ity that underlies tumorigenesis and the development of metastasis. Recently, our group developed a bioinformatics strategy termed Cancer Outlier Profile Analysis (COPA) in an effort to identify "oncogene outliers," or genes with marked overexpression in a fraction of cases, which characterize genes involved in high-level copy number changes or translocations, such as those described above. COPA identified the *Ets* transcription factors *ERG* and *ETV1* as outliers across multiple prostate cancer profiling studies. Characterizing cases with overexpression of *ERG* or *ETV1*, we demonstrated that these samples harbored recurrent gene fusions with the androgen-regulated gene *TMPRSS2*, and these fusions occur in the majority of prostate cancers (Tomlins et al., 2005). This approach was useful in the analysis of tumors without biologically relevant mouse models and may prove useful in mining mouse models of cancer as well.

In addition to using mouse models or bioinformatic strategies, other groups have used alternative integrative analyses to identify driving genetic events in tumorigenesis. For example, Garraway et al. performed an integrative analysis combining gene expression and copy number profiles of the NCI60 panel of cancer cell lines to identify and validate *MITF* as a lineage-specific oncogene amplified in a subset of melanomas (Garraway et al., 2005). Adler et al. also integrated expression and copy number profiles from breast cancers to identify coordinated amplification of *Myc* and *CSN5* as a regulator of the wound response profile that is predictive of poor outcome (Adler et al., 2006).

Amassing genomic scale data for human tumors is becoming relatively routine. The rapid advancement of high-throughput techniques suggests that complete genomic profiles of tumor samples may soon be realized. Integrative approaches will be needed to sift through this mass of data to identify the driving genetic lesions underlying cancer development and progression. The results of Zender et al. and Kim et al. demonstrate that integrative approaches based on defined mouse models can be used as one such method to identify driving oncogenic or metastatic events in human tumors.

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Differential utilization of two ATP-generating pathways is regulated by p53

A fundamental property of cancer cells is the preferential utilization of glycolysis over aerobic respiration to produce ATP. Renewed interest in understanding the mechanism underlying this metabolic shift in energy production is broadening our understanding of the relationship between cancer and cellular metabolism. In a recent article, Matoba et al. report that the p53 tumor suppressor regulates the expression of SCO2, a protein that is required for the assembly of cytochrome c oxidase (COX), a multimeric protein complex required for oxidative phosphorylation. The implication of these findings is that aerobic respiration is compromised in cells that lack functional p53.

In contrast to normal cells, cancer cells have a high glycolytic rate and produce high levels of lactate even in the presence of oxygen (Figure 1). This metabolic shift to a higher rate of aerobic glycolysis is commonly referred to as the Warburg effect. Because glycolysis produces energy (ATP) far less efficiently than aerobic respiration, tumor cells have a much higher rate of glucose uptake than normal cells (Figure 1). The physiological significance of the Warburg effect has been controversial since its discovery over 80 years ago, and now there is renewed and vigorous interest in understanding the relationship between cancer and altered energy metabolism. A commonly held view is that constitutive upregulation of glycolysis is likely to be an adaptation to hypoxia that develops as tumor cells grow progressively further away from their blood supply (Gatenby and Gillies, 2004). Interestingly, cells derived from tumors continue to utilize glycolysis in culture under normoxic

conditions, suggesting that stable genetic or epigenetic changes may account for this metabolic shift toward glycolysis.

The molecular mechanisms underlying the Warburg effect are uncertain and remain controversial. It is unclear if the defect lies within glycolysis, in oxidative phosphorylation, or in the pathways that modulate cellular metabolism. The shift from aerobic mitochondrial respiration to glycolysis in tumor cells could be the result of increased activity of glycolytic enzymes, decreased utilization of pyruvate by mitochondria, reduced capacity to transport cytosolic NADH into mitochondria through mitochondrial NADH/NAD+ shuttle pathways, impaired tricarboxylic cycle, or defects in respiration at various points in the electron transport chain.

HIF-1α, a protein that is stabilized under hypoxic conditions and associates with HIF-1β to form a heterodimeric transcription factor, has been implicated as an important regulator of glycolysis through its ability to increase the expression of genes encoding glucose transporters and glycolytic enzymes (Semenza, 2003). In addition to promoting glycolysis, HIF-1 has been shown to suppress both the TCA cycle and aerobic respiration by inducing pyruvate dehydrogenase kinase 1 (PDK1) (Kim et al., 2006; Papandreou et al., 2006). PDK1 phosphorylates and inactivates the TCA cycle enzyme, pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA. Deregulated c-MYC expression also enhances aerobic glycolysis by directly upregulating the expression of glycolytic genes independent of hypoxia (Kim et al., 2004). AKT activation increases glucose transport and glycolysis and renders cancer cells dependent on glucose availability for their survival (Elstrom et al., 2004). HIF-1 expression, PI3K/AKT activation, and c-MYC expression are common in many cancers.

Recent findings aimed at understanding the basis for constitutive aerobic glycolysis in cancer cells are provocative and insightful. A report by Matoba et al. (2006) invokes p53 as a determinant of ATP production through regulation of mitochondrial oxidative phosphorylation. They report that p53 regulates the expression of SCO2 (for synthesis of cytochrome c oxidase), a nuclear gene that encodes a copper binding protein that is required for the assembly of cytochrome c oxidase (COX). COX is a multimeric protein complex (complex IV of the respiratory chain) that is embedded in the inner mitochondrial membrane. It catalyzes the transfer of electrons from reduced cytochrome c to molecular oxygen in the terminal step of the respiratory chain and pumps protons from the mitochondrial matrix to the intermembrane space. COX is composed of 13 structural subunits; subunits I–III are encoded by the mitochondrial genome and constitute the catalytic core of the enzyme, and the remaining ten subunits are encoded by the nuclear genome. In humans, mutations in *SCO2* cause fatal infantile COX deficiency with the predominant symptoms being early onset hypertrophic cardiomyopathy and encephalopathy due to failure in holoenzyme assembly (Esteitie and Larsson, 2004).

Matoba et al. (2006) show that oxygen consumption (a measure of aerobic respiration) is lower in a preparation of liver mitochondria from p53-deficient mice compared with wild-type mice. A reduction in oxygen consumption is also seen in human HCT116 $p53^{-/-}$ cells compared with HCT116 $p53^{+/+}$ cells. ATP production is similar in $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells, but lactate production (a measure of glycolysis) is higher in the $p53^{-/-}$ cells. These data indicate that loss of p53 results in a metabolic shift away from aerobic respiration toward the production of glycolytic ATP. The missing link that connects p53 with aerobic respiration turns out to be SCO2, identified initially by SAGE analysis as a p53-regulated gene and subsequently by protein analysis. The protein data are striking and show that the basal level of SCO2 protein in unstressed HCT116 cells as well as in mouse liver mitochondria is dependent on p53 gene dosage. This is confirmed by siRNA-mediated knockdown of p53 expression in HCT116 cells, which results in a decrease in the level of SCO2 protein and a decrease in oxygen consumption. A clear and unambiguous conclusion from these experiments is that endogenous p53 in unstressed cells regulates the steady-state level of SCO2 protein to facilitate aerobic respiration. Whether this occurs through direct transcriptional activation of SCO2 is arguable. The presumed p53 response element in intron 1 of human SCO2 does not resemble a typical p53 consensus motif (only 14 of 20 residues are conserved), and it contains nucleotides at certain positions of the consensus that are rarely seen in well-characterized target genes (Miled et al., 2005). Since significant degeneracy can occur in the p53 consensus sequence of p53-responsive genes, a ChIP experiment would demonstrate whether p53 binds directly to this site in vivo. Matoba et al. (2006) also report that p53 null mice tire more easily than normal mice when subjected to a swim endurance test using an adjustable current swimming pool protocol. The interpretation provided is that p53 null mice exhibit a decrease in functional aerobic capacity. At the time of the endurance test, these mice were between 9 and 20 weeks of age. Most of these mice will die of lymphoma within 6 months of age and hence may have been very sick at the time of testing. So the results of this test need to be interpreted cautiously. This is a fascinating observation that reveals a novel and important phenotype of p53-deficient mice. Confirmation, possibly with neonatal mice, is needed.

Two previous studies had established a relationship between glucose metabolism and p53. Inhibition of glycolysis by glucose withdrawal was shown to serve as a signal for the phosphorylation and acti-

Glycolysis:

$$\text{glucose} + 2P_i + 2ADP + 2NAD+ \rightarrow 2\ \text{pyruvate} + 2ATP + 2NADH + 2H_2O$$

$$2\ \text{pyruvate} + 2NADH \rightarrow 2\ \text{lactate} + 2NAD+$$

Aerobic respiration:

$$\text{Glucose} + 6O_2 + 36P_i + 36\ ADP \rightarrow 6CO_2 + 36ATP + 42H_2O$$

Figure 1. ATP production by glycolysis and aerobic respiration

The first stage of glycolysis (the breakdown of glucose and its conversion to pyruvate) occurs in the cytoplasm and serves as an obligatory preparatory step for aerobic respiration, which occurs in mitochondria. Under aerobic conditions, pyruvate is not normally reduced to lactate but is oxidized directly to acetyl-CoA for entry into the tricarboxylic cycle (TCA or Krebs cycle). In addition, the cytoplasmic NADH produced during glycolysis is not oxidized by pyruvate but rather by the electron transport chain after shuttle-mediated entry of NADH into mitochondria. In the absence of oxygen, the conversion of pyruvate to lactate by lactate dehydrogenase regenerates NAD+, which is essential for glycolysis.

vation of p53 (Jones et al., 2005; Feng et al., 2005). It will be of interest to determine if SCO2 expression is further enhanced by p53 under stress conditions such as glucose deprivation.

Fantin et al. (2006) reported that suppression of aerobic lactate production upon shRNA-mediated knockdown of lactate dehydrogenase A (LDH-A) in neuinitiated mammary tumor cells resulted in increased oxygen consumption and increased mitochondrial oxidative phosphorylation. Hence, tumor cells retain the ability to generate ATP through aerobic respiration when aerobic glycolysis is blocked. Fantin et al. (2006) suggest that preferential utilization of NADH by LDH-A in the cytosol impedes mitochondrial respiration. Intriguingly, LDH-A-deficient tumor cells exhibited decreased proliferative and tumorigenic potential, supporting the long-held view that glycolysis provides tumor cells with a selective growth advantage. Fantin et al. (2006) note that some tumors had a strict LDH-A requirement and underwent apoptosis upon transient expression of LDH-A shRNA-expressing vectors. This indicates that reversion from aerobic glycolysis to mitochondrial respiration is not possible in all tumors and suggests that irreversible genetic or epigenetic changes may have occurred to prevent the metabolic switch. In light of the results by Matoba et al. (2006), it would be of considerable interest to determine if tumors with a strict LDH-A requirement have sustained mutations in p53 or in components of the p53 pathway.

In summary, after 80 years, the Warburg effect remains enigmatic. The paper by Matoba et al. (2006) resurrects the idea that enhanced aerobic glycolysis results from tumor-specific mutations that modulate oxidative phosphorylation. Importantly, this study introduces a new player—p53—into the field of cellular metabolism.

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