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Is targeting glycolysis with 2-deoxyglucose a viable therapeutic approach to bladder cancer?

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Original Article

Abstract

Purpose: Although several therapeutic options for bladder cancer are available, the poor efficacy and palpable side effects are a major concern. Establishing a more effective intervention is urgently demanded. Glycolysis is considered a strategic target and has been often investigated in various cancers. Particularly, 2-deoxyglucose (2DG), a glycolysis inhibitor, has been intensely studied and shown to be encouraging and promising. Accordingly, we investigated how targeting glycolysis with 2DG would be effective on bladder cancer cells. **Methods:** Bladder cancer 5637 cells were employed and cell viability was determined by MTT assay. To explore the anticancer mechanism of 2DG linked to glycolysis, two glycolytic parameters of hexokinase (HK) activity and ATP synthesis, metabolic signaling pathways, and induction of apoptosis were examined. Whether 2DG may potentiate several chemotherapeutic drugs being clinically used was also assessed for its possible chemosensitizing effect. **Results:** A dose-dependent study of 2DG showed a 23-80% reduction in cell viability. HK activity and cellular ATP level were decreased by ~46% and ~56% with 2DG, respectively, indicating the glycolysis inhibition. AMP-activated protein kinase was activated while protein kinase B was inactivated and also mammalian target of rapamycin was inhibited with 2DG. These modulations would lead to the growth cessation and the cell viability reduction. In fact, the down-regulation of anti-apoptotic bcl-2 and the up-regulation of pro-apoptotic Bax in 2DG-treated cells indicated induction of apoptosis. Moreover, chemotherapeutic drugs with poor cytotoxic activity were selectively sensitized with 2DG, resulting in a significantly improved cell viability reduction. **Conclusion:** 2DG has anticancer activity on bladder cancer cells and its anticancer mechanism involves the glycolysis inhibition, the modulations of certain signaling pathways, and induction of apoptosis. Additionally, 2DG has a chemosensitizing effect when combined with drugs. Thus, targeting glycolysis with 2DG appears to be an alternative, viable therapeutic approach to bladder cancer.

Keywords: 2-deoxyglucose, Anticancer, Chemosensitization, Glycolysis, Bladder cancer

1. Introduction

Bladder cancer is the second most common genitourinary malignancy with high morbidity and mortality rates in the United States.[1] Approximately 74,000 new cases were diagnosed and nearly 16,000 patients died in 2015.[2] Among several available therapeutic options for bladder cancer, intravesical administration of Bacillus Calmette-Guerin (BCG) is the most effective immunotherapy for superficial bladder cancer and carcinoma in situ (CIS).[3] This therapy has been shown to alter disease progression, reduce recurrence, and increase survival.[4] However, the therapeutic benefits of BCG are sometimes outweighed by its severe side effects, limiting its use in clinical practice.[3,4] One approach for establishing a safer, more effective therapeutic modality with fewer side effects is to disrupt the proliferative mechanism of bladder cancer substantially controlled by glycolysis.

The growth and development of tumor cells (as well as normal cells) must fulfill essential and critical cellular

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requirement, i.e. energy (ATP) metabolism. All kinds of cells require energy for cellular maintenance, activity, proliferation, and development. Peculiarly, cancer cells have been shown to preferentially depend on the inefficient glycolytic pathway (instead of TCA cycle) for ATP production (generating only 2 ATP moles/glucose) even in the presence of sufficient oxygen.[5] This is known as the "Warburg effect"[6] where cancer cells exhibit an extremely (~30-fold) higher rate of glycolysis compared to normal cells utilizing the highly efficient TCA cycle (generating 36 ATP moles/glucose).[7] The exact reason for this phenomenon is unknown but it could be attributed to the poor vasculature development with insufficient supplies of oxygen and nutrition in cancer cells.[6] In fact, as targeting glycolysis for cancer treatment has been explored as a feasible therapeutic approach,[8,9] a blocking of the glycolytic pathway may indeed have a significant impact on bladder cancer cell proliferation.

In particular, the glucose analog, 2-deoxyglucose (2DG), has been studied as a potential anticancer agent due to its ability to inhibit glycolysis via the hexokinase step.[5,10] Multiple preclinical (in vitro and in vivo) studies showed the efficacy of 2DG on several cancer cells such as leukemia, cervical cancer, breast cancer, prostate cancer etc., leading to the reduction in their cell growth/viability.[11,12] 2DG has been reported to have the sensitizing cytotoxic effects when combined with chemotherapeutic drugs or radiation in colon, breast, ovarian, and pancreatic cancers.[13,14,15] Additionally, 2DG in combination with chemotherapeutic drugs also demonstrated antitumor effects on osteosarcoma, lung, breast, and prostate cancers in the nude mouse xenograft models.[16,17] These anticancer/antitumor effects of 2DG are believed to be primarily due to the metabolic stress or cell death induced by the inhibition of glycolysis. Moreover, radiotherapy with 2DG in Phase I/II clinical trials on cerebral glioma showed the improved efficacy and was well tolerated by patients.[18]

Accordingly, we investigated if targeting glycolysis with 2DG would effectively control the proliferation of bladder cancer cells in vitro and also explored its underlying mechanism, focusing on glycolysis, metabolic signaling pathways, and apoptosis. This study suggests that 2DG would be an anticancer agent as well as an adjuvant agent (to be combined with chemotherapeutic drugs), offering a viable therapeutic option for bladder cancer. More details are described and discussed herein.

2. Methods and Materials

2.1. Cell culture

The human bladder cancer 5637 cells were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 µg/ml). For experiments, cells were seeded in 6-well plates or T-75 flasks at the initial cell density of 2 x 10^5 cells/ml and treated with given agents/drugs. Cell viability was then determined at specified times by MTT assay below.

2.2. MTT assay

MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay was used to determine cell viability following the vendor's protocol (Sigma-Aldrich, St. Louis, MO). Briefly, MTT reagent (1 mg/ml) was added to cells (in each well) in the 6-well plate, which was incubated for 3 h. After discarding MTT reagent, dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals (purple). Absorbance of formazan solution was read in a microplate reader, and cell viability was expressed by the % of sample readings relative to the control reading (100%).

2.3. Hexokinase (HK) assay

Hexokinase (HK) activity was determined by the HK Colorimetric Assay Kit (Biovision, Milpitas, CA) following the manufacturer's protocol with minor modifications. Control or agents-treated cells were lysed in HK buffer provided and supernatant (cell lysates) was obtained by centrifugation. The NADH standards and cell lysates (20 µg per sample) were prepared in the 96-well plate and the reaction was started by the addition of reaction mix (containing substrate). Immediately the plate was placed in a microplate reader and the absorbance changes with time were monitored at 450 nm for 20 min with 5-min intervals. All readings were calculated and normalized and then HK activity was expressed by the % of sample activity relative to the controls (100%).

2.4. Determination of cellular ATP level

The cellular ATP level was determined using the ATP Colorimetric Assay Kit (Biovision, Milpitas, CA) following the vender's protocol. Cells (2 x 10^5 cells/ml) in the 6-well plate were first lysed in ATP assay buffer and cell lysates were deproteinized with $HClO_4$ and neutralized with KOH. The ATP standards and samples (50 µl per sample) were prepared in the 96-well plate and the reaction was started by the addition of reaction mixture. The plate was then incubated at room temperature for 30 min in the dark. Absorbance (OD) at 570 nm was read on a microplate reader and ATP content was calculated by applying the sample readings to the ATP standards. The ATP level was then expressed by the % of sample readings relative to the controls (100%).

2.5. Western blot analysis

Briefly, cell lysates (10 µg) of control or 2DG-treated cells were resolved on 10% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The blot (membrane) was incubated with the primary antibodies against anti-phospho-AMPK, anti-phospho-Akt, anti-phospho-mTOR, anti-bcl-2, or anti-Bax (Santa Cruz Biotechnology, Santa Cruz, CA) for 90 min with agitation, followed by incubation with the appropriate secondary antibody conjugates for 30 min.

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The specific immunoreactive protein bands were detected by chemiluminescence following the manufacturer's protocol (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

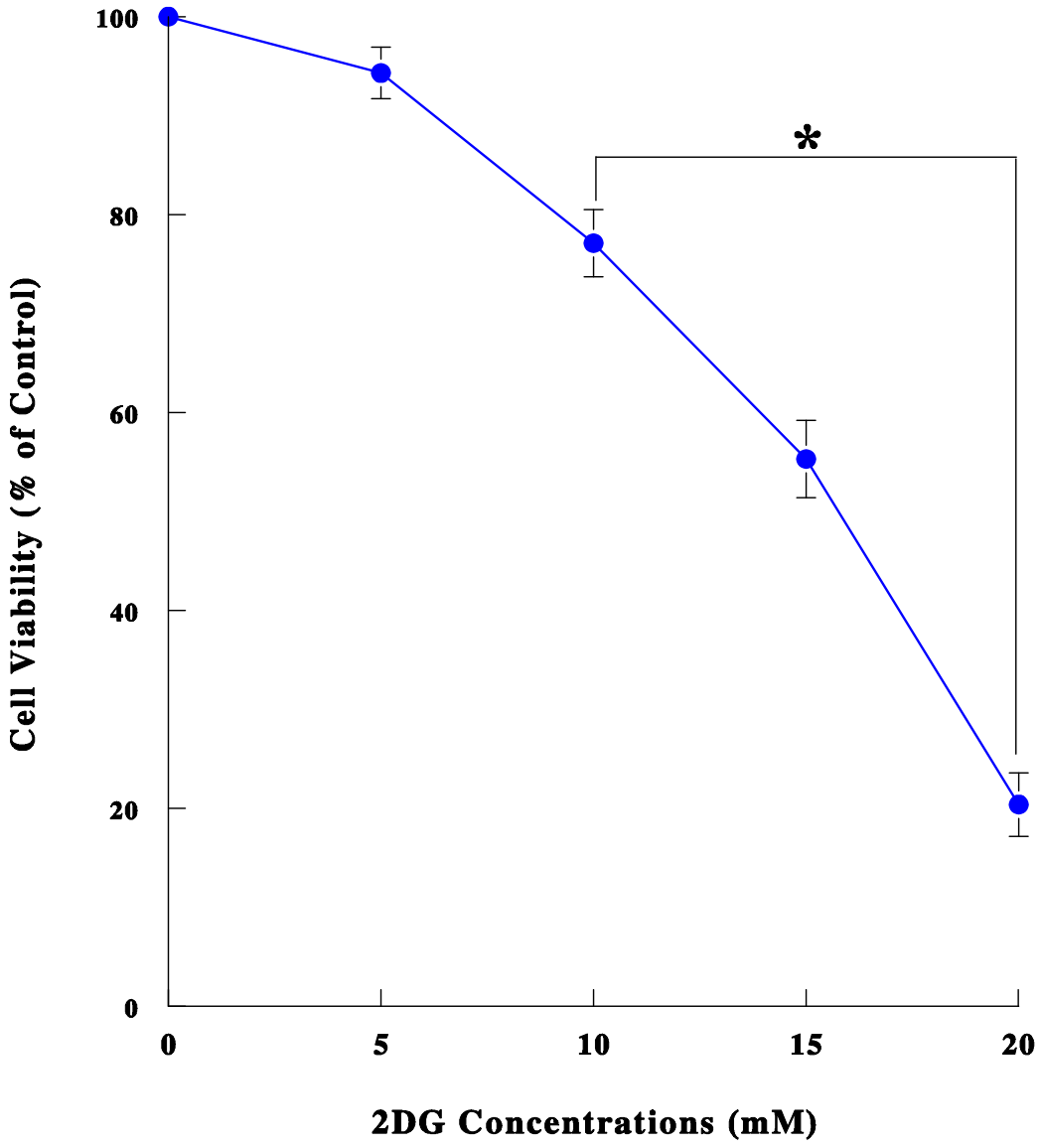

Figure 1: Dose-dependent effects of 2DG on 5637 cell viability. Cells were cultured with varying concentrations (0-20 mM) of 2DG and cell viability at 72 h was assessed by MTT assay. Cell viability was then expressed by the % of viable cells relative to that in control (100%). All data represent mean ± SD (standard deviation) from three independent experiments and (*$p < 0.05$ compared with controls).

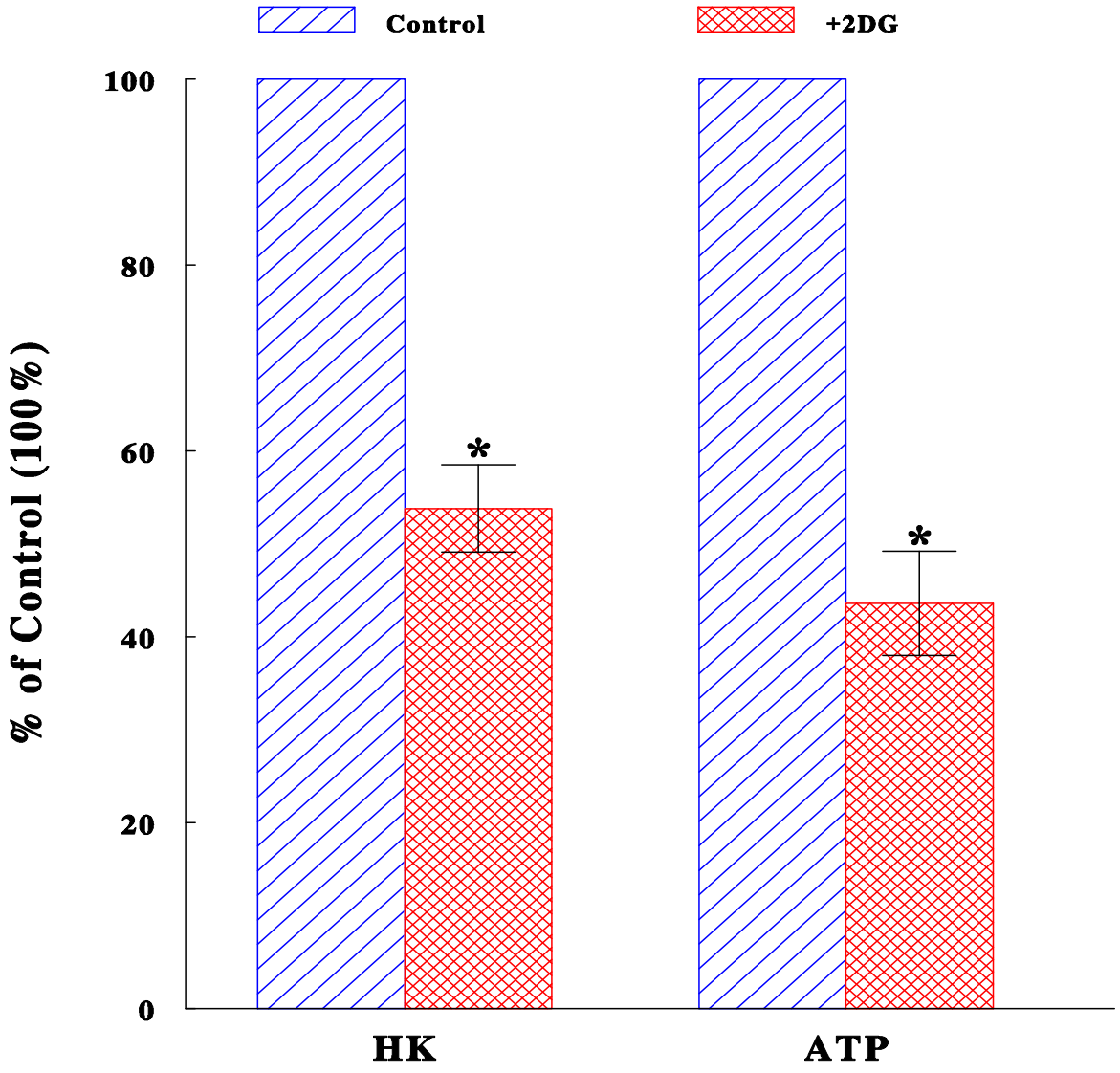

Figure 2: Effects of 2DG on HK activity and cellular ATP level. After cells were cultured with 2DG (15 mM) for 72 h, HK activity and ATP level were determined separately and were expressed by the % of HK activity and ATP level relative to respective control (100%). The data are mean ± SD from three separate experiments (*$p < 0.05$ compared with controls).

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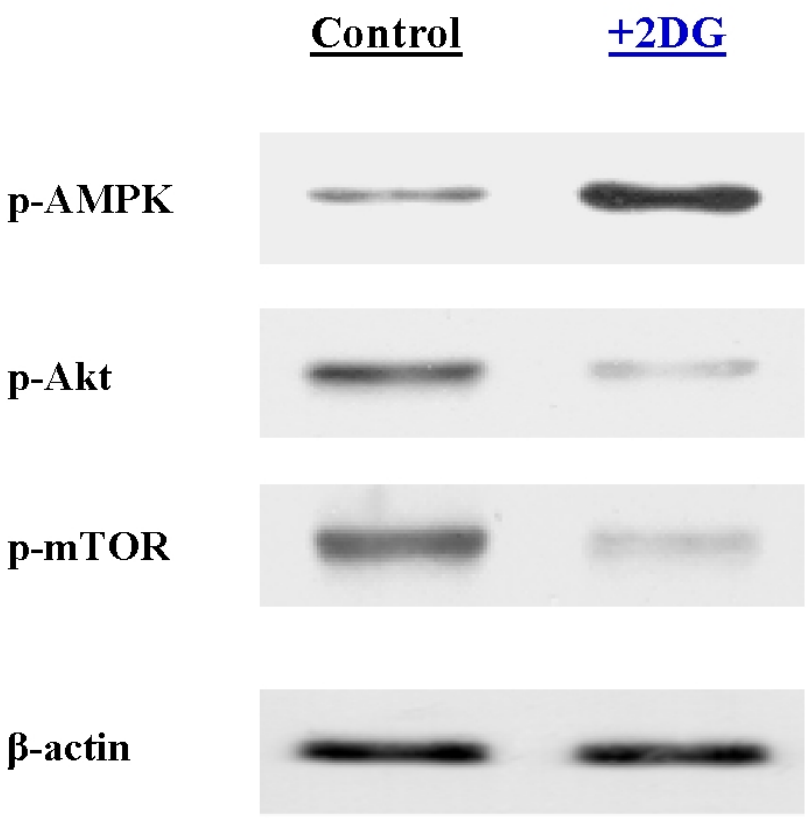

Figure 3: Effects of 2DG on specific signaling regulators. After cells were treated with 2DG (15 mM) for 72 h, the status of the key regulators in the specific signaling pathways such as p-AMPK, p-Akt, and p-mTOR was analyzed by Western blots. The elevated phosphorylation level of p-AMPK with 2DG indicates AMPK activation, while the reduced phosphorylation levels of p-Akt and p-mTOR indicate their inactivation. Beta-actin is used as a protein loading control. Further details are described in the text.

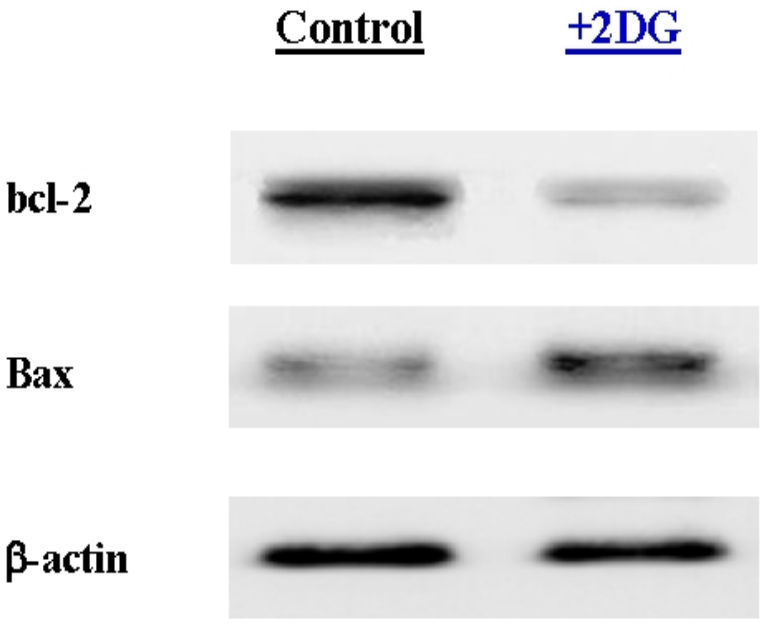

Figure 4: Effects of 2DG on apoptosis regulators. Protein expressions of bcl-2 and Bax in control and 2DG-treated cells at 72 h were analyzed using Western blots. Autoradiogram shows the decreased bcl-2 and the increased Bax expressions in 2DG-treated cells, indicating induction of apoptosis. Beta-actin is used as an internal control.

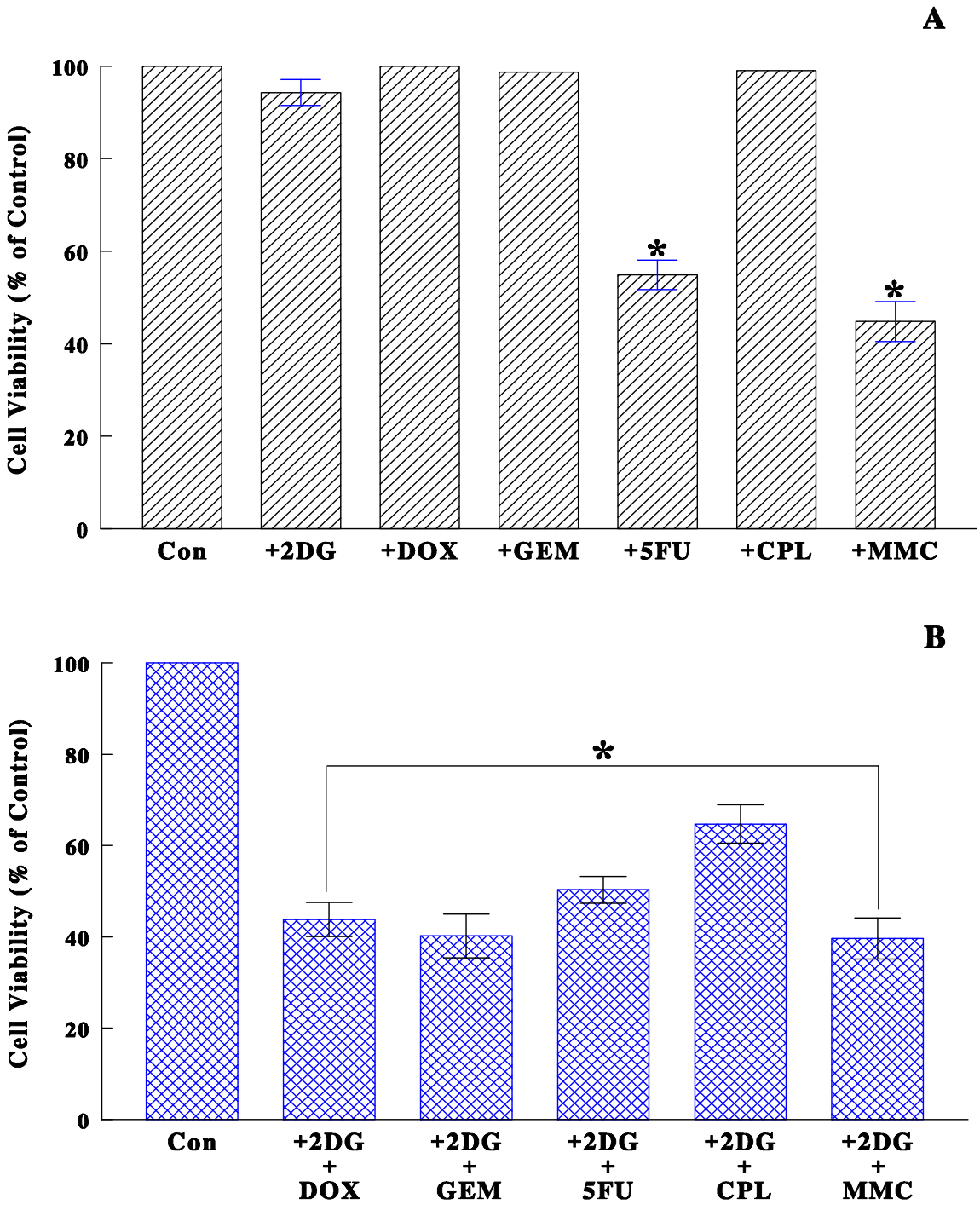

Figure 5: Chemosensitizing effect of 2DG. (A) Cells were treated with 2DG (5 mM), DOX (5 ng/ml), GEM (20 nM), 5FU (5 μg/ml), CPL (100 μM) or MMC (300 nM) for 72 h and cell viability was determined. (B) Cells were also treated with all these drugs combined with 2DG at given concentrations (as indicated) for 72 h and cell viability was determined. Cell viability was then expressed by the % of viable cells relative to that in control (100%). All data are mean ± SD from three separate experiments (*$p < 0.05$ compared with controls).

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2.6. Statistical analysis

All data were obtained from at least three independent experiments and presented as mean ± standard deviation (SD). Statistical differences between groups were assessed with either one-way analysis of variance (ANOVA) or the unpaired Student's t test. Values of $p < 0.05$ were considered to indicate statistical significance.

3. Results

3.1. Dose-dependent effect of 2DG on 5637 cell viability

To examine if 2DG may have anticancer effect on 5637 cells, a dose-dependent study was performed to assess cell viability that determines the % of viable cells following 2DG treatment. It should be noted that actual effects of 2DG would be seen when its concentrations go >10 mM because culture medium contains ~11 mM glucose, which could compete with 2DG. Cells were cultured with varying concentrations (0-20 mM) of 2DG for 72 h and cell viability was assessed by MTT assay. The results showed that 2DG led to a 23-80% reduction in cell viability once its concentrations exceeded ≥10 mM) (Figure 1). Thus, 2DG appears to have anticancer activity, significantly reducing cell viability. As 15 mM of 2DG is proximate to its IC_{50} (50% inhibitory concentration), this concentration was then used in the rest of our study.

3.2. Effect of 2DG on glycolysis

We next examined if 2DG-induced cell viability reduction could be associated with the glycolysis inhibition because 2DG is known to specifically inhibit HK involved in the irreversible committed step in glycolysis.[19] Cells treated with 2DG for 72 h were subjected to HK assay. Compared to controls (100%), HK activity declined to ~54% (i.e. a ~46% activity loss) with 2DG treatment (Figure 2). ATP assay revealed that the cellular ATP level was also down to ~44% (i.e. a ~56% reduction), due to HK inactivation by 2DG (Figure 2). Thus, the reduction in HK and ATP levels by 2DG indicates the inhibition of glycolysis, eventually leading to the growth cessation and the cell viability reduction.

3.3. Effects of 2DG on signaling pathways

As energy (ATP) reduction/depletion due to the glycolysis inhibition has been shown to affect metabolic signaling pathways,[20] cells treated with 2DG for 72 h were analyzed for three key regulators using Western blots. Such analysis revealed that AMP-activated protein kinase (AMPK)[21] was highly phosphorylated (activated) while serine/threonine protein kinase B (Akt)[22] was dephosphorylated (inactivated) by 2DG (Figure 3). Moreover, mammalian target of rapamycin (mTOR), working with Akt to promote cell proliferation,[23] was also dephosphorylated or inactivated (Figure 3). Thus, activation of AMPK concomitant with inactivation of Akt and mTOR by 2DG suggest that 5637 cells may ultimately undergo cell death as it has been shown in certain cancer cells.[21,22,23,24]

3.4. Induction of apoptosis by 2DG

It is also important to address if cell death induced by 2DG might be linked to apoptosis. Cells treated with 2DG for 72 h were analyzed for the status of two key apoptosis regulators, anti-apoptotic bcl-2 and pro-apoptotic Bax,[25] using Western blots. Analysis revealed that expression of bcl-2 was reduced or down-regulated while that of Bax was enhanced or up-regulated following 2DG treatment (Figure 4). Thus, these results indicate that 2DG could induce apoptosis in 5637 cells.

3.5. Chemosensitizing effect of 2DG

Chemotherapy is yet one of the most common therapeutic options for cancer patients despite the poor efficacy with considerable side effects.[26] We examined if 2DG could sensitize chemotherapeutic drugs, which were being clinically used. Those drugs included doxorubicin (DOX), gemcitabine (GEM), 5-flurouracil (5FU), cisplatin (CPL), and mitomycin C (MMC). Cells were treated with 2DG (5 mM), DOX (5 ng/ml), GEM (20 nM), 5FU (5 μg/ml), CPL (100 μM) or MMC (300 nM) for 72 h and cell viability was determined. The concentrations of these drugs used were the estimated maximum or above physiologically tolerable levels. Additionally, 5 mM (instead of 15 mM) of 2DG was used because it showed little effect but might demonstrate its sensitizing effect on drugs. Such studies showed that 2DG, DOX, GEM, and CPL alone had little effects, but 5FU and MMC alone led to a ~45% and ~55% cell viability reduction, respectively (Figure 5A). When these drugs were combined with 2DG at given concentrations, cell viability was significantly (~35-60%) reduced in all combinations (Figure 5B). However, it should be advised that anticancer effects of 5FU and MMC alone were not significantly improved in combination with 2DG (2DG+5FU and 2DG+MMC): cell viability of ~55% with 5FU and of ~45% with MMC alone was reduced to only ~50% and ~40% with 2DG combination (i.e. a merely 5% reduction), respectively. Hence, no significant potentiating effect of 2DG was found when combined with 5FU or MMC. Therefore, 2DG appears to selectively sensitize or potentiate chemotherapeutic drugs, resulting in the improved or greater cell viability reduction. This plausibly suggests that 2DG has a (selective) chemosensitizing effect.

4. Discussion

Glycolysis is the major metabolic pathway essential to cancer cells as described by the "Warburg effect".[6] Since 2-deoxyglucose (2DG) can interrupt glycolysis,[19] it has been considered an anticancer agent capable of inhibiting the growth and development of cancers. In fact, anticancer effect of 2DG has been studied on a

variety of cancer cells in vitro and in vivo.[12,14,15,16] We were then tempted to study if 2DG might have anticancer effect on bladder cancer cells, through the disruption of glycolysis. Additionally, the anticancer mechanism of 2DG was further explored, focusing on glycolysis and its related cellular/biochemical events such as metabolic signaling pathways and apoptosis.

A dose-dependent study showed that 2DG was indeed capable of significantly reducing cell viability. To have a better understanding of this 2DG-induced cell viability reduction, the effect of 2DG on glycolysis was examined because 2DG is a known glycolytic inhibitor. Such studies showed that HK activity and cellular ATP synthesis were significantly reduced by 2DG, indicating the inhibition of glycolysis. As a result, cellular energy (ATP) production would be critically reduced, eventually leading to a significant cell viability reduction (due to cell death).

Actually, energy (ATP) depletion, due to glycolysis inhibition (by 2DG), is shown to have a substantial impact on metabolic signaling pathways.[20] In fact, we found that AMPK and Akt, which were closely coupled with energy metabolism, were activated and inactivated by 2DG, respectively. AMPK is known as the master energy sensor that immediately responds to energy deficiency and halts glycolysis,[21] while Akt acts opposite to AMPK and is the key mediator of cell survival that stimulates glycolysis.[22] Both AMPK and Akt act as protein kinases and must be phopsphrylated to become active and functional.[22,23] Activation of AMPK during energy deficiency has been shown to be cytotoxic to a variety of cancer cells including lung, prostate, stomach, liver, breast, and glial cancer cells in vitro.[27] In contrast, Akt is activated through phosphatidyl inositol-3 kinase (PI3K) in response to several growth factors and this activation can regulate metabolic and nuclear processes for cell survival by stimulating glycolysis and inhibiting apoptosis.[22,28] In short, AMPK tends to inhibit glycolysis and promote apoptosis, while Akt stimulates glycolysis and inhibit apoptosis. Hence, AMPK and Akt can interact directly through two opposed mechanisms[21] and activation/inhibition of these two parameters would determine the cellular energy (ATP) metabolism, an anabolic or catabolic process. Moreover, activation of AMP and inhibition of Akt (due to ATP depletion) is also known to inhibit or down-regulate mTOR, the master orchestrator of cell proliferation, which is a protein kinase promoting protein synthesis and cell proliferation.[23] Therefore, AMPK activation and Akt/mTOR inactivation induced by 2DG in this study presumably lead to the growth cessation and ultimate cell death.

Along with the glycolysis inhibition and the modulations of metabolic signaling pathways through 2DG, 5637 cells were found to ultimately undergo apoptosis, indicated by specific modulations of bcl-2 and Bax.[25] Thus, 2DG-induced cell viability reduction would most likely result from apoptosis.

To find the clinical relevance of 2DG, we examined if 2DG would have a chemosensitizing effect, capable of improving/potentiating the drug efficacy. Such study showed that the combination of 2DG and various drugs (except for 5FU and MMC) led to a significantly improved cell viability reduction, although individual 2DG and drugs (except for 5FU and MMC) had only negligible effects. It is thus plausible that 2DG can be also considered as a potential adjuvant agent that has a selective chemosensitizing effect on drugs, improving the drug efficacy.

Lastly, it is important to address the safety of 2DG. Various animal and clinical studies reported that 2DG was safe and relatively non-toxic in animals and humans. For instance, the LD_{50} of 2DG was found to be ≥2 g/kg body weight for mice,[29] and Phase I/II clinical studies on gliomas and Phase I studies on advanced solid tumors as well as prostate cancer showed no adverse effects of 2DG with the improved antitumor effect found in those patients.[18,30,31] These clinical trials also involved the combinations of 2DG and chemotherapeutic drugs or radiation, which had been shown to have the better outcomes than monotherapy (2DG alone). A chemosensitizing effect of 2DG on several drugs seen in this study may also imply an advantage of combination therapy (over monotherapy) for bladder cancer. Thus, 2DG appears to be a safe, non-toxic agent that can be clinically used alone or combined with appropriate drugs/agents or interventions.

5. Conclusion

The present study demonstrates that 2DG has anticancer activity on bladder cancer 5637 cells and its anticancer mechanism involves diverse cellular effects such as, cell viability reduction, glycolysis inhibition, modulations of metabolic signaling pathways, and induction of apoptosis. Additionally, 2DG may have a chemosensitizing effect on certain drugs and could be used as an adjuvant agent. Therefore, targeting glycolysis using certain glycolytic inhibitors (e.g. drugs and agents such as 2DG) could be an alternative, viable therapeutic approach to bladder cancer. Further studies are warranted.

Conflict of Interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Abbreviations

BCG: Bacillus Calmette-Guerin
CIS: carcinoma in situ
2DG: 2-deoxyglucose

HK: hexokinase
ATP: adenosine triphosphate
TCA: tricarboxylic acid;
MTT:
(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide)
h: hour(s)
min: minute(s)
DMSO: dimethyl sulfoxide
SDS: sodium dodecyl sulfate
AMPK: AMP-activated protein kinase
Akt: serine/threonine protein kinase B
mTOR: mammalian target of rapamycin
PI3K: phosphatidyl inositol-3 kinase
DOX: doxorubicin
GEM: gemcitabine
5FU: 5-flurouracil
CPL: cisplatin
MMC: mitomycin C
IC_{50}: inhibitory concentration of 50%
LD_{50}: lethal dose 50%
SD: standard deviation
ANOVA: one-way analysis of variance.

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References

1. Kamat AM, Hahn NM, Efstathiou JA, *et al*. Bladder cancer. Lancet. 2016;388(10061): 2796-810.
2. Seigel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2015;65:5-29.
3. Shen Z, Shen T, Wientjes MG, *et al*. Intravesical treatments of bladder cancer: review. Pharm Res. 2008;25:1500-10.
4. Malmstrom PU. Intravesical therapy of superficial bladder cancer. Crit Rev Oncol Hematol. 2003;47:109-26.
5. Choi Y, Lee JH. The combination of tephrosin with 2-deoxy-D-glucose enhances the cytotoxicity via accelerating ATP depletion and blunting autophagy in human cancer cells. Cancer Biol Ther. 2011;12:989-96.
6. Warburg O. On the origin of cancer cells. Science. 1956;123:309-14.
7. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer. 2004;4:891-9.
8. Pelicano H, Martin DS, Xu RH, *et al*. Glycolysis inhibition for anticancer treatment. Oncogene. 2006;25:4633-46.
9. Xu RH, Pelicano H, Zhou Y, *et al.* Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia. Cancer Res. 2005;65:613-21.
10. Zhong D, Xiong L, Liu T, *et al.* The glycolytic inhibition of 2-deoxyglucose activates multiple prosurvival pathways through IGF1R. J Biol Chem. 2009;284:23225-33.
11. Munoz-Pinedo C, Ruiz-Ruiz C, Ruiz de Almodovar C, *et al*. Inhibition of glucose metabolism sensitizes tumor cells to death receptor-triggered apoptosis through enhancement of death-inducing signaling complex formation and apical procaspase-8 processing. J Biol Chem. 2003;278:12759-68.
12. Wang L, Wang J, Xiong H, *et al.* Co-targeting hexokinase 2-mediated Warburg effect and ULK1-dependent autophagy suppresses tumor growth of PTEN- and TP-53-deficiency-driven castration-resistant prostate cancer. EBioMedicine 2016;7:50-61.
13. Hernlund E, Strandberg Ihrlund L, Khan O, *et al.* Potentiation of chemotherapeutic drugs by energy metabolism inhibitors 2-deoxyglucose and etomoxir. Int J Cancer. 2008;123:476-83.
14. Hernlund E, Hjerpe E, Avall-Lundqvist E, *et al*. Ovarian carcinoma cells with low levels of β-F1-ATPase are sensitive to combined platinum and 2-deoxy-D-glucose treatment. Mol Cancer Ther. 2009;8:1916-23.
15. Coleman MC, Asbury CR, Daniels D, *et al.* 2-deoxy-D-glucose causes cytotoxicity, oxidative stress, and radiosensitization in pancreatic cancer. Free Radic Biol Med. 2008;44:322-31.
16. Maschek G, Savaraj N, Priebe W, *et al.* 2-deoxy-D-glucose increases the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers *in vivo*. Cancer Res. 2004;64:31-4.
17. Tagg SL, Foster PA, Leese MP, *et al.* 2-methoxyoestradiol-3,17-O,O-bis-sulpahamate and 2-deoxy-D-glucose in combination: a potential treatment for breast and prostate cancer. Br J Cancer. 2008;99:1842-8.
18. Mohanti BK, Rath GK, Anantha N, *et al*. Improving cancer radiotherapy with 2-deoxy-D-glucose: phase I/II clinical trials on human cerebral gliomas. Int J Radiati Oncol Biol Phys. 1996;35:103-11.
19. Miccoli L, Oudard S, Sureau F, *et al*. Intracellular pH governs the subcellular distribution of hexokinase in a glioma cell line. Biochem J. 1996;313:957-62.
20. Cheong JH, Park ES, Liang J, *et al.* Dual inhibition of tumor energy pathway by 2-deoxyglucose and metformin is effective against a broad spectrum of preclinical cancer models. Mol Cancer Ther. 2011;10:2350-62.
21. Priebe A, Tan L, Wahl H, *et al.* Glucose deprivation activates AMPK and induces cell

death through modulation of Akt in ovarian cancer cells. Gynecol Oncol. 2011;122:389-95.
22. Lee YK, Park OJ. Regulation of mutual inhibitory activities between AMPK and Akt with quercetin in MCF-7 breast cancer cells. Oncol Rep. 2010;24:1493-7.
23. Bolster DR, Crozier SJ, Kimball SR, *et al*. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. J Biol Chem. 2002;277:23977-80.
24. Lai E, Teodorp T, Volchuk A. Endoplasmic reticulum stress: signaling the unfolded protein response. Physiology. 2007;22:193-201.
25. Yip KW, Reed JC. Bcl-2 family proteins and cancer. Oncogene. 2008;27:6398-406.
26. Zhang HH, Guo XL. Combinational strategies of metformin and chemotherapy in cancers. Cancer Chemother Pharmacol. 2016;78:13-26.
27. Kuhajda FP. AMP-activated protein kinase and human cancer: cancer metabolism revisited. Int J Obes. 2008;32:S36-41.
28. MacFarlane M, Robinson GL, Cain K. Glucose – a sweet way to die: metabolic switching modulates tumor cell death. Cell Cycle. 2012;11:3919-25.
29. Landau BR, Lubs HA. Animal responses to 2-deoxy-D-glucose administration. Proc Soc Exp Biol Med. 1958;99:124-7.
30. Raez LE, Papadopoulos K, Ricart AD, *et al*. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. Cancer Chemother Pharmacolo. 2013;71:523-30.
31. Stein M, Lin H, Jeyamohan C, *et al.* Targeting tumor metabolism with 2-deoxyglucose in patients with castration-resistant prostate cancer and advanced malignancies. Prostate. 2010;70:1388-94.

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