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# AMP-Activated Protein Kinase: Friend or Foe in Cancer?

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## Keywords

AMP-activated protein kinase, AMPK, LKB1, STK11, tumor suppressor, tumor promoter

## Abstract

The AMP-activated protein kinase (AMPK) is activated by energy stress and restores homeostasis by switching on catabolism, while switching off cell growth and proliferation. Findings that AMPK acts downstream of the tumor suppressor LKB1 have suggested that AMPK might also suppress tumorigenesis. In mouse models of B and T cell lymphoma in which genetic loss of AMPK occurred before tumor initiation, tumorigenesis was accelerated, confirming that AMPK has tumor-suppressor functions. However, when loss of AMPK in a T cell lymphoma model occurred after tumor initiation, or simultaneously with tumor initiation in a lung cancer model, the disease was ameliorated. Thus, once tumorigenesis has occurred, AMPK switches from tumor suppression to tumor promotion. Analysis of alterations in AMPK genes in human cancers suggests similar dichotomies, with some genes being frequently amplified while others are mutated. Overall, while AMPK-activating drugs might be effective in preventing cancer, in some cases AMPK inhibitors might be required to treat it.

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## 1. INTRODUCTION

The AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis that is activated by increases in cellular AMP:ATP or ADP:ATP ratios (Carling 2017, Lin & Hardie 2017, Ross et al. 2016b). This occurs either when ATP synthesis is compromised (e.g., when cellular demand for oxygen or nutrients cannot be met by supply from blood vessels) or when ATP turnover is increased (e.g., during rapid cell proliferation). AMPK then acts to restore energy homeostasis by switching on alternative catabolic pathways that generate ATP, while switching off all major biosynthetic pathways and other ATP-consuming processes such as the cell cycle. AMPK may therefore play a particularly important role in tumor cells, where mutations can cause a rapid increase in cell proliferation, and where this may not initially be matched by a concomitant increase in the blood supply.

Given that the classical mechanism of AMPK activation by energy stress requires the tumor-suppressor kinase LKB1, and that AMPK switches off biosynthesis (i.e., cell growth) and the cell cycle (i.e., cell proliferation), the idea began to emerge that AMPK may mediate many of the tumor-suppressor actions of LKB1 (Hardie & Alessi 2013). However, more recent studies have suggested that, in some situations, AMPK promotes tumorigenesis by protecting tumor cells against the stresses they undergo during rapid growth or metastasis. The aim of this review is to reconcile these two apparently opposing viewpoints, and to address whether activators or inhibitors of AMPK would be efficacious in cancer, either for prevention or for treatment.

## 2. AMPK COMPLEXES: STRUCTURE AND CANONICAL ACTIVATION BY ENERGY STRESS

AMPK occurs in essentially all eukaryotes as heterotrimeric complexes comprising catalytic α and regulatory β and γ subunits. In humans and other mammals, there are multiple genes encoding isoforms of each subunit (**Table 1**). All isoform combinations can form complexes, so there are up to 12 distinct heterotrimeric complexes that display subtle differences in tissue/organ distribution or regulation (Ross et al. 2016b). These complexes also appear to differ in subcellular localization (e.g., Hudson et al. 2003, Kazgan et al. 2010, Salt et al. 1998), although the underlying mechanisms remain incompletely understood. As discussed in Section 7, genes encoding alternate subunit isoforms can also display different types of genetic change in different human cancers.

AMPK is only significantly active when phosphorylated at a conserved threonine, usually referred to as Thr172 (Hawley et al. 1996), within the activation loop of the kinase domain. Binding of AMP to the γ subunit activates AMPK by three complementary mechanisms: (*a*) promotion of Thr172 phosphorylation by upstream kinases, (*b*) inhibition of Thr172 dephosphorylation by protein phosphatases, and (*c*) allosteric activation of AMPK already phosphorylated on Thr172. All three effects are antagonized by binding of ATP (Davies et al. 1995, Gowans et al. 2013, Ross et al. 2016a). It has also been reported that AMPK can be activated by binding of ADP (Oakhill et al. 2011, Xiao et al. 2011), although research in our laboratory (Gowans et al. 2013, Ross et al. 2016a) has suggested that the effects of ADP are only significant for mechanism *b*. AMPK complexes containing different γ subunit isoforms display subtly different responses to the three regulatory nucleotides (**Table 1**) (Ross et al. 2016a).

In 2003, LKB1 was identified as the principal upstream kinase that phosphorylates Thr172 (Hawley et al. 2003, Shaw et al. 2004, Woods et al. 2003). Although it was known from genetic studies that LKB1 was a tumor suppressor (Alessi et al. 2006) and that its sequence contained a prominent serine/threonine kinase domain, AMPK was the first downstream target of the kinase activity of LKB1 to be identified. These findings also suggested that AMPK might have roles in cancer (Hardie & Alessi 2013).

**Table 1 Genes and proteins that generate heterotrimeric AMPK complexes in humans**

| Gene | Protein | Function | Features |
|---|---|---|---|
| *PRKAA1* | α1 | Catalytic | ■ Expressed ubiquitously |
| *PRKAA2* | α2 | Catalytic | ■ Usually expressed at lower levels than α1, except in skeletal muscle<br>■ Not expressed in cells of hematopoietic lineage |
| *PRKAB1* | β1 | Structural/glycogen-binding | ■ Expressed ubiquitously<br>■ Myristoylation required for lysosomal localization of AMPK<br>■ Glycogen-binding domain has low affinity for glycogen |
| *PRKAB2* | β2 | Structural/glycogen-binding | ■ Widely expressed, but highest levels in skeletal and cardiac muscle<br>■ Myristoylation required for lysosomal localization of AMPK<br>■ Glycogen-binding domain has high affinity for glycogen |
| *PRKAG1* | γ1 | AMP/ADP/ATP-binding | ■ Expressed ubiquitously<br>■ Thr172 dephosphorylation in γ1 complexes inhibited more potently by AMP than ADP |
| *PRKAG2* | γ2 | AMP/ADP/ATP-binding | ■ Widely expressed, but highest levels in cardiac and skeletal muscle<br>■ Thr172 dephosphorylation in γ2 complexes inhibited similarly by ADP and ATP |
| *PRKAG3* | γ3 | AMP/ADP/ATP-binding | ■ Expressed almost exclusively in skeletal muscle<br>■ Only very modest allosteric activation by AMP in γ3 complexes<br>■ Thr172 dephosphorylation in γ3 complexes inhibited more potently by AMP than ADP |

## 3. AMPK: NONCANONICAL ACTIVATION BY HORMONES AND GLUCOSE STARVATION

A second upstream kinase that phosphorylates Thr172 and activates AMPK by a noncanonical mechanism is the $Ca^{2+}$/calmodulin-dependent kinase, CaMKK2 (Hawley et al. 2005, Hurley et al. 2005, Woods et al. 2005). This mechanism explains how many hormones that trigger release of intracellular $Ca^{2+}$ can activate AMPK without altering AMP:ATP ratios (e.g., Stahmann et al. 2006, 2010; Yang et al. 2011). Another noncanonical activation mechanism occurs when mammalian cells are deprived of glucose. Although in some cells this leads to increases in cellular ADP:ATP and AMP:ATP ratios because of inhibition of glycolysis, in others (e.g., mouse embryo fibroblasts), glucose deprivation activates AMPK without detectable changes in nucleotide ratios, as long as an alternate carbon source such as glutamine is present. Moreover, even in cells where nucleotide ratios do change, some activation remains in cells expressing an AMP-insensitive mutant of AMPK (Zhang et al. 2017). These results confirm that AMPK can be activated by glucose deprivation, at least in part, by a noncanonical mechanism that is independent of adenine nucleotides. This mechanism requires the resident lysosomal proteins LAMTOR1 and the vacuolar ATPase, and it involves recruitment to the lysosome of LKB1 in complex with the adapter protein AXIN, where it interacts with AMPK. Glucose availability appears to be sensed by binding of the glycolytic metabolite fructose-1,6-bisphosphate to aldolase, which also interacts with the vacuolar ATPase (Zhang et al. 2017). Since many tumor cells have a high demand for glucose but a poor blood supply, this mechanism may be of particular relevance to cancer.

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## 4. AMPK: DOWNSTREAM TARGETS RELEVANT TO CANCER

In general, AMPK phosphorylates targets that either activate alternative catabolic pathways to generate more ATP or inhibit processes, especially biosynthetic pathways, that consume ATP. We first discuss the effects of AMPK on catabolism. AMPK activation acutely increases glucose uptake, not only via GLUT4 (Pehmoller et al. 2009) (expressed in insulin-sensitive tissues) but also via the more ubiquitously expressed GLUT1 (Barnes et al. 2002). The mechanism of GLUT1 activation appears to involve the degradation, induced by direct phosphorylation, of the arrestin family member TXNIP, thus inhibiting GLUT1 endocytosis and increasing the amount on the cell surface (Wu et al. 2013). AMPK also promotes glycolysis in some cell types by phosphorylation and activation of two of the four isoforms [PFKFB2 (Marsin et al. 2000) and PFKFB3 (Marsin et al. 2002)] of the enzyme that generates fructose-2,6-bisphosphate, a potent allosteric activator of the glycolytic enzyme phosphofructokinase. Interestingly, while expression of PFKFB3 is low in most adult cells (although it can be induced by proinflammatory stimuli), it is constitutively expressed in many tumor cells (Clem et al. 2008, Yi et al. 2019).

By phosphorylating these targets, AMPK may help to acutely promote the rapid glucose uptake and glycolysis typical of many cancer cells. However, in the longer term, AMPK tends to promote instead the oxidative metabolism provided by mitochondria, which is much more efficient in ATP production. Mechanisms by which AMPK promotes oxidative metabolism include: (*a*) activation of fatty acid oxidation via phosphorylation and inactivation of ACC2, the mitochondrial isoform of acetyl-CoA carboxylase—this reduces the level of malonyl-CoA, relieving inhibition of mitochondrial fatty acid uptake through CPT1 (carnitine:palmitoyl-CoA transferase 1) (Merrill et al. 1997); (*b*) increased expression of TCA (tricarboxylic acid) cycle enzymes (Winder et al. 2000) as well as mitochondrial biogenesis, in part by upregulation of the transcriptional coactivator PGC-1α (Zong et al. 2002); (*c*) induction of fission of damaged, dysfunctional mitochondria via phosphorylation of MFF (the mitochondrial fission factor) (Toyama et al. 2016), which may be necessary for their removal by mitophagy, which is independently enhanced by AMPK via phosphorylation of the autophagy-initiating kinase ULK1 (Egan et al. 2011, Herzig & Shaw 2017).

AMPK also acutely switches off most biosynthetic pathways via direct phosphorylation of key target enzymes, including: (*a*) fatty acid synthesis, via phosphorylation of the cytosolic ACC1 isoform of acetyl-CoA carboxylase (Fullerton et al. 2013, Munday et al. 1988); (*b*) cholesterol synthesis, via phosphorylation of 3-hydroxy-3-methylglutaryl-CoA reductase (Clarke & Hardie 1990); (*c*) triacylglycerol and phospholipid synthesis, via phosphorylation of glycerol-3-phosphate acyltransferase (Muoio et al. 1999); (*d*) glycogen synthesis, via phosphorylation of both isoforms of glycogen synthase (Bultot et al. 2012, Jorgensen et al. 2004); (*e*) nucleotide synthesis, via phosphorylation of phosphoribosyl pyrophosphate synthetase 1 (Qian et al. 2018); (*f*) ribosomal RNA synthesis, via phosphorylation of the RNA polymerase 1 transcription factor TIF-1A (Hoppe et al. 2009); and (*g*) protein synthesis, in part via inhibition of mTOR complex 1 (mTORC1) by phosphorylation of its upstream regulator TSC2 (Inoki et al. 2003) and its targeting subunit Raptor (Gwinn et al. 2008), and in part by phosphorylation and activation of elongation factor 2 kinase (Johanns et al. 2017).

Some of these pathways are also downregulated by AMPK at the transcriptional level. Inhibition of any of the above biosynthetic pathways is potentially important for the growth-restraining and anticancer effects of AMPK, but mTORC1 inhibition may be particularly important because tumor cells often display hyperactivation of mTORC1 (Zoncu et al. 2011).

In addition to inhibiting cell growth via these effects on biosynthesis, AMPK also inhibits cell proliferation by causing G1 cell cycle arrest. This was first demonstrated using the

pharmacological activator AICAR (Imamura et al. 2001) or by overexpressing a phosphomimetic mutant (T172D) of the AMPK-α2 kinase domain (Jones et al. 2005), which might have off-target or nonphysiological effects, respectively. However, it has recently been shown that cell cycle arrest induced by AMPK activation in a melanoma cell line was prevented by a CRISPR knockout of both AMPK-α1 and AMPK-α2 (Fogarty et al. 2016).

## 5. EVIDENCE FOR TUMOR-SUPPRESSOR ROLES FOR AMPK

The initial idea that AMPK was a tumor suppressor came from the findings that the principal upstream kinase required for AMPK activation in response to energy stress was a complex between LKB1 and two accessory subunits, STRADα/β and MO25α/β (Hawley et al. 2003). The gene encoding LKB1 (*STK11*) had been identified a few years earlier as having heterozygous loss-of-function mutations in Peutz-Jeghers syndrome, an inherited susceptibility to cancer in humans (Alessi et al. 2006). It was subsequently shown that biallelic somatic mutations in *STK11* also frequently occur in sporadic cancers, especially lung adenocarcinomas (Ji et al. 2007, Sanchez-Cespedes et al. 2002). The findings, reviewed above, that AMPK inhibited both macromolecular biosynthesis and the cell cycle, and that it inactivated the mTORC1 pathway, reinforced the idea that AMPK might exert most, if not all, of the tumor-suppressor functions of LKB1. In addition, following the discovery of the link between LKB1 and AMPK, the use of the AMPK activator metformin (Zhou et al. 2001) to treat type 2 diabetes was found to be associated with a reduced risk of cancer compared with other medications (Evans et al. 2005, Noto et al. 2012). Although this remains just a correlation with no proof of causality, these findings suggested that metformin might protect against cancer by activating AMPK in tumor progenitor cells. However, in addition to the α1 and α2 catalytic subunits of AMPK, LKB1 also acts upstream of at least 12 members of the AMPK-related kinase (ARK) family (Jaleel et al. 2005, Lizcano et al. 2004), raising the possibility that one or more ARKs exert some tumor-suppressor functions of LKB1. Indeed, expression of the transcription factor SNAIL1, which promotes the epithelial-to-mesenchymal transition required for invasion and metastasis by epithelial cancers, is repressed by LKB1 via a mechanism that is independent of AMPK but dependent on the ARKs MARK1/MARK4 (Goodwin et al. 2014).

With respect to the potential tumor-suppressor roles of AMPK itself, there are numerous reports that pharmacological activators of AMPK suppress growth and proliferation of tumor cells in vitro (e.g., Fogarty et al. 2016, Imamura et al. 2001, Jones et al. 2005, Rattan et al. 2005). However, many of these studies used activators with known AMPK-independent effects such as AICAR or metformin, and not all confirmed that AMPK was necessary for the effect by gene knockdown or knockout. In any case, cancer is a complex disorder involving interactions between tumor cells and surrounding stroma, as well as other processes such as angiogenesis and metastasis. It was therefore important to show that genetic loss of AMPK would prevent the development of cancer in vivo. One of the first approaches was in a mouse model of B cell lymphoma, in which tumors were induced by expression of c-Myc from a B cell–specific promoter (Faubert et al. 2012). These were crossed with mice that had a knockout of the *Prkaa1* gene encoding AMPK-α1, the sole catalytic subunit isoform expressed in cells of the hematopoietic lineage. Homozygous loss of *Prkaa1* correlated with accelerated development of B cell lymphomas, with heterozygous loss having an intermediate effect. These results suggested that expression of *Prkaa1* in the wild-type situation protected against B cell lymphoma, supporting the idea that AMPK can act as a tumor suppressor. One drawback with this study was that *Prkaa1* was knocked out globally rather than only in B cells. It was therefore not clear whether the effects of AMPK knockout were cell-autonomous, although that conclusion was supported by experiments in which wild-type or AMPK-deficient lymphoma cells were grown in irradiated wild-type recipient mice (Faubert et al. 2012).

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Another study crossed mice with global knockouts of the genes encoding p53 and AMPK-β1 *Prkab1*, the latter being the AMPK-β subunit isoform that predominates in thymocytes. AMPK-β1 knockout caused earlier onset of T cell lymphomas in both homozygous and heterozygous p53 knockouts, suggesting that β1 had a tumor-suppressor role (Houde et al. 2017). However, as with the previous study, AMPK-β1 knockout was global, so that it was not possible to conclude that the effect of AMPK knockout was a cell-autonomous effect in the tumor progenitor cells themselves.

Two recent studies have used mouse models to study the effects of knockout of AMPK genes specifically in the tumor progenitor cells. The first used prostate epithelial-specific knockouts of the tumor-suppressor gene *Pten* and the *Prkab1* gene encoding AMPK-β1 (Penfold et al. 2018). Although the knockout of *Prkab1* and *Pten* did not affect prostate size, it did result in a higher proliferative index and pathological grade, suggesting a tumor-suppressor role for AMPK-β1. A drawback with this model was that the prostate gland also expresses AMPK-β2, which might have partially compensated for lack of β1. The second study involved a model of T cell acute lymphoblastic leukemia/lymphoma (T-ALL) and utilized T cell–specific knockouts of *Pten* and the *Prkaa1* gene encoding AMPK-α1 (Vara-Ciruelos et al. 2018b). This model has the advantage that there is no expression of the other catalytic subunit isoform, α2, in T cells (Rolf et al. 2013). If *Prkaa1* and *Pten* were knocked out in developing T cells, lymphomas appeared earlier and were more aggressive (with a hazard ratio greater than 3). As in the study of B cell lymphoma described earlier (Faubert et al. 2012), AMPK loss was associated with mTORC1 hyperactivation; increased expression of HIF-1α, a transcription factor downstream of mTORC1; and of genes encoding glycolytic enzymes that are switched on by HIF-1α, leading to increased lactate production (Vara-Ciruelos et al. 2018b).

Since *Prkaa1* knockout in this study was T cell–specific, and since all other cells would have had normal AMPK-α1 expression, this mouse model also provided an excellent opportunity to test whether metformin might protect against tumor development by activating AMPK in the tumor progenitor cells. Disappointingly, oral metformin given prior to and during tumor formation had no effect on lymphoma development. However, this could be explained by the failure of thymocytes to take up metformin, so that AMPK was not activated in the thymus (Vara-Ciruelos et al. 2018b). Metformin is positively charged, hydrophilic, and only readily taken up by cells expressing transporters of the organic cation transporter family [especially OCT1 (Chen et al. 2014)], which may not to be expressed in thymocytes. It now seems probable that the lower incidence of cancer in subjects with type II diabetes taking metformin (Evans et al. 2005, Noto et al. 2012) is due to effects of metformin not on the tumor progenitor cells themselves, but on other tissues or organs that indirectly affect the tumor environment. One explanation is that metformin lowers insulin levels due to its ability to activate AMPK in the liver (which does express OCT1) and thus alters fatty acid metabolism so that consequent changes in storage of lipids in liver and muscle enhance their insulin sensitivity (Fullerton et al. 2013). Enhanced insulin sensitivity would lower insulin secretion and hence reduce the high insulin levels in many subjects with type II diabetes, which may otherwise have protumorigenic effects (Pollak 2012).

The failure of thymocytes to take up metformin prompted testing of the related biguanide phenformin, which is more hydrophobic than metformin and has been shown to be taken up by cells lacking OCT1 (Hawley et al. 2010). Intriguingly, oral phenformin protected against lymphoma development, but only when the developing T cells expressed AMPK-α1 (Vara-Ciruelos et al. 2018b). Thus, the protective effect of phenformin was both AMPK-dependent and cell-autonomous, requiring AMPK expression within the tumor progenitor cells themselves. Related to this, in a trial for treatment of advanced pancreatic cancer, which was one of the first randomized, placebo-controlled trials of metformin in human cancer to be reported, metformin had no beneficial effect when added to existing chemotherapy. However, the authors pointed out



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that metformin might not be taken up by the tumor cells, and suggested phenformin as a possible alternative (Kordes et al. 2015).

Other evidence supporting the idea that AMPK is a tumor suppressor comes from studies of E3 ubiquitin ligases involved in cellular degradation of AMPK subunits. MAGE-A3 and MAGE-A6 are two closely related, functionally redundant members of the melanoma-associated antigen (MAGE) family of proteins, normally expressed in testis but aberrantly reexpressed in many tumors (Pineda et al. 2015). They bind to the E3 ubiquitin ligase TRIM28, and a screen revealed AMPK-α1 as a prominent target for polyubiquitination by the MAGE-A3/A6:TRIM28 complex, with consequent proteasomal degradation. Consistent with this, knockdown of MAGE-A3/A6 or TRIM28 in tumor cells increased expression of AMPK-α1 and triggered the expected changes in metabolism and signaling, including mTORC1 inhibition. Finally, various human tumor cells that express MAGE-A3 or -A6 were found to have reduced levels of AMPK-α1 protein (Pineda et al. 2015).

Another ubiquitin ligase involved in cancer appears to target degradation of the other catalytic subunit isoform, AMPK-α2 (Vila et al. 2017). UBE2O is a hybrid ubiquitin ligase displaying both E2 and E3 functions. *Ube2o* knockout attenuated tumor development in genetically engineered mouse models of breast and prostate cancer, supporting the idea that UBE2O has tumor-promoting functions. A search for UBE2O-interacting proteins identified AMPK-α2 as a target for polyubiquitination and proteasomal degradation, and the levels of α2 (but not α1) were upregulated in tissues from *Ube2o*$^{-/-}$ mice. The human colon carcinoma line HCT116 grew less rapidly in mouse xenografts when UBE2O was knocked down using short hairpin RNA (shRNA), and this was reversed by concurrent knockdown of AMPK-α2 but not -α1. In humans, the *UBE2O* gene is located at 17q25, which is amplified in around 20% of breast, bladder, liver, and lung carcinomas. Using immunohistochemistry of human breast tumors, there was a negative correlation between expression of UBE2O and AMPK-α2, but a positive correlation between UBE2O expression and S6 phosphorylation, a marker for the mTORC1 pathway (Vila et al. 2017).

## 6. EVIDENCE FOR TUMOR-PROMOTER ROLES FOR AMPK

Despite the results described in the previous section, there is also evidence that, in certain contexts, AMPK can promote cancer by protecting tumor cells against stress. In one study, T-ALL was initiated in vitro by expressing mutant Notch1 in hematopoietic progenitor cells carrying a floxed AMPK-α1 gene (*Prkaa1*$^{fl/fl}$) and a tamoxifen-inducible Cre recombinase gene. The cells were amplified in irradiated mice and transferred to secondary irradiated recipients. After a period of time to allow engraftment, the mice were then treated with tamoxifen. Knocking out AMPK after establishment of T-ALL in this way resulted in a lower recovery of T-ALL cells and less severe disease (Kishton et al. 2016). Thus, while knocking out AMPK before development of disease accelerated onset and severity of T-ALL as described in Section 5 (Vara-Ciruelos et al. 2018b), knocking out AMPK after development of disease ameliorated it (Kishton et al. 2016). The implication is that, once cancer has arisen, AMPK switches from tumor suppression to tumor promotion.

Reduced survival of AMPK-deficient human tumor cells during stress has been observed in many in vitro studies. For example, LKB1-null tumor cells, or LKB1-expressing tumor cells with AMPK-α1 knocked down using shRNA, were more susceptible to cell death induced by glucose starvation or extracellular matrix detachment, suggesting that AMPK activation protected against these insults (Jeon et al. 2012). In another example, a synthetic lethal small interfering RNA screen was carried out to detect protein kinases required for survival of U2OS cells that overexpressed the Myc oncogene from a tamoxifen-inducible promoter. One of the top hits was AMPK-α1, which was also shown to be activated during Myc overexpression (Liu et al. 2012).



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One recent study, which utilized a genetically engineered, autochthonous mouse model of non-small-cell lung cancer, also concluded that AMPK acted as a tumor promoter by enhancing tumor cell survival (Eichner et al. 2019). Mice were generated in which Lox-Stop-Lox alleles of the oncogenic G12D mutant of KRas were combined with homozygous floxed alleles of *Tp53*, *Stk11*, or both *Prkaa1* and *Prkaa2* (encoding p53, LKB1, AMPK-α1, and -α2). In these mice, administration by nasal inhalation of lentiviral vectors encoding Cre recombinase would, in a few lung epithelial cells that were infected, cause expression of the KRas oncogene while knocking out p53, LKB1, or AMPK. Knockout of LKB1 enhanced tumor growth in tumors expressing mutant KRas, but by contrast, knockout of both AMPK-α1 and -α2 was found to cause reductions in the size and number of lung tumors, especially in tumors expressing mutant KRas and lacking p53. Overall, these results confirm that LKB1 is a tumor suppressor in non-small-cell lung cancer as expected, while the presence of either AMPK-α1 or -α2 promoted tumor growth (Eichner et al. 2019). This model represents a situation where loss of AMPK function would presumably have occurred simultaneously with tumorigenesis.

## 7. EVIDENCE FOR DISTINCT ROLES OF AMPK-α1 AND -α2 FROM ANALYSIS OF HUMAN CANCER GENOMES

One way to investigate human cancers is to analyze genetic changes in genes of interest using the cBioPortal database, which allows interrogation of the numerous whole-genome comparisons of human tumors and normal tissue that have been performed (Cerami et al. 2012, Gao et al. 2013). **Figure 1** shows analysis of alterations in the *STK11*, *PRKAA1*, *PRKAA2*, and *PRKAB2* genes, encoding LKB1, AMPK-α1, -α2, and -β2, respectively. The frequencies of genetic alterations in the other genes encoding AMPK subunits (*PRKAB1*, *PRKAG1*, *PRKAG2*, and *PRKAG3*), while still significant, were generally lower (not shown).

Since LKB1 is a well-established tumor suppressor, you would expect to see either mutations or deletions occurring in cancer. **Figure 1** shows that this generally is the case, although there are a few interesting exceptions where *STK11* is amplified. Confirming earlier reports (Ji et al. 2007, Sanchez-Cespedes et al. 2002), mutations in the *STK11* gene are particularly common in lung cancer, occurring in 15–20% of adenocarcinomas, 12% of non-small-cell cancers, and 10% of small-cell lung cancers.

A striking feature of the genetic alterations in the *PRKAA1* and *PRKAA2* genes (**Figure 1**) is that while the former is quite frequently amplified, suggesting a tumor-promoter function, the latter is more often mutated, suggesting a tumor-suppression function. Interestingly, following transformation with $HRas^{G12V}$ in vitro, mouse embryo fibroblasts from *Prkaa2*-null mice grew much more rapidly as allografts in vivo than wild-type cells, while cells from *Prkaa1*-null mice hardly grew at all (Phoenix et al. 2012). Thus, in this model, AMPK-α2 appeared to act as a tumor suppressor, while AMPK-α1 was necessary for tumor growth. Amplification of *PRKAA1* is particularly frequent in human lung adenocarcinomas, where LKB1 is also frequently mutated. At first this seems anomalous, given that LKB1 is required for AMPK activation in response to energy stress (Hawley et al. 2003). One caveat is that gene amplifications usually affect whole segments of chromosome rather than single genes, so it was possible that the *PRKAA1* gene was adjacent to an oncogene whose amplification was being selected for, with *PRKAA1* just being an innocent passenger. Arguing against this, however, is an analysis of concurrent genetic changes. For example, in 230 cases of lung adenocarcinoma (Cancer Genome Atlas Res. Netw. 2014), *PRKAA1* was amplified in 10% of cases, while *STK11* was mutated in 19%. However, these genetic changes were entirely mutually exclusive (**Figure 2**), whereas some overlap would be expected if the genes were acting independently ($P = 0.005$). By contrast, the *TP53* gene was mutated in almost every

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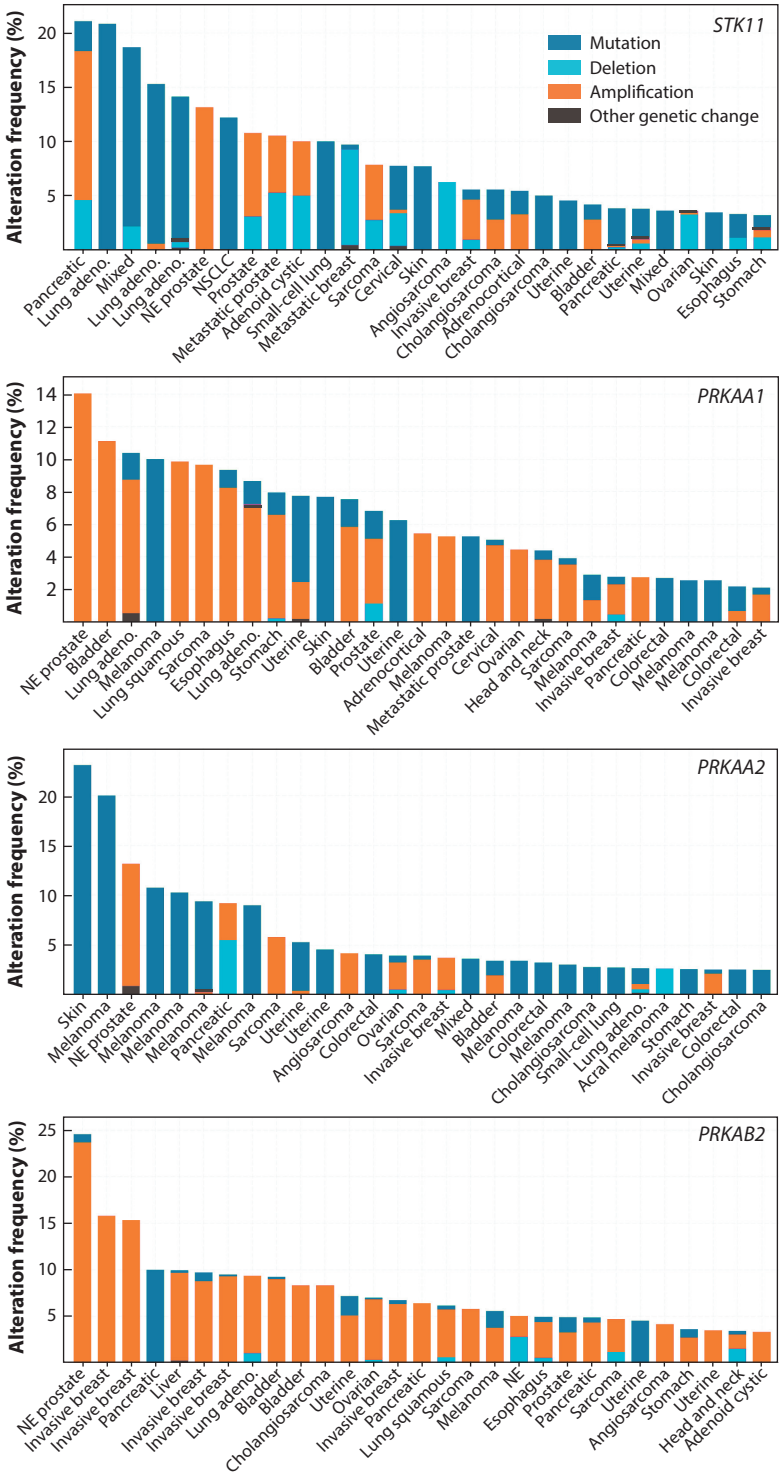


**Figure 1**

Genetic changes in AMP-activated protein kinase (AMPK) genes. The figure was generated using the cBioPortal database (Cerami et al. 2012, Gao et al. 2013) in early April 2019 using the "curated set of non-redundant studies" and the gene name given at the top right of each panel. Each vertical bar represents results from an individual cancer genome study, and only studies where the alteration frequency exceeded a threshold (2% or 3%, depending on the gene) are shown. Abbreviations: adeno., adenocarcinoma; NE prostate, castration-resistant prostate cancer with neuroendocrine features; NSCLC, non-small-cell lung cancer.

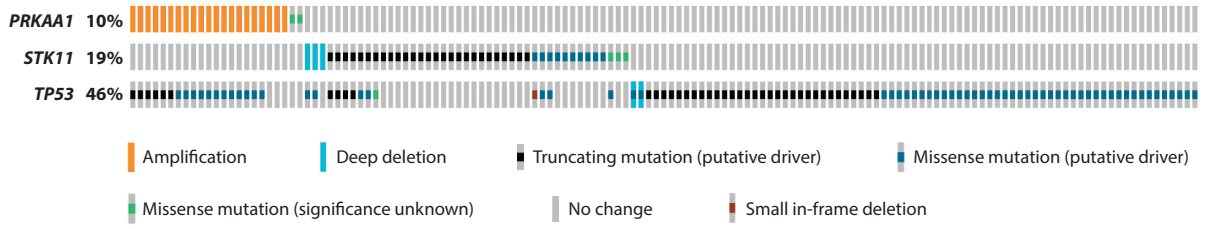


**Figure 2**

Co-occurrence of genetic changes in *PRKAA1*, *STK11*, and *TP53* in 230 cases of lung adenocarcinoma in the Cancer Genome Atlas (Cancer Genome Atlas Res. Netw. 2014). The figure was generated using the cBioPortal database (Cerami et al. 2012, Gao et al. 2013).

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case where *PRKAA1* was amplified, a co-occurrence that was also significant ($P < 0.001$). Overall, this suggests that *PRKAA1* amplification is being selected for in lung adenocarcinomas with normal LKB1 but mutant p53. Why *PRKAA1* amplification should only occur in tumors expressing normal LKB1 is fairly obvious, since there would not be much point in overexpressing AMPK-α1 if LKB1 were not available to activate it. However, why *PRKAA1* amplification should be selected for in p53-mutant tumors is less clear. The classical role of p53 is to protect cells by being upregulated during DNA damage and triggering either G1 arrest, so that the damage may be repaired, or apoptosis if the damage is severe (Speidel 2015). A possible clue to the co-occurrence of p53 mutations and AMPK-α1 amplification comes with recent findings that AMPK complexes containing the α1, but not the α2, isoform of AMPK are activated in the nuclei of various tumor cells in response to DNA damage induced by the anticancer drug etoposide (Vara-Ciruelos et al. 2018a). AMPK activation was also found to protect cells from death induced by etoposide, most likely because it causes G1 arrest and thus limits entry of cells into S phase, where they are particularly vulnerable to DNA damage. Thus, both p53 and AMPK are either upregulated or activated by DNA damage, and both protect cells by inhibiting entry into S phase. It is therefore possible that overexpression of AMPK-α1 may compensate for lack of p53 in *TP53*-mutant tumors, thus enhancing tumor cell survival.

Turning now to the gene encoding AMPK-α2, inspection of **Figure 1** shows that the top six cancers with the most frequent mutations in *PRKAA2* were all skin cancers or melanomas, where the frequency ranged from 10% to 22%. The reason for this preponderance of *PRKAA2* mutations in skin cancer is not clear, although it may have something to do with the relative expression of AMPK-α1 versus -α2 in the tumor progenitor cells. Comparison of point mutations within the coding regions of the *PRKAA1* and *PRKAA2* genes in studies of skin cancer and melanoma in the current database shows that there were only 10 mutations in *PRKAA1*, but no less than 80 in *PRKAA2*. The much higher burden of mutations in the *PRKAA2* gene in skin cancer suggests that it is being selected for loss-of-function mutations, i.e., that AMPK-α2 might be acting as a tumor suppressor in this disease.

Even more striking than the amplification of *PRKAA1* in cancers was the amplification of *PRKAB2*, encoding the AMPK-β2 subunit (**Figure 1**). In castration-resistant prostate cancer with neuroendocrine features (Beltran et al. 2016), *PRKAB2* was amplified in almost 25% of cases, and *STK11*, *PRKAA1*, and *PRKAA2* (and other AMPK genes, not shown) were also frequently amplified. These results suggest that AMPK might be a tumor promoter in that context.

## 8. CONCLUSIONS AND PERSPECTIVES

In summary, the available evidence suggests that AMPK can act as either a tumor suppressor or a tumor promoter, depending on context. We believe that the contrasting studies of different mouse

models of T-ALL are particularly instructive, with genetic loss of AMPK before the tumors arise exacerbating the disease (Vara-Ciruelos et al. 2018b), while loss of AMPK after the disease becomes established ameliorates it (Kishton et al. 2016). Before the disease arises, AMPK may oppose the switch to transformed lymphoblasts by inhibiting mTORC1 and other anabolic pathways, and by promoting oxidative metabolism rather than the glycolytic metabolism typical of proliferating cells. However, once cancer has arisen, AMPK may switch to being a tumor promoter because, while it might paradoxically slow the growth and proliferation of the tumor cells during nutrient and oxidative stress, it would also render them more likely to survive. A corollary is that while AMPK activators may provide protection against the development of cancer, AMPK inhibitors might be indicated to treat cancer after it has arisen. An example of the former is provided by the ability of oral phenformin to protect against development of T-ALL in an AMPK-dependent and cell-autonomous manner (Vara-Ciruelos et al. 2018b). Unfortunately, we do not yet have any specific inhibitors of AMPK that are well characterized. Although compound C (also known as dorsomorphin) has been quite widely used in the literature, it is far from a specific inhibitor of AMPK (Bain et al. 2007) (see also the Related Resources section) and its use is not recommended. Other AMPK inhibitors have been reported (Dite et al. 2018, Scott et al. 2015), but have not yet been widely used.

Why some AMPK genes (e.g., *PRKAA1*, *PRKAB2*) should be frequently amplified in human cancers, while others (e.g., *PRKAA2*) are more commonly mutated (**Figure 1**), remains unclear. However, answers to this question may be crucial in the design of new drugs, e.g., by developing compounds specific to certain isoform combinations.

There is currently no direct evidence that AMPK inhibitors would be efficacious in treating cancer. However, if (as we suspect) the amplification of AMPK genes in some cancers has been selected for because this enhances survival of tumor cells, then AMPK inhibitors may be particularly useful for treatment of those cases where these gene amplifications have occurred. In addition, since AMPK can protect cells against death induced by DNA damage, such as that caused by etoposide (Vara-Ciruelos et al. 2018a), AMPK inhibitors might be expected to enhance the efficacy of conventional cytotoxic chemo- and radiotherapies.

## SUMMARY POINTS

1. The AMP-activated protein kinase (AMPK) system senses cellular stress and acts to restore energy homeostasis by switching on alternate catabolic pathways and switching off cell growth and proliferation.
2. Before tumors have arisen, AMPK appears to act as a tumor suppressor by suppressing cell growth and proliferation, inhibiting mTORC1, and promoting the oxidative metabolism typical of quiescent rather than proliferating cells.
3. After tumors have arisen, AMPK may switch to being a tumor promoter, most likely by enhancing survival of tumor cells during stressful situations.
4. Analysis of genetic changes in human cancers supports these diverging roles of AMPK in cancer.
5. AMPK activators, such as phenformin, may be effective for prevention of cancer in high-risk individuals, whereas AMPK inhibitors may be effective in cancer treatment once it has arisen.

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## FUTURE ISSUES

1. Why are the genes encoding AMPK-α1 and -β2 often amplified in human cancers, whereas the gene encoding AMPK-α2 is more frequently mutated?
2. Are AMPK inhibitors effective in treatment of preexisting cancer, particularly in cases where AMPK genes have been amplified?
3. Do AMPK inhibitors synergize with conventional genotoxic cancer treatments (chemo- or radiotherapy)?

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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**Found that LKB1 represses expression of Snail1, which promotes epithelial-to-mesenchymal transition, invasion, and metastasis, via an AMPK-independent mechanism.**

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## RELATED RESOURCES

cBioPortal: **http://www.cbioportal.org/**. A database of genetic changes in human cancers.

MRC Kinase Profiling Inhibitor Database: **http://www.kinase-screen.mrc.ac.uk/kinase-inhibitors**. A database containing updated information about the specificity of kinase inhibitors, including compound C (dorsomorphin).

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