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Review

Tumor Cell Metabolism: Cancer's Achilles' Heel

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The essential hallmarks of cancer are intertwined with an altered cancer cell-intrinsic metabolism, either as a consequence or as a cause. As an example, the resistance of cancer mitochondria against apoptosis-associated permeabilization and the altered contribution of these organelles to metabolism are closely related. Similarly, the constitutive activation of signaling cascades that stimulate cell growth has a profound impact on anabolic metabolism. Here, we review the peculiarities of tumor cell metabolism that might be taken advantage of for cancer treatment. Specifically, we discuss the alterations in signal transduction pathways and/or enzymatic machineries that account for metabolic reprogramming of transformed cells.

Metabolic Reprogramming to the Advantage of Cancer Cells

The first tumor-specific alteration, altered metabolism, was discovered by the Nobel Prize winner Otto Warburg in the 1920s. The "Warburg phenomenon" consists of an increase in glycolysis that is maintained in conditions of high oxygen tension ("aerobic glycolysis") and gives rise to enhanced lactate production (Brahimi-Horn et al., 2007; Warburg et al., 1924). In addition, or alternatively, cancer cells use elevated amounts of glucose as a carbon source for anabolic reactions. Although the Warburg phenomenon is not universally applicable to all cancers (Funes et al., 2007), enhanced glucose uptake is sufficiently prevalent that it is taken advantage of to image cancer in clinics using the glucose analog 2-(^{18}F)-fluoro-2-deoxy-D-glucose (FDG) by positron emission tomography (PET). FDG-PET combined with computer tomography (PET/CT) has a >90% sensitivity and specificity for the detection of metastases of most epithelial cancers (Mankoff et al., 2007).

There are several reasons why enhanced glucose uptake for glycolytic ATP generation or anabolic reactions constitutes an advantage for tumor growth (Figure 1):

First, in conditions of aerobic glycolysis, cells can live in conditions of fluctuating oxygen tension (due to inconstant hemodynamics of distant blood vessels) that would be lethal for cells that rely on oxidative phosphorylation (OXPHOS) to generate ATP (Pouyssegur et al., 2006).

Second, cancer cells generate bicarbonic and lactic acids, lactate being the principal end product of aerobic glycolysis. Such acids condition their environment (Koukourakis et al., 2006), favor tumor invasion (Swietach et al., 2007), and suppress anticancer immune effectors (Fischer et al., 2007). Lactate that is produced by tumor cells can be taken up by stromal cells (via the monocarboxylate transporters MCT1 and MCT2) to regenerate pyruvate that either can be extruded to refuel the cancer cell or can be used for OXPHOS (Koukourakis et al., 2006). This arrangement generates a microecosystem in which anaerobic components (cancer cells) and aerobic components (nontransformed stromal cells) engage in complementary metabolic pathways, thus buffering and recycling products of anaerobic metabolism to sustain cancer cell survival and growth.

Third, tumors can metabolize glucose through the pentose phosphate pathway (PPP) to generate nicotinamide adenine dinucleotide phosphate (NADPH) that ensures the cell's antioxidant defenses against a hostile microenvironment and chemotherapeutic agents (Gatenby and Gillies, 2004). Moreover, NADPH can contribute to fatty acid synthesis. The nonoxidative part of the PPP (in which ribose 5-phosphate, an intermediate of the PPP, is fueled into glycolysis) is controlled by transketolase reactions, and the transketolase-1 isoform (TKL1) is overexpressed in multiple cancers (Foldi et al., 2007; Langbein et al., 2006).

Fourth, and most importantly, cancer cells use intermediates of the glycolytic pathway for anabolic reactions (for instance, glucose 6-phosphate for glycogen and ribose 5-phosphate synthesis, dihydroxyacetone phosphate for triacylglyceride and phospholipid synthesis, and pyruvate for alanine and malate synthesis) (Gatenby and Gillies, 2004). The embryonic isoform of pyruvate kinase (PK), which dephosphorylates phosphoenolpyruvate (PEP) to pyruvate, is highly expressed in tumors yet is absent from adult tissues except adipocytes (Christofk et al., 2008a). Interestingly, this isoform, PKM2, oscillates from high (tetrameric) to low (dimeric) activity (Christofk et al., 2008a; Mazurek et al., 2005). The low-active dimeric form of PKM2 provides the metabolic advantage that the phosphometabolites upstream of pyruvate accumulate and are then available as precursors for the synthesis of amino acids, nucleic acids, and lipids (Mazurek et al., 2005) while lactate production is avoided. This principle of "deviating" (at least part of the) intermediates from the glycolytic pathways toward anabolic reactions also applies to the metabolism of glycolytically derived pyruvate. In proliferating cancer cells, pyruvate may enter a truncated tricarboxylic acid (TCA) cycle. The net result of this truncated TCA cycle is that acetyl-CoA is exported from the mitochondrial matrix

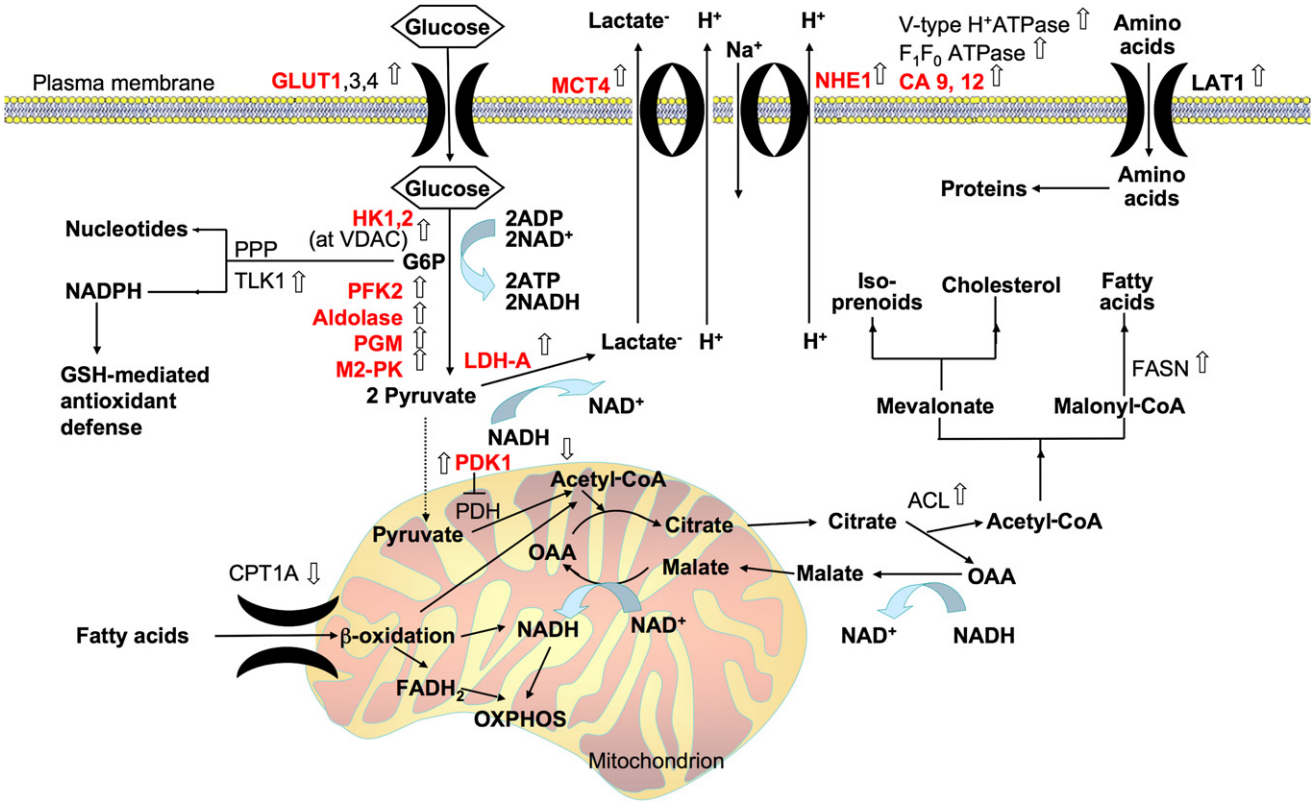

Figure 1. Metabolic Reprogramming in Cancer Cells

In normal cells, aerobic glycolysis implies the conversion of glucose via pyruvate into acetyl-CoA and its complete oxidation (through the mitochondrion-localized tricarboxylic acid [TCA] cycle and oxidative phosphorylation) to CO_2 and H_2O (which generates 38 ATP molecules per molecule of glucose). In contrast, in tumor cells, glycolysis tends to be aborted at either of two steps. First, aerobic glycolysis in tumor cells implies conversion of glucose into pyruvate (which generates only two ATP molecules per molecule of glucose) and subsequently into the waste product lactic acid. Second, in tumor cells, acetyl-CoA tends to be introduced into a truncated TCA cycle, with the net result that acetyl-CoA is exported into the cytosol and serves as a building block for cell growth and proliferation. In this truncated TCA cycle, citrate is preferentially exported to the cytosol via the tricarboxylate transporter. Once in the cytosol, citrate is cleaved by ATP citrate lyase (ACL) to generate oxaloacetate and acetyl-CoA. Oxaloacetate is reduced to malate, then reimported into mitochondria and reconverted to oxaloacetate in the matrix (while generating NADH that represses the TCA cycle), and it reacts with acetyl-CoA to complete the substrate cycle. Small arrows pointing up or down indicate cancer-associated upregulation/activation or downregulation/inhibition of enzymes, respectively. Alterations indicated in red can be caused by the activation of HIF-1. CA9 and CA12, carbonic anhydrases 9 and 12; CPT, carnitine palmitoyltransferase; GLUT, glucose transporter; GSH, glutathione; HIF, hypoxia-inducible factor; IDO, indoleamine 2,3-dioxygenase; HK, hexokinase; OXPHOS, oxidative phosphorylation; LAT1, L-type amino acid transporter 1; LDHA, lactate dehydrogenase isoform A; MCT, monocarboxylate transporter; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PI3K, phosphatidylinositol 3-kinase; PGM, phosphoglycerate mutase; PKM2, pyruvate kinase isoform M2; PPP, pentose phosphate pathway; SCO2, synthesis of cytochrome *c* oxidase 2; TLK, transketolase; VDAC, voltage-dependent anion channel.

(Figure 1) and becomes available for the synthesis of fatty acids, cholesterol, and isoprenoids. Indeed, fatty acid synthase (FASN), which synthesizes long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH, is upregulated or activated in many cancers (Wang et al., 2005). Similarly, choline kinase (ChoK), which forms phosphorylcholine, is often overexpressed in cancer (Glunde and Bhujwalla, 2007).

Thus, the entire metabolism (in particular glycolysis and the TCA cycle) is reorganized to augment anabolic reactions linked to cell growth and proliferation. However, it would be difficult to reconcile enhanced lactate production (which would result in a net loss of carbon that could have been used for anabolic reactions) with reduced PK activity (which rather would reduce pyruvate and hence lactate production) and a truncated TCA cycle (which would consume pyruvate) unless the net consumption of glucose was much higher in cancer cells than in their normal counterparts.

Mechanisms of Metabolic Reprogramming

The molecular mechanisms that underlie metabolic reprogramming of cancer cells are complex (Figure 2). Primary defects in OXPHOS have been invoked to explain the Warburg phenomenon because tumor mitochondria are often relatively small, lack cristae, and are deficient in the β-F1 subunit of the ATP(synth)ase (Lopez-Rios et al., 2007). Mitochondrial DNA (mtDNA) mutations may arise as a result of tumor progression (Brandon et al., 2006), but some mtDNA mutations might actively contribute to tumor progression. Thus, expression of a mutant mtDNA-encoded NADH dehydrogenase subunit 2 (associated with head and neck squamous carcinoma) as a nuclear, mitochondrion-targeted gene product concomitantly stimulates aerobic glycolysis, reactive oxygen species (ROS) production, and tumor growth (Zhou et al., 2007).

One of the principal mechanisms of aerobic glycolysis resides in the activation of hypoxia-inducible factor (HIF), a transcription factor that is activated by hypoxic stress but also by oncogenic,

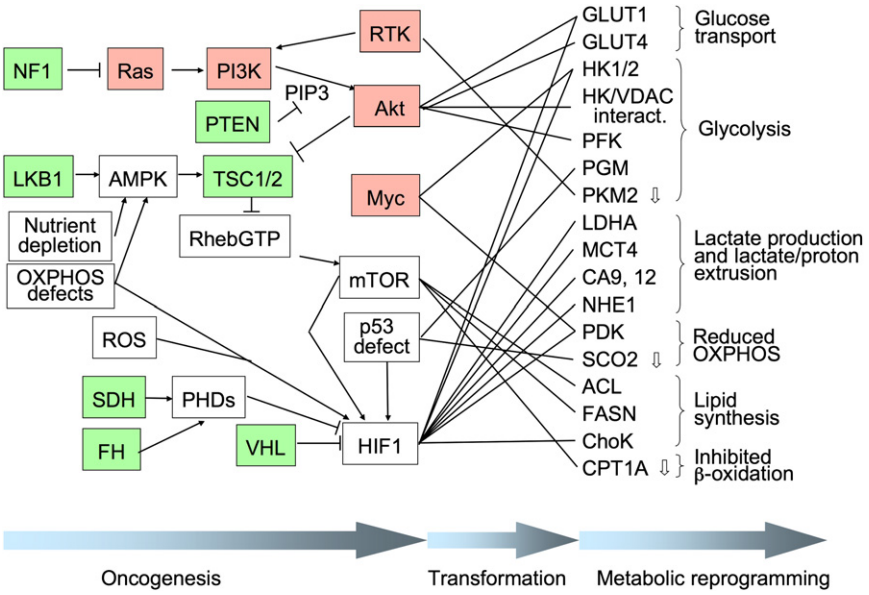

Figure 2. Molecular Mechanisms of Cancer-Specific Metabolic Reprogramming

As a result of oncogenic gain-of-function events (pink) or the loss of tumor suppressors (green) affecting the PI3K/Akt/mTOR/HIF axis and/or inactivation of the p53 system, a stereotyped pattern of metabolic changes is induced, leading to cancer-associated alterations in metabolism. Note that arrows connecting different proteins do not necessarily indicate direct interactions. ACL, ATP citrate lyase; AMPK, AMP-activated kinase; CA9 and CA12, carbonic anhydrases 9 and 12; ChoK, choline kinase; CPT, carnitine palmitoyltransferase; FH, fumarate hydratase; GLUT, glucose transporter; HIF, hypoxia-inducible factor; HK, hexokinase; OXPHOS, oxidative phosphorylation; LAT1, L-type amino acid transporter 1; LDHA, lactate dehydrogenase isoform A; MCT, monocarboxylate transporter; mTOR, mammalian target of rapamycin; NF, neurofibromin; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol triphosphate; PGM, phosphoglycerate mutase; PHD, prolyl hydroxylase; PKM2, pyruvate kinase isoform M2; SCO2, synthesis of cytochrome *c* oxidase 2; SDH, succinate dehydrogenase; TSC, tuberous sclerosis complex; VDAC, voltage-dependent anion channel; VHL, von Hippel-Lindau ubiquitin ligase.

inflammatory, metabolic, and oxidative stress (Harris, 2002; Semenza, 2007; Taylor and Pouyssegur, 2007). HIF-1 is a heterodimer composed of constitutive, stable β subunits and unstable α subunits, which are synthesized yet degraded under normoxic conditions due to the sequential action of oxygen-dependent prolyl hydroxylases (PHDs) and the VHL ubiquitin ligase. HIF-1 stimulates the conversion of glucose to pyruvate and lactate by upregulating glucose transporter (GLUT) isoform 1 (GLUT1), hexokinase (HK1 and HK2, which catalyze the initial step of glycolysis), and lactate dehydrogenase A (LDHA), as well as the lactate-extruding enzyme monocarboxylate transporter 4 (MCT4) (Pouyssegur et al., 2006; Semenza, 2007).

In addition, HIF-1 decreases the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase (PDH). For this, HIF-1 transactivates the gene encoding PDH kinase 1 (PDK1), which inhibits PDH (Kim et al., 2006; Papandreou et al., 2006). Acetyl-CoA is normally fed to the TCA cycle, producing the electron donors NADH and $FADH_2$, which donate electrons to the respiratory chain complexes I and II, respectively. Hence, by inhibiting PDH, HIF-1 compromises OXPHOS. In addition, HIF-1 facilitates the adaptation of mitochondria to hypoxia by transactivating the cytochrome *c* oxidase (COX) subunit COX4-2 and LON, a protease that degrades COX4-1 (Fukuda et al., 2007). HIF-1 counteracts the stimulatory action of Myc on mitochondrial biogenesis, thereby reducing mitochondrial mass (Zhang et al., 2007). In contrast, HIF-1 cooperates with c-Myc to promote aerobic glycolysis by induction of HK2 and PDK1 (Dang et al., 2008).

Besides by a decrease in O_2 tension, HIF-1 can be induced as a result of tumorigenic germline mutations of two enzymes of the TCA cycle: the mitochondrial matrix protein fumarate hydratase (FH) and the inner mitochondrial membrane protein succinate dehydrogenase (SDH). SDH is also a functional member of complex II of the respiratory chain. Loss-of-function mutations of FH or of SDH subunits (SDHB, SDHC, and SDHD) induce the accumulation of the TCA cycle intermediates fumarate or succinate, which competitively inhibit the α-ketoglutarate-dependent HIF-1α prolyl hydroxylase, the enzyme that usually targets HIF-1α for destruction in an oxygen-dependent fashion (Gottlieb and Tomlinson, 2005). These examples illustrate that induction of HIF-1 may contribute to the metabolic and oncogenic changes induced by primary mitochondrial dysfunctions.

Beyond the central role of HIF-1 activation, oncogenes and tumor suppressor genes determine metabolic reprogramming of cancer cells at several levels (Table 1), establishing multiple links between tumor cell biology and tumor biochemistry. Perhaps the most significant example of a switch from respiration to aerobic glycolysis is provided by the inactivation of the tumor suppressor p53, as discussed in the next section.

Hallmarks of Cancer Linked to Metabolic Change

Cancer cells differ from healthy cells due to a plethora of molecular changes (Hanahan and Weinberg, 2000; Zitvogel et al., 2006), many of which may be mechanistically linked to metabolic reprogramming (Figure 3).

Self-Sufficiency in Growth Signals

Growth factors usually activate receptor tyrosine kinases (RTKs), which then stimulate two key signal-transducing kinase pathways: the Ras→Raf→MAP kinase (ERK) pathway and the phosphatidylinositol 3-kinase (PI3K) pathway. ERK and PI3K converge to activate mTOR for stimulating cell growth. Most cancers harbor activating mutations of the master regulators (K-Ras, H-Ras, N-Ras, B-Raf, the p110a PI3K subunit, and RTKs) or their downstream effectors (such as the kinases Akt and PDK1), or inactivating mutations in negative regulators of these proteins (Shaw and Cantley, 2006) (Table 1). There are multiple mechanisms through which the constitutive activation of growth factor signals can cause cancer-associated metabolic reprogramming.

The activation of tyrosine kinases including RTK and Scr family kinases results in the phosphorylation of enzymes on tyrosine

Table 1. Metabolic Effects of Selected Oncogenes and Tumor Suppressor Genes

Gene	Effects	Disease	Reference
Oncogenes			
PI3K	Activates Akt via PIP3; reduces (via Akt) expression of the β-oxidation enzyme carnitine palmitoyltransferase 1A (CPT1A)	Ovarian and gastrointestinal cancer	Deberardinis et al. (2006)
Akt	Upregulates fatty acid synthase (FASN); activates mTOR complex 1	Breast and ovarian cancer	Wang et al. (2005)
Her2	Increases, through activation of PI3K, Akt, and mTOR, expression of FASN and acetyl-CoA carboxylase α (ACCα) at the translational level	Mammary carcinoma	Yoon et al. (2007)
Tyrosine kinases	Generate phosphotyrosines that can bind to pyruvate kinase isoform PKM2, converting it from a tetramer to a less active dimer	Multiple cancers	Christofk et al. (2008b)
E7 from HPV16	Binds PKM2, converting it from a tetramer to a less active dimer	Cervical carcinoma	Mazurek et al. (2005)
Tumor Suppressors			
p53	Required for expression of SCO2 and hence optimal OXPHOS; enhances the expression of TIGAR, a glycolysis inhibitor; reduces the expression of the glycolytic enzyme phosphoglyceromutase	Multiple cancers	Matoba et al. (2006); Bensaad et al. (2006); Kondoh et al. (2005)
VHL	Ubiquitin ligase required for degradation of HIF-1α	Clear cell renal carcinoma	Shaw and Cantley (2006)
TSC1 (hamartin) and TSC2 (tuberin)	Negative regulators of Rheb (which inhibits mTOR)	Tuberous sclerosis complex and lymphangioleiomyomatosis	Shaw and Cantley (2006)
PTEN	Negative regulator of class I PI3K	Cowden syndrome and prostate cancer	Shaw and Cantley (2006)
LKB1	Required for activation of AMPK	Peutz-Jeghers syndrome and sporadic lung adenocarcinoma	Shaw and Cantley (2006)
NF1	Negative regulator of RAS and PI3K-Akt pathway	Neurofibromatosis	Shaw and Cantley (2006)
PML	Negative regulator of mTOR complex 1	Promyelocytic leukemia and lung cancer	Shaw and Cantley (2006)
Succinate dehydrogenase subunits SDHB, C, and D	Accumulated succinate competitively inhibits HIF-1α prolyl hydroxylases (PHDs)	Paraganglioma (SDHB, C, and D) and pheochromocytoma (SDHB and D)	Gottlieb and Tomlinson (2005)
Fumarate hydratase (fumarase)	Accumulated fumarate inhibits PHDs	Leiomyomatosis and papillary renal carcinoma	Gottlieb and Tomlinson (2005)

residues. This indirectly modulates the activity of the cancer-specific isoform of pyruvate kinase, PKM2, which can bind to phosphotyrosine-containing peptides (Christofk et al., 2008b). Upon interaction between PKM2 and such phosphotyrosine-containing peptides, PKM2 releases its allosteric activator, FBP. This results in the inhibition of PKM2 activity as PKM2 transits from its active tetrameric state to its inactive dimeric state (Christofk et al., 2008b). This mechanism may contribute to the stimulation of anabolic reaction by oncogenic tyrosine kinases. The partial inhibition of PKM2 (which catalyzes the last step of glycolysis, upstream of pyruvate) allows the intermediates of glycolysis to "deviate" toward anabolic reactions and simultaneously avoids excessive pyruvate production. However, PKM2 inhibition alone is not sufficient to account for the cancer-specific reprogramming, which relies on additional features including a general increase in glycolytic flux.

Cell-autonomous overactivation of the PI3K/Akt system, downstream of RTKs, mediates an increase in glucose and amino acid flux through the plasma membrane that may be attributable in part to the activation of HIF-1α (Pouyssegur et al., 2006). Akt stimulates the expression of one glucose transporter (GLUT1) and induces the translocation of another (GLUT4) to the

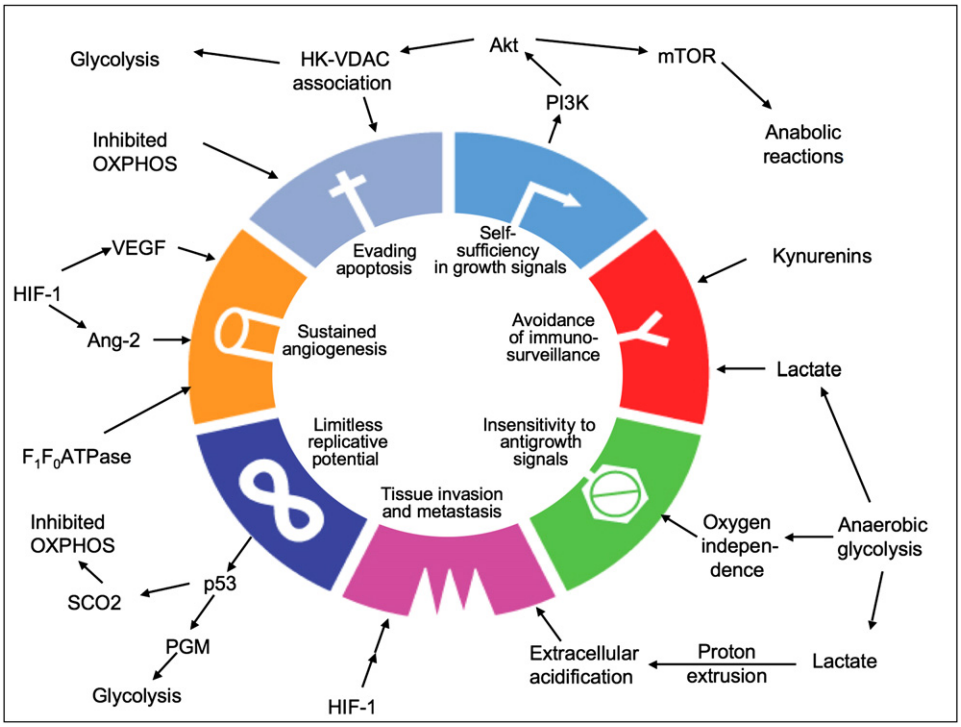

Figure 3. The Seven Hallmarks of Cancer and Their Links to Tumor Metabolism
The hypothetical links between different metabolic alterations and the seven nonmetabolic characteristics of neoplasia (circle) are depicted. Centripetal arrows (pointing from the inside outwards) indicate how the seven hallmarks of cancer can impinge on metabolism. Centrifugal arrows (pointing from the outside inwards) illustrate how neoplasia-associated metabolic reprogramming can contribute to the acquisition of the seven hallmarks. Ang-2, angiopoietin-2; GLUT, glucose transporter; HIF, hypoxia-inducible factor; HK, hexokinase; OXPHOS, oxidative phosphorylation; PGM, phosphoglycerate mutase; PI3K, phosphatidylinositol 3-kinase; SCO2, synthesis of cytochrome *c* oxidase 2; VDAC, voltage-dependent anion channel; VEGF, vascular endothelial growth factor.

plasma membrane. Akt stimulates glycolysis through the activating phosphorylation of 6-phosphofructo-2-kinase (PFK2) and fatty acid synthesis through the phosphorylation of ATP citrate lyase (Manning and Cantley, 2007). Through mechanisms that are not fully elucidated, Akt stimulates the association of HK1 and HK2 with mitochondria (Pastorino et al., 2005), thus linking residual ATP synthesis by mitochondria to the catalysis of the first, rate-limiting step of glycolysis. In addition, PI3K causes the transcriptional downregulation of carnitine palmitoyltransferase 1A (CPT1A) (Deberardinis et al., 2006), an enzyme located in the outer mitochondrial membrane that esterifies long-chain fatty acids to carnitine, thereby initiating the mitochondrial import of fatty acids and channeling them to β-oxidation. One of the net effects of PI3K activation is hence an inhibition of β-oxidation, which may contribute to the "glucose addiction" of cancer cells (Deberardinis et al., 2006). PI3K activation also induces FASN, and the activating phosphorylation of Akt and FASN correlates in tissue microarrays (e.g., in ovarian cancer) (Wang et al., 2005).

mTOR, a serine/threonine kinase, is activated downstream of the constitutively activated PI3K/Akt system. mTOR enhances cap-dependent protein translation and cell growth while inhibiting catabolic reactions mediated by autophagy. Several clinical trials are currently examining the therapeutic efficacy of mTOR inhibitors (Faivre et al., 2006). Increased activation of the PI3K/Akt/mTOR pathway leads to phosphorylation of 4E-BP1, an inhibitor of the eukaryotic translation initiation factor eIF4E, resulting in increased eIF4E activity and cap-dependent translation in cancers. eIF4E overexpression can be tumorigenic, and its inhibition can suppress the growth of xenografted human cancers (Graff et al., 2007). Conversely, mTOR is inhibited by hypoxia, and breast cancers often overexpress 4E-BP1 and eIF4G to increase the cap-independent translation of proangiogenic, hypoxia, and survival mRNAs (Braunstein et al., 2007). Hence, the contribution of cap-dependent versus cap-independent translation to cancer growth remains to be investigated in further detail.

Evading Apoptosis

Defective apoptosis not only is crucial for initial oncogenesis but contributes to chemotherapy resistance. Cancer cells often manifest resistance against mitochondrial membrane permeabilization (MMP), which is one of the decisive steps of apoptosis (Kroemer et al., 2007). One link between apoptosis inhibition and metabolic reprogramming may be provided by the association of HK with the voltage-dependent anion channel (VDAC). HK has been shown to associate more tightly with the outer mitochondrial protein VDAC in tumor cells than in normal control cells (Pedersen, 2007). This increased HK-VDAC interaction may be due to the constitutive activation of Akt. Akt induces the translocation of HK to the outer mitochondrial membrane where it binds to VDAC, presumably because Akt interferes with the glycogen synthase kinase 3 (GSK3)-mediated phosphorylation of VDAC (Pastorino et al., 2005) or because Akt phosphorylates HK (Miyamoto et al., 2008). When associated with VDAC, HK may

efficiently couple residual OXPHOS to the initial, rate-limiting step of glycolysis. In addition, HK can inhibit MMP, presumably through an effect on the permeability transition pore complex (PTPC) (which also involves VDAC). Accordingly, peptides that competitively disrupt the VDAC-HK interaction can induce MMP and subsequent apoptosis (Robey and Hay, 2006). On theoretical grounds, such VDAC-HK-dissociating agents would have a dual effect and hence revert the hyperglycolytic state while facilitating apoptosis induction.

Complete or partial OXPHOS defects may also induce apoptosis resistance. Total inhibition of the respiratory chain can suppress the activation of the proapoptotic Bcl-2 proteins Bax and Bak, either of which serves as a near obligate mediator of MMP (Tomiyama et al., 2006). Thus, severe OXPHOS defects, as are found in some cancers, might be automatically coupled to a blockade of MMP and hence an inhibition of apoptosis. OXPHOS defects also reduce the capacity of certain xenobiotics to elicit ROS generation in mitochondria, thereby abrogating their proapoptotic activity. This latter mechanism may explain the fact that ρ° cells (cells that lack mitochondrial DNA and hence OXPHOS) are resistant against a series of compounds that induce apoptosis by provoking futile redox cycles in mitochondria (Galluzzi et al., 2006; Kroemer et al., 2007). Thus, a state of deficient OXPHOS might automatically compromise the intrinsic pathway of apoptosis through a variety of distinct mechanisms.

Other links between mitochondrial alterations in cancer and disabled apoptosis are more indirect. A hyperpolarization of the inner mitochondrial transmembrane potential, as is frequently seen in cancer cells (perhaps secondary to defects in the F_1F_0 ATPase) (Galluzzi et al., 2006), can reduce the propensity of PTPC opening (Zoratti and Szabo, 1995). There is also a correlation between mitochondrial hyperpolarization and a relative deficiency in voltage-gated plasma membrane K^+ channels (Kv), enhancing cytosolic K^+ to a level that exerts a tonic inhibitory effect on caspases and apoptosis-inducing factor (AIF) (Bonnet et al., 2007). Pharmacological inhibition of PDK1, which is often overactivated in cancer, leads to reactivation of PDH and reportedly corrects both the activity of Kv channels and mitochondrial hyperpolarization, thereby inducing cancer cell apoptosis (Bonnet et al., 2007). Thus, PDK1 inhibitors may constitute yet another example of dual-hit agents that simultaneously reverse apoptosis resistance and metabolic reprogramming.

Limitless Replicative Potential

To ensure replicative potential, tumor cells often mutate or lose senescence-inducing proteins such as p53. Loss of p53 is also driven by Darwinian selection in a hypoxic environment, because hypoxia-mediated activation of p53 can trigger cell death (Culmsee and Mattson, 2005). As briefly stated above, inactivation of p53 can directly cause the Warburg phenomenon through several mechanisms. Thus, p53 positively regulates the expression of the protein synthesis of cytochrome *c* oxidase 2 (SCO2), which is required for assembling COX (Matoba et al., 2006). p53 negatively regulates phosphoglycerate mutase (PGM), the enzyme that converts 3-phosphoglycerate (3PG) to 2-phosphoglycerate (2PG) in glycolysis (Kondoh et al., 2005). Moreover, p53 transcriptionally activates TIGAR (TP53-induced glycolysis and apoptosis regulator), an isoform of PFK2 that inhibits overall phosphofructokinase activity, lowers the levels of FBP, and hence inhibits glycolysis (because FBP is an allosteric activator of the glycolytic enzyme 6-phosphofructo-1-kinase) while channeling glucose to the PPP (Bensaad et al., 2006). At present, there have been no systematic studies to determine through which dominant (SCO2-, PGM-, or TIGAR-dependent?) pathway inactivation of p53 impacts on cancer cell metabolism. Given the major contribution of p53 to cancer cell biology, this issue urgently awaits clarification.

Sustained Angiogenesis

Many tumors show increased expression of vascular endothelial growth factor (VEGF) as a result of activated signaling pathways (ERK, PI3K), an action that is amplified by hypoxia. Indeed, recent work indicates that the expression of VEGF is cooperatively induced by HIF-1 and c-Myc (Kim et al., 2007a). Another intriguing link between abnormal metabolism and cancer-mediated angiogenesis is provided by F_1F_0 ATPase. Both endothelial cells and tumor cells express the F_1F_0 ATPase (normally an inner mitochondrial membrane protein complex) at the cell surface, where it may extrude protons from the cytosol to the extracellular milieu and hence contribute to the net export of protons that is required to maintain aerobic glycolysis. An endogenous angiogenesis inhibitor, angiostatin, binds and inhibits surface F_1F_0 ATPase, causing intracellular acidification. Similarly, an antibody targeting the β catalytic subunit of F_1F_0 ATPase has an angiostatin-like antiangiogenic effect (Chi et al., 2007). These results hint at the possibility of using a single agent that targets the cell-surface F_1F_0 ATPase for subverting cancer-associated angiogenesis and metabolic reprogramming.

Tissue Invasion and Metastasis

One mechanistic link between tumor metabolism and invasion/metastasis is provided by HIF. HIF-1α activation causes the loss of E-cadherin (Esteban et al., 2006; Pouyssegur et al., 2006), the cadherin isoform that is required for the maintenance of intercellular contacts within epithelia and that is lost during the epithelium-mesenchyme transition (EMT). HIF-1α activation also causes the expression of the *met* proto-oncogene and of TWIST, both of which favor EMT (Pennacchietti et al., 2003; Yang et al., 2008), and induces two proteins that play a cardinal role in metastasis, the chemokine receptor CXCR4 (Igney and Krammer, 2002) and lysyl oxidase (LOX) (Erler et al., 2006). Thus, one single cause, HIF-1α activation, may entail both metabolic reprogramming and enhanced tissue invasion/metastasis. Accordingly, mtDNA mutations can stimulate the metastatic potential of cancer cells, presumably because enhanced ROS generation by mitochondria leads to the activation of HIF-1α (Ishikawa et al., 2008).

Aerobic glycolysis (and hence enhanced production of protons) causes acidification of the extracellular milieu due to the action of multiple proton-extruding enzymes. The monocarboxylate transporters MCT1 and MCT4 (which cotransport H^+ with monocarboxylate anions such as lactate) and the ubiquitous Na^+-H^+ exchanger are activated by growth factors, oncogenic transformation, hypoxia, and low intracellular pH (Counillon and Pouyssegur, 2000). In addition, cancer cells may use the surface V-type H^+ ATPase and/or the surface F_1F_0 ATPase as proton pumps. Tumor cells often express the exquisitely HIF-inducible carbonic anhydrase isoforms 9 and 12 (CA9 and CA12). CA9 and CA12 are transmembrane enzymes that hydrate extracellular CO_2, thereby generating membrane-impermeable H^+ and HCO_3^-. Rapid recapture of HCO_3^- by anion transporters

associated with CA9 as a "metabolome" (Morgan et al., 2007) ensures maintenance of a normal intracellular pH, which in turn is essential for cell survival (Pouyssegur et al., 2006; Swietach et al., 2007). Extracellular acidity supports invasion and metastasis, perhaps due to the pH-dependent activation of cathepsins and metalloproteinases that degrade extracellular matrix and basement membranes (Swietach et al., 2007). Irrespective of these mechanistic details, it may be expected that pharmacological inhibitors of the enzymes responsible for lactate production and/or proton extrusion would have an antimetastatic effect, in addition to reducing tumor growth.

Avoidance of Immunosurveillance

The metabolic microenvironment of tumor cells may inhibit the function of antitumor immune effectors such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells while attracting inflammatory cells that participate in tumor progression. Tumor-associated macrophages (TAMs) are often enriched in hypoxic and tumor perinecrotic areas and constitute a negative prognostic marker. Within TAMs, HIF-1 is essential to upregulate glycolysis so that the cells can migrate into tumor beds (Cramer et al., 2003). TAMs facilitate angiogenesis, promote tumor cell migration, and exert local immunosuppressive effects (Condeelis and Pollard, 2006). As a result, pharmacological HIF-1 inhibition should—theoretically—reduce TAM infiltration into tumors.

Acidification of tumor beds can inhibit the activity of NK cells (Lardner, 2001). Patients with high tumor burden have increased serum lactate levels (Fischer et al., 2007), and lactate may exert potent immunosuppressive effects, as seen in advanced cancer. Thus, lactate suppresses the proliferation, cytokine production, and cytolytic activity of CTLs. As a possible mechanism of this immunosuppressive effect, extracellular lactate blocks the capacity of CTLs to export intracellular lactate by the monocarboxylate transporter MCT1. The resulting lactate overload is incompatible with normal CTL functions (Fischer et al., 2007). Cancer cells often overexpress indoleamine 2,3-dioxygenase (IDO), an enzyme that catabolizes tryptophan (which is taken up by upregulated L-type amino acid transporter 1 [LAT1]) to the kynurenine pathway and oxidative stress. IDO overexpression has been implicated in cancer-associated anorexia/cachexia (Munn and Mellor, 2007), and kynurenine produced by tumor cells can kill CTLs by apoptosis (Puccetti and Grohmann, 2007). These examples illustrate that therapeutic interventions designed to correct abnormal tumor metabolism and, in particular, the inhibition of proton extrusion, lactate production, or IDO might reestablish a defective antitumor immune response.

Conclusions and Therapeutic Perspectives

As discussed above, alterations in cancer cell metabolism are intricately linked to the principal hallmarks of cancer. These links are established in several distinct scenarios. First, metabolic reprogramming may be the consequence of nonmetabolic oncogenic events. Thus, major oncogenic events (such as constitutive activation of growth factor pathways, constitutive activation of HIF-1, and inactivation of p53) may constitute the common cause of metabolic programming and well-studied hallmarks of cancer such as autonomous growth, resistance against apoptosis, limitless replication, and angiogenesis (Figure 2 and centrifugal arrows in Figure 3). Second, classical features of cancer may be conditioned by metabolic reprogramming. Thus, primary metabolic defects in OXPHOS may contribute to apoptosis resistance, while local extracellular emanations of the deregulated cancer cell metabolism may contribute to invasion, metastasis, and immunosuppression (centripetal arrows in Figure 3). Third, at an additional level of complexity, we must consider coevolution of the distinct traits of malignancy. The dual cause-effect relationship between tumor-specific metabolic and nonmetabolic hallmarks is likewise conditioned by the need of proliferating cancer cells to simultaneously subvert multiple cell-intrinsic and cell-extrinsic tumor suppressor mechanisms and hence to emerge from a selection process that forces the coevolution of all hallmarks of cancer, whether metabolic or nonmetabolic.

Irrespective of these theoretical and speculative considerations, the intimate relationship between cancer-associated metabolic reprogramming and all other cardinal features has therapeutic implications at several levels. First, therapeutic subversion of the nonmetabolic properties of cancer (interruption of cell-autonomous growth signals, restoration of apoptosis, inhibition of angiogenesis, etc.) may suppress cancer-specific metabolic programs and hence restore the normal state. This may explain the extraordinary performance of FDG-PET as a predictor of therapeutic outcome (Bonnet et al., 2007). Second, inhibition of the processes and enzymes that participate in metabolic programming may have a dramatic effect on tumors, not only by limiting cancer cell-specific bioenergetic flow and anabolic reactions but also by reversing the neoplastic phenotype and hence stopping growth, inducing apoptosis, and/or blocking angiogenesis and invasion.

Such "dual hits" are exemplified by the inhibition of HIF (which inhibits angiogenesis), reestablishment of p53 function (which restores apoptosis and senescence), or suppression of the PI3K/Akt/mTOR pathway (which inhibits growth), three interventions that should also normalize metabolic functions (Figure 3). Moreover, small inhibitors of enzymes that occupy a central role both in cancer-specific metabolism and in other hallmarks of cancer might be targeted for therapy (Table 2). For instance, low-glucose conditions and inhibitors of glycolysis preferentially induce apoptosis in "glucose-addicted" cells that carry active oncogenes (e.g., Ras, Her2, and Akt) or lack tumor suppressors (e.g., TSC1/2, LKB1, and p53) (Shaw, 2006). The HK2 inhibitor 3-bromopyruvate induces apoptosis in hepatocellular carcinomas in vitro and in vivo (Ko et al., 2004). This effect may involve the inhibition of glycolysis as well as the dissociation of the antiapoptotic interaction between HK2 and VDAC (Kim et al., 2007b), providing yet another example of a dual hit that has been validated at the experimental level, in tumor-bearing mice. Similarly, inhibition of PDK1 can restore PDH activity (and OXPHOS) while triggering apoptosis in tumor cells in vitro and in vivo (Bonnet et al., 2007).

The preclinical and clinical evaluation of metabolic inhibitors for cancer therapy is in its infancy, perhaps with the notable exception of mTOR antagonists (Faivre et al., 2006) and (unfortunately rather nonspecific) activators of AMPK that are given as oral antidiabetics and reduce the incidence of cancer (Evans et al., 2006). The lack of enzymatic inhibitors acting at an acceptable degree of specificity is one of the principal obstacles to evaluating whether inhibition of bioenergetic and anabolic pathways, alone or in combination therapies, might finally target the

Table 2. Potential Metabolic Targets for the Treatment of Cancer

Target	Desired Effects	Examples of Compounds	Reference
Glycolysis			
Glucose uptake	Inhibition of glucose transport or of the initial steps of glycolysis	2-deoxyglucose has radiosensitizing and chemosensitizing effects	Simons et al. (2007)
Hexokinase (HK1 and HK2)	Inhibition of enzymatic activity and dissociation from mitochondria	3-bromopyruvate has potent antitumor effects in vitro and in vivo	Kim et al. (2007b); Pedersen (2007)
Pyruvate dehydrogenase kinase 1 (PDK1)	Inhibition of PDK1 for deinhibition of pyruvate dehydrogenase	Dichloroacetate (DCA)	Bonnet et al. (2007)
Lactate dehydrogenase A (LDHA)	Inhibition	siRNA	Fantin et al. (2006)
Pyruvate kinase (PK) isoenzyme PKM2	Translocation of PKM2 to the nucleus for induction of apoptosis	Somatostatin and its derivative TT-232 (in vitro)	Stetak et al. (2007)
Fatty Acid Synthesis			
ATP citrate lyase (ACL)	Inhibition	SB-2049990 inhibits pancreatic cancer growth in nude mice	Hatzivassiliou et al. (2005)
Acetyl-CoA carboxylase (ACC)	Inhibition	Soraphen A induces apoptosis or autophagy in vitro	Beckers et al. (2007)
Fatty acid synthase (FASN)	Inhibition	Cerulenin and its derivative C57 inhibit human ovarian cancer cell growth in SCID mice	Wang et al. (2005)
Choline kinase (ChoK)	Inhibition	MN58b reduces phosphomonoesters in human cancer xenografts	Al-Saffar et al. (2006)
HIF			
HIF-1α prolyl hydroxylases (PHDs)	Activation of PHDs for inhibition of HIF, achieved by reversal of fumarate- or succinate-mediated inhibition of PHDs	Cell-permeating α-ketoglutarate derivatives reverse HIV activation in SDH- or FH-deficient cells in vitro	MacKenzie et al. (2007)
Hypoxia-inducible factor 1 (HIF-1)	Inhibition of DNA binding	Echinomycin	(Kong et al. (2005)
Reactive oxygen species (ROS)	Antioxidants neutralize ROS and reduce HIF-1 function via PHDs and VHL	N-acetylcysteine (NAC); vitamin C	Gao et al. (2007)
Hypoxia	Cytotoxic effects of components that are enriched in hypoxic cells	Tirapazamine (TPZ), a hypoxia-activated prodrug, is in clinical evaluation for combination chemotherapy	Brizel and Esclamado (2006)
Proton Extrusion			
Na^+/H^+ exchanger	Inhibition	Cariporide	Pouyssegur et al. (2006)
Bicarbonate/Cl^- exchanger	Inhibition	S3705	Pouyssegur et al. (2006)
MCT1 lactate/H^+ symporter	Inhibition	α-cyano-4-OH-cinnamate	Pouyssegur et al. (2006)
Carbonic anhydrases 9 and 12 (CA9 and CA12)	Inhibition	Sulfonamide indisulam	Thiry et al. (2006)
F_1F_0 ATP synthase	Inhibition	Angiostatin; antibodies	Chi et al. (2007)
Other			
AMPK	Activation	Biguanides (e.g., metformin) and thiazolidinediones (e.g., troglitazone) activate AMPK indirectly, probably through inhibition of OXPHOS, and reduce the risk of cancer in diabetic patients	Evans et al. (2006)
eIF4E	Inhibition of translation initiation mediated by eIF4E	Antisense oligonucleotide inhibits growth of human breast cancer xenografts	Graff et al. (2007)
L-type amino acid transporter 1 (LAT1)	Inhibition to reduce amino acid transport	2-aminobicyclo (2.2.1)-heptane 2-carboxylic acid inhibits tumor growth in a xenograft model	Nawashiro et al. (2006)

Achilles' heel of cancer. It can be anticipated that the development of highly specific, preferentially isoenzyme-selective metabolic inhibitors will generate an entirely novel armamentarium for our battle against cancer.

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REFERENCES

Al-Saffar, N.M., Troy, H., Ramirez de Molina, A., Jackson, L.E., Madhu, B., Griffiths, J.R., Leach, M.O., Workman, P., Lacal, J.C., Judson, I.R., and Chung, Y.L. (2006). Noninvasive magnetic resonance spectroscopic pharmacodynamic markers of the choline kinase inhibitor MN58b in human carcinoma models. Cancer Res. *66*, 427–434.

Beckers, A., Organe, S., Timmermans, L., Scheys, K., Peeters, A., Brusselmans, K., Verhoeven, G., and Swinnen, J.V. (2007). Chemical inhibition of acetyl-CoA carboxylase induces growth arrest and cytotoxicity selectively in cancer cells. Cancer Res. *67*, 8180–8187.

Bensaad, K., Tsuruta, A., Selak, M.A., Vidal, M.N., Nakano, K., Bartrons, R., Gottlieb, E., and Vousden, K.H. (2006). TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell *126*, 107–120.

Bonnet, S., Archer, S.L., Allalunis-Turner, J., Haromy, A., Beaulieu, C., Thompson, R., Lee, C.T., Lopaschuk, G.D., Puttagunta, L., Bonnet, S., et al. (2007). A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell *11*, 37–51.

Brahimi-Horn, M.C., Chiche, J., and Pouyssegur, J. (2007). Hypoxia signalling controls metabolic demand. Curr. Opin. Cell Biol. *19*, 223–229.

Brandon, M., Baldi, P., and Wallace, D.C. (2006). Mitochondrial mutations in cancer. Oncogene *25*, 4647–4662.

Braunstein, S., Karpisheva, K., Pola, C., Goldberg, J., Hochman, T., Yee, H., Cangiarella, J., Arju, R., Formenti, S.C., and Schneider, R.J. (2007). A hypoxia-controlled cap-dependent to cap-independent translation switch in breast cancer. Mol. Cell *28*, 501–512.

Brizel, D.M., and Esclamado, R. (2006). Concurrent chemoradiotherapy for locally advanced, nonmetastatic, squamous carcinoma of the head and neck: consensus, controversy, and conundrum. J. Clin. Oncol. *24*, 2612–2617.

Chi, S.L., Wahl, M.L., Mowery, Y.M., Shan, S., Mukhopadhyay, S., Hilderbrand, S.C., Kenan, D.J., Lipes, B.D., Johnson, C.E., Marusich, M.F., et al. (2007). Angiostatin-like activity of a monoclonal antibody to the catalytic subunit of F1F0 ATP synthase. Cancer Res. *67*, 4716–4724.

Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L., and Cantley, L.C. (2008a). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature *452*, 230–233.

Christofk, H.R., Vander Heiden, M.G., Wu, N., Asara, J.W., and Cantley, L.C. (2008b). Pyruvate kinase M2 is a phosphotyrosine binding protein. Nature *452*, 181–186.

Condeelis, J., and Pollard, J.W. (2006). Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell *124*, 263–266.

Counillon, L., and Pouyssegur, J. (2000). The expanding family of eucaryotic Na(+)/H(+) exchangers. J. Biol. Chem. *275*, 1–4.

Cramer, T., Yamanishi, Y., Clausen, B.E., Forster, I., Pawlinski, R., Mackman, N., Haase, V.H., Jaenisch, R., Corr, M., Nizet, V., et al. (2003). HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell *112*, 645–657.

Culmsee, C., and Mattson, M.P. (2005). p53 in neuronal apoptosis. Biochem. Biophys. Res. Commun. *331*, 761–777.

Dang, C.V., Kim, J.-W., Gao, P., and Yustein, J. (2008). The interplay between MYC and HIF in cancer. Nat. Rev. Cancer *8*, 51–56.

Deberardinis, R.J., Lum, J.J., and Thompson, C.B. (2006). Phosphatidylinositol 3-kinase-dependent modulation of carnitine palmitoyltransferase 1A expression regulates lipid metabolism during hematopoietic cell growth. J. Biol. Chem. *281*, 37372–37380.

Erler, J.T., Bennewith, K.L., Nicolau, M., Dornhofer, N., Kong, C., Le, Q.T., Chi, J.T., Jeffrey, S.S., and Giaccia, A.J. (2006). Lysyl oxidase is essential for hypoxia-induced metastasis. Nature *440*, 1222–1226.

Esteban, M.A., Tran, M.G., Harten, S.K., Hill, P., Castellanos, M.C., Chandra, A., Raval, R., O'Brien, T.S., and Maxwell, P.H. (2006). Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. Cancer Res. *66*, 3567–3575.

Evans, J.M., Ogston, S.A., Emslie-Smith, A., and Morris, A.D. (2006). Risk of mortality and adverse cardiovascular outcomes in type 2 diabetes: a comparison of patients treated with sulfonylureas and metformin. Diabetologia *49*, 930–936.

Faivre, S., Kroemer, G., and Raymond, E. (2006). Current development of mTOR inhibitors as anticancer agents. Nat. Rev. Drug Discov. *5*, 671–688.

Fantin, V.R., St-Pierre, J., and Leder, P. (2006). Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell *9*, 425–434.

Fischer, K., Hoffmann, P., Voelkl, S., Meidenbauer, N., Ammer, J., Edinger, M., Gottfried, E., Schwarz, S., Rothe, G., Hoves, S., et al. (2007). Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood *109*, 3812–3819.

Foldi, M., Stickeler, E., Bau, L., Kretz, O., Watermann, D., Gitsch, G., Kayser, G., Zur Hausen, A., and Coy, J.F. (2007). Transketolase protein TKTL1 overexpression: A potential biomarker and therapeutic target in breast cancer. Oncol. Rep. *17*, 841–845.

Fukuda, R., Zhang, H., Kim, J.W., Shimoda, L., Dang, C.V., and Semenza, G.L. (2007). HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell *129*, 111–122.

Funes, J.M., Quintero, M., Henderson, S., Martinez, D., Qureshi, U., Westwood, C., Clements, M.O., Bourboulia, D., Pedley, R.B., Moncada, S., and Boshoff, C. (2007). Transformation of human mesenchymal stem cells increases their dependency on oxidative phosphorylation for energy production. Proc. Natl. Acad. Sci. USA *104*, 6223–6228.

Galluzzi, L., Larochette, N., Zamzami, N., and Kroemer, G. (2006). Mitochondria as therapeutic targets for cancer chemotherapy. Oncogene *25*, 4812–4830.

Gao, P., Zhang, H., Dinavahi, R., Li, F., Xiang, Y., Raman, V., Bhujwalla, Z.M., Felsher, D.W., Cheng, L., Pevsner, J., et al. (2007). HIF-dependent antitumorigenic effect of antioxidants in vivo. Cancer Cell *12*, 230–238.

Gatenby, R.A., and Gillies, R.J. (2004). Why do cancers have high aerobic glycolysis? Nat. Rev. Cancer *4*, 891–899.

Glunde, K., and Bhujwalla, Z.M. (2007). Choline kinase alpha in cancer prognosis and treatment. Lancet Oncol. *8*, 855–857.

Gottlieb, E., and Tomlinson, I.P. (2005). Mitochondrial tumour suppressors: a genetic and biochemical update. Nat. Rev. Cancer *5*, 857–866.

Graff, J.R., Konicek, B.W., Vincent, T.M., Lynch, R.L., Monteith, D., Weir, S.N., Schwier, P., Capen, A., Goode, R.L., Dowless, M.S., et al. (2007). Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. J. Clin. Invest. *117*, 2638–2648.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Harris, A.L. (2002). Hypoxia–a key regulatory factor in tumour growth. Nat. Rev. Cancer *2*, 38–47.

Hatzivassiliou, G., Zhao, F., Bauer, D.E., Andreadis, C., Shaw, A.N., Dhanak, D., Hingorani, S.R., Tuveson, D.A., and Thompson, C.B. (2005). ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell *8*, 311–321.

Igney, F.H., and Krammer, P.H. (2002). Death and anti-death: tumour resistance to apoptosis. Nat. Rev. Cancer *2*, 277–288.

Ishikawa, K., Takenaga, K., Akimoto, M., Koshikawa, N., Yamaguchi, A., Imanishi, H., Nakada, K., Honma, Y., and Hayashi, J.-I. (2008). ROS-generating

mitochondrial DNA mutations can regulate tumor cell metastasis. Science *320*, 661–664.

Kim, J.W., Tchernyshyov, I., Semenza, G.L., and Dang, C.V. (2006). HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. *3*, 177–185.

Kim, J.W., Gao, P., Liu, Y.C., Semenza, G.L., and Dang, C.V. (2007a). Hypoxia-inducible factor 1 and dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. Mol. Cell. Biol. *27*, 7381–7393.

Kim, W., Yoon, J.H., Jeong, J.M., Cheon, G.J., Lee, T.S., Yang, J.I., Park, S.C., and Lee, H.S. (2007b). Apoptosis-inducing antitumor efficacy of hexokinase II inhibitor in hepatocellular carcinoma. Mol. Cancer Ther. *6*, 2554–2562.

Ko, Y.H., Smith, B.L., Wang, Y., Pomper, M.G., Rini, D.A., Torbenson, M.S., Hullihen, J., and Pedersen, P.L. (2004). Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochem. Biophys. Res. Commun. *324*, 269–275.

Kondoh, H., Lleonart, M.E., Gil, J., Wang, J., Degan, P., Peters, G., Martinez, D., Carnero, A., and Beach, D. (2005). Glycolytic enzymes can modulate cellular life span. Cancer Res. *65*, 177–185.

Kong, D., Park, E.J., Stephen, A.G., Calvani, M., Cardellina, J.H., Monks, A., Fisher, R.J., Shoemaker, R.H., and Melillo, G. (2005). Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res. *65*, 9047–9055.

Koukourakis, M.I., Giatromanolaki, A., Harris, A.L., and Sivridis, E. (2006). Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. Cancer Res. *66*, 632–637.

Kroemer, G., Galluzzi, L., and Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. Physiol. Rev. *87*, 99–163.

Langbein, S., Zerilli, M., Zur Hausen, A., Staiger, W., Rensch-Boschert, K., Lukan, N., Popa, J., Ternullo, M.P., Steidler, A., Weiss, C., et al. (2006). Expression of transketolase TKTL1 predicts colon and urothelial cancer patient survival: Warburg effect reinterpreted. Br. J. Cancer *94*, 578–585.

Lardner, A. (2001). The effects of extracellular pH on immune function. J. Leukoc. Biol. *69*, 522–530.

Lopez-Rios, F., Sanchez-Arago, M., Garcia-Garcia, E., Ortega, A.D., Berrendero, J.R., Pozo-Rodriguez, F., Lopez-Encuentra, A., Ballestin, C., and Cuezva, J.M. (2007). Loss of the mitochondrial bioenergetic capacity underlies the glucose avidity of carcinomas. Cancer Res. *67*, 9013–9017.

MacKenzie, E.D., Selak, M.A., Tennant, D.A., Payne, L.J., Crosby, S., Frederiksen, C.M., Watson, D.G., and Gottlieb, E. (2007). Cell-permeating alpha-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells. Mol. Cell. Biol. *27*, 3282–3289.

Mankoff, D.A., Eary, J.F., Link, J.M., Muzi, M., Rajendran, J.G., Spence, A.M., and Krohn, K.A. (2007). Tumor-specific positron emission tomography imaging in patients: [18F] fluorodeoxyglucose and beyond. Clin. Cancer Res. *13*, 3460–3469.

Manning, B.D., and Cantley, L.C. (2007). AKT/PKB signaling: navigating downstream. Cell *129*, 1261–1274.

Matoba, S., Kang, J.G., Patino, W.D., Wragg, A., Boehm, M., Gavrilova, O., Hurley, P.J., Bunz, F., and Hwang, P.M. (2006). p53 regulates mitochondrial respiration. Science *312*, 1650–1653.

Mazurek, S., Boschek, C.B., Hugo, F., and Eigenbrodt, E. (2005). Pyruvate kinase type M2 and its role in tumor growth and spreading. Semin. Cancer Biol. *15*, 300–308.

Miyamoto, S., Murphy, A.N., and Brown, J.H. (2008). Akt mediates mitochondrial protection in cardiomyocytes through phosphorylation of mitochondrial hexokinase-II. Cell Death Differ. *15*, 521–529. Published online December 7, 2007. 10.1038/sj.cdd.4402285.

Morgan, P.E., Pastorekova, S., Stuart-Tilley, A.K., Alper, S.L., and Casey, J.R. (2007). Interactions of transmembrane carbonic anhydrase, CAIX, with bicarbonate transporters. Am. J. Physiol. Cell Physiol. *293*, C738–C748.

Munn, D.H., and Mellor, A.L. (2007). Indoleamine 2,3-dioxygenase and tumor-induced tolerance. J. Clin. Invest. *117*, 1147–1154.

Nawashiro, H., Otani, N., Shinomiya, N., Fukui, S., Ooigawa, H., Shima, K., Matsuo, H., Kanai, Y., and Endou, H. (2006). L-type amino acid transporter 1 as a potential molecular target in human astrocytic tumors. Int. J. Cancer *119*, 484–492.

Papandreou, I., Cairns, R.A., Fontana, L., Lim, A.L., and Denko, N.C. (2006). HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab. *3*, 187–197.

Pastorino, J.G., Hoek, J.B., and Shulga, N. (2005). Activation of glycogen synthase kinase 3beta disrupts the binding of hexokinase II to mitochondria by phosphorylating voltage-dependent anion channel and potentiates chemotherapy-induced cytotoxicity. Cancer Res. *65*, 10545–10554.

Pedersen, P.L. (2007). Warburg, me and Hexokinase 2: Multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. J. Bioenerg. Biomembr. *39*, 211–222.

Pennacchietti, S., Michieli, P., Galluzzo, M., Mazzone, M., Giordano, S., and Comoglio, P.M. (2003). Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell *3*, 347–361.

Pouyssegur, J., Dayan, F., and Mazure, N.M. (2006). Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature *441*, 437–443.

Puccetti, P., and Grohmann, U. (2007). IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. Nat. Rev. Immunol. *7*, 817–823.

Robey, R.B., and Hay, N. (2006). Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt. Oncogene *25*, 4683–4696.

Semenza, G.L. (2007). Hypoxia-inducible factor 1 (HIF-1) pathway. Sci. STKE *2007*, cm8.

Shaw, R.J. (2006). Glucose metabolism and cancer. Curr. Opin. Cell Biol. *18*, 598–608.

Shaw, R.J., and Cantley, L.C. (2006). Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature *441*, 424–430.

Simons, A.L., Ahmad, I.M., Mattson, D.M., Dornfeld, K.J., and Spitz, D.R. (2007). 2-Deoxy-D-glucose combined with cisplatin enhances cytotoxicity via metabolic oxidative stress in human head and neck cancer cells. Cancer Res. *67*, 3364–3370.

Stetak, A., Veress, R., Ovadi, J., Csermely, P., Keri, G., and Ullrich, A. (2007). Nuclear translocation of the tumor marker pyruvate kinase M2 induces programmed cell death. Cancer Res. *67*, 1602–1608.

Swietach, P., Vaughan-Jones, R.D., and Harris, A.L. (2007). Regulation of tumor pH and the role of carbonic anhydrase 9. Cancer Metastasis Rev. *26*, 299–310.

Taylor, C.T., and Pouyssegur, J. (2007). Oxygen, hypoxia, and stress. Ann. N Y Acad. Sci. *1113*, 87–94.

Thiry, A., Dogne, J.M., Masereel, B., and Supuran, C.T. (2006). Targeting tumor-associated carbonic anhydrase IX in cancer therapy. Trends Pharmacol. Sci. *27*, 566–573.

Tomiyama, A., Serizawa, S., Tachibana, K., Sakurada, K., Samejima, H., Kuchino, Y., and Kitanaka, C. (2006). Critical role for mitochondrial oxidative phosphorylation in the activation of tumor suppressors Bax and Bak. J. Natl. Cancer Inst. *98*, 1462–1473.

Wang, H.Q., Altomare, D.A., Skele, K.L., Poulikakos, P.I., Kuhajda, F.P., Di Cristofano, A., and Testa, J.R. (2005). Positive feedback regulation between AKT activation and fatty acid synthase expression in ovarian carcinoma cells. Oncogene *24*, 3574–3582.

Warburg, O., Posener, K., and Negelein, E. (1924). Über den Stoffwechsel der Tumoren. Biochem. Z. *152*, 319–344.

Yang, M.H., Wu, M.Z., Chiou, S.H., Chen, P.M., Chang, S.Y., Liu, C.J., Teng, S.C., and Wu, K.J. (2008). Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat. Cell Biol. *10*, 295–305.

Yoon, S., Lee, M.Y., Park, S.W., Moon, J.S., Koh, Y.K., Ahn, Y.H., Park, B.W., and Kim, K.S. (2007). Up-regulation of acetyl-CoA carboxylase alpha and fatty

acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells. J. Biol. Chem. *282*, 26122–26131.

Zhang, H., Gao, P., Fukuda, R., Kumar, G., Krishnamachary, B., Zeller, K.I., Dang, C.V., and Semenza, G.L. (2007). HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. Cancer Cell *11*, 407–420.

Zhou, S., Kachhap, S., Sun, W., Wu, G., Chuang, A., Poeta, L., Grumbine, L., Mithani, S.K., Chatterjee, A., Koch, W., et al. (2007). Frequency and phenotypic implications of mitochondrial DNA mutations in human squamous cell cancers of the head and neck. Proc. Natl. Acad. Sci. USA *104*, 7540–7545.

Zitvogel, L., Tesniere, A., and Kroemer, G. (2006). Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat. Rev. Immunol. *6*, 715–727.

Zoratti, M., and Szabo, I. (1995). The mitochondrial permeability transition. Biochim. Biophys. Acta *1241*, 139–176.