A Different Way of Doing Things

Cancer cells exhibit altered metabolic processes that may serve as promising targets for new therapies.

By Kivanç Birsoy and David M. Sabatini | April 1, 2016

Cellular metabolism comprises an elaborate network of thousands of biochemical reactions that allow a cell to grow, divide, and respond to its environment. More than 100 years of research has identified some 3,000 enzymes and nutrient transporters, but only recently has it become clear that cancer cells exploit these metabolic components to support their own proliferation and survival.

Compared to nonproliferating normal cells, cancer cells have a number of different metabolic needs. Each time a cancer cell divides, it must replicate the components that make it up, including its DNA, organelles, and lipid membranes. The rapid proliferation of cancer cells requires an ample supply of building blocks for the production of these cellular components, and cancer cells have devised clever ways to ensure that this well does not run dry. Given that many cancer cells are dependent on such metabolic changes for survival, interest in targeting these pathways for treating tumors has surged in the last

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decade. Although only a few therapies have reached the market so far, basic research over the last 10 years has revealed many promising new targets, some of which have entered human testing, and there is already precedent for this approach in the clinic.

The building blocks of cancer

The idea that cancer cells exhibit an altered metabolism was first introduced by German biochemist Otto Warburg in the 1920s. Using calorimetric techniques he developed, Warburg measured the rates of two major energy-producing pathways in tumors and normal tissues: mitochondrial respiration and glycolysis.[1] He found that, unlike normal cells, which perform the less-efficient process of glycolysis only when oxygen is unavailable, cancer cells rely on glycolytic metabolism even in the presence of oxygen.

This phenomenon, termed aerobic glycolysis, has since been observed across several tumor types and is often accompanied by a greater dependence on glucose. Taking advantage of such increased glucose uptake by cancer cells, clinicians can inject patients with a radiochemical glucose analog called 18F-fluorodeoxyglucose (FDG) and track its location in the body using positron emission tomography (PET) to visualize cancer.

Although aerobic glycolysis is generally accepted as a metabolic hallmark of cancer, researchers still debate why cancer cells perform the less energetically efficient metabolic process. Warburg hypothesized that cancer cells harbor dysfunctional mitochondria and are thereby forced to rely exclusively on glycolytic metabolism for energy, but many cancer cells do perform mitochondrial respiration, suggesting that these organelles are functional. Moreover, some proliferating normal cells without any mitochondrial defects also display glycolytic metabolism and consume high levels of glucose.

An alternative hypothesis is that increased glycolysis may help cancer cells more easily accumulate the essential metabolic precursors they need for rapid cell proliferation. Intermediates of glycolysis can feed into the pentose phosphate pathway, for example, generating precursors for nucleotide and DNA biosynthesis. These intermediates can also provide the carbon backbones for making the diverse amino acids (e.g., serine and glycine) needed for nucleotide and protein synthesis. (See illustration.) The finding that cancer cells carry a low-activity form of pyruvate kinase, which catalyzes the last step of glycolysis, further supports the critical role of glycolytic intermediates in cell proliferation. Known as PKM2, the low-activity enzyme slows down the glycolytic process, allowing more biosynthetic intermediates to accumulate and to be siphoned off for biosynthesis.[2] Indeed, improving efficiency of PKM2 with small molecule drugs that activate the kinase decreases the availability of these upstream metabolic precursors and stunts cancer cell growth.[3]

Despite their increased dependence on glycolysis, most cancer cells still require active mitochondrial respiration to proliferate. This does not appear to stem from a need for energy, however, but rather the need for a single amino acid, aspartate, which is not only an important component of many proteins but

is a precursor for nucleotide synthesis as well. Cancer cells with respiration defects are starved for aspartate, and eventually stop proliferating. Using forward genetics and metabolomics approaches, our group4 and Matt Vander Heiden's lab at MIT[5] recently demonstrated that aspartate levels decrease dramatically when respiration is blocked in cancer cells. The addition of this single amino acid is sufficient to restore proliferation of respiration-defective cancer cells.

Mitochondrial metabolism is not only responsible for producing aspartate, but also many other amino acids, as well as lipids and nucleotides. Precursors for these building blocks are constantly made in the mitochondria by the Krebs cycle and exported to the cytoplasm for the synthesis of cellular components. In cancer cells with high glycolytic rates, however, only a portion of glucose enters the Krebs cycle; most is metabolized by aerobic glycolysis into lactic acid, which is excreted to the extracellular environment. Cancer cells therefore need to provide the Krebs cycle with alternative raw materials—nutrients other than glucose. Glutamine, in addition to its role in protein synthesis, is a major carbon and nitrogen source that cancer cells commonly use to supply the Krebs cycle and other metabolic activities.[6] It is therefore not surprising that some cancer types upregulate glutamine transporters and enzymes to capture and use glutamine more effectively. Small molecule inhibitors of glutamine metabolism are currently in clinical trials and might be an effective treatment for such glutamine-addicted tumors.

Hijacking metabolic pathways

Unlike the metabolism of single-celled microbes, which is largely controlled by extracellular nutrient availability, the metabolism of each cell within a multicellular organism must be coordinated with the needs of the whole individual. This is mediated in part by molecules circulating in the bloodstream, such as growth factors, which simultaneously stimulate cell proliferation and enable cells to take up glucose, glutamine and other nutrients. In cancers, genes encoding proteins involved in growth factor signaling are often mutated, leading to constitutive activation of these pathways.[7] As a result, cancer cells begin to accumulate nutrients independent of their availability and these growth factor signals.

One such pathway often affected in cancer cells is the phosphoinositide 3-kinase (PI3K) pathway, which mediates glucose metabolism in response to insulin. In normal physiology, insulin enhances glucose uptake in tissues such as muscle and fat through PI3K signaling. In many cancer cells, mutations in several components of the PI3K pathway lead to its aberrant activation, enabling the cells to take up high levels of glucose independent of insulin. Similarly, the transcription factor Myc, another key regulator of cell growth and proliferation in normal cells, is deregulated in many cancer cells, stimulating the expression of genes involved in uptake and use of glutamine.

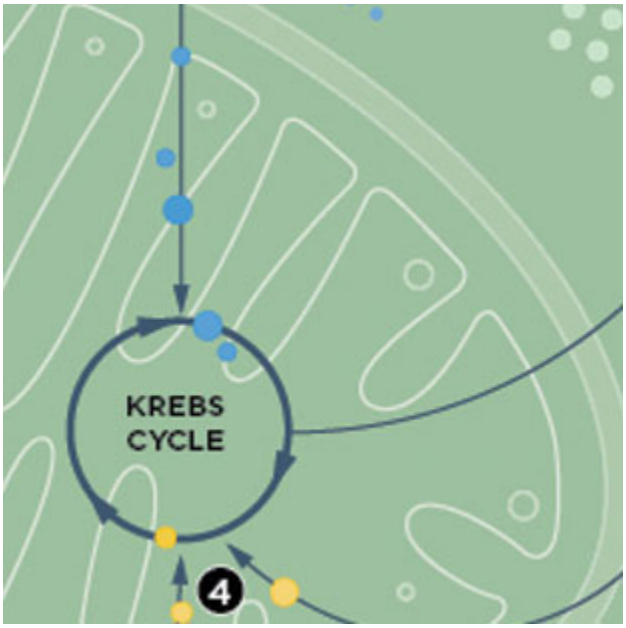
Research on cancer cell metabolism allows us to broaden our perspective on cancer, thinking of it not only as a genetic disease but as a disease of metabolic dysregulation.

In addition to the signaling components, there is growing evidence that metabolic enzymes can also be mutated and directly contribute to tumor formation. For example, genetic defects in the Krebs cycle enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH) lead to rare kidney and endocrine cancers. The genes encoding these enzymes behave as classic tumor suppressors—one mutant allele is usually inherited, while a second mutation occurs later in somatic cells, leading to cancer formation. Complete loss of these enzymes results in the accumulation of their upstream metabolites such as succinate and fumarate. Another Krebs cycle gene, *isocitrate dehydrogenase* (*IDH*), behaves as an oncogene; a mutation in a single allele is sufficient for cancer formation. This mutation, however, does not cause loss of activity but rather changes the enzyme's function in a way that results in the synthesis of an alternative metabolite called 2-hydroxyglutarate (2-HG).[8,9] While the underlying tumorigenic effects of these metabolic gene mutations

are not completely understood, the accumulation of the relevant metabolites (succinate, fumarate, and 2-HG) is thought to cause cancer by disrupting the epigenetic program of normal cells (i.e., by changing DNA methylation).[10]

The tumor environment

Another important factor that influences metabolism in cancer cells is the environment that they live in. In rapidly growing tumors, cancer cells are frequently starved for oxygen and nutrients, in part as a result of leaky and disorganized blood vessels. (See "The Forces of Cancer" from this issue.) One common cellular response to the low-oxygen conditions of a tumor is to activate a transcription factor called hypoxia inducible factor (HIF), which upregulates glycolytic enzymes and glucose transporters and switches the metabolism of cancer cells to glycolysis, enabling the cells to rely less on mitochondrial respiration and thus less on oxygen.



METABOLIC REPROGRAMMING: To support their unchecked proliferation, cancer cells often make metabolic adjustments to increase the supply of precursors to the necessary building blocks of the cell, such as amino acids, nucleotides, and lipids. Many of these altered metabolic pathways could serve as targets for novel anticancer therapies.
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Low oxygen also affects the function of metabolic enzymes that require molecular oxygen. For example, lipid desaturases employ oxygen to form the carbon-carbon double bonds that render fatty acid chains "unsaturated." These unsaturated fatty acids are critical components of the plasma membrane and contribute to its fluidity and permeability. By blocking the formation of unsaturated fatty acids, low oxygen levels lead to an accumulation of saturated fatty acids and prevent cell membranes from effectively controlling molecular transport, signaling, and cellular metabolic activities. To deal with this imbalance, many cancer cells import missing unsaturated fatty acids from their local environments.[11] In some cases, these cellular lipids can be directly transferred from nearby lipid-rich cells such as adipocytes.[12] Lipid saturation and import are therefore potential targets for killing cancer cells under low oxygen conditions.

Proliferating cancer cells must also adapt to deprivation of nutrients such as glucose and amino acids as a result of both impaired blood flow and rapid nutrient consumption. The glucose concentration in tumors is often much lower than in normal tissues. To deal with glucose deprivation, most cancer cells reversibly switch from glycolysis to mitochondrial respiration and rely on the electron transport chain to harness energy. As a result, blocking respiration can halt the proliferation of cancer cells under low glucose conditions.[13] A subset of pancreatic cancers takes a different approach to dealing with nutrient scarcity—taking up extracellular proteins and breaking them down into their component parts. Unlike amino acids, which are imported into cells via cell-membrane transporters, extracellular proteins enter cells through a process called macropinocytosis, in which bulk extracellular contents are taken up in large sacs.[14] These sacs then fuse with lysosomes, which digest the proteins and release free amino acids into the cytoplasm for protein synthesis. This process is stimulated by a mutant form of the protein K-ras, which generates signals to ignore environmental cues to stop dividing. It remains to be seen if disrupting this feeding mechanism could be a viable strategy to starve and slow down these hard-to-treat tumors.

Targeting cancer metabolism

Recent discoveries on how cancer cells exhibit metabolism distinct from that of normal cells are stirring excitement among researchers about the possibility of targeting these pathways to stunt cancer growth. While no new metabolism-based therapies have been approved yet, a handful of older cancer drugs suggest that such an approach could be a powerful complement to current treatments.

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Early attempts to develop metabolism-based cancer therapies focused on blocking new DNA synthesis. In the 1940s, Sidney Farber of Harvard Medical School observed that administration of folate, a vitamin critical in making new nucleotides, exacerbated leukemia progression. Farber speculated that interfering with folate metabolism, using synthesized folate analogs (anti-folate) to block the function of folate-requiring enzymes, could help stem cancerous growth. These agents were shown to be remarkably effective against leukemic tumors and formed the basis for the use of chemotherapy for cancers.[15] These days, anti-folate drugs (e.g. methotrexate) are routinely used as a part of standard chemotherapy for breast and blood cancers.

Another great example of exploiting cancer metabolism in the clinic involves the dependence of certain leukemias on the amino acid asparagine. Unlike normal cells, which are capable of making their own asparagine, these blood cancer cells are dependent on outside asparagine sources. In 1953, John Kidd of the Cornell Medical Center and his colleagues were the first to observe asparagine dependence of cancer cells, while testing the effect of guinea pig serum on cell proliferation.[16] The researchers found that adding guinea pig serum to cancer cell cultures strongly suppressed growth due to the presence of an enzyme called asparaginase that degrades extracellular asparagine. When injected into animals and humans, this enzyme depletes the amino acid from the blood, starving cancer cells of asparagine and killing them, while sparing normal cells. More-effective forms of asparaginase were later isolated from bacterial species and are currently part of the standard chemotherapy for the treatment of blood cancers. Human serum contains approximately 5,000 other metabolites, most with unknown cancer dependencies. Restricting the uptake or usage of these nutrients has the potential to disrupt cancer cell proliferation without affecting normal cells.

The metabolism of the whole organism can also influence tumor metabolism. As obesity and insulin resistance are generally associated with increased cancer risk, antidiabetic drugs such as metformin and phenformin have recently been explored for their anticancer effects. Several recent retrospective studies of metformin have uncovered substantial effects. For example, a study of diabetic individuals with pancreatic cancer showed a 15 percent increase in two-year survival among patients taking metformin.[17] A decrease in circulating insulin levels in people treated with metformin might be responsible for the lower

cancer incidence, as insulin, through the PI3K pathway, promotes cancer cell growth and nutrient uptake. However, it recently became clear that these diabetes drugs also exert antitumor effects directly, slowing down tumor growth by blocking the mitochondrial complex I component of the electron transport chain.[18] Adding a single yeast complex I protein that is resistant to metformin to tumors in animal models, researchers blunted the anticancer effect of metformin, suggesting the presence of a direct effect on mitochondrial respiration.[13,19] While the details of how metformin interacts with cancer cells is still debated, similar drug- repurposing approaches may be promising for cancer therapy.

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The revitalization of research on cancer cell metabolism offers exciting new therapeutic possibilities, some based on drugs already in use such as methotrexate and asparaginase, and many that will emerge with a greater understanding of metabolic vulnerabilities in individual cancers. Research on cancer cell metabolism also allows us to broaden our perspective on cancer and how to treat it, thinking of it not only as a genetic disease but as a disease of metabolic dysregulation. Although Warburg began to uncover the principles of cancer cell metabolism almost a century ago, it is only with resurgent interest and new technologies that we can put the whole puzzle together.

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References

1. O. Warburg et al., "Über den stoffwechsel der tumoren," *Biochem Zeitschrift*, 152:319-44, 1924.
2. M.G. Vander Heiden et al., "Understanding the Warburg effect: The metabolic requirements of cell proliferation," *Science*, 324:1029-33, 2009.
3. D. Anastasiou et al., "Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis," *Nat Chem Biol*, 8:839-47, 2012.
4. K. Birsoy et al., "An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis," *Cell*, 162:540-51, 2015.
5. L.B. Sullivan et al., "Supporting aspartate biosynthesis is an essential function of respiration in proliferating cells," *Cell*, 162:552-63, 2015.
6. ☐C.T. Hensley et al., "Glutamine and cancer: Cell biology, physiology, and clinical opportunities," *J Clin Invest*, 123:3678-84, 2013.
7. R.G. Jones, C.B. Thompson, "Tumor suppressors and cell metabolism: A recipe for cancer growth," *Genes Dev*, 23:537-48, 2009.
8. P.S. Ward et al., "The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate," *Cancer Cell*, 17:225-34, 2010.
9. ☐L. Dang et al., "Cancer-associated IDH1 mutations produce 2-hydroxyglutarate," *Nature*, 462:739-44, 2009. (Addendum published in *Nature*, 465:966, 2010.)

10. ☐M. Yang et al., "Oncometabolites: linking altered metabolism with cancer," *J Clin Invest*, 123:3652-58, 2013.
11. ☐J.J. Kamphorst et al., "Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids," *PNAS*, 110:8882-87, 2013.
12. K.M. Nieman et al., "Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth," *Nature Med*, 17:1498-503, 2011.
13. K. Birsoy et al., "Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides," *Nature*, 508:108-12, 2014.
14. C. Commisso et al., "Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells," *Nature*, 497:633-37, 2013.
15. S. Farber et al., "Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (Aminopterin)," *N Engl J Med*, 238:787-93, 1948.
16. J.G. Kidd, "Regression of transplanted lymphomas induced in vivo by means of normal guinea pig serum. II. Studies on the nature of the active serum constituent: histological mechanism of the regression: tests for effects of guinea pig serum on lymphoma cells in vitro: discussion," *J Exp Med*, 98:583-606, 1953.
17. N. Sadeghi et al., "Metformin use is associated with better survival of diabetic patients with pancreatic cancer," *Clin Cancer Res*, 18:2905-12, 2012.
18. M. Buzzai et al., "Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth," *Cancer Res*, 67:6745-52, 2007.
19. W. W. Wheaton et al., "Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis," *eLife*, 3:e02242, 2014.

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