Cancer cells exhibit altered metabolism compared with that of the surrounding tissue. There is hope that these reprogrammed metabolic pathways in tumors hold the key to advances for both cancer imaging and therapy. Translation of observations in cultured cancer cells to live tumors, however, has proven to be highly complex, and robust methods to analyze metabolic activity in primary human tumors are sorely needed. In this issue of the JCI, Sellers et al. use perioperative administration of isotope-labeled glucose to lung cancer patients to differentiate metabolic pathways between tumors and benign lung. They identify pyruvate carboxylation, a reaction that enables glucose-derived carbon to replenish TCA cycle intermediates, as a key component of anabolic metabolism in tumor cells.
In vivo analysis of lung cancer metabolism: nothing like the real thing

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The inner workings of tumor metabolism: probing intact human tumors
Interest in cancer metabolism is in the midst of a revival due to seminal discoveries revealing that signaling pathways perturbed in cancer regulate metabolism and that some metabolic enzymes function as bona fide tumor suppressors and oncogenes (1). Moreover, multiple lines of evidence indicate that cancer cells become dependent on the activation of particular metabolic pathways during malignant transformation. Metabolic reprogramming is most commonly studied by manipulating expression of oncogenes or tumor suppressors in cultured cell models and observing the subsequent changes in pathway activity or dependence. This paradigm has identified many novel regulatory mechanisms that link signal transduction to the metabolic network. Very few studies to date, however, have directly examined metabolic pathway utilization within intact human tumors. The paucity of metabolic data from patient tumors has produced a disconnect between reductionist models of cell-autonomous metabolism in culture and the much more complex and clinically relevant conditions experienced by cancer cells in vivo.

In this issue, Sellers et al. sought to close this gap by analyzing metabolism in intact tumors from patients with non–small-cell lung cancer (NSCLC) (2). Rather than simply measuring the abundance of metabolites in these tumors, the authors administered a bolus of 13C-labeled glucose a few hours before surgical resection. Fragments of tumor and nonmalignant lung tissue were flash-frozen immediately after resection. Metabolites extracted from these samples were then analyzed for 13C enrichment, which generated a blueprint of glucose handling in tumor and lung. This analysis confirms and extends findings from a previous study by Fan and colleagues (3), which showed that tumor metabolites bear hallmarks of enhanced glucose utilization through both glycolysis and the mitochondrial TCA cycle. Enhanced glycolysis in tumors is not surprising, as descriptions of this phenomenon date back to experiments performed by Otto Warburg in the 1920s (4). Enhanced activity of the TCA cycle was much less predictable, as a pervasive line of thinking for the past century has involved a “switch” from mitochondrial to glycolytic metabolism in tumors. Data from NSCLC tumors argue against a “switch” and instead strongly suggest that both pathways are enhanced in malignant tissue.

The TCA cycle as an anabolic pathway for tumor cell growth
As Sellers et al. point out, the TCA cycle serves a biosynthetic role in addition to its more familiar function in energy formation, and production of specific biosynthetic products may underlie the importance of the TCA cycle in tumors. Precursors for protein, lipid, and nucleic acid synthesis are produced in the TCA cycle, and export of these precursors from the cycle to supply macromolecular synthesis is a prominent feature of proliferating cancer cells in culture (5). Pyruvate carboxylation is one of several mechanisms by which carbon can be resupplied to the TCA cycle to offset precursor export (Figure 1A). Such processes, termed anaplerotic pathways, prevent TCA cycle intermediates from becoming depleted during cell growth. Although several nutrients can supply anaplerosis, glutamine has so far been the most widely studied in cancer cells. Glutamine is highly abundant in tissues and plasma. Conversion of glutamine to α-ketoglutarate in the mitochondria, a process initiated by the enzyme glutaminase (GLS), supplies anaplerosis in many cultured cancer cell lines and in a subset of tumors in mice (6, 7)(Figure 1A). Sellers et al. found that while GLS and pyruvate carboxylase (PC) are both expressed in NSCLC, only PC expression is systematically enhanced in the tumors relative to that in healthy lung tissue. Although glutamine metabolism was not directly assessed in vivo, the labeling signatures of TCA cycle intermediates following injection of

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Figure 1. Analysis of human lung cancer metabolism in vivo. (A) Simplified view of intermediary metabolism in NSCLC. Central carbon metabolism is outlined in blue, pathways feeding macromolecular synthesis are in green, and anaplerotic pathways are in red. (B) Proposed workflow to analyze metabolic and molecular heterogeneity in lung tumors. Presurgical imaging techniques, including FDG-PET and multi-parametric MRI, are used to identify regions of putative heterogeneity. The patient is then infused with a 13C-labeled substrate at the time of surgery, and samples of the tumor are selected based on presurgical imaging. Each sample is then analyzed individually for a variety of potentially informative features.
the glycolytic rate, because the majority of lactate is secreted from the tumor and lost from analysis. It is possible that applying sophisticated computational modeling to isotope tracer data, coupled with dynamic approaches that noninvasively monitor metabolic activity, will ultimately deliver quantitative flux measurements; a combinatorial approach along these lines has already been applied to cancer cells (15). Third, tracers besides 13C-glucose should now be used to identify novel, potentially targetable metabolic liabilities in vivo. It is unclear whether PC dependence is actionable in cancer, because severe reductions in PC activity cause lactic acidosis, hypoglycemia, and developmental abnormalities (16); however, other pathways are discoverable through isotope infusion. For example, modeling enrichment data from 13C-glucose infusions in patients with gliomas and metastatic brain tumors indicated that these tumors use substrates in addition to glucose to supply the TCA cycle (10). A search for alternative substrates then revealed that 13C-acetate could be oxidized by human brain tumors (17). Because oxidation in the normal brain is dominated by glucose, acetate metabolism may prove to be a targetable pathway.

Finally, extension of the approach described by Sellers et al. (2) may make it possible to understand metabolic variations within individual tumors. Solid tumors can be dramatically heterogeneous in terms of regional perfusion, contribution of stromal and immune cells to the microenvironment, and extent of clonal expansion of molecularly unique cell populations. All of these are predicted to influence metabolism and may impact the efficacy of therapies aimed at a particular enzyme or pathway. An emerging challenge, therefore, is to develop approaches that allow 13C enrichment to be merged with assessment of tumor histology and genetics. We speculate that pre-surgical metabolic imaging could be used to guide sample selection in patients on 13C infusion protocols, thereby enabling post-hoc correlation of metabolic activity with biological parameters (Figure 1B). 13C-F-fluorodeoxyglucose-PET (FDG-PET), which is commonly used to assess lung tumors, would identify localized areas of intense glucose uptake. Multiparametric MRI would also be particularly useful for this type of analysis (18), as it can be used to assess regional heterogeneity of perfusion, oxygenation, cellularity, necrosis, and other features relevant to cancer cell metabolism. Using these imaging features to inform sample selection would enable a direct test of the hypothesis that proliferating cells demand high anaplerotic activities and would allow examination of the relative contributions of tumor genetics and microenvironment to metabolic phenotypes.

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