Targeting Glucose Consumption and Autophagy in Myeloma with the Novel Nucleoside Analogue 8-Aminoadenosine

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Multiple myeloma, an incurable plasma cell malignancy, is characterized by altered cellular metabolism and resistance to apoptosis. Recent connections between glucose metabolism and resistance to apoptosis provide a compelling rationale for targeting metabolic changes in cancer. In this study, we have examined the ability of the purine analogue 8-aminoadenosine to acutely reduce glucose consumption by regulating localization and expression of key glucose transporters. Myeloma cells counteracted the metabolic stress by activating autophagy. Co-treatment with inhibitors of autophagy results in marked enhancement of cell death. Glucose consumption by drug-resistant myeloma cells was unaffected by 8-aminoadenosine, and accordingly, no activation of autophagy was observed. However, these cells can be sensitized to 8-aminoadenosine under glucose-limiting conditions. The prosurvival autophagic response of myeloma to nutrient deprivation or to nucleoside analogue treatment has not been described previously. This study establishes the potential of metabolic targeting as a broader means to kill and sensitize myeloma and identifies a compound that can achieve this goal.

Multiple myeloma (MM) is a plasma cell malignancy with a yearly incidence of 14,000 in the United States and accounts for 10% of deaths from hematological malignancies (1). Multiple myeloma remains incurable with a median survival of 3–5 years. Current therapeutic options extend longevity, but patients eventually succumb to the disease due to the development of drug resistance (2).

Nucleoside analogues are antimetabolites that play a pivotal role in the treatment of a spectrum of hematological malignancies. Our laboratory has extensively characterized the novel purine nucleoside analogue 8-chloroadenosine and congener 8-aminoadenosine (8-NH₂-Ado) (3–5), which are highly effective in tissue culture models of multiple myeloma as well as a spectrum of other hematological and solid cancers. 8-Chloroadenosine is currently in phase I clinical trial. The general mechanism of action of these antimetabolites involves RNA and/or DNA termination, ATP depletion, and subsequent induction of apoptosis (6), but a comprehensive analysis of their cellular effects has not been reported.

In this study, we describe a novel feature in the mechanism of action of purine nucleoside analogue 8-NH₂-Ado. We find that 8-NH₂-Ado inhibits glucose consumption that is associated with an activation of autophagy. This ability to reduce glucose consumption is particularly important, given the dependence of many tumor cells on aerobic glycolysis and the ensuing increase in glucose consumption to meet energetic and biosynthetic demands. Normal cells typically rely on oxygen to metabolize glucose via the glycolytic and oxidative phosphorylation pathways to generate ATP. In contrast, many tumor cells demonstrate enhanced glycolysis and lactate production, utilizing the less efficient mode of ATP production even in the presence of oxygen (7). This phenomenon, termed aerobic glycolysis, was first described by Otto Warburg in the 1930s and was originally thought to be a result of defects in oxidative phosphorylation (OXPHOS) (8). Recent studies have shown that tumor cells do contain functional mitochondria (9) yet still produce excessive lactate, suggesting that the enhanced glycolytic flux may confer a growth advantage. In support of this notion, tumor cells forced to revert to oxidative phosphorylation display reduced tumorigenicity (9). Clinicians have capitalized on the ubiquity of these metabolic alterations in cancer (7), including myeloma (10, 11), for diagnostic and prognostic purposes through the use of [¹⁸F]fluorodeoxyglucose positron emission tomography. The significance of the glycolytic phenotype in neoplastic pathogenesis has been explored in a variety of studies. Large scale gene expression analyses reveal the selective up-regulation of genes encoding constituents of the glycolytic pathway across numerous forms of cancer, including myeloma (12). An intimate association between transcriptional control of metabolic genes and the activity of classical oncogenes and tumor suppressors, including Myc, p53, and Hif1α, is well documented (13). Recently, requisite events preceding the switch from OXPHOS to aerobic glycolysis in immortalized cells have been shown to involve expression of the embryonic form of pyruvate kinase, correlating with the ability to form tumors in xenograft studies (14). These studies have stimulated a renewed
interest in strategies that target metabolism and cellular bioenergetics unique to cancer cells.

Here we report that 8-NH$_2$-Ado acutely regulates glucose uptake prior to loss of mitochondrial membrane potential. Glucose deprivation-induced bioenergetic stress is buffered by the cellular activation of autophagy, providing the rationale for combining 8-NH$_2$-Ado with inhibitors of autophagy. In cells more resistant to 8-NH$_2$-Ado, artificial reduction of glucose consumption robustly sensitizes these cells to 8-NH$_2$-Ado without the activation of autophagy. This finding suggests that tumor cells with deficient autophagic responses may be uniquely susceptible to interference with glucose utilization pathways.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The MM.1S cell line was developed in our laboratory. U266, ARH 77, and RPMI 8226 cell lines were from ATCC. All cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, 2.5 μg/ml fungizone, and 0.5 μg/ml plasmocin (InvivoGen, San Diego, CA) and maintained in a 37 °C incubator with 5% CO$_2$.

**Patient Samples**—Multiple myeloma cells were freshly isolated from bone marrow aspirates after informed consent. Mononuclear cells were isolated with Ficoll/Histopaque 1077 (Sigma), followed by CD138+ enrichment with microbeads and automated magnetic cell sorting using an AutoMacs cell sorter (Miltenyi Biotec, Auburn, CA).

**Chemicals and Reagents**—8-NH$_2$-adenosine was purchased from R.I. Chemicals Inc. (Orange, CA). Gemcitabine and bortezomib (Millennium Pharmaceuticals) were obtained from Northwestern Memorial Hospital pharmacy. 3-Methyladenine, cytochalasin B, chloroquine, saponin, 2-deoxyglucose, and 1,1-dimethylbiguanide hydrochloride (metformin) were from Sigma. Tetramethylrhodamine ethyl ester perchlorate was from R.I. Chemicals Inc. (Orange, CA). Glyceraldehyde-3-phosphate dehydrogenase antibody was used to determine cell growth. Cells (10,000–25,000 cells/ml) were left untreated or treated with 3 μM 8-NH$_2$-Ado for the indicated times. 20 nM tetramethylrhodamine ethyl ester perchlorate was added to all wells at a final concentration of 100 nM. Cells were incubated at 37 °C for 30 min, followed by PBS wash and resuspended in PBS containing 2% fetal bovine serum and 20 nM tetramethylrhodamine ethyl ester perchlorate, and samples were analyzed by flow cytometry.

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**Cell Proliferation Assays**—CellTiter 96® AQ’ueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) was used to determine cell growth. Cells (10,000–25,000 cells/well) were cultured in 96-well plates, and 490-nm absorbance measured was proportional to the number of live cells.

**Annexin V/DAPI Staining**—Subsequent to specific treatments, cells were washed in PBS and stained with Annexin V-fluorescein isothiocyanate/propidium iodide (PI) according to the manufacturer’s instructions. Data analysis was performed with the FCS Express version 3 (De Novo Software, Los Angeles, CA).

**HPLC Analysis of Intracellular 8-NH$_2$-ATP and ATP**—Nucleotides were extracted using perchloric acid and neutralized with KOH according to the protocol described in Ref. 15. 1 × 10$^7$ cells were harvested after treatment and washed with PBS, and acid extraction of cell pellet was performed with 220 μl of 0.3 M cold perchloric acid. After vortexing tubes were kept on ice for 15 min. Acidic supernatant was isolated by centrifugation at 14,000 rpm for 30 s and neutralized with 100 μl of 0.5 N KOH. Samples were stored at −80 °C and eluted isocratically from a TSK gel DEAE-2 SW (240 × 4.6 mm, 5-μm particle size, TOSOH Corp., Tokyo, Japan) with a 0.06 M Na$_2$HPO$_4$ (pH 6.9) acetonitrile buffer (60:40) at a flow of 1.0 ml/min. The eluant was monitored at 261 nm, and concentrations of ATP and 8-NH$_2$-ATP were determined from an external standard curve, which was linear from 0.01 to 10 nm for both compounds. Intracellular concentrations were calculated by determination of cell volume. The volume of cells was determined by measuring mean cell diameter using a Beckman Coulter Vi-Cell® XR cell viability analyzer.

Mitochondrial Membrane Potential—0.5 × 10$^6$ cells were treated with 3 μM 8-NH$_2$-Ado for the indicated times. 30 min prior to the harvest time, potentiometric dye tetramethylrhodamine ethyl ester perchlorate was added to all wells at a final concentration of 100 nM. Cells were incubated at 37 °C for 30 min, followed by PBS wash, and resuspended in PBS containing 2% fetal bovine serum and 20 nM tetramethylrhodamine ethyl ester perchlorate, and samples were analyzed by flow cytometry.

**Oxygen Consumption Measurements in Intact Cells**—The respiration rate was measured with an Oxymeter (Hansatech Instruments) in 2 × 10$^6$ (MM.1S) or 5 × 10$^6$ (U266) cells/ml of PBS. After obtaining a stable rate, sodium cyanide was added at a concentration of 5 μM, and the resulting respiration rate was recorded. Oxygen consumption rates were expressed in nmol of oxygen consumed/10$^6$ cells.

**Glucose Consumption Assays**—Cellular glucose consumption correlating to [3H]2-deoxyglucose uptake was determined by modification of the protocol described previously (16). Briefly 5 × 10$^6$ MM.1S cells were left untreated or treated with 3 μM 8-NH$_2$-Ado for 5 h. Cells were then harvested and resuspended in glucose-free medium with or without drug. And the end of 5 h, cells were harvested and resuspended in 1 ml of glucose-free RPMI 1640 containing 0.2 μCi/ml 2-deoxy-D-[1-3]H glucose (specific activity 8 Ci/mmol; GE Healthcare) and 0.5 μM 2-deoxyglucose, with or without cytochalasin B (5 μM final concentration), for 25 min. The uptake was stopped by pelleting cells and resuspending them in ice-cold PBS supplemented with 5 mM 2-deoxyglucose. Cell pellets were lysed in 3% SDS, and 3H counts/min in the total lysate were measured using a liquid scintillation counter.

The rate of cellular glucose consumption was also determined using the AmpLEX Red® glucose/glucose oxidase kit (Invitrogen) following manufacturer’s instructions. Glucose concentrations in medium samples taken at the start and end of the experiments were used to determine cellular uptake.

**Immunoblot Analysis**—Cellular lysates were prepared with the Complete Lysis-M buffer (Roche Applied Science) supplemented with protease and phosphatase inhibitor mixture tablets (Roche Applied Science) prepared according to the manufacturer’s instructions. Protein concentrations were determined by a Bio-Rad protein assay. Proteins were separated on precast Tris-glycine gels (Invitrogen) transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Bedford, MA), following which membranes were blocked in casein. Primary antibody incubations were over-
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night at 4 °C, followed by 1-h room temperature incubations with horseradish peroxidase-linked anti-rabbit secondary antibody (Cell Signaling Technology, Inc., Danvers, MA) or anti-mouse secondary antibody (Amersham Biosciences). Blots were developed using the Enhanced Chemiluminescence Plus Western blotting detection reagent (Amersham Biosciences).

Immunofluorescence Microscopy—Cells were washed once in PBS, and ~65,000 cells were spun onto coated microscope slides (Shandon Cytospin™ using a Shandon Cytospin™ 2 cytocentrifuge (Thermo Electron Corp., Pittsburgh, PA). Slides were fixed in 3% paraformaldehyde, permeabilized with 0.03% saponin in PBS, and blocked with 10% normal goat serum. All primary and secondary antibody dilutions were made in PBS containing 10% normal goat serum and 0.03% saponin. Cells were stained overnight with 1:100 dilutions of antibodies to human GLUT1, GLUT4, LC3, or Golgin-97 with detection using anti-rabbit IgG-Alexa Fluor 594 or anti-mouse IgG-Alexa 488 (Invitrogen) for 60 min. Cells were mounted with Ultra Cruz mounting medium (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) containing DAPI for counterstaining. Cells were visualized with a 63 × (1.4 numerical aperture) oil objective LSM-510 Meta Carl Zeiss confocal microscope. Image analysis was performed using the Zeiss Axiovision LE image browser.

Acridine Orange Staining—Acidic vesicular organelles (AVOs) were detected by incubating cells with acridine orange (17). In stained cells, acidic compartments fluoresced in bright red, quantified using fl3 mode (>650 nm), and base-line green/fluorescein isothiocyanate fluorescence was measured utilizing the fl1 channel (500–550 nm). To assess the percentage of cells forming AVOs, cells were incubated with 0.1 μg/ml acridine orange (Molecular Probes) for 15 min at 37 °C just prior to harvesting at the end of a treatment period.

Transient Transfections—MM.1S cells were transiently cotransfected with 0.5 μg of mCherry RFP vector (obtained from Drs. C. Clevenger, F. Fang, and J. Kobinski, Northwestern University, Chicago, IL) and either 2.5 μg of GLUT1 (GeneCopoeia™, Germantown, MD) or empty vector pCDNA 3.0 (Invitrogen). Transfections were carried out using Amaza Nucleofector kit V (Amaza Biosystems, Cologne, Germany). Cells were incubated for 24 h before RFP-positive cells were sorted by Dako Moflo cell sorter.

Statistical Analyses—All data presented are derived from at least three independent determinations, unless otherwise noted. Two-tailed p values were calculated using paired or unpaired t tests as indicated, with GraphPad Prism software (San Diego, CA). p values of <0.05 were considered to be statistically significant.

RESULTS

Sensitivity of Myeloma Cell Lines to 8-NH₂-Ado—Sensitivity to 8-NH₂-Ado was determined by assessment of cellular proliferation (Fig. 1, A1) in response to a range of concentrations of 8-NH₂-Ado in the MM.1S, RPMI 8226, U266 (myeloma) and ARH 77 (B lymphoblastoid) cell lines. The MM.1S cells were most sensitive to the growth-inhibitory effects of 8-NH₂-Ado followed by ARH 77, RPMI 8226 and U266 cells. Studying two differentially sensitive cell lines represents an ideal system to investigate unappreciated aspects of the mechanism of action of 8-NH₂-Ado, particularly those that directly influence cell death. Therefore, we selected U266 and MM.1S cells for further study and verified the differential toxicity of 8-NH₂-Ado by directly measuring cell death with the same dose range of 8-NH₂-Ado (Fig. 1, A2). The IC₅₀ in MM.1S and U266 cells is 1.5 and 8.88 μM, respectively. We have previously shown in MM.1S cells that 8-NH₂-Ado rapidly decreases intracellular ATP with a concomitant accumulation of 8-NH₂-ATP (5). Studies in adenosine kinase-deficient cells indicate that the toxic effects of 8-NH₂-Ado require adenosine kinase activity to convert 8-NH₂-Ado to 8-NH₂-ATP (5). To ensure that decreased accumulation of the active compound was not responsible for the relative resistance of the U266 cell line, we first measured ATP and 8-NH₂-ATP levels following treatment in both cell lines.

Reduction in Intracellular ATP Is More Pronounced in the MM.1S Cells, whereas 8-NH₂-ATP Accumulation Is Equivalent—We determined intracellular 8-NH₂-ATP and ATP levels in a time course assay of MM.1S and U266 cells treated with 3 μM 8-NH₂-Ado. Although the intracellular concentration of 8-NH₂-ATP reaches similar levels in both cell types, we observed a greater drop in ATP levels in the MM.1S cells (Fig. 1, B1 and B2). The similar accumulation of 8-NH₂-ATP in the less sensitive U266 cells rules out the possibility that deficient uptake of pro-drug and subsequent conversion to 8-NH₂-ATP accounts for lower toxicity. However, we were interested in determining the cause of the steeper drop in ATP levels in the MM.1S cells. Competition between phospho-derivatives of 8-NH₂-Ado and their corresponding endogenous counterparts (e.g. ADP and AMP) for enzymes involved in adenosine phosphorylation probably accounts for the decreased ATP levels in U266 cells. The observation that 8-NH₂-ATP accumulation is mirrored in the MM.1S cells while ATP levels decline to a greater extent suggested to us that a bioenergetic pathway may be additionally and selectively impaired in the MM.1S cells. Therefore, we investigated the effects of 8-NH₂-Ado on two other ATP-generating pathways: OXPHOS and glycolysis.

Two Indices of Mitochondrial Function Reveal No Alterations at Early Time Points following 8-NH₂-Ado Treatment—To ask whether a selective inhibition of oxidative phosphorylation may account for the steeper drop in ATP levels in the MM.1S cells, we evaluated mitochondrial functionality by measuring mitochondrial membrane potential (∆ψₘ) during a time course of 8-NH₂-Ado treatment. At 5 h post-drug treatment, a time point at which there is significant loss of intracellular ATP in both cell lines, there was no observed reduction of ∆ψₘ (Fig. 1C). At later time points, there was a selective decrease in ∆ψₘ in the MM.1S cells, which was probably a secondary effect of cell death caused by mitochondrial outer membrane permeabilization and cytochrome c release. The observation that ATP concentrations decrease before ∆ψₘ declines suggests that loss of mitochondrial function is not the cause of the bioenergetic deficit. To validate this conclusion, we also measured oxygen consumption in MM.1S and U266 cells treated with 8-NH₂-Ado.
FIGURE 1. 8-NH₂-Ado treatment impacts cellular bioenergetics. A1, dose response of 8-NH₂-Ado in myeloma cell lines MM.1S, ARH 77, U266, and RPMI 8226. Cells were exposed to the indicated concentrations of 8-NH₂-Ado for 48 h, and cell proliferation was determined by the MTS assay (Promega). Results are averages of quadruplicate wells and representative of two independent experiments ± S.D. A2, dose response of MM.1S and U266 cells to 8-NH₂-Ado. Cells were treated with 8-NH₂-Ado for 48 h, and cell death was analyzed by AnnexinV DAPI staining and flow cytometry. Results were derived from two independent experiments, performed in duplicate, ± S.E.

B1 and B2, 8-NH₂-ATP accumulation and ATP reduction in 8-NH₂-Ado-treated MM.1S and U266 cells. MM.1S and U266 cells were treated with 3 μM 8-NH₂-Ado for various times. Intracellular concentrations of ATP (B1) and 8-NH₂-ATP (B2) were determined by HPLC analysis of acid extracts, as described in Ref. 45. Results are derived from a minimum of two independent experiments, performed in duplicate, with two HPLC runs per sample, ± S.E. C, time course analysis of mitochondrial membrane potential in MM.1S and U266 cells treated with 8-NH₂-Ado. MM.1S cells were treated with 3 μM 8-NH₂-Ado for various times, following which mitochondrial membrane potential was measured by tetramethylrhodamine ethyl ester perchlorate fluorescence. Results are from two independent experiments performed in duplicate ± S.D. D1 and D3, cellular oxygen consumption is unchanged in MM.1S and U266 cells treated with 8-NH₂-Ado. MM.1S and U266 cells were treated with 3 μM 8-NH₂-Ado, and oxygen consumption was measured by loading untreated (control; C) or treated cells in an oxygraph chamber. D2 and D4, following stabilization of oxygen consumption, sodium cyanide (5 μM final concentration) was injected into the chamber, and subsequent oxygen consumption was determined to obtain a ratio of mitochondrial to total oxygen consumption. For MM.1S, n = 7, and for U266, n = 4, ± S.E. Unpaired t test analysis did not indicate statistically significant differences between control and treated cells.
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Additionally, to confirm that there is no change in mitochondrial respiration in the MM.1S and U266 cells, we measured oxygen consumption following cyanide treatment. Cyanide is a potent inhibitor of mitochondrial respiration, and any oxygen consumption measured post-cyanide treatment is considered non-mitochondrial. Utilizing cyanide, we calculated the ratio of mitochondrial oxygen consumption to total oxygen consumption for untreated and 8-NH₂-Ado-treated cells. This ratio was not significantly different between treated and untreated cells (Fig. 1, D2 and D4) in either MM.1S or U266 cells. These results confirm that changes in mitochondrial respiration are not responsible for the greater decline in intracellular ATP seen in the MM.1S cells.

An alternative mechanism that could explain this occurrence could be changes in flux through the glycolytic pathway. Glucose metabolism via glycolysis is thought to provide a rapid (albeit inefficient) supply of ATP to proliferating tumor cells. To investigate whether 8-NH₂-Ado elicits changes in glucose metabolism, we measured glucose consumption in MM.1S and U266 cells treated with 8-NH₂-Ado.

8-NH₂-Ado Decreases Glucose Uptake in the MM.1S Cell Line—To evaluate glucose uptake, we treated MM.1S cells with 3 μM 8-NH₂-Ado for 5 h. During the final 1 h, cells were switched to glucose-free medium, followed by incubation with 2-deoxy-o-[³H]glucose (0.2 μCi/ml) and 0.5 μM 2-deoxyglucose for 25 min. Untreated cells were incubated in the absence or presence of 5 mM cytochalasin B (CytB). Cell pellets were lysed, and ³H counts/min were measured in a liquid scintillation counter. Results are normalized to untreated control, set to 100%. n = 3, ±S.E., with two replicates per experiment.

Myeloma Cells Less Sensitive to 8-NH₂-Ado Resist Changes in Glucose Consumption—We assessed the 8-NH₂-Ado-induced regulation of glucose consumption in the panel of cell lines previously characterized for their sensitivity to 8-NH₂-Ado. Glucose uptake was analyzed in the four cell lines.

At 5 h of 8-NH₂-Ado treatment, we observed no change in base-line oxygen consumption (Fig. 1, D1 and D3) in both the MM.1S and U266 cells, in accordance with the Δψᵢₒ studies.
cell lines at 5 and 17 h after 8-NH₂-Ado treatment. Glucose consumption was determined by measuring glucose depletion from media by cells cultured in the absence or presence of 8-NH₂-Ado. A 40% decline in glucose consumption was observed in MM.1S cells treated with 3 mM 8-NH₂-Ado for 5 h (Fig. 2, B1), thus corroborating previous results evaluating [³H]2-deoxyglucose uptake. RPMI 8226 and ARH 77 cells (Fig. 2, B2 and B4), which are more resistant to 8-NH₂-Ado, required higher concentrations of 8-NH₂-Ado (i.e. 6 mM) to induce decreases in glucose consumption comparable with those seen in the sensitive MM.1S cells. Even at the higher 6 mM dosage of 8-NH₂-Ado, the U266 cells exhibited only a slight decrease in glucose consumption, which diminished over time (Fig. 2, B3), consistent with the observed resistance to 8-NH₂-Ado. Given the connections between ATP generation and glucose consumption within the context of malignancy, these observations suggest that 8-NH₂-Ado-induced glucose limitation could facilitate the greater decline in ATP in the sensitive MM.1S cells.

**Regulation of Glucose Uptake Is Unique to 8-NH₂-Ado**—To determine if the reduction in glucose consumption is a generalized phenomenon preceding myeloma cell death or represents a unique property of 8-NH₂-Ado, we examined glucose uptake in the presence of clinically relevant therapeutics that act through distinct mechanisms. We examined the effect of the myeloma therapeutic bortezomib (Velcade; PS-341) and the nucleoside analogue gemcitabine on glucose transport rates in comparison with 8-NH₂-Ado. At the concentrations used, these compounds elicit higher toxicity than 8-NH₂-Ado (18, 19) but do not demonstrate an inhibition of glucose consumption comparable with that seen with 8-NH₂-Ado (Fig. 2C), thereby suggesting that regulation of glucose consumption is not a generalized phenomenon associated with cytotoxic therapeutics.

**8-NH₂-Ado Treatment Causes Aggregation of GLUT4 in MM.1S Cells**—To examine the functional role of diminished glucose consumption in the toxicity of 8-NH₂-Ado, we first investigated potential mechanisms that may be responsible for the decline in glucose consumption in the MM.1S cell line. We examined expression and subcellular localization of GLUT1 and GLUT4, two key glucose transporters present in B cells. Immunoblot analysis of whole cell lysates of MM.1S cells treated with 8-NH₂-Ado for 5 and 24 h indicates a time-dependent decrease in GLUT1 expression at 5 h, whereas at 24 h there was a down-regulation of both transporters (Fig. 3, A1 and A2). Given the rapid, robust inhibition of glucose uptake and the relatively modest reduction in GLUT1 protein levels at early time points, we hypothesized that another regulatory mechanism may be involved. We then determined the subcellular localization of GLUT1 and -4 by immunofluorescence microscopy, since the trafficking of these proteins to the cell

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**FIGURE 3.** Selective regulation of GLUT4 localization by 8-NH₂-Ado in MM.1S cells. A1 and A2, MM.1S and U266 cells were left untreated (control; C) or treated for 5 or 24 h with 3 mM 8-NH₂-Ado, following which whole cell lysates were analyzed for GLUT1 or -4 expression by immunoblot analysis. Densitometric scanning of GLUT1 or -4 expression was normalized to glyceraldehyde-3-phosphate dehydrogenase and expressed as a percentage of the untreated control. Results are from n = 3, ± S.E. B1 and B2, 8-NH₂-Ado induces aggregation of GLUT4 in the MM.1S cells. MM.1S and U266 cells were left untreated (C) or treated with 3 mM 8-NH₂-Ado for 5 h, washed, and spun onto slides by cytoospin. Cells were stained for GLUT1, GLUT4, and the trans-Golgi network (with anti-Golgin-97) and visualized by confocal immunofluorescence microscopy. The arrows indicate aggregation of GLUT4 and depletion of plasma membrane stain for GLUT4 in MM.1S cells treated with 8-NH₂-Ado. Results are representative of two independent experiments.
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FIGURE 4. Overexpression of GLUT1 in MM.1S cells does not impact 8-NH2-Ado toxicity. MM.1S cells were co-transfected with a plasmid encoding GLUT1 or empty vector along with an RFP control vector. A, GLUT1 overexpression by immunoblot analysis in total transfected population. Representative immunoblot is demonstrated (n = 2). B, rescue of glucose consumption in GLUT1-transfected cells. Glucose uptake 5 h post-8-NH2-Ado treatment was measured in the total transfected cell population as described previously (n = 3, ± S.E.). C, cell death in GLUT1-transfected, 8-NH2-Ado-treated MM.1S cells. RFP-positive cells were sorted, treated with 1 µM 8-NH2-Ado for 24 h, and analyzed for cell death by flow cytometric determination of AnnexinV/DAPI-positive cells (n = 3, ± S.E.).

surface also impacts their functionality. Untreated MM.1S (Fig. 3, B1) and U266 cells (Fig. 3, B2) showed a homogenous cytoplasmic and plasma membrane distribution of GLUT1 and -4. Treatment of MM.1S cells with 8-NH2-Ado caused the selective aggregation of GLUT4 within the trans-Golgi network, as evidenced by co-localization of GLUT4 with anti-Golgin-97, a trans-Golgi network protein (20) (Fig. 3, B1). A corresponding reduction in the plasma membrane and cytoplasmic distribution of GLUT4 in MM.1S cells was also detected. Conversely, we detected minimal translocation of GLUT1 (Fig. 3, B1) in the MM.1S cells. The localizations of GLUT1 and -4 were not regulated by 8-NH2-Ado in the U266 cells at either time (Fig. 4, A1, A2, and B2). Therefore, abrogation of GLUT4 plasma membrane localization is temporally associated with the selective decrease in glucose uptake seen in the MM.1S cell line, suggesting a causal relationship between these events.

Restoration of Glucose Consumption during 8-NH2-Ado Treatment in MM.1S Cells via GLUT1 Overexpression Elicits Only a Mild Cytoprotective Effect—To directly assess the role of the 8-NH2-Ado-induced decrease in glucose consumption in the MM.1S cells, we overexpressed GLUT1 to reverse this effect during treatment. We chose to exogenously express GLUT1 to restore glucose transport rates due to the sustained plasma membrane localization of this protein during 8-NH2-Ado treatment. If GLUT4 were overexpressed, this protein would presumably aggregate similarly to the endogenous protein and not affect glucose uptake. Furthermore, we utilized a lower concentration of 8-NH2-Ado, which was more suitable for the extended duration of these experiments. Increased GLUT1 expression (Fig. 4A) effectively reversed the 8-NH2-Ado-induced decline in glucose consumption (Fig. 4B). Given the strong connections in the literature between glycolytic inhibition and tumor cell death, we anticipated that restoration of glucose consumption during 8-NH2-Ado treatment would partially abrogate the toxicity of the drug in the MM.1S cells. This result would have indicated that regulation of glucose transporter functionality accounts for the relative sensitivity of the MM.1S cells compared with the U266 cells. Surprisingly, the protective effect of GLUT1 overexpression and restoration of glucose consumption during 8-NH2-Ado treatment was much less than anticipated and did not reach statistical significance (Fig. 4C). Considering that the maintenance of glucose metabolism is critical to the growth and survival of tumor cells, we hypothesized that the deleterious effects of glucose deprivation during 8-NH2-Ado treatment of MM.1S cells may be counteracted by a cellular response to metabolic stress. One process known to be activated under conditions of such stress is autophagy. Therefore, we sought to determine whether an increase in autophagic activity may be involved.

Decreased Glucose Consumption Elicited by 8-NH2-Ado Activates Autophagy—Nutrient deprivation can be associated with the activation of the process of macroautophagy (herein referred to as autophagy) (21) as a survival mechanism allowing cells to catabolize intracellular components to sustain critical bioenergetic and synthetic needs. This protective response results in resistance to the toxic effects of drugs that elicit cell death by creating metabolic stress (22). To determine whether an autophagic response is associated with 8-NH2-Ado treatment, we examined 8-NH2-Ado-treated MM.1S cells for changes in the LC3 (microtubule-associated protein-1 light chain-3) protein (23). Upon induction of autophagy, cytoplasmic LC3-I is post-translationally processed to a membrane-bound form, LC3-II, which accumulates in autophagosomes. Therefore, LC3-II levels represent an ideal marker for the initiation of autophagy. After 2.5 h of 3 µM 8-NH2-Ado treatment, we detected the formation of LC3-II protein by immunoblot analysis (Fig. 5A) and by the appearance of LC3-II punctate staining, characteristic of membrane-bound LC3-II (Fig. 5B). As an additional indicator of autophagic activity, we stained with acridine orange (a lysosomal marker), which fluoresces bright red under the acidic conditions in autophagolysosomes (17). Treatment of MM.1S cells with 1 µM 8-NH2-Ado over a longer period caused the appearance of a population with a high AVO content (Fig. 5, C1 and C2), indicating the activation of
FIGURE 5. 8-NH$_2$-Ado treatment induces autophagy in the MM.1S cell line. A, immunoblot analysis of LC3-I processing to LC3-II in MM.1S cells. Cells were treated with 3 μM 8-NH$_2$-Ado for the indicated times (h), following which whole cell lysates were analyzed for LC3 by immunoblot analysis. Glyceraldehyde-3-phosphate dehydrogenase expression was used as a loading control. Results are representative of n = 3, ±S.E. B, immunofluorescence analysis of LC3 localization in MM.1S cells treated with 8-NH$_2$-Ado, showing appearance of a punctate staining pattern. Cells were treated with 3 μM 8-NH$_2$-Ado for the indicated times and stained with anti-LC3 antibody. The arrows point to a punctate staining pattern. Results are an average of two independent repeats. C1 and C2, activation of autophagy by detection of AVO-containing cells. Acridine orange (AO) was used to detect cells with increased abundance of AVOs. In acridine orange-stained cells, acidic compartments fluoresce bright red, quantified using fl3 mode (>650 nm), and base-line green/fluorescein isothiocyanate fluorescence was measured utilizing the fl1 channel (500–550 nm). MM.1S cells were treated or not with 1 μM 8-NH$_2$-Ado for 24 h before incubation for 15 min with acridine orange. The AVO-high population represents cells undergoing autophagy. In C2, the percentage of cells in which autophagy was detected by the presence of AVOs is depicted. n = 3, ±S.E. D1 and D2, p62 protein levels are selectively reduced in the AVO-high population. MM.1S cells were treated with 1 μM 8-NH$_2$-Ado for 24 h before acridine orange staining and separation of AVO-high and AVO-low cells by fluorescence-activated cell sorting. Untreated cells were stained but not separated. The three cell populations were lysed and subjected to immunoblot analysis of p62 protein levels. In D2, p62 expression values were normalized to an endogenous control (n = 3, ±S.E.).
To confirm that the AVO-high population demonstrates activation of autophagy, we sorted the AVO-high and AVO-low populations and determined expression levels of p62 protein, which is decreased upon activation of autophagy (24). p62 is responsible for the aggregation of polyubiquitylated proteins with LC3, thereby enabling their degradation in the autophagosome (24). The AVO-high population induced with 8-NH₂-Ado exhibits a reduction in p62 expression in comparison with the AVO-low population (as shown in Fig. 5, D1 and D2). We also treated cells with inhibitors of autophagy. 3-Methyladenine (3-MA) inhibits Class III phosphatidylinositol 3-kinase Vps34, the activity of which is required for autophagosome formation. Consistent with its activity at proximal steps in the pathway, this compound completely blocked the appearance of the AVO-high population during 8-NH₂-Ado treatment (Fig. 6 A). Chloroquine (CQ), an inhibitor of lysosomal acidification, acts further downstream in the autophagic pathway by inhibiting the fusion of the autophagosomes with lysosomes (25) or by inhibiting the acid-dependent degradation of autophagosomal contents (26). In accordance with its mechanism of action, we observed an increase in the number of cells in the AVO-high population during CQ co-treatment, indicative of impaired processing of autophagosomes (Fig. 6 A). In summation, the aforementioned studies suggest that the AVO-high population represents cells actively undergoing autophagy in response to incubation with 8-NH₂-Ado.

**FIGURE 6.** Blocking autophagy in MM.1S cells with chloroquine or 3-MA enhances 8-NH₂-Ado-induced cell death, suggesting a pro-survival role for autophagic activation. A, autophagy inhibitors modulate acridine orange staining and increase 8-NH₂-Ado-induced apoptosis. MM.1S cells were treated with the indicated combinations of drugs for 24 h before incubation with acridine orange, AnnexinV-APC, and DAPI to concurrently assess autophagic activity and cell death. Concentrations of drugs are as follows: 1 μM 8-NH₂-Ado, 10 μM CQ, and 2 mM 3-MA. Representative data are shown from one of three independent experiments. B, autophagy inhibitors enhance the toxicity of 8-NH₂-Ado in MM.1S cells. Shown is a quantification of results from experiments in A (n = 3, ±S.E.).
of 3-MA on autophagosome formation (Fig. 6, A and B). These results confirm that the activation of autophagy in response to 8-NH₂-Ado is a prosurvival response.

**Inhibition of Glucose Consumption by 8-NH₂-Ado Is Required for Induction of Autophagy and Sensitization to CQ in MM.1S Cells**—To connect the inhibition of glucose uptake by 8-NH₂-Ado and the autophagic response of MM.1S cells, we again restored glucose uptake during 8-NH₂-Ado and assessed markers of autophagy. This time we employed a pharmacological approach to reverse the decline in glucose consumption by pretreating cells with metformin. This anti-diabetic drug increases glucose consumption in muscle cells by promoting plasma membrane localization of GLUT1 and GLUT4 (27). Co-treatment with metformin restored glucose uptake in MM.1S cells to base-line levels (Fig. 7A). Metformin treatment also reversed the appearance of the LC3-II protein (Fig. 7B) and the AVO-high population (Fig. 7C and C2) induced by 8-NH₂-Ado treatment. Importantly, metformin treatment reverses the enhancement in cell death induced by CQ co-treatment with 8-NH₂-Ado (Fig. 7D). Therefore, blocking the activation of autophagy enhances the efficacy of 8-NH₂-Ado by potentiating the stress of glucose deprivation. Overexpression of GLUT1 in MM.1S cells also reversed the enhancement by CQ of 8-NH₂-Ado-induced cell death (Fig. 7E). Taken together, these results confirm that activation of autophagy is a direct survival response to the inhibition of glucose consumption elicited by 8-NH₂-Ado.

**Limiting Glucose Consumption Sensitizes Resistant U266 Cells to 8-NH₂-Ado**—We were interested in determining the effect of artificially reducing glucose consumption in the resistant U266 cells and evaluating sensitivity to 8-NH₂-Ado. We cultured U266 cells in glucose-limiting medium (glucose-free medium with 10% serum) and assayed for sensitivity to 8-NH₂-Ado. The effective glucose concentration in medium containing 10% serum was determined to be 0.7 mM, far lower than the 11 mM glucose of standard medium. Indeed culture in low glucose-containing medium sensitizes U266 cells to 8-NH₂-Ado with a 1.75-fold enhancement of drug-induced cell death (Fig. 8A). The U266 cells did not demonstrate activation of autophagy based on the lack of an AVO-high cell population and the insignificant regulation of p62 expression (Fig. 8, B1, B2, C1, and C2). Treatment of U266 cells in complete or low glucose-containing medium with 8-NH₂-Ado does not sensitize them to CQ or 3-MA treatments, consistent with the fact that no autophagic response is detected in these cells (Fig. 8D). We assayed a variety of compounds known to activate autophagy (including rapamycin and valproic acid) for use as positive controls in the U266 cells, but all autophagy markers were negative (data not shown). This suggests that the lack of activation of autophagy in the U266 cells may compromise the ability to buffer metabolic stresses.

**Primary Multiple Myeloma Cells Exhibit Enhanced Sensitivity to 8-NH₂-Ado with CQ Co-treatment**—We further substantiated the utility of combining 8-NH₂-Ado with CQ in myeloma patient samples. CD138+ plasma cells obtained from myeloma patients were treated with a dose range of 8-NH₂-Ado with and without CQ co-treatment. Analogous to the MM.1S cell line, CQ co-treatment was found to enhance sensitivity of myeloma primary cells to 8-NH₂-Ado (Fig. 9). This observation underscores the therapeutic potential of targeting cellular bioenergetics and the ensuing process of autophagy in myeloma.

**DISCUSSION**

Nucleoside analogues have been used extensively in the treatment of a spectrum of hematological malignancies and more recently for solid tumors (28). Continued understanding of the mechanisms of action and pathways of resistance to these first line cancer chemotherapeutics can elucidate cancer-specific molecular targets. In this study, we set out to determine whether cellular bioenergetics could account for the greater sensitivity of a representative cell line to 8-NH₂-Ado. Although we found that glucose transport inhibition by 8-NH₂-Ado was unrelated to this increased sensitivity, this phenomenon did enable us to identify strategies to increase the activity of this compound in two different cellular contexts.

We previously established that 8-NH₂-Ado toxicity is associated with a substantial decrease in intracellular ATP levels (5). Given the more pronounced decline in ATP levels in the sensitive MM.1S cells compared with the resistant U266 cells, we examined several parameters of cellular bioenergetics in these two cell lines following 8-NH₂-Ado treatment. We first measured changes in $\Delta \phi_m$ and mitochondrial oxygen consumption in these cell lines in response to 8-NH₂-Ado as indicators of OXPHOS activity. We noted no difference in these parameters between MM.1S and U266 cells during the first 5 h following treatment. In the absence of changes in cellular respiration, a potential upstream metabolic pathway that might account for a differential decline in intracellular ATP levels between the two cell lines could be glycolysis.

Our data demonstrate the specific regulation of glucose uptake in myeloma cell lines. We have determined aggregation of GLUT4 within the endosomal trans-Golgi network that may
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A

Glucose uptake (% of untreated control)

1.25 mM Met 5 mM 8-NH2-Ado Met + 8-NH2-Ado

B1

3 uM 8-NH2-Ado: - + + + + + + + + + + + + + + +
1.25 mM Metformin: - - - - - + + + + + + + + + + + + +
Hours of Treatment: 0 1 2 2.5 3 1 2 2.5 3

LC3-I 19
LC3-II 14
GAPDH 37

B2

Fold change LC3-II (normalized to control)

Duration of treatment (Hrs)

8-NH2-Ado

8-NH2-Ado + Met

C1

C 8-NH2-Ado

Met 8-NH2-Ado + Met

FL3/Texas Red

FL1/FITC

AVO low

AVO high

C2

% AVO high cells

C 8-NH2-Ado 8-NH2-Ado + Met

D

Cell death (% Annexin +)

C Met CQ 8-NH2-Ado + Met + CQ 8-NH2-Ado + CQ

E

Cell death (% Annexin +)

Drug induced - baseline

CQ 8-NH2-Ado 8-NH2-Ado + CQ

Empty GLUT1

*
potentially account for the decrease in glucose consumption induced by 8-NH$_2$-Ado. Although the exact role for localization of GLUT4 in the trans-Golgi network is unknown, studies in muscle and adipose tissue indicate that transit into the trans-Golgi network may allow for storage and/or routing of GLUT4 into an insulin-responsive compartment (29, 30). We cannot rule out the roles of other downstream effectors of glycolysis and glucose consumption (i.e. allosteric regulation of phosphofructokinase 1 by phosphorylation of phosphofructokinase-2) (31) and hexokinase II (32) contributing to 8-NH$_2$-Ado-induced reduction of glucose consumption.

The overexpression of GLUT1 and restoration of glucose consumption in the MM.1S cells during 8-NH$_2$-Ado treatment led to an insignificant reversal of cell death elicited by 8-NH$_2$-Ado. Metabolic stress can be counteracted by the activation of autophagy, which aids in the release of critical biosynthetic intermediates by catabolizing cytoplasmic macromolecules. The decrease in glucose consumption stimulated by 8-NH$_2$-Ado treatment exerts energetic stress in the MM.1S cell line, which precipitates an activation of autophagy. The glucose deprivation-induced activation of autophagy in MM.1S cells promotes resistance, as evidenced by the increased cell death seen when 8-NH$_2$-Ado is administered in combination with inhibitors of autophagy. Using two different approaches (i.e. pretreatment with metformin or overexpression of GLUT1), we were able to demonstrate reversal of CQ-induced hypersensitivity to 8-NH$_2$-Ado. The stimulatory effect of metformin on tumor cell glucose transport described herein is of direct clinical significance, since this effect could potentially antagonize the activity of therapeutics, such as genotoxic agents and mTOR inhibitors, that have been shown to be more efficacious in tumor cells exhibiting decreased glycolytic flux (33–35).

Our data suggest two distinct responses elicited upon regulation of glucose consumption in different myeloma cell types, as outlined in Fig. 10. The sensitive MM.1S cells demonstrate a rapid decline in glucose consumption that does not facilitate cell death and is only responsible for activating autophagy. This active process of protecting cells against nutrient deprivation can be taken advantage of only when induced (i.e. the protective effects are afforded only in the context of a decline in glucose). Therefore, the metabolic stress associated with glucose deprivation can only translate to enhanced cell death upon concomitant inhibition of autophagy. Restoring glucose consumption to basal rates does not increase cell death, because the cell has not experienced nutrient deprivation or activated autophagy. Future work will focus on determining the particular metabolites generated by autophagy that can compensate for glucose restriction. Interestingly, we have determined that methyl pyruvate is incapable of compensating for 8-NH$_2$-Ado-induced glucose deprivation. This suggests that tricarboxylic acid cycle stimulation is not the critical downstream effector of glucose metabolism in our system. It is tempting to speculate that increased abundance of glutamine following protein catabolism in autophagolysosomes could be playing a role, given the overlapping ability of both glucose and glutamine to stimulate a distinct set of metabolic reactions. For example, alternative pathways exist for the generation of NADPH, biosynthesis of nucleotides, and supply of tricarboxylic acid cycle intermediates that can be stimulated by either molecule (36).

In contrast to the above studies, culture of the less sensitive U266 cells in glucose-limiting medium sensitizes these cells to the cytotoxic effects of 8-NH$_2$-Ado. Several studies in the IL-3-dependent FL5.12 cell line have demonstrated the protective effects of glucose metabolism during IL-3 withdrawal (37–39). In the aforementioned study, reduction in glucose consumption was a cause of, rather than a consequence of, declining mitochondrial function, and IL-3-deprived cells were rescued from growth factor withdrawal-induced apoptosis by overexpression of GLUT-1. The magnitude of glycolytic inhibition, which was proportional to growth factor withdrawal or glucose deprivation, correlated to the induction of apoptosis (37). These studies highlight two key observations pertinent to this study in that glucose consumption is not entirely driven by homeostatic demands and the decline in glucose consumption preceded the onset of apoptosis. Studies in the U266 cells indicate that a deficiency in autophagic activity may render cells incapable of buffering glucose deprivation-induced metabolic stress. The fact that many malignancies develop mechanisms to suppress autophagy may provide a unique opportunity for sensitization to classical chemotherapeutics with inhibitors of glucose metabolism (e.g. lonidamine or 2-deoxyglucose).

Multiple myeloma is characterized by a deregulation of glycolytic genes (12). This observation and the effectiveness of [18F]fluorodeoxyglucose positron emission tomography in the detection of myeloma (10, 11) suggest a dependence of myeloma on increased glucose consumption and glycolysis. Glucose can contribute to maintaining mitochondrial integrity via the generation of the NADH- and FADH$_2$-reducing equivalents (40, 41). Glucose also helps maintain the association of hexokinase II with the mitochondria, thereby preventing the release of cytochrome C (32, 42). Besides generating bioenergetic equivalents, such as ATP, glucose also serves to generate...
FIGURE 8. Sensitivity of U266 cells to 8-NH$_2$-Ado is directly enhanced under glucose-limiting conditions. A, artificial glucose restriction sensitizes U266 cells to 8-NH$_2$-Ado. U266 cells were cultured in complete medium (11 mM glucose; black bars) or in glucose-limiting medium (0.7 mM glucose; gray bars) for 48 h with and without 3 $\mu$M 8-NH$_2$-Ado. Cell death was quantitated by AnnexinV/DAPI staining and flow cytometric analysis ($n = 3$, ±S.E.). B1 and B2, U266 cells do not exhibit increased acridine orange staining during 8-NH$_2$-Ado treatment regardless of glucose availability. U266 cells were treated or not with 3 $\mu$M 8-NH$_2$-Ado in full or glucose-limiting medium for 24 h before incubation with acridine orange (AO) and flow cytometry analysis. One representative experiment of three is shown. C1 and C2, p62 protein levels remain unaltered following 8-NH$_2$-Ado treatment in U266 cells. Cells were treated as in B1 and B2 and lysed for immunoblot analysis of p62 expression. The decline in expression was not statistically significant ($n = 3$, ±S.E.). D, neither CQ nor 3-MA co-treatment affects toxicity of 8-NH$_2$-Ado in U266 cells cultured in full or low glucose medium. U266 cells were treated for 24 h with the indicated combinations of drugs in complete medium (11 mM glucose; black bars) or glucose-limiting medium (0.7 mM glucose; gray bars). CQ was used at 10 $\mu$M, whereas 3-MA was used at 2 mM. Cells were harvested and stained with AnnexinV/DAPI and analyzed by flow cytometry ($n = 3$, ±S.E.).
biosynthetic intermediates for nucleotide and fatty acid synthesis (36) and is also able to regulate various effectors controlling cell death (i.e. prosurvival Mcl-1 (43), proapoptotic BAD (44), and proapoptotic Bax (38)). The association of glycolysis and glucose consumption in immortalized B cells (37), leukemia and lymphoma cell lines, and other cancer cell lines (33, 45) is well established. The contribution of the glycolytic phenotype to increased resistance to apoptosis-inducing agents (7, 46) supports the utility in targeting glucose consumption.

Our results also suggest that further delineation of the role of autophagy in various therapeutic settings is warranted. It is important to determine whether activation of this pathway in tumor cells following drug treatment is associated with cell death or survival, since it may provide an opportunity to enhance the selectivity and potency of a given therapeutic. Indeed, chloroquine has been reported to increase the activities of doxorubicin, cyclophosphamide, and inhibitors of Akt in certain types of cancer (47–49). This notion is particularly intriguing when applied to myeloma, a malignancy characterized by progressive chemoresistance. Alternatively, compounds that have been shown to induce autophagic cell death could be prime candidates for use in malignancies harboring well-defined mutations in components of the apoptotic pathway, thereby necessitating the engagement of alternative pathways to cell death.

Our study has demonstrated the effectiveness of 8-NH₂-Ado as a pleiotropic compound that is able to elicit cell death along with an unrelated inhibition of glucose transport, a feature that appears to be unique to this compound among other myeloma therapeutics tested. The activation of autophagy can be self-destructive in time or provide an addi-

**FIGURE 9.** Primary myeloma cells exhibit increased sensitivity to 8-NH₂-Ado when co-treated with chloroquine. CD138⁺ cells were isolated from bone marrow aspirates of three myeloma patients. Next, cells were seeded in 96-well plates with the indicated concentrations of 8-NH₂-Ado with and without 10 μM CQ. Cells were incubated for 48 h before determining live cell numbers by an MTS assay. Results are expressed as a percentage of control, untreated cells (n = 1).

**FIGURE 10.** Schematics depicting pathways contributing to cell death following 8-NH₂-Ado treatment in MM.1S and U266 cells. **A**, MM.1S cells exhibit decreased glucose uptake during 8-NH₂-Ado treatment, which causes metabolic stress. This stress is alleviated by autophagy activity, thereby blocking the contribution of glucose deprivation to cell death. However, autophagy inhibitors block this protective response, resulting in much higher levels of cell death upon co-treatment. Furthermore, autophagy induction following 8-NH₂-Ado treatment can be blocked by restoring glucose consumption via GLUT1 overexpression or metformin co-treatment. **B**, U266 cells exhibit no decrease in glucose consumption in response to 8-NH₂-Ado. However, artificial reduction of glucose consumption sensitizes these cells to 8-NH₂-Ado due to the lack of protective autophagic activity in this cell line.
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A tional strategy to sensitize myeloma cells to 8-NH2-Ado. Future studies will focus on defining metabolic signatures in myeloma in order to predict responsiveness to genotoxic drugs and inhibitors of autophagy as well as to enable identification of specific effectors of glucose deprivation-mediated cell death. Understanding cellular metabolism in myeloma is a promising avenue that could yield potent combinatorial strategies based on interfering with glucose utilization during administration of existing therapeutics.

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