AMPK in cancer: Tumor friend or foe?

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Background

AMP-activated protein kinase (AMPK) is a downstream target of the tumor suppressor and serine/threonine kinase, LKB1. LKB1 phosphorylates conserved threonine residues in the activation loops not only of AMPK but also of several AMPK-related kinases, activating all of them. AMPK (but not the AMPK-related kinases) is switched on in an LKB1-dependent manner by energy stress, and acts to conserve ATP by switching off biosynthetic pathways and progress through the cell cycle, while switching on ATP-producing catabolic pathways. In the longer term, it promotes the more energy-efficient oxidative catabolic pathways utilized by quiescent cells, rather than the glycolytic catabolism typical of cells undergoing rapid growth and proliferation. AMPK is, therefore, potentially a "friend" in cancer by inhibiting cell growth and proliferation and opposing the metabolic changes in tumor cells, and may mediate many of the tumor suppressor effects of LKB1. However, there would also be selection pressure for the pathway to be downregulated in tumor cells and this can indeed occur, either by genetic loss of LKB1 or by other mechanisms. Paradoxically, in those cases where the pathway has NOT been down-regulated, it may act as a "foe" in cancer by enhancing survival of the tumor cells during treatment with cytotoxic agents. These concepts are discussed below.

Discussion

Ten years ago, searches for the upstream kinase that activated AMPK resulted in identification of LKB1 (1, 2), while a search for downstream targets of LKB1 resulted in the reciprocal identification of AMPK (3). This provided direct links between AMPK, an energy-sensor known to regulate cell metabolism, and LKB1, the product of a tumor suppressor gene in which heterozygous mutations cause a human cancer susceptibility called Peutz-Jeghers syndrome. AMPK is activated by phosphorylation of a conserved threonine residue (Thr172) within the activation loop of the kinase domain. In intact cells, this occurs in response to stresses causing increases in cellular ADP:ATP and AMP:ATP ratios. LKB1 appears to be constitutively active (4), but Thr172 is rapidly dephosphorylated such that only a small proportion of AMPK remains in its active, phosphorylated form under basal conditions (5). However, binding of AMP to AMPK during energy stress enhances Thr172 phosphorylation, while binding of either AMP or ADP inhibits Thr172 dephosphorylation (5). These dual effects cause a sensitive switch to the phosphorylated form, and the activation this causes is amplified by a third effect of AMP binding, a >10-fold allosteric activation (5). Thus, although LKB1 itself does not appear to be regulated (4), it is required for AMPK activation during energy stress.
Stimuli that activate AMPK include stresses inhibiting ATP production such as hypoxia or glucose deprivation, and stresses accelerating ATP turnover such as muscle contraction (6). AMPK is also activated by the drug metformin (7), currently the primary therapy for treatment of Type 2 diabetes, because it causes a mild inhibition of the mitochondrial respiratory chain (8). Although metformin has AMPK-independent effects (9), recent studies in mice suggest that its ability to improve insulin sensitivity is mediated by effects of AMPK to reduce cellular lipid stores (10).

Once activated, AMPK promotes catabolic pathways that generate ATP, while switching off cell cycle progress (11, 12) and most anabolic pathways that consume ATP, including biosynthesis of lipids, proteins and RNA required for cell growth (6). Thus, it exerts a cytostatic effect that limits cell growth and proliferation. This may explain some of the tumor suppressor effects of LKB1, although the AMPK-related kinases may also contribute. However, consistent with an important role for AMPK are recent findings that genetic loss of the AMPK-α1 isoform in mice accelerates the development of lymphomas induced by Myc over-expression in B cells (13). This was associated with a metabolic shift towards aerobic glycolysis, consistent with the idea that AMPK activation favors the more energy-efficient oxidative metabolism used by quiescent cells, rather than the glycolytic metabolism (the Warburg effect) often utilized during rapid cell growth.

Since AMPK activation exerts a cytostatic effect, tumor cells in which the pathway has been downregulated would have a growth advantage and might undergo positive selection. The commonest cause of downregulation appears to be genetic loss of LKB1, which occurs frequently in lung and cervical cancer (14–16). Mutations in the AMPK subunits themselves appear to be less common, although expression of the α2 isoform is frequently downregulated in hepatocellular carcinoma (17), while in tumor cells where the Akt pathway is activated due to loss of PTEN, AMPK is downregulated because Akt phosphorylates AMPK-α1 at a site that inhibits subsequent phosphorylation at Thr172 (18).

The original finding of the LKB1-AMPK link led to retrospective analyses suggesting that cancer incidence was lower in diabetics taking metformin rather than other medications (19). It is currently unclear whether this is mediated by AMPK activation and, even if it is, whether it is a direct effect on the tumor cells themselves or an indirect effect due to lowering of plasma insulin and/or glucose, through the insulin-sensitizing effects of metformin on the liver (20). A recent study found that phenformin (a biguanide drug related to metformin) was more effective in treatment of a mouse lung cancer model in which the AMPK pathway had been rendered non-functional by genetic ablation of LKB1 (21). In this case phenformin, which is more cell-permeable and a more potent mitochondrial inhibitor than metformin (8), may be acting as a cytotoxic agent that induces cell death in the tumors because, unlike surrounding normal cells, they lack a functional LKB1-AMPK pathway to protect them against mitochondrial inhibition. Thus, while the pathway may be a tumor suppressor and "friend" in cancer by restricting the growth of tumor cells, in some circumstances it may, paradoxically, be a "foe" in that tumors that have retained a functional AMPK pathway may be harder to kill using cytotoxic agents. In such cases, AMPK inhibitors might even be useful adjuncts to cytotoxic therapies.

LKB1 and AMPK Signaling in Cancer
Future Directions

Given the evidence that AMPK exerts at least some of the tumor suppressor effects of LKB1, the testing of AMPK activators for the prevention and/or treatment of cancer is an exciting prospect. There are currently over 100 patents for novel compounds that activate AMPK, while there are already close to 200 clinical trials of metformin in cancer listed on the ClinicalTrials.gov website. Many are small scale pilot trials, but there is one large phase 3 trial for early stage breast cancer, scheduled for completion in 2016, where metformin or placebo are being administered for six years as an adjuvant therapy (22).

As discussed in the previous section and shown in Fig. 1, there are at least three possible mechanisms to explain the apparent protective effect of metformin in cancer: (i) a direct cytostatic effect via activation of AMPK in tumor cells; (ii) a cytotoxic effect due to ATP depletion in neoplastic cells that have lost the LKB1-AMPK pathway, while surrounding normal cells are protected; (iii) by an indirect mechanism on the liver. In the latter case, the drugs activate AMPK and reverse liver insulin resistance by reducing lipid storage (10), thus reducing hepatic glucose output. The reduced hepatic glucose output in turn reduces insulin secretion from the β cells of the pancreas, and thus reverses the hyperinsulinemic (and hyperglycemic) environment of the preneoplastic cells that might otherwise promote their growth and proliferation.

Fig. 1. Three potential mechanisms by which the biguanide drugs metformin or phenformin reduce the incidence of cancer: (i) they activate AMPK in preneoplastic cells, exerting a cytostatic effect; (ii) by inhibiting the mitochondrial respiratory chain, they deplete ATP and selectively kill neoplastic cells that have lost the LKB1-AMPK pathway, while surrounding normal cells are protected; (iii) by an indirect mechanism on the liver. In the latter case, the drugs activate AMPK and reverse liver insulin resistance by reducing lipid storage (10), thus reducing hepatic glucose output. The reduced hepatic glucose output in turn reduces insulin secretion from the β cells of the pancreas, and thus reverses the hyperinsulinemic (and hyperglycemic) environment of the preneoplastic cells that might otherwise promote their growth and proliferation.
depletion that kills tumor cells in which the LKB1-AMPK pathway has undergone changes that reduce or eliminate its function; (iii) an indirect effect of metformin in the liver to lower plasma glucose (and hence insulin release from the pancreas) thus reducing the hyperglycemic and hyperinsulinemic environment of preneoplastic cells. These mechanisms are not mutually exclusive, although (i) and (ii) would not co-exist in the same tumor. There is already some support for mechanisms (ii) and (iii) from mouse xenograft studies (20), and for mechanism (ii) from a genetically-engineered mouse model (21), although both studies were performed using models in which LKB1, rather than AMPK, was ablated. Further work is warranted in which AMPK itself is knocked out specifically in the cells that give rise to the tumors. If mechanism (i) is correct, metformin/phenformin should only enhance tumor-free survival when the tumors still express a functional LKB1-AMPK pathway, whereas if mechanism (ii) is correct they will enhance survival only in tumors that have lost the pathway. Since metformin or phenformin do not lower plasma glucose in animals with normal insulin sensitivity, mechanism (iii) would be supported if the drugs enhanced survival to a greater extent in mice that were insulin-resistant than in insulin-sensitive controls. Distinguishing between these mechanisms in mouse models would provide valuable information to aid the design of future clinical trials of metformin, phenformin and other AMPK activators in humans.

Finally, it has recently been shown that AMPK is activated in cell-free assays and in vivo by salicylate, which binds to the same site as a synthetic activator, A-769662 (23). Salicylate is the natural plant product from which the synthetic drug aspirin (acetyl salicylate) was developed, and regular use of aspirin, like metformin, is associated with a reduced incidence of cancer (24). Aspirin is a potent inhibitor of the cyclo-oxygenases involved in prostaglandin synthesis, and it has been generally assumed that its protective effects in cancer are connected with that. However, when aspirin is taken orally it is broken down within minutes to salicylate, which has a half-life of many hours and whose peak plasma concentration is much higher than that of aspirin. This raises the interesting possibility that some of the anticancer effects of aspirin might be mediated by activation of AMPK by salicylate, rather than by inhibition of cyclo-oxygenases by aspirin itself.

References


