

REVIEW

ROS homeostasis and metabolism: a critical liaison for cancer therapy

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Evidence indicates that hypoxia and oxidative stress can control metabolic reprogramming of cancer cells and other cells in tumor microenvironments and that the reprogrammed metabolic pathways in cancer tissue can also alter the redox balance. Thus, important steps toward developing novel cancer therapy approaches would be to identify and modulate critical biochemical nodes that are deregulated in cancer metabolism and determine if the therapeutic efficiency can be influenced by changes in redox homeostasis in cancer tissues. In this review, we will explore the molecular mechanisms responsible for the metabolic reprogramming of tumor microenvironments, the functional modulation of which may disrupt the effects of or may be disrupted by redox homeostasis modulating cancer therapy.

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INTRODUCTION

Otto Warburg and co-workers showed in the 1920s that cancer tissues can metabolize, even in aerobic conditions, ~10-fold more glucose to produce lactate than normal tissues, and this is known as aerobic glycolysis or the Warburg effect.¹ For the biosynthetic demands of continuous proliferation and survival of cancer cells to be satisfied, many genetic and epigenetic changes in oncogenes and tumor suppressor genes, such as HIF-1 α , AKT, Myc, PI3K/AKT, Ras, p53, PTEN and LKB1, responsible for tumorigenesis, can directly regulate the expression, regulation and activity of important components of metabolic pathways and exert a direct impact on metabolism, most remarkably, on glucose uptake and aerobic glycolysis.^{2–6} Although cancer cells display a diverse range of metabolic profiles, the metabolic phenotype resulting from the Warburg effect is considered a widespread cancer-associated trait. This increased aerobic glycolysis has been erroneously thought to be a consequence, not a cause or contributing factor, in cancer and has been suggested as evidence that mitochondrial function in cancer cells is compromised. Despite enhanced glycolysis, most cancer cells also maintain mitochondrial respiration to produce a significant fraction of

ATP,^{7–9} and functional mitochondria are essential for the survival of cancer cells.^{2,10}

In recent years, therapeutic strategies targeting substantially altered cellular metabolism as a possible area for the development of novel anti-cancer agents have again been intensively investigated. The basis of metabolism correction strategies for cancer therapy, exploiting the heavy reliance of cancer cells on specific metabolic enzymes or processes, and finding a possible therapeutic window that we can use to eradicate cancer cells rather than normal non-transformed cells, involves the manipulation of redox homeostasis in cancer cells. The principles of current strategies exploiting metabolic perturbations for cancer therapy are nicely explained in a recent review.⁸

Studies have identified that cancer cells can become vulnerable to high reactive oxygen species (ROS) levels.¹¹ Indeed, increased sensitivity and apoptosis of cancer cells from increased ROS in response to depletion of ATP from manipulation of glycolytic enzymes and chemotherapeutics or radiotherapy have been reported from preclinical studies,^{12–14} and the design of new therapeutic approaches combining chemotherapeutic reagents and glycolysis inhibitors is being studied. Therefore, the general application of combined

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therapies targeting cancer metabolism with inhibitors of metabolic enzymes or oncogenes and disabling key anti-oxidant systems with ROS inducers such as chemotherapeutic reagents appears to show promise and is speculated to be a promising strategy to overcome drug resistance.

However, we still lack solid evidence that such approaches are widely applicable in human patients, especially on a long-term basis. As far as we are aware, much of our current understanding of ROS is from studies with isolated mitochondria and cells *in vitro*, and the influence of high ROS levels induced by therapeutic interventions on cancer cellular metabolism with respect to cancer cell survival appears to have been studied surprisingly little.^{15,16} Studies have indicated that metabolic changes and ROS production are intertwined in cancer cells.¹¹ Notably, evidence indicates that oxidative stress and hypoxia can control metabolic reprogramming of tumor microenvironments and that perturbed metabolic pathways can also alter redox balance.^{10,17–20} Furthermore, we know that cancer cells can develop heightened anti-oxidant systems to survive in high oxidative stress environments (Figure 1).^{21,22} For example, Ras- and Myc-driven cancer cells with their heightened anti-oxidant system are among the most difficult to treat.²³ These findings indicate that decisions to use particular reagents, such as chemotherapeutics to increase intracellular ROS to reach a toxic level or anti-oxidants to disturb redox balance, may depend on the type and stage of cancer and the level of endogenous ROS, the activation of ROS-induced survival pathways and metabolic perturbations in cancer.

Thus, it would be logical to assume that altered glycolytic metabolism owing to therapeutic interventions combining ROS inducers and specific inhibitors of metabolic targets can be both an oncogenesis and treatment resistance mechanism and also that successful cancer therapy would require a thorough understanding of the molecular mechanisms responsible for the metabolic reprogramming of the tumor microenvironment,

which may be different between multiple neoplasms and even between individuals diagnosed with the same cancer type.

In this review, we provide a summary of published reports on the molecular mechanisms responsible for the metabolic reprogramming of tumor microenvironments, the functional modulation of which may disrupt the effects of or may be disrupted by redox homeostasis modulating cancer therapy.

GLUCOSE METABOLISM IN CANCER CELLS

It is now clear that the spectrum of metabolic reprogramming in cancer cells goes beyond the Warburg effect and includes increased flux through the pentose phosphate pathway (PPP), high glutamine consumption, reduction/oxidation (redox) imbalance (either oxidative or reductive depending on cancer type,^{24,25} and elevated rates of lipid biosynthesis including fatty acid synthesis and the mevalonate pathway (Figure 2).^{5,8,24–26} Although recent studies have revealed the importance of fatty acids and proteins as fuel sources for cancer cells to proliferate and survive,^{27–30} extensive studies have been conducted on the use of glycolysis as a major fuel source for cancer cells. To address metabolic reprogramming, we will focus on glucose metabolism in this review. Notably, activation of oncogenes and suppression of tumor suppressors are mostly responsible for metabolic rewiring of cancer cells, and functional mutations found in metabolic genes are relatively scarce.¹⁴

The conversion of fructose-6-phosphate (F6P) to fructose 1,6-bisphosphate (F1,6BP) by phosphofructokinase-1 (PFK1), a rate-limiting and irreversible reaction in glycolysis, is a primary control point in glycolysis (Figure 2). PFK1, the prominent rate-limiting enzyme in glycolysis, is an allosteric enzyme. Fructose 2,6-bisphosphate (F2,6BP), the most potent allosteric activator of PFK1, is produced by PFK2 and critically regulates the glycolytic rate. The affinity of PFK1 for F6P is increased upon binding of F2,6BP to PFK1, and ATP-mediated inhibition of PFK1 is lost. Among all PFK2 enzymes, 6-phosphofructo-2-kinase (PFKFB3), with high kinase activity and almost no phosphatase activity, is generally found to be overexpressed in human cancers.^{31,32} Thus, PFKFB3 preferentially drives the synthesis of F2,6BP. Fructose-1,6-bisphosphatase 1 (FBP1), a rate-limiting enzyme in the gluconeogenesis pathway, catalyzes the hydrolysis of F1,6BP to F6P. The FBP1-mediated reaction is the reverse of the rate-limiting glycolytic reaction catalyzed by PFK1, and FBP1 can antagonize glycolysis. In cancer cells with overexpressed PFKFB3, negative feedback from the Krebs cycle is not effective on glycolysis, and cancer cells can keep high levels of PFK1 activity and glycolysis. PFKFB3 and F2,6BP promote cell cycle progression and suppress apoptosis via Cdk1-mediated phosphorylation and subsequent degradation of p27, a tumor suppressor gene, indicating that a certain glycolysis enzyme can also function as an oncogenic driver.³¹ Snail represses FBP1 expression by binding to the FBP1 promoter, thereby enhancing glycolysis.³³ Thus, Snail not only induces the epithelial–mesenchymal transition but also suppresses mitochondrial oxidative metabolism.^{2,34}

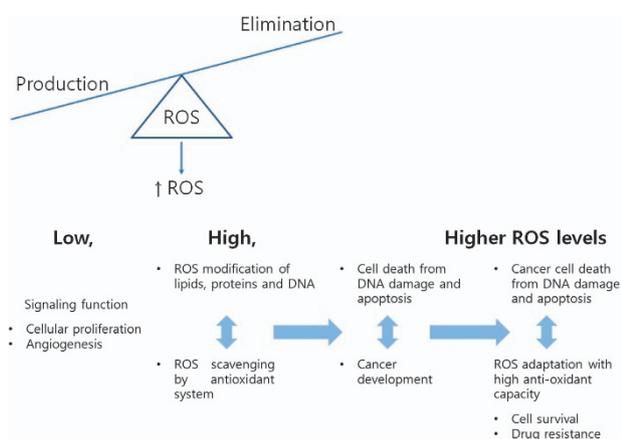


Figure 1 REDOX adaptation in cancer. Low levels of ROS can activate various signaling pathways to stimulate cell proliferation and survival. Adaptation to persistent and high levels of ROS can promote cancer development, survival of cancer cells and resistance to chemotherapeutics.

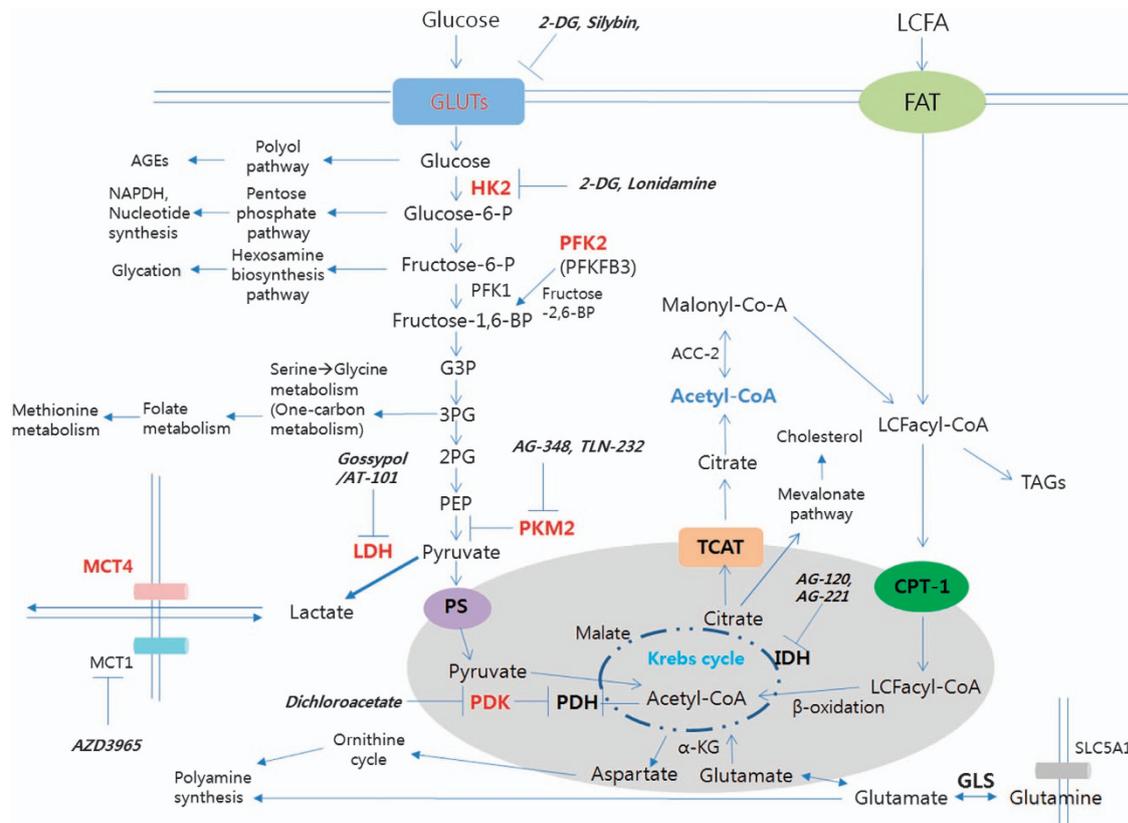


Figure 2 Metabolic targets at a glance. GLUTs, HK2, PFK2, PKM2, LDH, PDK and MCT4 shown in red can be upregulated from stabilization of HIF-1 α . Representative inhibitors of metabolic nodes entering clinical trials are shown in bold. ACC, acetyl-CoA carboxylase; AGE, advanced glycation end product; CPT-1, carnitine palmitoyltransferase 1; FAT, fatty acid translocase; G3P, glyceraldehyde 3-phosphate; GLS, glutaminase; GLUT, glucose transporter; HK2, hexokinase 2; LCFA, long chain fatty acid; LCFAcyl-CoAs, long chain fatty acyl-CoAs; MCT, monocarboxylate transporter; PEP, phosphoenolpyruvate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PS, pyruvate symporter; SLC5A1, sodium glucose co-transporter; TAG, triacylglyceride; TCAT, tricarboxylic acid transporter; 2-DG, 2-deoxyglucose; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate.

Enhanced glycolysis in cancer cells does not necessarily increase glycolytic flux in downstream metabolic pathways. Cancer cells allow the buildup of glycolytic intermediates for biosynthesis by slowing down the last step of glycolysis that is catalyzed by pyruvate kinase M2 (PKM2). The increased generation of the PKM2 isoform from alternative splicing of the PK gene, an event shown to be under the regulation of the oncogenic transcription factor Myc commonly deregulated in cancer, indicates that PKM2 expression may be a potential oncogenic driver.^{3,35} Unlike PKM1 that can efficiently promote glycolysis, PKM2, preferentially expressed in cancer cells in an inactive dimeric state, is inefficient at promoting glycolysis.^{6,36} PKM2 exists as either an inactive dimer or a more active tetramer, and the transition between the two conformations is subject to post-translational modifications (PTMs).³⁷ Oncogenic tyrosine kinases phosphorylate tyrosine 705 on PKM2 and promote the formation of the inactive dimer.³⁸ High levels of glucose-induced acetylation of PKM2³⁹ and ROS-mediated direct oxidation of a cysteine residue on PKM2⁴⁰ decreases its activity. These data suggest that increased expression of inactive PKM2 in cancer increases glucose flux into PPP flux, NADPH and glutathione levels and cancer cell

proliferation. Notably, the inhibitory function of PKM2 recently came under dispute; there are reports that inhibition or activation of PKM2 can increase or decrease cell proliferation and tumorigenesis, respectively.^{12,37,41}

Isocitrate dehydrogenase (IDH) is an enzyme that catalyzes the reversible oxidative decarboxylation of isocitrate, producing α -ketoglutarate and carbon dioxide in the Krebs cycle, also known as the tricarboxylic acid cycle. In cancer cells, acetyl-coA enters the Krebs cycle and generates citrate, which is utilized by malic enzyme and IDH1 to produce NADPH.¹⁴ Interestingly, oncogenic IDH mutations result in complex genetic and epigenetic changes including DNA methylation and metabolism.^{24,42} Gain-of-function mutations of IDH found in gliomas have the capacity to catalyze the NADPH-dependent reduction of α -ketoglutarate to 2-hydroxyglutarate and not to produce NADPH.^{43,44} 2-Hydroxyglutarate is a competitive inhibitor of α -ketoglutarate-dependent dioxygenases, including histone demethylases and the TET family of 5-methylcytosine hydroxylases, ultimately leading to genome-wide histone and DNA methylation alterations.^{45,46}

HIF-1 is a transcription factor comprised of HIF-1 α and HIF-1 β subunits. Under a hypoxic environment, HIF-1 α is

stabilized and activates its target genes. HIF-1 critically determines the metabolic shift from glucose oxidation to aerobic glycolysis in cancer. HIF-1 transcriptionally activates pyruvate dehydrogenase kinases (PDKs).^{47–49} Inactivation of pyruvate dehydrogenase (PDH) from phosphorylation by PDKs prevents pyruvate from entering the mitochondrial Krebs cycle, reduces mitochondrial respiration and prevents the excessive production of ROS.⁵⁰ HIF-1 also enhances lactate dehydrogenase (LDH)-mediated pyruvate-to-lactate conversion.⁵¹ LDH-A is a direct transcriptional target of HIF and is highly inducible by hypoxia.⁵² HIF-1 also increases

GLUT1, GLUT3, MCT4 and PKM2 to increase glucose uptake, to increase the export of lactate and to uncouple glycolysis and oxidative phosphorylation, respectively (Figure 2).^{53,54} Thus, HIF1 can suppress mitochondrial respiration and also ROS production. HIF-1 induced expression of NADH dehydrogenase (ubiquinone) 1 subcomplex subunit 4-like 2 (NDUFA4L2), and suppression of electron transport chain complex I activity can inhibit oxygen consumption and mitochondrial ROS production.⁵⁵ HIF-1-mediated metabolic reprogramming is responsible for the survival of metastatic cancers during their colonization in lungs by reducing

Table 1 Representative inhibitors of metabolic enzyme and oncogenes that entered clinical trials (from <https://clinicaltrials.gov/>)

<i>Compound name</i>	<i>Protocol number</i>	<i>Status</i>	<i>Study completed or last updated year</i>
<i>AMPK activator</i>			
Metformin	NCT01433913	Phase II completed	2015
	NCT01941953	Phase II completed	2015
	NCT01210911	Phase II completed	2010
<i>GLUTs inhibitor</i>			
2-DG	NCT00633087	Phase I/II terminated	2011
	NCT00096707	Phase I	2008
<i>HIF1 inhibitor</i>			
EZN-2968, Antisense Oligonucleotide Inhibitor	NCT01120288	Phase I completed	2014
RO7070179, HIF1A mRNA antagonist	NCT01251926	Phase I completed	2014
PX-478	NCT02564614	Phase I recruiting	2016
BAY87-2243	NCT00522652	Phase I completed	2010
	NCT01297530	Phase I terminated	2012
<i>IDH inhibitor</i>			
AG-120, AG-221	NCT02677922	Phase I/II recruiting	
	NCT02073994	AG-120, phase I recruiting	
	NCT02074839	AG-120, phase I recruiting	
	NCT02632708	Phase I recruiting	
	NCT01915498	Phase I active, not recruiting	
	NCT02577406	AG-221, phase III recruiting	
<i>LDH inhibitor</i>			
Gossypol(AT-101)	NCT00848016	Phase II completed & results	2014
	NCT00540722	Phase II completed	2013
	NCT00666666	Phase II completed	2013
<i>MCT1 inhibitor</i>			
AZD3965	NCT01791595	Phase I recruiting	2016
	NCT01278615	Phase II terminated	
<i>PDK inhibitor</i>			
Dichloroacetate	NCT01029925	Phase II terminated	2013
	NCT00540176	Phase II completed	2014
<i>PKM2 inhibitor</i>			
TLN-232	NCT00422786	Phase II completed	2008
<i>Myc inhibitor</i>			
Quarfloxin	NCT00780663	Phase II completed	2010
	CX-3543	NCT00955786	Phase I completed

cytotoxic ROS levels.⁵⁰ It is also known that ROS can activate the HIF-1 α promoter via a functional NF- κ B site, indicating the negative feedback regulation of ROS by HIF-1.^{56,57} Genetic ablation of adenosine monophosphate-activated protein kinase activates mTOR signaling with stabilization of HIF-1 α and results in activation of aerobic glycolysis.⁵⁸ This finding indicates that the cellular energy status is closely linked to aerobic glycolysis.

A recent report that tumor-associated mutant p53 drives the Warburg effect indicates that p53 mediates suppression of the Warburg effect.⁵⁹ p53 regulates genes that can balance the utilization of respiratory and glycolytic pathways, and p53-deficient cells show higher rates of glycolysis and decreased mitochondrial respiration than those of wild-type cells.⁶⁰ p53 downregulates expression of glucose transporter 1 (GLUT1) and GLUT4.⁶¹ In addition, p53 also downregulates PDK2 to increase the entry of pyruvate into mitochondria for oxidative metabolism,⁶² and p53-altered cellular metabolism is an important cause of increased ROS production in cancer cells.¹¹ Physical interaction of p53 with glucose 6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the PPP, to inhibit the formation of the active G6PD dimer suppresses glucose consumption and NADPH and GSH production.^{63,64} p53-induced glycolysis and apoptosis regulator (TIGAR) is a fructose-2,6-bisphosphatase. By lowering F2,6BP levels, p53 via p53-induced glycolysis and apoptosis regulator reduces the activity of PFK1. Thus, p53 can decrease the rate of glycolysis⁶⁵ and counteracts the Warburg effect.⁶²

Myc activates PDK1 and LDH-A^{66,67} under normoxic conditions or in conjunction with HIF-1 α under hypoxic conditions. Myc also drives glucose metabolism in triple-negative breast cancer cells by direct repression of thioredoxin-interacting protein (TXNIP).⁶⁸ TXNIP is a potent negative regulator of glucose uptake, aerobic glycolysis and glycolytic gene expression. Thus, its repression by Myc provides an alternate route to Myc-driven glucose metabolism. The mutation of TP53, which is a defining molecular feature of triple-negative breast cancer, has been shown to enhance the correlation between Myc suppression of TXNIP and death from breast cancer. A Myc-driven metabolic shift to glycolytic, pentose-phosphate and glutaminolysis pathways has also been found in activated T lymphocytes in addition to cancer cells.^{69,70}

TARGETING METABOLISM IN CANCER CELLS

Several cancer-associated alterations in catabolic and anabolic metabolic pathways, including glycolysis, the Krebs cycle, glutaminolysis and fatty acid oxidation, have been studied as potential drug targets, and identification of agents that selectively eradicate cancer cells based on metabolic alterations has gained immense interest.^{3,71,72} These approaches have led to the development of several molecules that are now entering clinical trials (Table 1),^{2,3,5,6,12,73,74} and readers are referred to excellent reviews with detailed summaries of metabolic targets for cancer therapy.^{5,6} Proteins that are possible therapeutic targets include the glycolytic enzymes^{6,75} (hexokinase-2,⁷⁶ phosphoglycerate kinase-1,^{77,78} phosphoglycerate mutase,⁷⁹

PDK,⁸⁰ and PKM2,^{36,38}) lipid synthesis/fatty acid metabolism targets (ATP citrate lyase,⁸¹ fatty acid synthase,⁸² monoglyceride lipase⁸³ and carnitine palmitoyltransferase 1 (CPT1),⁸⁴) and the PPP proteins (glucose-6-phosphate dehydrogenase,⁶³ transaldolase and transketolase).⁸⁵ Several other glycolytic enzymes and transporters, including PFKFB3,³² GAPDH,⁸⁶ LDH-A,⁸⁷ GLUT1 and GLUT4^{88,89} and monocarboxylate transporter 4 (MCT4), may become candidates for anticancer therapy.⁹⁰

Although performed *in vitro* or at preclinical stages, successful modulation of oxidative stress by targeting cancer cell metabolism has been reported as follows. Inhibition of CPT1 with etomoxir impairs NADPH production and promotes oxidative stress-induced cell death in human glioblastoma cells.⁹¹ Inhibition of PDKs could upregulate the activity of the pyruvate dehydrogenase complex and rectify the balance between the demand and supply of oxygen, which could lead to cancer cell death.⁸⁰ Inhibition of glycolysis and PPP with the disruption of thioredoxin systems showed selective cancer targeting in pancreatic and breast cancer cells.⁹² Inhibition of LDH-A with FX11 impaired malignant progression of lymphoma and pancreatic xenografts by inducing oxidative stress.⁸⁷ Inhibition of glycolysis and PPP through 2-deoxy-d glucose and 6-aminonicotinamide induced oxidative stress and sensitized cancer cells to radiotherapy.⁹³ Deprivation of glutamine through the inhibition of GLS1 decreases the GSH level to alter the redox balance and increase chemosensitivity in cancer cells.^{92,94} There are other reports that inhibition of the redox pathway can effectively eradicate cancer cells. NOV-002 and canfosamide targeting S-glutathionylation, PX-12 targeting thioredoxin and arsenic derivatives with unknown mechanisms demonstrated anti-cancer activity by increasing oxidative stress.⁹⁵

Have our attempts to correct genetic or epigenetic changes reversed metabolic reprogramming in cancer cells, and does the metabolic reprogramming affect responses to anti-oncogene targeted therapies? The answers appear to be 'No' and 'Yes', respectively. Recent reports show that rewiring of metabolic circuits in cancer cells results in resistance to several oncogene-targeted therapies and that the combined use of glycolytic inhibitors is somewhat successful in avoiding the resistance.^{12,96–101} Importantly, these findings indicate that altered glycolytic metabolism can be both an oncogenesis and treatment-resistance mechanism and that simple reversion of genetic or epigenetic causes responsible for the metabolic reprogramming may not be effective in cancer cells that have been metabolically fully rewired during the progression of tumorigenesis. The metabolic changes that occur in cancer cells were previously considered secondary to the transformation process and also completely different from non-transformed normal cells. However, studies have revealed that changes in cellular metabolism and epigenetic and genetic changes in malignant transformation are intertwined and cannot be dissociated from other facets of malignant transformation.^{102,103} To add complexity, a recent report that circulating cancer cells increase mitochondrial biogenesis and oxidative phosphorylation, without slowing the glycolysis rate,

to increase viability and invasiveness¹⁰⁴ indicates that cancer cells can adapt to ATP demand by enhancing oxidative phosphorylation and that the balance between mitochondrial productivity and detoxification of ROS can be easily shifted based on various cellular needs. Therefore, methods to correct glycolysis-shifted metabolic reprogramming in localized primary or metastatic cancer may be effective but may also affect the viability and invasive properties of circulating cancer cells with increased oxidative phosphorylation (the metabolic therapy may reduce localized cancer burden at the cost of increasing the incidence of metastasis).

TARGETING METABOLIC SYNERGY BETWEEN CANCER AND STROMAL CELLS IN THE TUMOR MICROENVIRONMENT

Put simply, the tumor microenvironment is comprised of cancer and stromal cells such as cancer associated fibroblasts (CAFs), immune cells and blood vessels.¹⁰⁵ Cancer aggressiveness is driven by metabolic synergy between cancer and stromal cells.¹⁰⁶ Although cancer research has previously focused on cancer cells, the role of stromal cells in cancer is becoming a new central focus. Metabolic synergy, which induces the efficient utilization of catabolites by cancer cells, results from differential expression of catabolite transporters in stromal and cancer cells.¹⁰⁷ For example, cancer and stromal cells secrete lactate, and extracellular lactate directly increases ROS levels in neutrophils, CD34+ vasculogenic stem cells, and cancer cells.^{108,109}

Microenvironments of cancer tissues are found to produce ROS levels that are higher than normal intracellular ROS levels.^{22,110,111} It has been determined that cancer-associated metabolic alterations (Warburg effect) are not a strictly uniform feature of malignant cells. They differ across distinct cancers and are found even in non-transformed cells in tumor microenvironments.^{112,113} Furthermore, non-transformed stromal, endothelial, and immune cells outnumber their neoplastic counterparts in cancer.^{5,114} Because rapid cell proliferation requires accelerated production of basic cellular building blocks for assembling new cells, differences in metabolism between cancer cells and non-transformed stromal and endothelial cells together can fuel cancer growth by lactate shuttling, maximally producing substrates for biosynthesis.^{89,107,115} The provision of lactate to oxidative cancer cells by lactate shuttling or by other unknown mechanisms may compensate for the lack of intracellular lactate and anti-oxidative resources in cancer cells.¹¹⁶ The tumor microenvironment strongly affects the metabolic status of cancer cells, and the supporting role of CAFs in the metabolic reprogramming of cancer cells is being emphasized.^{8,17} Cancer cells induce aerobic glycolysis in neighboring fibroblasts by providing a hypoxic ROS-rich microenvironment.^{107,117} Induced fibroblasts differentiate to myofibroblasts and upregulate MCT4 to secrete lactate and pyruvate that transforms normal stroma to ultimately help cancer cells grow (reverse Warburg effect). Lactate uptake by cancer cells can increase the surrounding stromal pH and can

protect cells from harsh acidic microenvironments. Lactate shuttling between normoxic and hypoxic cancer cells or adjacent stromal cells induces a switch from glycolysis to oxidative phosphorylation and increases the generation of intracellular ROS.⁸⁹ However, it also increases the survival of cancer cells by increasing anti-apoptotic mechanisms.¹¹⁸ These findings imply that metabolic adaptations in tumor microenvironments may obscure the direct tumoricidal effect of high-level ROS easily observable in *in vitro* culture systems. Alterations in oncogenes and cancer suppressor genes direct cellular metabolism to satisfy the biosynthetic demands for continuous cancer cell proliferation. However, CAFs are considered to be free from the alterations observed in oncogenes or tumor suppressors, and it is known that the induction of metabolic reprogramming in CAFs is driven by hypoxic status of the microenvironment.^{17,119} Stromal cells such as endothelial cells and other cancer-associated cells are also considered free of genetic changes and have an important role in ROS metabolism within the tumor microenvironment. Functional changes in their mitochondria induced by cancer cells can be considered reversible.¹²⁰ Thus, modulation of ROS in stromal cells may be more effective for both preventing myofibroblastic differentiation of fibroblasts into CAFs and reducing stabilization of HIF1- α and the resulting increase of GLUT1, GLUT3, MCT4 and PKM2 than in cancer cells where genetic changes are primarily and permanently responsible for the irreversible metabolic reprogramming. It has been shown that PDGF signaling is increased by ROS-induced oxidative inactivation of protein tyrosine phosphatases.^{121,122} These findings indicate that PDGF signaling induced by ROS may at least be responsible for the proliferation of a PDGFR- β + population of CAFs and that modulation of PDGF signaling may become a potential target in the future.

TARGETING METABOLIC CHANGES IN BLOOD VESSELS

Angiogenesis is a hallmark of hypoxic cancer mass, and intensive studies have been performed to target cancer angiogenesis. Chemical inhibition of vascular endothelial growth factor (VEGF) signaling is the only clinically approved anti-angiogenesis-based strategy, but resistance from genetic mutations is acquired within months after treatment initiation, seriously limiting its benefit.^{123–125} The endothelium is a single layer of endothelial cells (ECs) lining the blood vessel lumen and is in direct contact with blood.¹²⁶ Surprisingly, ECs predominantly rely on glycolysis rather than oxidative metabolism for ATP production.^{127,128} Over 80% of ATP is produced from conversion of glucose into lactate in the physiological state. Less than 1% of pyruvate from glycolysis enters mitochondria for oxidative metabolism and subsequent ATP production.¹²⁷ However, ECs can also switch to oxidative metabolism of glucose, amino acids and fatty acids.^{129,130} This is strikingly similar to the aerobic glycolysis observed in cancer cells, and compounds targeting cancer cellular metabolic reprogramming show similar effects in cancer cells and ECs.^{127,131,132} Currently, it is not clear whether metabolic phenotypes in ECs are modulated by cancer cells or CAFs.

Importantly, the switch to an angiogenic phenotype (as occurs in cancer) is even more metabolically demanding and mediated by an increase in aerobic glycolysis in EC metabolism.¹²⁸ Stabilization of HIF-1 α can also induce pro-angiogenic signaling pathways such as NF- κ B, IL-8 and VEGFR2, leading to increased angiogenesis.^{133,134} LDH-B is upregulated in the endothelium, and VEGF signaling increases glycolytic flux by inducing expression of GLUT1 and PFKFB3.¹²⁷ Indeed, inhibition with 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) or EC-specific gene knockdown of PFKFB3 inhibits cancer growth *in vivo* by inhibiting glycolytic flux.¹³⁵ Recently, 3PO has been shown to reduce pathological angiogenesis in a variety of disease models.^{128,132,135}

Targeting endothelial MCT1 can reduce the import of lactate released from hypoxic cancer cells and stromal cells in cancer microenvironments (lactate shuttling), which can stabilize HIF-1 α and increase angiogenesis through the NF- κ B/IL-8 pathway.¹³⁴ Inhibition of MCT1 suppresses angiogenesis and reduces cancer growth in mice.^{133,134} These observations are encouraging for validation in clinical trials. It should be noted that the therapeutic strategy of targeting glucose metabolism in ECs to inhibit cancer angiogenesis is very recent, but evidence suggests that it is possible.

CELLULAR RESPONSE TO MODULATION OF ROS IS DIFFICULT TO PREDICT

There are conflicting reports about the potency of cellular anti-oxidant systems and the efficiency of ROS targeting cancer therapies and even an increased risk of cancer in a long-term trial of redox modulation.^{3,15,110,111,136–138} Furthermore, the lack of any comprehensive study of the redox status of various cancer cell types critically limits the validation of speculation about the contribution of redox shifts to phenotypes across a broad spectrum of cancer cell types.¹³⁹ Consequently, reports have confusingly shown that ROS can be cytotoxic and also tumorigenic, can activate or inhibit telomerase activity and that it can also contribute to metabolic reprogramming, invasion and metastasis of cancer cells.¹⁹ Notably, the related therapeutic effects at the bedside appear disappointingly controversial, and neither increasing nor decreasing levels of ROS show uniform therapeutic effects.¹⁴⁰

ROS have dual functions, implying antagonism between different types and levels of ROS (Figure 1).¹³⁷ Low levels of ROS can activate various signaling pathways to stimulate cell proliferation, survival and even extend the lifespans of *C. elegans*, invertebrates and mice.^{141–143} Because excess levels of ROS irreversibly damage cellular macromolecular components and result in cell death, both the mitochondrial competence and detoxification of ROS are known to be critical for cancer cell viability.⁹ ROS can also mediate motility and invasive properties of cancer cells, contribute to extracellular matrix remodeling, increase neo-angiogenesis and induce the metabolic reprogramming of both cancer and stromal cells.¹⁷ To detoxify ROS, cells express ROS scavenging enzymes.¹¹ The mitochondrial manganese superoxide dismutase (MnSOD or

SOD2), the expression of which is induced upon accumulation of ROS in hypoxic cancer cells, converts superoxide to the less reactive hydrogen peroxide. Hydrogen peroxide is broken down into water and dioxygen by enzymatic and non-enzymatic anti-oxidants. The balance between the production and elimination of ROS leads to redox homeostasis. Other important defensive mechanisms include peroxiredoxin, NADPH-dependent thioredoxin and glutathione/glutaredoxin systems.¹⁴ For detailed descriptions of ROS features, readers are referred to other excellent reviews.^{11,16,110,111,139,144}

It has been generally speculated that an anti-ROS strategy can prevent tumorigenesis by suppressing oncogenic functions of ROS and that increasing ROS will preferentially eradicate vulnerable cancer cells.¹¹ Indeed, disruption of ROS maintenance and the resulting redox imbalance in cancer cells has been regarded as an important principle in cancer therapeutics, including chemotherapy because cancer cells are generally considered to be more vulnerable to disruption of redox balance and mitochondrial function than those of normal cells.^{110,145–147}

However, previous speculation that cancer cells are already flooded with high levels of ROS and that reducing ROS scavenging activity to increase ROS toxicity will uniformly eradicate cancer cells, appears to be an oversimplification. Cancer cells develop heightened anti-oxidant systems to survive in a high oxidative stress environment,^{21,22} and this is regarded as an important drug resistance mechanism. A heightened anti-oxidant system is thought to be the reason that Ras- and Myc-driven cancer cells are among the most difficult to treat.²³ Although numerous mechanisms are responsible for cancer resistance to chemotherapy, glycolytic phenotypes also count as a resistance mechanism. Increased glucose consumption supports cell proliferation and even enhances anti-oxidant capacity by activating the PPP pathway and keeping pyruvate away from mitochondrial oxidation to avoid the generation of excess ROS, implying that metabolic reprogramming can inherently increase anti-oxidant capacity that favors survival of cancer cells against high levels of ROS.³ Indeed, stabilization of HIF1- α , cysteine oxidation of PKM2 by increased ROS, activation of hexokinase 2 and major NADPH-producing PPP, *de novo* serine metabolism and NAD⁺ production from conversion of pyruvate into lactate by LDH, all upregulated along with the Warburg effect, can also presumably downregulate cytotoxic ROS.^{40,50,148–150} Furthermore, export of increased lactate leads to an acidic tumor microenvironment, which accounts for the resistance to many chemotherapy drugs from decreased uptake of the drugs and increased NADPH and GSH levels to maintain redox status.^{12,151,152} These mechanisms can potentially reduce the efficiency of chemotherapies expected to exploit vulnerabilities of cancer cells with presumably high ROS levels.

Recent findings that regulation of cellular signaling by redox homeostasis is highly sophisticated and that modulation of metabolism and ROS may produce conflicting results depending on whether cancer cells are resident in a tumor microenvironment or circulating in blood also emphasize that

benefits from the regulation of ROS are not easily predictable.¹⁰⁴ Nuclear respiratory factor 2 (Nrf2), a potent regulator of redox homeostasis, regulates ROS levels in normal cells by increasing expression of anti-oxidant genes. However, the activating mutations of Nrf2 or treatment of cancer cells with anti-oxidants can not only reduce ROS levels but also activate oncogenic activities.¹⁵³ Furthermore, another report that treatment with antioxidants, *N*-acetylcysteine and vitamin E increases cancer cell proliferation by reducing ROS, DNA damage, and even p53 expression in mouse and human lung tumor cells¹³⁸ again emphasizes that consequences from the modulation of ROS are hard to predict.

INFLUENCE OF OXIDATIVE STRESS ON METABOLIC ENZYMES

For predicting cellular response to ROS, it is important to understand how changes in ROS levels and redox imbalance can affect macromolecules in the progression of cancer. A significant body of evidence supports a crucial role for ROS in cellular functions and shows that ROS can interact with and modify biological macromolecules including DNA, lipids and proteins.¹⁹ However, surprisingly little is known about the cellular targets of ROS or how redox signaling is integrated in the oncogenic response.¹⁵⁴ ROS and/or redox imbalance-sensitive PTMs of metabolic enzymes, oncogenes, tumor-suppressor transcription factors and signaling molecules may

play considerable roles in metabolic reprogramming. The PTMs on these proteins identified to be modulated by redox changes in cancer cells are phosphorylation, nitration, cysteine oxidation, glutathionylation, acetylation, methylation and SUMOylation (Figure 3),^{19,155–159} and studies showed acetylation of most enzymes functioning in glycolysis and the Krebs cycle.^{160,161} Oxidative stress induced with menadione affects the activities of core metabolic enzymes, such as PDH, IDH, fumarase, GAPDH, malic enzyme and citrate synthase, as well as levels of various metabolites from glycolysis and the Krebs cycle.¹⁶² Hypoxia induces O-GlcNAcylation at serine 529 of PFK1 and inhibits PFK1 activity.¹⁶³ The S529 glycosylation of PFK1 redirects glucose flux toward oxidative PPP, leading to increased NADPH production and concentration of glutathione. It has long been known that ROS can activate Akt.^{164,165} Akt can phosphorylate serine 466 of PFK2, leading to activation of PFK1 by the PFK2 product F2,6BP.¹⁶⁶ Furthermore, ROS can induce oxidation of cysteine 358 of PKM2 and result in decreased PKM2 activity.⁴⁰ Importantly, it has been hypothesized that SUMOylation of key metabolic enzymes and glucose transporters may shift cellular metabolic strategies toward increased flux through the glycolytic pathway during periods of hypoxic stress.^{167–169} Mitochondrial aconitase catalyzes the reversible conversion of citrate to isocitrate. Oxidative stress causes various PTMs on aconitase resulting in functional impairment, and subcellular localization of the enzyme is shifted from mitochondria to the cytosol, resulting in slowing of the Krebs cycle.¹⁷⁰

Increased ROS levels can reduce oxidative phosphorylation via various mechanisms, including stabilization of HIF1- α , and cysteine modifications, including S-glutathionylation of complex I proteins, and affect cellular signaling from inactivation of phosphatases, including PTP1b, PTEN and MAPK phosphatases, favoring aerobic glycolysis and survival.^{171–174} Furthermore, ROS-dependent activation of receptor tyrosine kinases^{175,176} and stabilization of HIF1- α by ligand-induced activation of multiple receptor tyrosine kinases¹⁷⁷ have been shown. Thus, oxidative stress induced by chemotherapeutic interventions can aggravate metabolic reprogramming patterns in cancer cells by stabilization of HIF1- α and also possibly by PTMs, such as acetylation, and oxidation of metabolic enzymes, such as PKM2.

Transient oxidation of thiols in protein tyrosine phosphatases (PTPs) and other structurally related phosphatases, such as the tumor suppressor PTEN, leads to their reversible inactivation by the formation of either an intramolecular disulfide bridge or a sulfenyl-amide bond.^{173,178,179} Conversely, oxidation of some non-receptor protein tyrosine kinases leads to their activation, either by direct SH modification or indirectly by concomitant inhibition of PTPs that guides sustained activation of protein tyrosine kinases. PDK1 phosphorylates and inactivates PDH and consequently the pyruvate dehydrogenase complex.¹⁸⁰ It is known that PDK1 is commonly tyrosine phosphorylated in human cancers by diverse oncogenic tyrosine kinases.¹⁸¹ Although it is known that oncogenic tyrosine kinases, including fibroblast growth

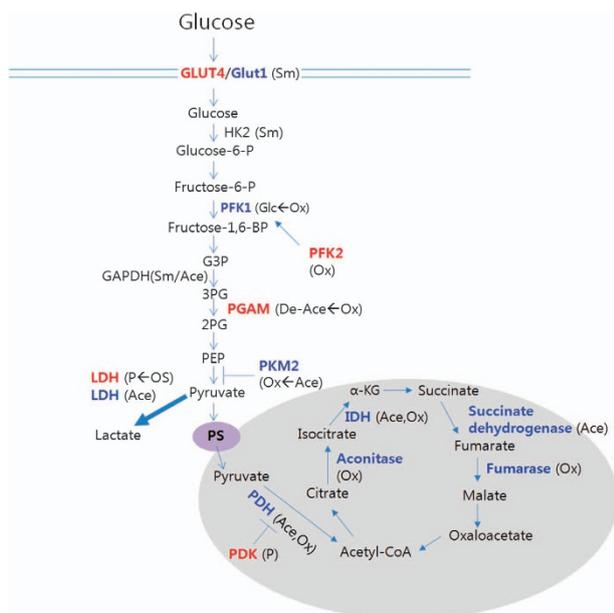


Figure 3 PTMs affecting enzymatic activities in glucose metabolism. Enzyme activities that are increased or decreased upon specific PTMs are shown in red and blue, respectively. We use arrows to indicate if any specific inducer is known for a PTM (inducer → resultant PTM). Whether sumoylation increases enzyme activities of HK2 and GAPDH is not clear; however, induced sumoylation promotes glycolysis, and the two enzymes are found to be SUMOylated.¹⁶⁷ Ace, acetylation; De-Ace, deacetylation; Glc, glycosylation; OS, oxidative stress; Ox, oxidation; P, phosphorylation; PGAM, phosphoglycerate mutase; Sm, SUMOylation.

factor receptor 1 (FGFR1), BCR-ABL and FLT3-ITD, can phosphorylate diverse metabolic enzymes such as PKM2, LDH-A and PDK1,¹⁵⁹ whether and how oxidative stress can affect the phosphorylation of these enzymes are still not clear. Interestingly, direct oxidation of cysteine 488 of FGFR1 decreases its activity.¹⁸² FGFR1 phosphorylates tyrosine 10 and 83 of LDH-A and enhances LDH-A enzyme activity to promote the Warburg effect and tumor growth by regulating NADH/NAD(+) redox homeostasis.¹⁸³ FGFR1 can also inhibit PKM2 by direct phosphorylation of PKM2 tyrosine 105.¹⁸⁴

All of these findings suggest that changes in ROS levels can affect metabolic rewiring directly or indirectly and also that ROS modulation of metabolic enzymes can be another potential mechanism of resistance to chemotherapeutic approaches, which are not easily observable *in vitro*. Thus, the current strategy of cancer therapy, based on the long-held speculation of supposedly higher ROS levels in cancer cells, aimed at finding a possible therapeutic window that can be used to eradicate cancer cells rather than normal non-transformed cells appears to warrant further studies. The caveat is that the studies cited that form the basis of our understanding about metabolic reprogramming and cellular response to oxidative stress have been acquired primarily from cancer cell lines rather than from intact cancer tissues.¹⁸⁵ Considering our ignorance regarding the role of ROS and anti-oxidant systems in different tumor microenvironments, the lack of reliable tools to evaluate *in vivo* levels of ROS, and the heterogeneity of tumor microenvironments comprised of cancer, stromal, endothelial, immune and even circulating cancer cells,¹⁰⁵ the general administration of oxidative stress inducers for cancer therapy should be weighed carefully.

CONCLUSIONS

In conclusion, for the successful implementation of cancer therapy, potential therapeutic targets will need to be identified based on their roles in cancer metabolism coupled with cancer-specific expression/isoforms, potential mutations and oncogenic control mechanisms as well as based on the prediction of whether functional reversal of specific metabolic pathways will counteract the effects of ROS modulation and vice versa. The parameters that we need to clarify may include cancer type and staging, tissue type in which the cancer originated (the metabolic profile of tumors depends not only on the type of genetic lesion but also on the tissue in which the mutation arises¹⁸⁶), duration and types of metabolic or ROS modulation, further genetic changes acquired from treatment, composition of the microenvironment, degree of metabolic synergy, the current lack of biomarkers to represent *in vivo* ROS levels, incomplete and confusing understanding of the role of ROS in cancer progression, *in vivo* validation of prior *in vitro* findings, identification of cellular targets of ROS and the competence of mitochondrial function in cancer cells.

A breakthrough will likely come from the advent of a technology that can detect *in vivo* redox status across a wide range of cancer cells at different stages as well as other non-transformed cells in the tumor microenvironment and

from the development of small molecules that can locally target specific biochemical nodes. We expect the feasibility of combined approaches targeting ROS and metabolism for successful cancer therapy to become an exciting topic of study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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