

Glucose Deprivation-Induced Oxidative Stress in Human Tumor Cells

A Fundamental Defect in Metabolism?

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ABSTRACT: Recently, glucose deprivation-induced oxidative stress has been shown to cause cytotoxicity, activation of signal transduction (i.e., ERK1, ERK2, JNK, and Lyn kinase), and increased expression of genes associated with malignancy (i.e., bFGF and c-Myc) in MCF-7/ADR human breast cancer cells. These results have led to the proposal that intracellular oxidation/reduction reactions involving hydroperoxides and thiols may provide a mechanistic link between metabolism, signal transduction, and gene expression in these human tumor cells. The current study shows that several other transformed human cell types appear to be more susceptible to glucose deprivation-induced cytotoxicity and oxidative stress than untransformed human cell types. In a matched pair of normal and SV40-transformed human fibroblasts the cytotoxic process is shown to be dependent upon ambient O₂ concentration. A theoretical model to explain the results is presented and implications to unifying modern theories of cancer are discussed.

INTRODUCTION

For 70 years it has been noted that cells that have undergone neoplastic transformation (cancer cells) demonstrate altered metabolism when compared to untransformed (normal) cells.¹⁻⁴ The most pronounced and almost universal metabolic disruptions appear to involve metabolism of glucose and the loss of regulation between glycolytic metabolism and respiration.¹⁻⁴ In general it has been found that cancer cells exhibit increased glycolysis and pentose phosphate cycle activity, while demonstrating only slightly reduced rates of respiration.¹⁻⁴ Initially these metabolic differences were thought to arise as a result of "damage" to the respiratory mechanism and tumor cells were thought to compensate for this defect by increasing glycolysis.¹ However, studies of the mechanism involved in these metabolic changes focused on ATP production and energy metabolism,¹ which turned out not to be a

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fruitful line of investigation for understanding carcinogenesis or designing cancer therapies.

Recently, it was discovered that glucose deprivation causes cytotoxicity in the MCF-7/ADR human multidrug-resistant breast carcinoma cell line.⁵⁻⁸ Glucose deprivation-induced cytotoxicity in this model system was found to be preceded by the rapid activation of several signal transduction pathways (within 10 min) including extracellular regulated protein kinases (ERK1/ERK2), Lyn kinase (a *src* family kinase), and c-Jun N-terminal kinase (JNK).^{7,8} Likewise the activities of MEK, Raf, Ras, and PKC were found to increase rapidly in glucose-deprived MCF-7/ADR cells.^{5,7,8} In addition glucose-deprivation of MCF-7/ADR caused an increase in the DNA-binding activity of the AP-1 transcription factor as well as an increase in the expression of cellular homologues of oncogenes (c-Fos, c-Jun, c-Myc) and the angiogenic factor, basic fibroblast growth factor (bFGF).^{5,6} Finally, it has been shown that over-expression of the mitochondrial protein, Bcl-2, protects MCF-7/ADR from glucose deprivation-induced cytotoxicity, suggesting that mitochondrial metabolism might be involved in the process that caused cytotoxicity.⁶ These results show that removal of glucose from these human tumor cells results in cytotoxicity as well as activation of signal transduction pathways and increased expression of genes thought to be involved with neoplastic transformation. These results support the hypothesis that alterations in glycolytic metabolism could be linked by some process to signal transduction and gene expression associated with the malignant phenotype.

Since these original observations, studies to determine if oxidation/reduction reactions mediate glucose deprivation-induced cytotoxicity as well as the process linking glycolytic metabolism to alterations in signal transduction and gene expression were accomplished.^{9,10} The fact that ERK1/ERK2 are members of the mitogen-activated protein kinase (MAPK) family which had been previously shown to be activated by oxidants (such as H₂O₂) and suppressed by reductants,^{11,12} lead to the hypothesis that reductants such as N-acetylcysteine (NAC) could suppress activation of MAPKs induced by glucose deprivation in MCF-7/ADR. This hypothesis was supported by the findings that MAPK activation during glucose deprivation could be inhibited by treatment with the thiol antioxidant NAC.⁹ These studies went on to show that glucose deprivation-induced cytotoxicity, activation of Lyn kinase and JNK as well as increases in steady state levels of mRNA coding for bFGF and c-Myc could all be inhibited by treatment with 1 mM NAC.^{9,10}

The fact that a thiol-containing antioxidant was capable of inhibiting glucose deprivation-induced activation of signal transduction, increased expression of genes thought to be involved with maintenance of the malignant phenotype, and cytotoxicity lead to the hypothesis that oxidative stress was responsible for the effects seen during glucose deprivation in MCF-7/ADR. This hypothesis was supported by experiments showing that glucose deprivation resulted in the stimulation of glutathione (GSH) synthesis (2-fold), an increase in steady state levels of intracellular oxidized glutathione (GSSG) content (3- to 10-fold), and an increase in intracellular prooxidant production (2- to 4-fold).^{9,10} A causal link between glucose deprivation-induced oxidative stress and glucose deprivation-induced cytotoxicity, activation of signal transduction, and increased gene expression was supported by the fact that NAC not only inhibited changes in signal transduction and gene expression, but entered the cells in the reduced form and inhibited parameters indicative of oxidative

stress and cytotoxicity.^{9,10} Finally, the hypothesis that metabolism of O_2 to hydroperoxides was the source of increased prooxidant production was supported by the fact that pyruvate, an intracellular hydroperoxide scavenger,¹³ inhibited increased prooxidant production as well as cytotoxicity during glucose deprivation.⁹

After the formation of glucose 6-phosphate (via hexokinase) the major pathways of glucose metabolism include glycolysis and the pentose phosphate cycle.⁴ Glycolysis results in the formation of pyruvate and the pentose phosphate pathway results in the formation of NADPH.⁴ Pyruvate, in addition to being a substrate for the formation of acetyl-CoA and energy metabolism via the tricarboxylic acid (TCA) cycle and mitochondrial oxidative phosphorylation, has been shown to scavenge H_2O_2 and other hydroperoxides.¹³ NADPH, by virtue of being the source of reducing equivalents for the glutathione/glutathione peroxidase/glutathione reductase system, has also been shown to participate in the metabolic decomposition of H_2O_2 and organic hydroperoxides.¹⁴ Therefore, in addition to its well known role in energy production, glucose metabolism appears to be integrally related to the metabolic detoxification of intracellular hydroperoxides formed as byproducts of oxidative metabolism. In fact, increasing glucose concentrations in tissue culture media has been shown to render CHO cells resistant to H_2O_2 -induced cytotoxicity.¹⁵ Because glucose metabolism appears to be involved with the detoxification of intracellular hydroperoxides, we propose the theoretical model shown in FIGURE 1, to explain the results observed during glucose deprivation in MCF-7/ADR cells.

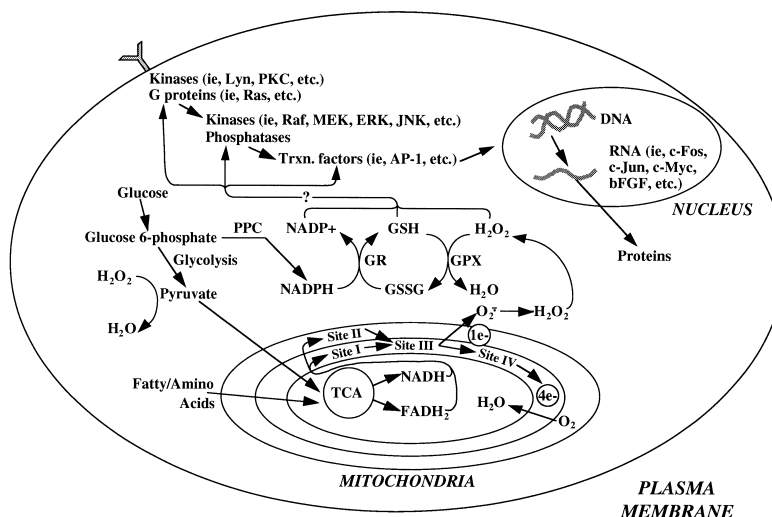


FIGURE 1. Theoretical model outlining of pathways contributing to the observed effects of glucose deprivation-induced oxidative stress in MCF-7/ADR. TCA = Tricarboxylic Acid Cycle; PPC = Pentose Phosphate Cycle; GR = Glutathione Reductase; GPX = Glutathione Peroxidase, ? = possibly thioredoxin, glutaredoxin, Ref-1, or direct oxidation/reduction.

During glucose deprivation steady state levels of intracellular prooxidants (presumably hydroperoxides) appear to increase immediately.¹⁰ This suggests that hydroperoxides are being produced by ongoing metabolic processes and that the metabolic decomposition of these prooxidants is compromised by the removal of glucose probably via a decrease in intracellular NADPH and pyruvate (FIG. 1). We hypothesize that prooxidant production is occurring via mitochondrial electron transport chain activity because in the absence of glucose, fatty acids and amino acids would provide alternative substrates for the TCA cycle leading to the production of NADH and FADH₂ as the source of electrons for mitochondrial ATP production.⁴ During mitochondrial respiration, O₂ acts as the terminal acceptor of electrons, with the 4-electron reduction of O₂ yielding H₂O. However, there exists a finite probability (which differs in different tissues) that 1-electron reduction of O₂ to yield superoxide will occur,¹⁶ probably at Site I (NADH-dehydrogenase) or Site III (ubiquinone-cytochrome b) of the electron transport chain.¹⁶ Superoxide then rapidly dismutates to form H₂O₂. It has been estimated during normal respiration 1–4% of O₂ consumption results in superoxide and hydrogen peroxide production.¹⁶ Therefore, decreased peroxide scavenging (by pyruvate and NADPH dependent pathways) during glucose deprivation, could result in increased steady state levels of prooxidants (i.e., H₂O₂) produced as by products of mitochondrial electron transport chain activity (FIG. 1).

Increased steady state levels of prooxidants during glucose deprivation cause oxidative stress and cytotoxicity as evidenced by the accumulation of GSSG and increased clonogenic cell killing, which are inhibited by the thiol antioxidant (NAC) as well as a peroxide scavenger (pyruvate^{9,10}). The cells appear to respond to glucose deprivation-induced oxidative stress by increasing the synthesis of glutathione^{9,10} but in the absence of substrates necessary for the regeneration of NADPH, glutathione cannot be maintained in the reduced state.¹⁰ In support of this notion glutamate rescues MCF-7/ADR cells from glucose deprivation-induced cytotoxicity and suppresses prooxidant production, presumably via the capacity of glutamate dehydrogenase to generate NADPH during the conversion of glutamate to α -ketoglutarate.^{4,9} Because the thiol reductant (NAC) inhibits: 1) increased prooxidant production, 2) accumulation of oxidized glutathione, 3) increased synthesis of glutathione, and 4) activation of signal transduction and gene expression, we hypothesize that oxidation reactions involving thiols and hydroperoxides are causally involved with the intracellular signaling processes activated during glucose deprivation (FIG. 1).

Glucose deprivation-induced signal transduction cascades are believed to originate in either a receptor-dependent or a receptor-independent fashion. The former case is mediated through an interaction of cellular membrane receptors or G protein coupled receptors with protein kinases. The latter case is mediated through activation of non-receptor protein kinases. Activation of up-stream molecules (i.e., Lyn kinase, PKC, Ras) leads to the activation of a series of intermediary kinases (i.e., Raf, MEK) that ultimately activate cytosolic kinases (i.e., ERKs and JNKs). These cytosolic kinases then phosphorylate substrates (i.e., c-Fos and c-Jun) leading to the activation of transcription factors (i.e., AP-1) which bind target genes leading to the synthesis of new gene products (FIG. 1). Signal transduction cascades are believed to stand poised ready to sense intra- or extra-cellular stimuli and when activated are

believed to redirect cellular function to respond to the stimulus. Our results, using MCF-7/ADR cells, suggest that alterations in oxidative metabolism caused by the removal of glucose trigger a number of these signaling cascades (i.e. Lyn kinase, PKC, Ras, Raf, MEK, ERKs, and JNK) resulting in the activation of transcription factors (i.e., AP-1) and increased expression of cellular homologues of oncogenes (i.e., c-Myc, c-Fos, and c-Jun) as well as angiogenic factors (i.e. bFGF) (FIG. 1).

At present it is unknown how glucose deprivation-induced oxidative stress activates signal transduction and gene expression, but a consideration of the literature reveals three distinct possibilities. First, it is well known that proteins involved in signaling cascades (i.e., Lyn kinase, Ras, AP-1) contain critical sulfhydryl residues that are sensitive to oxidation/reduction reactions and appear to modulate the transmission of signals via alterations in the activity of the signaling proteins.¹⁷⁻²⁰ It is therefore possible that direct oxidation reactions occurring at critical sulfhydryl residues could result in changes in activity of these signaling cascades.^{17,20} Secondly, it is known that alterations in the steady state of oxidized/reduced ratios of NADP⁺/NADPH and GSH/GSSG can alter the oxidation reduction status of thiol residues in thioredoxin and glutaredoxin that are capable of transmitting these signals to oxidation/reduction sensitive sites on kinases and transcription factors involved in signaling cascades.^{20,21} Finally, it is also known that proteins [i.e., redox factor-1 (Ref-1)] containing critical sulfhydryl residues can interact with thioredoxin to transmit redox signals to thiol containing transcription factors such as AP-1, thereby altering their DNA-binding activity.²⁰ We hypothesize that these types of alterations in thiol redox status occurring on kinases, phosphatases, G-proteins, and/or transcription factors “sense” changes in intracellular oxidation/reduction reactions during glucose deprivation of MCF-7/ADR cells and result in the activation of signal transduction cascades leading to increases in gene expression (FIG. 1). In this way alterations in the flow of electrons through redox-sensitive signaling circuitry could represent one means by which alterations in electron transport chain activity are coupled to alterations in gene expression necessary to redirect cellular function.

While the model in FIGURE 1 is partially supported by the data gathered using the MCF-7/ADR human breast carcinoma cells, the generality of these results to other normal and tumor cell lines remains largely unknown.¹⁰ Furthermore, it is unclear if alterations in glycolytic metabolism that have been noted for many years in transformed cells¹⁻⁴ relate to their susceptibility to glucose deprivation-induced cytotoxicity and oxidative stress.

The studies presented here were designed to determine:

1. if glucose deprivation induces cytotoxicity and oxidative stress in a variety of transformed human cell types;
2. if untransformed and transformed human cells are differentially susceptible to glucose deprivation-induced cytotoxicity and oxidative stress; and
3. if glucose deprivation-induced cytotoxicity and oxidative stress are dependent upon O₂-metabolism.

To accomplish these goals two matched pairs of SV40-transformed and untransformed human fibroblasts as well as HT29 human colon carcinoma cells and aortic smooth muscle cells were deprived of glucose. GSSG content was used as an index of oxidative stress and clonogenic cell survival was used as an endpoint indicative of

cytotoxicity. To begin to determine if the metabolism of O₂ was involved in the cytotoxic process, selected experiments were done in 21% O₂ vs. 4% O₂.

MATERIALS AND METHODS

Cells and Culture Conditions

IMR90 (rep# I90 PO4) untransformed (normal) human fibroblasts and their SV40-transformed counterparts [designated IMR90 SV40 (rep# AG02804C)] were obtained from the Coriell Institute and maintained in Eagle's MEM supplemented with 1 × MEM vitamins, 1 × MEM essential amino acids, 2 × non-essential amino acids and 20% fetal bovine serum (Hyclone). GM00037F untransformed (normal) human fibroblasts and their SV40-transformed counterparts (GM00637G) were obtained from the Coriell Institute and maintained in Eagle's MEM with 10% fetal bovine serum. HT29 human colon carcinoma cells were obtained from ATCC and maintained in McCoy's 5A media supplemented with 10% fetal bovine serum. Untransformed human aorta smooth muscle cells were obtained from ATCC (#CRL-1999) and maintained in F12K media supplemented with 2 mM glutamine, 0.01 mg/ml insulin, 0.01 mg/ml transferrin, 10 ng/ml sodium selenite, 0.02 mg/ml endothelial cell growth supplement, and 10% fetal bovine serum. All stock cultures were maintained in 5% CO₂ and air in a humidified 37°C incubator in the absence of antibiotics.

Glucose Deprivation Conditions and Cell Survival Experiments

For experiments, cells were plated in 60 mm tissue culture dishes and grown for 2 to 4 days until each dish contained 1–2 × 10⁶ cells in the presence of antibiotics (penicillin/streptomycin). At the beginning of each experiment the cells were rinsed with phosphate buffered saline (PBS) to remove glucose and placed in media containing all other additives except glucose and dialyzed fetal bovine serum as previously described.^{9,10} Control cultures were treated identically except glucose was added at the normal concentration found in media (1 g/L). Cells were then placed in an incubator and harvested at the times indicated. For experiments in less than ambient O₂ (21% O₂ vs. 4% O₂), a Heraeus Instruments model 6000 (BB6220) tri-gas incubator was utilized. The desired O₂ concentrations were verified using both the internal detector in the incubator as well as external gas analyzers calibrated to certified gas standards. Following exposure to glucose deprivation, clonogenic cell survival was assayed as previously described⁹ and surviving fraction of glucose deprived cells was normalized to the respective control plus glucose.

Biochemical Analysis

Following treatment cells were scrape-harvested in PBS at 4°C, centrifuged, the PBS discarded and the cell pellets frozen at –80°C. Samples were thawed and homogenates were prepared as described.^{9,10} Total glutathione (GSH + GSSG), glutathione (GSH), and glutathione disulfide (GSSG) were determined using a spectrophotometric recycling assay and normalized per mg protein as previously described.^{9,10}

RESULTS

FIGURE 2 shows the results of experiments where normal and transformed cells were exposed to 48 hours of glucose deprivation and clonogenic cell survival was assayed. IMR90 and GM00037F normal human fibroblasts deprived of glucose for 48 hours demonstrated no clonogenic cell killing, relative to their respective control (FIG. 2). The untransformed human aortic smooth muscle cells deprived of glucose for 48 hours demonstrated a slight (20%) reduction in clonogenic cell survival (relative to their control), but this did not reach statistical significance ($p > 0.05$, FIG. 2). In contrast, the transformed cells (IMR90 SV40, GM00637G, and HT29) demonstrated significant ($p < 0.05$) clonogenic cell killing following 48 hours of glucose deprivation (FIG. 2). The glucose-deprived IMR90 SV40 demonstrated $\approx 50\%$ cell killing, while GM00637G as well as HT29 demonstrated $> 90\%$ cell killing, relative to their respective controls (FIG. 2). These results indicate that, while differences exist between the transformed cell lines, they are in general more susceptible to glucose deprivation-induced cytotoxicity than the untransformed cells tested.

Glutathione analysis following 48 hours of glucose deprivation in untransformed cells (IMR90, GM00037F, and aortic smooth muscle) showed no change in total glutathione content (GSH + GSSG) (FIG. 3A). Furthermore, GSSG was below the limit

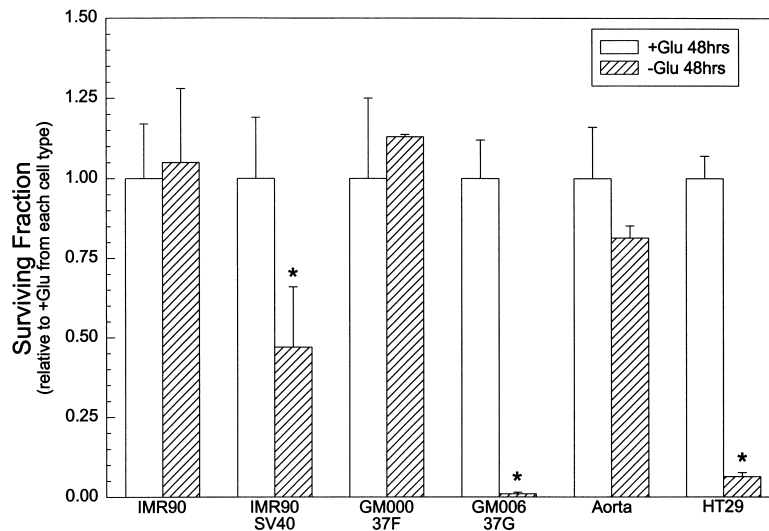


FIGURE 2. Clonogenic cell survival data showing the differential susceptibility of untransformed (IMR90, GM00037F, Aorta) and transformed (IMR90 SV40, GM00637G, HT29) human cell types to glucose deprivation-induced cytotoxicity. Errors represent ± 1 standard deviation (SD). Asterisks indicate significant differences between +Glu and -Glu from each cell line ($p < 0.05$, paired t-test). Data from IMR90 and IMR90 SV40 represent the mean of at least 12 determinations from 3 separately treated dishes in 2 separate experiments. Data from all other cell types are the mean of at least 4 determinations from each treatment group in one experiment.

of detection in the glucose-deprived untransformed cells (FIG. 3B). In contrast, glucose deprivation of transformed cell types (IMR90 SV40, GM00637G, and HCT) resulted in increased total glutathione, GSSG, and GSH (FIG. 3A,B,C). The fact that total and GSSG increased in the glucose-deprived transformed cells, supports the previously proposed hypothesis that these cells were experiencing oxidative stress and responding to that stress by increasing the synthesis of glutathione.^{9,10} However, it appears that in the absence of glucose, the transformed cells were unable to maintain the newly synthesized glutathione in the reduced state, as evidenced by the accumulation of GSSG. Furthermore, during glucose deprivation, GSSG accumulated

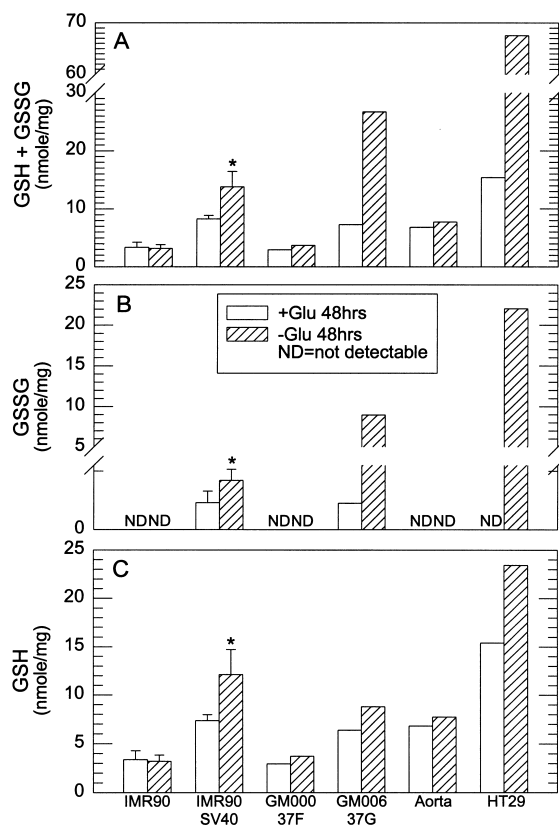


FIGURE 3. Glutathione analysis of untransformed (IMR90, GM00037F, Aorta) and transformed (IMR90 SV40, GM00637G, HT29) cell types exposed to glucose deprivation in FIGURE 2. Data points from IMR90 and IMR90 SV40 are the mean of 6 determinations done in 2 separate experiments (errors = ± 1 SD, asterisks indicate significant differences between +Glu and -Glu, paired *t*-test, $p < 0.05$). Data from other cell types are single determinations in one experiment.

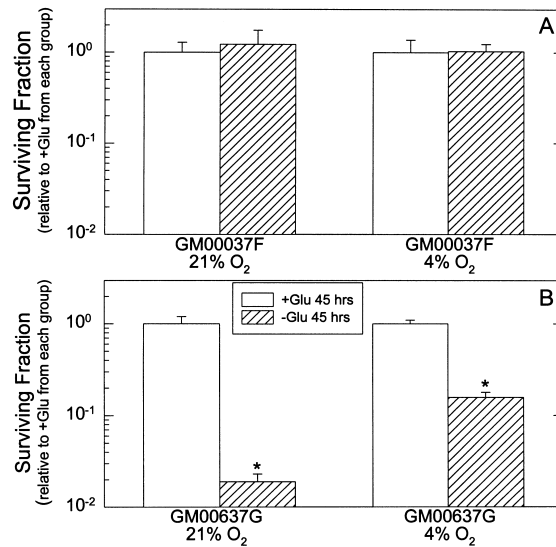


FIGURE 4. Clonogenic cell survival data showing the effect of O₂ concentration (21% vs. 4%) on glucose deprivation-induced cytotoxicity in untransformed (A) vs. SV40-transformed (B) human fibroblasts. Errors represent ± 1 SD. Asterisks indicate significantly different from all other treatment groups within a cell type ($p < 0.05$, paired *t*-tests). Data points are the mean of 4 determinations from each treatment group in one experiment.

to a greater extent in the transformed cell types which were the most susceptible to glucose deprivation-induced cytotoxicity (GM00637G and HT29 vs. IMR90 SV40, FIGS. 2 and 3). These results support the hypothesis that glucose deprivation-induced oxidative stress occurred in the transformed cells and contributed to the cytotoxicity noted in FIGURE 2.

In an attempt to determine if glucose deprivation-induced cytotoxicity was dependent on the metabolism of O₂, experiments were performed in 4% vs. 21% O₂ (FIG. 4). Confirming the results shown in FIGURE 2, glucose deprivation caused no significant clonogenic cell killing in the untransformed GM00037F human fibroblasts at either 21% O₂ or 4% O₂ (FIG. 4A). Again confirming the results shown in FIGURE 2, the glucose-deprived SV40-transformed cells (GM00637G) demonstrated significant clonogenic cell killing, with only 2% survival after 45 hours in 21% O₂ (FIG. 4B). When the SV40-transformed cells were glucose-deprived in the presence of 4% O₂, clonogenic cell killing was significantly less than was seen at 21% O₂ (15% survival vs. 2% survival, respectively; FIG. 4B). These results confirm that glucose deprivation was selectively cytotoxic to SV40-transformed human fibroblasts and show that the process leading to cytotoxicity was dependent upon ambient O₂ concentration. These results support the hypothesis that glucose deprivation-induced cytotoxicity is mediated by a process involving the metabolism of O₂.

DISCUSSION

The results in the current report extend the previous observations using MCF-7/ADR human breast carcinoma cells into three potentially important areas. First, it appears that glucose deprivation–induced cytotoxicity and oxidative stress occurs in several human transformed cell lines in addition to MCF-7/ADR (IMR90 SV40, GM00637G, and HT29). These transformed cells were derived from spontaneous transformation *in vivo* (MCF-7/ADR and HT29) as well as viral transformation events mediated by SV40 *in vitro* (IMR90 SV40 and GM00637G). These data allow for the speculation that susceptibility to glucose deprivation–induced cytotoxicity and oxidative stress is not limited to a single type of transformation event or transformed human cell type. This opens the possibility that glucose deprivation–induced cytotoxicity and oxidative stress could represent a general phenomenon common to several different types of cancer cells. Secondly, transformed cells appear to be more susceptible to glucose deprivation–induced cytotoxicity and oxidative stress than non-transformed cells (including two matched sets of normal and virally transformed fibroblasts). These results allow for the speculation that normal cells may be less susceptible to glucose deprivation–induced cytotoxicity and oxidative stress than cancer cells. This also supports the speculation there may be a fundamental defect in oxidative metabolism in cancer cells that could be exploited to gain a therapeutic advantage when trying to kill cancer cells while sparing normal tissues. Finally, it appears in one transformed cell line (GM00637G), glucose deprivation–induced cytotoxicity is dependent upon the metabolism of O₂. This result is consistent with the speculation that a defect in the transformed cell's respiratory mechanism may give rise to the reactive specie(s) responsible for glucose deprivation–induced cytotoxicity and oxidative stress. The data presented here are consistent with the metabolic pathways outlined in the model shown in FIGURE 1. However, the effect of glucose deprivation on signal transduction and gene expression in the vast majority of transformed human cell lines remains unknown.

Perhaps the most thought provoking and speculative considerations that can be derived from the recent work with glucose deprivation (refs. 5–10, FIGS. 2–4) pertain to a historical perspective on understanding of the origin of cancer cells. Warburg suggested that cancer was a metabolic disease in which respiration was “damaged” and glycolysis was increased to compensate for defective respiration.¹ Warburg hypothesized that this defect in metabolism gives rise to the cancerous phenotype. Oberley *et al.*²² proposed that cancer cells had a defect in the metabolism of superoxide (produced as a byproduct of mitochondrial respiration) that could trigger immortalization, uncontrolled cell proliferation, and the development of the malignant phenotype. During this same period of time it was also discovered that environmental insults and chemicals that lead to increases in prooxidant production could act as both initiators and promoters of the carcinogenic process.^{23,24} In addition it was shown that antioxidant enzymes and vitamins could inhibit both the initiation and promotion of carcinogenesis.^{23–25} Finally it was suggested that tumor cells produce large amounts of prooxidants (presumably hydroperoxides)²⁶ and that prooxidants appear to be involved with both stimulation of cell proliferation during mitogenesis as well as the generation of mutations and genomic instability, which are hallmarks of the transformed phenotype.^{27–29} Therefore, it appears that transformed cells demonstrate

metabolic abnormalities involving glucose and respiration as well as alterations in intracellular oxidation/reduction reactions towards a more prooxidant environment. Furthermore, prooxidants appear to accelerate mutagenesis as well as carcinogenesis, and antioxidants appear to be anticarcinogens.

More recently the theory that cancer is a genetic disease has gained widespread support.^{30,31} In this model carcinogenesis is thought to be a multi-step process in which genetic mutations gradually accumulate over time, eventually resulting in immortalization, the loss of control of cell proliferation, and progression to the malignant phenotype. Mutations in, and altered expression of, cellular homologues of oncogenes (i.e., Ras, c-Fos, c-Jun, and c-Myc) associated with growth and development as well as tumor suppressor genes (i.e., p53) have been suggested to contribute to the process of neoplastic transformation.³⁰⁻³⁴ Many of these genes have also been suggested to be involved in signal transduction pathways (*src* family kinases, Ras, etc.). In addition several of the mutations associated with conversion of non-oncogenic genes into their oncogenic counterparts appear to involve changes in oxidation/reduction sensitive regulatory sites (particularly thiol residues) on the respective proteins (i.e., v-Fos, v-Jun, and mutant p53).³⁴⁻³⁶ Furthermore, changes in the redox status of thiols have long been associated with the process of cell division and cancerous liver tissue has been shown to have an altered pattern of reduced thiols when compared to normal liver (reviewed in ref. 37). In addition it has been suggested that genes coding for proteins that block the process of programmed cell death (apoptosis) (i.e. Bcl-2), may permit cells, carrying a potentially oncogenic defect and destined to die via programmed cell death, to survive and continue down the path to neoplastic transformation.³⁸ Bcl-2 has also been suggested to regulate the intracellular localization of respiratory proteins and thiols as well as protecting cells from oxidative stress and glucose deprivation-induced cytotoxicity.^{6,38-41} Therefore, it appears that a common thread linking metabolic and genetic theories of cancer involves glucose metabolism, respiration, mutations in redox regulated proteins governing growth and development, and intracellular oxidation/reduction reactions involving hydroperoxides and thiols.

Given this interesting historical commonality between theories of cancer and the model for explaining recent results obtained with glucose deprivation (FIG. 1), it is now possible to propose a scenario incorporating critical aspects of both genetic and metabolic theories of cancer. All O₂ metabolizing cells are thought to produce a relatively constant low level of prooxidants (i.e., superoxide, hydrogen peroxide, etc.) as by products of electron transport chain activity that is balanced by cellular antioxidant capacity to maintain a viable non equilibrium steady state environment that is predominantly reducing. NADPH and the glutathione/glutathione peroxidase/glutathione reductase system are thought to represent major sources of reducing equivalents necessary to maintain this environment in a reduced state. If cancer cells have a defective respiratory mechanism (either increased prooxidant production and/or decreased cellular antioxidant capacity) that leads to increases in steady state levels of hydroperoxide production, they may exist in a relatively prooxidant intracellular environment (with respect to normal cells). Metabolism of glucose could be up regulated to produce more pyruvate and NADPH to compensate for this defect thereby rescuing the transformed cell from a respiratory-dependent cell death. Although not lethal (because of increased metabolism of glucose), the ensuing prooxidant environment produced by

defective respiration may be capable of stimulating seemingly uncontrolled cell division via aberrant activation of redox regulated signal transduction pathways and/or cell cycle regulatory proteins as well as inhibition of processes involved with differentiation (i.e., DNA methylation). In addition this prooxidant environment may also contribute to mutagenesis, genomic instability, and cellular heterogeneity, which are hallmarks of the progression to the malignant phenotype. Likewise, genetic mutations that result in the loss of redox control of cell cycle regulatory and/or signal transduction proteins that govern growth and development may cause these proteins to become activated or inactivated at inappropriate redox potentials leading to uncontrolled cell growth and the inability to differentiate. In addition mutations in redox regulated tumor suppressor genes (i.e., p53) may predispose cells to undergo malignant transformation more readily (without a preexisting defect in respiration) or escape cell death in the presence of defective in respiration. In fact, apoptosis mediated by p53 has recently been suggested to involve oxidative stress in cancer cells.⁴² Finally, over expression of proteins (such as Bcl-2), which could rescue cells from a lethal respiratory defect, might allow the defective cells to continue progressing towards malignant transformation. Cancer might therefore represent a constellation of metabolic and/or genetic diseases where the common theme is uncoupling of normal cellular processes that govern cell growth and development caused by the inappropriate flow of electrons from metabolic oxidation/reduction reactions to redox sensitive proteins governing signal transduction and gene expression.

While the current data linking glucose derivation-induced cytotoxicity, activation of signal transduction, and gene expression to metabolic oxidative stress in human tumor cells opens many theoretical possibilities with potentially far reaching implications to the understanding of malignancy, the generality and predictive power of the results remains largely unknown. Many future studies will be required to test the validity of these concepts.

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