

## Review

## New aspects of the Warburg effect in cancer cell biology

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## ABSTRACT

Altered cellular metabolism is a defining feature of cancer [1]. The best studied metabolic phenotype of cancer is aerobic glycolysis – also known as the Warburg effect – characterized by increased metabolism of glucose to lactate in the presence of sufficient oxygen. Interest in the Warburg effect has escalated in recent years due to the proven utility of FDG-PET for imaging tumors in cancer patients and growing evidence that mutations in oncogenes and tumor suppressor genes directly impact metabolism. The goals of this review are to provide an organized snapshot of the current understanding of regulatory mechanisms important for Warburg effect and its role in tumor biology. Since several reviews have covered aspects of this topic in recent years, we focus on newest contributions to the field and reference other reviews where appropriate.

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## 1. Introduction

## 1.1. History of the Warburg effect

In the 1920s, Otto Warburg made his seminal observation that tumor cells metabolize more glucose to lactate than normal cells. By directly measuring lactate production and oxygen consumption

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**Table 1**  
Applications for FDG-PET in selected cancers.

Cancer	<sup>18</sup> F-FDG phenotype	Applications
Lung cancer*	High	D, S, RS, TM
Breast cancer**	High	D, S, RS, TM
Colorectal cancer***	High	D, S, RS, TM
Melanoma	High	S, RS, TM
HG-non Hodgkin lymphoma	High	S, RS, TM
LG-non Hodgkin lymphoma	Low to moderate	S, RS, TM
Hodgkin's lymphoma	High	S, RS, TM
Endometrial cancer	High	S, RS, TM
Soft tissue and bone sarcoma	High	S, RS, TM
Hepatocellular carcinoma****	Low to high	Not established
Renal cell carcinoma	Moderate	Not established
Pancreatic cancer***	Variable	Not established
Primary prostate cancer	Low	Not established
Metastatic prostate cancer	High	Not established
Bladder	High	Not established

*Exceptions and Abbreviations:* Low FDG phenotype in \*Brochoalveolar CA, \*\*lobular breast CA, \*\*\*mucin producing variants, \*\*\*\*grade-dependent; D = diagnosis, S = staging, RS = restaging, TM = therapy monitoring, HG = high grade, LG = low grade.

rates in thin slices of rat liver carcinoma and normal liver tissue, Warburg and colleagues found that normal liver tissue exhibited the Pasteur effect (inhibition of lactate production in the presence of oxygen), whereas tumor tissue maintained lactate production regardless of oxygen tension [2,3]. Sustained lactic acid production in the presence of oxygen was also observed in several human carcinomas. Warburg determined that cancer tissue consumes ten-fold more glucose than can be accounted for by respiration, and the amount of lactic acid produced is two orders of magnitude greater than the amount produced by normal tissue [2]. For a detailed description of Dr. Warburg's life and research, we direct the reader to an eloquent recent review by Dang and colleagues [4].

### 1.2. Translating the Warburg effect to the clinic: usefulness of FDG-PET imaging for cancer patients

FDG-PET (2-deoxy-2-[<sup>18</sup>F]fluoro-D-glucose positron emission tomography) is a molecular imaging technique that exploits cancer cells' preferential utilization of aerobic glycolysis. Similar to glucose, FDG enters cancer cells via glucose transporters GLUT1 and GLUT3 and is subsequently phosphorylated by hexokinase to FDG-6-phosphate. While glucose-6-phosphate undergoes further isomerization to fructose-6-phosphate in the glycolytic pathway or oxidation to 6-phosphogluconolactone in the pentose phosphate pathway, FDG-6-phosphate cannot be further catabolized due to lack of an oxygen atom at the C-2 position. FDG-6-phosphate is unable to diffuse out of cells, and the rate of dephosphorylation occurs slowly; therefore, it becomes trapped and accumulates at a rate proportional to glucose utilization. Thus, FDG uptake depends on both glucose transporter expression and hexokinase activity and provides a way to assess glucose uptake rates in cells [5]. Tumors that take up more glucose than surrounding tissues can be non-invasively visualized in cancer patients by FDG-PET.

FDG-PET has been used in cancer patients since the 1980s and is now a widely used clinical imaging tool in oncology. It is approved for disease diagnosis, staging, restaging, and therapy monitoring in many but not all cancers [5]. Table 1 shows a current list of select cancers for which FDG-PET has established applications [6]. Despite its widespread use in clinical oncology, some cancers remain difficult to image by FDG-PET, such as hepatocellular carcinoma, prostate cancer, and pancreatic cancer. It is possible that these tumor types do not exhibit the Warburg effect metabolic phenotype and instead rely on alternative carbon sources than glucose to fuel proliferation. Alternatively, these tumor types may be difficult to image by FDG-PET for imaging-related reasons such as poor perfusion of the tumor by the probe, low density of tumor cells in

the tumor tissue (high stromal cell to tumor cell ratio) or high background signal (such as bladder signal in the case of prostate cancer). Some cancers, such as well-differentiated hepatocellular carcinoma, exhibit high expression levels of glucose-6-phosphatase, which by dephosphorylating FDG-6-phosphate, allows efflux of FDG from cancer cells [7]. Further work is needed to address these issues in FDG-PET negative cancers. However, the large number of tumor types for which FDG-PET has proven utility has helped spur interest in the Warburg effect and its regulation and role in tumor biology.

## 2. Regulation of the Warburg effect

Accumulating evidence indicates that every major oncogene and tumor suppressor can affect metabolic regulation. However, the molecular mechanisms by which the cancer metabolic phenotype is accomplished by oncogenes and tumor suppressors in different tumor types varies. Below we outline some of the better characterized mechanisms by which the Warburg effect is established in cancer cells. This topic was also recently reviewed by Mak and colleagues [8].

### 2.1. Transcriptional regulation of the Warburg effect

One well-defined mechanism by which cancer cells establish the Warburg effect is via transcriptional upregulation of glycolytic enzymes. Elevated expression of glucose transporters and glycolytic enzymes are found in numerous cancers and may contribute to tumor progression [9–11]. The hypoxia-inducible factor 1 (HIF1) transcription factor complex, a heterodimer comprised of HIF1 $\alpha$  and constitutively expressed HIF1 $\beta$ , upregulates expression of glucose transporters and most of the glycolytic enzymes [12]. HIF1 also upregulates expression of pyruvate dehydrogenase kinases (PDKs), which phosphorylate and inactivate the mitochondrial pyruvate dehydrogenase complex, the enzyme that controls entry of glucose-derived pyruvate into the tricarboxylic acid (TCA) cycle [13,14]. Under normoxia in non-transformed tissues, HIF1 $\alpha$  is constantly degraded via oxygen-dependent hydroxylation by prolyl hydroxylases which results in recognition by the E3 ubiquitin ligase von Hippel-Lindau tumor suppressor (VHL). In hypoxia, HIF1 $\alpha$  is stabilized, allowing HIF1 to modulate metabolic adaptation to low molecular oxygen levels. Under these conditions, HIF1 increases cellular glycolysis while simultaneously reducing pyruvate flux into the TCA cycle, oxidative phosphorylation rates, and oxygen consumption [15]. Importantly, stabilization of HIF1 $\alpha$  protein is a common occurrence in tumors even under non-hypoxic conditions, and upregulation can be caused by enhanced transcription downstream of the PI3K/AKT/mTOR pathway. Therefore, loss-of-function mutations in the machinery that regulates HIF1 $\alpha$  degradation, such as in the tumor suppressor VHL, as well as oncogenic activation of PI3K/Akt signaling can lead to increased HIF1 activity and capacity for glycolysis in cancer cells. See a recent review by Semenza for more details on HIF1 regulation and impact on cancer metabolism [12].

The MYC oncogene is another important transcriptional regulator of the metabolic phenotype in cancers. A recent study found that MYC overexpression is the molecular alteration most associated with high FDG uptake in human breast cancer [11]. Among the thousands of genes upregulated by MYC are several glucose transporters and glycolytic enzymes, as well as PDK1 and lactate dehydrogenase A, which enhance the Warburg effect by increasing flux of glucose through the glycolytic pathway while attenuating entry of pyruvate into the TCA cycle [16–18]. MYC also activates transcription of genes involved in glutamine metabolism, such as glutamine transporters and glutaminase-1, which converts

glutamine to glutamate [19]. Glutamate can be converted to alpha-ketoglutarate and enter the TCA cycle, thus providing the carbons for TCA intermediates important for fatty acid and amino acid biosynthesis. However, glutamate can also be directly converted to glutathione and contribute cellular redox state maintenance. Regulation of cancer cell metabolism by MYC is an active area of study recently reviewed [20].

The tumor suppressor p53, best known for its role in the DNA damage response and apoptosis, is another important transcription factor that regulates metabolism. p53 suppresses glycolysis by elevating expression of TP53-induced glycolysis and apoptosis regulator (TIGAR), a fructose-2,6-bisphosphatase [21]. Additionally, p53 promotes oxidative phosphorylation by enhancing expression of synthesis of cytochrome c oxidase-2, which is required for assembly of the cytochrome c oxidase complex of the electron transport chain [22]. Therefore, loss of p53 expression in tumor cells may facilitate the Warburg effect by simultaneously increasing flux through glycolysis and decreasing oxidative phosphorylation. For a thorough review of p53's role in metabolism (see a recent review by Vousden and Ryan [23]).

It will be of interest to determine whether other transcription factors known to affect glucose metabolism in non-transformed tissues contribute to aerobic glycolysis in cancer. For example, the orphan nuclear receptor estrogen-related receptor alpha (ERR $\alpha$ ), which regulates genes important for mitochondrial biogenesis and oxidative metabolism [24], was recently shown to be essential for increased glucose metabolism and proliferation during effector T cell activation [25]. Another recent study showed that *Drosophila* ERR drives a metabolic gene expression program that coordinates carbohydrate metabolism with proliferation during development [26]. Additionally, elevated ERR $\alpha$  expression correlates with poor clinical outcomes in breast cancer and compounds that antagonize ERR $\alpha$  activity reduce proliferation of breast cancer cells with heightened ERR $\alpha$  expression [27]. Further work is needed to delineate the role of ERR $\alpha$ , and other transcription factors that regulate metabolic gene expression, in promoting the Warburg effect metabolic phenotype in cancer.

## 2.2. Metabolic isoform switching contributes to the Warburg effect

Alternative isoforms of metabolic enzymes and transporter proteins have evolved over time to confer additional functions and mechanisms of regulation to benefit cell proliferation. Some of these alternative isoforms of glycolytic enzymes can be exploited by cancer cells to enforce the Warburg effect. One such example necessary for the Warburg effect is the splicing switch to the M2 isoform of pyruvate kinase (PKM2). Pyruvate kinase, active as a tetramer in cells, catalyzes the final rate-limiting step of the glycolytic pathway, conversion of phosphoenolpyruvate and ADP to pyruvate and ATP. Four different isoforms of pyruvate kinase are found in mammals: PKL, found in the liver and kidneys; PKR, found in erythrocytes; PKM1, found in many tissues including muscle and brain; and PKM2, found in self-renewing cells including embryonic and adult stem cells [28]. PKM2 is also expressed in tumor cells, and unlike its alternative splice variant, PKM1, promotes the Warburg effect metabolic phenotype and tumorigenesis [29]. A recent study by Luo et al. found that PKM2, but not PKM1, functions as a transcriptional coactivator of HIF1 [30]. They showed that PKM2 directly interacts with HIF1 $\alpha$  and enhances HIF1 binding and p300 recruitment to hypoxia gene response elements to promote transactivation of target genes. Hydroxylation of PKM2 by prolyl hydroxylase 3 enhances binding of PKM2 to HIF1 and its coactivator function. Also, PKM2 itself is a HIF1 target gene. Therefore, in addition to its role as a glycolytic enzyme, PKM2 promotes the Warburg effect by participating in a positive feedback loop that

enhances HIF1 activity and reprogramming of metabolism in cancer cells [30].

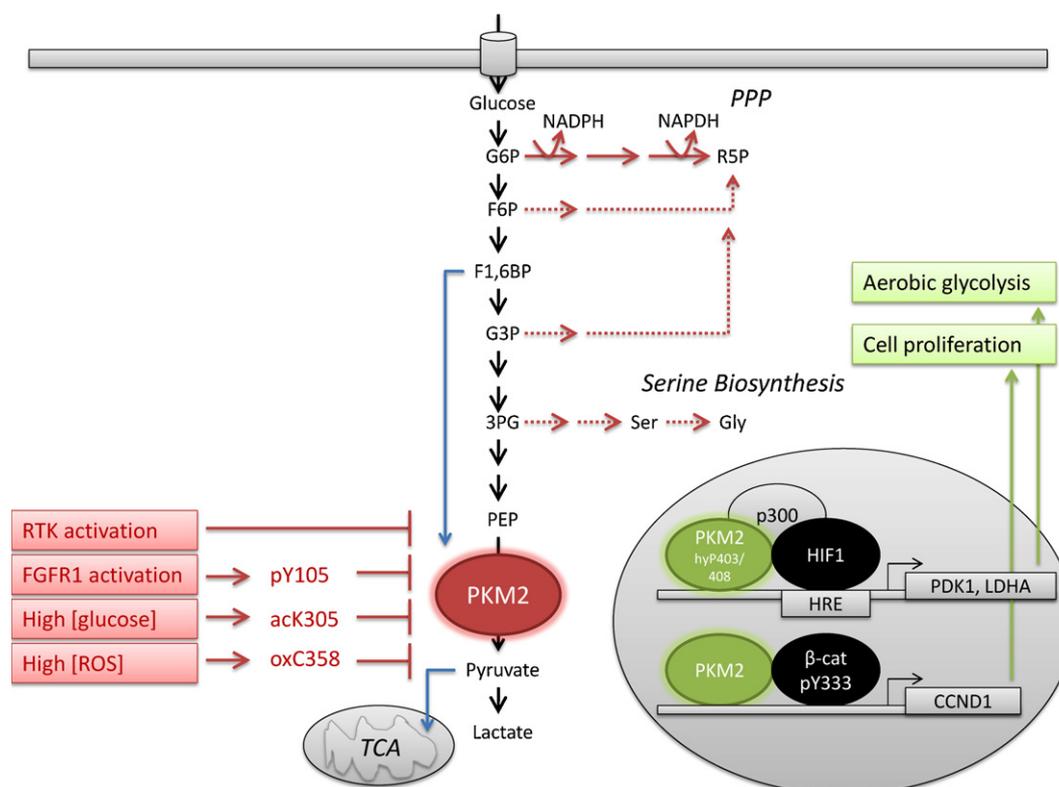
Unlike its splice variant PKM1, PKM2 is allosterically activated in a feed-forward regulatory loop by F1,6BP and is inhibited by growth factor signaling through interaction with tyrosine-phosphorylated proteins [31,32]. A recent study by Yang et al. identified a phosphotyrosine-dependent interaction of PKM2 with  $\beta$ -catenin [33]. They found that EGF-induced activation of c-Src results in phosphorylation of  $\beta$ -catenin at Y333 which enables PKM2 to bind  $\beta$ -catenin in the nucleus of cancer cell lines. Interaction of PKM2 with  $\beta$ -catenin is required for expression of cyclin D1 downstream of EGFR activation, as well as tumor cell proliferation and tumor development. Thus, PKM2 contributes to tumor development via enhancing the Warburg effect as well as by enabling  $\beta$ -catenin transactivation downstream of EGFR signaling [33].

Another metabolic enzyme that exhibits isoform switching in tumor cells is 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB). The bifunctional PFKFB enzyme family controls intracellular levels of fructose-2,6-bisphosphate (F2,6BP), an allosteric activator of phosphofructokinase-1 (PFK-1) which is a rate-limiting enzyme and highly regulated control point in the glycolytic pathway. PFKFB enzymes are encoded by four genes, PFKFB1–4, and can phosphorylate F6P to F2,6BP and dephosphorylate F2,6BP to F6P. Both the PFK-2 (kinase) and FBPase (bisphosphatase) reactions are catalyzed on the same polypeptide [34]. PFKFB1, PFKFB2 and PFKFB4 were originally found expressed in liver/muscle, kidney/heart, and testes, respectively, and displays nearly equal kinase:phosphatase ratios [35]. The PFKFB3 gene encodes the inducible PFK2/FBPase [36–38], which is rapidly induced by inflammatory stimuli [36] and hypoxia [39,40]. Unlike the other PFKFB isozymes, PFKFB3 displays a markedly increased kinase:bisphosphatase activity ratio of  $\sim$ 740:1, suggesting constitutive pro-glycolytic activity. Multiple groups have found elevated PFKFB3 levels in human solid tumors [36,37,41]. PFKFB3 promotes glycolytic flux and tumor growth in Ras-transformed mouse fibroblasts [42]. A recent study found that oncogenic activation of the JAK/STAT5 pathway, via the JAK2V617F point mutation commonly found in patients with myeloproliferative neoplasms, causes increased PFKFB3 expression and aerobic glycolysis [43]. PFKFB3 is required for the Warburg effect metabolic phenotype in human erythroleukemia cells expressing JAK2V617F as well as for proliferation and tumor formation [43]. PFKFB3 is also required for growth and proliferation of CD44+CD24–breast cancer cells that often exhibit JAK2/STAT3 pathway activity [44]. For a detailed overview of PFKFB regulation of glucose metabolism in cancer, see the recent review by Yalcin et al. [34].

Some studies suggest a preference for LDH-A expression over LDH-B in cancer. Likewise, specific members of the monocarboxylate transporter (MCT) family of proteins responsible for lactate and other monocarboxylate transport across the plasma membrane are found elevated in cancers [45–47]. Further work is needed to delineate whether specific forms of LDH, MCT, and other metabolic proteins have acquired special functions or mechanisms of regulation that benefit tumor metabolism and growth.

## 2.3. Post-translational regulation of the Warburg effect

Phosphorylation events on metabolic enzymes resulting from oncogenic signaling network activation can contribute to aerobic glycolysis. One example is phosphorylation of hexokinase and PFK-2 by AKT [48,49], which occurs downstream of PI3K activation. AKT signaling also promotes GLUT expression and plasma membrane localization [49]. Therefore PI3K/AKT pathway activation can promote the Warburg effect by simultaneously stimulating glucose uptake, trapping in the cell, and commitment to further catabolism by glycolysis.



**Fig. 1.** PKM2 integrates diverse signals to modulate metabolic flux and cell proliferation. When cytosolic PKM2 is activated by F1,6BP, glycolytic intermediates are diverted into the TCA cycle (blue arrows). However, PKM2 can be inhibited by receptor tyrosine kinase activation, direct phosphorylation by FGFR1, acetylation in the presence of high glucose concentrations, and oxidation upon elevated ROS levels (red arrows). PKM2 inhibition promotes redirection of glycolytic intermediates into biosynthetic pathways important for proliferation (solid red arrows indicate proven flux changes, dotted red arrows indicate hypothesized flux changes). PKM2 also functions as a transcriptional coactivator to promote aerobic glycolysis and proliferation in cancer cells. Nuclear PKM2 enhances HIF1 binding to hypoxia response elements (HRE) promoting transcription of HIF1 target genes and interacts with tyrosine-phosphorylated  $\beta$ -catenin to promote transcription of cyclin D1. Thus both cytosolic and nuclear PKM2 contribute to altered metabolism and proliferation in cancer.

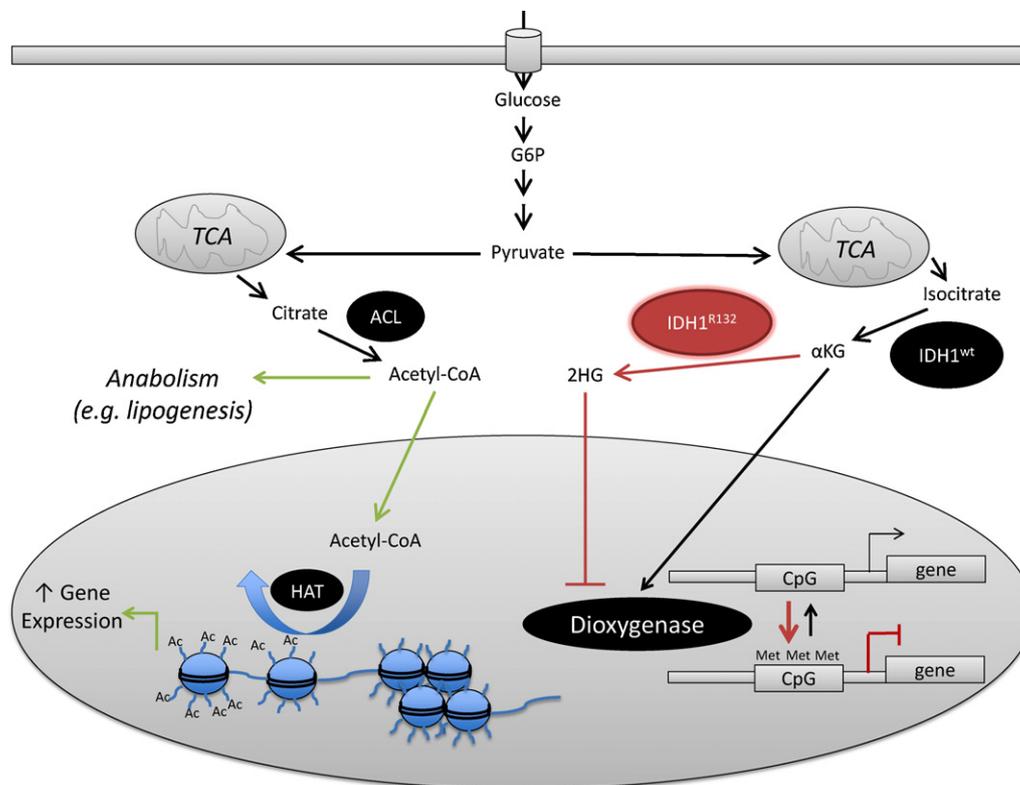
Recent studies have identified additional post-translational events on metabolic proteins resulting from growth factor signaling pathway activation, as well as nutrient availability and oxidative stress. In addition to the hydroxylation event on PKM2 mentioned previously [30], PKM2 can be phosphorylated [50], acetylated [51], and oxidized [52] to regulate activity. Decreased PKM2 activity as a result of oncogenic signaling pathway activation is thought to contribute to tumor cell proliferation by enabling use of upstream glycolytic intermediates for biosynthesis [32,53,54]. Consistent with this model, these newly identified modifications on PKM2 also reduce enzymatic activity but do not affect PKM1 activity. Phosphorylation of PKM2 on Y105 by oncogenic fibroblast growth factor receptor type 1 inhibits PKM2 activity by disrupting binding of the allosteric activator F1,6BP which disrupts tetramer association. Expression of Y105F mutant PKM2 in cancer cells reduces lactate production, increases oxygen consumption, and reduces growth of tumor xenografts [50]. Interestingly, a recent study by Lv et al. found that acetylation of PKM2 on K305 stimulated by high glucose concentrations reduces PKM2 enzymatic activity by lowering affinity for PEP and promoting degradation of PKM2 via chaperone-mediated autophagy [51]. Expression of an acetylation mimetic K305Q version of PKM2 leads to increased levels of glycolytic intermediates and NADPH and enhances cancer cell proliferation and tumor growth. Lastly, Anastasiou et al. recently showed that PKM2 oxidation on C358 in high ROS concentrations reduces PKM2 activity by impairing tetramer association. They found that ROS-dependent inhibition of PKM2 leads to enhanced flux of G6P into the pentose phosphate pathway which provides NADPH required for glutathione reductase to generate GSH for ROS detoxification. Indeed, expression of an oxidation-deficient C358S

mutant PKM2 results in lowered GSH levels, increased susceptibility to ROS-induced cell death, and decreased tumor growth. These results suggest that PKM2 inhibition via oxidation during oxidative stress contributes to cell survival when ROS accumulate [52].

Together these studies suggest that PKM2 acts as an important integrator of signals from growth factor signaling pathways, nutrient availability, and oxidative stress to regulate flux through glycolysis into central carbon and biosynthetic pathways (Fig. 1). Thus fine-tuned regulation of PKM2 activity enables metabolic reprogramming under varied conditions. Interestingly, large-scale mass spectrometry-based studies have identified many post-translational events on other metabolic enzymes that may also contribute to metabolic flux regulation in cancer cells [55–58]. A recent proteomic analysis by Zhao et al. of lysine acetylation in human liver tissue mapped acetylation sites on the majority of enzymes involved in intermediary metabolism, including glycolysis, the TCA cycle, and fatty acid metabolism. Nutrient availability influenced acetylation status of the metabolic enzymes, and several specific acetylation events were shown to modulate enzymatic activity [58]. Data from this study implies that acetylation of multiple components of metabolic pathways in response to nutrient availability can together impact metabolic regulation. Further work is needed to study these modification events in the context of cancer to build a systems level understanding of metabolic reprogramming in tumors.

#### 2.4. MicroRNA regulation of the Warburg effect

MicroRNAs (miRNAs) are endogenous ~22 bp RNAs that mediate temporal and tissue-specific eukaryotic gene regulation by



**Fig. 2.** Metabolism can directly influence gene expression programs. A schematic representation of how metabolism can intrinsically influence epigenetics resulting in durable and heritable gene expression programs in progeny. On the left side, histones are tightly packed thereby decreasing transcriptional complex accessibility. As intracellular acetyl-CoA concentration increases to meet anabolic requirements (green arrows), histone acetyltransferases (HAT) acetylate histones (Ac) providing increased accessibility to transcription factors resulting in heightened gene expression. On the right side of the figure, mutations in cytosolic IDH1, or mitochondrial IDH2, result in novel enzymatic activity driving the conversion of  $\alpha$ -ketoglutarate ( $\alpha$ KG) to 2-hydroxyglutarate (2HG) (red arrows). 2HG is a competitive inhibitor of  $\alpha$ KG – dependent dioxygenases leading to preferential methylation of genomic elements (Met) and silencing of genes. A similar pattern of histone methylation has also been observed in IDH mutant cells. ACL = ATP citrate lyase.

targeting mRNAs for cleavage or translational inhibition [59]. Since the discovery that miRNAs are aberrantly expressed in cancer [60,61], accumulating evidence suggests that miRNAs contribute to tumor growth by modulating levels of oncogenes and tumor suppressors [62]. Not surprisingly, some miRNAs have been shown to regulate cancer metabolism. miRNA-23a and miRNA-23b, which are suppressed by MYC, repress mitochondrial glutaminase expression [19]. Therefore, MYC enhances glutaminase and glutamine metabolism, an important carbon and nitrogen source for biosynthesis in cancer cells, by repressing miRNA-23a/b expression. A recent study by Eichner et al. found that ERBB2 signaling leads to miRNA-378 expression, which promotes the Warburg effect by inhibiting expression of  $ERR\gamma$ , a binding partner for PGC-1 $\beta$ , leading to reduced transcription of tricarboxylic acid cycle genes. miRNA-378 expression, which correlates with progression in human breast cancer tissues, causes increased lactate production, decreased respiration, and increased proliferation of breast cancer cell lines [63]. Another recent study has implicated miRNA-210 in metabolic reprogramming in cancer. Favaro et al. found that miRNA-201, which is induced by hypoxia, represses the mitochondrial iron sulfur scaffold protein ISCU resulting in decreased mitochondrial complex 1 activity, aconitase activity, increased lactate production and hypoxic cell survival [64]. Future studies will undoubtedly uncover additional miRNAs important for aerobic glycolysis in cancer.

### 2.5. Genomic regulation of the Warburg effect

Growing evidence suggests that genetic aberrations in metabolic genes can contribute to metabolic reprogramming

important for cancer growth. Recent studies have found that the gene for phosphoglycerate dehydrogenase (PHGDH) lies within a genomic region of recurrent copy number gain in cancers, including melanoma and breast cancer [65–67]. PHGDH catalyses the first step in the serine biosynthesis pathway, which diverts glycolytic carbon into serine and glycine biosynthesis. Suppression of PHGDH expression in breast cancer cell lines with the PHGDH gene amplification impairs cell proliferation and decreases flux of glucose carbons into serine biosynthesis [65,66]. These data suggest that certain cancers depend on diversion of glucose carbons into de novo serine biosynthesis. Other examples of genetic alterations resulting in metabolic flux changes, including mutations in IDH1/2 and FH, are discussed below (see Section 2.7). Expanding genomic data sets from normal and cancer tissues resulting from The Cancer Genome Atlas and from the advent of new generation sequencing technologies will likely enable discovery of additional mutations, deletions, and amplifications in metabolic genes that contribute to the Warburg effect in cancer.

### 2.6. ATP consumption in futile cycles promotes the Warburg effect

To support proliferation, cancer cells must strike a balance between energy production and biosynthesis of macromolecules. Excess ATP resulting from increased glycolysis or OXPHOS can reduce glycolytic flux by allosteric inhibition of PFK. Recent studies suggest that cancers evade this feedback inhibition to ensure continued glucose uptake and metabolism for biosynthesis by lowering ATP levels through increased ATP hydrolysis and by decoupling glycolysis from ATP production. Fang et al. found that the ER UDPase ENTPD5, which is elevated in tumors and cell lines with active

AKT, works together with cytidine monophosphate kinase-1 and adenylate kinase-1 to constitute an ATP hydrolysis cycle providing a major source of ATP consumption in PTEN-deficient cells. Knockdown of ENTPD5 decreases lactate production and tumor growth, whereas ectopic expression of ENTPD5 increases lactate production [68]. These data suggest that ATP consumption involving ENTPD5 promotes the Warburg effect in cancer. Vander Heiden et al. recently showed that proliferating cells exhibit an alternate enzymatic activity to pyruvate kinase that converts PEP to pyruvate without generating ATP. This enzymatic activity was shown to contribute up to one-half the amount of pyruvate generated from PEP [69]. Further work is needed to elucidate the precise enzyme(s) involved in this activity and to determine its contribution to metabolic flux. This topic was reviewed in more detail recently by Locasale and Cantley [70].

### 2.7. Mitochondrial (dys)function and the Warburg effect

As delineated above, the molecular mechanisms driving the Warburg effect are highly heterogeneous. Thus, it should not be surprising that dysfunction of tumor cell mitochondria can also result in an increased reliance on aerobic glycolysis. Indeed, Otto Warburg first hypothesized nearly 80 years ago that glycolysis under normoxic conditions (termed aerobic glycolysis) must be attributable to an intrinsic mitochondrial perturbation leading to a loss of OXPHOS capacity during carcinogenesis [71]. While this hypothesis has fallen out of favor due to strong evidence that a majority of cancers retain normal OXPHOS capacity [72–76], it should be noted that intrinsic mitochondrial dysfunction has been identified in an array of studies [77]. One such mechanism centers on the co-optation of mitochondrial uncoupling proteins to decrease mitochondrial membrane potential. Mitochondrial uncoupling was initially identified as a critical adaptive response to cold to generate heat for the preservation of core body temperature [78,79]. Dissipation of membrane potential by mitochondrial uncoupling proteins, notably UCP1, results in the generation of metabolic heat, providing an important source of energy for adaptive thermogenesis. Of course, the generation of metabolic heat comes at the expense of ATP production from OXPHOS and cells must rely on increased glycolysis to meet energetic demands. Heightened expression of uncoupling proteins, in particular (UCP2), has been observed in several cancers including, colon, liver, breast, thyroid and acute myeloid leukemia [80,81] and positively correlates with tumor grade in breast cancers [82]. In support of an important role for mitochondrial uncoupling in cancer pathogenesis, enforced expression of UCP2 promoted chemo-resistant tumor growth in vivo [83], whereas loss-of-function inhibited cellular transformation in vitro [84]. The functional consequences of mitochondrial uncoupling appear to be several-fold including an increased reliance on glycolysis, decreased ATP production (further reinforcing glycolytic flux), decreased ROS production, decreased apoptotic sensitivity and increased chemotherapeutic resistance [83,84].

Disruption of OXPHOS can also occur through mutations in TCA cycle and electron transport chain (ETC) proteins. Mutations in TCA cycle enzymes succinate dehydrogenase (SDH), which also functions as complex II of ETC), isocitrate dehydrogenase (IDH2) and fumarate hydratase (FH) have been documented in a small, but increasing number of cancers (reviewed in [77,84,85]). Other changes in mitochondrial respiratory function have been proposed as important contributors to the Warburg effect in cancer cells. Principally, disruption of mitochondrial DNA (mtDNA) copy number, genetic mutations in mtDNA stability, and epigenetic alterations of genomic DNA encoding mitochondrial proteins have all been documented in human tumors [77,85]. Mutations in these proteins result in decreased TCA cycle function and accumulation of citric cycle intermediates. In the case of IDH2, or its

cytosolic counterpart IDH1, mutated proteins acquire novel enzymatic activity resulting in accumulation of a novel metabolite 2-HG [86–88] which appears to mediate significant changes to the genome [89,90]. This intriguing observation is further explored below. Disruption of TCA and ETC function clearly contributes to metabolic reprogramming of cancer cells both directly through the necessary adaptation to decreased OXPHOS function, and likely through the accumulation of so called oncometabolites (e.g., 2-HG). Understanding the oncogenic or pro-tumor signals generated by TCA disruption and oncometabolites remains enigmatic. And it will be important to determine whether changes in mitochondrial function are primary drivers of cellular transformation, or if these mutations provide a competitive advantage to cells by supporting tumor growth and survival in tumors.

## 3. Role of the Warburg effect in cancer

### 3.1. Can the Warburg effect and cancer metabolism be programmed?

While the Warburg effect metabolic phenotype was initially identified in cancer tissue, it is now well appreciated that rapidly dividing normal tissues, such as ES cells and lymphocytes, employ aerobic glycolysis to meet their energetic and biosynthetic requirements during expansion (reviewed in [91]). These observations support the notion that aerobic glycolysis is a preferred metabolic program under conditions of rapid cellular expansion. However, it remains unclear how the Warburg effect is initiated and maintained; these need not be the same signals in cancer versus normal tissues which lack dysregulated signaling. One critical signaling axis for metabolic programming of normal cells is the PI3K/AKT/mTOR pathway downstream of growth receptors. Genetic and pharmacologic models have clearly identified mTOR signaling in controlling cellular growth and metabolism (reviewed in [92]). Perturbation of this pathway results in altered glucose transport and flux through the glycolysis, lipogenic and nucleotide biosynthetic pathways. However, upregulated PI3K/mTOR signaling is subject to negative feedback and likely not maintained throughout cellular proliferation. In support of this notion, T lymphocytes can maintain robust proliferative responses after removal from mitogenic signals [93]. Thus, it appears that some cells can be programmed by mitogenic signals to complete a proliferative program without continuous growth receptor signaling. A logical supposition is that the underlying metabolic programs that support cellular growth should likewise be programmed to meet cellular energetic and biosynthetic requirements.

One attractive hypothesis is that upstream growth signals imprint an anabolic program on dividing cells through epigenetic modifications. Acetyl-CoA is a citric acid cycle intermediate that serves as a critical building block of numerous macromolecular products including lipids and amino acids. Acetyl-CoA also serves as a critical donor to histone acetyltransferases that epigenetically mark the genome contributing to genetic accessibility by transcriptional complexes. In this manner, mitochondrial production and homeostasis of acetyl-CoA could serve to link metabolism to gene expression programs. In support of this concept, work from Thompson and colleagues demonstrated that genetic or pharmacologic inhibition of ATP-citrate lyase (ACL), a critical enzyme in the conversion of citrate to acetyl-CoA, inhibited cancer cell proliferation and tumor growth [94]. Subsequently, they demonstrated that the acetyl-CoA pool in the cells is derived from glucose via the enzymatic action of ACL. Moreover this metabolite flux was necessary for the histone acetyltransferase (HAT) mediated acetylation of histones [95]. Quite interestingly, the observed histone acetylation was restricted to key metabolic genes that are involved in

glycolysis and anabolism. It has yet to be determined the mechanism by which specificity of epigenetic modifications at metabolism genes is achieved in mammalian cells. Elegant mechanistic work in yeast by the Tu laboratory extended on this concept and demonstrated that acetylation of histones at a broad array of genes associated with anabolism occurs with entry into growth [96]. Using an isotopomer enrichment metabolomics approach, they demonstrated that a variety of nutrients could serve as carbon donors for acetyl-CoA synthesis. Moreover, they observed significant and rapid incorporation into acetylation reactions on histones, effectively linking nutrient uptake to epigenetics. Importantly, growth rates correlated with cellular acetyl-CoA levels suggesting that acetyl-CoA homeostasis could serve as a metabolic signal to cell cycle machinery, although this has yet to be shown. Nevertheless, these data raise the possibility that modulating acetyl-CoA levels may offer a novel strategy for controlling dysregulated growth in cancer and other hyperproliferative diseases.

Another emerging story of epigenetic modifications to the genome mediated by tumor metabolism centers on the recent identification of mutations in the isocitrate dehydrogenase enzymes (IDH1 and IDH2) in cancer (Fig. 2). Mutations in these enzymes have been identified in upwards of 80% of low-grade gliomas and 30% of AML [97–99]. Quite strikingly, the mutations are single amino acid substitutions at an arginine residue in the active site of the enzyme (e.g. R132 for IDH1 and R172 in IDH2 in gliomas or R140 for IDH2 in AML). The mutations occur in a homologous portion of the IDH1 and IDH2 enzymes and cells are always heterozygous, retaining 1 copy of the WT IDH enzyme. These data indicate that there is strong pressure on this enzyme to acquire a specific mutant activity rather than a generalized gain- or loss-of-function. Indeed, recent studies have demonstrated that rather than generating  $\alpha$ -ketoglutarate and NADPH from isocitrate, mutated IDH proteins consume NADPH and produce the novel metabolite 2-hydroxyglutarate (2-HG) [86,88]. These changes in enzymatic activity appear to mediate metabolic reprogramming of cancer cells resulting in significant alterations to the genome. The molecular mechanism by which cellular metabolism is reprogrammed is not well understood, but appears to be dependent on 2-HG accumulation resulting in epigenetic modifications. Several studies provide evidence that 2-HG levels influence DNA and histone methylation state in cancer tissue by interfering with the function of the  $\alpha$ -KG-dependent dioxygenases (e.g. TET family of 5-methylcytosine hydroxylases) [89,90]. (For a more in depth treatment of this subject, we refer the readers to a companion review by Yue Xiong and colleagues [117]) Future studies will be required to prove that aberrant epigenetic modifications mediated by metabolism are an essential part of driving cancer cell fate and function. Nevertheless, these data help solidify a relationship between metabolism and gene expression and may provide important insights as to how metabolic information is conveyed to daughter progeny.

### 3.2. The Warburg effect and cancer microenvironment

Tumor tissue is highly heterogeneous, comprised of a mixture of normal and neoplastic cells in variable states of structural organization. Cells that contribute to tumor parenchyma include components of vasculature, immune cells (e.g., macrophage), fibroblasts and cancer cells (reviewed in [100]). Not unreasonably, it has been hypothesized that the metabolic phenotype of cancer cells may also be a functional consequence of spatial heterogeneity in nutrient and oxygen availability within the tumor proper. Those cells closer to vasculature would have increased molecular oxygen and nutrients, thus they are more likely to engage in oxidative metabolism. In contrast, tumor cells that are more distant from vasculature would be more glycolytic. An alternative model posits that a symbiotic metabolic relationship exists amongst tumors cells

themselves and between normal and neoplastic cells in the tumor parenchyma. One such as example of this symbiotic relationship centers on lactate, the canonical byproduct of the aerobic glycolysis. In these studies, hypoxic tumor tissue produces lactate which is excreted and delivered in trans to adjacent normoxic tumor cells [101]. Uptake of lactate was mediated by the monocarboxylate transporter 1 (MCT1) and shuttled into oxidative phosphorylation via the conversion of lactate to pyruvate by lactate dehydrogenase B (LDHB). These data demonstrate how by-products of one cell could serve as a carbon source for anabolism and energetics in neighboring tumor cells. Analogously, work on epithelial cancer microenvironments revealed that cancer associated fibroblasts (CAFs) acquire a unique metabolic program that incorporates the many elements of the cancer metabolic phenotype, including the Warburg effect [102,103]. In this model, cancer cells induce a metabolic or oxidative stress on neighboring CAFs resulting in the acquisition of a glycolytic phenotype. Subsequently, the CAFs provide nutrients (lactate or pyruvate) in trans to tumor cells that drive anabolism for cellular growth. Interesting work from Collier and colleagues demonstrated that “quiescent” fibroblasts maintain a heightened anabolic program despite undergoing replicative arrest [104]. It is not entirely clear why these cells maintain this heightened metabolic program; nevertheless it is easy to hypothesize that the fibroblast metabolic program could be “highjacked” by neighboring cancer cells to provide requisite metabolites in trans. Additionally, recent studies on metastatic disease revealed that metastatic cancer cells in the omentum receive energetic support from neighboring adipocytes by driving the AP2-mediated transfer of fatty acids from normal fat cells to tumor cells [105]. Thus, catabolic programs in normal cells within the tumor parenchyma can play a pivotal role in supporting the anabolic program of cancer. Indeed, recent studies indicate that despite robust anabolic programs in tumors, addition of lipolytic capabilities robustly increase tumorigenesis [106]. Although speculative, the symbiotic relationship between normal cells and tumor cells could help to explain cancer associated cachexia by driving a generalized catabolic program in tumor bearing individuals.

### 3.3. Does aerobic glycolysis contribute to chemotherapeutic resistance or susceptibility?

Given that many cancers exhibit altered metabolism, it should not be surprising that there has been increased effort to therapeutically target these pathways as a means to decrease tumor growth or alter behavior. This topic has been recently reviewed [107,108] and will not be covered herein; rather we will focus on the question as to whether metabolic adaptation by cancer cells intrinsically contributes to conventional chemotherapeutic or radio-resistance. The Warburg effect shifts the metabolic program of cancer cells away from mitochondrial respiration, thus it could be reasonably hypothesized that a decreased reliance on mitochondrial function would translate into increased resistance to apoptotic stimuli mediated by the intrinsic apoptotic pathway. However, studies examining the influence of metabolism on chemotherapeutic resistance provide data that both support and refute this hypothesis. Studies interrogating apoptotic susceptibility to chemotherapeutic agents in mtDNA depleted (rho minus) cancer cells demonstrated that loss of mitochondrial gene expression mediates resistance to an array of apoptotic stimuli and cytotoxic agents [109–112]. However, conclusions drawn from these studies must be tempered by the acknowledgment that rho minus cells poorly recapitulate the genetic and metabolic program of tumors in situ. In contrast, examination of temozolamide-resistant glioma cells revealed a decrease in glucose consumption and lactate production when compared to parental cells [113]. The acquisition of chemo-resistance was correlated with heightened mitochondrial coupling efficiencies and

reduced ROS generation ameliorating oxidative stress from drug treatment. Thus, a shift away from aerobic glycolysis provided a measurable selective advantage under chemotherapeutic pressure. Interestingly, these studies demonstrated that the chemotherapeutic resistance was associated with remodeling of the ETC resulting in changes in specific activity of respiratory complexes as well as alterations in mtDNA copy number [113,114]. It will be of interest to determine if chemotherapy-induced remodeling of ETC is a permanent feature of chemotherapeutic resistant cells or if the cells can reacquire their metabolic program after drug pressure is removed.

Finally, the question of metabolic plasticity is important as we begin to examine the impact of targeted therapeutics on cancer cells. Targeted pharmacologic inhibition of growth signaling pathways has provided impressive results in the clinic and holds significant therapeutic promise. One potential complication in the use of these drugs is the strong influence they can have on a cancer cell's metabolic program. Many of the signaling pathways involved in oncogenesis directly or indirectly engage the Warburg effect and anabolic metabolism to support oncogene-directed tumor growth. As delineated above, there is emerging evidence that changes in metabolic programs intrinsically contribute to chemotherapeutic resistance. A logical conclusion from these studies is that therapeutics targeting growth signaling pathways may select for resistant cancer cells that have shifted cellular metabolism away from aerobic glycolysis toward OXPHOS. Alternatively, inhibition of growth signaling pathways by targeted therapeutics may result in resistant cancer cells that reacquire a glycolytic phenotype by an alternate mechanism (discussed in more detail in Section 2). Further work is needed to advance our understanding of metabolic plasticity in cancer and determine whether it plays a role in therapeutic resistance.

### 3.4. Metabolism of cancer stem cells

While the concept of bona fide cancer stem cells (CSCs) remains controversial, there is strong evidence to indicate that a subset of cancer cells are endowed with the capacity to initiate tumor formation [100]. Oft times defined as tumor-initiating cells (TICs), these cells appear to be critical for the ability of tumors to resist conventional radio- or chemotherapeutics and repopulate the tumor during and after treatments. Whether TICs have distinct metabolic programs from the bulk of tumor cells is not well established. A major barrier to these studies is the reliable identification of cancer stem cells in situ. Recent studies from Pajonk and colleagues exploited observable differences in proteosomal activity to prospectively identify CSCs in patient-derived glioma cell lines and interrogate their metabolic state [115]. In contrast to the bulk of glioma cells, CSCs exhibited an increased reliance on OXPHOS for energetics with a marked shift away from aerobic glycolysis. Of potential importance, these CSCs appear to have increased radio-resistance and lower free radical production. These studies are in accordance with the observations that tighter mitochondrial coupling, reduced ROS production, and an increased reliance on respiration are correlated with resistance to the DNA alkylating agent temozolamide in glioma [113,114]. However, it has not been determined if selection of chemotherapeutic resistant glioma cells in this model system drives CSC enrichment. Studies by Rich and colleagues have demonstrated that radio-therapy selectively enriches for glioma CSCs in vivo [116], thus it seems plausible that the population of cancer cells which survive therapy have distinct metabolic programs. Many more studies are required to determine if the observed relationship between reliance on oxidative metabolism and chemotherapeutic resistance in TICs is specific to glioma or if this is a generalizable principle that can be applied to many types of cancers.

## 4. Conclusion

In conclusion, the cancer metabolic program is an important component of tumor growth and survival. Renewed interest in this program has yielded significant progress over the last decade toward identifying genetic and biochemical events underlying “how” and “why” a cancer cell engages aerobic glycolysis. Importantly, these observations have reinforced our understanding of the molecular heterogeneity by which the Warburg effect and cancer metabolic phenotype is achieved. This heterogeneity also highlights difficulties in ongoing efforts to target cancer metabolism as a therapeutic approach (reviewed in [107,108]). While these efforts are exciting and appear to have therapeutic potential, cumulative lessons in oncology have demonstrated that cancers have tremendous plasticity enabling therapeutic resistance and tumor recurrence. Nevertheless, continued efforts to understand the cancer metabolic program should continue to yield important insights into cancer biology and uncover novel therapeutic strategies.

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