Reactive oxygen species (ROS) are a key determinant of cancer’s metabolic phenotype

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Cancer cells exhibit a wide range of metabolic phenotypes, ranging from strict aerobic glycolysis to increased mitochondrial respiration. The cause and utility of this metabolic variation is poorly understood. Given that cancer cells experience heavy selection within their microenvironment, survival requires metabolic adaptation to both extracellular and intracellular conditions. Herein, we suggest that reactive oxygen species (ROS) are a key determinant of cancer’s metabolic phenotype. Intracellular ROS levels can be modified by an assortment of critical parameters including oxygenation, glucose availability and growth factors. ROS act as integrators of environmental information as well as downstream effectors of signaling pathways. Maintaining ROS within a narrow range allows malignant cells to enhance growth and invasion while limiting their apoptotic susceptibility. Cancer cells actively modify their metabolism to optimize intracellular ROS levels and thereby improve survival. Furthermore, we highlight distinct metabolic phenotypes in response to oxidative stress and their tumorigenic drivers.

Introduction

There is a high degree of metabolic heterogeneity across various types of cancers. Nucleotide synthesis and glycolysis are often upregulated while changes in oxidative phosphorylation can be more variable. Otto Warburg was the first to characterize cancer as having a more glycolytic metabolism despite the availability of oxygen, labeled the “Warburg effect.” Aerobic glycolysis may be a cardinal feature of many tumors, but it does not represent the full spectrum of cancer metabolism.

Oxidative phosphorylation is not exclusive to benign cells and many studies in the past decade have observed a high rate of respiration in cancer cells. A related variation known as the “Reverse Warburg” involves cancer-associated fibroblasts adopting a glycolytic metabolism, excreting lactate into the microenvironment for the cancer cells to use aerobically. Furthermore, the role of glutamine as an alternative form of energy production has also been investigated extensively.

The heterogeneity of cancer metabolism is present on several levels. First, different types of cancers are often characterized by a more predominant form of metabolism. For example, renal cell carcinomas predominately exhibit the Warburg effect while breast cancer cells are more variable and have a greater tendency toward oxidative phosphorylation. Subtypes of cancer are characterized by different metabolic phenotypes such as in breast cancer where triple negative tumors are more glycolytic than ER-positive tumors. Even cancer cells within the same individual can vary when comparing metastatic lesions with primary tumors or while examining different sections of the same primary tumor. These types of variations are likely influenced by differing microenvironments, which may either induce cellular adaptations or select for well-adapted variants present in the tumor population. A common situation is where hypoxic cells near a tumor’s necrotic core become more glycolytic than cells at the vascularized periphery. Individual cancer cells demonstrate plasticity and can change their metabolic phenotype over time. Metabolic switching is observed in response to a variety of cancer treatments.

The cause of metabolic variation in cancer cells is poorly understood. One possibility is that random mutation events result in different tumorigenic drivers, which may have differing downstream effects on metabolism. Although this may be true early on in tumorigenesis, it is unlikely these early metabolic phenotypes remain unchanged. Successful tumor cells must survive and thrive under heavy selection within the body. Progression to advanced disease presents a variety of obstacles including hypoxia, acidosis, anoikis as well as survival in both the vasculature and distant organs. Therefore, metabolic phenotypes are not retained by-products of initial driver mutations but rather reflections of changing intracellular and extracellular conditions. Cancer cells must optimize their metabolism to achieve optimal fitness for their given microenvironment.

Herein, we suggest that reactive oxygen species (ROS) are a key determinant of cancer’s metabolic phenotype. Studies have shown that cancer cells have higher steady-state ROS levels. Intracellular ROS levels can be altered by oxygen saturation, energy availability and growth factor activity. ROS act as both environmental sensors and downstream effectors of...
signaling pathways. Cancer cells actively alter their metabolism to optimize ROS levels and thereby improve survival.

**Sources of ROS Generation That Can Facilitate Cancer Progression**

It has long been noted that cancer cells have higher steady-state ROS levels.\(^8\)\(^-\)\(^10\) The role of this pro-oxidative state within cancer cells has been controversial. Elevated ROS can trigger apoptotic mechanisms or directly impair and damage structures within the cell. This view of oxidative stress as merely a detrimental side effect of tumorigenesis is brought into question by evidence that ROS signaling may benefit cancer cells and is actively induced. For example, cancer cells require ROS to maintain telomerase activity.\(^10\) Active telomerase is a critical hallmark of cancer since it facilitates immortalization of the cell line. ROS can have differing effects depending on the type, duration of exposure and oxidative dosage. Although oxidative stress increases apoptosis and necrosis at high doses, it can promote cell survival when administered modestly.\(^11\) Temporally, acute ROS was found to lower breast cancer cell growth, while chronic exposure increased tumorigenic potential.\(^12\) Redox signaling pathways are necessary for cell-to-cell disassociation, migration and invasion.\(^13\)\(^-\)\(^17\)

Since cancer cells are often characterized by a high rate of proliferation compared to untransformed cells, ROS accumulation can be partially attributed to increased growth. Activated Ras and a variety of growth factors have been shown to enhance intracellular ROS including EGF, FGF, PDGF and TNF-α.\(^18\)\(^-\)\(^23\) The ROS generated by receptor-ligand interactions can mediate growth signaling itself.\(^24\) Furthermore, the growth process may be intrinsically tied to increased ROS generation due to higher metabolic activity and increased energy requirements. ROS are produced during disulfide bond formation through oxidative folding of proteins.\(^25\) In addition, fatty acid synthesis depletes the cell of NADPH, a necessary cofactor for antioxidant enzymes.

One of the main intracellular sources of ROS is the NADPH oxidase family of proteins (NOX). These membrane-bound proteins generate cytosolic ROS in response to a variety of stimuli including growth factors, oncogenic RAS and hypoxia.\(^22\)\(^-\)\(^24\)\(^-\)\(^28\) Therefore, NOX proteins translate environmental information into bursts of ROS production, effectively using ROS as a signaling intermediate. Oxidative species produced by NOX often facilitate survival and diminish apoptotic pathways.\(^22\) Expression of these proteins can provide the cells with a method of controlling and enhancing ROS production to activate beneficial downstream pathways.

In the context of cancer, these ROS-producing NOX proteins have been implicated in facilitating the invasion process.\(^8\) Cancer cells have even been manipulated to migrate directionally by using ROS-inducing light.\(^29\) Other evidence supporting the role of NOX in motility is their specific localization to invadopodia structures within the cell membrane. These membrane protrusions are specific to cancer and facilitate movement and invasion.\(^14\)\(^,\)\(^30\) Many of the effects of ROS signaling through NOX may be mediated by activation of Src kinase.\(^16\) This includes the ROS-induced production of matrix-degrading metalloproteinases.\(^31\)\(^,\)\(^32\)

Mitochondria are the other main source of intracellular ROS.\(^33\) Oxidative phosphorylation intrinsically produces ROS at various points along the electron transport chain.\(^34\) The baseline ROS produced by mitochondria can be enhanced by hypoxia, p53 or RAS activation, serum deprivation and apoptosis.\(^37\) The main sources of ROS are summarized in Figure 1. Despite the connection between apoptosis and overwhelming ROS production, moderate mitochondrial ROS can be beneficial to tumors. Experimental knockdown of the TRAP1 protein was found to increase respiration, ROS production and Src activation.\(^35\) Cells with higher respiration were found to be more invasive in this context. Studies where mitochondrial ROS was lowered through catalase overexpression or the addition of the SOD2-mimetic “MitoTempo” have also noted a decrease in cell migration and growth.\(^36\)\(^-\)\(^38\)

ROS production appears to be a critical function of mitochondria, which do not need to be functionally intact to perform this role. Mutant mitochondrial proteins result in electron transport chain inefficiencies and excess ROS, yet they can promote migration and invasion.\(^39\)\(^,\)\(^40\) The fact that a dysfunctional electron transport chain may be beneficial to tumorigenesis suggests that it is the increased production of ROS and not ATP that makes these cells more aggressive. Ishikawa et al. demonstrated that transferring defective mitochondrial DNA from a highly metastatic tumor cell line to a poorly metastatic cell line caused less respiration, but increased ROS production and metastatic potential.\(^41\) Treatment of these recipient cells with antioxidants suppressed this increase in metastatic potential, suggesting a causal relationship.

Another relevant publication examined the effects of complete mitochondrial DNA deletion on the ability of tumors to establish themselves in vivo.\(^32\) Cancer cells without mtDNA could not establish tumors if injected intravenously, yet these same cells became capable after first being injected subcutaneously and allowed to grow for some period of time. The authors found that these glycolysis-dependent cells acquired host mtDNA from the tumor microenvironment and progressively regained respiratory function in their transition from a primary tumor to circulating cells and finally to metastatic lesions. One might assume that the reason cells without mtDNA could not metastasize was due to a lack of mitochondrial energy production. Yet, no increase in ATP levels was found as the cells acquired more mtDNA from the environment. The authors did note an increase in mitochondrial ROS between the mtDNA null cells and those allowed to grow in vivo and re-establish respiration. Our interpretation of the study by Tan et al. supports the notion that invading cells require some degree of oxidative phosphorylation for the purpose of maintaining baseline ROS signaling.
Glycolysis Reduces Overabundant ROS to Increase Survival and Proliferation

Although ROS are a functional side effect of increased metabolism, overabundance is limiting to cell division. Proceeding through the cell cycle with high ROS results in DNA damage and poor clonogenic fitness. This was indeed found to be the case in cells with overactivated c-Myc, where an increase in proliferation was associated with higher ROS production and DNA damage.\(^4^{3}\) The poor clonogenic survival of these cells was rescued by antioxidant administration. When confronted with the possibility of extensive oxidative damage, cells shut down mTOR which is a key regulator of anabolic processes that result in proliferation.\(^4^{4–46}\) Conversely, active mTOR upregulates both growth and glycolysis.\(^4^{7–50}\)

A glycolytic metabolism is highly correlated with tumor proliferation. Knockdown or pharmacological inhibition of glycolytic enzymes reduces growth in a variety of cancers.\(^5^{0–53}\) Forced induction of respiration has led to slower growth rates both \textit{in vitro} and \textit{in vivo}.\(^6,54,55\) Conversely, lower oxidative phosphorylation due to mutant mitochondria increases both glycolysis and growth.\(^5^{6}\) The effects of glycolysis on proliferation are more profound under hypoxic conditions and while observing \textit{in vivo} models.\(^6,57,58\)

The connection between glycolysis and growth has often been attributed to cells attempting to divert glucose molecules toward anabolic pathways. After importing a glucose molecule, a cell can incorporate the carbon as biomass instead of burning it off as carbon dioxide in the mitochondria. However, the notion that glycolysis conserves glucose carbons is contradicted by the fact that the majority of imported glucose carbon is quickly excreted in the form of lactate. Recent work by Hosios \textit{et al.} has shown that cancer cell lines acquire only \(10\%\) of their dry mass from glucose with the majority coming from a variety of absorbed amino acids.\(^5^{9}\) The relationship between the induction of glycolysis and growth must go beyond using glucose for anabolic processes. What advantage does glycolysis offer if less energy is produced per glucose molecule when compared to oxidative phosphorylation? Although glycolytic shunts are important for nucleotide synthesis during proliferation, this does not explain the massive amount of glucose that is shifted down the central glycolytic pathway and excreted as lactate. One explanation is that glycolysis allows rapid ATP production while reducing the growth-limiting ROS associated with oxidative phosphorylation.

Switching to a more glycolytic metabolism causes a decrease in ROS.\(^6^{0}\) In a highly hypoxic environment, oxidative phosphorylation is unable to generate ATP and inefficiently creates excess radicals. The deficiency of ATP under these circumstances can also stimulate excess ROS secretion by NOX enzymes resulting in apoptosis. Since the mitochondria are a key source of ROS, diverting fuel away from oxidative phosphorylation is a highly efficient anti-oxidation mechanism. Furthermore, glucose can be diverted to the pentose phosphate pathway which generates the cofactor NADPH, needed for the antioxidant activity of glutathione.

Knockdown experiments support the role of glycolysis in reducing ROS levels. Shutting down a key enzyme in the glycolytic pathway such as LDHA results in the expected shunting of pyruvate toward the mitochondria along with higher oxygen consumption. A simultaneous increase in ROS production and decreased proliferation were found to accompany this knockdown.\(^7,6^{1}\) Forced induction of respiration causes an increase in ROS, ultimately resulting in less growth both \textit{in vivo} and \textit{in vitro}.\(^5^{4}\) The causal relationship between ROS and glycolysis

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**Figure 1.** A summary of the main factors affecting the balance of ROS. On the left side of the diagram are circumstances that increase intracellular ROS, while the right side shows ROS-lowering factors. [Color figure can be viewed at wileyonlinelibrary.com]
was highlighted in fibroblasts. In response to H₂O₂ treatment, cells increased glycolytic flux and NADPH content. Inhibition of this glycolytic compensatory response resulted in higher ROS and subsequent cell death.⁶²

A key mediator of glycolysis activation is the Hypoxia-inducible factor 1 (HIF-1), which can be activated by both hypoxia and ROS itself. Expression of mutant mitochondrial proteins was found to cause a switch to glycolysis due to ROS-dependent HIF expression, resulting in increased growth.⁶⁶ However, HIF-1 is not necessary for a glycolysis-mediated decrease in oxidation. This was demonstrated in HIF-1 knockout cells which showed increased ROS generation under hypoxic conditions. Subsequent forced expression of the downstream enzyme PDK1 effectively enhanced glycolysis while simultaneously lowering ROS and preventing apoptosis.⁶³ The induction of glycolysis by PDK1 instead of HIF-1 suggests that it was the switch toward glycolysis itself that caused a reduction in ROS and not another downstream target of HIF-1. Inhibiting PDK1 directly causes increased oxygen consumption and an increase in ROS even in normoxia.⁶⁷

Perhaps the strongest evidence on the importance of glycolysis as a mechanism of limiting ROS is found in the case of mitochondrial mutations. Transfection of cells with mutant mitochondrial proteins causes both an increase in ROS production and a compensatory increase in glycolysis.⁶⁹ One might initially assume that the increase in glycolysis is a form of maintaining energy production with defective mitochondria. Unexpectedly, exogenous antioxidants caused these cells to switch back to aerobic metabolism. Production of ATP was of secondary importance in this situation. Rather, the cells upregulated glycolysis to limit ROS production and would readily switch back to oxidative phosphorylation.

Antioxidant Enzymes and Glutaminolysis Can Facilitate Advanced Malignancies

Although a certain level of ROS may improve motility and invasion, cancer cells often reach apoptotic levels of oxidative stress within distant organs. Piskounova et al. showed that cells within metastatic lesions have substantially higher levels of mitochondrial ROS than cells in circulation or the subcutaneous primary tumor. The foreign environment of the target organ in advanced cancers and lack of any established vasculature can increase ROS-mediated cell death. The metastatic tumors themselves attempt to counteract the higher oxidative stress by increasing their NADPH content.⁶⁴

Important antioxidant enzymes in cancer include glutathione-s-transferase (GST), glutathione peroxidase (GPx), glutamate cysteine ligases, superoxide dismutates (SODs), catalase and thioredoxin.⁶⁵ Inhibition of antioxidant pathways compromises the cancers ability to handle oxidative stress and results in cell death.⁶⁶,⁶⁷ Regulators of the antioxidant response also control metabolic phenotypes. Wild-type p53 is known to promote respiration. However, it can also increase antioxidant capacity through TIGAR, which facilitates survival under starvation conditions or other stressors. Treatment with H₂O₂ or chemotherapy can increase TIGAR expression while antioxidants have the opposite effect.⁵⁴,⁶⁸ Glioblastoma was found to highly express TIGAR, which was necessary for survival while restricting both glucose and oxygen.⁷⁹,⁷⁰ Likewise, the mitochondrial biogenesis factor PGC-1 increases antioxidant enzyme expression to reduce ROS and compensate for higher oxidative phosphorylation.

Nrf2 is another transcription factor heavily implicated in both metabolism and redox. It is activated by both ROS and oncogenes such as Kras, Braf and Myc.⁷¹ It induces the production of the NADPH cofactor through the pentose phosphate pathway (PPP), which simultaneously enhances proliferation through increased nucleotide synthesis.⁷²–⁷⁵ Nrf2 is notable in that it not only increases the expression of antioxidant enzymes like glutathione, but it also facilitates the availability of key precursors including glutamine and cysteine.⁷⁶ Treatment with H₂O₂ directly results in the increased import of cysteine, the rate-limiting protein for the formation of glutathione.⁷⁷

Aggressive cancers in particular are found to have a dependence on glutamine availability.⁷⁸–⁸⁰ While glutaminolysis does play an important role in replenishing metabolic intermediates, its importance for redox is underappreciated. Glutamine can increase antioxidant capacity since it is a precursor for glutathione and can also help import cysteine through the xCT antiporter.⁷⁷ In addition, glutamine can be used to generate NADPH through the malate pathway, providing a multitude of substrates for the glutathione system. Inhibition of glutamine metabolism sensitizes lung cancer cells to radiation.⁸¹ Similarly, the folate pathway can also support detoxification through two mechanisms. First, SHMT2 is involved in the synthesis of another glutathione precursor in glycine. Subsequently, the enzyme MTHFD1 can produce additional reducing equivalents in the form of NADPH. Inhibition of MTHFD1 was found to substantially reduce metastatic burden in a mouse model.⁶⁴

Cancer Metabolism is Contextual: A ROS-Dependent Model

Both extracellular and intracellular factors can affect cancer’s metabolic phenotype, resulting in survival adaptations in the tumor population. The central integrator of these parameters is the intracellular ROS level, which rapidly provides the cell with information on a variety of stressors. ROS status is affected by the tumor’s respiration activity, mitochondrial abundance, nutrient availability, oxygen content, growth factors, redox capabilities and exogenous therapeutics. A variety of signaling pathways are controlled by the cell’s oxidation status, which can increase either survival or cell death depending on the level of exposure.⁹ The differing experimental effects of ROS on cancer mirror the controversies regarding metabolism. Recent studies suggest that antioxidants accelerate tumor migration and metastasis.⁵⁴,⁸²–⁸⁴ In direct contrast, other work has found that antioxidant
administration prevents cancer proliferation, invasion, migration and metastasis.\textsuperscript{36,37,83–90}

The differing effects of ROS modulation are context dependent. Cells with low oxidation status may seek to enhance their ROS signaling for a survival and motility advantage. In contrast, cells under high stress will aim to reduce ROS to avoid damage. It is essential for cancer cells to optimize their ROS levels to maintain tumor progression. Inhibition of both ROS generation or antioxidant capacity in the same transformed cells was found to reduce proliferation both \textit{in vitro} and \textit{in vivo}.\textsuperscript{68} Likewise, NOX-generated ROS has dose-dependent effects with moderate levels increasing proliferation and high levels causing cell death.\textsuperscript{91}

The necessity for tightly controlled buffering is evident by the opposing effects of many metabolic regulators. Both p53 and PGC-1a increase oxidative phosphorylation, but at the same time upregulate compensatory antioxidant enzymes. Another example is activated Wnt, which induces both the ROS-promoting Rac1 and the ROS-inhibiting TIGAR.\textsuperscript{68} Conventional thinking emphasizes ROS as a by-product of cellular metabolism. However, achieving ideal ROS signaling may in fact be the driver of the heterogeneity we observe in cancer’s metabolic phenotypes. Although metabolism in early tumorigenesis is likely determined by the cancer’s oncogenic drivers, selection pressures force cells to adapt to their extracellular and intracellular conditions. ROS are both a measure of stress and an effector of response mechanisms. The importance of maintaining ROS equilibrium is highlighted in experiments where glucose depleted cells were rescued through overexpression of mitochondrial catalase, SOD or the addition of NAC.\textsuperscript{92–94} Although these starved cells were deficient in ATP, the more pertinent parameter resulting in apoptosis was their elevated ROS level.

Cells with low ROS may increase oxidation through upregulation of NOX or oxidative phosphorylation, thereby enhancing downstream signaling pathways. The antioxidant NAC has been shown to induce a rapid oxidation response within the mitochondria due to increased respiration.\textsuperscript{95} This may highlight the cell attempting to increase its suddenly lower ROS levels. High doses of NAC paradoxically increase mitochondrial mass and mitochondrial ROS.\textsuperscript{96} The benefits of mitochondrial ROS are dependent on the cell’s overall level of stress, which can alter energy demands and NOX activity. Overexpression of catalase (lowering hydrogen peroxide) or addition of the antioxidant MitoTempo (lowering superoxide) inhibited survival and migration under normal conditions.\textsuperscript{36–38} In contrast, glucose-deprived cells have improved survival with the overexpression of mitochondrial catalase or SOD.\textsuperscript{93,94} These findings suggest some cells at baseline benefit from mitochondrial ROS, but its reduction may be important under stressful circumstances where ROS may already be too high, such as glucose deprivation.

As cancers progress to more advanced stages they are more likely to encounter environmental stressors at both the primary and metastatic sites. An inhospitable environment can drive metabolism in differing directions. Hypoxic cells transition to a Warburg metabolism due to the ROS generated by NOX and the mitochondria under low oxygen.\textsuperscript{97–99} Both HIF-1 and ERK1/2 can facilitate the switch to a glycolytic metabolism in response to ROS.\textsuperscript{100} This is suggestive of the underlying compensatory function of the Warburg effect. In contrast, ROS induced by low glucose can increase respiration and ATP generation.\textsuperscript{101} How opposing types of metabolism are both activated by intracellular oxidation is not well understood, but likely involves an integration of multiple sensory pathways. HIF-1 and PGC-1 can both be activated by ROS yet they promote opposite metabolic phenotypes and reciprocally inhibit each other.\textsuperscript{54,102,103} The energy sensor AMPK may be a one of the molecular switches that determines the cellular response to ROS. While AMPK usually increases oxidative phosphorylation during starvation through PGC-1, it can also activate HIF-1 and thereby glycolysis under an assortment of different stressors.\textsuperscript{62,102,104–107} Another consequence of AMPK activation is the induction of a more quiescent phenotype due to inhibition of the growth regulator mTOR.\textsuperscript{105} Whether glycolysis or oxidative phosphorylation is induced, the AMPK stress response ensures the cell will not proliferate rapidly. Measuring the balance between mTOR and AMPK activation may be a useful measure of tumor dormancy.

**Metabolic Phenotypes in Response to Elevated ROS**

Since tumors have higher baseline ROS due to growth and environmental stressors throughout the dissemination process, an overabundance of oxidation presents a greater obstacle than a deficiency. When comparing primary and metastatic cells, lesions at distant sites experienced higher oxidative stress.\textsuperscript{64} Treatment with the antioxidant NAC enhanced metastatic burden but not growth of subcutaneous tumors, suggesting they have lower ROS. Different cell lines demonstrate different methods of combating stress.\textsuperscript{108} We propose that cancer cells may reduce ROS through three primary mechanisms. First, tumors may increase their detoxification capacity by upregulating key antioxidant enzymes. Alternatively, cells can lower ROS production by either upregulating glycolysis and thereby reducing oxidative phosphorylation or becoming quiescent to reduce metabolic demand. We speculate that cancer cells exhibit two general phenotypes summarized in Figure 2. Many tumors reside on a spectrum between the metabolic extremes we describe here, with even highly glycolytic tumors still performing respiration. Pre-existing mutations may influence which phenotype is expressed, for example cells with mutant mtDNA will perform glycolysis while tumors retaining wild-type P53 may favor oxidative phosphorylation. However, environmental factors may have an even greater influence on metabolic phenotype and subsequently the differing ways these cells respond to elevated ROS.

In the first theoretical phenotype, cancer cells are characterized by high oxidative phosphorylation, antioxidant
enzymes expression and slow proliferation.\textsuperscript{70,109} This phenotype is favorable when nutrients are scarce (active AMPK) but oxygen levels are not limiting. These cells depend on the ROS-dependent activation of P53 or PGC-1, which concurrently increase both respiration and antioxidant capacity. However, this metabolic response may be insufficient at higher doses of oxidative damage. While moderate doses of ROS have been shown to increase PGC-1 and respiration in a variety of cell types, high ROS has the opposite effect.\textsuperscript{103,109,110} By reducing growth, these cells can decrease ROS production. AMPK activation diminishes fatty acid synthesis and thereby conserves NADPH.\textsuperscript{111} Circulating tumor cells are largely quiescent and were shown to specifically upregulate PGC-1, indicating a more aerobic metabolism compared to both primary and metastatic tumors in the same organism.\textsuperscript{112} A further increase in ROS during starvation conditions may then enhance autophagy, which is inhibitory to growth. Through recycling organelles including the mitochondria, autophagy reduces cellular metabolism and thereby limits ROS production.\textsuperscript{113,114}

The second phenotype we propose involves a high level of glycolysis which may be more viable for proliferating cells when glucose is abundant. Earlier lines of thought suggested glycolysis was primarily a means of accumulating glucose carbons for biomass accumulation.\textsuperscript{115} In contrast, we propose glycolysis in proliferating cells is primarily a method of producing energy while avoiding mitochondrial ROS. This is supported by the fact that amino acids and not glucose, are the primary building blocks for cancer growth and biomass accumulation.\textsuperscript{59} Cells can reduce energetic and redox requirements by absorbing organic molecules for biosynthesis instead of having to generate them \textit{de novo} through glycolytic pathways.

Both glycolysis and antioxidant upregulation may provide the greatest survival advantage and allow cancers to thrive under extreme conditions. This is possible through the ROS-dependent induction of both HIF-1 and the transcription factor Nrf2, which itself increases HIF-1 activation.\textsuperscript{116–118} This relationship is found during intermittent hypoxia exposure, where NOX is responsible for activating both Nrf2 and subsequently HIF-1. In this somewhat paradoxical relationship, a stress-induced increase in ROS activates more cytoplasmic ROS production by NOX, which finally signals for the elimination of ROS through increased glycolysis and antioxidant enzymes. Although the pathway may seem convoluted, it illustrates how intertwined ROS are with metabolism and their importance as a determinant of phenotype.

**Conclusions and Treatment Implications**

Diversity in cancer metabolism is facilitated not only by mutations, but also by intrinsic plasticity and adaptation. Piskounova \textit{et al.} found that metastatic tumor lesions have lower mitochondrial mass than primary tumors.\textsuperscript{64} Transplanting either primary or metastatic tumors from one mouse into another resulted in the same metabolic pattern forming. The site of transplantation determined the mitochondrial mass
not the origin of the tumor cell. Many cancer cells display the capacity for reversible metabolic changes that depend on the environment and not the site of origin. Understanding the factors that determine cancer metabolism and facilitate tumor survival will allow us to pinpoint therapeutic targets and predict mechanisms of resistance.

Since each patient’s cancer is unique, metabolic and redox profiling of biopsies can help guide individualized treatments. Assays previously used for the direct measurement of ROS include the oxidation of dihydroethidine (DHE) to measure superoxide levels and CDCFH2 for hydrogen peroxides. However, the unstable and reactive nature of ROS make these unsuitable for biopsy analysis. A more stable downstream marker of ROS would be the level of advanced oxidation protein products. In addition, analysis of NADPH/NAD+ ratios as well as the expression of specific antioxidant enzymes would assist with an understanding of a cancer’s adaptive antioxidant response. Currently a standardized range for oxidation status within cancer cells does not exist, therefore normal surrounding tissue can be used as a comparative control. New PET imaging technologies are emerging that may help facilitate ROS measurement without biopsies.119,120

If baseline ROS status is low, treatment with an antioxidant may further deplete the cancer of necessary ROS signaling. Knowing if cells have higher baseline ROS levels can be helpful in anticipating responsiveness to ROS-inducing therapy.121 Many traditional chemotherapies increase oxidation, while cancers with multi-drug resistance may be targeted by a selective agent like Tesmilifene which increases ROS in cells expressing drug efflux pumps.122 Furthermore, we can combine ROS induction with an inhibition of the compensatory metabolic responses thus causing synergistic oxidation. For highly proliferative cancers, one could treat with ROS-inducing agents combined with inhibitors of glycolysis such as 2-DG and DCA. In tumors with limited nutrient availability and subsequent increased respiration, inhibitors of oxidative phosphorylation would be more effective. Since these cells would also be dependent on AMPK to maintain quiescence, they may also be susceptible to AMPK inhibitors.

In conclusion, ROS and metabolism are heavily intertwined entities. We have identified ROS levels as a central determinant of cancer’s metabolic phenotype, alongside other environmental factors. Just as each patient is a unique individual that requires a personalized approach, the tumors they develop have different metabolic drivers and intracellular conditions. Through a deeper understanding of the implications of ROS levels on metabolism, we can better determine appropriate treatments through rationally targeting both redox pathways and metabolic adaptations.

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