A Mitochondrial Power Play in Lymphoma

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http://dx.doi.org/10.1016/j.ccr.2012.09.023

Deregulated energetics is a hallmark of malignancy, but metabolic heterogeneity among individual tumors is unknown. A study by Caro et al. in this issue of *Cancer Cell* demonstrates that a subset of lymphomas is defined by reliance on mitochondrial energy generation and is selectively killed when this pathway is impaired.

Altered metabolism was among the first cancer biomarkers (Koppenol et al., 2011). By 1925, Otto Warburg had observed that tumors rapidly took up glucose and converted it to lactic acid, which was released into the milieu. This was a departure from the other organs he studied, which imported less glucose, oxidized a higher fraction of it to carbon dioxide, and secreted much less lactate. This remarkable phenotype, called the "Warburg effect," provides the rationale for two modern imaging technologies in cancer diagnosis: ^{18}fluoro-2-deoxyglucose positron emission tomography (FDG-PET) to identify tumors by rapid glucose import and ^{1}H magnetic resonance spectroscopy to identify areas of elevated lactate. Over the last two decades, the Warburg effect has been mechanistically tied to the molecular basis of transformation, as tumor-promoting mutations in many different oncogenes and tumor suppressors have been demonstrated to stimulate glycolysis (Ward and Thompson, 2012).

Cells have two ways to produce adenosine triphosphate (ATP) for energy: glycolysis and oxidative phosphorylation (OxPhos) (Figure 1). In glycolysis, glucose is converted to pyruvate, generating NADH from NAD^+ and ATP from ADP. If the pyruvate is reduced to lactate, NAD^+ is regenerated and glycolysis continues. Although glycolysis is rapid, it is deemed inefficient because most of the energy that could be generated from glucose is lost when the cell secretes lactate. In contrast, OxPhos is highly efficient. When substrates like pyruvate are oxidized in the mitochondria, reducing equivalents are delivered to the electron transport chain, creating a proton gradient coupled to ATP synthesis (Figure 1). This system yields nearly 20 times as many ATP molecules per glucose as the Warburg effect. Other oxidizable substrates, like fatty acids, produce an even higher energy yield. Warburg considered tumor glycolysis to be a metabolic anomaly, famously postulating that it was the consequence of irreversible defects in respiration (i.e., OxPhos) which were the root cause of cancer (Warburg, 1956). This model has been disproven as a general principle, because most cancer cells do not have static defects in their respiratory machinery, and more pointedly, because nonmalignant cells also display the Warburg effect if stimulated to grow (Wang et al., 1978). Nevertheless, the concept that glycolysis is a universal feature of aggressive tumor growth, and that OxPhos is counter-productive to tumorigenesis still pervades the literature.

It was therefore surprising when a bioinformatics study on diffuse large B cell lymphoma (DLBCL) revealed that some 30% of these tumors belonged to a subset defined by high expression of genes involved in OxPhos (Monti et al., 2005). DLBCL is the most common non-Hodgkin's lymphoma in Western populations and is characterized by rapid growth. Several attempts have been made to classify DLBCL by molecular typing, particularly through shared patterns of gene expression. One such approach revealed three major DLBCL subtypes: the OxPhos subset; the B cell receptor (BCR) subset, characterized by expression of genes involved in B cell receptor signaling; and the host response (HR) subset, expressing markers of infiltrating inflammatory cells (Monti et al., 2005). Now, a study in this issue of *Cancer Cell* (Caro et al., 2012) examines the metabolic phenotypes of these subtypes and found that OxPhos cell lines and primary biopsies contained elevated expression of many mitochondrial proteins. OxPhos cells displayed enhanced glucose oxidation relative to lactate formation, better defenses against oxidative stress, and a robust ability to oxidize fatty acids in the mitochondria. Providing exogenous fatty acids stimulated survival and growth in OxPhos cells, but not in cells from other subtypes. Overall, cells from OxPhos tumors generated a higher fraction of their ATP in the mitochondria, and this activity was required for cellular fitness.

Importantly, inhibition of mitochondrial metabolism selectively killed OxPhos cells. The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ), which regulates the expression of many genes encoding mitochondrial proteins, was overexpressed in OxPhos DLBCLs. PPARγ antagonists suppressed fatty acid oxidation and killed OxPhos cells in culture. Inhibitors of fatty acid oxidation had the same effect, as did silencing of an enzyme required to synthesize the antioxidant glutathione. None of these treatments affected non-OxPhos cells.

The work illustrates several key principles in cancer metabolism. First, it demonstrates significant metabolic heterogeneity among tumors of the same type. This point should be emphasized, because it tempers the paradigm that tumors are monolithic in their preference for glycolytic energy production. Second, metabolic heterogeneity is programmed, and it persists when cells are adapted to culture. This argues strongly against the concept that the glycolytic phenotype of most cancer cell lines is

an unavoidable consequence of culture conditions. Even the suppression of OxPhos in other DLBCL subtypes was programmed, because these cells could be stimulated to activate fatty acid oxidation simply by silencing Syk, a kinase in BCR signaling. Third, high rates of OxPhos can support growth in cancer cell lines. This may also be true in vivo. Although a complete clinical description of the OxPhos DLBCL is lacking, patients with these tumors do not appear to survive any longer than those with other types (Monti et al., 2005). The aggressive course of DLBCL as a whole suggests that a preference for OxPhos does not prevent rapid cell growth in the large subset of tumors using that metabolic platform. Thus, metabolic programming to use the mitochondria rather than glycolysis as the major site of energy formation is an effective strategy for cancer cell growth.

The findings also emphasize that cancer cells, like normal cells, use multiple pathways concurrently to produce energy (Figure 1). The vast majority of cancer cells use both glycolysis and OxPhos together, although the balance between the two varies widely (Zu and Guppy, 2004). DLBCL cells are no exception. Despite their ability to oxidize pyruvate and fatty acids in the mitochondria, OxPhos cells still consumed glucose and secreted a modest amount of lactate. Conversely, the non-OxPhos cells still produced half of their ATP in the mitochondria (Figure 1). Along these lines, a large majority of DLBCL tumors are readily detected by FDG-PET, and this likely includes OxPhos tumors. Thus, avid uptake of glucose analogs may occur even when the mitochondria are fully active. Another recent study on metabolic flux in glioblastoma reached this same conclusion (Maher et al., 2012). Besides ATP, mitochondria produce biosynthetic precursors, reactive oxygen species, and other factors that stimulate cell growth and may contribute to tumorigenesis (Weinberg et al., 2010).

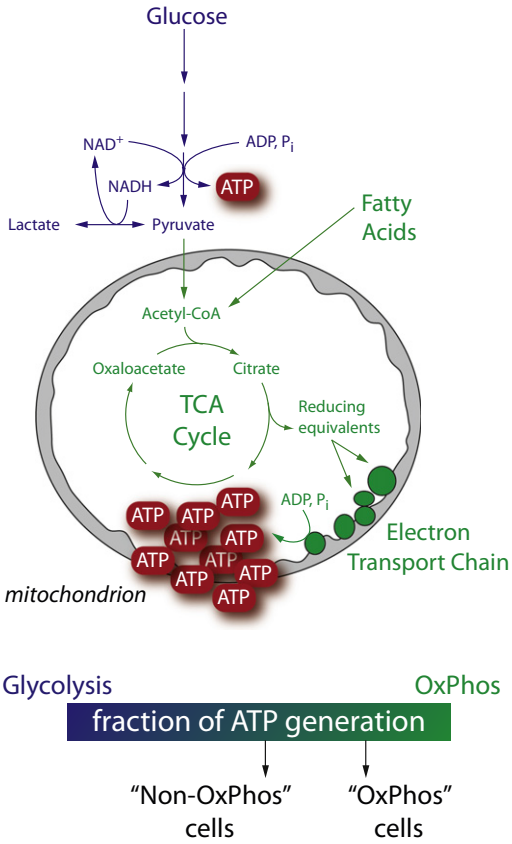

Figure 1. Variable Reliance on Oxidative Phosphorylation in DLBCL

Cells produce ATP from glycolysis (blue pathway) and oxidative phosphorylation (OxPhos, green). Cancer cell lines vary according to what fraction of their total ATP comes from each pathway, as shown on the spectrum at the bottom. A subset of DLBCL tumors was defined by high expression of genes related to OxPhos. These cells produced most of their ATP from OxPhos and required this pathway for survival and growth. Non-OxPhos cells, which did not share this gene expression signature, produced a higher fraction of their ATP from glycolysis, and were resistant to OxPhos inhibition. Abbreviation: TCA, tricarboxylic.

It remains to be seen whether the OxPhos phenotype is driven by tumorigenic mutations or other features of DLBCL biology, and whether it bestows some context-specific advantage to this subset. Regardless of the mechanism, it is encouraging that molecular phenotyping led to the discovery of metabolic vulnerabilities in DLBCL. In a sense, the new work fortifies Warburg's historical claim that metabolism is an "actionable" biomarker (even if he would have been surprised by these particular results). PPARγ antagonism may provide a starting point for targeting OxPhos tumors, and it would also be interesting to determine whether OxPhos predicts sensitivity to therapies currently in use for cancer. New approaches to image tumor metabolism noninvasively (Kurhanewicz et al., 2011) may soon make it possible to stratify patients to therapeutic regimens solely by probing metabolism in vivo.

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