Inhibition of Akt Potentiates 2-DG–Induced Apoptosis via Downregulation of UPR in Acute Lymphoblastic Leukemia

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Abstract

The ability to pair the regulation of metabolism and cellular energetics with oncogenes and tumor suppressor genes provides cancer cells with a growth and survival advantage over normal cells. We investigated the mechanism of cell death induced by 2-deoxy-D-glucose (2-DG), a sugar analog with dual activity of inhibiting glycolysis and N-linked glycosylation, in acute lymphoblastic leukemia (ALL). We found that, unlike most other cancer phenotypes in which 2-DG only inhibits cell proliferation under normoxic conditions, ALL lymphoblasts undergo apoptosis. Bp-ALL cell lines and primary cells exhibited sensitivity to 2-DG, whereas T-ALL cells were relatively resistant, revealing phenotypic differences within ALL subtypes. Cotreatment with D-mannose, a sugar essential for N-linked glycosylation, rescues 2-DG–treated ALL cells, indicating that inhibition of N-linked glycosylation and induction of ER stress and the unfolded protein response (UPR) is the predominant mechanism of 2-DG’s cytotoxicity in ALL. 2-DG–treated ALL cells exhibit upregulation of P-AMPK, P-Akt, and induction of ER stress/UPR markers (IRE1α, GRP78, P-eIF2α, and CHOP), which correlate with PARP cleavage and apoptosis. In addition, we find that pharmacologic and genetic Akt inhibition upregulates P-AMPK, downregulates UPR, and sensitizes ALL cells to remarkably low doses of 2-DG (0.5 mmol/L), inducing 85% cell death and overcoming the relative resistance of T-ALL. In contrast, AMPK knockdown rescues ALL cells by upregulating the prosurvival UPR signaling. Therefore, 2-DG induces ALL cell death under normoxia by inducing ER stress, and AKT and AMPK, traditionally thought to operate predominantly on the glycolytic pathway, differentially regulate UPR activity to determine cell death or survival. Mol Cancer Res; 10(7); 969–78. ©2012 AACR.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and adolescents and is a leading cause of cancer-related deaths in these patients (1). Current clinical practices have had only minimal impact on cure rates for patients with resistant phenotypes or after relapse (2, 3). A number of ALL phenotypes exhibit mutations that lead to inactivation or constitutive activation of oncogenic pathways such as LKB1, PTEN, PI3K/Akt and RAS, which have been linked to the regulation of energy metabolism in general and glucose metabolism in particular (4–6). T-ALL is known to have a high rate of PTEN mutations that lead to constitutive activation of Akt (7). Our laboratory has previously shown that the master energy regulator AMPK has significant feedforward and feedback cross-talk with these pathways and that AMPK is a suitable target for ALL therapy (8, 9). Consequently, although targeting glucose metabolism in ALL has not been explored, the above-described biology of ALL lymphoblasts suggests a significant therapeutic potential.

The increased glycolytic rate exhibited by cancer cells compared with their nonmalignant counterparts, even in the presence of sufficient oxygen, was first described by Otto H. Warburg (10). This unique alteration in glucose metabolism provides a biochemical basis for targeting glycolysis as a selective anticancer treatment strategy. Indeed, the glucose analog 2-deoxy-D-glucose (2-DG) has shown activity against solid tumors in preclinical models and early phase human clinical trials (11), but its role as an antileukemic agent has not been explored. 2-DG can induce cell growth arrest and cell death by inhibiting 2 key glycolytic enzymes, hexokinase and phosphoglucone isomerase (PGI; ref. 12). Following facilitated diffusion into cells through glucose transporters, 2-DG is converted to 2-DG-6-phosphate by hexokinase, but unlike glucose-6-phosphate, 2-DG-6-phosphate cannot be further metabolized by PGI, and its accumulation inhibits the glycolytic pathway (13). Inhibition of glycolysis has been shown to be a main mechanism of 2-DG’s cytoxicity in hypoxic cells of solid tumors (14). However, because of its structural similarity with mannose, 2-DG has also been...
shown to be a potent inhibitor of N-linked glycosylation (12, 15). Indeed, 2-DG can be incorporated in place of mannose into lipid-linked oligosaccharide (LLO) chains (15), but unlike mannose, 2-DG cannot accommodate the required complex branching structure of these molecules, leading to premature termination of LLO synthesis, and accumulation of misfolded, nascent ER-synthesized proteins (16).

The 2-DG–induced accumulation of misfolded glycoproteins in the ER lumen leads to ER stress and induction of the unfolded protein response (UPR), a protective physiologic response aimed at reducing the presence of unfolded proteins and consequently ER stress (11, 12). The UPR is mediated via 3 ER transmembrane receptors: protein kinase dsRNA- and consequently ER stress (11, 12). The UPR is mediated response aimed at reducing the presence of unfolded proteins and unfolded protein response (UPR), a protective physiologic (16).

Chen et al. uncovered that unlike most cancer phenotypes, which do not undergo cell death when treated with relatively low doses (≤8 mmol/L) of 2-DG under normoxic as opposed to hypoxic conditions, ALL cells undergo apoptosis primarily through activation of ER stress. Moreover, we found differential sensitivity to inhibition of N-linked glycosylation versus aerobic glycosylation within ALL subtypes, in which Bp-ALL cells show significantly greater sensitivity to 2-DG than T-ALL cells. We showed that the decreased sensitivity of T-ALL is conferred by Akt and AMPK differentially modulate the ability of ALL cells to effectively activate UPR, which buffers the proapoptotic effects of 2-DG–induced glycoprotein misfolding and ER stress.

Material and Methods

Cell culture and reagents

The ALL cell lines CCRF-CEM (T-ALL), Jurkat (T-ALL), and NALM6 (B precursor-ALL) were maintained in RPMI-1640 medium (Cellgro) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich) and antibiotics (penicillin, 100 IU/mL; streptomycin, 100 µg/mL; Cellgro). SupB15 cells (Bp-ALL, BCR-ABL t(9;22)) were maintained in Iscove’s Dulbecco’s modified Eagle’s medium plus heat-inactivated FBS and antibiotics. Primary ALL cells were obtained from patients with ALL at the University of Miami following Institutional Review Board–approved informed consent. Primary cells were cocultured using a human bone marrow stromal cell feeder layer (2.5 × 10⁶ cells/75 cm²) immortalized by human telomerase reverse transcriptase (hTERT) transfection (provided by Dr. D. Campana, SJCRH, Memphis, TN). 2-DG, 2-fluoroxyo-t-glucose, and t-mannose were from Sigma-Aldrich, and Akt Inhibitor X was from Calbiochem.

Cell viability and apoptosis assays

Cell viability was assayed by trypan blue exclusion using the Vi-CELL XR analyzer (Beckman Coulter). Cell death was determined either using the Vi-CELL XR or by flow cytometry analysis of Annexin V–fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining using BD Pharmigen FITC Annexin V apoptosis detection kit. Labeled cells were then analyzed by BD LSR II flow cytometer in the appropriate channel and FACSDiva software version 4.1.2 (BD Biosciences). Events positive for Annexin V and PI staining were gated and used to define the population of dead/dying cells. The level of cell death is expressed as the percentage of death measured in untreated cells. Apoptosis was expressed as a percentage (%) of cells stained with Annexin V–FITC/PI in the population (mean ± SEM, n = 3). Synergy analysis was determined using Chou’s combination index (CI) analyses using the following equation: CI = [D1_combination/D1_single] + [D2_combination/D2_single]. Assessment of synergy was carried out by quantitating drug interaction with the Calcsyn computer program (Biosoft). CI values of <1, 1, and >1 indicate synergy, additivity, and antagonism, respectively (21). Statistical differences in cell proliferation or cell death were assessed by one-way ANOVA followed by the Newman–Keuls multiple comparison test or by unpaired Student t test using GraphPad PRISM.

Construction of stable shRNA-expressing cell lines

To downregulate the expression of Akt1 and GRP78, we used lentiviral particles generated from cotransfection of p8.2vpdeltaR, PVSVG-pseudotyped lentiviral and pLKO.1 plasmids encoding specific hairpin RNA sequences in Heko-293 cells (Clontech). The hairpin oligonucleotides for short hairpin RNA (shRNA)-Akt1 (22) and GRP78 (23) were hybridized and the duplex ligated into pLKO.1puuro (Addgene plasmid #10878). Negative control lentiviral shRNAs (sc-108080) were obtained from Santa Cruz Biotechnology. To knockdown AMPKα1, lentiviral particles containing up to 5 different constructs encoding target-specific 19 to 25 nt shRNA were used (sc-296730V; Santa Cruz Biotechnology). Stable ALL cell lines expressing each one of these shRNAs were developed by transducing CCRF-CEM and NALM6 cells by spinoinoculation and selection with 1 µg/mL puromycin as described elsewhere (24). Each stable
shRNA-expressing cell line was validated for knockdown by immunoblotting.

Protein extracts and immunoblots

Cells were harvested at the specified time points, washed with 1× PBS, and sonicated in 50 mmol/L Tris-HCl (pH 7.4) containing protease inhibitors (Thermo). Proteins (50 μg per lane) were resolved by SDS-PAGE electrophoresis and transferred onto polyvinylidene difluoride membranes. β-Actin was used as a loading control. The immunoblots presented are representative of 3 independent experiments.

Results

2-DG induces growth inhibition and cell death in ALL cells under normoxia

To evaluate the clinical relevance of glycolytic inhibitors in childhood ALL, we first determined the effects of 2-DG and 2-FDG on ALL proliferation and cell death under normoxic (pO2 = 21%) and hypoxic (pO2 = 0.5%) conditions. For this, ALL cell models for T-ALL (CCRF-CEM and Jurkat, both PTEN mutants), Bp-ALL (NALM6 and SupB15, a chemotherapy-resistant BCR-ABL[t(9;22)]-expressing cell line), and representative primary ALL cells (PATIENT) were treated with a range of doses of 2-DG or 2-FDG for up to 72 hours. As shown in Fig. 1, 2-DG (0.5–4 mmol/L) induced growth inhibition (Fig. 1A) and cell death (Fig. 1B) under normoxic conditions in all ALL cell models and primary ALL cells tested. Significantly lower cytotoxicity was observed when cells were treated under hypoxic conditions (not shown). Although growth inhibition was comparable across T- and B-lineage phenotypes, T-ALL cells (CCRF-CEM and Jurkat) exhibited relative resistance to 2-DG–induced cell death compared with B-lineage ALL cells (Fig. 1B). Primary Bp-ALL cells tested exhibited sensitivity to 2-DG under normoxic conditions in the same range as observed in the representative cell models. In contrast, treatment with equimolar concentrations of the more selective glycolytic inhibitor 2-FDG had negligible effects on CCRF-CEM cells and induced less cell death than 2-DG in NALM6 and SupB15 cells under normoxia (Fig. 1C).

It has been reported that in cancer phenotypes in which low-dose treatment with 2-DG (<4 mmol/L) induces cell death under normoxia, 2-DG acts through inhibition of N-linked glycosylation (12, 14). D-Mannose has been shown to reverse both the growth inhibitory and cytotoxic effects associated with inhibition of N-linked glycosylation but cannot reverse the cytotoxic effects triggered by inhibition of glycolysis (25, 26). In addition, 2-FDG is known to preferentially inhibit glycolysis with minimal effects on N-linked glycosylation. On the basis of the profile of 2-DG and 2-FDG–induced cytotoxicity in ALL cells, we postulated...
that inhibition of N-linked glycosylation by 2-DG leads to cell death in T-ALL, and although it seems to be the predominant mechanism leading to cytotoxicity in Bp-ALL, inhibition of glycosylation also contributes to cell death in the latter phenotype. Consequently, we cotreated CCRF-CEM, NALM6, and SupB15 cells with 2-DG and D-mannose and evaluated the effects on cell death. Our results indicated that mannose completely reverses the cytotoxic effects of 2-DG in CCRF-CEM at 4 and 8 mmol/L doses of 2-DG, but only at 4 mmol/L. 2-DG dose in NALM6 (Fig. 1D). Partial reversal was seen in NALM6 at higher dose of 2-DG (8 mmol/L) and in SupB15 cells at both doses (Fig. 1D). Therefore, inhibition of N-linked glycosylation is the predominant mechanism by which low-dose 2-DG induces cell death in ALL cells. However, phenotypic differences exist, and it is possible that in Bp-ALL, inhibition of aerobic glycolysis also contributes to 2-DG–induced cytotoxicity.

2-DG induces changes in the expression and phosphorylation/activation of Akt, AMPK, and ER stress/UPR-associated signaling proteins in ALL cell lines

To further understand the molecular mechanisms by which 2-DG induces cell death in ALL, we examined the expression of critical signaling proteins associated with energy metabolism in general, glucose metabolism in particular, and cell proliferation. As shown in Fig. 2A, 2-DG induced significant activation of P-AMPK (T172) in both cell lines. Consistent with increased P-AMPK expression, P-mTOR (S2448) and its downstream target P-p70S6K (T389) were downregulated by 2-DG through the inhibitory effect of AMPK on mTOR activity (27), and the downregulation of mTOR signaling was greater in NALM6 cells as compared with CCRF-CEM cells (Fig. 2A). The PTEN-mutant CCRF-CEM cells exhibited both high basal expression and sustained increased expression of P-Akt (S473) (6 vs. 24 hours), whereas in NALM6 cells, the basal expression of P-Akt (S473) was lower, and the strong upregulation of P-Akt (S473) observed at 6 hours subsided by 24 hours. Our data show a strong correlation between the high basal and sustained expression of P-Akt following treatment with 2-DG in CCRF-CEM cells and their relative resistance to this agent (Fig. 1B). We postulated that PTEN inactivation in CCRF-CEM cells and subsequent hyperactivation of the Akt pathway leads to relative resistance of T-ALL cells to 2-DG. Because of the high prevalence of inactivating mutations in patients with T-ALL (7), this mechanism of resistance is of clinical relevance.

Inhibition of N-linked glycosylation by 2-DG has been shown to induce accumulation of misfolded nascent glycoproteins in the ER lumen, leading to ER stress and UPR activation (25, 28). We analyzed the expression of ER stress/UPR markers in CCRF-CEM and NALM6 cells treated with 2-DG. In both cell lines, 2-DG (4 mmol/L) increased the expression of GRP78, GRP94, IRE1α, P-eIF2α (S51), and CHOP, confirming induction of ER stress/UPR, and their increased expression correlated with PARP cleavage, a marker for apoptosis (Fig. 2B). These data suggested that...
2-DG induces ALL cell death through ER stress/UPR-mediated mechanisms. The changes in Akt/mTOR and UPR signaling observed in these cell lines were replicated using representative primary ALL cells, validating their clinical relevance (Fig. 2C; representative Bp-ALL sample). To confirm that the putative inhibition of N-linked glycosylation by 2-DG led to induction of ER stress/UPR, we treated the cell line models with 2-DG in the presence or absence of mannose. Western blots revealed that the addition of mannose (10 mmol/L) resulted in downregulation of GRP78, P-eIF2α (S51), and CHOP in both CCRF-CEM and NALM6 cells (Fig. 2D), suggesting that mannose relieves ER stress-inducing cytotoxicity in ALL cells by reversing 2-DG-induced inhibition of N-linked glycosylation.

**Inhibition of Akt sensitizes ALL cells to 2-DG–induced cell death via a UPR-mediated mechanism**

On the basis of upregulation of P-Akt (S473) in response to 2-DG and the relative resistance of cells, harboring inactive PTEN mutations and constitutive activation of Akt, we hypothesized that Akt plays a central prosurvival role in ALL cells treated with 2-DG. To test this, we treated CCRF-CEM cells with a pharmacologic inhibitor of Akt and examined its effects on 2-DG–mediated cell death. Figure 3A shows that inhibition of Akt signaling using the Akt inhibitor X (AIX, 10 μmol/L) sensitized CCRF-CEM cells to even a very low dose of 2-DG (0.5 mmol/L). This combination led to more than 85% apoptotic death as compared with very modest effects with each agent alone (Fig. 3A; 2-DG–8.1%; AIX–6.9%, P < 0.001). In addition, we found that this combination exhibited synergism (CI = 0.36), as determined using the equation by Chou (21). Similar effects, albeit of a lesser magnitude, were observed in NALM6 (CI = 0.60; data not shown). Western blot analysis of CCRF-CEM cells treated with both agents (2-DG–0.5 and 10 mmol/L; AIX–10 μmol/L) showed significant downregulation of P-Akt (S473) and the UPR markers GRP78, IRE1α, P-eIF2α (S51), and CHOP, whereas the level of P-AMPK (T172) was significantly increased (Fig. 3B).

To confirm these results, we used a genetic approach based on the lentiviral delivery of shRNAs to specifically knockdown Akt1 expression in ALL cells. We found that shAkt1-expressing ALL cells treated with 2-DG (0.5, 1.5, and 5.0 mmol/L) exhibited significant increase in apoptotic death compared with shRNA scramble controls (shSCR; P < 0.001 for CCRF-CEM; P < 0.01 for NALM6; Fig. 3C). As expected, Western blotting showed downregulation of
Akt1 in shAkt1-transduced cells compared with shSCR control cells. Moreover, as was the case in the AIX-treated cells, genetic downregulation of Akt lead to increased expression of P-AMPK (T172) and decreased expression of UPR markers IRE1α, GRP78, P-eIF2α (S51), and CHOP, compared with cells treated with 2-DG alone (Fig. 3D). Taken together, our results showed that Akt signaling is critical for ALL cell proliferation and survival in response to 2-DG treatment and supports the role of Akt as a positive regulator of the prosurvival arm of the UPR (29).

Inhibition of AMPK restores UPR function and rescues ALL cells from ER stress–mediated cell death following treatment with 2-DG alone or in combination with Akt downregulation

During the course of our investigations, we uncovered that 2-DG induced P-AMPK (T172) in ALL cells (Fig. 2A) and that inhibition of Akt further enhanced AMPK activation (Fig. 3). To determine the role of AMPK in 2-DG–treated ALL cells, we used shRNAs to downregulate AMPKα1 in CCRF-CEM and NALM6 cells. Cells were treated with 2-DG (0.5 and 2.5 mmol/L) ± AIX (10 μmol/L), and the expression of AMPK, Akt, UPR markers, and cell death were examined. We found that downregulation of AMPK rescued CCRF-CEM and NALM6 cells from cell death induced by the combination of 2-DG + AIX (Fig. 4A and B; P < 0.0001 and P < 0.001 for shAMPK vs. shSCR cell death in CCRF-CEM and NALM6, respectively). Importantly, these effects were accompanied by increased induction of UPR markers in both AMPK knockdown cell lines treated with 2-DG + AIX (Fig. 4C and D), indicating that AMPK knockdown restored the ability of ALL cells to effectively engage the UPR.

Inhibition of GRP78 sensitizes ALL cells to 2-DG–induced cell death

To definitively establish that the ability of ALL cells to effectively engage the UPR is central to the ability of ALL cells to buffer 2-DG–induced cytotoxicity, we used a genetic approach to inhibit the main effector of UPR signaling—GRP78 (17, 18) and evaluated apoptotic death in 2-DG–treated ALL cells. Downregulation of GRP78 sensitized ALL cell models to equimolar concentrations of 2-DG, leading to a significant increase in cell death compared with shSCR-expressing cells (Fig. 5B, P < 0.001 for CCRF-CEM/shAMPK vs. CCRF-CEM/shGRP78; Fig. 5C, P < 0.01 for NALM6/shAMPK vs. NALM6/shGRP78). These data confirmed that the ability of ALL cells to effectively engage the UPR is essential to buffer the proapoptotic effects of 2-DG.

Discussion

Reprogramming tumor metabolism has recently been proposed as an emerging hallmark of cancer (30). This includes reprogramming of glucose metabolism in general and the glycolytic pathway in particular, to allow cancer cells...
to generate ATP through aerobic glycolysis, whereas this switch is limited in normal cells (31). Although there is substantial evidence to support increased glycolysis in tumor versus normal tissue, recently there have been reports indicating that glycolysis is closely linked with the cell cycle and that increased glucose metabolism is a consequence of growth signaling within a cell (normal or tumor) and not necessarily as a result of "rewiring" of a cancer cell (32). Nonetheless, increased glucose metabolism seems to be a hallmark of cancer, which is likely due to their uncontrolled growth signaling within a cell (normal or tumor) and not necessarily as a result of "rewiring" of a cancer cell (32). Although the role of UPR is to alleviate sustained ER stress by inhibiting protein translation, activating chaperones, and enhancing proteosomal degradation of misfolded proteins (17), the persistent ER stress will turn on the proapoptotic arm of UPR regulated by CHOP (19). Our data indicate that 2-DG induces ALL cell death via a mechanism mediated by ER stress/UPR as evidenced by increased expression of several UPR markers, including CHOP, which correlates with PARP cleavage and evidence of cell death shown by Annexin V/PI staining. Moreover, we show that the addition of D-mannose not only rescues 2-DG cytotoxicity, suggesting that 2-DG induces cell death in Bp-ALL cells via inhibition of both N-linked glycosylation and glycolysis. Conversely, the more selective glycolytic inhibitor 2-FDG induced cell death in Bp-ALL, but not in T-ALL, consistent with increased dependence on glycolysis by Bp-ALL as compared to T-ALL (37). Our data support the notion that phenotypic differences in the regulation of glucose metabolism lead to the differential sensitivity of T- versus Bp-ALL cells to 2-DG and that further elucidation of these phenotypic differences may lead to lineage-specific therapeutic strategies.

Because of its structural similarity to mannose, a sugar central to N-linked glycosylation of proteins in the ER lumen, 2-DG can enter the glycosylation pathway and disrupt the elongation of polysaccharide chains linked to nascent proteins, resulting in accumulation of misfolded proteins in the ER lumen (12, 25) and induction of UPR (38). Although the role of UPR is to alleviate sustained ER stress by inhibiting protein translation, activating chaperones, and enhancing proteosomal degradation of misfolded proteins (17), the persistent ER stress will turn on the proapoptotic arm of UPR regulated by CHOP (19). Our data indicate that 2-DG induces ALL cell death via a mechanism mediated by ER stress/UPR as evidenced by increased expression of several UPR markers, including CHOP, which correlates with PARP cleavage and evidence of cell death shown by Annexin V/PI staining. Moreover, we show that the addition of D-mannose not only rescues 2-DG–treated cells but also correlates with the downregulation of UPR markers. Therefore, our data are consistent with published reports indicating that inhibition of glycolysis modulates prednisolone resistance in ALL and that very high doses of 2-DG (40 mmol/L) induce cytotoxicity in lymphoma cell lines (39, 40).

Mammalian cells exhibit redundancy in the regulatory pathways that coordinate essential physiologic processes aimed at buffering proapoptotic signals. This redundancy is significant in pathways that pair cellular energetic

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**Figure 5.** Downregulation of GRP78 sensitizes 2-DG-treated ALL cells. Western blot analysis of GRP78 (A) in stably transduced CCRF-CEM and NALM6 cells expressing shRNAs against GRP78 (shGRP78) and treated with 2-DG (0.5–2.5 mmol/L) for 24 hours under normoxia. Level of apoptosis in stably transduced CCRF-CEM (B) and NALM6 (C) cells expressing either scramble shRNAs (shSCR) or shRNAs against GRP78 (shGRP78) and treated with 2-DG (0.5, 1.0, and 2.5 mmol/L) for 72 hours under normoxia. * and # denote P < 0.001 and P < 0.01, respectively, for shSCR versus shGRP78.
We propose that it is the inhibition of the prosurvival UPR response by AMPK that significantly potentiates apoptosis in response to 2-DG treatment, rather than CHOP-mediated apoptosis, induced in response to prolonged UPR and extensive ER stress. Indeed, shRNA-mediated inhibition of AMPK rescued ALL cells from 2-DG–induced cytotoxicity and correlated with restoration of the essential UPR-signaling factors. Therefore, restoration of effective UPR is needed for ALL cells to buffer and reduce the proapoptotic signals mediated by sustained 2-DG–induced ER stress. The prosurvival role of the UPR in determining the cellular response to 2-DG in ALL was further confirmed using shRNA inhibition of GRP78, which also sensitized ALL cells to 2-DG. On the basis of these data, we propose a model for the mechanism of 2-DG–induced cell death via ER stress/UPR-mediated apoptosis in which AMPK and Akt modulate the UPR (Fig. 6).

In summary, we show for the first time that 2-DG (at low dose) induces cell death in ALL cells under normoxia by triggering ER stress/UPR-mediated apoptosis. The sensitivity of ALL cells to 2-DG under normoxia places them among the few cancer phenotypes that behave this way, which is important when considering 2-DG as a potential antileukemic agent as leukemia cells circulate freely through normoxic and hypoxic environments. The resistance to glycolytic inhibitors observed in PTEN-mutant T-ALL cells underscores the role of Akt signaling as a prosurvival pathway that buffers ER stress/UPR-mediated cell death. The crosstalk between the AMPK and Akt pathways and glucose metabolism represents synthetically lethal interactions and supports further consideration of 2-DG, alone or in combination with selected targeted agents, as an antileukemic agent for future translation into clinical trials for resistant/refractory or relapsed ALL.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: J. DeSalvo, J.N. Kuznetsov, J. Du, G.M. Leclerc, G.J. Leclerc, T.J. Lampidis, J.C. Barredo


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Study supervision: G.M. Leclerc, G.J. Leclerc, T.J. Lampidis, J.C. Barredo

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![Diagram](https://example.com/diagram.png)

**Figure 6.** Proposed mechanism of action for 2-DG in ALL cells under normoxia. 2-DG interferes with N-linked glycosylation, leading to accumulation of misfolded glycoproteins, which triggers ER stress and induction of UPR. Binding of the misfolded glycoproteins to the central UPR regulator GRP78 will result in its dissociation from the UPR effectors (IRE1α, ATF6, and PERK), allowing them to fully engage UPR. GRP78 also acts as a major chaperone during protein folding, removes excess misfolded proteins from the ER lumen for proteosomal degradation, and functions to suppress prosapoptotic pathways triggered by CHOP. Separately, 2-DG inhibits glycolysis, decreases ATP levels, and leads to activation of AMPK. Our data show that AMPK activation blocks expression of UPR signaling and significantly potentiates apoptosis in 2-DG–treated ALL cells. Akt promotes UPR signaling and cell survival via downregulation of AMPK. Therefore, we hypothesize that it is the inhibition by AMPK of the prosurvival GRP78-mediated UPR signaling that significantly potentiates apoptosis in response to 2-DG plus Akt treatment, rather than the classical CHOP–mediated apoptotic signaling induced by extensive ER stress and prolonged UPR.
References


19. 2-DG Induces UPR-Mediated Cell Death in ALL


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