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Genetic analysis of the Warburg effect in yeast

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A B S T R A C T

We recently discovered that the Warburg effect, defined by the dramatically enhanced metabolism of glucose to pyruvate, even in well-oxygenated cancer cells, can occur as a consequence of mutations that enhance lipid biosynthesis at the expense of respiratory capacity. Specifically, mutations in the E1 subunit of either of two respiratory enzymes, pyruvate dehydrogenase (PDC) or α-ketoglutarate dehydrogenase (KGDC), change substrate specificity from the 3-carbon α-ketoacid pyruvate, or the 5-carbon α-ketoacid α-ketoglutarate, to the 4-carbon α-ketoacid oxaloacetate (OADC). These mutations result in OADC-catalyzed synthesis of malonyl-CoA (MaCoA), the essential precursor of all fatty acids. These mutants arose as spontaneous suppressors of a yeast $acc1^{cs}$ cold-sensitive mutation encoding an altered form of AcCoA carboxylase (Acc1) that fails to produce MaCoA at the restrictive temperature (16°C). Notably, these suppressors are respiratory defective *as a result of the same nuclear mutations* that suppress $acc1^{cs}$. These mutants also suppress sensitivity to Soraphen A, a potent inhibitor of Acc1 activity, at normal temperature (30°C). To our knowledge, OADC activity has never been identified in eukaryotic cells. Our results offer a novel perspective on the Warburg effect: the reprogramming of energy metabolism in cancer cells as a consequence of mutational impairment of respiration to meet the fatty acid requirements of rapidly proliferating cells. We suggest OADC activity is a common feature of cancer cells and represents a novel target for the development of chemotherapeutics.

Abbreviations: Acc1, AcCoA carboxylase; FAS, fatty acid synthase complex; KGDC, pyruvate dehydrogenase complex; PDC, pyruvate dehydrogenase complex; OADC, oxaloacetate dehydrogenase complex; MaCoA, malonyl-CoA.

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Introduction

Enormous strides have been made over the past several decades in our understanding of cancer biology. Hanahan and Weinberg published a comprehensive review of the "hallmarks" of cancer in 2000 (Hanahan and Weinberg, 2000). These hallmarks, however, addressed exclusively regulatory and signaling processes. The Warburg effect was not mentioned in this review. Only in the past decade or so has there been a resurgence of interest in the Warburg effect and its role in cancer. In their updated review, Hanahan and Weinberg included two additional hallmarks of cancer: (i) defense against immune destruction; and (ii) modifications in energy metabolism (Hanahan and Weinberg, 2011). The latter – aberrant energy metabolism – is a trait common to all cancers that was initially described in the 1920s (reviewed in (Warburg, 1956b)). Understanding the Warburg effect, specifically how and why cancer cells metabolize glucose by "aerobic glycolysis," rather than by respiration, is critically important to reach a fundamental understanding of cancer. Efforts directed toward this goal are imperative, as cancer remains a major health problem worldwide. Indeed, cancer recently surpassed heart disease as the leading cause of death in the United States (Twombly, 2005).

Warburg recognized that aerobic glycolysis is not a consequence of oxygen deprivation, but occurs even in well-oxygenated tumor cells (Warburg, 1956a, b). Indeed, the Warburg effect has been exploited clinically since 1976 as the basis of the PET (positron emission tomography) scan that detects uptake of the radiolabelled, glucose analog 2–^{18}F-2-deoxyglucose (FDG) by cancer cells (Fowler and Ido, 2002). The Warburg effect seems to be a common metabolic thread that runs through all or nearly all cancers. Nonetheless, despite having been identified more than 90 years ago, the biochemical basis of the Warburg effect remains poorly understood and largely unexplained.

The Warburg effect established that the limiting factor in cancer cell growth is not energy production in the form of ATP, but metabolic intermediates derived from glucose that serve as building blocks for cell proliferation (Jiang and Deberardinis, 2012). During the past decade, attempts have been made to rationalize the Warburg effect based on diversion of carbon metabolism away from energy production and toward the synthesis of metabolic intermediates. As a notable example, Cantley and colleagues identified a splice site isoform of pyruvate kinase (PK M2) that is important for tumor metabolism and proliferation (Christofk et al., 2008). They proposed that PK M2 restricts the terminal reaction in glycolysis, thereby facilitating exit of upstream glycolytic intermediates into other pathways essential for cell growth (Vander Heiden et al., 2009). However, the pathways and metabolites critical for rapid cell growth and division remain undefined.

α-Ketoacid dehydrogenase complexes

All cells contain α-ketoacid dehydrogenase complexes that catalyze the irreversible oxidative decarboxylation of α-ketoacids yielding CoA derivatives and reduced NADH. The most well-characterized of these enzymes is the pyruvate dehydrogenase complex (PDC), an extraordinary complex composed of three enzymatic activities (E1, decarboxylase; E2, transacetylase; and E3 dehydrogenase) and five cofactors (thiamine, CoA, NAD^+, FAD and lipoid acid) that link glycolysis with the citric acid cycle by catalyzing oxidation of the 3-carbon α-ketoacid, pyruvate, to produce AcCoA (Patel et al., 2014):

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{AcCoA} + \text{HCO}_3^- + \text{NADH} + \text{H}^+ \quad (1)$$

The citric acid cycle includes an analogous reaction catalyzed by the α-ketoglutarate dehydrogenase complex (KGDC). KGDC catalyzes oxidation of the 5-carbon α-ketoacid, α-ketoglutarate, to succinyl-CoA:

$$\alpha - \text{ketoglutarate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{succinyl} - \text{CoA} + \text{HCO}_3^- + \text{NADH} + \text{H}^+ \quad (2)$$

An analogous α-ketoacid dehydrogenase complex recognizes 5-carbon and 6-carbon α-ketoacids involved in branched chain amino acid metabolism (Kumaran et al., 2013). Remarkably, no analogous

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