Cancer Signaling Drives Cancer Metabolism: AKT and the Warburg Effect

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The Warburg effect, the propensity of some cells to metabolize glucose to lactate in the presence of oxygen (also known as aerobic glycolysis), has long been observed in cancer and other contexts of cell proliferation, but only in the past two decades have significant gains been made in understanding how and why this metabolic transformation occurs. In 2004, *Cancer Research* published a study by Elstrom and colleagues that provided one of the first connections between a specific oncogene and aerobic glycolysis. Studying hematopoietic and glioblastoma cell lines, they demonstrated that constitutive activation of AKT promotes an increased glycolytic rate without altering proliferation or oxygen consumption in culture. They proposed that it is this effect that allows constitutive AKT activation to transform cells and found that it sensitizes cells to glucose deprivation. In the years since, mechanistic understanding of oncogenic control of metabolism, and glycolysis specifically, has deepened substantially. Current work seeks to understand the benefits and liabilities associated with glycolytic metabolism and to identify inhibitors that might be of clinical benefit to target glycolytic cancer cells.

See related article by Elstrom and colleagues, Cancer Res 2004;64:3892–9

Oncogenic transformation induces many changes to cell function that promote tumorigenesis. Work over the past two decades has highlighted how oncogene signaling can reprogram cellular metabolism and how these changes support cancer-cell proliferation (1). High glucose uptake and glycolytic conversion to lactate under oxygen-replete conditions, known as the Warburg effect due to its early documentation by Otto Warburg, has been observed for decades in cancer cells and other proliferating cell types (2). However, the underlying causes of this phenomenon and its contributions to proliferation were unclear. In their study from 2004, Elstrom and colleagues (3) found that activation of the protein kinase AKT is likely to be one cause of the elevated glycolytic metabolism widely observed in cancer cells.

Mutations in a number of oncogenes and tumor suppressors can result in the growth factor–independent activation of AKT, which is a critical regulator of various metabolic enzymes and pathways (1). The three isoforms of AKT are activated in response to exogenous growth factors by class I phosphoinositide-3-kinase (PI3K), which produces the lipid second-messenger phosphoinositide-3,4,5-trisphosphate (PIP_3). AKT engages PIP_3 for recruitment to the plasma membrane, where it is activated. Although activating mutations in AKT are found in some cancers, more common oncogenic mutations that activate growth factor receptor-tyrosine kinases (RTK), PI3K itself, or RAS can all promote AKT activity, as do inactivating mutations in the tumor-suppressor PTEN, which serves as a PIP_3 phosphatase to turn off PI3K-AKT signaling (**Fig. 1**). Thus, aberrant activation of AKT is a frequent event in human cancers.

In their article, Elstrom and colleagues provide some of the first evidence that oncogene signaling can directly alter cancer-cell metabolism as well the metabolic dependencies of cancer cells (3). Prior studies had indicated that exogenous expression of either AKT or the antiapoptotic protein BCL-X_L is able to prevent apoptosis in hematopoietic cells but only AKT is able to transform them, leading to the question of how else AKT might act as an oncogene. The authors observed that a constitutively active version of AKT (myr-AKT), which is recruited to the plasma membrane via a myristoylation sequence rather than via PIP_3 binding, enhanced the rate of glycolysis without substantially changing mitochondrial metabolism as measured by oxygen consumption. Intriguingly, this elevated glycolysis rendered the cells more sensitive to glucose withdrawal, indicating that uncontrolled AKT activation also led to a vulnerability in these cells.

Several studies preceding this one proposed that growth factor signaling could promote cell growth and survival by altering glycolytic flux. In lymphocytes, for example, the cytokine IL3 is able to stimulate AKT activity and to induce proliferation and survival. Depletion of IL3 attenuates glycolysis and leads to lymphocyte atrophy and apoptosis accompanying a reduction in ATP production and glycolytic metabolite levels (4). Surprisingly, culturing lymphocytes in the presence of reduced levels of IL3 primed them for survival upon either total IL3 withdrawal or glucose deprivation, an effect attributed to reduced glycolysis under low IL3 levels (5). One intriguing possibility is that reduced AKT activity in cells grown under low IL3 conditions decreases glycolytic flux and allows cells to avoid the dependence on glucose and glycolysis that occurs when AKT activity is high. A subsequent study looked at the role of oncogene-induced glucose uptake on the survival of breast epithelial cells following detachment from the extracellular matrix (ECM; ref. 6). Notably, overexpression of the RTK HER2, a frequent transforming event in breast cancer, was found to promote survival upon ECM detachment by activating the PI3K-AKT pathway and increasing glycolysis. Thus, although direct oncogenic activation of AKT is less common in cancer, other oncogenic alterations can converge on this pathway to enhance glycolysis and other cell survival mechanisms to promote transformation.

Although Elstrom and colleagues demonstrate that glucose uptake and lactate production are increased by constitutively-active AKT, they did not identify the mechanism by which the induction of glycolysis occurs. In the years around and since publication of this

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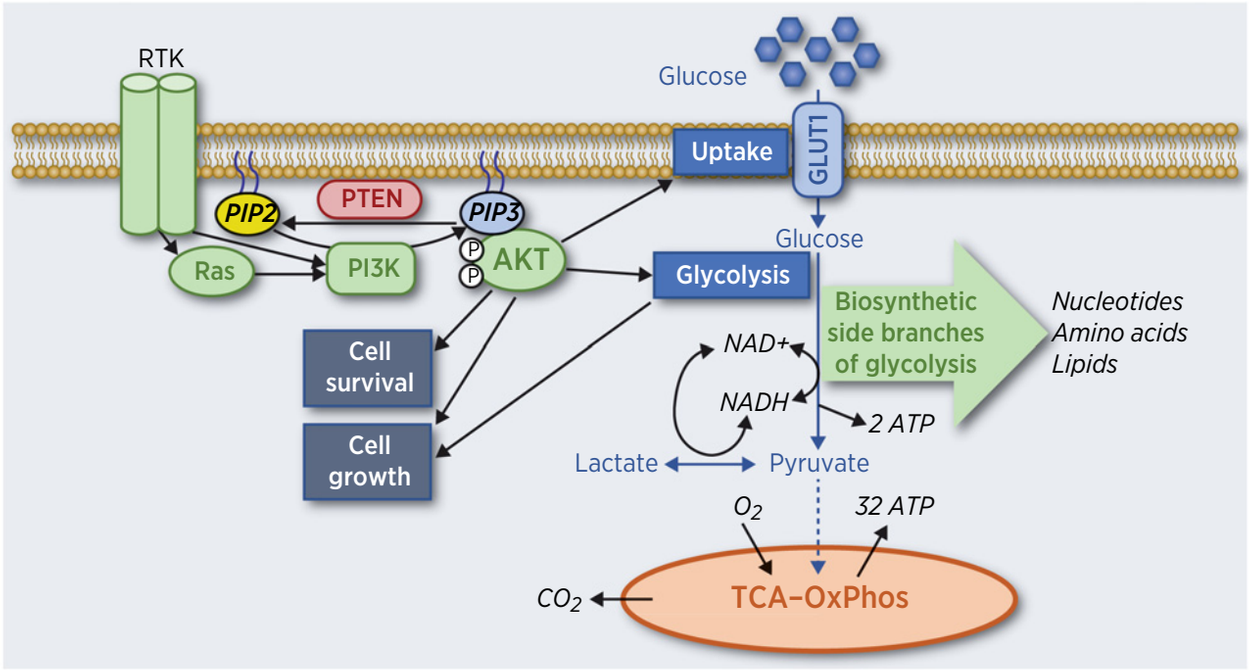

Figure 1.

AKT regulates glycolytic metabolism downstream of oncogene signaling. AKT is activated by phosphorylation following its recruitment to the plasma membrane by binding to PIP_3, which is produced by PI3K. Activating mutations in AKT itself or upstream oncogenes, including RTK, PI3K, and RAS, or inactivating mutations in the tumor-suppressor PTEN lead to constitutive activation of AKT in numerous cancers. AKT promotes cell survival and growth in addition to stimulating glucose uptake and glycolysis. Following import via GLUT1 or other glucose transporters, glucose is catabolized to produce pyruvate, which can be further metabolized in the mitochondria or converted to lactate and excreted. Glycolysis produces two molecules each of ATP and NADH per molecule of glucose and can supply precursors for macromolecule biosynthesis to support cell growth. Oxidative metabolism of pyruvate through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OxPhos) in the mitochondria produces additional ATP from pyruvate, while lactate production allows cells to regenerate NAD^+.

article, several mechanisms by which AKT can stimulate glycolysis in various settings have been elucidated, including by increasing glucose uptake and through the direct or indirect regulation of specific glycolytic enzymes (**Fig. 1**).

AKT activation can enhance glucose uptake in cancer cells through regulation of the major glucose transporter GLUT1. Constitutively active AKT promotes the accumulation of GLUT1 on the plasma membrane to enable glucose uptake, and oncogenic activation of the PI3K-AKT pathway drives glucose uptake (1). AKT has been found to phosphorylate and to inhibit the thioredoxin-interacting protein (TXNIP), a protein that negatively regulates trafficking of GLUT1 to the plasma membrane, providing a posttranslational mechanism by which AKT stimulates GLUT1 translocation and glucose uptake in cancer cells (7). Underscoring the importance of GLUT1 to growth factor–stimulated glycolysis, exogenous overexpression of GLUT1 has been found to enhance lymphocyte viability following growth factor deprivation, which would otherwise result in decreased glycolysis and induction of apoptosis (5).

Beyond increasing glucose uptake, AKT regulates several glycolytic enzymes through posttranslational and transcriptional mechanisms (1). Among the enzymes directly phosphorylated by AKT is phosphofructokinase-fructose-bisphosphatase 2 (PFKFB2 or PFK2), one of the earliest AKT substrates identified. In a study that predates the study by Elstrom and colleagues, PFKFB2 was shown to be phosphorylated and activated by AKT and related protein kinases (8). PFKBP2 regulates glycolysis by producing an allosteric activator of the glycolytic enzyme phosphofructokinase 1 (PFK1). Longer term AKT activation, as might occur following oncogenic activation of the PI3K-AKT pathway, can lead to the transcriptional induction of *GLUT1* and genes encoding nearly every glycolytic enzyme via hypoxia-inducible factor 1α (HIF1α). mTORC1 activation downstream of AKT can promote elevated levels of HIF1α, in the absence of hypoxia, leading to the induction of aerobic glycolysis (9). Although Elstrom and colleagues indicated that HIF1 was not differentially expressed in their cell system, induction of HIF1α targets may contribute to elevated glycolysis in other contexts of AKT activation. More research is needed to define the specific mechanism(s) that drive AKT-mediated aerobic glycolysis in various cancer settings.

In the experimental setting used by Elstrom and colleagues, myr-AKT was found to drive glucose consumption without changes in oxygen consumption, suggesting that the increased glucose carbon being taken up was not being oxidized in the mitochondria, representing a classic example of the Warburg effect. While AKT activation may explain one major mechanism to induce this metabolic phenomenon, the precise benefit it provides to cancer cells and other proliferating cells has been difficult to fully understand. Glycolysis generates ATP and provides biosynthetic precursors in the form of glycolytic intermediates for metabolic pathways contributing to amino acid, lipid, and nucleotide synthesis, the building blocks of cellular proteins, membranes, and nucleic acids. Nevertheless, it is unclear why cells consume such large amounts of glucose only to convert most of it to lactate. Glycolysis yields just two molecules of ATP per molecule of glucose converted to lactate, much less than would be generated following total oxidation of glucose in the mitochondria (**Fig. 1**), suggesting that ATP

production may not be limiting for cell proliferation (10). Additionally, the fact that a large fraction of consumed glucose carbon is excreted as lactate indicates that increased glucose uptake does not yield an equivalent increase in biomass accumulation. Thus the direct generation of these metabolic products from glucose may not explain why cells engage in aerobic glycolysis. Recent studies have suggested that cellular demand for the redox cofactor NAD^+ is a primary driver of aerobic glycolysis to favor production of lactate from pyruvate rather than mitochondrial oxidation to carbon dioxide (10). Glycolysis converts two molecules of NAD^+ to two molecules of NADH per molecule of glucose catabolized, and Elstrom and colleagues reported elevated glycolytic NADH production in cells expressing myr-AKT. NAD^+ can be regenerated via the mitochondrial electron transport chain or upon pyruvate conversion to lactate by lactate dehydrogenase (LDH), and its regeneration is critical for cancer cell growth. It is possible that the AKT-induced glycolytic flux produces NADH in excess of the mitochondrion's ability to oxidize it, necessitating regeneration of NAD^+ via lactate production. The seemingly wasteful conversion of a majority of glucose carbon to lactate may therefore indicate that a high rate of glycolysis is, itself, the important contributor to biosynthesis and proliferation (2). Production of lactate to regenerate NAD^+ allows cells to sustain rapid glucose uptake and entry into glycolysis, thus enabling a fraction of this carbon to flow into biosynthetic side-branches of glycolysis, promoting macromolecular synthesis for cell growth.

The prevalence of high glycolytic metabolism in cancer has clinical relevance. Based on their unusually high consumption of glucose, tumors can often be imaged by fluorodeoxyglucose-positron emission tomography (FDG-PET), in which uptake of a radioactive glucose analogue is monitored. AKT-driven glucose uptake may be one factor influencing the degree to which tumors are FDG-PET positive. Notably, acute treatment of mice with PI3K inhibitors causes xenograft tumors to become FDG-PET negative due to diminished plasma-membrane localization of GLUT1 (11), and clinical trials with the PI3K inhibitor alpelisib have also observed reduced FDG-PET signal accompanying decreased tumor size with prolonged treatment (12). It is possible that PI3K inhibitors impede tumor growth, in part, by disrupting AKT-driven glucose metabolism, however, systemic glucose homeostasis through insulin signaling is also regulated by PI3K and AKT, and treatment with PI3K inhibitors often results in hyperglycemia as an on-target toxicity (1, 12). There is also interest in potentially targeting glycolysis itself for cancer therapy. Expressing constitutively active AKT makes cells more sensitive to glucose deprivation, suggesting that activation of the PI3K–AKT pathway exposes a metabolic vulnerability in cancer cells (3). Inhibitors have been developed that target glucose transporters as well as early glycolytic enzymes such as hexokinase. These inhibitors would effectively starve cells of glucose and might be of benefit in cancers with hyperactivated PI3K-AKT signaling. Other inhibitors have been developed to target later stages of glycolysis, for example by inhibiting LDH or lactate transporters. Future studies are needed to determine whether a therapeutic window exists for the use of such compounds in humans.

In the years since Elstrom and colleagues first noted that oncogenic activation of the PI3K–AKT pathway promoted glycolytic metabolism in cancer cells, our understanding of oncogenic control of metabolism has expanded significantly. For example, numerous mechanisms linking AKT to cellular metabolism have now been identified, leading to a more complete understanding of how and why glycolysis and other metabolic pathways are upregulated so often in cancer (1, 2). Future work will undoubtedly determine how these metabolic alterations create opportunities for therapeutic intervention in the context of specific oncogenic mutations.

Authors' Disclosures

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