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COMMENTARY

Metformin as an anticancer drug: A Commentary on the metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides

The prevalence of type 2 diabetes and cancer are increasing worldwide. Our readers will be well aware of that type 2 diabetes is associated with an increased risk of several types of cancers, and will be paying attention to the recent reports that the use of certain antidiabetes drugs; that is, pioglitazone, might increase the risk of certain cancers[1]. In short, diabetes and cancer have something common. What might be the mechanism(s) behind this commonness? This is an important question, as any new knowledge on these huge health problems is worth knowing; and now a common antidiabetic drug, metformin, comes center stage.

Type 2 diabetes is characterized by defects in glucose homeostasis and proper insulin action, and cancer can be described as genetic alterations compared with the normal cells that render cells to proliferate without limitation. Among the mechanisms linking diabetes and cancer, one could think of the roles of hyperinsulinemia, hyperglycemia and inflammatory cytokines, such as tumor necrosis factor-alpha, C-reactive protein and interleukin-6. However, there is no evidence supporting the long-term use of insulin increasing the risk of cancer. For cytokines and inflammation, mitochondrial dysfunction might be the link. In fact, mitochondrial dysfunction could induce a inflammatory response, which in turn might be a result of exposure to environmental pollutants. We will not discuss this issue here again, but readers are reminded that environmental pollutants could cause both type 2 diabetes and cancers[2]. How about hyperglycemia or abnormal glucose metabolism?

In living cells, glucose plays a major role to energy metabolism, taken up by specific glucose transporters (GLUT). Once inside the cell, it is converted to pyruvate through the glycolytic pathway generating a small amount of energy in the form of adenosine triphosphate (ATP). Pyruvate is then transported into the mitochondria, enters the tricarboxylic acid cycle and is oxidized through in the mitochondria respiratory chain (oxidative phosphorylation system [OXPHOS]), generating ATP. This aerobic process is a major source of energy supporting life. Mitochondria are frequently dysfunctional in type 2 diabetes, but most of the ATP in patients with type 2 diabetes is generated through OXPHOS.

Cancer cells, meanwhile, tend to synthesize more ATP through glycolysis than normal cells do. This metabolic shift to aerobic glycolysis is a hallmark of cancer, and is applied to a common clinical test for it, positron emission tomography. Recent studies have suggested that this metabolic shift could be to facilitate the uptake and incorporation of more nutrients into cell building blocks, such as nucleotides, amino acids and lipids, which are required for highly proliferating cells. Mitochondrial dysfunction in cancer cells might be behind this phenomenon, which is well appreciated after Otto Warburg proposed it could be the primary cause[3]. All in all, the mechanisms underlying the dysregulated cellular metabolism of cancer cells remain poorly understood. Whatever the mechanisms are, blocking these metabolic alterations is now emerging as a new therapeutic approach of cancer, and as such, some of the metabolic enzymes involved in the glycolytic pathway are currently considered as therapeutic targets. Glucose deprivation is currently considered as one of such therapeutic options.

Some cancer cells show different sensitivity of inhibition to cell proliferation or cell death under low-glucose culture conditions. In other words, some cancer cells use OXPHOS as major source of energy metabolism, and others are heavily dependent on glucose as a major energy source. Therefore, a better understanding of the roles of glycolysis and OXPHOS as a source of energy in cancers might be useful in developing new therapeutic agents. Then, the question boils down to the specific therapeutic target according to different aspects of the metabolic alterations of each cancer cell.

To answer some of these questions, Birsoy *et al.*[4] devised a continuous-flow culture system for maintaining proliferating cells in reduced, but steady, glucose concentrations for long periods of time. The media of a defined glucose concentration is continuously fed into a suspension culture, while spent media is removed at the same rate, creating a stable condition for long-term culture, and producing reliable results. Furthermore, the authors carried out a competitive proliferation assay with a pooled collection of 28 patient-derived cancer cell lines to determine whether all cancer cells respond similarly to long-term low-glucose culture. From the data using various cancer cell lines, they showed that

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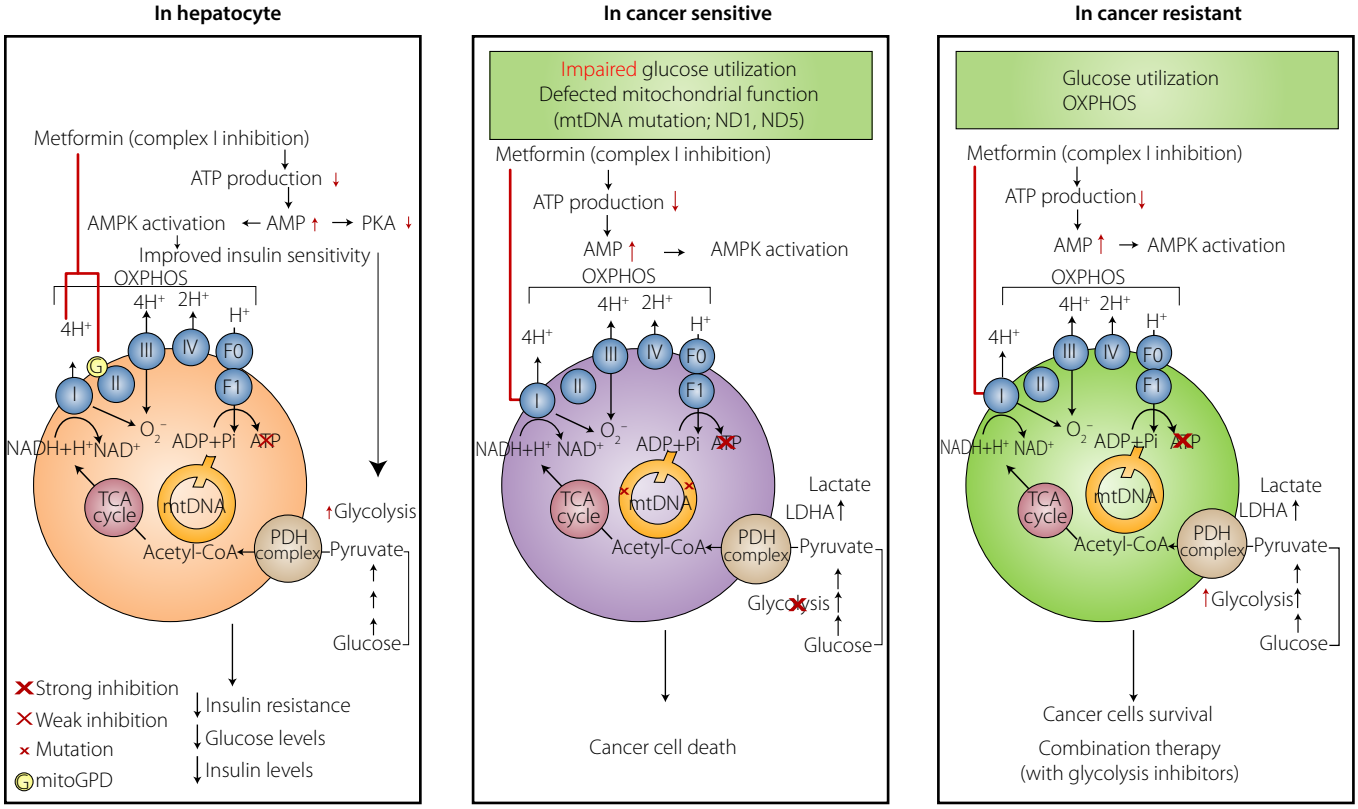

Figure 1 | Model of metformin action in the hepatocyte and cancer cells. Metformin inhibits mitochondrial complex I, blocks adenosine triphosphate (ATP) production and results in an accumulation of adenosine monophosphate (AMP), which in turn activates the glycolytic pathway. Accumulation of AMP activates AMP kinase (AMPK), which contributes to the improved insulin sensitivity. Besides, metformin suppresses mitochondrial glycerophosphate dehydrogenase (mitoGPD), which catalyzes the oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate in hepatic cells, alters the mitochondrial and cytosolic redox state, and reduces reactive oxygen species production, mechanisms linked to inhibiting gluconeogenesis. Cancer cells produce most ATP through the oxidative phosphorylation system (OXPHOS), but some ATP is generated through glycolysis. Cancer cells with deficiencies in glucose utilization or complex I are sensitive to metformin, but cancer cells without those deficiencies are not, in which the combination with glycolysis inhibitors is effective in inhibiting cancer cell growth. ADP, adenosine diphosphate; LDHA, lactic dehydrogenase; NAD, nicotinamide adenine; NADH, nicotinamide adenine dehydrogenase; PDH, pyruvate dehydrogenase; PKA, cyclic AMP-dependent protein kinase; TCA, tricarboxylic acid.

the difference of sensitivity in response to low glucose did not correlate with known oncogenic mutation(s).

To investigate the metabolic processes that mediate the response to glucose limitation, the authors also used a pooled ribonucleic acid (RNA) interference screen of 2,752 human metabolic enzymes and small molecule transporters (total 15,997 short hairpin RNA). They identified 64 genes whose suppression preferentially inhibited cell proliferation in high (28 genes) or low (36 genes) glucose through the experiment. What they found was that genes for OXPHOS function and encoding the GLUT1 glucose transporter were required to survive in low-glucose conditions, suggesting that the glucose transporter and OXPHOS are key metabolic processes required for optimal proliferation of cancer cells under glucose limitation.

The biguanide class of drugs, including metformin and the more potent phenformin, have been known as inhibitors of mitochondrial OXPHOS (complex 1). When Birsoy *et al.*[4] applied treated cancer cells with biguanides, the drug was found to be more effective in low-glucose sensitive cancer cell lines. The authors claim these results suggest better strategies for cancer therapy than the previously proposed combined inhibition of OXPHOS and glycolysis[5]. Furthermore, the results render the importance of considering glucose concentrations when evaluating the sensitivity of cancer cells to biguanides or other OXPHOS inhibitors.

Diabetologists have been using metformin in the treatment of diabetes since 1975. It has gained attention for its pleiotropic effects, including its anticancer effect, which has been well documented; for example, in the case of polycystic ovary syndrome (PCO)[6]. Metformin treatment markedly improves the insulin resistance of PCO patients, but also prevents development of endometrial cancer,

which develops almost 10-fold more than in the control subjects. From the study of Birsoy *et al.*[4], we now know why therapeutic use of metformin might inhibit cancer development in patients with type 2 diabetes: it might reduce development of cancer by inhibiting OXPHOS.

This line of reasoning raises another important question: if subjects with type 2 diabetes have mitochondrial dysfunction, and mitochondrial dysfunction is involved in the pathogenesis of cancer, why would further inhibition of mitochondrial function with metformin prevent cancer development, rather than enhance it?

We might get a clue again from the study of Birsoy *et al.*[4]; they reported that the anticancer effects of metformin are dependent on glucose utilization and the type of mitochondrial (dys)function of cancer cells. In low-glucose media, cell lines with mitochondrial deoxyribonucleic acid (mtDNA) encoded complex I mutations or impaired glucose utilization were more sensitive to phenformin compared with control cancer cell lines. Interestingly, overexpression of GLUT3 almost overcomes the effects of phenformin on proliferation and oxygen consumption of cells with impaired glucose utilization. Besides, phenformin sensitivity is restricted to cells with intermediate levels of mitochondrial dysfunction in cancer cells. Cells lacking mtDNA (143B Rho), thus with severe mitochondrial dysfunction, are insensitive to phenformin, but sensitive to low glucose. Therefore, the authors suggested that the glucose-utilization gene signature described earlier and the mutation in mtDNA-encoded complex I subunits might be used as biomarkers for identifying tumors to metformin treatment. As metformin accumulates in the inside of mitochondria and inhibits complex 1 of the mitochondrial respiratory chain, it might inhibit the development of cancer in those subjects with certain mitochondria.

Very recently, Madiraju *et al.*[7] reported that metformin inhibits mitochondrial glycerophosphate dehydrogenase (mitoGPD), and thus alters the mitochondrial and cytosolic redox state, and reduces reactive oxygen species production. It is not clear how metformin inhibits complex 1 and mitoGPD or if the two mechanisms are interrelated. We are fully aware that there are other possible mechanisms for the anticancer effect of metformin, including stimulation of adenosine monophosphate-activated protein kinase (AMPK) and its upstream regulator, liver kinase B1 (LKB1), although they could well be secondary to its inhibitory effect on the mitochondrial function and the reduction of free radicals through inhibition of mitoGPD, as suggested by Madiraju *et al.*[7]. Figure 1 summarizes these complex relationships.

Understanding the aberrant mechanisms of cancer energy metabolism through the mass analysis of genetic and metabolic features using various cancer cell lines will be of great interest to those scientists developing new therapeutics for cancer. Furthermore, cancers are very heterogeneous in nature and they are constantly evolving[8]. Although one could not classify cancers simply according to their energy metabolism, the results of Birsoy *et al.*[4] clearly show that the mitochondrial state of cancer cells is important in cancer therapeutics, and thereby helps diabetologists in improving the overall health of their patients, including the prevention of cancers.

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DISCLOSURE

The authors declare no conflict of interest.

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