Cancer's sweet tooth

Even in the presence of an adequate oxygen supply, many tumors metabolize the majority of the glucose they take up through glycolysis. It has been a long-held belief that this glycolytic phenotype is due to cancer-specific defects in mitochondrial oxidative phosphorylation. In this issue of *Cancer Cell*, Fantin et al. now report that most tumor cells have a substantial reserve capacity to produce ATP by oxidative phosphorylation when glycolysis is suppressed. These new data add to mounting evidence that the high rate of glycolysis exhibited by most tumors is required to support cell growth rather than to compensate for defect(s) in mitochondrial function.

Before the introduction of free oxygen into the atmosphere, life on earth depended on glycolysis for energy production. With the rise of atmospheric oxygen, cells evolved the ability to use oxidative phosphorylation (OXPHOS) to produce more energy per metabolite than the more ancient anaerobic pathway. Most present day cells have developed the ability to differentially utilize these two ATP-generating pathways depending on physiologic circumstances. Pasteur was the first to observe the reciprocal relationship between these processes when he found that glycolysis was inhibited in the presence of oxygen (Pasteur, 1861). In contrast, Warburg observed that some tumors preferentially utilized glycolysis instead of OXPHOS in the presence of oxygen (Warburg, 1930). Since Warburg's pioneering studies, aerobic glycolysis has been observed in a wide variety of cancers. The high rate of glucose utilization by such tumors can be visualized clinically by positron emission tomography (PET) imaging of tumor uptake of ^{18}F-2-deoxyglucose. Given the inefficiency of glycolysis in producing ATP, the high rate of glycolysis by tumor cells has long puzzled cancer biologists. In this issue of *Cancer Cell*, Fantin et al. provide new insight into this problem by examining the effects of lactate dehydrogenase A (LDH-A) suppression on cancer cell metabolism, growth, and tumorigenicity (Fantin et al., 2006).

The enzyme LDH mediates a redox reaction at the end of glycolysis in which pyruvate is reduced to lactate when the cytosolic NADH/NAD$^+$ ratio is high. This reaction permits the regeneration of NAD$^+$, needed as an electron acceptor to maintain cytosolic glucose catabolism. To sustain glycolysis when LDH-A is suppressed, the cells must compensate by oxidizing glycolytic NADH through the electron transport chain. Fantin et al. found that LDH-A inhibition in *neu*-initiated mammary epithelial tumor cell lines resulted in enhanced OXPHOS. The data demonstrate that the glycolytic shift in these tumor cells is not due to a defect in the ability to carry out oxidative phosphorylation. In fact, the efficiency with which the tumor cell mitochondria produced ATP through electron transport chain activity was identical to the ATP production efficiency of mitochondria from nontransformed epithelial cells.

Although there was a compensatory increase in mitochondrial respiration, Fantin et al. observed that inhibition of LDH-A suppressed the proliferative and tumorigenic potential of cancer cells. There are several potential explanations for these observations that are in keeping with the hypothesis that a high rate of glycolysis is advantageous to growing cells. First, although OXPHOS produces more energy per molecule of glucose than glycolysis, glycolysis is capable of producing ATP considerably faster than OXPHOS as long as glucose supplies are unlimited. Growing cells have an enormous demand for ATP to fuel their growth, and glycolysis is much better suited to meeting this demand (Pfeiffer et al., 2001). In contrast, the rate at which ATP can be generated from glucose by oxidative metabolism is limited by the slow rate of NADH shuttling from the cytosol to the mitochondrial electron transport chain (Figure 1). Consistent with this, when LDH-A was suppressed, the compensatory increase in oxidative phosphorylation was unable to keep up with the cancer cell's metabolic demands, and both the mitochondrial membrane potential and ATP levels of the cell declined.

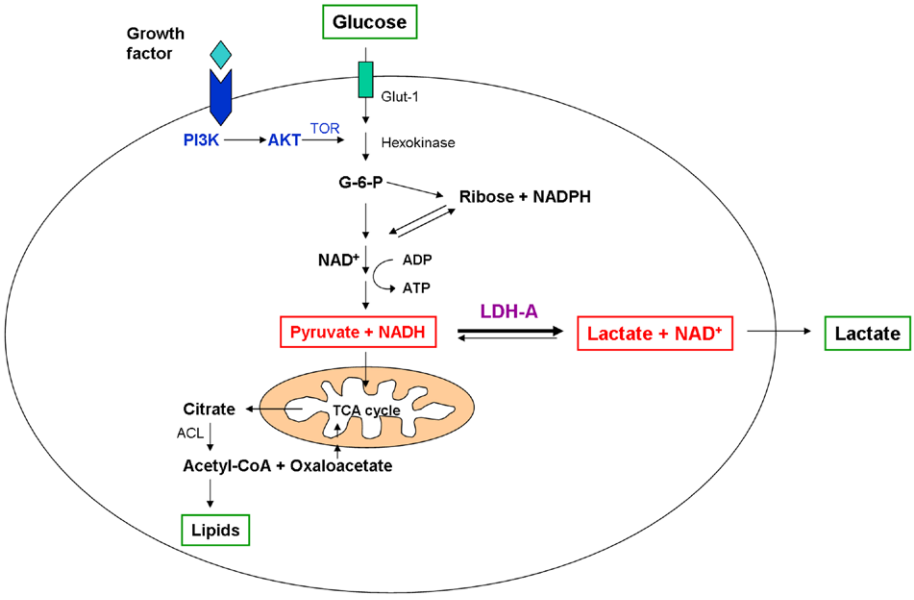

Figure 1. Cancer cells exhibit constitutive aerobic glycolysis

In nontransformed cells, glucose uptake is regulated by receptor activation of PI3K. Activating mutations in PI3K or Akt lead to glucose uptake in excess of the cell's requirements, resulting in excess production of pyruvate and NADH through glycolysis. In cancer cells, some of the pyruvate produced in excess of that needed to maintain the mitochondrial membrane potential is shunted into lipid synthesis through a truncated TCA cycle. The remaining pyruvate is converted to lactate by LDH-A, regenerating NAD$^+$ needed to maintain glycolysis. When glycolysis is suppressed by LDH-A shRNA, cancer cells compensate with increased oxidative phosphorylation. The slower rate through which oxidative phosphorylation generates ATP does not appear to be able to keep up with a cancer cell's bioenergetic needs, leading to a decrease in mitochondrial membrane potential, cessation of cell growth, and inhibition of tumorigenesis.

A second potential reason for why suppression of glycolysis results in decreased cell growth and tumorigenicity is that glucose derivatives contribute to more than ATP generation during cell growth. Glycolytic metabolites have been shown to be required for both fatty acid production and the maintenance of nonessential amino acid pools during growth. The generation of NADPH, a cofactor for many synthetic reactions, is produced in growing cells by oxidative degradation of glucose by glucose-6-phosphate dehydrogenase (G6PD). The resulting ribose is disposed of by returning the carbon to the glycolytic pathway using the transaldolase/transketolase complex.

The increase in OXPHOS upon LDH-A suppression demonstrates that the tumor cells preferentially maintain their metabolism through glycolysis despite a considerable residual capacity to engage in OXPHOS. What can account for this preference in the absence of a defect in oxidative phosphorylation? It has been suggested that the preferential use of glycolysis by tumor cells provides the cells with a competitive advantage under conditions of hypoxia (Gatenby and Gillies, 2004). Consistent with this proposal, cells with attenuated levels of LDH-A were found to have a reduced ability to grow under conditions of hypoxia. In tumors exposed to hypoxia, the transcription factor HIF-1α is activated and induces the transcription of glycolytic enzymes (Dang and Semenza, 1999). Fantin et al. propose that the high rate of tumor glycolysis results from oncogene-directed signaling pathways that upregulate HIF-1α such as those initiated by Ras, Src, and Her-2/Neu. In addition to increasing the expression of glycolytic genes, HIF-1α transcription leads to repression of pyruvate utilization by mitochondria. Recently, two groups have shown that HIF-1α also induces the expression of pyruvate dehydrogenase kinase 1 (PDK1) (Kim et al., 2006; Papandreou et al., 2006). When expressed, PDK1 phosphorylates and inactivates mitochondrial pyruvate dehydrogenase (PDH). This suppression of substrate entry into the citric acid cycle would further enhance the dependence of cells on glycolysis for ATP production.

At first blush, oncogene induction of HIF-1α appears to explain both the enhanced rate of glycolysis and the repressed state of oxidative phosphorylation observed in tumors. However, there are two recent observations that challenge this simple interpretation. First, constitutive activation of HIF-1α, while inducing glycolysis, appears to suppress tumor growth (Mack et al., 2003). Second, the ability of tumors to produce sufficient quantities of cytosolic acetyl-CoA to fuel the synthesis of lipids needed for membrane synthesis and isoprenoids for lipid modification of signaling proteins depends on continued pyruvate degradation by mitochondrial PDH. The resulting mitochondrial acetyl-CoA is combined with oxaloacetate to form citrate. When mitochondrial respiration is low, the citrate moves down its concentration gradient into the cytosol by means of the tricarboxylic acid transporter. In the cytosol, the enzyme ATP-citrate lyase (ACL) converts citrate into acetyl-CoA and oxaloacetate. The oxaloacetate is returned to the mitochondria to complete a truncated citric acid cycle, and the remaining acetyl-CoA provides a glucose-derived substrate for cytosolic fatty acid synthesis (Bauer et al., 2005). This glucose-dependent production of cytosolic acetyl-CoA contributes to the ability of glycolysis to fuel tumor growth, as ACL inhibition in cancer cells results in the suppression of fatty acid synthesis and tumor growth (Hatzivassiliou et al., 2005). The ability of glucose to fuel lipid synthesis in this manner would be inhibited by HIF-1α induction of PDK1.

What then explains the high rate of glycolysis observed in tumors? Recently, Elstrom et al. (2004) have proposed that the high rate of glycolysis is driven by oncogene-directed increases in glucose uptake and metabolism. Oncogenic activation of the PI3K/Akt/Tor pathway results in coordinated upregulation of glucose transporters, enhanced glucose capture through glucose phosphorylation by hexokinase, and commitment of glucose to degradation by activation of PFK-1 and/or G6PD. In this model, oncogenic signaling drives glucose uptake and metabolism in excess of cellular needs. Because most tumor cells cannot store carbon as glycogen or triglyceride, the excess carbon from glycolysis must be secreted as lactate, and this requires LDH-A activity. As Fantin et al. found, interrupting this mechanism of disposal increases pyruvate metabolism through mitochondrial oxidation, but LDH-A attenuation also arrested cell proliferation. This effect on cell proliferation is unexpected and raises further interesting questions about the connections between glucose metabolism and cancer cell physiology. For example, what is the mechanism of the effect on proliferation? Is this due to interference with biosynthesis, to signaling effects of accumulated metabolic intermediates like pyruvate, or to other mechanisms?

In summary, the paper by Fantin et al. (2006) adds to the growing evidence that aerobic glycolysis contributes to cancer cell growth and tumorigenicity. Warburg's original hypothesis that aerobic glycolysis results from tumor-specific mutations in oxidative phosphorylation no longer seems tenable. Nevertheless, these new insights raise as many questions as they answer. The time has come for cancer biologists to dust off their biochemistry textbooks. It seems there are a few chapters that still need to be written.

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DOI 10.1016/j.ccr.2006.05.012