Chapter 2
The Warburg Phenomenon and Other Metabolic Alterations of Cancer Cells

Abstract The altered metabolism of cancer cells was recognized and pioneered by the elegant works of Otto Warburg in the 1920s and popularized in the 1950s. Presently, it is well recognized that over sixty percent of all cancers are glycolytic. The Warburg effect or phenomenon is discussed, and the molecular understanding of some of Warburg's statements is provided with regard to modern knowledge of carcinogenesis. In addition, molecular explanation of aerobic glycolysis of the cancer cell is detailed. Other metabolic alterations of the cancer cell including glutaminolysis and lipogenesis are discussed. The altered citrate metabolism specifically associated with malignant transformation of peripheral zone prostate epithelial cells, and the potential clinical applications of such metabolic alterations conclude this chapter.

2.1 Introduction

> Cancer cells originate from normal body cells in two phases. The first phase is the irreversible injury of respiration.
>
> - Otto Warburg (1956)

One of the main distinguishing features between the normal and cancer cell is in their intermediary metabolism. Cancer cells have an altered metabolism, a characteristic that was recognized decades ago by Otto Warburg, for which initial molecular explanation was offered by Peter L. Pedersen and others. These bioenergetics and metabolic features do not only permit cancer cells to survive under adverse conditions such as hypoxia, but enable their proliferation, progression, invasiveness, and subsequent distant metastasis. Compared to normal cells, malignant transformation is associated with an increased rate of intracellular glucose import, and a higher rate of glycolysis associated with reduced pyruvate oxidation and increased lactic acid production. In addition, the cancer cell has increased gluconeogenesis, increased glutaminolytic activity, reduced fatty acid oxidation, increased de

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novo fatty acid synthesis, increased glycerol turnover, modified amino acid metabolism, and increased pentose phosphate pathway activity. These metabolic alterations are useful and have been explored for diagnostic, prognostic, and therapeutic targeting in cancer management. This chapter addresses some tenets of the Warburg phenomenon or effect in regard to current scientific understandings of cancer biology. Molecular explanations of the Warburg effect are examined, as well as other different cancer cell metabolic changes. Finally, the unique metabolism of prostate cancer (PCa) cells and the clinical utility implications are addressed. Uncovering the intricate and myriad mechanisms employed by the cancer cell to achieve these metabolic switches also holds tremendous companion diagnostic and therapeutic opportunity.

2.2 Bioenergetics of Normal Cells

The metabolism of the basic energy substrates (carbohydrates, proteins, and lipids) in normal cells generates several metabolic intermediates that are used to synthesize nucleic acids, nonessential amino acids, glycogen, and other biomolecules required for normal body functioning. Importantly, because energy is critical to all biologic processes, metabolism partly results in the production of acetyl CoA that is oxidized in the tricarboxylic acid (TCA) or Krebs cycle to produce reducing equivalents for energy production by the respiratory chain. Carbohydrates are broken down to glucose, which is taken up by cells and metabolized via the fundamental biochemical process known as glycolysis (Fig. 2.1). As well, glucose in the blood can arise from noncarbohydrate sources such as gluconeogenesis. Glucose enters the cell through specific glucose transporters. In the cytosol, several enzymes catalyze glycolysis that ends with the production of pyruvate and two molecules of ATP per glucose. Pyruvate enters the mitochondria and is converted to acetyl CoA by pyruvate dehydrogenase (PDH). Acetyl CoA condenses with oxaloacetate (OAA) to form citrate, which is completely oxidized in the TCA cycle to generate reducing equivalents for the respiratory chain. The respiratory chain produces ~34 more ATP molecules per glucose, bringing the total number of ATPs produced per complete oxidation of glucose to ~36, which accounts for over 90% of the energy requirements of the normal cell. Thus, in normal cellular intermediary metabolism, glycolysis, TCA cycle, and respiratory chain activities are tightly linked and regulated.

2.3 The Crabtree Effect

In 1926, Herbert G. Crabtree made an observation on the utility of carbohydrates by tumors (Crabtree 1926). He observed that, for normal cells, the presence of glucose slightly increased respiration or had no effect on oxygen consumption. On the contrary, glucose decreased oxygen uptake by tumor cells. This respiratory

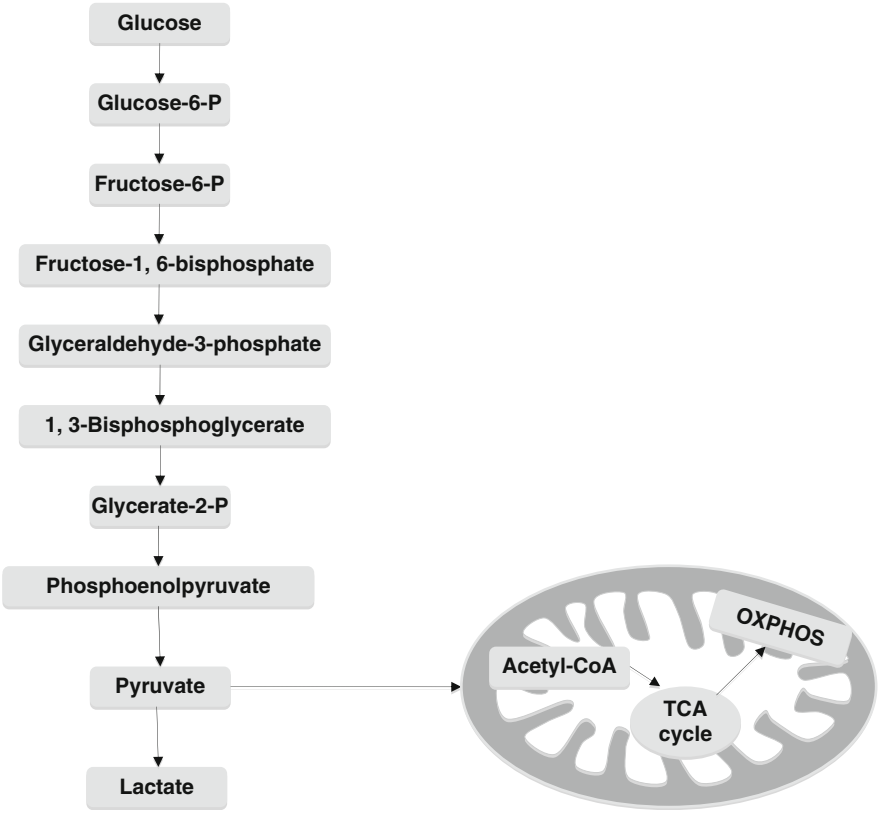

Fig. 2.1 *Glycolysis*. The sequence of reactions that produce pyruvate is known as glycolysis. Under anaerobic conditions, glycolysis produces minimal energy in the form of ATP. This mode of glycolysis or fermentation is usually not coupled to the TCA cycle and OXPHOS. However, under aerobic conditions, glycolysis, TCA cycle activity, and OXPHOS generate considerable energy for cellular functions

inhibition of cancer cells by glucose is called the Crabtree effect, named after Herbert Crabtree. It is now known that this metabolic transformation of cancer cells is not a specific feature of carcinogenesis, but appears to be a requirement of rapidly dividing cells such as proliferating thymocytes, spermatozoa, intestinal mucosal cells, renal cells, and embryonic stem (ES) cells (Wojtczak 1996). This phenomenon is also reported in bacteria and yeast. Apart from rapid proliferation, an important characteristic shared by all these cells, as will be expected from respiratory impairment, is a high rate of glycolysis. Another observation of the Crabtree effect is the initial increase in respiration following the provision of glucose. Indeed, it appears that other hexoses can induce this effect in cancer cells as well.

The mechanism of the Crabtree effect is not completely understood; however, several explanations have been provided. These include the following: (1) A competition between glycolysis and OXPHOS for available ADP + Pi can cause

respiratory impairment by increased glucose availability (Sussman et al. 1980). (2) Increased lactic acid production with decreasing cytosolic pH and inhibition of oxidative enzymes (Heinz et al. 1981). (3) Disruption of coupled respiration by calcium; increased mitochondrial calcium levels by glucose caused an increased association of the inhibitory subunits of F_1F_0 to the ATP synthase to inhibit coupled respiration (Wojtczak 1996). (4) Glucose metabolism increases ROS production that damages mitochondrial membranes and depresses respiration (Yang et al. 1997). (5) It is possible that the Crabtree effect might be regulated by multifaceted mechanisms in cancer cells, possibly involving changes in ATP/ADP ratio, Pi, glucose-6-phosphate, cytosolic pH among others (Rodriguez-Enriquez et al. 2001). Irrespective of the mechanism, cancer cells are glycolytic and this is associated with partial mitochondrial impairment (i.e., suppressed respiration, less oxygen consumption, and low ATP production).

2.4 The Warburg Phenomenon

While it can be conceived that fast-growing cancer cells will require more energy than normal cells, it is ironic to realize that, in contrast to normal cells, cancer cells use a primitive inefficient reaction, aerobic glycolysis, to generate considerable amounts of their energy. A possible reason for this bioenergetic alteration is the requirement to produce other metabolic end products to support their rapid growth and proliferation under low oxygen tension, and the possible adaptation to evade death in toxic environments or due to the effects of cytotoxic agents.

Probably the most important treatise ever provided for mitochondrial dysfunction and its possible causative role of cancer is that provided several decades ago by the Nobel Laureate and Biochemist, Otto Warburg. In his series of experiments on respiration and metabolism of cancer cells, coupled with his in-depth analysis of reported works from other investigators at the time, by an approach reminiscent of what Watson and Crick employed in deciphering the DNA double helical structure, Otto Warburg was able to unwaveringly hypothesize that neoplastic transformation originated as a consequence of irreversible damage to mitochondrial respiration. Cancer cells are, therefore, compelled to rely on the inefficient glycolytic mode of ATP synthesis (2 ATPs/glucose), rather than respiration that produces substantially more ATP/glucose (~36 ATPs/glucose). Warburg observed that, in conditions of normal oxygen tension, normal cells produced most of their energy via mitochondrial respiration. In contrast, over 50% of cancer cell energy was generated in the cytosol via glycolysis, with the remainder from the mitochondrial respiratory chain. This bioenergetically inefficient glycolytic reliance of cancer cells for most of their energy production is not primarily due to lack of oxygen, because it operates even in the presence of adequate oxygenation. The bioenergetically inferior nature of glycolysis implies that cancer cells must adopt a mode of increased glucose import to meet their energy demands.

In his seminal presentation in German on May 25th 1955 to the German Central Committee for Cancer Control, which was later translated into English and published in Science, Otto Warburg made several important landmark observations with regard to the origin of cancer, as implied in the title of his publication *On the origin of cancer cells* (Warburg 1956). Extracts from this publication are presented below and discussed in the context of modern scientific understanding of the development and progression of cancer.

> The irreversible injury of respiration is followed, as the second phase of cancer formation, by a long struggle for existence by the injured cells to maintain their structure, in which a part of the cells perish for lack of energy, while another part succeed in replacing the irreversibly lost respiration energy by fermentation energy. Because of the morphological inferiority of fermentation energy, the highly differentiated body cells are converted by this into undifferentiated cells that grow wildly – the cancer cells.

The body of evidence in support of respiratory damage to cancer cells is currently overwhelming and compelling. Almost every cancer investigated to date demonstrates some components of mtDNA mutations, ranging from somatic single nucleotide mutations, polymorphisms, large-scale deletions, to content alterations, in association with varying levels of respiratory dysfunction and ROS production. Given that mitochondria contributes 13 subunits for four of the five respiratory chain complexes, significant heteroplasmic levels of functionally important mutations will inhibit or slow down respiratory chain activity of cancer cells. Severe damage to some cells should trigger apoptosis (which will be consistent with Warburg's statement *in which a part of the cells perish for lack of energy*).

Glycolysis, TCA cycle, and the respiratory chain are functionally tightly coupled. The TCA cycle is mainly regulated by substrate availability, and inhibited by product accumulation and other cycle intermediates. Loss of respiration will ultimately lead to the accumulation of reduced nicotinamide adenine dinucleotide (NADH) and other critical regulators of the TCA cycle such as OAA, succinyl CoA, and citrate. NADH and succinyl CoA inhibit citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate, which are TCA cycle rate-limiting enzymes. As well NADH inhibits PDH while citrate inhibits citrate synthase. Clearly then, respiratory damage will reduce pyruvate conversion to acetyl CoA and a generalized reduction in TCA cycle activity. Even in the presence of oxygen, glycolysis will become the obvious mode for the cancer cell to obtain sufficient energy for cellular functions (*while another part succeeds in replacing the irreversibly lost respiration energy by fermentation energy*).

> . . .fermentation – the energy-supplying reaction of the lower organisms – is morphologically inferior to respiration. Pasture said in 1876 in the description of these experiments, if there should arise in the mind of an attentive hearer a presentiment about the causes of those great mysteries of life which conceal under the words youth and age of cells. Today, after 80 years, the explanation is as follows: the firmer connection of respiration with structure and the looser connection of fermentation with structure.

Aging is undeniably associated with structural and functional decline in all tissues, but in humans, it is very evident in the skin as a loss of rigidity and elasticity,

which shows as wrinkles. Part of the reason that as we age we lose structure is because of apoptotic cell loss, presumably as a consequence of severe damage to the mitochondrial genome. Indeed, Harman and colleagues proposed several decades ago that the aging process is caused by ROS-mediated damage to macromolecules, giving birth to the free radical theory of aging (Harman 1956). A refinement of this theory by Miquel et al. (1983) led to the mitochondrial theory of aging. While there are several theories on the aging process, the free radical/mitochondrial theory is well studied and has achieved popularity. The basic tenet of this theory is the mitochondria serving as the source as well as target of ROS. The mitochondrial genome and structure can be damaged by ROS. Mitochondrial dysfunction, partly as a consequence of mtDNA mutations, is a hallmark of the aging process. Indeed, experiments have shown that cutaneous aging is associated with significant age-dependent differences in the mitochondrial functions of keratinocytes, and that the aged skin is functionally anaerobic (Prahl et al. 2008). Consistent with this recent observation in the skin is the statement by Warburg about *the looser connection of fermentation with structure*.

> The mysterious latency period of the production of cancer is, therefore, nothing more than the time in which the fermentation increases after a damaging of the respiration. This time differs in various animals; it is especially long in man and here often amounts to several decades, as can be determined in the cases in which the time of the respiratory damage is known – for example, in arsenic cancer and irradiation cancer.

Cancer is an aging disease and therefore, a majority of cancers are diagnosed in people after middle age. Indeed, if we live long enough, almost all of us will develop some form of cancer. Chemical carcinogens have characteristically lengthy periods, averaging decades in some cases, between exposure and appearance of first primary tumors. Carcinogens act via several mechanisms. However, they can covalently bind to DNA to form adducts and cause mutations in the genome. In many circumstances, these DNA adducts and mutations are repaired to maintain genomic integrity. Given the poor repair capability of the mitochondrial system, mutations will likely accumulate in this genome overtime after exposure. Nonetheless, chromosomal instability and mutations in oncogenes, tumor suppressor, and caretaker genes can occur and initiate cancer. But, the noted effects of chemical carcinogens in causing increased ROS production and apoptosis strongly implicate the mitochondrial respiratory injury in chemical carcinogenesis.

> The first notable experimental induction of cancer by oxygen deficiency was described by Goldblatt and Cameron, who exposed heart fibroblasts in tissue culture to intermittent oxygen deficiency for long periods and finally obtained transplantable cancer cells, whereas in the control cultures that were maintained without oxygen deficiency, no cancer cells resulted.

Intermittent oxygen exposure simulates ischemic-reperfusion, and in chronic states, can injure cells. Ischemia is associated with hypoxia, increased glycolysis, lactic acidosis, decreased intracellular pH, increased ROS production, and calcium signaling. Reperfusion following ischemia causes most of the cellular damage by exposing ischemic cells to acute oxygen burst, which can induce excessive

mitochondrial ROS production in association with the depletion of antioxidants. The massive ROS in mitochondria of such cells can inevitably cause mtDNA damage and impairment of respiration with subsequent mitochondrial depletion and initiation of tumorigenesis.

The events of ischemic reperfusion are well studied in organ transplants. Myocardial infarction is a major problem with heart transplants. Ischemic pre or postconditioning is usually performed to prevent or reduce the occurrence of myocardial ischemic injury. Ischemic preconditioning involves inducing short sublethal ischemic episodes interspersed with reperfusion prior to prolonged ischemic insult (Murry et al. 1986). On the other hand, ischemic postconditioning involves short ischemic episodes at the beginning of reperfusion (Zhao et al. 2003). Both manipulations are found to offer protection to ischemic injury via activation of the pro-survival PI3K/AKT signaling pathway (Tong et al. 2000; Mocanu et al. 2002; Hausenloy et al. 2005), probably through the reduction in the levels of the tumor suppressor protein, phosphatase and tensin homolog (PTEN) (Cai and Semenza 2005). It is worth noting that chronic activation of the PI3K/AKT signaling pathway can cause unwanted cellular growth and malignant transformation. Thus, could this be the mechanism by which Goldblatt and Cameron obtained their transplantable cancer cells via chronic intermittent oxygen deficiency?

> Rajewsky and Pauly have recently shown that the respiration linked with the grana can be destroyed with strong doses of X-rays, while the small part of the respiration that takes place in the fluid protoplasm can be inhibited very little by irradiation. Carcinogenesis by X-rays is obviously nothing else than a destruction of respiration by elimination of the respiring grana.

Ionizing radiation generates considerable amounts of ROS. Ogawa et al. (2003) found that ROS formation occurred immediately after cellular irradiation and continued for several hours, resulting in oxidative DNA damage. Perhaps, this high ROS production was due to mtDNA damage following irradiation. Excess ROS can trigger mitochondrial apoptosis; however, depending on the specific types and levels of ROS, some cells can evoke alternative survival pathways. Among the intracellular targets of ROS damage, the mitochondrial genome is more vulnerable. Importantly, the mitochondrial genome is packed with coding genes without introns; hence, damage easily involves coding genes, leading to respiratory impairment (*respiration linked with the grana*[1] *can be destroyed with strong doses of X-rays*). As cancer is a genetic disease, and the nuclear genome is less likely to sustain the major insults from ionizing radiation, the conclusion reached by Warburg that *Carcinogenesis by X-rays is obviously nothing else than a destruction of respiration by elimination of the respiring grana*, is quite interesting and compelling, and should not be viewed with skeptism.

> On the other hand, we have found that the fermentation of the body cells is greatest in the very earliest stages of embryonal development and that it then increases gradually in the course of embryonal development. Under these conditions, it is obvious – since

[1]Grana refers to mitochondria.

> ontogeny is the repetition of phylogeny – that the fermentation of body cells is the inheritance of undifferentiated ancestors that have lived in the past at the expense of fermentation energy

Recent works provide ample evidence in support of the fact that cancer cells are identical to their undifferentiated ancestors or ES cells, not only in their metabolism, but also in the molecular pathways they invoke. Cancer cells and normal human ES cells share similar properties such as self-renewal, pluripotency, and teratoma formation. In support of these characteristics, several cancer cells express molecular signatures that define the pluripotent state as well. These molecular markers include *POU5F1* (*OCT4*), *NANOG, SALL4, TDGF1 (CRIPTO), LECT1, BUB1, SOX2,* and *LIN28*. Indeed, Ben-Porath et al. (2008) demonstrated the preferential overexpression of genes characteristic of ES cells in histologically poorly differentiated tumors. In breast cancer, the ES-like gene signature expression was associated with poor outcome. Apart from these pluripotent markers, it is clear that several signaling pathways employed for embryonic tissue patterning are equally deregulated in cancer cells. These include myriads of signaling networks, including the HH, WNT, NOTCH, BMP, and FGF pathways. Therefore, Otto Warburg's statement that "it is obvious – since ontogeny is the repetition of phylogeny – that the fermentation of body cells is the inheritance of undifferentiated ancestors that have lived in the past at the expense of fermentation energy", is very interesting in the light of our understanding of ontogeny and oncology, and we are only beginning to understand the molecular underpinnings of these observations.

2.5 Molecular Basis of the Warburg Phenomenon

Advances in molecular biology continue to shed tremendous amount of light on the physiologic relevance of the Warburg effect to cancer biology. The well-established increased intake of glucose by cancer cells has found widespread clinical utility in the imaging of cancer using ^{18}(F)-Fluorodeoxyglucose (2-fluoro-2-deoxy-D-glucose – FDG) in positron emission tomography (PET) scans. A glucose analog, 2-fluoro-2-deoxy-D-glucose, is, therefore, taken up at higher concentrations in cells with increased glucose transport such as malignant cells. Intracellular FDG is phosphorylated by hexokinases (HK) to FDG-6-phosphate, which cannot be metabolized further in glycolysis. Thus, FDG-6-phosphate accumulates in the cell and the radioactive isotope fluorine-18 enables imaging of the cell before radioactive decay. The technology is used in the diagnosis, staging, and monitoring of several cancers, including lymphoma, melanoma, and cancers of the colon, breast, and lung. Increased glucose intake by cancer cells is associated with poor prognosis (Lopez-Rios et al. 2007). FDG-PET is also used for diagnosis of Alzheimer's disease. Apart from imaging, the emerging understanding of the molecular

networks controlling the Warburg effect is beginning to reveal exploitable molecular targets for cancer prevention and therapy. It is becoming more evident that aerobic glycolysis is an adaptive mechanism involving several coordinated pathways that maintain the phenotypic features of cancer cells, including survival in hypoxic conditions, metastatic propensity, and evasion from apoptosis. A few interconnected signaling pathways and dysfunctional mutations provide enough evidence for the glycolytic phenotype of cancer cells, and an overview of these events is provided in Table 2.1.

Table 2.1 Control of glycolysis in cancer cells

Regulatory factors	Function	Role in cancer cell	Glycolytic regulation
Hexokinase	Glycolytic enzyme; Converts glucose to glucose-6-phosphate	Expression is increased in several cancers	Rate-limiting enzyme and first to phosphorylate glucose once inside the cell. Enables more glucose entry into the cell via its concentration gradient
PI3K/AKT	Oncogene signaling pathway	Activated in cancer cells by several mechanisms, including *PTEN* mutations	Increases expression and plasma membrane clustering of glucose transporters Increases expression and activity of hexokinase II Promotes association of hexokinase with mitochondria Increases expression of phosphofructokinase
MYC and MondoA	Transcription factors		Induce expression of glycolytic genes such as hexokinase II, enolase, lactate dehydrogenase, and phosphofructokinase
HIF	bHLH transcription factors	Stabilized in cancer even under normoxic conditions	Increases expression of glucose transporters Induces expression of all glycolytic enzymes Induces expression of both lactate dehydrogenase and monocarboxylate transporter 4 Inhibits pyruvate dehydrogenase through activation of PDK1
P53	Tumor suppressor gene; guardian of the genome	Mutated in several cancers	Through loss of TIGAR and SCO2

2.5.1 HK and Glycolysis

The classical role of HK in providing an initial molecular explanation to aerobic glycolysis deserves special attention. The HK gene family comprises four isoforms named HK I–IV (reviewed in Mathupala et al. 2006; Pedersen 2007). All the HKs have structural and functional similarity but different kinetics with respect to substrate utility. HKs I–III are 100 kDa proteins with low Michaelis constants (Km) (~0.02–0.03 mM; i.e., they have high affinities for glucose even at very low glucose concentrations). On the contrary, HK IV, also known as glucokinase, is a 50-kDa protein with only one catalytic site, instead of two as in HK II. Therefore, it has a high Km (~5–8 mM) for glucose that is several hundred times that of HKs I–III. The HKs also differ in their regulatory properties, expression patterns, and subcellular localizations. HK IV is almost exclusively expressed by adult hepatocytes. On the other hand, HK II is silenced in many normal mammalian tissues, except in muscle, fat, and lung tissues where low amounts are expressed (Wilson 1997, 2003).

Work by several groups, including the Pedersen's laboratory that sought explanation for the Warburg's observations, provided the first critical molecular explanation of the Warburg effect. These groups demonstrated that an early metabolic event in carcinogenesis of liver and pancreatic cells was the increased expression of high affinity HK II, and to a lesser extent, HK I in association with the downregulation of HK IV (Rempel et al. 1994; Mathupala et al. 1997; Mayer et al. 1997; Pedersen et al. 2002). In the presence of ATP, HK II catalyzes the rate-limiting and committed step of glucose metabolism in the cell, that is, the ATP-dependent phosphorylation of glucose to glucose-6-phosphate. This reaction does not only create a concentration gradient for the influx of glucose into the cell but also dictates the fate of intracellular glucose. HK I and II directly interact with mitochondria at specific voltage-dependent anion channels (VDACs) (Bustamante and Pedersen 1977). VDACs and ANTs move adenine nucleotides between the mitochondrial matrix and the cytosolic compartments. The interaction of HK II with VDAC can serve dual functions; first, it can inhibit the functional role of VDAC in shuttling ATP from the inter-membrane space into the cytosol, and second, it can interfere with BAX/BAK interaction with VDAC and therefore, inhibit apoptosis. Thus, the elevated levels of HK II in hepatomas and other cancers could contribute to an increase in apoptotic resistance as well as a build-up of ATP at VDAC. The accumulated ATP can be harnessed by HK II to phosphorylate and therefore, commit glucose for metabolism inside the cell. A normal mitochondrial function should couple the glycolytic end product, pyruvate, under aerobic conditions, to the TCA cycle and respiratory chain for energy production. However, defects in the mitochondrial genome, or other signaling pathways in cancer cells that adversely affect mitochondrial respiration imply that the pyruvate produced by glycolysis will be converted to lactate, even in the presence of oxygen (the Warburg phenomenon: i.e., the irreversible damage to cancer cell respiration and therefore, the dependence of cancer cells on glycolysis for energy production even in the presence of adequate oxygen).

2.5.2 The PI3K/AKT Signaling Pathway and Glycolysis

Several oncogene signaling cascades are deregulated in cancer cells, and all appear to control altered metabolism (Table 2.1), apoptosis, and several other phenotypic features of cancer cells.

Phosphoinositide 3-kinase (PI3K) is a heterodimeric protein with two functional subunits, an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The PI3K signaling pathway is activated by prosurvival signals such as cytokines, growth factors, hormones, and oncogenic Ras. Upon activation by G protein-coupled receptors or tyrosine kinase receptors, the 85 kDa subunit interacts with phosphorylated tyrosine residues on the receptor through a Src-homology 2 (SH2) domain. The 110 kDa catalytic subunit then transfers a phosphate group to membrane phospholipids. Sequentially, this process involves the phosphorylation of phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P2)/PIP2 into a second messenger, phosphatidylinositol (3,4,5)-triphosphate (PtdIns(3,4,5)P3)/PIP3. The intracellular second messenger recruits and activates 3-phosphoinositide-dependent kinase 1 (PDK1) that activates protein kinase B (AKT/PKB), which then triggers downstream signaling events.

Protein kinase B/AKT is a serine/threonine kinase that was discovered as a cellular homologue of a viral oncogene. Protein kinase B is downstream of the PI3K signaling cascade, and is activated by phosphorylation. Possibly, the most frequent mode of PKB/AKT activation is through PTEN loss of function. The *PTEN* tumor suppressor is a negative regulator of PI3K. PTEN, which are ubiquitously expressed in cells, dephosphorylate PtdIns(3,4,5)P3 back to PtdIns(4,5)P2 and therefore, block PI3K/AKT signal activation. Heterozygous *PTEN* knockout mice develop numerous tumors. PTEN are mutated in a vast majority of human cancers, and thus, cause PI3K/AKT activation in the absence of growth factor receptor stimulation. Protein kinase B/AKT is amplified and activated in several cancers. Constitutive activation of PKB/AKT in cancer cells can occur indirectly via growth factor stimulation or amplification of the PI3K pathway.

Once phosphorylated and activated, PKB/AKT phosphorylate target intracellular substrates, such as CREB, E2F, NF-κB in the nucleus, and caspase 9, BAD, and GSK-3β in the cytosol. Protein kinase B/AKT has pleiotropic functions. This signaling pathway primarily promotes cell proliferation and survival. It also regulates glucose uptake and hexokinase activity as well as the maintenance of mitochondrial membrane potential. The functions of PKB/AKT are mediated by several downstream components of the signaling cascade. Importantly, PKB/AKT activates mTOR (mammalian target of rapamycin) that is deregulated in several cancers as well.

Protein kinase B/AKT pathway stimulates metabolic conversion of cancer cells toward aerobic glycolysis in several different ways. First, PKB/AKT signaling induces increased expression and plasma localization of glucose transporters, and this increases glucose import and consumption by cancer cells. Second, activated PKB/AKT stimulates increased expression and activity of HK II and

thus, phosphorylation of glucose, which in turn will facilitate more glucose entry into cells along its concentration gradient. Several growth factors promote association of HK with mitochondria by using PI3K/AKT/mTOR pathway. Finally, PKB/AKT signaling induces the expression of another glycolytic enzyme, phosphofructokinase.

Interestingly, PKB/AKT signaling can be activated by mitochondrial respiratory dysfunction (e.g., mtDNA mutations). Decreased respiration from mitochondrial dysfunction inactivates PTEN, leading to PKB/AKT activation. Mitochondrial DNA depletion also activated PKB/AKT signaling accompanied by glycolysis, increased invasiveness, and evasion from apoptosis/anoikis (Moro et al. 2008). Thus, apart from growth factor stimulation or amplification of PI3K and *PTEN* mutations or downregulation, loss of mitochondrial respiratory functions that are pervasive in cancers augments PKB/AKT activation.

2.5.3 The MYC Oncogene and Glycolysis

The MYC oncogene is a transcription factor that controls growth, proliferation, and death of cells via the regulation of several genes. MYC forms a complex with its partner, MAX, and the activated complex binds to E-box DNA motifs (canonical CACGTG, CATGTG) to induce transcription of target genes. Overexpression of MYC is demonstrated in several cancers.

MYC induces glycolysis in a number of ways. Overexpression of MYC increases the metabolism of glucose via the activation and expression of several glycolytic enzymes (Osthus et al. 2000). Hexokinase II, glyceraldehyde-3-phosphate dehydrogenase, enolase 1, pyruvate kinase, and lactate dehydeogenase are highly overexpressed in several cancers (Altenberg and Greulich 2004; Mathupala et al. 2006). MYC has been shown to bind genes-encoding glycolytic enzymes such as HK II, enolase, and lactate dehydrogenase A (Kim and Dang 2005). Lactate dehydrogenase converts pyruvate to lactate, a metabolic pathway that is very active in glycolytic cancer cells. Lactate dehydrogenase expression is directly induced by oncogenes such, as MYC, and indirectly by activation of HIF1α. The activity of MYC leads to increased ROS production that could damage mtDNA, impair respiratory chain activity, and thus, facilitate aerobic glycolysis via several established mechanisms.

Another transcription factor that functions similar to MYC in the regulation of glycolysis is MondoA. MondoA is a basic helix-loop-helix leucine zipper transcription factor that heterodimerizes with its partner, M1x (Billin et al. 1999). This complex shuttles between the mitochondrial outer membrane and the nucleus. It appears that the complex senses the mitochondrial energy requirements of the cells, and relays this to the nucleus to influence the required gene expression. In the nucleus, MondoA:M1x complex interacts with CACGTG E-box motifs to activate the transcription of target genes. Three key glycolytic enzymes, lactate dehydrogenase A, HK II, and 6-phosphofruto-2-kinase/fructose-2,6-bisphosphatase 3, are direct targets of MondoA:M1x complex (Billin et al. 2000; Sans et al. 2006).

2.5.4 *Hypoxia-Inducible Factor Pathway and Glycolysis*

The growth of cancer cells is associated with different levels of oxygen deficiencies. Because solid tumors grow rapidly, cells at the periphery will have access to vasculature and therefore, more likely to have adequate oxygenation. However, progressively deep into the tumor will be cells exposed to decreasing levels of oxygen that could range from hypoxia to even anoxia. The reason for the reduced oxygen is primarily due to poor blood supply from rapid growth that outstrips vascular supply. Indeed, as little as 300 cancer cells can induce hypoxia as a consequence of inadequate vascularization. One mechanism to survive these adverse conditions is the induction and stabilization of hypoxia-inducible factors (HIFs), which partly restore new blood vessels to the tumor.

The HIFs are transcription factors with basic helix-loop-helix and Per/ARNT/Sim (PAS) domains. They were first identified as factors that regulate increased expression of erythropoietin in response to hypoxia and hence, so named (Semenza and Wang 1992). The two family members, HIFα and HIFβ become active upon the formation of heterodimers. Hypoxia-inducible factor α has three subunits, namely, HIF1α, HIF2α, and HIF3α. Hypoxia-inducible factor 2α is ∼48% homologous to HIF1α, and is expressed and stabilized under hypoxic conditions as well (Tian et al. 1997). On the contrary, HIF3α is a dominant negative regulator of HIF because it dimerizes with HIF1β to form a transcriptionally inactive heterodimer, thus, reducing the activity of HIFs. Hypoxia-inducible factor β is an aryl hydrocarbon receptor nuclear translocator (HIF1β/ARNT) with two homologues, ARNT2 and ARNT3. All three HIF1β homologues are constitutively expressed and can heterodimerize with HIFα subunits (Maynard and Ohh 2004). Whereas both HIF1α and 2α are functionally active and can interact with hypoxia-responsive elements (HREs: canonical CCATG sequences) in gene promoters, it appears HIF1α preferentially induces HREs in glycolytic gene promoters (Hu et al. 2007).

2.5.4.1 Regulation of Hypoxia-Inducible Factor

Regulation by Hypoxia: Hypoxia-inducible factor 1α is stabilized under hypoxic conditions. Thus, in normoxia, the levels of HIF1α are low in cells because of ubiquitin-mediated proteasome degradation. In this process, the protein is first hydroxylated on proline 402 and 564 in the oxygen-dependent degradation (ODD) domains by HIF1α prolyl hydroxylases. The von Hippel–Lindau (VHL) protein products (Schofield and Ratcliffe 2005) in a complex with Cul-2, Elongin B, and Elongin C (Linehan et al. 2003) recognize the hydroxylated proteins. The VHL protein is an E3 ubiquitin ligase (together with NEDD8) that mediates the proteasome degradation of hydroxylated HIF1α. Under hypoxic conditions, HIF1α cannot effectively be hydroxylated by prolyl hydroxylases as their Km for oxygen is very high. Therefore, under such circumstances, HIF1α is stabilized and enters the nucleus to form an active transcription factor by heterodimerizing with HIF1β subunits. The heterodimeric complex then binds to HREs and induces the expression

of several genes, including those involved with glucose metabolism, angiogenesis, tumor invasion, and survival. In normal physiology, hydroxylation is the primary mode of HIF1 regulation. However, hypoxia and pseudohypoxia are hallmarks of fast-growing solid tumors. Cancer cell microenvironment can stabilize HIF1α via hydroxylation. It has recently become evident, that apart from hypoxia, the levels and activity of HIF1α can be increased under normoxic conditions by cancer cells (pseudohypoxia).

Regulation by Mutations in VHL Protein: VHL is a tumor suppressor gene and mutations in this gene are associated with the VHL syndrome where patients develop tumors in multiple organs, including the kidneys. VHL gene mutations in renal cell carcinomas cause constitutive stabilization of HIF1α and HIF2α even under normoxic conditions (Kaelin 2002) because of loss or degradation of these molecules. This leads to loss of tumor suppressor activity of VHL, and constitutive transcription of several tumor promoting target genes.

Regulation by Oncogene Signaling: Oncogene signaling is common in almost all cancers. Ras-MAPK pathway activation caused increased levels of HIF1α in several model systems (Sheta et al. 2001). Similarly, the expression of Src resulted in the accumulation of HIF1α in nomoxic conditions (Jiang et al. 1997), and the activation of PKB/AKT increases HIF1α protein translation by AKT/FRAP/mTOR pathway (Laughner et al. 2001; Plas and Thompson 2005). Finally, receptor tyrosine kinase signaling through MAPK/ERK1/2 can phosphorylate and activate HIF1α. Thus, an important function of activated oncogenic pathways in cancer is to promote tumor progression via HIF pathway activation.

Regulation by TCA Cycle Enzymes: Enzymes of the TCA cycle have been recently shown to provide a feedback mechanism to stabilize HIF1α. Mutations in succinate dehydrogenase and fumarate hydratase genes cause paragangliomas and leiomyomas, respectively (see Chap. 4). The functions of the two enzymes in the TCA cycle result in the generation of reducing equivalents for the respiratory chain. Therefore, loss of enzyme activity leads to reduced mitochondrial respiration and stalled TCA cycle activities that lead to the accumulation of succinate and/or fumarate, which can cause a condition known as pseudohypoxia (see Sect. 4.2.3). During hydroxylation of the proline residues in HIF1α, the prolyl hydroxylase converts α-ketoglutarate or 2-oxoglutarate and oxygen to succinate and carbon dioxide (Kaelin 2005). Accumulated succinate or fumarate can leak into the cytosol and inhibit the activity of HIF1α prolyl hydroxylases. Tumors with loss of fumarate hydratase activity are highly vascularized with increased HIF1α expression (Pollard et al. 2005a, b), and increased expression of glycolytic enzymes (Vanharanta et al. 2006). Succinate dehydrogenase mutations in tumors cause HIF pathway activation and gene expression profiling of pheochromocytomas with *SDHB, SDHD,* or *VHL* mutations reveals a pseudo-hypoxic HIF gene signature pattern (Dahia et al. 2005). Pyruvate and lactate have also been shown to stabilize HIF1α (Lu et al. 2005).

Regulation by Mitochondrial Reactive Oxygen Species: It has been suggested that mitochondrial ROS is an important signal that mediates HIF1α stabilization. Mitochondria may function as oxygen sensors and signal under low oxygen tension to stabilize HIF1α by releasing ROS into the cytosol (Simon 2006). Consistent with

this suggestion, ROS from complexes II and III have both been implicated in HIF1α stabilization (Guzy et al. 2005, 2008; Klimova and Chandel 2008). Hydrogen peroxide, in particular, can oxidize Fe^{2+} in the Fenton reaction to Fe^{3+} associated with the production of hydroxyl radical. Because Fe^{2+}, but not Fe^{3+} is a cofactor for HIF1α prolyl hydroxylases, decreased Fe^{2+} will decrease the activity of this enzyme and therefore, reduced hydroxylation of HIF1α for ubiquitination and degradation, leading to its stabilization.

2.5.4.2 Regulation of Glycolysis and Mitochondrial Functions by Hypoxia-Inducible Factor

HIFs contribute to the glycolytic phenotype of cancer cells in several ways (Fig. 2.2). There is more glucose in the blood than in cells; however, the intake of

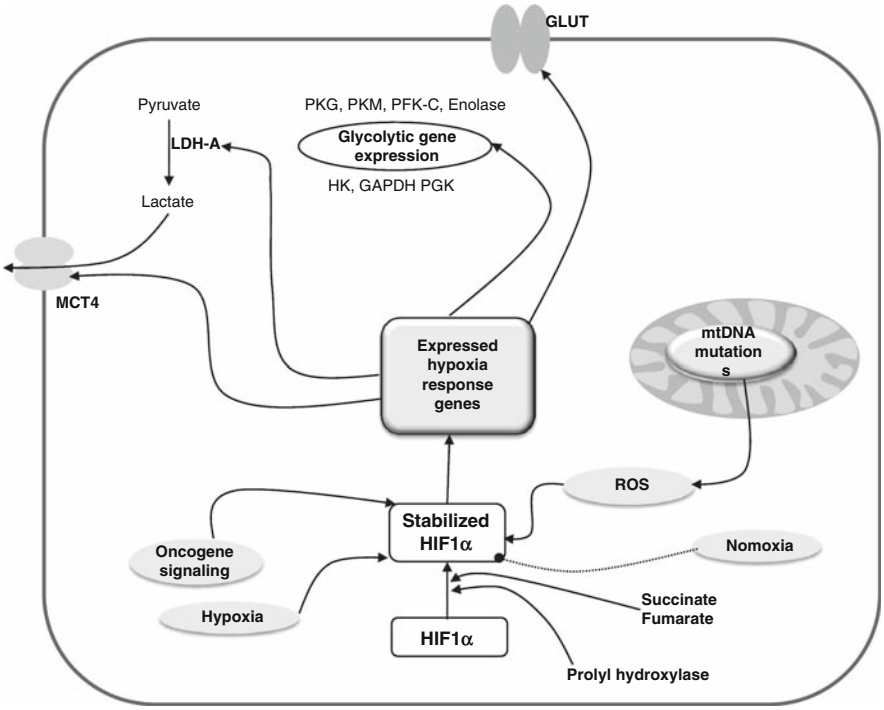

Fig. 2.2 *Regulation of glycolysis by HIF activity*. In nomoxia, HIF1α is hydroxylated, ubiquitinated, and destroyed. However, cancer-cell phenotype, such as hypoxia, oncogene signaling, and accumulation of tricarboxylic cycle intermediates (e.g., succinate and fumarate), stabilize HIF1α. Stabilized HIF1α has several glycolytic effects, including the activation of several glycolytic enzyme gene expressions. Other important genes induced by stabilized HIF1α are glucose transporters (GLUT) to import glucose into the cell, lactate dehydrogenase to convert pyruvate to lactase, and monocarboxylate transporter 4 (MCT4) to remove toxic lactate from the cell into the extracellular space

glucose into cells requires specific transporters. There are 13 members of the glucose transporter proteins; however, GLUT1 and GLUT3 appear to mediate most intracellular import of glucose because of their high affinity for glucose and the number of copies of these per cell (Brown 2000). Hypoxia-inducible factor 1 targets and induces GLUT1 and GLUT3 expression. Once in the cell, glucose is phosphorylated by HK to glucose-6-phosphate, the initial step of glycolysis. However, glucose-6-phosphate can adopt other fates in the cell. It can enter the pentose phosphate shunt to produce ribose for nucleic acid biosynthesis, or used to synthesize glycogen for storage or for a biosynthesis of structural glycoproteins. However, stabilized and activated HIF facilitates glycolysis by its induction of HK I and importantly, HK II, as well as all glycolytic enzymes, thereby biasing the metabolism of glucose-6-phosphate towards glycolysis.

Under normal cellular oxygenation, pyruvate from glycolysis enters the mitochondria and is converted to acetyl CoA by PDH. Acetyl CoA is metabolized in the TCA cycle to produce reducing equivalents for energy production by the respiratory chain. Because of impaired respiration of cancer cells, pyruvate and NADH accumulate in the cytosol. Stabilized HIF1 in cancer or hypoxic cells induces lactate dehydrogenase and plasma membrane monocarboxylate transporter 4 (MCT4). Lactate dehydrogenase converts pyruvate and NADH to lactate and NAD+. The lactate is then shuttled into the extracellular space by MCT4 while the NAD+ is fed back into glycolysis to be used by glyceraldehyde-3-phosphate dehydrogenase.

Hypoxia inducible factor 1 depresses mitochondrial respiration in addition to stimulating glycolysis. Indirectly, HIF1 inhibits PDH, the enzyme that catalyzes the irreversible conversion of pyruvate to acetyl CoA that is subsequently metabolized by the TCA cycle to produce NADH and $FADH_2$ for the electron transport chain. Pyruvate dehydrogenase kinase 1 (PDK1) is one of the four family members of protein kinases that phosphorylate and inactivate PDH (Patel and Korotchkina 2001), thereby attenuating the entry of carbon from pyruvate into the TCA cycle. PDK1 expression results in the inhibition of pyruvate dehydrogenase α (PDHα) subunit through phosphorylation. It is now well established that PDK1 is a direct target of HIF1 in cancer cells (Papandreou et al. 2006). Consistent with this finding, PDK1 is overexpressed in tumors in which HIF1 is stabilized (Koukourakis et al. 2005). Knockdown of PDK 1 with short hairpin RNA reduced PDHα phosphorylation and restored PDH activity. This treatment also reversed the Warburg effect, decreased normoxic HIF1α stabilization, reduced hypoxic cell survival, decreased cancer cell invasiveness, and inhibited tumor growth (McFate et al. 2008).

Oxygen insufficiency and/or depressed respiration will favor glycolysis as a means to obtain energy. Two examples illustrate this in cancer cells.

- In renal carcinomas with VHL mutations in which HIF1 is chronically stabilized, a different mechanism of modulating mitochondrial respiration is postulated. This mechanism involves the MYC oncogene. In normal cells, MYC dimerizes with MAX, and the active complex regulates increased expression of genes, including TFAM, which controls mitochondrial transcription and

replication (Li et al. 2005). In chronic hypoxia, HIF1 activates a different MYC family member, MAX interactor 1 (MXI1), a negative regulator of MYC. MAX interactor 1 displaces MAX and therefore, inactivates MYC, thereby reducing TFAM expression, mitochondrial biogenesis, and oxygen consumption (Zhang et al. 2007).

- Hypoxia-inducible factor 1 also regulates mitochondrial utilization of oxygen through its modulation of cytochrome c oxidase (COX) expression and activity. COX is the final respiratory chain complex that donates electrons to oxygen. Fukuda et al. (2007) demonstrated in mammalian cells that oxygen controls COX4-1 and COX4-2 expression. These two isoforms modulate COX activity differently. Indeed, in hypoxia, HIF1 induces the expression of COX4-2 isoform and mediates the destruction of COX4-1 by inducing the expression of LON, a mitochondrial protease that degrades COX4-1. It is suggested that COX4-2 enables more efficient utilization of limited oxygen in hypoxic conditions.

2.5.5 P53 and Glycolysis

In addition to its well-known functions, such as maintenance of genomic integrity, cell cycle arrest, and apoptosis, recent evidence suggests that p53 regulates mitochondrial respiration and glycolysis. Bensaad et al. (2006) identified TP53-induced glycolysis and apoptosis regulator (TIGAR) as a p53 target gene. In wildtype cells, TIGAR expression is normal and functions to decrease the levels of fructose-2, 6-bisphosphate, which suppresses glycolysis by diverting glucose-6-phosphate to the pentose phosphate pathway. Pentose phosphate pathway activation increases the levels of NADPH, which increases the levels of reduced glutathione to scavenge ROS. Therefore, in normal TIGAR expressing cells, there is an overall decrease in ROS. Loss of p53 and therefore, TIGAR expression, causes increased glycolysis at the expense of pentose phosphate pathway, as well as increased ROS production.

Matoba et al. (2006) also identified a synthesis of cytochrome oxidase 2 (SCO2) as a p53 downstream target. Expression of SCO2 was observed to mirror the levels of p53, decreasing in levels from p53+/+ to p53+/− to p53−/− cells. SCO2 is required for the assembly of COX complex. Interestingly, expression of SCO2 in p53−/− cells restored respiration and disruption of SCO2 in p53+/+ cells impaired respiration. Loss of p53 leads to loss of SCO2 expression and improper COX assembly and a consequent loss of respiration. Loss of respiration will force cells to depend on glycolysis for energy production, and also activate PKB/AKT, setting up an amplification loop for glycolysis.

Phosphoglycerate mutase (PGM) is a glycolytic enzyme that catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate. The activity of PGM is high in cancers of the lung, colon, and liver. Kondoh et al. (2007) demonstrated that p53 suppresses the expression of PGM. Therefore, loss of p53 in cancer cells will lead to increased PGM activity and enhanced glycolysis.

2.6 Glutamine Metabolism in Cancer Cells

Glutamine is the most abundant naturally occurring amino acid in the body. It can be synthesized from metabolic intermediates such as α-ketoglutarate and OAA. However, in catabolic conditions, such as injury or chronic illness, glutamine becomes conditionally essential and has to be ingested to meet the demands of the stressed body.

Glycolysis may function to meet the energy demands of the cancer cell, especially in hypoxic conditions. However, adaptive accelerated glutamine metabolism by cancer cells appears to provide substrates for increased lipogenesis and nucleic acid biosynthesis that are critical to the proliferative phenotype of the cancer cell (Fig. 2.3). Cancer cells exhibit increased glutamine intake and metabolism that far exceeds its role as the amino group donor for many biosynthetic pathways, including being the precursor for the synthesis of proline, ornithine, and arginine.

The majority (∼60%) of glutamine in the cancer cell is used to generate lactate and alanine in a reaction that involves the malic enzymes and hence, NADH

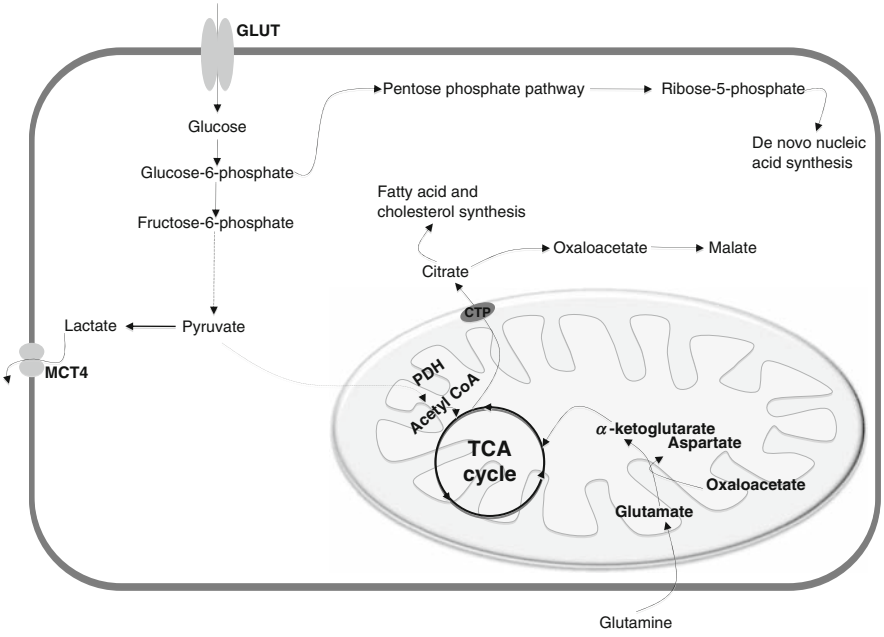

Fig. 2.3 *Increased glycolysis, glutaminolysis, and lipogenesis of the cancer cell.* Increased glucose uptake powers glycolysis, but because of the inefficient utilization of glycolytic end products by the cancer cell, pyruvate is converted to lactate that is removed from the cancer cell by the increased activity of MCT4. Cancer cells take in more glutamine that feeds the tricarboxylic acid (TCA) cycle leading to more citrate production. Citrate is transported into the cytosol mediated by citrate transport proteins (CTP). Cytosolic citrate is converted to acetyl CoA that supports lipid and cholesterol biosynthesis

production. NADH is a critical requirement for lipogenesis. Glutamine metabolism also generates OAA via an anaplerotic reaction (DeBerardinis et al. 2007). The OAA feeds into the TCA cycle and is used to produce citrate. Because, the citrate cycle is truncated in cancer cells, citrate is exported into the cytosol via the activity of citrate transport proteins (CTPs). To augment the transport process, the activity of CTP is increased in cancer cells. Cytosolic citrate is used for lipogenesis. Thus, glutamine metabolism provides both NADH and citrate for increased lipogenesis of the cancer cell. Glutamine carbon in the TCA cycle has been detectable in aspartate, which can be used to synthesize nucleotides, aspartate, and arginine (DeBerardinis et al. 2007).

MYC regulates the transcription of genes involved in mitochondrial glutamine metabolism, independent of PI3K/AKT signaling pathway (Wise et al. 2008). Glutamine metabolism was adequate to supply energy for cancer cells, in association with the reduced utilization of glucose in the TCA cycle as well as glucose for mitochondrial-dependent synthesis of phospholipids (Wise et al. 2008).

2.7 Lipid Metabolism in Cancer Cells

In addition to aerobic glycolysis and increased glutaminolysis, cancer cells have altered lipid metabolism and elevated de novo fatty acid biosynthesis. Studies have shown that irrespective of the concentration of extracellular lipid, fatty acids in cancer cells are mainly synthesized de novo. The reason for this is unclear, but could include perpetuation of the malignant phenotype such as increased proliferation and evasion from apoptosis.

Cytosolic acetyl CoA for lipogenesis appears to come from at least two sources. First, in the TCA cycle, mitochondrial acetyl CoA condenses with OAA to form citrate that can enter the cytosol where it is converted back to acetyl CoA for lipogenesis. Perhaps, the cancer cell adapts increased glutaminolysis in order to accelerate citrate production via the supply of OAA generated in an anaplerotic reaction. Second, lipogenesis can depend on uptake by cancer cells of acetate from blood. In the cell, acetate is converted to acetyl CoA by cytosolic acetyl coenzyme A synthase. In support of this source of substrate for lipogenesis, plasma acetate levels were found to be lower in cancer patients compared to healthy control subjects (Pomare et al. 1985).

Lipogenesis is established in cancer cells as a result of increased expression and activity of a number of lipogenic enzymes, including fatty acid synthase (FASN), ATP citrate lyase, acetyl CoA carboxylase α (ACCα), and Spot14. FASN was first identified as breast cancer-associated protein OA-519. It is overexpressed in a variety of other cancer cells. FASN is a multifunctional enzyme that catalyzes the synthesis of palmitate from acetyl CoA and malonyl coenzyme A. This is a major enzyme that mediates the anabolic synthesis of fatty acids from carbohydrates in cancer cells. FASN is mainly expressed in normal hepatocytes where it mediates the de novo synthesis of lipids for export to adipose tissues for storage or to actively

metabolizing tissues for use in energy production. Many normal tissues rely on circulating lipids and hence, do not express FASN. Many cancers, especially aggressive tumors constitutively express very high levels of FASN. Meanwhile, expression of FASN is an early event in carcinogenesis, and the levels mirror disease progression from early to late stage cancers, and correlates with cancer prognosis.

A complex regulatory network controls the expression of FASN. It is well established that steroid hormones induce its expression in hormone-dependent prostate, breast, and endometrial cancer cells, and this appears to involve MAPK, MEK1/MEK2, and PI3K/AKT signaling pathways. The PI3K/AKT pathway plays an important role in lipogenesis. Activated PKB/AKT signaling stimulates lipogenic gene expression via activation and nuclear localization of the lipogenic transcription factor, sterol response element binding protein-1. Furthermore, activated PI3K/AKT pathway stimulates fatty acid synthesis by a direct activation of ATP citrate lyase and inhibition of β-oxidation by downregulating the expression of CPT1A. FASN expression activates PKB/AKT in a subset of thyroid cancers and inhibition of FASN suppresses growth and induces cancer cell death (Uddin et al. 2008).

ACCα is a rate-limiting enzyme that catalyzes the formation of malonyl CoA from acetyl CoA and carbon dioxide. ACCα is highly expressed in breast and PCa cells, and when inhibited, results in a marked reduction in lipogenesis and induction of apoptosis. Spot14 regulates several lipogenic enzymes, including ACCα and FASN. Spot14 levels in breast cancer correlate with invasive phenotype and poor outcome.

2.8 Citrate Metabolism by Prostate Glandular Epithelial Cells

The intermediary metabolism of the prostate gland is uniquely different from other body cells. PCa development is associated with an early metabolic switch from citrate secreting normal epithelial cells to citrate oxidizing cancer cells. Lower zinc levels in the transforming cells as a consequence of decreased expression of zinc transporters mediate this metabolic switch. In view of these observations, Leslie C. Costello and Renty B. Franklin have advocated for the exploitation of this metabolic transformation for the early detection, diagnosis, chemoprevention, and treatment of PCa. The altered metabolism of PCa cells is summarized here. For an in-depth discussion on this topic, see the numerous reviews by L. C. Costello and R. B. Franklin (Costello and Franklin 2000, 2006; Costello et al. 1999, 2004, 2005).

Benign Prostate Epithelial Cells: The intermediary metabolism of the human prostate gland is not identical in all glandular epithelial cells because of their different embryonic sources (Costello and Franklin 2000). According to McNeal, the prostate gland can be divided into three zones. The part that surrounds the proximal urethra is called the transition zone, and accounts for 10% of all PCas. The central zone surrounds the transition zone and extends to the angle of the urethra

and base of the bladder. Only ~5% of all PCas originate from this region. The bulk of the prostate gland consists of the peripheral zone, which contains ~75% of prostatic glandular tissue. Consistent with this histology, the peripheral zone is the site of most prostate malignancies and the part of the gland that is metabolically important. The epithelial cells in the peripheral zone are highly specialized citrate secreting cells. In contrast to cells in the central zone, the peripheral zone cells accumulate very high levels of zinc (3–10-fold higher) (Costello and Franklin 2000). This is due mainly to the normal activity of the zinc uptake transporter in these cells (Franklin and Costello 2007). The presence of high zinc levels in the mitochondria of the peripheral zone cells inhibits the activity of mitochondrial aconitase (m-aconitase), thereby blocking citrate oxidation in the Krebs cycle. The accumulated citrate is secreted in the prostatic fluid. Thus, normal prostate glandular epithelial cells have truncated Krebs cycle, low citrate oxidation, low respiration, and are therefore, bioenergetically inefficient (~60% less ATP production compared to PCa cells).

PCa Cells: Apart from the prostate gland, normal epithelial cells completely oxidize citrate in the TCA cycle to produce reducing equivalents for energy production in the respiratory chain. On the other hand, most tumor cells demonstrate the Warburg effect (i.e., bioenergetically inefficient aerobic glycolysis). Therefore, tumorigenesis, in general, is associated with a metabolic switch from energy-efficient benign cells to energy-inefficient tumor cells. On the contrary, and indeed very intriguingly, an early metabolic switch from rather energy-inefficient benign cells to energy-efficient tumor cells marks the malignant transformation of peripheral zone prostate glandular epithelial cells. An early indication of this metabolic alteration is the downregulation of the gene that encodes the zinc uptake transporters in the transforming epithelial cells (Desouki et al. 2007). This event leads to a dramatic reduction in zinc levels (70–80% lower than that in normal peripheral zone cells) in these cells. The low zinc levels in the mitochondria of cancer cells remove the inhibition of zinc on m-aconitase and therefore, enable the complete oxidation of citrate through the TCA cycle. This metabolic pathway generates 36 ATP/glucose metabolized, which is over two times higher than that of the normal peripheral zone cells that only produce ~14 ATPs per glucose metabolized. Thus, malignant prostate cells completely oxidize citrate and hence, generate more energy than their benign counterparts. Indeed, citrate levels in PCa cells are significantly lower than in benign epithelial cells. It should be noted that part of the citrate in these transforming cells is used for lipogenesis, which is a necessary requirement of rapidly dividing cells.

2.9 Clinical Implications of Altered PCa Metabolism

PCa is the most commonly diagnosed malignancy in men. However, mortality is low compared to many other cancers. One reason for the low mortality is that PCa is a slow-growing tumor, and importantly, it is completely curable when diagnosed

early. Because of the indolent nature of PCa, it is more frequently diagnosed in men over the age of 50 years, although autopsy series have revealed the presence of this tumor in men as young as 20 years of age. The long latency of PCa development provides an adequate window for early detection and chemoprevention. The metabolic transformation of PCa cells is an early event in PCa evolution. hZIP gene downregulation and therefore, derepression of the activity of m-aconotase to oxidize citrate, are all observed in the very early stages of PCa development. The metabolic transformation can be targeted in imaging and/or biochemical assay development for the detection and management of PCa. They also have a tremendous implication in the treatment and prevention of PCa.

2.9.1 Diagnostic Imaging of PCa

Accurate local cancer staging, intraprostatic localization, and assessment of tumor volume, are all important factors considered in making PCa treatment decisions. Currently, a combination of clinical parameters, such as the extent of disease on biopsy, number of positive cores, Gleason score, and serum PSA, are used in the initial assessment of patients. Conventional pelvic imaging using MRI/CT scans are also useful, but they are insensitive and costly procedures.

The dramatic decrease in citrate levels observed in PCa is associated with increased choline and creatine levels. Importantly, these changes are not observed in normal prostate or benign prostatic hyperplasia. Magnetic resonance spectroscopy is an imaging modality that uses strong magnetic field to assess metabolites in tissue. This technique is developed to obtain the metabolic information of citrate and choline, and the citrate–choline ratio has served as a sensitive detection method of PCa. The metabolic spectra can be coupled with MRI to give precise in situ anatomic and metabolic map of the prostate for accurate intraprostatic localization and assessment of tumor volume. Currently, endorectal MRI/MRSI are standardized and used to improve PCa diagnosis and staging (Jung et al. 2004).

Compared to TRUS-guided prostate biopsy for cancer detection, endoMRI/MRSI was more sensitive in tumor detection. In a study involving 42 men with at least two previous negative biopsies, 31 of them had metabolic abnormalities reminiscent of PCa that was revealed by endoMRI/MRSI (Prando et al. 2005). Umbehr et al. (2008) performed a systemic review and meta-analysis of MRI/MRSI in PCa diagnosis. They found that for men suspected of PCa, the sensitivity and specificity of MRI/MRSI were 82 and 88%, respectively. Importantly, in low-risk patients, MRS/MRSI was not sensitive, but was highly specific (91%) at detecting PCa. This finding is probably because MRI/MRSI can detect tumors that are 0.5 cm or larger, and is better at detecting Gleason score 8–10 tumors than lower Gleason score tumors (Zakian et al. 2005).

2.9.2 Screening for PCa Using Biofluids

The screening for early detection of PCa still relies on PSA and digital rectal examination of men 50–70 years. However, the PSA test is not specific, because serum PSA is also elevated in other nonmalignant prostatic conditions such as prostatitis and benign prostatic hyperplasia. Therefore, there has been a need to identify and validate accurate biomarkers for PCa screening. Yet, no such biomarker exists at present. The specific PCa-associated metabolic transformations appear very attractive for an accurate detection of PCa using biofluids such as PMF or first-catch postDRE urine (Costello and Franklin 2009). The normal PMF citrate (~40–150 mM) and zinc (~8–10 mM) levels are several folds higher than in PCa (citrate; ~0.2–2 mM, and zinc; ~0.4–0.8 mM). These figures strongly suggest that biochemical assays developed and validated for PCa screening using these metabolites should increase sensitivity and specificity over PSA and possibly other emerging biomarkers.

2.9.3 PCa Prevention and Treatment

An early event in PCa development is hZIP downregulation leading to low zinc levels and hence, increased activity of m-aconitase and citrate oxidation. Another important role of zinc is the induction of apoptosis in PCa cells. Zinc treatment of PCa cells increases the BAX/BCL-2 ratio leading to BAX-mediated cytochrome c release and induction of apoptosis via caspase activation (Feng et al. 2008; Franklin and Costello 2009). Thus, can restoration of zinc in premalignant cancer cells reverse them to normal states or will repopulation of PCa cells with normal zinc levels eliminate them via apoptosis? There are models to increase zinc levels in PCa cells.

- The simplest approach will be to increase zinc levels in the extracellular milieu of prostate cells through zinc supplementation. Unfortunately, the epidemiologic literature does not support the basic science findings. In some studies, high doses and prolonged zinc supplementation have been associated with an increased risk of PCa. An Italian study suggested that increased zinc intake adversely influenced the risk for developing advanced PCa (Gallus et al. 2007). However, in other reports, moderate to low levels of zinc, especially in the elderly, is effective against PCa. Gonzalez et al. (2009) showed that the risk of developing significant PCa was reduced with increased zinc intake. Larger and well-designed studies on the effect of zinc in PCa are therefore, necessary to clarify its role in PCa prevention.
- Reactivate the expression of the zinc transporters. This approach is currently not possible because more work needs to be done to uncover the mechanisms by which these transporters are shut down in PCa.

- Because one of the major aims is to inhibit citrate oxidation by m-aconitase, the activity of this enzyme can be targeted in PCa cells. Inhibiting its expression is probably inappropriate to reduce the activity of the enzyme, because protein levels do not account for the decreased enzymatic activity in normal prostate cells (Singh et al. 2006). Hence, an effective agent should directly target the enzymatic activity of m-aconitase. Fluoroacetate can inhibit m-aconitase, but it will need PCa-specific targeting as it is toxic to normal cells.

2.10 Conclusion

The cancer cell metabolism is unquestionably altered. The initial theories of Warburg and his contemporaries are being unequivocally explained by modern science. Not only is the cancer cell glycolytic, but also this metabolic phenotype is intricately linked to other metabolic conversions such as increased de novo lipid synthesis and glutaminolysis. Some of these metabolic changes continue to be invaluable in clinical diagnostics, and are opening up targets for chemotherapy. Part of the reason we are still incapable of fighting cancer successfully is because of our incomplete knowledge on the metabolic changes of the cancer cell. The future should establish a complete metabolic and metabolomic map of the cancer cell. Undoubtedly, such developments will prove very useful in the diagnostic and selective elimination of cancer cells from the human body.

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