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Research Paper

Combined inhibition of glycolysis, the pentose cycle, and thioredoxin metabolism selectively increases cytotoxicity and oxidative stress in human breast and prostate cancer

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ABSTRACT

Inhibition of glycolysis using 2-deoxy-D-glucose (2DG, 20 mM, 24–48 h) combined with inhibition of the pentose cycle using dehydroepiandrosterone (DHEA, 300 μM, 24–48 h) increased clonogenic cell killing in both human prostate (PC-3 and DU145) and human breast (MDA-MB231) cancer cells *via* a mechanism involving thiol-mediated oxidative stress. Surprisingly, when 2DG+DHEA treatment was combined with an inhibitor of glutathione (GSH) synthesis (L-buthionine sulfoximine; BSO, 1 mM) that depleted GSH > 90% of control, no further increase in cell killing was observed during 48 h exposures. In contrast, when an inhibitor of thioredoxin reductase (TrxR) activity (Auranofin; Au, 1 μM), was combined with 2DG+DHEA or DHEA-alone for 24 h, clonogenic cell killing was significantly increased in all three human cancer cell lines. Furthermore, enhanced clonogenic cell killing seen with the combination of DHEA+Au was nearly completely inhibited using the thiol antioxidant, N-acetylcysteine (NAC, 20 mM). Redox Western blot analysis of PC-3 cells also supported the conclusion that thioredoxin-1 (Trx-1) oxidation was enhanced by treatment DHEA+Au and inhibited by NAC. Importantly, normal human mammary epithelial cells (HMEC) were not as sensitive to 2DG, DHEA, and Au combinations as their cancer cell counterparts (MDA-MB-231). Overall, these results support the hypothesis that inhibition of glycolysis and pentose cycle activity, combined with inhibition of Trx metabolism, may provide a promising strategy for selectively sensitizing human cancer cells to oxidative stress-induced cell killing.

Introduction

Cancer cells, relative to normal cells, demonstrate up regulation of glucose metabolism and a loss of regulation between glycolysis and aerobic respiration [1–3]. Growing evidence supports the hypothesis that tumor cells have altered mitochondrial metabolism leading to increased steady-state levels of intracellular reactive oxygen species (ROS) including superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) [4–10]. It has also been hypothesized that cancer cells compensate for increases in steady-state levels of ROS by increasing glycolysis and pentose cycle activity to provide reducing equivalents for hydroperoxide metabolism (Fig. 1) [4–9]. Glucose provides electrons for hydroperoxide metabolism *via* the activity of the pentose cycle to regenerate nicotinamide adenine dinucleotide phosphate (NADPH) to serve as the electron donor for glutathione (GSH) and thioredoxin (Trx) dependent peroxidase activity as well as through glycolysis to form pyruvate that can directly react to detoxify hydroperoxides through a decarboxylation reaction (Fig. 1) [8,11,12].

Consistent with the hypothesis that cancer cells have increased glycolysis and pentose cycle activity as a mechanism of protection against increased fluxes of hydroperoxides, inhibition of these pathways through glucose deprivation is known to cause selective oxidative stress and cytotoxicity in cancer cells versus normal cells [9,13,14]. The glucose analog, 2-deoxyglucose, inhibits glycolysis and cannot be fully oxidized in the pentose cycle, regenerating only half as much NADPH as a molecule of glucose [15]. Previous studies have demonstrated that 2DG treatment disrupts the $NADP^+$/NADPH balance [16,17], is cytotoxic to tumor cells *in vitro*

Abbreviations: 2DG, 2-deoxy-D-glucose; NAC, *N*-acetylcysteine; GSH, glutathione; GSSG, glutathione disulfide; DHEA, dehydroepiandrosterone; Au, auranofin; G6PDH, glucose-6-dehydrogenase; ROS, reactive oxygen species; Trx, thioredoxin; TrxR, thioredoxin reductase

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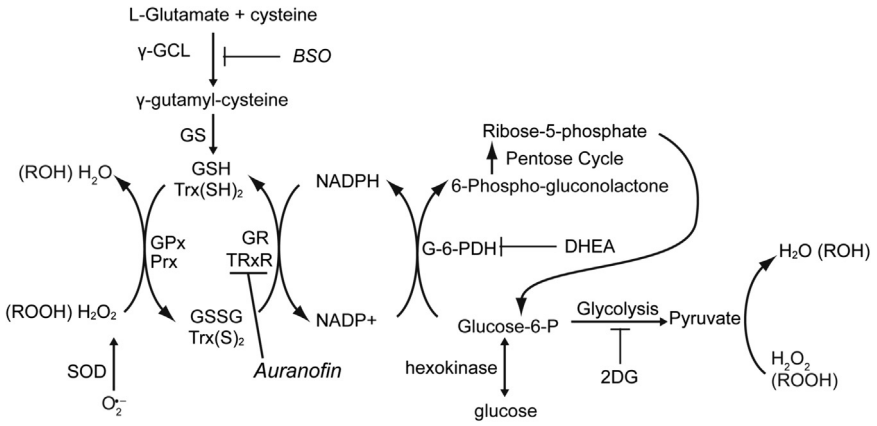

Fig. 1. The pathways involving glucose and hydroperoxide metabolism believed to be involved with protection of cancer cells from metabolic oxidative stress (inhibitors of Trx and GSH metabolism are shown in italics). 2DG competes with glucose for uptake into the cells competitively inhibiting pyruvate production and the pentose cycle after glucose-6-phosphate-dehydrogenase (G6PD). DHEA inhibits G6PD. The GSH and Trx dependent systems participate in the detoxification of H_2O_2 and organic hydroperoxides. NADPH is a source of reducing equivalents for the Trx/GSH-dependent systems. BSO inhibits glutamate cysteine ligase (γ-GCL) preventing glutathione synthesis. Auranofin is the inhibitor of thioredoxin reductase (TrxR), which reduces the oxidized Trx to the reduced form. These inhibitors were used alone and in combination to increase the cancer cell oxidative stress, resulting in cancer cell cytotoxicity.

[18,19] and enhances the inhibition of tumor growth by agents that kill cancer cells via an oxidative stress mechanism *in vivo* [16,20,21].

Glucose-6-phosphate dehydrogenase (G6PDH) is the rate limiting enzyme in the oxidation of glucose through the pentose phosphate pathway. G6PDH catalyzes the chemical reaction of D-glucose-6-phosphate to 6-phospho-D-glucono-lactone regenerating NADPH (Fig. 1) [22]. Studies have shown that G6PDH expression and activity is increased in tumor tissues compared with normal cells [23,24] and is strongly related to cellular oxidative stress responses [25]. Dehydroepiandrosterone (DHEA) is an endogenous primate steroid precursor that has been shown to be an inhibitor of mammalian G6PDH [26,27]. It has been shown that treatment with DHEA leads to a 30–40% decrease of NADPH/$NADP^+$ ratio, which may compromise cellular hydroperoxide metabolism [26].

GSH and Trx are cellular thiol redox cofactors that participate in redox sensitive signaling pathways, scavenging hydroperoxides and allowing for the maintenance of cellular redox potential. Studies have demonstrated that these antioxidant systems are up regulated in multiple cancer types compared to matched non-cancerous tissue [28–32]. In this regard up-regulation of GSH and Trx metabolism in breast and prostate cancer is correlated with disease progression and poor patient outcomes [29,32]. The rate limiting step in GSH synthesis is glutamate cysteine ligase, which is inhibited by buthionine sulfoximine (Fig. 1; BSO). Trx is maintained in the reduced state by thioredoxin reductase (Fig. 1; TrxR). Auranofin (Au; Fig. 1) is a potent inhibitor of both cytosolic and mitochondrial TrxR [33,34]. We have previously demonstrated that simultaneous inhibition of the GSH and Trx pathways results in cancer cell death via metabolic oxidative stress [35–37].

To determine drug combinations that were less toxic to normal versus cancerous human cells that could selectively cause metabolic oxidative stress induced cell-killing in cancer cells, the current study focused on combining pharmacological agents that inhibit glycolysis and the pentose cycle (2-DG and DHEA) with inhibitors of thiol-dependent hydroperoxide metabolism (BSO and Au). Treatment of human prostate and breast cancer cells with either 2DG or DHEA was found to decrease clonogenic cell survival and cell killing was further enhanced by combining both agents. Although this decrease in cancer cell survival was associated with disruptions in GSH metabolism, depleting GSH using BSO did not further enhance clonogenic cell killing. In contrast, inhibiting Trx metabolism using Au resulted in significantly increased clonogenic cell death when combined with DHEA or 2DG+DHEA that was reversed using NAC a small molecule thiol antioxidant. Importantly, normal human mammary epithelial cells (HMEC) were not as sensitive to 2DG, DHEA, and Au induced cell killing as their cancer cell counterparts (MDA-MB-231). These results support the hypothesis that cancer cells are more dependent on glucose as well as hydroperoxide metabolism than are normal cells and that combining inhibitors of glycolysis and the pentose cycle with Au may represent a promising approach for selectively causing oxidative stress-induced cell killing in breast and prostate cancer cells.

Results

DHEA inhibits G-6-PDH activity and enhances 2DG cell killing in breast and prostate cancer cells

We have previously determined that 2DG inhibits cancer cell growth through an oxidative stress mechanism in multiple cancer cell lines including MDA-MB231 breast cancer cells [20,35,37–39]. To test the hypothesis that an inhibitor of G6PDH could further enhance metabolic oxidative stress caused by 2DG (Fig. 1) MDA-MB231 breast cancer cells, PC-3 and DU145 prostate cancer cells were treated with 2DG and/or DHEA for 24 and 48 h followed by clonogenic cell survival assay (Fig. 2). DHEA inhibits the activity of human recombinant G6PDH with an IC50 of ~330 μM *in vitro* [27]. In the current studies 300 μM DHEA significantly inhibited G6PDH activity 35–50%, in all three of the cancer cell lines (Table 1). 20 mM 2DG was used to ensure that a relevant ratio of 2DG to glucose (≈ 1.8) was used to competitively inhibit glucose metabolism in the cells grown in RPMI 1640 medium, which contains 11 mM glucose. As expected, treatment with 2DG or DHEA decreased surviving fractions of all 3 cell lines by 10–20% or 20–40% after 24 or 48 h, respectively (Fig. 2A–C). Interestingly, treating the cells with DHEA in combination with 2DG significantly inhibited clonogenic cell survival compared to treatment with 2DG or DHEA alone, at both 24 and 48 h, in all three cancer cell lines tested (Fig. 2A–C). These results support the hypothesis that simultaneous disruption of glucose metabolism using both a glycolysis inhibitor (2DG) and an inhibitor of the pentose cycle (DHEA) enhanced cancer cell killing.