The LKB1-AMPK Pathway—Friend or Foe in Cancer?

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Use of the biguanide metformin, an AMPK activator, is associated with a reduced incidence of cancer in diabetics, but it has been unclear whether this requires AMPK. In this issue of *Cancer Cell*, Shackelford and colleagues show, paradoxically, that biguanides are more effective in the treatment of mouse tumors that lack a functional LKB1-AMPK pathway.

Ten years ago, LKB1 was found to be the crucial upstream kinase required for activation of AMP-activated protein kinase (AMPK) (reviewed in Hardie, 2004). This finding placed a protein kinase known to be a tumor suppressor (LKB1) upstream of a kinase involved in regulation of metabolism in response to energy stress (AMPK). AMPK was already known to be activated by the anti-diabetic drug metformin. Putting these findings together, Morris and Alessi analyzed retrospective data from a cohort of patients with type 2 diabetes (T2D) and found that use of metformin rather than other medications was associated with a 30% lower cancer incidence (Evans et al., 2005). This association has since been observed in other diabetic cohorts, but the mechanism by which metformin might protect against cancer has been the subject of much debate. In a study reported in this issue of *Cancer Cell*, Shackelford et al. (2013) use a mouse model to show that the related drug phenformin appears, paradoxically, to be more effective in the treatment of non-small cell lung cancer (NSCLC) if the tumors lack a functional LKB1-AMPK pathway, suggesting that the latter can sometimes be a ''foe'' rather than a ''friend'' in cancer.

Metformin and phenformin, first used over 50 years ago for the treatment of T2D, are related biguanide drugs. Phenformin was withdrawn in most countries because of the serious but rare side effect of lactic acidosis, whereas metformin has become the drug of first choice in T2D. Both are cations that accumulate in mitochondria due to the charge gradient across the inner membrane, where they inhibit complex I of the respiratory chain (Owen et al., 2000). This causes depletion of cellular ATP and increases ADP and AMP, which is why they activate AMPK (Hawley et al., 2010). However, given the ubiquitous role of these nucleotides in cell function, it is not surprising that many AMPK-independent effects are now being documented.

At least three mechanisms can be proposed to explain the effects of metformin on cancer (Figure 1). Mechanism 1 is based on its known insulin-sensitizing and anti-hyperglycemic effects. Humans with T2D have an increased cancer incidence, and their high plasma insulin and/or glucose levels, which provide a cellular environment conducive to tumor growth, might be the culprits. By reversing hyperglycemia, and hence hypersecretion of insulin, metformin lowers plasma insulin as well as glucose. This could account in part for the protective effects of metformin in diabetics, but it cannot explain the effects in rodent models where the animals were not insulin-resistant. Two alternative hypotheses, both involving direct effects of metformin on tumor cells themselves, can be proposed in such cases. In mechanism 2, metformin activates AMPK in the tumor or pre-tumor cells, restraining their growth and proliferation. In addition, AMPK has been shown to oppose the Warburg effect, the switch from oxidative metabolism to glycolysis commonly observed in tumor cells (Faubert et al., 2013). Mechanism 3 is based on findings that LKB1-deficient cells are more prone to apoptosis in response to metabolic stress (Shaw et al., 2004) and that metformin causes a greater depletion of ATP in cells that are AMPK-deficient and therefore have defective energy homeostasis (Foretz et al., 2010). Up to 30% of NSCLCs (Ji et al., 2007) and smaller proportions of other cancers display loss-of-function mutations in LKB1. In such tumors, the ATP-depleting properties of metformin might promote cell death.

Although it focues on phenformin rather than metformin, the new study by Shackelford et al. (2013) helps to distinguish between mechanisms 2 and 3. Starting with a K-Ras mutant and LKB1 null NSCLC cell line, they found that phenformin had a larger effect than metformin on ATP levels, while only phenformin triggered apoptosis. Similar results were obtained in other NSCLC lines, where apoptosis triggered by phenformin correlated strictly with LKB1 loss but not with other mutations. They went on to use a mouse model of NSCLC in which expression of mutant K-Ras, either alone or combined with loss of either LKB1 or p53, was selectively triggered in lung epithelial cells by intratracheal administration of viral vectors encoding Cre recombinase (Ji et al., 2007). As observed previously, the tumor burden was greatly increased when mutant K-Ras was combined with the loss of LKB1 or p53. In the tumors that still expressed LKB1, phenformin caused more AMPK phosphorylation than metformin, so further experiments focused on phenformin (although these results did confirm that both drugs were reaching the tumor cells and causing energy stress). They next tested two protocols, one giving phenformin in drinking water 6 weeks after tumor initiation and the other by daily oral gavage after 3 weeks. In the former, phenformin seemed to cause modest reductions in tumor burden and increased markers of tumor apoptosis in all three genotypes, although these effects were only statistically significant in the LKB1 null tumors. In the latter, phenformin enhanced overall survival of the mice by several weeks, while tumor volume and fluorodeoxyglucose uptake (a marker of

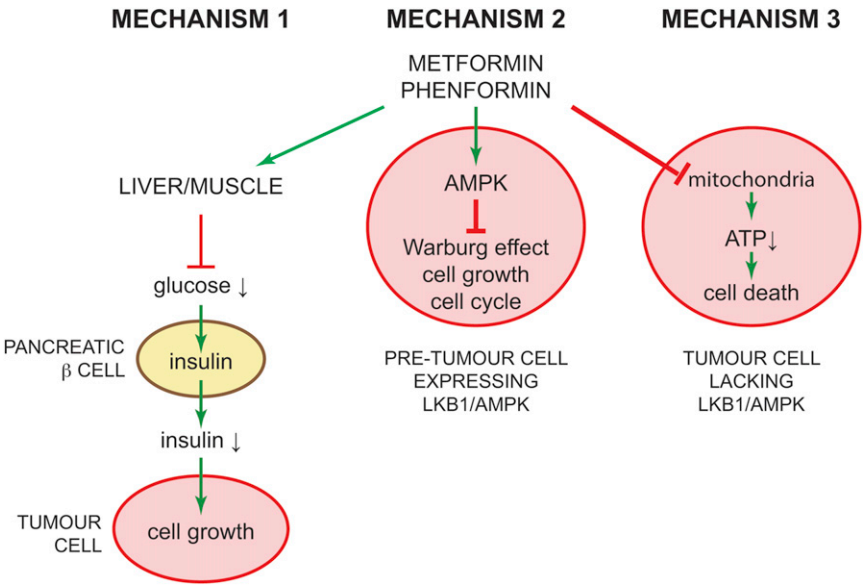

Figure 1. Three Alternate Mechanisms to Explain Protective Effects of Biguanides in Cancer
In mechanism 1, which would only operate in insulin-resistant individuals, the drugs lower plasma glucose, and thus increase the secretion of insulin from the pancreas. The consequent drop in plasma insulin and glucose reverses the favorable environment for tumor cell growth. In mechanism 2, they activate AMPK in the pretumor cell, exerting a cytostatic effect and preventing the metabolic switch to the Warburg effect. In mechanism 3, they selectively reduce ATP levels in tumor cells with a nonfunctional LKB1-AMPK pathway due to their inability to respond to energy stress, thus triggering cell death. All three mechanisms might operate in different cases of cancer, while mechanisms 1 and 2 or 1 and 3, could conceivably coexist in a single case.

the Warburg effect) were lower after 4 weeks of treatment, but, crucially, all of these effects were only seen in the tumors that had lost LKB1.

Because the mice used in these studies were not diabetic or insulin-resistant, phenformin did not reduce plasma glucose or insulin, ruling out mechanism 1. In addition, because the beneficial effects were only observed in the LKB1 null cancers, they had to be direct, cell-autonomous effects on the tumor cells themselves. Overall, the results clearly support mechanism 3 (i.e., that cells that have lost a functional LKB1-AMPK pathway are more sensitive to cell death induced by phenformin) and not mechanism 2 (i.e., that it works by activating AMPK in the tumors). In this mechanism, phenformin is effectively acting as a cytotoxic agent, but an attractive feature is that normal cells would be resistant because they have a functioning LKB1-AMPK pathway. This raises the exciting prospect that biguanides might be particularly useful for the treatment of those tumors where the LKB1-AMPK pathway is downregulated. There are mechanisms by which this happens, other than by simple genetic loss of LKB1; for example, although mutations in the LKB1 gene appear to be rare in breast cancer, immunohistochemical analyses suggest that AMPK activation is frequently downregulated (Hadad et al., 2009).

These results do not rule out the occurrence of mechanisms 1 and 2 in other situations, particularly where biguanides may be used to prevent rather than treat cancer. Another caveat is that the beneficial effects on tumor burden and fluorodeoxyglucose uptake observed after 4 weeks of phenformin treatment were no longer significant after 6 weeks, suggesting that phenformin resistance developed. This might arise as the cells start to make more of their ATP by glycolysis and become less dependent on mitochondrial function. All of the mice did also eventually succumb to cancer despite treatment, so phenformin is unlikely to be effective as a single therapy.

One of the most interesting features of this study was the focus on the use of phenformin rather than metformin. The effects of metformin on tumor burden or survival were not tested, although its smaller effects on AMPK in the tumors expressing LKB1, and its lack of effect on apoptosis in the cell culture models suggest that it would have been less effective than phenformin. A possible explanation of the greater effectiveness of phenformin is that it is more cell-permeable than metformin, and its uptake into cells is less dependent on expression of organic cation transporters (Hawley et al., 2010). Although phenformin was withdrawn for use in T2D because of cases of lactic acidosis, these were rare (<1 case per 1,000 patient years), and this frequency of side effect may be more acceptable for the treatment of cancer rather than diabetes.

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