

Review

Energy Homeostasis and Cancer Prevention: The AMP-Activated Protein Kinase

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Abstract

Caloric restriction has long been recognized as an extremely effective cancer preventive. Current population demographics suggest that caloric excess and obesity will lead to increased cancer incidence, underscoring the need to elucidate the molecular mechanisms that couple dysregulated energy homeostasis to aberrant cell growth. The AMP-activated protein kinase (AMPK) is a critical monitor of cellular energy status, largely studied for its importance in metabolic regulation. AMPK also controls processes relevant to tumor development, including cell cycle progression, protein synthesis, cell growth, and survival. Several tumor suppressors impinge on AMPK signaling, and activation of the kinase inhibits tumor growth. However, AMPK can also promote cancer in some settings, necessitating a more complete understanding of the complexities of this signaling network. Because dysregulated energy balance is a nexus for multiple chronic diseases of aging, drugs that target these pathways may find broad utility in aging populations.

The National Cancer Institute, Division of Cancer Prevention has published a series of reviews on mechanism-based targets for cancer-preventive intervention. Recent reviews include examination of AKT (1), mammalian target of rapamycin (mTOR; ref. 2), and epigenetic modulators (3). Here, the potential to exploit AMP-activated protein kinase (AMPK) activators for chemoprevention is reviewed.

Background

AMPK is an energy-sensing serine/threonine kinase present in all eukaryotes. The enzyme is a heterotrimeric complex consisting of a catalytic α subunit and regulatory β and γ subunits with multiple genes encoding each subunit. At the cellular level, AMPK is activated by metabolic stressors that deplete ATP and increase AMP (e.g., exercise, hypoxia, glucose deprivation). At the level of the organism, enzyme activity is also under the control of hormones and cytokines, such as adiponectin and leptin (4–6).

AMPK is regulated by the cellular AMP/ATP ratio and by phosphorylation by upstream kinases. AMP controls enzyme activity by allosteric activation and renders AMPK less susceptible to dephosphorylation by protein phosphatases (4, 5). Activation also requires phosphorylation on Thr172

in the AMPK α subunit. The two most well-documented upstream kinases are LKB1 and calmodulin-dependent protein kinase kinase β (CaMKK β ; Fig. 1). LKB1 is a tumor suppressor (see below); it is constitutively active and transduces signals generated by changes in cellular energy status (4, 5). CaMKK β is switched on by increased Ca²⁺ levels and functions in the absence of changes in cellular AMP. Unlike the ubiquitously expressed LKB1 (7), CaMKK β is found largely in brain, testis, thymus, and T cells, suggesting cell-type specific effects. However, in cells that do express CaMKK β , increases in cytoplasmic Ca²⁺ should lead to increased ATP demand because Ca²⁺ will be immediately removed from the cytoplasm by ATP-driven pumps (8). Other upstream kinases have been identified, such as transforming growth factor β 1-activated kinase 1; however, their physiologic relevance is unclear (4, 9).

Activation of AMPK suppresses metabolic functions that use ATP and increases activities that generate ATP. For example, activation increases glucose uptake, fatty acid oxidation, and mitochondrial biogenesis, and decreases synthesis of fatty acids, sterols, glycogen, and proteins. AMPK affects control both directly by phosphorylation of protein targets and indirectly via transcriptional regulation (4, 9).

AMPK and Cancer

Connections at the Level of the Organism

Links between AMPK and cancer can be made both at the level of the organism and at the molecular level. Decreased AMPK activation is implicated in human metabolic disorders associated with increased cancer risk. Prominent examples include obesity, diabetes, and the metabolic syndrome (10). Although the contribution of AMPK to the etiology of these disorders is unclear, pharmacologic AMPK activators are

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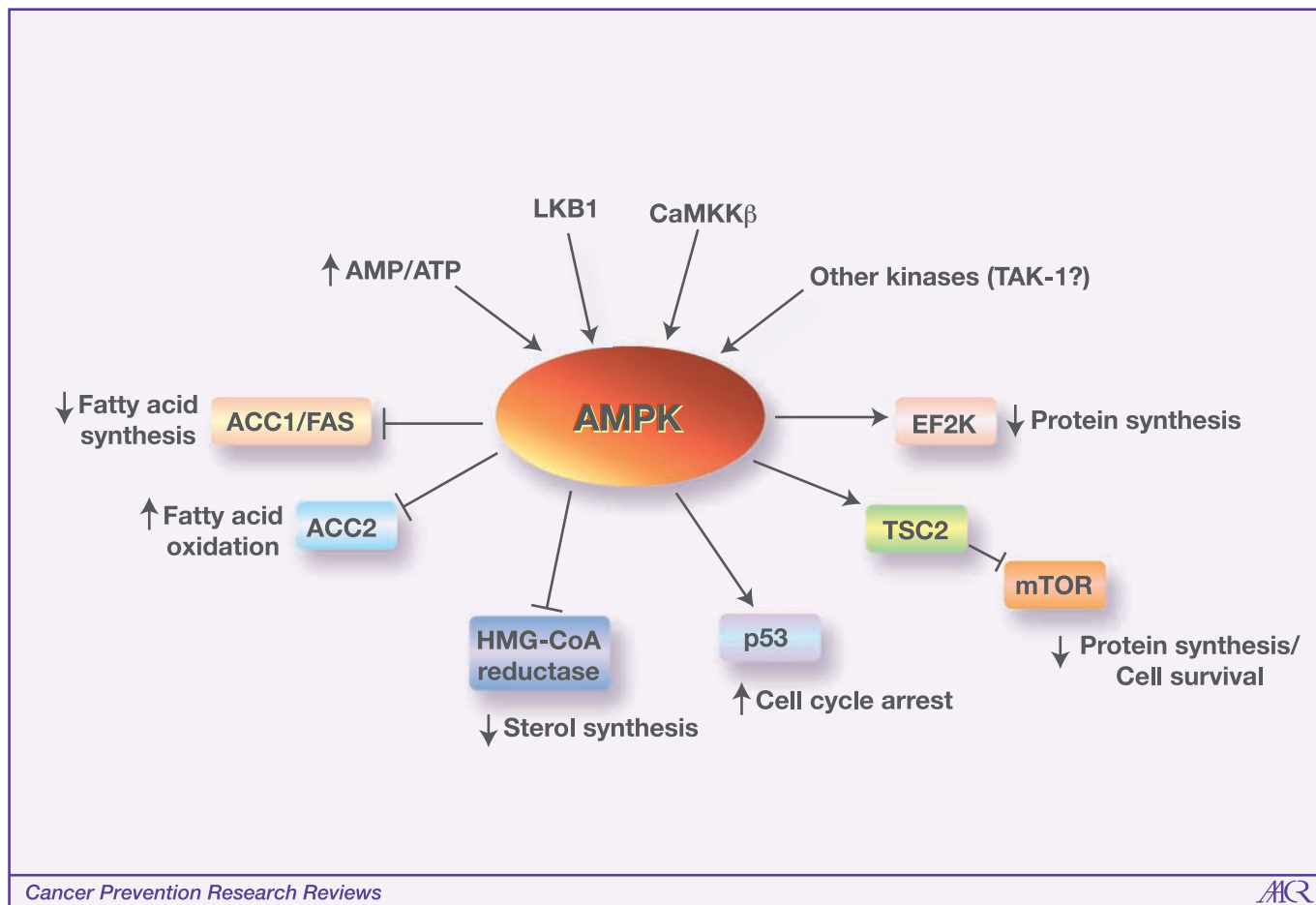


Fig. 1. AMPK signaling. AMPK is activated by phosphorylation on Thr172 in the α subunit by LKB1 (stimulated by AMP binding to AMPK), CaMKK β , and perhaps other kinases. Activation leads to down-regulation of ATP-consuming pathways (e.g., synthesis of proteins, sterols, and fatty acids) and up-regulation of ATP-producing pathways (e.g., fatty acid oxidation and glucose uptake). Selected downstream effectors, particularly those associated with tumor development, are shown (see text for details).

effective in their treatment (9). AMPK is also activated by metabolic stressors linked to decreased tumor development, such as exercise and caloric restriction (6, 10). AMPK activators mimic the effects of exercise in animal models (11). Energy restriction regimens competent to suppress mammary tumorigenesis in rodents increase the levels of activated AMPK in the tumors that do form (12).

Connections at the molecular level

The tumor suppressor LKB1 is an important upstream activator of AMPK. Germ-line *LKB1* mutations are associated with Peutz-Jegher syndrome, which predisposes carriers to benign hamartomas and a variety of malignant epithelial tumors (13). *LKB1* mutations are generally rare in sporadic tumors, with the notable exception of non-small-cell lung cancers. Up to half of these cancers harbor homozygous inactivating *LKB1* mutations (13). Most of these mutations occur in patients with a history of smoking (13). Loss of LKB1 function occurs early in the disease process, as evidenced by detection in adenomatous atypical lung hyperplasias (13). Preliminary data suggest that LKB1 expression is also lost in a subset of high-grade *in situ* ductal breast carcinomas and invasive lesions (14). LKB1 signals to numerous kinases, and the role

of AMPK in LKB1 tumor suppressor function is far from clear (15). However, AMPK activators inhibit the growth of *LKB1*-null tumor cells (16, 17) and the effects of LKB1 on epithelial cell polarity and mitosis are at least partially mediated by AMPK (18, 19). Another upstream regulator of AMPK is the AKT kinase, which negatively controls AMPK by decreasing the AMP/ATP ratio (20, 21).

AMPK signals to multiple pathways that regulate cell growth and proliferation. Prominent among these is the mTOR complex 1 (mTORC1), which is blocked by activation of AMPK (Fig. 2). mTORC1 integrates signals from growth factors and nutrients to control protein synthesis and is positively associated with oncogenesis (22). mTORC1 is composed of four subunits: mTOR, G β L (also known as mLST8), proline-rich AKT substrate of 40 kDa (PRAS40), and regulatory associated protein of mTOR (RAPTOR). mTORC1 is switched on by activation of phosphoinositide-3-kinase (PI3K)/AKT signaling (22). AKT stimulates mTORC1 by phosphorylating and inhibiting the tumor suppressor tuberous sclerosis complex (TSC). TSC2 contains a GTPase-activating protein domain that inactivates the GTPase RHEB (ras homologue enriched in brain). RHEB binds to and directly stimulates mTORC1 activity. When TSC2 is phosphorylated by AKT, it inhibits

the GTPase-activating protein activity of TSC, which leads to mTORC1 activation (22). AKT can also activate mTORC1 in a TSC2-independent manner by phosphorylating and suppressing the negative regulatory mTORC1 component PRAS40 (23, 24).

Like AKT, AMPK regulates mTORC1 by both TSC2-dependent and TSC2-independent mechanisms. Activation of AMPK directly phosphorylates TSC2 at serine residues distinct from AKT. This positively regulates TSC2 by promoting its GTPase-activating protein activity, which turns off RHEB and inhibits mTORC1 (25). The second mechanism by which AMPK suppresses mTORC1 involves the mTORC1 component RAPTOR (22). AMPK directly phosphorylates RAPTOR on two conserved serine sites, suppressing mTORC1 activity (26). Thus, AMPK can directly phosphorylate both TSC2 and RAPTOR to inhibit mTORC1 (26). Importantly, activation of AMPK by hypoxia, low glucose, or other energy stressors inhibits mTORC1 activation, even in the presence of growth factors and stimulation by AKT (reviewed in ref. 27). Blockade of mTORC1 by AMPK is vital in determining apoptotic or growth arrest in cells in response to glucose deprivation or hypoxia (27).

The tumor suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN) also impinges on AMPK signaling. PTEN negatively regulates PI3K/AKT signaling by limiting recruitment of AKT to the plasma membrane, which is necessary for AKT activation (Fig. 2). Recent studies show that stimulating AMPK suppresses tumorigenesis in mice with reduced levels of LKB1 that are PTEN deficient. Mice carrying a hypomorphic *LKB1* allele do not develop tumors; however, the decrease in LKB1 levels markedly accelerates tumor development in animals that are also *PTEN*^{+/-}. Pharmacologic AMPK activators significantly delay tumor onset in these mice (see also below ref. 28).

AMPK also signals to the p53 tumor suppressor. In response to glucose deprivation, AMPK phosphorylates and activates p53. This promotes cell cycle arrest in G₁-S, but cells rapidly reenter the cell cycle when glucose levels are restored. Cell cycle arrest occurs despite continued amino acid availability and active mTOR. On the other hand, p53-null cells continue cycling and subsequently undergo apoptosis (26, 29). Inhibition of tumor cell growth by pharmacologic AMPK activators increases the expression of p53 and the cyclin-dependent kinase inhibitors p21^{CIP} and p27^{KIP1} (17, 30, 31). AMPK-induced cell

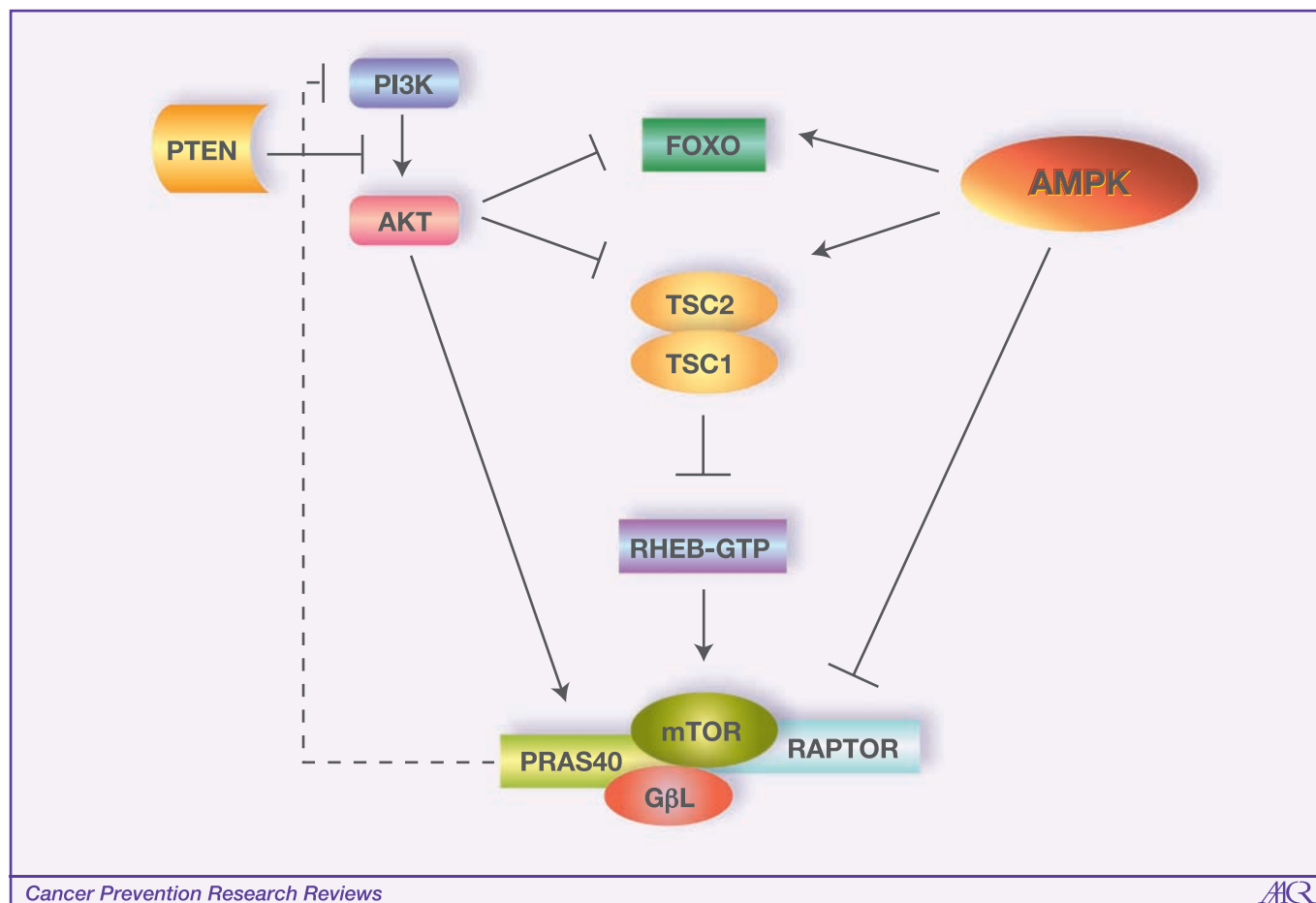
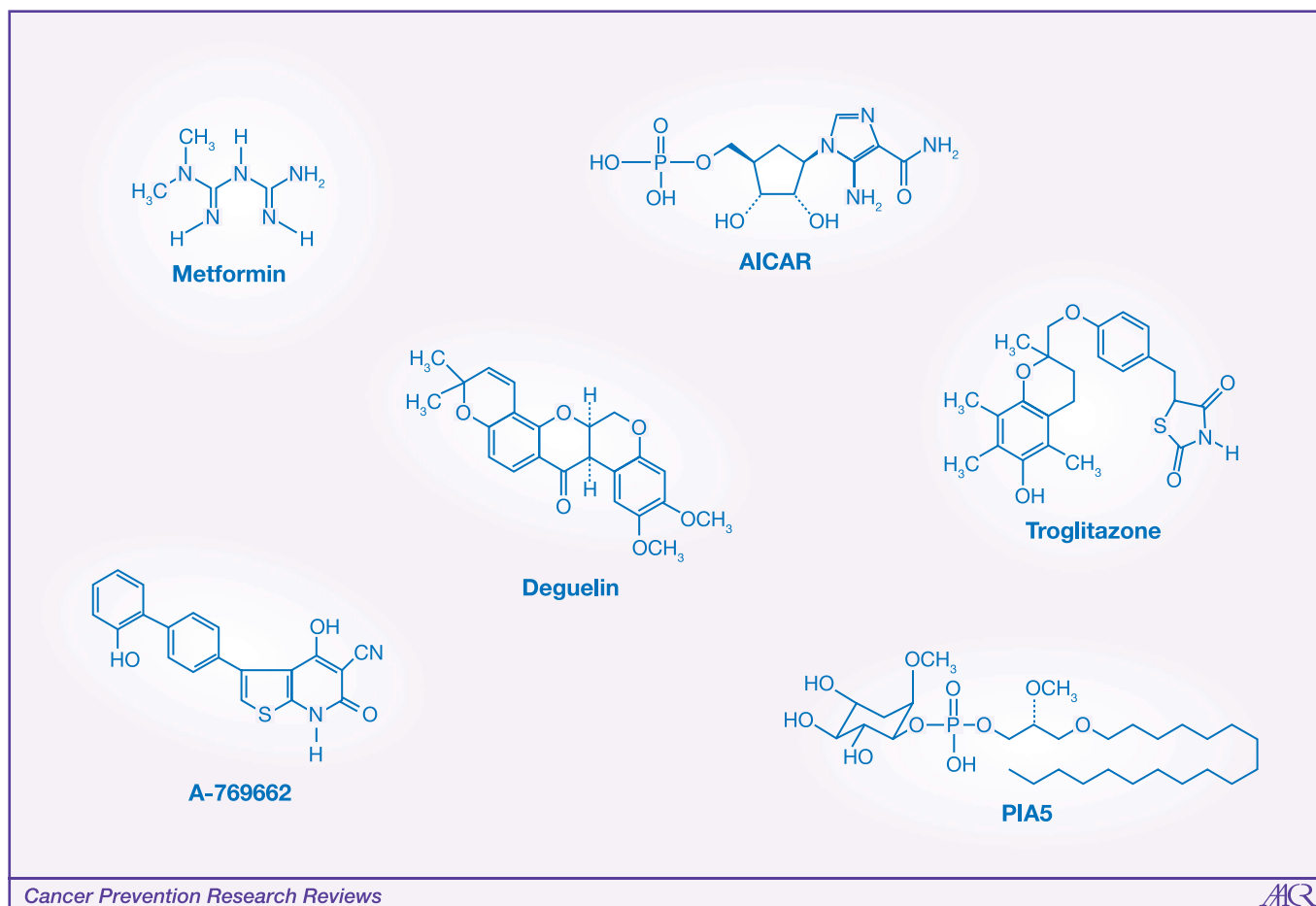


Fig. 2. Cross talk between the AMPK and PI3K/AKT cascades. TSC1/2 integrates AMPK and PI3K/AKT signaling to mTORC1. AMPK positively regulates TSC2, which suppresses RHEB and blocks mTORC1 activation. AMPK also directly blocks the mTORC1-positive regulatory subunit RAPTOR. AKT acts in the opposite manner. When activated by PI3K, AKT suppressed TSC2, which leads to mTORC1 activation. It also directly activates mTORC1 by activating the mTORC1 positive regulatory subunit PRAS40. The tumor suppressor PTEN blocks AKT activation by PI3K. A negative feedback loop shuts down PI3K/AKT on chronic stimulation of mTORC1. AMPK and AKT also signal reciprocally to FOXO. Not shown is the regulation of AMPK by AKT whereby AKT suppresses AMPK by decreasing the AMP/ATP ratio.



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Fig. 3. AMPK activators.

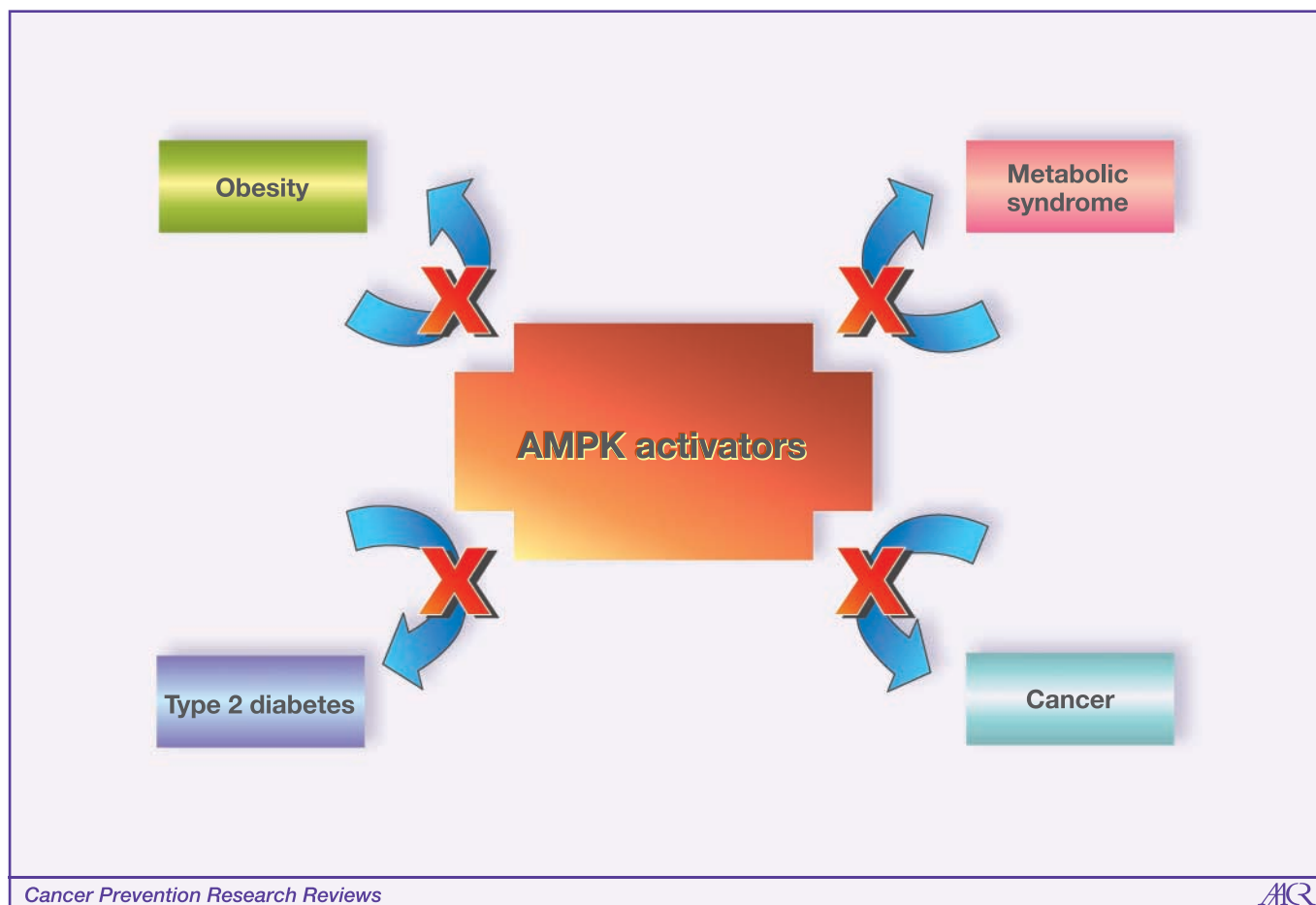
cycle arrest may also be contingent on blockade of nuclear export of the RNA-binding protein human antigen R. The latter is required to stabilize mRNAs of cell cycle regulators such as cyclins A and B1 (32). AMPK regulates additional signaling molecules relevant to tumorigenesis, including the FOXO family. AMPK directly phosphorylates and stimulates the transcriptional activity of FOXO3 (33). AMPK/FOXO signaling mediates the antiaging effects of dietary restriction in worms

under some conditions (34). As AKT can phosphorylate and inhibit FOXO activity, this introduces another level of cross talk between PI3K/AKT and AMPK signaling (Fig. 2).

A number of AMPK-regulated enzymes classically implicated in cellular metabolic control are also linked to cancer. Among these are fatty acid synthase (FAS), acetyl-CoA carboxylases (ACC), and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. ACC1, ACC2, and HMG-CoA reductase are

Table 1. Possible clinical targets for cancer prevention with AMPK-activating drugs

AMPK pathway target	Clinical target	Reference
ACC (↑)	Breast	(74)
AKT/mTOR (↑)	Breast, colon, head and neck, lung, prostate, lung, skin (melanoma)	(1)
FAS (↑)	Breast, ovary, prostate	(43–45)
HMG-CoA (↑)	Breast, colon, prostate, skin (melanoma)	(84)
IGF-I/IGF receptor (↑)	Breast, colon, prostate	(84)
LKB1 (↓)	Breast, lung	(13, 14)
TP53 (↓)	Lung, esophagus, head and neck	(84)
BRCA1 (↓)	Breast, ovary	(74)
PTEN (↓)	Endometrium	(73)



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Fig. 4. AMPK activators may treat or prevent multiple chronic diseases of aging.

directly phosphorylated by AMPK; FAS is controlled at the level of transcription by ACC1 (8). All of these enzymes are negatively regulated by AMPK activation (4). HMG-CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis and is inhibited by statin drugs. Statins prevent cancer in experimental settings (35) and may lower cancer risk in humans (36), although this is far from established (37). Phosphorylation of ACC1 and ACC2 by AMPK decreases fatty acid synthesis and increases fatty acid oxidation, respectively (4). ACC was one of the first AMPK targets identified and its phosphorylation is often used as a marker of AMPK activity (e.g., refs. 17, 38, 39). Knockdown of ACC1 with small interfering RNA (siRNA) can inhibit growth and induce apoptosis in tumor cells (40). Activation of AMPK also suppresses expression of FAS, another key enzyme in fatty acid biosynthesis (41). AMPK does not directly phosphorylate FAS but activates unknown intermediary kinase(s) (42). It is overexpressed in numerous cancers and in preneoplastic colon, stomach, esophagus, oral cavity, prostate, and breast lesions (41). Increased FAS expression correlates with tumor progression in the breast (43), lung (44), and prostate (45). FAS inhibitors prevent mammary cancer in *NEU-N* transgenic mice (41), although the contribution of AMPK to these effects is unknown. However, the antitumor actions of the FAS inhibitor C93 are rescued by AMPK blockade, implicating AMPK (46).

Effects of Pharmacologic AMPK Activators on Transformed Cells

5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR; Fig. 3) is widely used experimentally to activate AMPK. AICAR inhibits the growth of established tumor cells *in vitro* (colon, breast, prostate, gastric, and glioma; refs. 16, 17, 31) and *in vivo* (rat gliomas, human breast, and colon xenografts; refs. 16, 17, 47). In cells, AICAR is phosphorylated and converted to the AMP mimic ZMP (AICAR monophosphate). AICAR arrests tumor cells in S phase, increases expression of p21^{CIP}, p27^{KIP1}, and p53, and inhibits PI3K/AKT/mTOR signaling (16, 17, 21, 31, 47). AICAR's antiproliferative effects are independent of cellular LKB1 status (16, 17) and have been confirmed, both by genetic and chemical means, to depend on AMPK (17). Although highly valuable as an experimental tool, AICAR is not entirely specific for AMPK (4).

The biguanide metformin is another important AMPK activator. Metformin is used to treat type 2 diabetes. It acts indirectly on AMPK via a mechanism that is not well understood but is thought to result from inhibition of complex I of the respiratory chain. This should increase the cellular AMP/ATP ratio and activate the enzyme. Although increases in the AMP/ATP ratio have not been verified with metformin, these changes are evident in cells treated with the more potent metformin analogue phenformin (5). Other studies show that activation of AMPK by

metformin can be mediated by mitochondrial reactive nitrogen species and the c-SRC/PI3K pathway (48).

Metformin exerts its insulin-sensitizing effects in mice by activating AMPK in the liver in an LKB1-dependent fashion (49). Although metformin is an insulin sensitizer in glucose responsive cells (e.g., liver, muscle), it suppresses insulin- and insulin-like growth factor-I (IGF-I)-stimulated growth of human breast cancer cells. AMPK α 1 siRNA rescues cells from the antiproliferative actions of the drug, confirming that the effects in tumor cells are AMPK dependent (39). Growth suppression is associated with reduced protein synthesis and, at the molecular level, with inhibition of mTOR signaling (39). The antiproliferative effects of metformin are not restricted to the breast because it also limits the growth of cultured ovarian, colon, and prostate cancer cells (39). Consistent with metformin's dependence on functional LKB1 to activate AMPK in insulin-responsive cells (49), the drug does not affect the proliferation of LKB1-null tumor cells (39, 47).

In vivo, metformin (and AICAR) suppresses the growth of $p53^{-/-}$ colon tumor xenografts but has no overall effect on the growth of $p53^{+/+}$ xenografts (47). Clusters of apoptotic cells are seen only in drug-treated animals that harbor $p53^{-/-}$ tumors, but not in vehicle-treated $p53^{-/-}$ tumors or $p53^{+/+}$ tumors (+/- metformin). To test if metformin-induced regional cell loss can result from response to local areas of nutrient deficiency (which could be the consequence of low perfusion), survival of cultured $p53^{+/+}$ and $p53^{-/-}$ colon cancer cells under conditions of nutrient deprivation was studied. Metformin induces death in $p53$ -deficient cells in the absence (but not in the presence) of glucose. In $p53^{+/+}$ (but not $p53^{-/-}$ cells), it induces autophagy, increases fatty acid β oxidation, and stimulates glycolysis. The effects of metformin on glycolysis are completely blocked in AMPK $^{-/-}$ cells (47). Thus, p53 loss seems to compromise the ability of tumor cells to execute AMPK-induced metabolic changes that foster survival under nutrient-limiting conditions (47).

Although metformin inhibits the growth of both estrogen receptor α (ER α)-positive and ER α -negative breast cancer cells *in vitro*, new studies show that it enhances the growth of ER α -negative MDA-MB-435 xenografts *in vivo*. The protumorigenic effects of metformin *in vivo* are associated with increased vascular endothelial growth factor expression and intratumoral microvascular density. *In vitro* studies confirm that metformin induces vascular endothelial growth factor in an AMPK-dependent fashion in ER α -negative tumor cells (50). It is notable that mice need ~10-fold the dose of metformin required by humans to lower IGF-I levels (51); the dose used in the aforementioned xenograft studies (750 mg/kg/d) is ~25- to 50-fold higher than a typical human dose (1,000-2,000 mg/d).

Like metformin, thiazolidinediones are used clinically to treat type 2 diabetes. Thiazolidinediones are ligands for the nuclear hormone receptor family member peroxisome proliferator-activated receptor γ (PPAR γ). They bind to PPAR γ on adipocytes, which triggers transcriptional events that culminate in release of adiponectin into the circulation. This activates AMPK in muscle, liver, and perhaps other cells (5). Thiazolidinediones also have more direct, acute effects at the cellular level. They can activate AMPK within minutes in mammalian tissues in conjunction with increasing the AMP/ATP ratio (52), likely via inhibition of complex I of the

respiratory chain (5). AMPK has been implicated in the anti-tumor effects of thiazolidinediones in at least one study (53). In non-small-cell lung cancer lines, the antiproliferative effects of the thiazolidinedione rosiglitazone are partially reversed by AMPK α siRNA (54).

Unlike either AICAR or metformin, phosphatidylinositol ether lipid analogues can directly activate purified AMPK. In cells, however, they act via a mechanism that is not well delineated but seems to be dependent on CaMKK β but not LKB1. Phosphatidylinositol ether lipid analogues suppress the growth of LKB1-mutant non-small-cell lung cancer xenografts in conjunction with intratumoral activation of AMPK. They are not selective for AMPK as they also independently inhibit AKT and activate p38 α . Studies in AMPK $\alpha^{-/-}$ mouse embryo fibroblasts show that phosphatidylinositol ether lipid analogue-induced cell death (both apoptotic and nonapoptotic) is in part AMPK dependent (55).

Cancer-Preventive Effects of Pharmacologic AMPK Activators

In addition to influencing the growth of fully transformed cells, metformin can prevent mammary and pancreatic cancers in model studies. In HER2/NEU transgenic mice, it increases mammary tumor latency and decreases tumor size (56). Tumors that develop in these mice are generally ER α negative (although this was not specifically examined in this study), presenting an apparent contrast to the promoting activity seen in ER α -negative xenografts (50). Metformin can also suppress the development of carcinogen-induced pancreatic tumors and premalignant lesions in hamsters on a high-fat diet (57). Unfortunately, the importance of AMPK activation in these systems has not been examined. Two human studies suggest that metformin reduces cancer risk (58) and mortality (59) in type 2 diabetics.

Metformin also suppresses tumor development in PTEN $^{+/-}$ mice that harbor a hypomorphic LKB1 allele (28). LKB1 $^{fl/+}$ PTEN $^{+/-}$ mice have moderately reduced levels of hepatic LKB1 protein, but this decrease does not affect the ability of metformin (or other AMPK activators studied; see below) to stimulate AMPK. Tumors form in LKB1 $^{fl/+}$ PTEN $^{+/-}$ mice by 4 months of age, with 100% of mice affected by 8 months of age. Metformin (300 mg/kg bw/d) delays tumor onset by 1 month and full penetrance until 12 months of age. The effects of phenformin and a newly developed AMPK activator, A-769662, were also studied. A-769662 activates the enzyme allosterically and by inhibiting dephosphorylation of AMPK on Thr172 (60, 61). None of the mice dosed with phenformin or A-769662 had tumors at 6 months of age, compared with 60% of controls. The first tumors developed in both of these treated groups by 7 months of age, and by 10 months, 60% of phenformin-treated and 80% of A-769662-treated mice had tumors. By the end of the experiment, at the age of 12 months, 20% of phenformin and 10% of A-769662 groups remained tumor-free. The types and morphology of tumors that developed in animals treated with all three drugs were similar to those in controls (mostly lymphomas and intestinal polyps; ref. 28).

AMPK activation may also contribute to the chemopreventive actions of thiazolidinediones. For example, thiazolidinediones rosiglitazone and troglitazone suppress IGF-I-driven

formation of mouse skin tumors. Because this tissue does not express PPAR γ , additional mechanisms may be in play. Modeling studies in cultured normal murine keratinocytes show that troglitazone activates AMPK in response to IGF-I. The drug further suppresses IGF-I-stimulated proliferation and mTOR signaling, both of which are partially reversed by expression of dominant negative AMPK (53). These findings show that thiazolidinediones can activate AMPK directly in IGF-I-stimulated skin cells; however, they do not preclude the possibility that indirect AMPK activation (via PPAR γ -induced adiponectin release) plays a role in tumor suppression *in vivo*.

Tumor prevention by the natural product deguelin has been more directly linked to AMPK. Deguelin inhibits tobacco-induced carcinogenesis in the lung *in vitro* and *in vivo* (62, 63); these effects had previously been tied to blunting PI3K/AKT signaling (62, 63). Deguelin is also known to alter cellular energy balance, prompting the exploration of control at the level of AMPK (21). In cultured premalignant human bronchial cells, the anti-transforming and proapoptotic actions of deguelin are dependent on suppression of the protein survivin. The latter is an antiapoptotic regulator downstream of mTORC1. Deguelin decreases ATP, activates AMPK, and suppresses AKT activation and mTOR/survivin signaling. Blockade of AMPK chemically or genetically restores survivin expression, implicating AMPK in deguelin action. AKT is also involved as overexpression of constitutively active AKT inactivates AMPK and restores survivin expression (21). These findings are consistent with the ability of AKT to negatively regulate AMPK by decreasing the AMP/ATP ratio (20).

A number of other well-known chemopreventives are competent to activate AMPK. Examples include resveratrol (38), epigallocatechin-3-gallate (64), genistein (65), and selenium (66). In some cases, effects on AMPK have only been shown in nontarget cells such as adipocytes (65). Moreover, these agents modulate numerous signaling pathways relevant to tumor development (67), and the contribution of AMPK to their preventive actions is unknown.

Because AMPK is activated in response to decreased energy or glucose levels, it is well positioned to participate in the biological responses to calorie restriction, including decreased cancer risk (68, 69). The cancer-preventive effects of calorie restriction have been linked to down-regulation of insulin/IGF-I signaling (70). AMPK acts in opposition to insulin/IGF-I both at the level of the organism (reducing circulating insulin/IGF-I) and at the cellular level (suppressing mTOR signaling; ref. 39). Feeding metformin (187 mg/kg bw/d) to mice mimics the effects of calorie restriction on gene expression (71).

Strategies for Developing AMPK Activators for Cancer Prevention

Identifying clinical target populations

Identifying patients with a high probability of responding to AMPK activators is critical for successful prevention strategies. Although evidence of decreased AMPK activity in precancerous lesions would provide clues to possible target populations, to our knowledge, these studies have not been conducted. Patients with *TP53*-null precancerous lesions might be a good initial experimental cohort because AMPK activators can selectively inhibit the growth of *p53*-null xenografts (47). *LKB1*-deficient precancerous lung lesions may be

another target for selected AMPK activators (55). Notably, although some *LKB1* function is required for suppression of tumor formation by metformin and phenformin, these drugs are competent to inhibit tumor growth in *LKB1* hypomorphic mice (28). This highlights the importance of clarifying the level of *LKB1* activity necessary for AMPK activation by various drugs (72).

As predicted, studies have now confirmed the preventive activity of AMPK-activating drugs on tumors driven by *PTEN* deficiency (28). Functional loss of *PTEN* is generally a relatively late event in human tumorigenesis; however, *PTEN* mutations are prevalent in both precancerous and cancerous endometrial lesions (73). These findings also support the broader use of AMPK activators to prevent tumor progression in precancerous lesions with hyperactivated PI3K/AKT/mTORC1 (Table 1). The existence of a negative feedback loop in which long-term mTORC1 activation inhibits AKT (Fig. 2; ref. 22) emphasizes the need for model studies before clinical trials. Such studies should be used to define cohorts with a high probability of response and to identify dosing biomarkers and AMPK targets that predict efficacy.

An interesting target population may be women who harbor mutations in the *BRCA1* tumor suppressor. Although these women are at significantly higher risk for both breast and ovarian cancer, adequate chemoprevention strategies have not been developed. AMPK activators may act as *BRCA1* mimics (74). This notion is predicated on the observation that normal *BRCA1* and AMPK act similarly toward *ACC1*. Activation of AMPK phosphorylates and inhibits *ACC1*, whereas *BRCA1* stabilizes the phosphorylated, inactive *ACC1*. Moreover, breast cancer-associated *BRCA1* mutations disrupt this *BRCA1*/*ACC1* interaction (74). Interestingly, *BRCA1*/2-mutant tumors have a higher frequency of *TP53* mutations, and metformin selectively inhibits the growth of *p53*-null tumors (47). However, *BRCA1*-mutant tumors are also generally ER α negative (75), and as discussed above, high metformin doses can enhance the growth of ER α -negative xenografts (50).

Drug development issues

Preventive agents will likely be administered for extended periods of time, underscoring the need for both chronic safety and ease of administration. AICAR is in phase III clinical development for ischemia-reperfusion injury after coronary bypass graft surgery (76). However, it is not entirely specific for AMPK; it also has limited oral bioavailability, restricting its use to conditions amenable to acute i.v. dosing (76). Oral metformin is currently used by more than 120 million people worldwide to treat type 2 diabetes (8). The drug is very well tolerated; the most common side effects are gastrointestinal in nature, although lactic acidosis occurs rarely (9). Lactic acidosis (and perhaps some gastrointestinal effects) likely results from metformin's inhibition of complex I of the respiratory chain. Indeed, severe lactic acidosis was the reason for withdrawal of the more potent phenformin as a clinical antidiabetic (9). Direct activators, such as A-769662, may be devoid of these side effects. Design of direct AMPK activators will be greatly enhanced by the recent publication of the crystal structure of AMPK subunits (77, 78).

On-target effects of AMPK activation may also be detrimental. Activating mutations in complexes containing the

AMPK γ 2 isoform can cause heart disease. It will be important to examine the cardiac effects of AMPK-activating drugs, with particular attention to isoform-specific actions (9). Clearly, the possible tumor-promoting effects of AMPK must be carefully assessed. In addition to the finding that metformin selectively promotes growth of ER α -negative tumors, AMPK may be essential for tumor growth in other settings (e.g., refs. 79, 80). For example, the ability of *ras*-transformed, AMPK-deficient mouse embryo fibroblasts to grow as xenografts is dramatically suppressed compared with their AMPK wild-type counterparts. Tumors can apparently take advantage of AMPK to survive their hypoxic and glucose-deficient microenvironments (80).

Maximal benefit in terms of both efficacy and toxicity may be achieved by combining AMPK activators with other drugs. One setting where AMPK activators might be exploited for this purpose is in combination with AKT inhibitors. These drugs are promising chemopreventives, but adverse effects on glucose homeostasis may limit their utility. AMPK-activating antidiabetics may relieve these effects and perhaps augment efficacy (1). Concurrent targeting of additional key nodes in the PI3K/AKT/mTOR signaling cascade, such as PI3K and mTORC1, may also be efficacious, as has been observed for combinations of AKT and mTOR-specific inhibitors (81). The effectiveness of the latter combination is apparently due to the ability of AKT inhibitors to override the negative feedback mTORC1/AKT loop described above (22). The increased endurance seen in sedentary mice treated with pharmacologic AMPK activators is dependent on PPAR δ , suggesting that the effects of AMPK can also be augmented by PPAR δ ligands (11).

Future Directions and Conclusions

Signaling pathways initially tied to cell metabolism are increasingly recognized as integral to the tumorigenic process, with AMPK emerging as a central control point. The availabil-

ity of large cohorts chronically treated with metformin presents an important and unique opportunity for cancer prevention research. Well-designed human studies should be initiated in patients who have been treated with the drug to determine the effects on cancer risk. These studies, together with animal and mechanistic experiments, will help address the numerous questions that remain about the complexities of AMPK biology.

One of the most fundamental issues to be resolved is the differential signaling cascades that allow the kinase to both positively and negatively regulate insulin/IGF-I action in diverse cell types. Nevertheless, it is evident that dysregulated energy metabolism is a common feature of many chronic diseases of aging (82). A clearer understanding of the mechanisms that drive obesity, type 2 diabetes, atherosclerosis, the metabolic syndrome, and cancer will foster significant opportunities to develop a single or a limited number of drugs that target molecular pathways common to these diseases (Fig. 4). Treatment regimens that take advantage of this strategy will diminish the need for the use of multiple medications in aging populations. This should lead to increased compliance, minimize drug interactions, and reduce side effects. Additionally, it will decrease medication costs, which are becoming a major financial burden on both individuals and health care systems. This research could have even greater effect as "chronic diseases of aging" are no longer limited to aging cohorts and are reaching epidemic proportions in younger populations (83).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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