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## REVIEW

# Is Cancer a Metabolic Disease?

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Although cancer has historically been viewed as a disorder of proliferation, recent evidence has suggested that it should also be considered a metabolic disease. Growing tumors rewire their metabolic programs to meet and even exceed the bioenergetic and biosynthetic demands of continuous cell growth. The metabolic profile observed in cancer cells often includes increased consumption of glucose and glutamine, increased glycolysis, changes in the use of metabolic enzyme isoforms, and increased secretion of lactate. Oncogenes and tumor suppressors have been discovered to have roles in cancer-associated changes in metabolism as well. The metabolic profile of tumor cells has been suggested to reflect the rapid proliferative rate. Cancer-associated metabolic changes may also reveal the importance of protection against reactive oxygen species or a role for secreted lactate in the tumor microenvironment. This article reviews recent research in the field of cancer metabolism, raising the following questions: Why do cancer cells shift their metabolism in this way? Are the changes in metabolism in cancer cells a consequence of the changes in proliferation or a driver of cancer progression? Can cancer metabolism be targeted to benefit patients? (*Am J Pathol* 2014, 184: 4–17; <http://dx.doi.org/10.1016/j.ajpath.2013.07.035>)

## Discoveries of Otto Warburg

Otto Warburg's pioneering work in the 1920s established that tumor cells exhibit altered metabolism. Warburg discovered an important distinction between the relative use of different modes of energy production in normal cells and tumors. In normal tissues, most of the pyruvate formed from glycolysis enters the tricarboxylic acid (TCA) cycle and is oxidized via oxidative phosphorylation. In tumors, in contrast, the pyruvate is largely converted to lactic acid and energy is produced anaerobically.<sup>1</sup> This finding seemed counterintuitive. Surely, a rapidly proliferating cancer cell would prefer the 36 ATPs that can be claimed by complete oxidation of a glucose molecule to the two ATPs available through glycolysis. Furthermore, this shift in metabolism in which pyruvate is converted to lactate and secreted, rather

than being oxidized, occurred in tumors even when there was sufficient oxygen to support mitochondrial function. The conversion of most pyruvate to lactate through fermentation, even when oxygen is present, is called aerobic glycolysis or the Warburg effect.

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## Evidence that Aerobic Glycolysis Promotes Tumorigenesis

Since these early discoveries, rapid consumption of glucose and secretion of lactate have been discovered to be a characteristic of many types of tumors. By using the imaging agent 2-<sup>18</sup>F]fluoro-2-deoxy-D-glucose, coupled with positron emission tomography (PET), primary and metastatic lesions can be identified with a specificity and sensitivity near 90%.<sup>2</sup> Furthermore, glucose uptake assessed with PET correlates with poor prognosis in oral squamous cell carcinoma,<sup>3</sup> gastric cancer,<sup>4</sup> and neoplasms of other tissues.<sup>5</sup> Tumor-produced lactate concentrations also correlate with shorter survival and increased metastases in cervical and head and neck cancer.<sup>6–8</sup> Overall, the association between a glycolytic phenotype and poor prognosis, along with the consistency of the phenotype and its usefulness for diagnosis, supports a model in which metabolic changes are a reproducible characteristic of cancer cells and may even promote disease progression.

In this review, we consider the way in which cancer cells rewire their metabolism with a focus on a few key questions. What is the metabolic phenotype of cancer cells and how is it achieved molecularly? How do oncogenes and tumor suppressors coordinate and enforce the metabolic changes that occur with cancer? Is the metabolic phenotype of cancer cells a reflection of their rapid growth? Why do tumor cells undergo this dramatic shift (ie, what advantage would an inefficient energy production program confer)? Are metabolic changes drivers of cancer progression or do they just come along for the ride? And finally, is the cancer metabolic profile sufficiently distinct from that of normal cells that it can be targeted therapeutically?

## Molecular Basis for the Cancer Cell Metabolic Phenotype

### Cancer Cells Reengineer Glycolysis

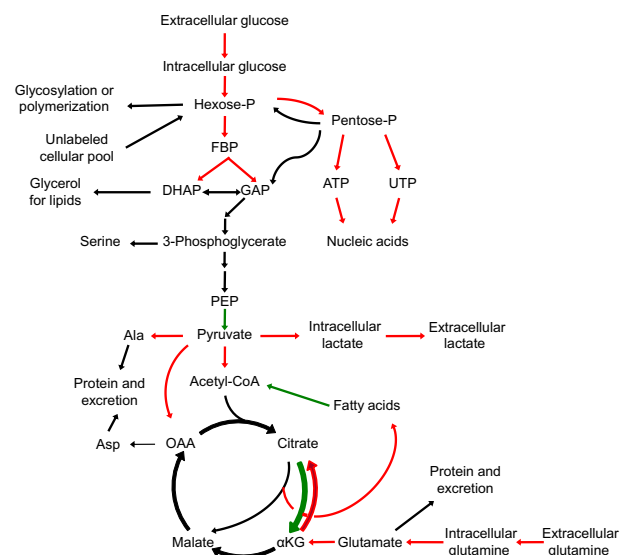
Cancer cells evade the mechanisms that normally regulate glycolytic flux using multiple different strategies. The levels of many different glycolytic enzymes are induced in tumors<sup>9</sup> (Figure 1 and Table 1). In addition, cancer cells subvert the feedback mechanisms that normally allosterically inhibit rate-controlling steps in glycolysis. For instance, phosphofructokinase (PFK) is inhibited by ATP; when the cell is energy rich, glycolysis should decrease. However, when glucose is abundant, the metabolite fructose 2,6-bisphosphate is formed from fructose 6-phosphate by 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatases (PFKFBP1-4), and fructose 2,6-bisphosphate can override ATP-mediated PFK inhibition. In tumor cells, high levels of glucose transport<sup>2,10,11</sup> and hexokinase activity<sup>10,24,25</sup> lead to elevated levels of fructose 2,6-bisphosphate, which allosterically activates PFK. The specific PFK isozymes overexpressed in cancer cells are less sensitive to allosteric inhibition by ATP and more strongly activated by fructose 2,6-bisphosphate.<sup>31</sup> Cancer cells also

trick themselves and generate cues that there are higher levels of blood glucose than actually exist by overexpressing PFKFBPs, increasing the levels of fructose 2,6-bisphosphate and, thus, driving glycolysis.<sup>34</sup> As a result of these different mechanisms of activation, PFK activity is much higher in cancer cells than normal tissue.<sup>31</sup>

Cancer cell lines and tumors also reexpress the embryonic isoform (PKM2) of pyruvate kinase (PK).<sup>39</sup> PKM2 is distinguished from other PK isoforms because it can associate with tyrosine-phosphorylated peptides,<sup>68</sup> an association that results in a transition to a dimeric form with low affinity for its substrate, phosphoenolpyruvate.<sup>69</sup> The less active PKM2 allows for a diversion of glycolytic metabolites to serine and glycine biosynthetic pathways.<sup>70</sup> Phosphorylated PKM2 can also translocate to the nucleus, phosphorylate histone H3, and act as a transcriptional co-activator that induces expression of genes involved in glycolysis.<sup>71</sup>

The shunting of pyruvate to secreted lactate in tumors is associated with elevated levels of lactate dehydrogenase (LDH)<sup>48</sup> and monocarboxylate transporters (MCTs) that cotransport lactate and a proton out of the cell.<sup>52</sup> Elevated LDH levels have been discovered in Burkitt's lymphoma<sup>48</sup> and non-small cell lung cancer,<sup>72</sup> whereas increased MCT levels have been detected in ovarian,<sup>73</sup> prostate,<sup>52</sup> gastric,<sup>74</sup> and cervical<sup>75</sup> carcinomas. The shift of pyruvate toward lactate production and away from oxidative phosphorylation also reflects decreased activity of the pyruvate dehydrogenase complex, which can result from induction of the inhibitory pyruvate dehydrogenase kinases (PDKs).<sup>42</sup>

There is substantial evidence that elevated glucose consumption and increased lactate secretion in tumors contribute to their growth. Patients with type 2 diabetes have high levels of blood glucose and an increased risk of developing cancers



**Figure 1** Cancer metabolism. Scheme shows central carbon metabolism. Metabolic reactions that tend to be faster in tumors are identified in red, whereas reactions that tend to be slower in tumors are identified in green. DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; KG,  $\alpha$ -ketoglutarate; OAA, oxaloacetate; PEP, phosphoenolpyruvate.

**Table 1** Metabolic Changes in Tumors and Activated Lymphocytes

Metabolic step	Cancer cells	Primary tumors	Functional importance	Potential target	Activated lymphocytes	Potential oncogene target
Glucose uptake/ glucose transporters	Increased <sup>10</sup>	Increased <sup>2,11</sup>	Yes <sup>12,13</sup>	Yes <sup>14</sup>	Increased <sup>15–18</sup>	Induced by MYC, <sup>19,20</sup> AKT, <sup>15</sup> and HIF <sup>21</sup> and repressed by p53 <sup>22,23</sup>
Hexokinase	Hexokinase II increased <sup>24,25</sup>	Hexokinase II increased <sup>25</sup>	Yes <sup>26</sup>	Yes <sup>27</sup>	Increased <sup>17,28</sup>	Induced by MYC <sup>29</sup> and AKT <sup>30</sup>
Phosphofructokinase	Liver isozyme induced <sup>31</sup>	Liver isozyme increased <sup>31</sup>	Yes <sup>32</sup>	Yes <sup>32</sup>	Increased <sup>17</sup>	Induced by MYC <sup>20</sup> and AKT <sup>33</sup>
6-Phosphofructo-2- kinase	Induced <sup>34</sup>	Increased <sup>34</sup>	Yes <sup>35</sup>	Yes <sup>36</sup>	Increased <sup>37</sup>	Induced by p53 <sup>38</sup>
Pyruvate kinase	Shift to PKM2 <sup>39</sup>	Shift to PKM2 <sup>39</sup>	Yes <sup>39–41</sup>	Yes <sup>39–41</sup>	Increased <sup>17,28</sup>	
Pyruvate dehydrogenase kinase		Increased <sup>42</sup>	Yes <sup>43,44</sup>	Yes <sup>44,45</sup>		Increased by HIF <sup>46</sup> and repressed by p53 <sup>47</sup>
Lactate dehydrogenase		Increased <sup>48</sup>	Yes <sup>49,50</sup>	Yes <sup>51</sup>	Increased <sup>28</sup>	Increased by MYC <sup>50</sup>
Monocarboxylate transporters	Increased <sup>52</sup>	Increased <sup>52</sup>	Yes <sup>53</sup>	Yes <sup>53</sup>	Increased <sup>28</sup>	Repressed by p53 <sup>54</sup>
Lactate secretion		Increased <sup>49</sup>	Yes <sup>49,50</sup>		Increased <sup>15</sup>	Increased by MYC <sup>19</sup> and repressed by p53 <sup>22</sup>
ATP citrate lyase		Increased <sup>55</sup>	Yes <sup>56</sup>	Yes <sup>56</sup>		Activated by AKT <sup>57</sup> Increased by MYC <sup>60</sup>
Glutamine consumption/ glutamine transporters	Increased <sup>58</sup>				Increased <sup>17,28,59</sup>	
Glutaminase	Increased <sup>61</sup>		Yes <sup>62</sup>	Yes <sup>19,62</sup>	Increased <sup>17,59</sup>	Increased by MYC <sup>61</sup>
Glutamate dehydrogenase			Yes <sup>63</sup>	Yes <sup>63</sup>	Increased <sup>59</sup>	
Glutamate oxaloacetate transaminase			Yes <sup>63</sup>	Yes <sup>60,63,64</sup>	Increased <sup>28,59</sup>	
Oxidative phosphorylation	May increase <sup>65–67</sup>		Yes <sup>67</sup>	Yes <sup>67</sup>	Increased <sup>18</sup>	Induced by MYC <sup>67</sup> and p53 <sup>22</sup>

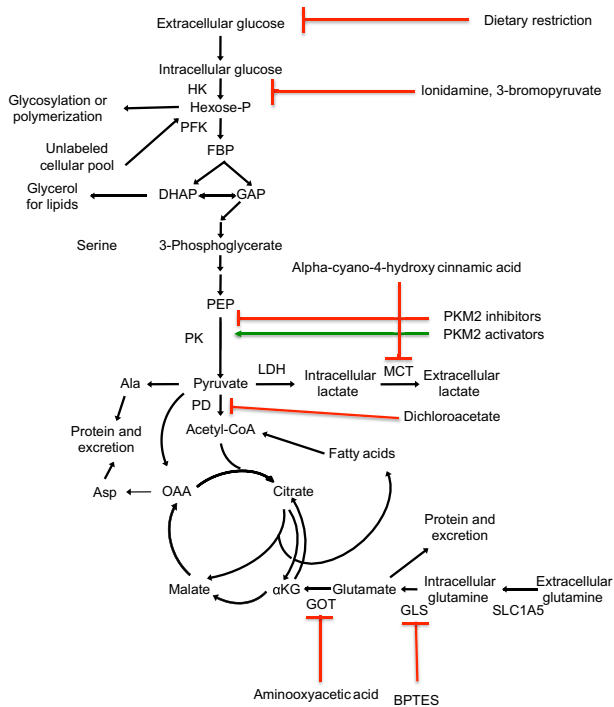
of the pancreas, liver, colon, gastrointestinal tract, breast, and endometrium.<sup>76</sup> Inhibiting expression of a glucose transporter GLUT1,<sup>12</sup> PKM2,<sup>40</sup> LDH,<sup>49</sup> or PDK<sup>43</sup> results in reduced tumorigenicity in xenograft models. Reducing the levels of 6-phosphofructo-2-kinase suppresses glycolytic flux, growth in soft agar, and tumor growth in mice.<sup>35</sup> Knocking down the  $\beta$ -catalytic subunit of the mitochondrial H<sup>+</sup>-ATP synthase results in a higher glycolytic rate and a more aggressive tumor-forming phenotype.<sup>77</sup> Taken together, these studies highlight the importance of the glycolytic phenotype for tumor progression.

Multiple approaches to reducing glycolytic flux are being considered as potential cancer therapies (Figure 2 and Table 1). In one strategy, patients eat low-carbohydrate diets, thus starving their tumors of glucose, and it was shown to be promising in a recent pilot study.<sup>14</sup> Pharmacological approaches are also being attempted. Lonidamine, a derivative of indazole-3-carboxylic acid that inhibits hexokinase, reduces cancer cell proliferation, and sensitizes xenograft tumors to death by radiation and other compounds.<sup>27</sup> An inhibitor of PFKFB3, 3-(3-pyridinyl)-1-

(4-pyridinyl)-2-propen-1-one, decreases intracellular concentrations of fructose 2,6-bisphosphate, suppresses glucose uptake, reduces the growth of cells from multiple types of cancer *in vitro*, and inhibits the growth of established tumors *in vivo*.<sup>36</sup> Dichloroacetate, a pyruvate mimetic that inhibits pyruvate dehydrogenase kinase, increases pyruvate dehydrogenase activity and the oxidation of glucose, reduces the proliferation of breast cancer cell lines, inhibits proliferation, and slows xenograft tumor growth.<sup>44</sup> In a pilot study, dichloroacetate resulted in radiological regression in three of five patients with glioblastoma multiforme.<sup>45</sup> In sum, there are substantial data to suggest that impeding glycolysis, or redirecting pyruvate toward oxidative pathways and away from its conversion to lactate, inhibits tumor growth.

#### Glutamine Is the Major Anaplerotic Source for Cancer Cells

Some cancer cells also run the TCA cycle in a pattern that distinguishes them from most non-transformed cells. In some cancer cells, pyruvate from glycolysis enters a truncated TCA



**Figure 2** Metabolic approaches to treating cancer. Scheme shows some of the compounds being explored as anticancer agents and the metabolic reactions that they target. **Red lines** indicate inhibition; **green lines**, activation. BPTES, bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; GLS, glutamine synthetase; GOT, glutamate oxaloacetate transaminase; HK, hexokinase; MCT, monocarboxylate transporters; OAA, oxaloacetate; PD, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PK, pyruvate kinase.

cycle that ends as citrate is shuttled from the mitochondrial matrix to the cytosol.<sup>78</sup> Citrate is cleaved by ATP citrate lyase (ACL) to provide acetyl-CoA that can be used for fatty acid synthesis. Disruption of ACL impairs tumor growth.<sup>56</sup> This truncated TCA cycle results in a flow of metabolites out of the TCA cycle (cataplerosis) that needs to be balanced by an influx of metabolites (anaplerosis). In many cancer cells, glutamine fulfills this role: it is converted to glutamate and then to the TCA intermediate,  $\alpha$ -ketoglutarate.<sup>79</sup> Although glucose is the precursor for 90% of secreted lactate in cancer cells, oxidative conversion of glutamine accounts for as much as 40% of TCA cycle intermediates<sup>79</sup> and  $\geq 30\%$  of the ATP generated.<sup>61,79</sup> To meet the glutamine requirements, some cancer cells dramatically increase glutamine consumption through induction of glutamine transporters.<sup>58</sup> Cancer cells also induce enzymes that metabolize glutamine, such as glutaminases, that convert glutamine to glutamate (glutaminase1 and glutaminase C)<sup>61</sup> and glutamate oxaloacetate transaminases that convert glutamate to  $\alpha$ -ketoglutarate.<sup>80</sup>

Glutamine withdrawal results in the death of some cancer cells,<sup>60</sup> which is surprising because glutamine is a nonessential amino acid that can be synthesized from glucose. The strict requirement of some tumors for glutamine makes glutaminolysis enzymes attractive anticancer targets.

Glutaminase inhibitors, such as bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide, reduce cancer cell growth, transformation, and tumorigenesis.<sup>19,62</sup> Transaminase inhibitors have also been suggested as anticancer agents because glutamine-derived carbons are more likely to enter the TCA cycle through transamination in cancer cells, whereas normal cells tend to rely more heavily on glutamate dehydrogenase.<sup>80</sup> Transaminase inhibitor, aminoxyacetic acid, has a cytotoxic effect specifically on cancer cells,<sup>60,63,64</sup> with little effect on healthy cells.<sup>64</sup> Treatment with aminoxyacetic acid reduced the growth of breast cancer cells in a mouse xenograft model without any obvious dose-limiting toxicities.<sup>64</sup>

## Reevaluation of the Warburg Effect

Warburg hypothesized that the shift from respiration to aerobic glycolysis in cancer cells reflects defective mitochondrial respiration.<sup>1</sup> In support of this model, tumors tend to down-regulate the expression of genes involved in oxidative phosphorylation in general,<sup>81</sup> and specifically, the  $\beta$ -F1 subunit of the ATP(synth)ase.<sup>82</sup> In addition, mutations in mitochondrial DNA have been observed in multiple tumor types.<sup>83</sup> Furthermore, experiments in which the levels of mitochondrial components are modulated have largely reinforced the importance of the glycolytic phenotype for tumor growth *in vivo*.<sup>77</sup> Taken together, the findings of the functional importance of high glycolytic rates and mitochondrial abnormalities in tumors have contributed to the prevailing paradigm that tumors generate most of their ATP through glycolysis.

However, this model is being reevaluated for several reasons. First, recent studies have indicated that some tumor cell lines do perform oxidative metabolism.<sup>65,66,84</sup> In some studies, respiration actually increases in tumor mitochondria.<sup>65,67</sup> In one study, glycolysis contributed 50% to 70% of ATP for some cancer cell lines, consistent with Warburg's findings, but as little as 10% of cellular ATP in other cell lines.<sup>67</sup> Furthermore, there are studies that indicate that mitochondrial activity and oxidative phosphorylation support tumor growth.<sup>85,86</sup> In particular, overexpression of the mitochondrial citrate transporter has been shown to increase tumor growth in xenograft models, whereas inhibition of the mitochondrial citrate transporter, which enhances glycolysis, actually reduces tumor growth.<sup>87</sup> Further supporting such a model, some human and rodent tumors are susceptible to death induced by highly specific respiratory inhibitors.<sup>67</sup>

The Warburg effect is also being reconsidered by investigators who have argued that some of the cells within a tumor actually consume, rather than secrete, lactate. Lactic acid recycling occurs in normal physiological conditions as contracting skeletal muscle supplies lactate to the liver. The liver uses gluconeogenesis to convert lactate back to glucose that is released into the bloodstream and absorbed by muscle, thus completing the Cori cycle. In the tumor microenvironment,

oxidative tumor cells (eg, those near blood vessels) have been proposed to consume lactate secreted by tumor cells that are engaging in aerobic glycolysis.<sup>53</sup> Absorbed lactate can be converted to pyruvate and used to fuel oxidative phosphorylation in these well-oxygenated cells. The reliance of aerobic cells within a tumor on lactate as a fuel may preserve the available glucose for the hypoxic cells that strictly require it.<sup>53</sup>

### Metabolism of the Tumor Stroma

It has also been proposed that cells within the host tissue, the stroma, and not the tumor cells, perform aerobic glycolysis. Stromal cells, for example, the fibroblasts, in the tumor microenvironment can actively support malignant transformation<sup>88</sup> and metastasis.<sup>89</sup> A hypothesis has been proposed that the tumor stroma is glycolytic and that stromal cells express MCTs that exude lactate, whereas tumor cells perform oxidative metabolism and express transporters that consume lactate.<sup>90,91</sup> The proposed model is that tumor growth is fueled by lactate, ketones, and glutamine provided by stromal cells that are then absorbed by cancer cells and used for oxidative phosphorylation. It has been further suggested that the PET avidity observed by tumors reflects 2-deoxy-glucose uptake by nearby stromal and inflammatory cells rather than the cancer cells themselves.<sup>84</sup> This model has been called the reverse Warburg effect because the increased glycolysis occurs in the surrounding stromal cells, rather than the tumor cells.<sup>91</sup> From this perspective, cancer is viewed as a parasitic disease that steals energy-rich metabolites from the host organism.<sup>91–93</sup>

### Summary of Molecular Mechanisms of Cancer Metabolism

In summary, although studies have recently questioned the glucose flux paradigm,<sup>87,91</sup> the prevailing model is that there is higher flux of glucose through most metabolic pathways in tumor cells compared with normal cells. More glucose is transmitted to metabolic intermediates, lactate, citrate, and fatty acid synthase, and possibly even more to oxidative phosphorylation.<sup>78</sup> Meeting all of these conditions would seem to require a large increase in glucose uptake in tumors. PET imaging has confirmed the increased glucose consumption in many, but not all, tumors, and glucose consumption rates exceed the amounts that can be easily explained by needs for energy or metabolites.<sup>2</sup> Glutamine consumption follows a similar pattern of excess consumption.<sup>79</sup> We consider now the mechanisms that enforce this metabolic shift and possible explanations for its occurrence.

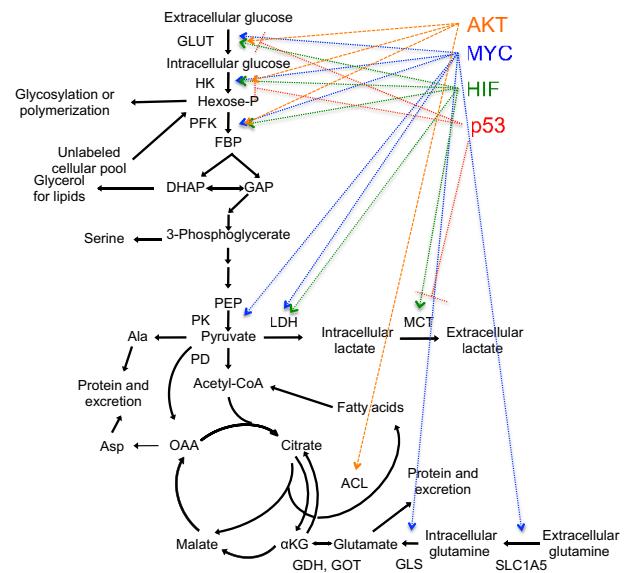
### Oncogenes and Tumor Suppressors Enforce the Metabolic Shift

The key to understanding the mechanism(s) affecting changes in metabolism in tumors lies in the discovery that

oncogenes and tumor suppressors consistently activated or deleted in tumors are important regulators of metabolism.<sup>78,94</sup> The oncogenic molecules AKT, MYC, and hypoxia-inducible factor-1 (HIF-1) can all contribute to the metabolic shift that occurs during carcinogenesis (Figure 3 and Table 1), whereas the tumor suppressor p53 acts to minimize the glycolytic phenotype and its loss contributes to aerobic glycolysis and the tumor metabolic phenotype. In tumors, multiple oncogenic mutations likely cooperate with each other to result in a phenotype in which cells absorb nutrients to meet or even exceed the bioenergetic demands of cell growth and proliferation.

### PI3K/AKT

In non-transformed cells, the phosphatidylinositol-3-kinase (PI3K) pathway is activated in response to growth signals.<sup>15</sup> In a sizable fraction of all cancers, the PI3K pathway is constitutively activated through mutation or amplification,<sup>95</sup> resulting in constitutive activation of AKT kinase and a growth-promoting metabolic program. AKT activation increases the glycolytic rate, in part by increasing GLUT1 expression<sup>15</sup> and translocation of GLUT1 to the plasma membrane.<sup>16</sup> AKT causes the glycolytic enzyme, hexokinase, to associate with the mitochondrial outer membrane.<sup>30</sup> AKT also performs an activating phosphorylation of PFK that releases its inhibition by ATP.<sup>33</sup> Finally, AKT promotes the conversion of citrate to fatty acids by phosphorylating and activating ACL.<sup>57</sup> By simultaneously reducing the expression of carnitine palmitoyltransferase 1A,<sup>96</sup> an enzyme that



**Figure 3** Metabolic effects of oncogenes and tumor suppressors. Scheme shows the metabolic reactions in central carbon metabolism affected by AKT (orange), MYC (blue), HIF (green) and p53 (red). **Arrows** indicate activation; **lines**, repression. DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; GDH, glutamate dehydrogenase; GLS, glutamine synthetase; HK, hexokinase; KG,  $\alpha$ -ketoglutarate; LDH, lactate dehydrogenase; OAA, oxaloacetate; PEP, phosphoenolpyruvate.

initiates the esterification and breakdown of long-chain fatty acids, AKT may eliminate a potential nutrient source and contribute to the glucose addiction of some cancer cells. Thus, activation of the PI3K/AKT pathway can be a powerful mechanism for altered tumor cell metabolism.

## MYC

Deregulated expression of c-MYC, an early serum response transcription factor, is one of the most common oncogenic events in cancer.<sup>97</sup> Although MYC has well-established roles in the regulation of cell proliferation, differentiation, and apoptosis, MYC also drives the accumulation of cellular biomass by regulating nucleotide biosynthesis, ribosome and mitochondrial biogenesis, and metabolism.<sup>98</sup> In an MYC-inducible human Burkitt's lymphoma model, glucose consumption, lactate production, glutamine uptake, and glutamine incorporation into the TCA cycle were all induced by MYC.<sup>19,20,50</sup> The induction of LDH by MYC has been specifically demonstrated to be functionally important for tumor growth, because MYC-dependent tumors exhibit reduced proliferative capacity and ability to grow in soft agar when LDH expression is reduced.<sup>50</sup> MYC also promotes glutamine metabolism by inducing the expression of glutamine transporters<sup>60</sup> and by up-regulating levels of glutaminase indirectly via repression of the miRNA miR-23.<sup>61</sup> As a result, some MYC-transformed cells have an absolute requirement for glutamine to maintain continuous replenishment of TCA cycle intermediates.<sup>19,60,99</sup>

## HIF

The oxygen-sensitive HIF-1 transcription factor is a heterodimer composed of constitutively expressed  $\beta$  subunits and oxygen-sensitive  $\alpha$  subunits.<sup>100</sup> In well-oxygenated cells, HIF-1 $\alpha$  is hydroxylated, which facilitates its ubiquitination and degradation by the proteasome. In hypoxic conditions, HIF-1 is stabilized and activated. During tumorigenesis, localized hypoxic regions in which HIF-1 is stabilized may develop. This results in the expression of HIF-1 target genes, such as angiogenesis factors that increase oxygen delivery to hypoxic tissues.<sup>67</sup> HIF-1 also facilitates the activation of an oxygen-independent mode of energy extraction (ie, glycolysis in oxygen-deprived cancer cells by inducing many enzymes in the glycolytic pathway).<sup>21</sup> HIF-1 $\alpha$  also promotes aerobic glycolysis by transcriptionally inducing PDK,<sup>46</sup> thus reducing the oxidative stress expected to occur if the electron transport chain were active. Hypoxic tumors, which induce HIF-1 and glycolysis most strongly, tend to be more invasive and metastatic than those with normal oxygen levels.<sup>13</sup> Furthermore, high HIF-1 is associated with higher mortality.<sup>101</sup> Thus, hypoxia experienced by tumors promotes HIF-1 expression, which, in turn, coordinates a transition to an aerobic glycolytic phenotype.

## p53

The p53 tumor suppressor is also being reconsidered from a metabolic perspective. The role of p53 in orchestrating cell cycle arrest, apoptosis, or senescence in response to DNA damage or cellular stress has been thought to explain its role as a tumor suppressor.<sup>102</sup> More recently, p53, like MYC, has been discovered to be an important regulator of cellular metabolism. *p53*<sup>-/-</sup> Cells have higher rates of glycolysis, produce more lactate, and exhibit decreased mitochondrial respiration compared with wild-type cells,<sup>22</sup> indicating that wild-type p53 suppresses an aerobic glycolysis phenotype. p53 Functions that might enforce these metabolic changes include down-regulation of glucose transporters,<sup>23</sup> up-regulation of a fructose-bisphosphate-phosphatase that lowers levels of fructose 2,6-bisphosphate,<sup>38</sup> repression of lactate transporters,<sup>54</sup> repression of PDKs,<sup>47</sup> induction of the mitochondrial oxidation regulator, synthesis of cytochrome c oxidase 2,<sup>22</sup> and competition with HIF-1 for limiting amounts of a shared transcriptional co-activator.<sup>103</sup>

A recent article has critically tested the importance of the role of p53 in metabolism in the prevention of tumorigenesis. Cells with three p53 lysine mutations (*p53*<sup>3KR</sup>) lack the normal functions of p53 in cell-cycle arrest, senescence, or apoptosis, but retain the ability to suppress glycolytic rates and maintain low reactive oxygen species (ROS) levels.<sup>104</sup> Although *p53*-null mice rapidly develop thymic lymphomas leading to death, surprisingly, *p53*<sup>3KR/3KR</sup> mice do not exhibit early-onset tumor formation.<sup>104</sup> These findings suggest that less conventional functions of p53, such as inhibiting the metabolic shift to aerobic glycolysis and reducing ROS levels, are critical for the ability of p53 to suppress early-onset spontaneous tumorigenesis.

The studies previously described demonstrate that p53 can modulate metabolism. Recent studies have shown that the availability of carbohydrates can, in turn, affect p53 levels. Glucose restriction has been reported to specifically induce deacetylation and degradation of mutant, but not wild-type, p53 both *in vitro* and *in vivo*.<sup>105,106</sup> Because wild-type p53 inhibits tumor growth and mutant forms of p53 can promote tumorigenesis,<sup>107</sup> the findings suggest that there may be reciprocal regulation between diet and metabolism on the one hand, and p53 status on the other, that affects tumor growth.

## Cancer Metabolic Phenotype

### Activated Lymphocytes Share Metabolic Properties with Cancer Cells

The metabolic program of cancer cells, although different from that of most normal, differentiated cells, shares significant similarities with some proliferating cells, including activated lymphocytes. Mature, resting lymphocytes rely on oxidative metabolism of glucose and glutamine for the energetic needs.<sup>28</sup> Recognition of their corresponding antigen

results in activation of the lymphocytes and is accompanied by a dramatic shift in metabolism.<sup>108</sup> Activated lymphocytes increase in size, divide rapidly, consume glucose and glutamine in excess of what can be easily explained by their need for biosynthesis or ATP, and secrete the extraneous material as lactate.<sup>15,17,18</sup> Many of the molecular changes that occur when lymphocytes are activated are similar to those that occur in tumors, including increased activity of glucose transporters,<sup>15,16</sup> glycolytic enzymes,<sup>17,28</sup> PFKFBP3,<sup>37</sup> lactate dehydrogenase,<sup>28</sup> and MCTs.<sup>28</sup> To compensate for the loss of citrate from the TCA cycle, glutamine consumption increases when lymphocytes are activated,<sup>17,59</sup> and this is associated with higher levels of glutamine transporters<sup>17,28,59</sup> and enzymes involved in glutaminolysis (Table 1).<sup>17,28,59</sup> The increased glucose flux in activated lymphocytes also results in higher levels of oxidative phosphorylation.<sup>18</sup> The similarity between the metabolic profile of tumor cells and activated lymphocytes suggests that this metabolic pattern and may be associated more generally with rapid cell division.

### Not All Proliferating Cells Use Aerobic Glycolysis

In addition to lymphocytes, many fast-growing unicellular organisms, including the baker's yeast *Saccharomyces cerevisiae*, rely on glucose fermentation during proliferation, even when oxygen is available.<sup>109</sup> However, despite the similarities between tumors, activated lymphocytes, and fermenting yeast, respiration can sustain fast cell growth. Some tumor cells rely on oxidation to generate ATP,<sup>65,66</sup> and some aerobic yeasts, such as *Yarrowia lipolytica*, rely on respiration for growth.<sup>110</sup> Conversely, nondividing cells can preferentially rely on glycolysis. Hematopoietic stem cells, which are largely quiescent, have higher glycolytic activity, lower mitochondrial activity,<sup>111</sup> and higher PDK activity,<sup>112</sup> compared with their more proliferative descendants. In a primary human fibroblast model system, a shift between proliferation and quiescence was not found to be associated with a dramatic difference in glycolytic rate.<sup>113</sup> Finally, recent studies report that the shift to glycolysis in lymphocytes is not necessary for proliferation or survival, but rather supports cytokine secretion.<sup>114</sup> Thus, in some model systems, the metabolic changes observed in tumors occur with a shift to a high proliferative rate, but this transition is not always observed when proliferative rate changes; even if it does occur, it may not facilitate faster proliferation.

## The Advantages of the Tumor Cell Metabolic Profile to the Tumor

### Rapid ATP

Why is a less efficient catabolic pathway so strongly induced in tumor cells? One suggestion is that aerobic glycolysis is advantageous because it provides ATP more rapidly than oxidative phosphorylation.<sup>66</sup> However, some

cancer cells actually recover a significant fraction of their ATP from oxidative phosphorylation.<sup>66</sup> Furthermore, it is not clear that ATP levels, or the speed which ATP can be extracted, is actually limiting for cellular growth.<sup>94</sup> Even rapidly dividing mammalian cells have been found to maintain high ratios of ATP/ADP.<sup>39</sup> And, signaling pathways exist that allow cells to increase low ATP levels by activating catabolic pathways that generate ATP.<sup>94</sup> For these reasons, the rationale that cells shift to aerobic glycolysis to recover rapid ATP is being reconsidered, and other interpretations for the Warburg effect have been offered.

### Carbon Skeletons for Growth

Although there may not be selective pressure for generating ATP, per se, one can imagine selective pressure for the rate of cellular proliferative expansion.<sup>94</sup> Organisms in which immune cells can respond to the presence of invaders by rapidly mounting an immune response ought to be less likely to succumb to infection and, therefore, be more fit. Increased glycolysis in tumor cells provides a constant supply of metabolic intermediates that can be diverted to support cell growth.<sup>94</sup> Furthermore, because glucose is one of the two main nutrients that the cell consumes, it is needed to provide all of the molecules necessary for cell growth.

To make a fatty acyl chain, a single glucose molecule can provide five times the ATP required, whereas seven glucose molecules are needed to generate the necessary NADPH through the pentose phosphate pathway.<sup>94</sup> If all of the available glucose were converted efficiently and completely to ATP in mitochondria, there would not be any glucose to provide acetyl-CoA to make fatty acids. There would also be no glucose available to divert from glycolysis for the synthesis of NADPH, nonessential amino acids, or ribose needed for generating nucleotides. Furthermore, complete oxidation of each glucose molecule would result in high ATP levels that would feedback and shut down glycolysis.<sup>94</sup> The fact that rapidly proliferating lymphocytes and yeast also rely heavily on glycolysis over oxidative phosphorylation could support the argument that the cancer metabolism phenotype is the metabolic profile that channels glucose among the available pathways in a way that facilitates rapid proliferation and growth.<sup>109</sup>

But, one might reasonably wonder, if the goal of cancer cells is to increase their biomass, then why do they secrete and waste 90% of the glucose carbons they consume?<sup>18,79,109</sup> There are several possible explanations. One possibility is that the cell needs a high rate of flux through glycolysis to ensure that metabolic intermediates can be siphoned off to anabolic pathways without dramatically affecting the sizes of the metabolite pools.<sup>109,115</sup> Another important consideration is that achieving a high level of glycolytic flux actually requires NAD<sup>+</sup> to be regenerated, which is achieved by converting pyruvate into lactate.<sup>109</sup> Furthermore, the secreted lactate is not, in fact, lost. As previously described, aerobic tumor cells might absorb the extracellular lactate released by

glycolytic cells, convert it to pyruvate, and use it as a fuel for mitochondrial oxidative phosphorylation.<sup>53</sup>

### Optimization of Fitness

A somewhat different perspective is to view the Warburg effect as an extension of a pattern of metabolic pathway use that exists in simpler model organisms. As growth rate, cell size, and ribosomal content increase, there is often an associated shift toward metabolic pathways with less efficient energy recovery.<sup>116</sup> This has been interpreted as a tradeoff between two different catabolic pathways, one of which is more expensive to generate, but generates more ATP, and the other uses less enzyme, but produces less energy. At low extracellular substrate concentrations, intracellular substrate is expensive, so an efficient catabolic method is necessary. At higher substrate concentrations, however, the catabolic pathway that requires less energy to produce its components becomes more valuable. Thus, a pathway that seems wasteful in that all possible ATP is not recovered from each nutrient, may be cheap in terms of the resources needed to construct the pathway, and may actually be the more desirable pathway when cells are in a nutrient-rich environment. A logical extension of the argument to cancer cells might be to recognize that performing oxidative phosphorylation requires the generation and maintenance of entire organelles, the mitochondria, complete with their own genomes and ribosomes, and an expensive-to-maintain membrane potential. Respiration, from this perspective, is a costly catabolic path that requires a substantial investment, but is useful for efficiently extracting ATP when nutrients are scarce. When nutrients are abundant, the less resource-intensive process of glycolysis might be more desirable. Thus, if resources are not limiting, cells may benefit from engaging a cheap, but seemingly wasteful, metabolic program.

Despite these cogent arguments, there are still unanswered questions about the metabolic phenotype of cancer cells. For instance, if the cancer cell phenotype is designed to facilitate cell growth, then why do cancer cell lines have higher glucose, lactate, and glutamine fluxes per unit area of cell membrane, higher hexokinase activity, and higher pentose phosphate pathway activity than nonmalignant cells growing at the same rate?<sup>117</sup> Are other benefits conferred on the tumor by this metabolic strategy in addition to simply a faster growth rate?

### Minimizing ROS

The use of aerobic glycolysis allows cells to expend less energy in the generation and maintenance of mitochondria and protects tumor cells from ROS that would be generated by performing oxidative phosphorylation in conditions of limited oxygen. In addition, both the glucose and the glutamine consumed by cancer cells can be metabolized to generate NADPH,<sup>79</sup> a necessary cofactor for the

replenishment of the cell's most important antioxidant, reduced glutathione. The importance of the pentose phosphate pathway and ROS detoxification in tumor cell growth was highlighted in a recent study in which hypoxia was found to induce glycosylation and inhibition of PFK, leading to redirection of glycolytic intermediates into the pentose phosphate pathway.<sup>32</sup> Blocking PFK glycosylation reduced cancer cell proliferation *in vitro* and impaired tumor formation *in vivo*. Thus, reducing ROS levels and protecting against ROS-mediated cell death may represent an advantage conferred by a Warburg effect metabolic phenotype.

### Protection against Apoptosis

In addition to controlling ROS levels, the aerobic glycolysis phenotype of cancer cells may also protect them from apoptosis by inhibiting the release of pro-apoptotic factors from the mitochondria through the mitochondrial permeability transition pore. The ease with which this pore opens depends on the mitochondrial membrane potential generated as hydrogen ions are transferred out of the inner mitochondrial membrane during oxidative phosphorylation. The low flux through the electron transport chain in cancer cells results in mitochondria with higher membrane potential<sup>45</sup> and a higher threshold for transition pore opening, thus suppressing apoptosis. If the hyperpolarization in cancer mitochondria is reversed by forcing pyruvate into the mitochondria, glucose oxidation increases, mitochondrial membrane potential decreases, and cancer cells undergo more cell death.<sup>45</sup> Thus, active electron transport flux may facilitate mitochondria-mediated cell death, and cancer cells may maintain viability, in part, by minimizing respiration.

High levels of glycolysis also protect against apoptosis via hexokinase. Hexokinases can be found physically associated with the outer surface of mitochondria.<sup>24</sup> Some tumor cells have higher levels of hexokinase<sup>24,25</sup> and a tighter association between hexokinase and the mitochondrial membrane.<sup>118</sup> The localization of hexokinase to the mitochondria, which is facilitated by active AKT,<sup>29</sup> inhibits the release of apoptosis-inducing factors, and suppresses apoptosis.<sup>119</sup> Thus, aerobic glycolysis may provide a survival advantage for tumor cells that helps to explain its prevalence in human cancers.

### Adaptation to the Tumor Microenvironment

Another possibility is that aerobic glycolysis is selected for in tumors because they are found in a hypoxic environment. According to this model, as a tumor grows, cells will be found further and further from the blood supply and  $pO_2$  levels decline even more rapidly with distance from blood vessels than glucose levels. Lack of oxygen will reduce mitochondrial respiration and lead to a decline in mitochondrial ATP. Lower ATP levels are expected to relieve allosteric inhibition of PFK and PK and promote glycolysis.



Hypoxia also induces HIF-1 $\alpha$  stabilization and activity, which will promote glycolysis and the growth of new blood vessels. Even if new blood vessels are formed, the solid tumor microenvironment will still be characterized by disorganized microvasculature and cycles of normoxia-hypoxia.<sup>120</sup> Aerobic glycolysis would continue to benefit cells in this environment. Thus, the tumor microenvironment, in this model, induces an aerobic glycolysis metabolic profile and then provides a selective advantage for tumor cells with high glycolytic metabolism. Aerobic glycolysis would provide a strong selective advantage during metastasis as well and, indeed, cells pretreated with hypoxia are more likely to survive during metastasis than their normoxic counterparts.<sup>121</sup>

There are a few questions surrounding this model. Some studies have questioned whether oxygen levels in the tumor microenvironment are, in fact, lower than the  $K_m$  for the rate-limiting enzymes in oxidative phosphorylation.<sup>67</sup> Others have questioned the implied timing of the model, and argued that cancer cells activate a glycolytic metabolism even before they are exposed to hypoxic conditions.<sup>94</sup> In addition, the aerobic glycolysis metabolic profile is not limited to hypoxic tumors.<sup>94</sup> Leukemic cells and lung tumors found in airways are highly glycolytic, even though they are exposed to oxygen.<sup>94</sup> Furthermore, although the tumor microenvironment might select for cells with an aerobic glycolysis phenotype, tumor cells maintain the metabolic phenotypes in culture under normoxic conditions. This may reflect the stabilization of HIF-1 $\alpha$  and the persistent effects on gene expression of the combination of HIF-1 $\alpha$ , oncogenes, and tumor suppressors. Thus, a more inclusive model might be that, in response to a combination of microenvironmental conditions, including hypoxia, and the activity of oncogenes and tumor suppressors, cancer cells acquire a metabolic phenotype that is stable and heritable, persists even when oxygen is available, and provides a selective advantage in the tumor environment and during metastasis.

### Functional Role of Secreted Lactate

A final proposed explanation for the Warburg effect is that lactate secreted from tumor cells has an important functional role in promoting tumorigenesis. In support of this explanation, much of the glucose consumed by cancer cells is converted to lactate,<sup>18,79,109</sup> and high levels of lactate are associated with a poor tumor prognosis.<sup>7</sup> MCTs cotransport lactate and a hydrogen ion out of the cell, resulting in an acidification of the local environment. The ensuing decrease in pH might promote cancer cell invasion and metastasis by killing normal host cells, thus generating space for the tumor and possibly releasing nutrients that the tumor can consume. A low pH might also stimulate invasion<sup>122</sup> and metastasis<sup>123</sup> by activating pH-sensitive metalloproteinases and/or cathepsins that degrade proteins in the extracellular matrix and basement membranes.<sup>124</sup> Furthermore, as previously described, secreted lactate has been proposed to provide nutrients to surrounding cells.<sup>53</sup> Lactate secreted by cancer

cells has also been proposed to feed nontumor, stromal cells.<sup>125</sup> Thus, from the perspective of lactate recycling, the cancer can be considered a microecosystem in which the different tumor components engage in complementary metabolic pathways that allow for the recycling of the waste product metabolites of aerobic glycolysis to support tumor growth.<sup>53,84,125</sup>

Finally, the secretion of lactic acid has also been proposed to play a role in suppressing the host anticancer immune response.<sup>126</sup> The metabolism of cytotoxic T lymphocytes, like that of the tumor cells, requires lactate secretion to drive high rates of glycolysis. In an advanced tumor, the high levels of lactate in the microenvironment may impede the ability of immune cells to export the intracellular lactate because secretion depends on a concentration gradient between intracellular and extracellular lactate. The resulting lactate overload reduces the ability of the T cells to secrete cytokines,<sup>126</sup> thus reducing the defense normally provided by the host immune response.

## Conclusions

### The Role of Metabolic Changes in Cancer

For many years, cancer was considered fundamentally a disease of uncontrolled cell proliferation. Although metabolic changes were acknowledged to occur in cancer cells, it was considered a secondary phenomenon. More recently, the metabolic changes that occur during cancer are being reconsidered as more central to the disease itself. So, is cancer a disease of metabolism? Are the proliferation changes primary and the metabolic changes come along for the ride, or vice versa? One possible model is that oncogenes and tumor suppressors make cancer cells hyperproliferative, and the coordinated shift in metabolism is a consequence. For instance, MYC would be expected to promote proliferation, whereas the loss of p53 may protect cells from senescence. Because these molecules also affect metabolism, metabolic changes would ensue.

A variation on this model would stress that the effects of oncogenes and tumor suppressors on proliferation are closely associated with metabolic changes that are also necessary to promote cell growth. The similarity in the changes between cancer cells and rapidly proliferating immune cells,<sup>15,17,18</sup> and even yeast,<sup>109</sup> supports a model in which altered metabolism provides the building blocks needed to form new cells. From this perspective, inappropriate cell proliferation would still be considered the primary driver of the tumorigenesis phenotype, and the metabolic changes are considered a coordinated and complementary program that supports the higher proliferative rate. Treating cell proliferation will, as a consequence, reverse the metabolic phenotype. A dramatic demonstration in support of this view is the ability of the tyrosine kinase inhibitor, imatinib, to normalize glucose metabolism in leukemic cells.<sup>127</sup>

An alternative model would propose that changes in metabolism are necessary to support biomass accumulation and drive the cancer phenotype. This argument is based on the premise that the aerobic glycolysis phenotype per se, and not just increased growth rate, contributes to tumorigenesis, a statement supported by the findings that glycolytic tumors are more invasive and more likely to cause the patient's death.<sup>6</sup> This argument might stress that the excessive lactate secreted by tumor cells indicates that glucose carbons are not required just for biomass accumulation, but rather that secreted lactate likely actively promotes tumorigenesis, possibly by suppressing the host immune response or promoting invasion or metastasis. This argument would also stress that the changes in metabolism in tumor cells are more extreme than,<sup>117</sup> and somewhat distinct from,<sup>24</sup> those observed in most proliferating cells, some of which do not demonstrate the aerobic glycolytic phenotype of activated lymphocytes.<sup>113</sup> For example, the association of hexokinase with mitochondria is observed in hepatoma cells, but not in normal liver, even when it is regenerating.<sup>24</sup> Glucose transporters are induced in pancreatic cancer, but not mass-forming pancreatitis.<sup>11</sup> Finally, one might argue, well-established oncogenes and tumor suppressors repeatedly observed as amplified, mutated, or deleted in tumors, such as those previously reported, RAS<sup>128</sup> and JAK2<sup>V617F</sup>,<sup>129</sup> are being discovered to have direct effects on metabolism.

An extreme version of this model would argue that all of the more classically accepted attributes of tumors actually derive from the metabolic phenotype of tumor cells.<sup>130</sup> Then, is an aerobic glycolytic phenotype sufficient to transform a cell in the absence of other nonmetabolic cancer attributes? It seems unlikely—many immune cells temporarily adopt an aerobic glycolysis phenotype in response to antigen exposure. When they no longer receive inflammatory signals, they revert to the resting state and rarely form tumors.<sup>108</sup> On the other hand, a p53 mutant that can counter aerobic glycolysis and ROS production, but cannot induce apoptosis, senescence, or cell cycle arrest, retains the ability to suppress tumorigenesis.<sup>104</sup> These recent findings with p53 support a model in which metabolic changes are critical drivers of tumorigenesis, and highlight the need for more studies to clarify this issue.

### The Prospects for Targeting Cancer through Metabolism

The first anticancer agents targeted metabolic pathways required for proliferation (eg, by depleting pools of nucleotide precursors).<sup>131</sup> Successful anticancer agents designed more recently have largely focused on a specific activated oncogene. These targeted therapies have been extremely successful in achieving a rapid remission of some tumors, but unfortunately, for many patients, the disease recurs. Metabolism-based therapeutics might have advantages over gene-based therapies. Although most genes are important

drivers of only a subset of tumor types, some of the shifts in metabolism observed in tumors are common to tumors derived from many different tissues. In addition, it may be more challenging, although certainly not impossible, for a tumor to acquire mutations that confer resistance to an anti-metabolism therapy than a gene-based therapy.<sup>108</sup> If the metabolic characteristics of tumors are essential for the tumor's growth and survival, targeting the tumor's metabolism could have a dramatic effect on tumor viability.

However, there are drawbacks to a metabolism-based approach to therapy as well. Metabolism-based therapies face a major hurdle of non-specific toxicity: the same metabolic pathways are required for the survival of all cells. Activated immune cells might be expected to be especially vulnerable to anticancer therapies, which is especially concerning because these are the cells that would normally target the tumor.<sup>108</sup> Neurons consume large amounts of glucose, and peripheral neuropathy has been detected as the dose-limiting toxicity for some anti-glycolytic therapies.<sup>45</sup>

Nevertheless, there is some reason to be hopeful about the prospects of metabolic targeting. A combination of energy metabolism inhibitors with other antitumor drugs could represent a powerful new approach to treatment.<sup>78</sup> Energetic collapse due to blocked glycolysis could make other physical and chemical anticancer agents more effective (eg, by reducing the effectiveness of efflux transporters and allowing drugs to accumulate to higher effective doses). Alternatively, forcing cancer cells to reactivate the mitochondria might strengthen the therapeutic activity of antineoplastic treatments that depend on the induction of free radicals.

There is also hope that tumor-specific metabolic programs can be exploited for therapy. Some tumors organize the TCA cycles so that they are addicted to glucose for anaplerosis and survival,<sup>99</sup> whereas other tumors are glutamine dependent.<sup>60,99</sup> Tumors characterized by a strict reliance on either glucose or glutamine may be targetable through this metabolic vulnerability. There may be opportunities to target cancer-specific isozymes<sup>24,119</sup> or pathways that are relied on more heavily by cancer cells than normal cells (eg, the conversion of glutamine to glutamate through transamination).<sup>64</sup> PKM2 is another attractive target; both allosteric activators and inhibitors of PKM2 reduce tumor growth.<sup>40,41</sup> Further studies that elucidate the molecular basis for distinguishing cancer cell metabolism from a proliferative phenotype, and the range of metabolic profiles in different types of cancer cells, will allow for prioritization among the targets that have been identified and will likely suggest even more targets for exploration.

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