Cell Metabolism
Review

The Biology of Cancer: Metabolic Reprogramming Fuels Cell Growth and Proliferation

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Cell proliferation requires nutrients, energy, and biosynthetic activity to duplicate all macromolecular components during each passage through the cell cycle. It is therefore not surprising that metabolic activities in proliferating cells are fundamentally different from those in nonproliferating cells. This review examines the idea that several core fluxes, including aerobic glycolysis, de novo lipid biosynthesis, and glutamine-dependent anaplerosis, form a stereotyped platform supporting proliferation of diverse cell types. We also consider regulation of these fluxes by cellular mediators of signal transduction and gene expression, including the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR system, hypoxia-inducible factor 1 (HIF-1), and Myc, during physiologic cell proliferation and tumorigenesis.

Introduction

In mammals, cell proliferation is required for embryogenesis, growth, proper function of several adult tissues, and tumorigenesis. A primary focus of research on cell proliferation has been understanding the mechanisms that regulate the proliferative state, work that has led to identification of growth-factor signal transduction pathways and transcriptional networks enabling cells to initiate and maintain cell cycling. But the onset of proliferation introduces important problems in cellular metabolism as well, because each passage through the cell cycle yields two daughter cells and requires a doubling of total biomass (proteins, lipids, and nucleic acids). This poses a profound metabolic challenge that must be met if cells are to respond to proliferative stimuli.

Proliferating cells often take up nutrients in excess of bioenergetic needs and shunt metabolites into pathways that support a platform for biosynthesis (Bauer et al., 2004). Signals that stimulate cell proliferation must also participate in the reorganization of metabolic activity that allows quiescent cells to begin to proliferate (Figure 1). Over the past several decades, a consistent picture of intermediary metabolism has emerged from studies on diverse types of proliferating cells. Metabolism in these cells differs from quiescent cell metabolism by high rates of glycolysis, lactate production, and biosynthesis of lipids and other macromolecules (Figure 2). In this review, we focus on the roles of these metabolic activities and the replenishment of intermediates for the tricarboxylic acid (TCA) cycle (anaplerosis) during proliferation. We also discuss current concepts regarding how signal transduction pathways influence cell metabolism.

Most of the work cited below involves proliferating lymphocytes or tumor cells. Lymphocytes and other hematopoietic cells are excellent models for the study of metabolic regulation because quiescent cells can be stimulated to proliferate in vitro and the signaling mechanisms behind cell proliferation are well characterized. Tumor cells are also useful because a wide variety of cell lines are available and the genetic mechanisms leading to tumorigenesis are often known. It should be stressed that "tumor metabolism" is not synonymous with the metabolism of cell proliferation. While proliferation is required for tumors to grow, many factors within the tumor microenvironment can influence cellular metabolism, resulting in heterogeneous metabolic activity. Our interest in tumor cells as discussed here involves the metabolic activities that promote their growth and proliferation.

Proliferating Cells Use Aerobic Glycolysis

In the 1920s, Otto Warburg published the seminal observation that rapidly proliferating ascites tumor cells consume glucose at a surprisingly high rate compared to normal cells and secrete most of the glucose-derived carbon as lactate rather than oxidizing it completely, a phenomenon known as the "Warburg effect" (Warburg, 1925, 1956b). This observation presented a paradox that still has not been completely resolved: Why do proliferating cells, which ostensibly have a great need for ATP, use such a wasteful form of metabolism? Warburg proposed that tumor cells harbor a permanent impairment of oxidative metabolism resulting in a compensatory increase in glycolytic flux (Warburg, 1956a). But later studies on proliferating primary lymphocytes revealed similar patterns, in which more than 90% of glucose carbon was converted to lactate, ruling out the possibility that aerobic glycolysis is unique to tumor cells or that the Warburg effect only develops when oxidative capacity is damaged (Brand, 1985; Hedeskov, 1968; Roos and Loos, 1973; Wang et al., 1976). Indeed, many highly proliferative tumor cell lines that have been carefully studied do not have defects in oxidative metabolism (Moreno-Sanchez et al., 2007).

So why does the Warburg effect occur? Clearly, the high glycolytic rate provides several advantages for proliferating cells. First, it allows cells to use the most abundant extracellular nutrient, glucose, to produce abundant ATP. Although the yield of ATP per glucose consumed is low, if the glycolytic flux is high enough, the percentage of cellular ATP produced from glycolysis can exceed that produced from oxidative phosphorylation (Guppy et al., 1993; Warburg, 1956b). This may be due to the high rate of ATP production during glycolysis compared to

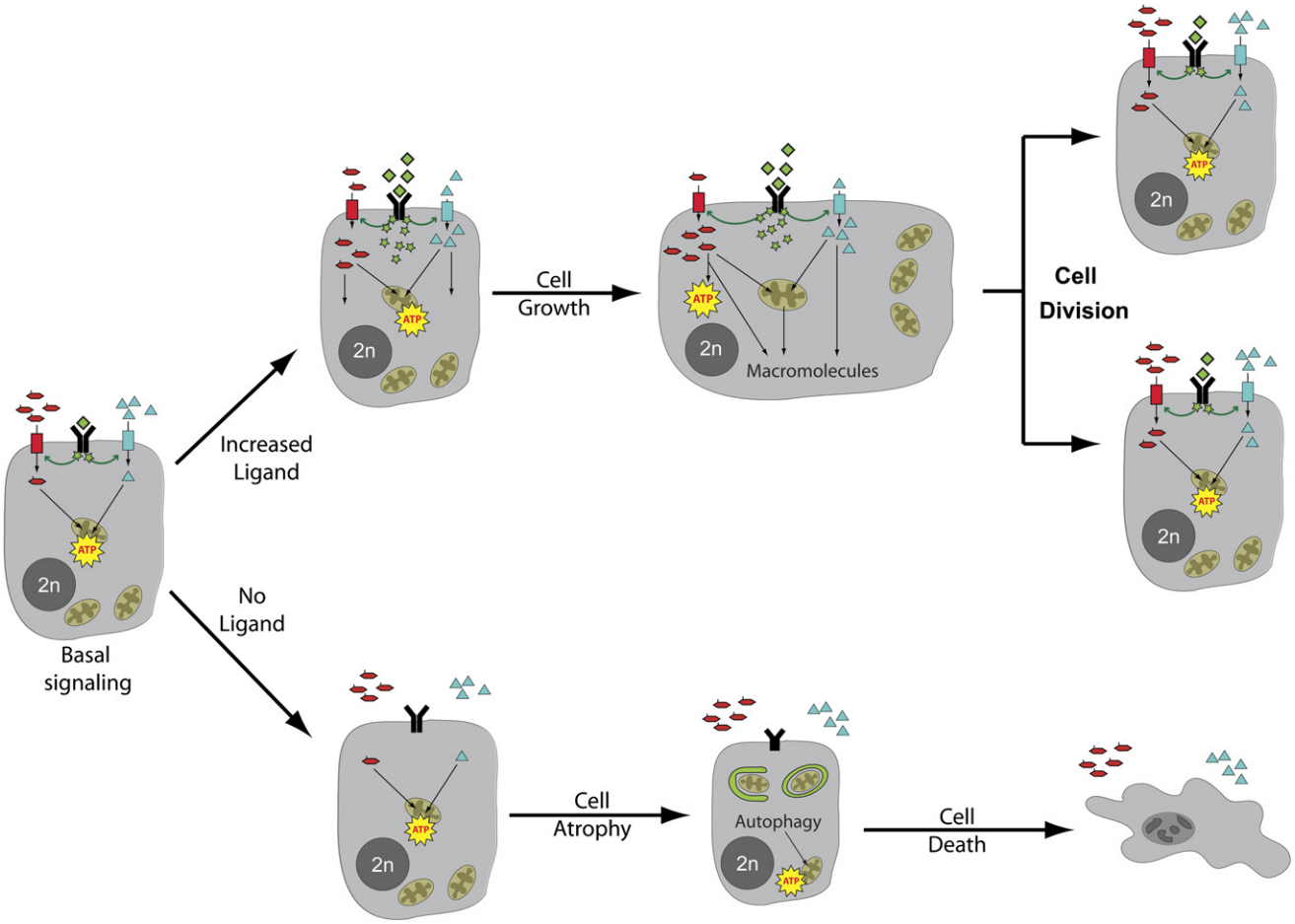

Figure 1. Growth-Factor Signaling Regulates the Uptake and Metabolism of Extracellular Nutrients
At rest, basal levels of lineage-specific growth-factor signaling (green) allow cells to take up sufficient nutrients like glucose (red) and amino acids (blue) in order to provide the low levels of ATP production and macromolecular synthesis needed to maintain cellular homeostasis. In the absence of any extrinsic signals (no ligand), mammalian cells lose surface expression of nutrient transporters. To survive in the absence of the ability to take up extracellular nutrients, growth-factor-deprived cells engage in autophagic degradation of macromolecules and organelles. This is a finite survival strategy, ultimately resulting in cell death. In contrast, increases in ligand signaling instruct cells to begin taking up nutrients at a high rate and to allocate them into metabolic pathways that support production of ATP and macromolecules like proteins, lipids, and nucleic acids. These activities culminate in a net increase in cellular biomass (growth) and, ultimately, the formation of daughter cells.

oxidative phosphorylation (Pfeiffer et al., 2001). Second, glucose degradation provides cells with intermediates needed for biosynthetic pathways, including ribose sugars for nucleotides; glycerol and citrate for lipids; nonessential amino acids; and, through the oxidative pentose phosphate pathway, NADPH. So the Warburg effect benefits both bioenergetics and biosynthesis.

What remains controversial about the Warburg effect is why the rate of lactate production is so high when more of the pyruvate could presumably be oxidized to enhance ATP production. One explanation is simply that glycolysis outpaces the maximal velocity of pyruvate oxidation, so that cells must instead eliminate pyruvate using high-flux mechanisms. Oxidation of pyruvate requires import into the mitochondrial matrix, followed by activity of highly regulated enzymes like the pyruvate dehydrogenase (PDH) complex, whose activity is influenced by phosphorylation, free CoA levels, and the NAD^+/NADH ratio, all of which may limit its activity relative to glycolytic flux. Glycolytic flux may exceed the V_{max} of PDH by more than an order of magnitude during cell proliferation, implying the need for a high-capacity system to avoid accumulation of pyruvate (Curi et al., 1988). In proliferating cells, expression of lactate dehydrogenase A (LDH-A) solves this problem by rapidly consuming pyruvate, regenerating NAD^+ in the face of a relentless glycolytic flux while yielding a product (lactate) that can easily be secreted (Figure 2). LDH-A is induced by oncogenes (c-*myc*, *HER2/neu*, and others) and by mitogen stimulation in lymphocytes, and it participates in xenograft tumorigenicity, implying a prominent role in cell proliferation (Fantin et al., 2006; Marjanovic et al., 1990; Shim et al., 1997).

A further advantage of the high glycolytic rate is that it allows cells to fine tune the control of biosynthetic pathways that use intermediates derived from glucose metabolism. When a high-flux metabolic pathway branches into a lower-flux pathway, the ability to maintain activity of the latter is maximized when flux through the former is highest. In proliferating cells, this has been proposed as a way to resolve the apparent paradox

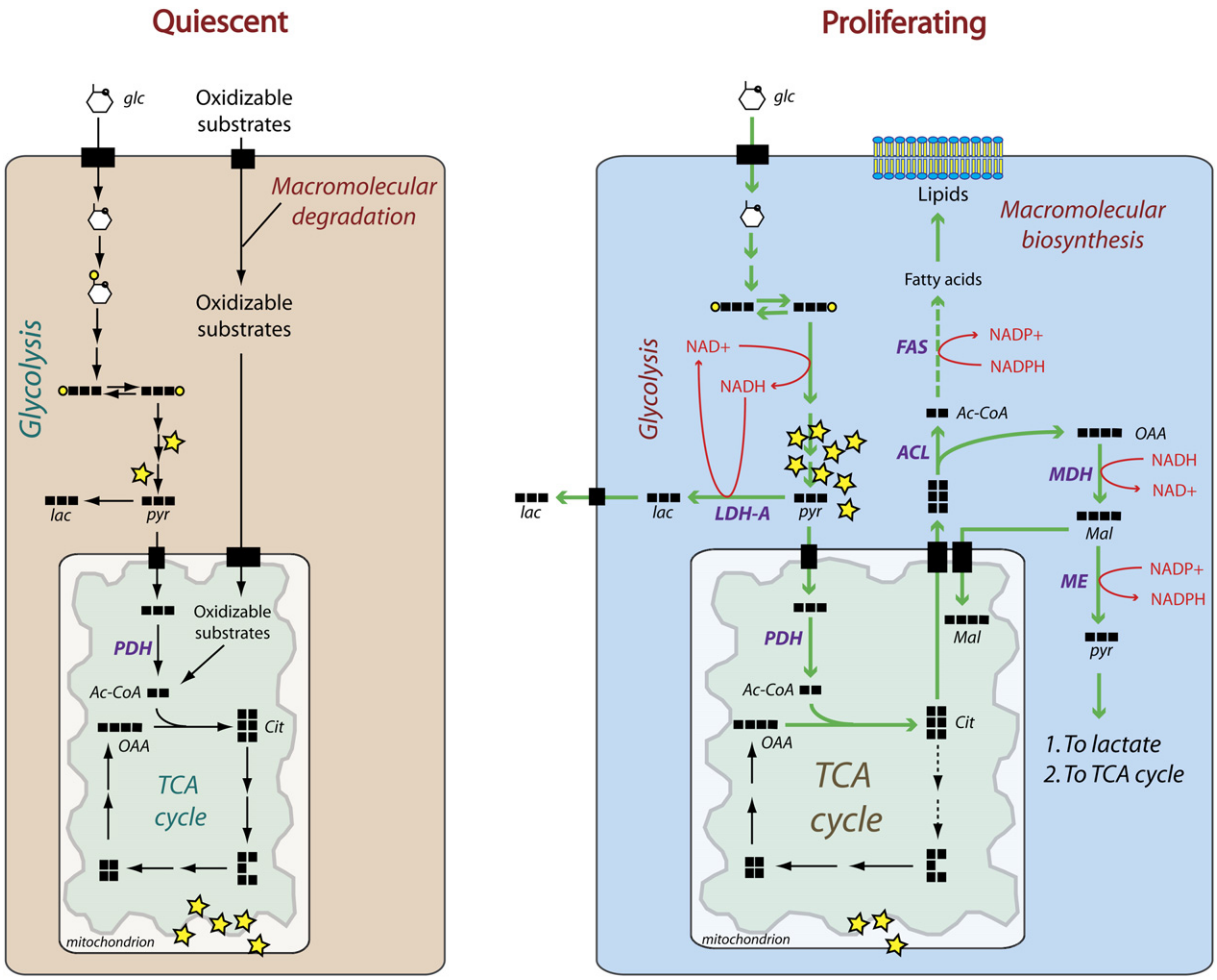

Figure 2. Carbon Flux Differs in Quiescent versus Proliferating Cells

Quiescent cells (left) have a basal rate of glycolysis, converting glucose (glc) to pyruvate (pyr), which is then oxidized in the TCA cycle. Cells can also oxidize other substrates like amino acids and fatty acids obtained from either the environment or the degradation of cellular macromolecules. As a result, the majority of ATP (yellow stars) is generated by oxidative phosphorylation. During proliferation (right), the large increase in glycolytic flux generates ATP rapidly in the cytoplasm, reducing the cytoplasmic NAD^+/NADH ratio. Most of the resulting pyruvate is converted to lactate (lac) by lactate dehydrogenase A (LDH-A), which regenerates NAD^+ from NADH. The NAD^+ allows glycolysis to persist, and the lactate is secreted from the cell. Some of the pyruvate is converted to acetyl-CoA (Ac-CoA) by pyruvate dehydrogenase (PDH) and enters the TCA cycle, where it is converted into intermediates like citrate (cit) that can be used for macromolecular biosynthesis. Citrate is required for the synthesis of fatty acids and cholesterol used to generate lipid membranes for daughter cells. After export to the cytoplasm, citrate is cleaved by the enzyme ATP citrate lyase (ACL). The resulting acetyl-CoA is used by fatty acid synthase (FAS) to synthesize lipids, while the oxaloacetate (OAA) is converted to malate (mal) by malate dehydrogenase (MDH), utilizing the low cytosolic NAD^+/NADH ratio. Malate can either be returned to the mitochondria during citrate-malate antiport or be converted to pyruvate by malic enzyme (ME), generating NADPH to be used in fatty acid synthesis.

between the need for glucose-derived carbon for macromolecular synthesis and the high rate of lactate production (Newsholme et al., 1985). Low-flux pathways in this model include those that use glycolytic intermediates as biosynthetic precursors. The very high rate of glycolysis allows cells to maintain biosynthetic fluxes during rapid proliferation but results in a high rate of lactate production.

The TCA Cycle Provides Proliferating Cells with Biosynthetic Precursors

To synthesize lipids, proteins, and nucleic acids, cells use precursors derived from TCA cycle intermediates. Therefore, a key role of the TCA cycle in proliferating cells is to act as a hub for biosynthesis. This is an important difference from the metabolism of nonproliferating, oxidative tissues like the heart, where the traditional view of the TCA cycle is that it serves to derive maximal ATP production from oxidizable substrates, generating two CO_2 molecules per turn. During cell proliferation, however, much of the carbon that enters the TCA cycle is used in biosynthetic pathways that consume rather than produce ATP. This results in a continuous efflux of intermediates (cataplerosis).

Synthesis of lipids (fatty acids, cholesterol, and isoprenoids) is a prime example of cataplerosis in proliferating cells. Glucose is a major lipogenic substrate using the pathway highlighted in green in the right panel of Figure 2. This pathway transfers mitochondrial citrate out to the cytosol to be converted to oxaloacetate (OAA) and the lipogenic precursor acetyl-CoA. The lipogenic enzymes ATP citrate lyase and fatty acid synthase are induced in tumor cells and proliferating hematopoietic cells, and their

activity is required for proliferation (Bauer et al., 2005; Hatzivassiliou et al., 2005; Kuhajda et al., 1994; Pizer et al., 1996). This may be because a large percentage of fatty acids in the membranes of proliferating cells are synthesized de novo rather than scavenged from the extracellular environment (Kannan et al., 1980; Ookhtens et al., 1984) or because some crucial cellular lipid pool requires de novo synthesis. The export of citrate for lipid synthesis impacts overall function of the cycle, resulting in what some have called a "truncated" cycle because of the relative decrease in the fraction of mitochondrial citrate that is oxidized (Hatzivassiliou et al., 2005; Parlo and Coleman, 1984). The high flux of mitochondrial citrate to cholesterol synthesis has been studied in hepatoma cells, where proliferation is proportional to the rate of citrate efflux and inversely proportional to citrate-stimulated respiration (Parlo and Coleman, 1984, 1986). Therefore, in these cholesterol-rich cells, TCA truncation appears to support cell proliferation. Other TCA cycle intermediates are used for biosynthesis of different macromolecules. OAA and α-ketoglutarate (α-KG) supply intracellular pools of nonessential amino acids to be used in the synthesis of proteins and nucleotides. These activities also contribute to cataplerosis in proliferating cells engaged in macromolecular biosynthesis.

In rare cases, the TCA cycle enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH) behave genetically as tumor suppressors. Familial paraganglioma can be caused by mutations in *SDHB*, *SDHC*, or *SDHD*, three of the four SDH subunits (Astuti et al., 2001; Baysal et al., 2000; Niemann and Muller, 2000). In affected families, a mutation in any of these genes imposes a dominantly inherited tumor risk, with loss of the wild-type allele in tumors. Similarly, *SDHB* and *SDHD* mutations can cause pheochromocytoma (Astuti et al., 2001; Gimm et al., 2000), and mutations in *FH* cause a dominant syndrome of uterine fibroids, leiomyomata, and papillary renal cell cancer (Tomlinson et al., 2002). Interestingly, cells from some paragangliomas have no residual SDH activity, implying severe impairment of TCA cycling in those tumors (Gimenez-Roqueplo et al., 2001). Despite this, the cells not only survive but accumulate at a pathologic rate. These examples are interesting exceptions to the general finding that tumor cells contain functional TCA cycles. Further investigations may reveal compensatory metabolic pathways that support this form of tumor cell growth.

Anaplerosis Allows Proliferating Cells to Use the TCA Cycle for Biosynthesis

In order to sustain TCA cycle function in the face of cataplerosis, cells must have a matching influx of intermediates to resupply "lost" OAA (anaplerosis). Citrate export for fatty acid synthesis demonstrates this necessity: formation of another citrate molecule requires an OAA produced from pyruvate or amino acids. Anaplerosis is a critical feature of growth metabolism because it gives cells the ability to use the TCA cycle as a supply of biosynthetic precursors. A high anaplerotic flux is a more specific indicator of cell growth than a high glycolytic flux, because the latter can be initiated by hypoxia and other stresses independently of macromolecular synthesis.

There are several mechanisms that cells can use to produce anaplerotic activity. The simplest uses pyruvate carboxylase (PC), which generates OAA directly from pyruvate. Mitogens enhance PC activity in lymphocytes, suggesting that PC might be part of the proliferative metabolic program in those cells (Curi et al., 1988). But in MCF-7 breast carcinoma cells, estrogen stimulation suppresses PC activity while enhancing proliferation (Forbes et al., 2006). Furthermore, most hepatomas have decreased PC expression and activity compared to normal liver (Chang and Morris, 1973; Hammond and Balinsky, 1978), and the ratio of PC/PDH activity is decreased in glioma and neuroblastoma cells compared to normal glia and neuronal tissue (Brand et al., 1992). Therefore, PC does not appear to be a universal component of anaplerotic flux during cell proliferation.

An alternative source of anaplerosis is through metabolism of amino acids, particularly glutamine, the most abundant amino acid in mammals. Proliferating cells metabolize glutamine in multiple pathways for bioenergetics and biosynthesis (Eagle et al., 1956; Kovacevic and McGivan, 1983). Cells can partially oxidize glutamine in a manner analogous to the partial oxidation of glucose during aerobic glycolysis (Reitzer et al., 1979). This pathway ("glutaminolysis") adds to cellular production of NADPH and lactate (Figure 3). Unlike aerobic glycolysis, however, glutaminolysis uses several steps of the TCA cycle, leading to general recognition of the fact that glutamine is a source of energy for proliferating cells. It is equally important that mitochondrial glutamine metabolism can produce OAA, providing a source of anaplerosis in growing cells (Figure 3). Evidence from a variety of cell types supports this conclusion. Estrogen stimulation induces glutaminolysis in breast cancer cells (Forbes et al., 2006), while mitogen stimulation has similar effects in lymphocytes (Brand, 1985). Nuclear magnetic resonance (NMR) spectroscopy using ^{13}C-labeled substrates has revealed the use of glutamine as the major anaplerotic precursor in proliferating glioma cells in both rats (Portais et al., 1996) and humans (DeBerardinis et al., 2007). Impressively, glutamine deprivation from fibroblast cultures essentially eliminates pools of the TCA cycle intermediates fumarate and malate (Yuneva et al., 2007). Together, these observations suggest that glutamine metabolism allows cells to maintain a sufficient anaplerotic flux to use a sizable fraction of TCA cycle intermediates as precursors for biosynthetic pathways. Importantly, glutamine's central role in multiple pathways of intermediary metabolism that produce glutamate and α-KG (Figure 3) makes it a convenient molecule for cells to use as a source of carbon for the TCA cycle.

Regulation of Metabolic Activity in Proliferating Cells

Normal mammalian cells do not proliferate autonomously but instead enter the cell cycle only when instructed to do so by growth factors and downstream signaling pathways, which influence gene expression and cell physiology. Given that proliferation relies on the metabolic activities discussed above, it is not surprising that growth-factor-stimulated signal transduction regulates these activities as well. Traditional views of intermediary metabolism hold that metabolic activities are largely regulated through allosteric effects of metabolites on rate-limiting enzymes, giving pathways self-regulatory capacity and introducing control at branch points between intersecting pathways. While many of these mechanisms are at work in proliferating cells, efforts to understand the impact of signal transduction on cell proliferation have revealed a variety of effects directed at metabolic fluxes. For example, during proliferation of tumor cells and

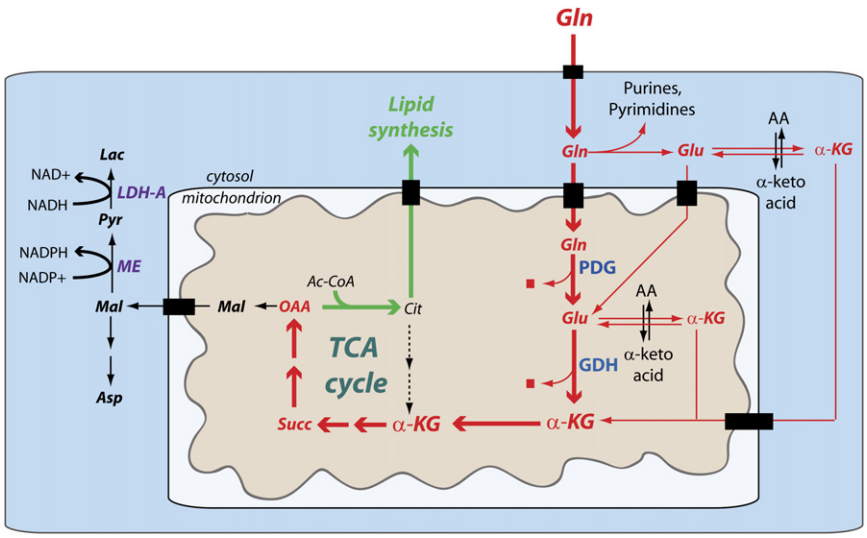

Figure 3. Glutamine-Dependent Anaplerosis Allows Proliferating Cells to Use TCA Cycle Intermediates as Precursors for Biosynthesis

The proliferating cell shown here is using citrate for lipid synthesis (green arrows), resulting in loss of oxaloacetate from the TCA cycle. OAA replenishment (anaplerosis) is derived from the complex metabolism of glutamine (Gln, red arrows). In the cytosol, glutamine donates nitrogen to purines and pyrimidines, resulting in the formation of glutamate (Glu). Glutamate donates its amino group to α-keto acids to form nonessential amino acids and α-ketoglutarate (α-KG), which can enter the mitochondria. Glutamine can also be converted to glutamate in the mitochondrial matrix by phosphate-dependent glutaminase (PDG), which releases glutamine's amido group as free ammonia (red square). Mitochondrial glutamate can be converted to α-KG by glutamate dehydrogenase (GDH, forming another ammonia molecule) or intramitochondrial aminotransferases. During anaplerosis, α-KG enters the TCA cycle and produces OAA. In addition to its use as a source of OAA, glutamine carbon can be converted to lactate (glutaminolysis). This process generates both NADPH and NAD^+ in the cytoplasm. Ammonia generated during glutamine metabolism is mostly secreted from the cell. Other abbreviations: Asp, aspartate; Succ, succinate; AA, amino acid.

lymphocytes, growth-factor signaling suppresses β-oxidation of fatty acids, minimizing futile cycling and maximizing lipid synthesis (Buzzai et al., 2005; DeBerardinis et al., 2006). In hematopoietic cells, this requires a specific inhibitory effect of the PI3K/Akt signaling pathway on the expression of carnitine palmitoyltransferase IA, the rate-limiting enzyme in β-oxidation (DeBerardinis et al., 2006). Therefore, growth-factor signaling can reorganize metabolic fluxes independently of traditional allosteric mechanisms of pathway regulation.

Generating high fluxes of glycolysis and glutaminolysis largely depends on increasing cellular uptake of glucose and glutamine. Proliferating cells rely on growth-factor signaling to generate these fluxes because a primary effect of signaling is to enhance nutrient capture from the extracellular environment (Figure 1). In fact, in the absence of growth-factor signaling, mammalian cells rapidly lose nutrient transporter expression and cannot maintain sufficient cell-autonomous nutrient uptake for basal bioenergetics and replacement macromolecular synthesis. Instead, they turn to a form of "self-cannibalism" termed autophagy, which provides a limited supply of substrates generated from macromolecular degradation to maintain ATP production for cell survival (Figure 1) (Lum et al., 2005).

The mechanisms that integrate signal transduction and cell metabolism are largely conserved between normal cells and cancer cells. The major difference is that in normal cells, initiation of signaling requires extracellular stimulation, while cancer cells often have mutations that chronically enhance these pathways, allowing them to maintain a metabolic phenotype of biosynthesis independently of normal physiologic constraints. In other words, cancer cells have increased metabolic autonomy. Below, we discuss a few mechanisms that integrate cell signaling and key aspects of metabolism during physiologic cell proliferation and tumorigenesis. Together, activities of the PI3K/Akt/mTOR pathway and effects of the transcription factors HIF-1α and Myc appear to regulate complementary aspects of cellular metabolism (Figure 4).

The PI3K/Akt/mTOR Pathway Is a Master Regulator of Aerobic Glycolysis and Cellular Biosynthesis

The PI3K/Akt/mTOR pathway is a highly conserved, widely expressed system used by cells to respond to growth factors (Franke et al., 2003). Binding of a growth factor to its surface receptor activates PI3K, resulting in phosphorylation of phosphatidylinositol lipids at the plasma membrane. These are involved in recruitment and/or activation of downstream effectors, particularly the serine/threonine kinases Akt and mTOR. Activation of the PI3K/Akt/mTOR pathway in growth-factor-dependent cells and tumor cells enhances many of the metabolic activities that support cellular biosynthesis (Figure 4). First, it permits cells to increase the surface expression of nutrient transporters, enabling increased uptake of glucose, amino acids, and other nutrients (Barata et al., 2004; Edinger and Thompson, 2002; Roos et al., 2007; Wieman et al., 2007; Xu et al., 2005). Second, through effects on gene expression and enzyme activity, Akt increases glycolysis and lactate production and is sufficient to induce a Warburg effect in either nontransformed cells or cancer cells (Elstrom et al., 2004; Plas et al., 2001; Rathmell et al., 2003). Third, activation of this pathway enhances the biosynthesis of macromolecules. PI3K and Akt stimulate expression of lipogenic genes and lipid synthesis in numerous cell types (Bauer et al., 2005; Chang et al., 2005), while mTOR is a key regulator of protein translation (Gingras et al., 2001).

In normal cells, activation of the PI3K system is tightly controlled by dephosphorylation of phosphatidylinositol species by the phosphatase PTEN. But in malignancies, activity of the pathway can be augmented through a variety of mechanisms, which together constitute one of the most prevalent classes of mutations in human tumors (Table 1). These mutations activate PI3K, eliminate activity of negative regulators (e.g., PTEN),

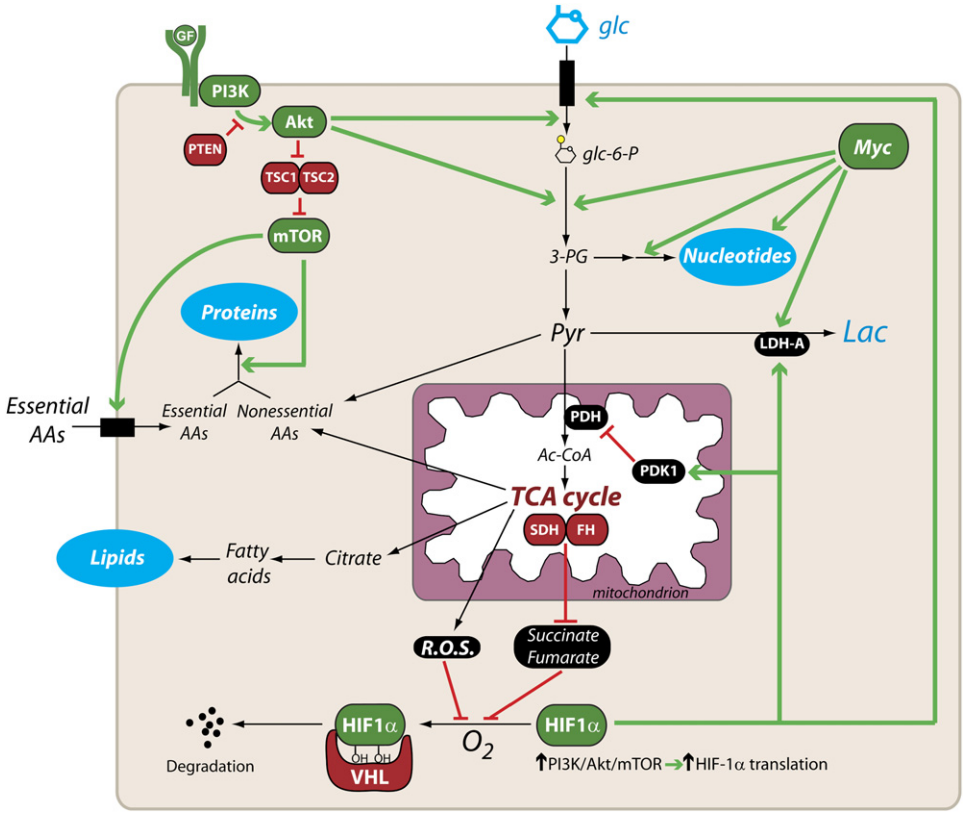

Figure 4. A Signaling Network to Regulate Metabolism in Proliferating Cells

The model shows some of the prominent aspects of metabolism in proliferating cells, including glycolysis; lactate production; the use of TCA cycle intermediates as macromolecular precursors; and the biosynthesis of proteins, nucleotides, and lipids. The PI3K/Akt/mTOR pathway, HIF-1α, and Myc participate in various facets of this metabolic phenotype. The binding of a growth factor (GF) to its surface receptor brings about activation of PI3K and the serine/threonine kinases Akt and mTOR (top left). Constitutive activation of the pathway can occur in tumors due to mutation of the tumor suppressors *PTEN*, *TSC1*, and *TSC2*, or by other mechanisms (see text). Metabolic effects of the PI3K/Akt/mTOR pathway include enhanced uptake of glucose and essential amino acids and protein translation. The transcription factor HIF-1α (bottom) is involved in determining the manner in which cells utilize glucose carbon. Translation of HIF-1α is enhanced during growth-factor stimulation of the PI3K/Akt/mTOR pathway. In the presence of oxygen, HIF-1α is modified by prolyl hydroxylases, which target it to a ubiquitin ligase complex that includes the tumor suppressor VHL. This association results in constitutive normoxic degradation of the HIF-1α protein. Hypoxia, mutation of *VHL*, or accumulation of reactive oxygen species (ROS) or the TCA cycle intermediates succinate and fumarate impair HIF-1α degradation, allowing it to enter the nucleus and engage in transcriptional activity. Transcriptional targets include genes encoding glucose transporter 1 (GLUT1), LDH-A, and PDK1. The combined effect on glucose metabolism is to increase both glucose utilization and lactate production, as PDK1 inhibits conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase (PDH). The transcription factor Myc (top right) increases expression of many metabolic enzymes, including glycolytic enzymes, LDH-A, and several enzymes required for nucleotide biosynthesis. Abbreviations: PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; TSC, tuberous sclerosis complex; mTOR, mammalian target of rapamycin; glc-6-P, glucose-6-phosphate; 3-PG, 3-phosphoglycerate; PDK1, pyruvate dehydrogenase kinase 1; SDH, succinate dehydrogenase; FH, fumarate hydratase; HIF-1α, hypoxia-inducible factor 1α; VHL, von Hippel-Lindau.

or introduce enhanced activities to stimulate the system (BCR-ABL, *HER2/neu* amplification, etc). Regardless of the mutation, activation of Akt is likely the most important signaling event in terms of cell metabolism, because Akt is sufficient to drive glycolysis and lactate production and to suppress macromolecular degradation in cancer cells (Buzzai et al., 2005; Elstrom et al., 2004).

HIF-1 Signaling Regulates Glucose Metabolism in Response to Hypoxia and Growth Factors

Decreased oxygen availability (hypoxia) stimulates cells to consume glucose and produce lactate. In mammalian cells, this response is coordinated by the hypoxia-inducible factor 1 (HIF-1) transcription factor complex (Gordan and Simon, 2007; Semenza, 2003). HIF-1's targets include genes encoding glucose transporters, glycolytic enzymes, and LDH-A (O'Rourke et al., 1996; Semenza et al., 1994). HIF-1 activity requires the subunit HIF-1α, which is expressed under the control of growth-factor signaling, in particular the PI3K/Akt/mTOR pathway (Cramer et al., 2003; Jiang et al., 2001; Majumder et al., 2004). During normoxia, HIF-1α undergoes a posttranslational modification by prolyl hydroxylation, which promotes association with the von Hippel-Lindau (VHL) tumor suppressor, targeting HIF-1α for ubiquitination and degradation (Figure 4). During hypoxia, prolyl hydroxylation is inhibited by a process involving reactive oxygen species (ROS) generated in the mitochondria, resulting in stabilization of the HIF-1α protein and transcriptional activity of the HIF-1 complex (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005).

Constitutive cellular stabilization of HIF-1α during normoxia can occur in tumors as a result of mutations in the tumor suppressor *VHL*. Other mutations in SDH and FH stabilize HIF-1α by interfering with prolyl hydroxylation, which is inhibited by accumulation of succinate or fumarate (Isaacs et al., 2005; Pollard

Table 1. Selected Tumorigenic Mutations that Activate PI3K or Its Effectors

Gene	Mutation	Cancer	Frequency	Reference
PIK3CA	Activating point mutations	Breast	25%	Bachman et al. (2004)
		Colon	>30%	Samuels et al. (2004)
	Amplification	Head and neck	>35%	Pedrero et al. (2005)
Akt2	Amplification	Ovary	12%	Bellacosa et al. (1995)
		Head and neck	30%	Pedrero et al. (2005)
PTEN	Mutation, loss of heterozygosity	Glioma	≤40%	Knobbe et al. (2002); Ohgaki (2005)
BCR-ABL	Fusion kinase arising from chromosomal translocation	Chronic myelogenous leukemia	>90%	Kurzrock et al. (2003)
		Acute lymphocytic leukemia	20%	Kurzrock et al. (2003)
HER2/neu	Gene amplification	Breast	25%	Slamon et al. (1989)
EGFR	Gene amplification, increased expression	Lung (non-small cell)	>50%	Cappuzzo et al. (2005)

et al., 2007; Selak et al., 2005). In tumors with mutations in *VHL*, *FH*, or SDH subunits, constitutive (normoxic) expression of HIF-1 target genes likely contributes to aerobic glycolysis.

Although HIF-1's role in promoting glycolysis is clear, recent data suggest that it does not promote biosynthesis at the cellular level. HIF-1 induces expression of pyruvate dehydrogenase kinase 1 (PDK1), which phosphorylates and inhibits the PDH complex (Kim et al., 2006; Papandreou et al., 2006). This limits entry of glycolytic carbon into the TCA cycle and increases conversion of pyruvate to lactate. This adaptation may be important for cell survival during hypoxia, but it would impose a barrier to proliferating cells, which rely on the availability of TCA cycle intermediates for biosynthesis. Recent studies in hematopoietic cells support this hypothesis (Lum et al., 2007). In these cells, growth-factor stimulation is required for cells to express HIF-1α, which in turn is required to regulate the intracellular fate of glucose-derived carbon. During normoxia, reducing HIF-1α expression with RNA interference increases lipid synthesis, cell size, and rate of proliferation. Together, these observations argue for more general metabolic functions of HIF-1 than its conventional role as a reactionary mediator during tissue hypoxia, extending its influence into the arena of growth-factor-regulated orchestration of intermediary metabolic fluxes. In this context, it appears to act as a rheostat on mitochondrial metabolism, fine tuning entry of carbon into the TCA cycle. Perhaps during the large increase in glycolytic flux that occurs during growth-factor stimulation, this allows cells to match TCA cycle flux with maximal electron transport chain capacity so as to diminish oxidative stress.

Does c-Myc Regulate Metabolic Activities Needed for the G1/S Transition?

The metabolic activity that distinguishes cell growth (i.e., increase in cell biomass per se) from proliferation is duplication of the genome, which requires a massive commitment to nucleotide biosynthesis by the cell. Compared to glycolytic flux, the regulation of de novo nucleotide biosynthetic pathways by cell signaling is poorly understood. These complex pathways rely on coordination of multiple fluxes involving glucose, glutamine, several nonessential amino acids, and the cellular one-carbon pool.

The *myc* family of genes (c-*myc*, L-*myc*, s-*myc*, and N-*myc*), commonly amplified in human tumors, encode transcription factors that regulate growth and cell-cycle entry by inducing expression of genes required for these processes. In normal cells, mitogen stimulation leads to a burst of c-Myc expression in G1 phase, facilitating entry into S phase in part by activating expression of cyclins and CDK4 (Adhikary and Eilers, 2005). Like other oncogenic transcription factors, targets of c-Myc include glycolytic enzymes and LDH-A (Osthus et al., 2000; Shim et al., 1997). However, c-Myc also induces expression of enzymes involved in nucleotide and one-carbon metabolism, without which cells could not successfully complete S phase (Figure 4). These include inosine 5′-monophosphate dehydrogenase (Guo et al., 2000), serine hydroxymethyltransferase (Nikiforov et al., 2002), adenosine kinase, adenylate kinase 2, and phosphoribosyl pyrophosphate amidotransferase (O'Connell et al., 2003). These data suggest that c-Myc reinforces the effects of growth-factor signaling on glucose metabolism and also exerts control over specialized metabolic activities needed to duplicate the genome.

In addition, recent work has demonstrated that some c-Myc-transformed cells have an absolute requirement for glutamine in order to maintain viability (Yuneva et al., 2007). Depriving these cells of glutamine results in depletion of TCA cycle intermediates, suggesting an increased need for glutamine-based anaplerosis during c-Myc activity. Perhaps this is a consequence of the metabolic shift toward de novo nucleotide biosynthesis, which requires glutamine as a nitrogen source and glucose as a carbon source. The resulting increased availability of glutamine carbon skeletons coupled with the reduced availability of glucose carbon might limit the utility of PC as an anaplerotic mechanism during peak nucleotide biosynthesis.

Future Directions: Cell Proliferation, Signal Transduction, Metabolism, and Systems Biology

As summarized above, the emerging view of metabolic regulation in proliferating cells is that signal transduction pathways

and transcriptional networks participate in a major reorganization of metabolic activities into a platform that supports bioenergetics, macromolecular synthesis, and ultimately cell division. Efforts to integrate modern concepts of signal transduction with cellular metabolism are still in their infancy. The current challenge is to develop broad, systems-based approaches devoted to integrating information from previously disparate areas of inquiry so that a more complete understanding of the metabolic phenotype of cell proliferation will emerge. This will require a new set of tools combining, at a minimum, molecular biology and metabolic flux analysis so as to determine the impact of manipulating signaling mediators on specific and global metabolic activities.

One area that needs to be addressed is the regulation of anaplerosis and of mitochondrial metabolism in general. This important matter has so far escaped the scrutiny directed at aerobic glycolysis in the 80-plus years since Warburg's observations. The models of cell metabolism proposed here predict that biosynthetic fluxes using TCA cycle intermediates are matched on a mole-per-mole basis by anaplerotic fluxes. Determining whether this hypothesis is correct and how such fluxes are regulated will be an important piece in the biological puzzle of cell proliferation.

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