). Phosphorylation of AMPKβ1 at Ser182 has not been shown to affect the kinase activity, but is associated with nuclear localization (93). Another study of total AMPKβ1 in ovarian cancer also found that higher protein expression was associated with early tumor stage ($P = 0.008$), lower tumor grade ($P = 0.013$), and absence of metastasis ($P = 0.008$; ref. 84). This same research group previously demonstrated that higher expression of the gene encoding AMPKα2, measured by quantitative PCR, was associated with improved overall ($P = 0.030$) and disease-free ($P = 0.014$) survival in a hospital-based series of 76 ovarian cancer patients, although gene expression of the α1, β1, β2, γ1, and γ2 subunits was not associated with outcomes (91). Zupa and colleagues, in addition to the findings for p-AMPKα1 and p-ACC listed in Table 1, reported an association between higher protein expression of p-AMPKβ1 at Ser108, indicative of AMPK activation (93), and short-term versus long-term survival ($P = 0.0286$) among 28 pathologic stage N0 NSCLC patients (82). Lastly, Vetvik and colleagues found that tumor expression of the gene encoding AMPKβ1 was positively

Table 1. Population-based studies of AMPK activation in tumor tissue, clinicopathologic features, and prognosis

Author, year (ref.)	Cancer site	Country	Population	Age range, y	Time period of diagnosis	Cases, *n*	Median follow-up, y	Antibody used for AMPK activation; method	Main findings[a]		
									Overall, cancer-specific, and PFS	Tumor grade and stage	Other clinicopathologic features
Park, 2009 (71)	Prostate	USA	Patients with paraffin-embedded arrayed prostate cancer specimens	NS	NS	244	NA	p-ACC (Ser79, Cell Signaling Technology); IHC		No association of p-ACC with Gleason grade (data not shown).	
Tennakoon, 2013 (72)	Prostate	USA	Patients with archival tissue collected from radical prostatectomy	NS	NS	61	NS	p-AMPKα (Thr172; Santa Cruz Biotechnology); IHC	Higher p-AMPK associated with biochemical recurrence ($P = 0.017$).	No association of p-AMPK with Gleason score or disease stage at time of surgery.	
Choudhury, 2014 (63)	Prostate	UK	Patients with paraffin-embedded arrayed prostate cancer specimens	NS	NS	213	NA	p-AMPKα (Thr172); IHC		Higher p-AMPK associated with higher Gleason score ($P = 0.0251$).	
Hadad, 2009 (73)	Breast	Scotland	1. Patients enrolled in the Adjuvant Breast Cancer (ABC) clinical trial	34–76	1992–2000	117	6.1	p-AMPKα (Thr172; Cell Signaling Technology); IHC	No association of p-AMPK with OS (data not shown).	Higher p-AMPK associated with lower histological grade ($P = 0.010$ and 0.021 for cohorts 1 and 2, respectively).	Higher p-AMPK associated with fewer positive axillary nodes ($P = 0.021$ and 0.087 for cohorts 1 and 2, respectively). No association with tumor size (data not shown).
			2. Patients with primary, previously untreated breast cancer from Tayside University Hospitals	28–89	1997–2002	237	5.0				
Zhang, 2014 (74)	Breast	UK	1. Discovery cohort	31–70	1998–2006	166	9.0	p-AMPKα (Thr172; Cell Signaling Technology); IHC	No association of p-AMPK with OS (data not shown).	No association of p-AMPK with tumor grade or stage (data not shown).	No association of p-AMPK with tumor size or lymph node status (data not shown).
			2. Validation cohort	18–72	1986–1998	609	11.2				
			Both comprised of stage I–III invasive breast cancers from patients treated by wide local excision and radiotherapy								
Su, 2014 (75)	Head and neck	Taiwan	Patients with surgically resected squamous cell carcinoma of the head and neck	30–89	1998–2010	118	up to 2012	p-AMPKα (Thr172; Cell Signaling Technology); IHC	Higher p-AMPK associated with improved OS in univariate ($P = 0.018$), but not multivariate ($P = 0.188$), analyses.	Higher p-AMPK associated with lower T stage ($P = 0.020$). No association of p-AMPK with tumor differentiation ($P = 0.200$).	No association of p-AMPK with surgical margin status ($P = 0.253$) or lymph node status ($P = 0.369$).
								p-ACC (Ser79; Cell Signaling Technology); IHC	Higher p-ACC associated with worse OS in univariate ($P = 0.021$) and multivariate ($P = 0.018$) analyses.		
Baba, 2010 (76)	Colorectal	USA	Incident cases in Nurses' Health Study and Health Professionals Follow-up Study	≤59 (20%); 60–69 (42%); ≥70 (38%)	1976–2004	718	10.8	p-AMPKα (Thr172; Cell Signaling Technology); IHC	No association of p-AMPK with cancer-specific survival ($P = 0.56$) in all patients combined. Higher p-AMPK associated with improved cancer-specific survival among P-MAPK3/1–positive ($P = 0.0006$), but not P-MAPK3/1–negative ($P = 0.45$), patients.	Higher p-AMPK associated with lower tumor grade ($P = 0.0009$). No association of p-AMPK with tumor stage ($P = 0.16$).	No association of p-AMPK with tumor border ($P = 0.80$).
Zulato, 2014 (77)	Colorectal	Italy	Patients with metastatic colorectal cancer treated with FOLFIRI-bevacizumab	28–74	2007–2011	48	2.0	p-AMPKα (Thr172; Cell Signaling Technology); IHC	Higher p-AMPK associated with improved OS ($P = 0.0002$). No association with PFS ($P = 0.231$).		
								p-ACC (Ser79; Cell Signaling Technology); IHC	Higher p-ACC associated with improved OS ($P = 0.0007$) and improved PFS ($P = 0.011$).		
Kang, 2012 (78)	Gastric	South Korea	Patients receiving a combination regimen of cisplatin and S-1	22–71	2006–2010	73	2.2	p-AMPKα (Cell Signaling Technology); IHC	Higher p-AMPK associated with worse relapse-free survival ($P = 0.022$). No association with OS ($P = 0.102$).	No association of p-AMPK and overall pathologic stage ($P = 0.955$), T stage ($P = 0.708$), N stage ($P = 0.807$), or histology ($P = 0.142$).	No association of p-AMPK with tumor size ($P = 0.600$)

(Continued on the following page)

Table 1. Population-based studies of AMPK activation in tumor tissue, clinicopathologic features, and prognosis (Cont'd)

Author, year (ref.)	Cancer site	Country	Population	Age range, y	Time period of diagnosis	Cases, *n*	Median follow-up, y	Antibody used for AMPK activation; method	Main findings[a]		
									Overall, cancer-specific, and PFS	Tumor grade and stage	Other clinicopathologic features
Kim, 2013 (79)	Gastric	South Korea	Patients who underwent surgical gastrectomy	24–85	2003–2006	621	Up to 10 years	p-AMPKα (Thr172; Cell Signaling Technology); IHC	Higher p-AMPK associated with improved OS ($P = 0.024$) and disease-free survival ($P = 0.030$).	Higher p-AMPK associated with lower tumor stage ($P = 0.000$).	Higher p-AMPK associated with absence of lymph node metastasis ($P = 0.000$).
Zheng, 2013 (80)	Liver	China	Patients who underwent radical resection	<50 (56%); ≥50 (44%)	2005–2009	273	2.7	p-AMPKα (Thr172; Cell Signaling Technology); IHC	Higher p-AMPK associated with improved OS ($P = 0.00029$) and longer time to recurrence ($P = 0.00071$).	Higher p-AMPK associated with lower pathologic tumor stage (0.00014) and lower Edmondson grade (0.00324).	Higher p-AMPK associated with complete tumor encapsulation ($P = 0.00235$) and absence of distant metastasis ($P = 0.00281$). No association of p-AMPK with tumor size ($P = 0.775$) or multiplicity ($P = 0.0932$).
William, 2011 (81)	Lung	USA	Patients who underwent surgical resection for NSCLC	32–90	1997–2005	463	4.1	p-AMPKα (Thr172; Cell Signaling Technology); IHC	Higher p-AMPK associated with improved OS ($P = 0.0009$) and recurrence-free survival ($P = 0.0007$) in all patients, and in patients with adenocarcinoma ($P = 0.0001$ and 0.001, respectively). No association of p-AMPK with OS ($P = 0.35$) or recurrence-free survival ($P = 0.11$) in patients with squamous cell carcinoma.	No association of p-AMPK with overall pathologic stage ($P = 0.45$), T stage ($P = 0.61$), or N stage ($P = 0.66$).	
Zupa, 2012 (82)	Lung	Italy	Patients who underwent surgical resection for NSCLC	43–83	1993–2005	47	NS	p-AMPK α1 (Ser485; Cell Signaling Technology); RPPA	Higher p-AMPK α1 at Ser485 (prevents AMPK activation) associated with worse OS ($P = 0.0041$) among 28 pathologic stage N0 patients.		
								p-ACC (Ser79; Cell Signaling Technology); RPPA	Higher p-ACC associated with worse OS ($P = 0.0256$) among 28 pathologic stage N0 patients.		
Nanjundan, 2010 (83)	Lung	USA	Patients who underwent surgical resection for NSCLC	48–81	NS	46	NS	p-AMPKα (Thr172; Cell Signaling Technology); RPPA	No association of p-AMPK with recurrence or survival (data not shown).		
								p-ACC (Ser79; Cell Signaling Technology); RPPA	Higher p-ACC associated with disease recurrence ($P = 0.010$). No association with survival (data not shown).		
Li, 2014 (84)	Ovary	USA	Patients included on a commercially available ovarian cancer tissue array (OVC1021, Pantomics Inc.)	NS	NS	97	NA	p-AMPKα (Thr172; Cell Signaling Technology); IHC		Higher p-AMPK associated with lower tumor stage (data not shown).	
Tsavachidou-Fenner, 2010 (85)	Kidney	USA	Patients with metastatic renal cell carcinoma who underwent nephrectomy, post-treatment with bevacizumab—erlotinib or bevacizumab alone	Median: 61	NS	37	NS	p-AMPKα (Thr172; Cell Signaling Technology)[b]; RPPA	Higher p-AMPK associated with improved OS ($P = 0.0003$).		
Cancer Genome Atlas Research Network, 2013 (86)	Kidney	USA	Clear-cell renal cell carcinoma patients included in the publicly available TCGA database	NS	NS	411	Up to 10 years	p-AMPKα (Thr172); RPPA	Higher p-AMPK associated with improved OS ($P < 0.0001$).		
								p-ACC (Ser79); RPPA	Higher p-ACC associated with worse OS ($P < 0.01$).		

Abbreviations: NA, not applicable; NS, not specified; p-ACC, phosphorylated *acetyl-CoA carboxylase*; p-AMPK, phosphorylated AMP-activated protein kinase; *P-MAPK3/1*, extracellular signal-regulated kinase (ERK)1/2; RPPA, reverse-phase protein array.

[a]Color code: green, improved survival or favorable clinical features associated with AMPK activation; red, worse survival or unfavorable clinical features associated with AMPK activation; gray, null results.

[b]Personal communication.

correlated with advanced tumor stage, but not with the number of affected lymph nodes, in specimens from 60 colorectal cancer patients (92).

With the exception of Zupa and colleagues and Vetvik and colleagues, these studies suggest that higher tumor expression of specific AMPK subunits may be related to favorable clinicopathologic features and improved outcomes among cancer patients. Additional studies are warranted to confirm these findings in larger study populations and across cancer sites.

Differential expression of AMPK/ACC in tumor versus normal tissue has been reported in a few neoplasms, including liver (80), ovarian (90, 91), thyroid (94), cervical (95), brain (47), skin (87), prostate (63, 71, 72, 96), and colorectal cancer (92). In hepatocellular carcinoma, protein expression of p-AMPKα1 at Thr172 was downregulated in 62% of tumor versus distant normal liver tissue (80). In ovarian specimens, protein expression of p-AMPKβ1 at Ser182 was significantly higher ($P = 0.038$) in carcinoma compared with borderline tumors and normal ovaries (90). Li and colleagues also found higher expression of the genes encoding AMPKα2, β1, β2, γ1, and γ2 ($P \leq 0.001$), but not AMPKα1 ($P = 0.320$), in primary cancer versus normal ovarian tissue (91). In papillary thyroid carcinoma patients, protein expression of total AMPKα, p-AMPKα1 at Thr172, and p-ACC at Ser79 was elevated ($P < 0.001$) in carcinoma versus paired nonneoplastic tissue (94). Similarly, protein expression of AMPKα1 was significantly higher ($P < 0.001$) in tumor versus normal epithelium in cervical cancer patients (95). In a small study of brain cancer, high protein expression of p-ACC at Ser79 was seen in all glioblastoma specimens compared with the absence of expression in normal brain (47). In melanoma patients, total AMPKα1 protein expression was increased in primary melanoma versus dysplastic nevi ($P < 0.005$), but slightly decreased in metastatic versus primary melanoma specimens ($P < 0.05$) (87). In prostate cancer patients, both p-AMPKα1 at Thr172 and p-ACC at Ser79 were expressed in tumor tissue, compared with no detectable expression in nonpaired benign prostatic hyperplasia samples (63). Two additional prostate studies reported elevated expression of p-AMPKα1 at Thr172 and p-ACC at Ser79 ($P < 0.001$) in prostate tumor versus nonneoplastic tissue (71, 72). Utilizing the Oncomine database, the gene encoding AMPKβ1 was expressed at greater levels in metastatic versus primary prostate cancer in publicly available data from 4 studies (96). Lastly, expression of the gene encoding AMPKβ1 was significantly higher in colorectal cancer versus adjacent mucosa (92). Taken together, these studies support that AMPK dysregulation contributes to neoplastic transformation.

In summary, AMPK expression/activation varies by tumor stage and histology, clinical outcomes, and tissue type (normal, tumor, and metastatic). Most of the studies in tumor tissue support a role of AMPK activation, measured by phosphorylation at Thr172, in delaying tumor progression. However, comparing tumor with nonneoplastic tissue suggests that AMPK may be involved in tumor initiation. Thus, evidence from human studies also underscores the dual role of AMPK in carcinogenesis.

AMPK-activating drugs in humans: metformin, phenformin, and aspirin

Several review articles and meta-analyses on metformin and cancer risk have been published in recent years. A 2012 meta-analysis of randomized controlled trials among participants with or at risk of type 2 diabetes did not find reduced cancer incidence for treatment with metformin versus placebo/usual care or active comparators [$n = 9$ studies; summary relative risk (RR), 1.02; 95% confidence interval (CI), 0.82–1.26; ref. 97]. Meta-analyses of observational studies among diabetics have shown a reduced risk of cancer associated with metformin use: the fixed-effect summary RRs (95% CI) were 0.70 (0.67–0.73) for 9 cohort studies (98), 0.90 (0.84–0.98) for 13 case–control studies (98), and 0.73 (0.61–0.88) for 21 cohort and case–control studies combined (99). However, both meta-analyses exhibited significant between-study heterogeneity, with Thakkar and colleagues reporting random-effects model estimates that were attenuated (summary RR, 0.85; 95% CI, 0.65–1.11) among cohort studies, but retained significance (summary RR, 0.71; 95% CI, 0.57–0.88) among case–control studies (98). Inconsistent results may be due to variations in metformin dose, duration of metformin use, length of follow-up, type of comparison group (diabetics taking nonmetformin antidiabetic medications, diabetics on alternative therapy, or nondiabetics), outcome assessed (incident cancer or cancer mortality as a surrogate), variation by cancer site, systematic biases, or confounding. Of particular concern are potential time-related biases that may arise when evaluating metformin and cancer risk (100). A recent meta-analysis of observational and randomized studies attempted to account for major biases and confounders, still finding a significant, though attenuated, reduction in cancer incidence among studies without time-related biases ($n = 8$ studies; summary RR, 0.90; 95% CI, 0.89–0.91) and among studies adjusted for body mass index ($n = 11$ studies; summary RR, 0.82; 95% CI, 0.70–0.96; ref. 101). Observational studies published after these meta-analyses have been either consistent with reduced cancer risk (102, 103) or null (104–106). Overall, the literature suggests that metformin either reduces or has no effect on cancer risk, though very few studies have addressed metformin use in the nondiabetic population. Future clinical trials of metformin therapy in the general population should provide vital data on the potential use of metformin as a chemopreventive agent.

Metformin use may also influence disease progression after a cancer diagnosis. In observational studies, metformin has been associated with a decreased risk of disease recurrence, overall mortality, or cancer-specific mortality in patient cohorts of prostate cancer (107, 108), multiple myeloma (109), liver cancer (110), ovarian/endometrial cancer (110–112), bladder cancer (113, 114), and breast cancer (115, 116). Two additional studies of prostate cancer patients who underwent radical prostatectomy found no significant associations between metformin use and time to biochemical recurrence or longer-term outcomes (117, 118). Two additional studies of breast cancer patients were null for metformin use and overall or cancer-specific survival (119, 120). Numerous clinical trials of metformin as an adjuvant therapy to cancer treatment are under way, as indicated on ClinicalTrials.gov. Combined with the observational data, these new clinical trials will shed light on the potential therapeutic role of metformin in cancer survivors.

In addition, a limited number of "window-of-opportunity" (i.e., phase 0) trials have been conducted to evaluate metformin administration in the time window between cancer diagnosis and surgery. These studies show mixed results for tumor p-AMPKα at Thr172 expression before and after metformin use (range, 850–2250 mg/day): p-AMPKα protein expression was increased in

1 study of endometrial cancer patients (121), decreased in another study of endometrial cancer patients (122), and unchanged in 2 studies of endometrial (123) and prostate (124) cancer patients. Thus, a direct link between short-term metformin use and AMPK activation in targeted tissue is unclear. Larger studies of longer duration and varying dosage of metformin use across various cancer types are needed to determine whether metformin acts through the AMPK pathway to influence tumor growth and progression.

Phenformin, a metformin analogue, is also a potent indirect activator of AMPK and was administered as antidiabetic medication starting in the mid-1900s. However, increased risk of lactic acidosis, often fatal, led to the withdrawal of phenformin by the FDA in 1977 (125). Phenformin has a longer half-life and displays more potent antineoplastic activity compared with metformin in *in vitro* and *in vivo* preclinical studies (126). *In vitro* studies of the antitumorigenic effects of metformin are often at supraphysiological concentrations that may be unattainable in humans; thus, phenformin may offer an alternative for chemoprevention or adjuvant therapy for cancer patients. Phenformin continues to be available in some parts of the world. In a recent cohort study of biguanide use and colorectal cancer risk in Denmark, phenformin comprised 0.5% of biguanide prescriptions (127). The investigators analyzed all biguanides as a group and found an increased risk of colorectal cancer among biguanide users compared with nondiabetics, and risk estimates were inconsistent when biguanide users were compared with diabetics on other oral antidiabetic drugs. These results conflict with the much of the current literature suggesting a reduced risk or null association for biguanide treatment and colorectal cancer incidence (99).

More recently, salicylate, the metabolic derivative of aspirin, has been shown to directly activate AMPK (69). Aspirin has long been known to exhibit antineoplastic properties, though whether these properties are mediated by AMPK is unknown. Algra and colleagues summarized the results for any aspirin use and long-term cancer incidence, reporting summary RRs (95% CI) of 0.88 (0.84–0.92) among 150 case–control studies and 0.87 (0.83–0.91) among 45 cohort studies for risk of all cancer types, with the most consistent findings for reduced risk of colorectal cancer (128). Rothwell and colleagues summarized the results for regular aspirin use and cancer incidence and mortality among randomized controlled trials for the primary prevention of cardiovascular disease, reporting summary RRs (95% CI) of 0.88 (0.80–0.98) for cancer risk among 6 trials and 0.85 (0.76–0.96) for cancer deaths among 34 trials (129). This group also found that aspirin use among patients with nonmetastatic adenocarcinoma at diagnosis was associated with a reduced risk of subsequent metastasis (summary RR, 0.45; 95% CI, 0.28–0.72) and cancer death (summary RR, 0.50; 95% CI, 0.34–0.74) among 5 randomized trials of daily aspirin for the prevention of vascular events (130). Additional observational studies support an association between regular aspirin use after diagnosis and improved survival outcomes among breast (131, 132), colorectal (refs. 133–137; reviewed in ref. 138), and prostate cancer (139, 140) patients, while other studies do not (141–144). Overall, the current evidence from long-term observational and randomized studies is strongly suggestive of a potential role for aspirin in the primary and secondary prevention of cancer.

In summary, observational and randomized studies suggest a potential benefit of AMPK-activating drugs for chemoprevention and/or improving cancer survival. These findings are in agreement with associations between AMPK activation levels in tumor tissue and more favorable clinicopathologic features and survival outcomes observed in several cancer types (Table 1). In future studies, it will be important to understand to what extent AMPK activation mediates the ability of these drugs to reduce cancer risk, and to define their action in the context of the metabolic status of the individual, concurrent medication use, and the natural history of cancer.

Conclusions

The duplicitous role of AMPK activation in cancer cells is context-specific and affects the outcome of AMPK modulation. More sophisticated genetic manipulation of AMPK is necessary to understand its biochemical and cell biology function in the different contexts. In addition, knowledge of long-term outcomes in healthy individuals and cancer patients in relation to AMPK status is necessary to inform the potential use of AMPK modulators in the clinical setting. Thus, the road toward a deeper understanding of AMPK's role in cancer and its therapeutic exploitation is still under construction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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