COMMENTARY

In vivo analysis of lung cancer metabolism: nothing like the real thing

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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.

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 ^{13}C-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 ^{13}C 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 labeling 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
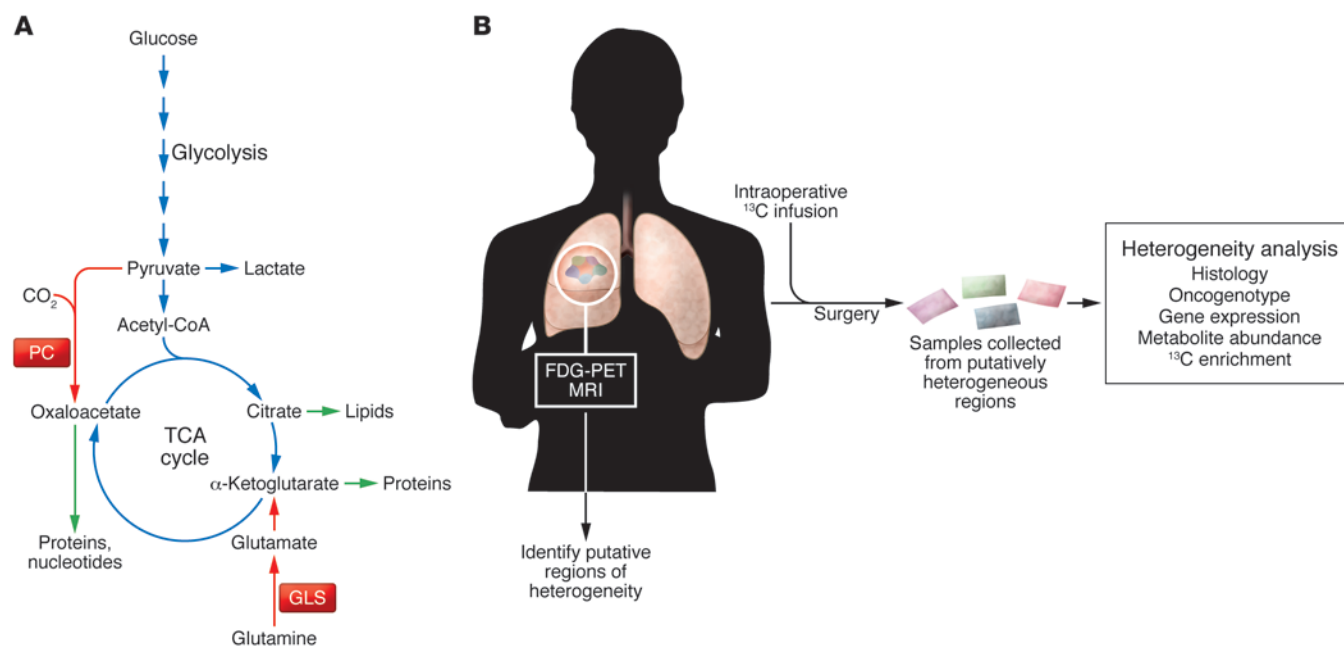
Figure 1. Analysis of human lung cancer metabolism in vivo. (A) Simplified view of intermediary metabolism in NSCLC. Central carbon metabolism is outlined in blue, pathways feeding macromolecular synthesis are in green, and anaplerotic pathways are in red. **(B)** Proposed workflow to analyze metabolic 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 ^{13}C-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.

^{13}C-glucose was consistent with enhanced PC-dependent anaplerosis in the tumors. Sellers et al. complemented their in vivo approach with analysis of freshly prepared slices of tumor and lung tissues that were cultured ex vivo with ^{13}C-labeled nutrients. These experiments demonstrated that both GLS and PC were active in tumor tissue; indeed, glutamine was a more prominent source of anaplerosis. However, only PC-dependent labeling was enhanced in the tumor relative to that observed in normal lung. Silencing PC expression in NSCLC cell lines altered TCA cycle activity and suppressed cell growth in culture and in xenografts (2).

The study by Sellers and colleagues adds to a number of recent reports that emphasize the prominence of mitochondrial metabolism in tumors, both in mice (7–9) and humans (10). The apparent preference for pyruvate carboxylation over glutamine-dependent anaplerosis is similar to the metabolic phenotypes of human-derived, high-grade orthotopic gliomas implanted from patients directly into the mouse brain (9). In this system, infusions with ^{13}C-glucose and ^{13}C-glutamine indicated the presence of PC activity but little, if any, glutamine catabolism, and cells

isolated from these glioma tumors did not require glutamine for survival or growth. Thus, while anaplerosis may be a fundamental feature of rapid cell proliferation, cancer cells and tumors seem to possess preferences for using either glutamine or pyruvate to supply anaplerosis. These preferences may be hard-wired by the oncogenotype, because enhanced MYC clearly induces a preference for glutamine in some systems (11–13). Interestingly, although glutamine-dependent, MYC-driven cancer cells can be coaxed into a glutamine-independent state in culture, this adaptation requires PC expression (14). Other tumor cell lines use PC as the preferred anaplerotic enzyme in culture and cannot acutely adapt to rely on glutamine when PC is silenced, similar to the NSCLC cell lines studied by Sellers et al (14).

Moving forward

A major strength of the Sellers et al. study is that it demonstrates the feasibility of a multidimensional approach to analyze metabolism with isotope tracers in primary tumor tissue from a large number of patients (more than 80 participated in one or more facets of the study). The authors' success in using a combination of periop-

erative ^{13}C-glucose infusions coupled with ex vivo tumor slices bodes well for additional studies in a variety of solid tumors. Hopefully, these techniques will further reduce the dependence on monolayer culture and murine models as the sole systems to study metabolic flux in cancer. However, several analytical and biological questions remain to be addressed to fully capture the potential of these approaches. First, two very different strategies have been used to deliver ^{13}C-labeled nutrients to cancer patients: the bolus method used by Sellers et al. (2) and a continuous infusion to approximate isotopic steady state in both the plasma and tumor (10). Each method has its advantages, including the practicality and low cost of the bolus and the opportunity to achieve sustained, high ^{13}C enrichment with infusions. A rigorous comparison of these two methods, preferably side by side in the same model, would help clarify the strengths and pitfalls of each delivery approach. Second, at the moment, these methods provide only a qualitative rather than quantitative view of pathway utilization and cannot formally measure metabolic rates. For example, although lactate labeling can be measured precisely, it is not equivalent to measuring

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 ^{13}C-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 ^{13}C-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 ^{13}C-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 ^{13}C 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 ^{13}C infusion protocols, thereby enabling post-hoc correlation of metabolic activity with biological parameters (Figure 1B). ^{18}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.

Acknowledgments

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