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Original Article

Targeting cancer cell metabolism: The combination of metformin and 2-Deoxyglucose regulates apoptosis in ovarian cancer cells via p38 MAPK/JNK signaling pathway

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Abstract: Targeting cancer cell metabolism is a new promising strategy to fight cancer. Metformin, a first-line treatment for type 2 diabetes mellitus, exerts anti-cancer and anti-proliferative action. 2-deoxyglucose (2-DG), a glucose analog, works as a competitive inhibitor of glycolysis. In this study, we show for the first time that metformin in combination with 2-DG inhibited growth, migration, invasion and induced cell cycle arrest of ovarian cancer cells in vitro. Moreover, metformin and 2-DG could efficiently induce apoptosis in ovarian cancer cells, which was achieved by activating p38 MAPK and JNK pathways. Our study reinforces the growing interest of metabolic interference in cancer therapy and highlights the potential use of the combination of metformin and 2-DG as an anti-tumor treatment in ovarian cancer.

Keywords: Metformin, 2-Deoxyglucose, apoptosis, cell cycle arrest, p38 MAPK/JNK signaling pathway

Introduction

Ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause for cancer death among women in the world [1]. The majority of the cancer cases are diagnosed with advanced disease due to the lack of effective screening strategies and typical early signs and symptoms associated with this disease. Metastases are already present when diagnosed at advanced disease stages, leading to poor prognosis. Despite the encouraging developments in chemotherapy, surgery, and sometimes radiotherapy, the likelihood of long-term disease-free survival is still very low (15-20%) [2-4]. Identification of new anti-cancer drugs is essential, enabling preventative and therapeutic advances to be made in this rare but deadly disease.

Targeting cancer metabolism has become a major area of investigation, which is largely based on the principle of Warburg Effect [5]. As Otto Warburg proposed, even with adequate oxygen, cancer cells display increased glucose uptake and production of lactate. This suggests that rapidly growing tumors depend on aerobic glycolysis to maintain energy balance [6-8]. However, recent studies suggest that both mitochondrial glucose oxidation and glycolysis are necessary to support the rapid and aggressive growth of tumors [9, 10]. Therefore, therapeutic strategies targeting both mitochondrial oxidative phosphorylation and glycolysis may be more effective in the metabolism interference of tumors.

Metformin, an anti-hyperglycemic agent, has been the first-line treatment for type II diabetes mellitus for decades. Despite the extensive use of metformin as an anti-diabetic drug for 40 years, in 2001 it was first reported of its potential anti-cancer effect in mammals [11]. In addition, the first report of a reduced risk of cancer in patients with type II diabetes treated with metformin was published only 10 years ago [12]. Since then, many articles suggested that metformin exhibited a strong and consistent

anti-proliferative action on different cancer cell lines and xenografts or transgenic mice, including ovarian cancer [13-15]. As related to metabolism, metformin is identified as a new class of complex I and ATP synthase inhibitor [16], and it acts directly on mitochondria to limit respiration, which causes energy inefficient and reduces glucose metabolism through the citric acid cycle [17].

As a glucose analog, 2-deoxyglucose (2-DG) can be readily taken up by glucose transporters and works as a competitive inhibitor of glycolysis [18]. As reported in multiple tumor types, the combination of metformin with 2-DG can impair cell metabolism and cause the death of tumor cells [19-22]. These effects have been caused by simultaneous metabolism interference in glycolysis (with 2-DG) and oxidative phosphorylation (with metformin). Based on these reported studies, the combination of 2-DG and metformin may be effective in some cancer types, however, it has not yet been tested in ovarian cancer.

The present study was performed to clarify whether combination of metformin and 2DG can lead to a stronger inhibitory effect on ovarian cancer cell viability and to elucidate the possible molecular mechanisms.

Materials and methods

Cell lines and agents

Epithelial ovarian cancer cell lines, SKOV3 and hey cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum and 100 units/mL penicillin, 100 mg/mL streptomycin at 37°C and 5% CO_2. Metformin and 2-Deoxyglucose were purchased from Sigma Chemical Co. (St. Louis, MO). SB203580 and SP600125 were purchased from Beyotime Institute of Biotechnology (China). Metformin and 2-DG were dissolved in culture media. SB203580 and SP600125 were dissolved in DMSO, and the final concentration of DMSO was 0.075%.

Cell proliferation assay

Cell proliferation was measured by the CCK-8 assay kit (Dojindo Japan). Briefly, cells were seeded in 96-well culture plates. 24 hours later, cells were starved in FBS-free medium for 12 hours before metformin or 2-DG treatment. After incubation for indicated time, 10 μl of CCK-8 reagent was added to each well and the absorbance was measured at 450 nm. All experiments were repeated at least three times.

Glucose uptake and lactic assays

Cells (4×10^5 per well) were seeded in 6-well plates. 24 hours later, cells were starved in FBS-free medium for 12 hours before treated with the indicated agents. After 48 hours, the media was collected and measured for glucose and lactic acid using glucose kit and lactic acid kit (Jiancheng China) according to manufacturer's protocol. Counts were normalized to protein concentration.

ATP measurement

The level of ATP in cells was determined using the ATP Bioluminescence Assay Kit (Beyotime Technology China). Briefly, Cells (4×10^5 per well) were seeded in 6-well plates. 24 hours later, cells were starved in FBS-free medium for 12 hours before treated with indicated agents for 48 hours. Then cells were lysed with a lysis buffer, followed by centrifugation at 10,000 × g for 2 min at 4°C. Finally, the level of ATP was determined by mixing 50 μl of the supernatant with 50 μl of luciferase reagent, which catalyzed the light production from ATP and luciferin. The emitted light was linearly related to the ATP concentration and measured using a luminometer. Counts were normalized to protein concentration.

Wound healing assay

Cells were seeded in 6-well plates and grew to about 90% confluence. Medium was removed and cell monolayers were wounded by manually scraping the cells with a 200 ul pipette tip. After being washed with PBS twice, cells were added with indicated drugs and then incubated at 37°C in fresh medium. Images of wound closure were evaluated at the indicated times with an inverted microscope. The migration rate was quantified with measurements of the distance between cells. The migration rate was calculated by the following formula: migration rate = migration distance/original distance.

Invasion assay

Cells treated with indicated drugs after 24 hours were harvested and seeded into the upper Transwell chamber (pre-applied with 50

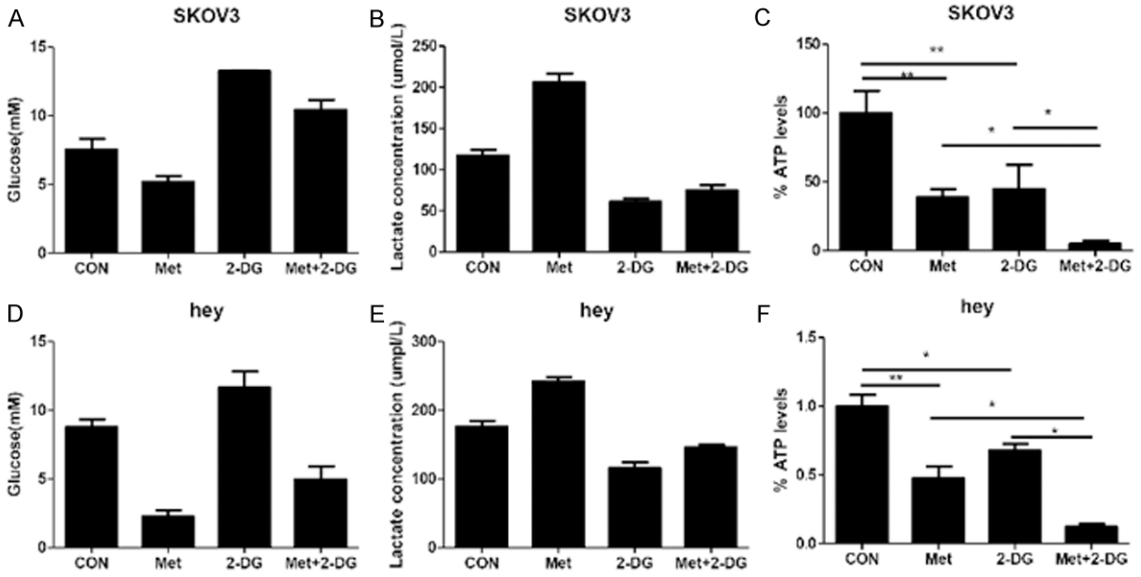

Figure 1. Effects of metformin and/or 2-DG on cell metabolism and intracellular ATP concentration in SKOV3 and hey cell lines. A and D: Glucose concentration in culture medium after treatment with metformin (10 mM) and/or 2-DG (10 mM) for 24 h. B and E: Lactate concentration in culture medium after treatment with metformin (10 mM) and/or 2-DG (10 mM) for 24 h. C and F: Intracellular ATP concentration in cells treated for 24 h. Results are mean of three independent experiments, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

uL Matrigel) with 1×10^5 cells/well in 200 mL of serum-free medium, and 60omL medium containing 20% FBS was added into the lower chamber. After 48 hours, the upper surface of the membrane was wiped with a cotton swab. Cells on the lower surface of the membrane were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. Five random areas of the membrane were photographed and counted under an inverted microscope.

Cell cycle analysis

Cell cycle analysis was performed using cell cycle analysis kit (LIANKE BIOTECH China). Briefly, Cells (4×10^5 per well) were seeded in 6-well plates. 24 hours later, cells were starved in FBS-free medium for 12 hours before treated with indicated agents for 48 hours. Then, the adherent cells were washed once with PBS, trypsinized, and collected by centrifugation at 1000 bpm for 5 min. The cells (10^6 cells per sample) were fixed in 4 ml of cold 75% ethanol at -20°C overnight. After centrifugation at 2000 bpm for 10 min, cell pellets were incubated with 1 ml propidium iodide at room temperature for 30 min. Cell cycle distribution was analyzed by measuring DNA content using flow cytometry.

Apoptosis assay

Apoptotic cells were identified by Annexin V/Dead Cell Apoptosis Kit (Invitrogen USA). Briefly, Cells (4×10^5 per well) were seeded in 6-well plates. 24 hours later, cells were starved in FBS-free medium for 12 hours before treated with indicated agents for 48 hours. Then, cells were harvested and washed twice with ice-cold PBS. Cells were resuspended in annexin-binding buffer and 5 μl Annexin V-FITC and 1 μl PI were added before incubating at room temperature for 15 mins in the dark. FITC and PI fluorescence were analyzed by flow cytometry.

Western blot analysis

Cells were harvested and lysed in RIPA buffer containing PMSF and phosphotransferase inhibitor (Beyotime Technology China). Equal amounts of protein lysates were electrophoretically separated on SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 5% non-fat dried milk for 1 hour at room temperature and then incubated with primary antibodies in 4°C overnight. After incubation with a HPS secondary antibody for 1 hour at room temperature, the protein bands were detected using the ECL detection system

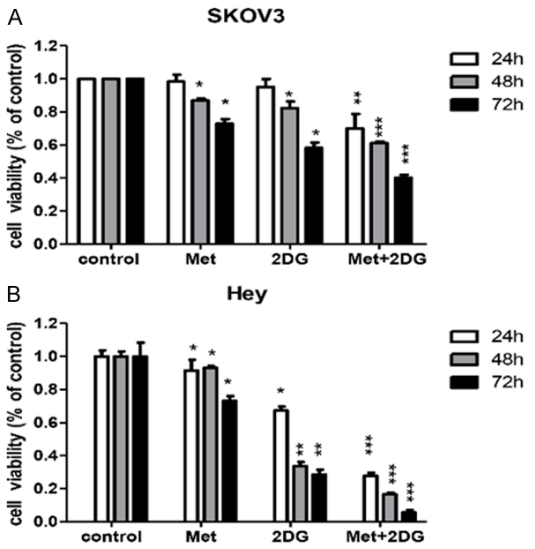

Figure 2. Effects of metformin and/or 2-DG on cell viability in SKOV3 and hey cell lines. Ovarian cancer cells were cultured with metformin (10 mM) and/or 2-DG (10 mM). Cell viability was assessed by CCK-8 assay after 24, 48 and 72 hours. Results are mean of three independent experiments, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

(Millipore USA). Antibodies used were listed above: antibodies against cleaved-caspase 3, CDK-4, cyclin D1, p38 MAPK, p-p38 MAPK, JNK and p-JNK were purchased from Cell signaling technology. Antibodies against Bcl-2 and β-actin were purchased from Abcam.

Statistical analysis

Results were presented as means ± SEM. Statistical analyses were carried out using Stata 12.0 software. Student's t test or oneway ANOVA was chosen to analyze statistical differences. $P < 0.05$ was considered statistically significant.

Results

Metformin and 2-DG combination resulted in ATP depletion

To determine whether metformin and 2-DG affect ovarian cancer cell metabolism, we analyzed their effects on glucose consumption and lactate secretion in SKOV3 and hey cells. Metformin (10 mM) accelerated glucose depletion and consequently decreased lactate concentration (one of the end products of aerobic glycolysis) in both SKOV3 and hey cells (**Figure 1A**, **1B**, **1D** and **1E**). On the contrary, as a competitive inhibitor of glycolysis, 2-DG (10 mM) decreased lactate production and prevented metformin-induced lactate production in SKOV3 and hey cells (**Figure 1A**, **1B**, **1D** and **1E**). To determine the effects of metformin and 2DG on cellular energetics more directly, we assayed ATP levels after treatment for 24 hours (**Figure 1C** and **1F**). Metformin and 2-DG alone decreased intracellular ATP concentration by about 60% in SKOV3 and 40% in hey cells. Importantly, the combination of the two agents robustly diminished ATP concentration by about 90% in both cell lines. Altogether, these results suggested that combination of metformin and 2-DG inhibited the two main sources of cellular ATP, thus initiated a strong metabolic stress in ovarian cancer cells.

Metformin and 2-DG combination inhibited ovarian cancer cell growth, migration and invasion

In order to examine the potential of cellular metabolism as a therapeutic target in ovarian cancer, we investigated the effect of the combination of metformin and 2-DG on ovarian cancer cells: SKOV3 and hey. To assess the effects of metformin and 2-DG on cell proliferation, CCK-8 assay was used to measure cell viability after 24, 48 and 72 hours of treatment (**Figure 2A**, **2B**). In both SKOV3 and hey cells, combination of metformin with 2-DG resulted in a significant reduction in cell growth than single agent over the time course. Taken together, these results demonstrated that metformin and 2DG exhibited a synergistic interaction in all the ovarian cancer cell lines tested. Moreover, wound healing assay was used to test cell migration in SKOV3 and hey cells. As shown in **Figure 3A**, **3B**, **3E** and **3F**, although metformin or 2-DG alone decreased ovarian cancer cell migration, the combination significantly enhanced the effect as compared to either treatment alone. Meanwhile, Transwell assay was carried out to determine effects of metformin and 2-DG on cell invasion. As shown in **Figure 3C**, **3D**, **3G** and **3H**, metformin and 2-DG combination significantly inhibited cell invasion as compared to either treatment alone, though each treatment exhibited invasion inhibiting effect on both SKOV3 and hey cells.

Metformin and 2-DG combination increased ovarian cancer cell apoptosis and G0/G1 arrest

To determine whether the increased anti-proliferative effect was due to increased apoptosis

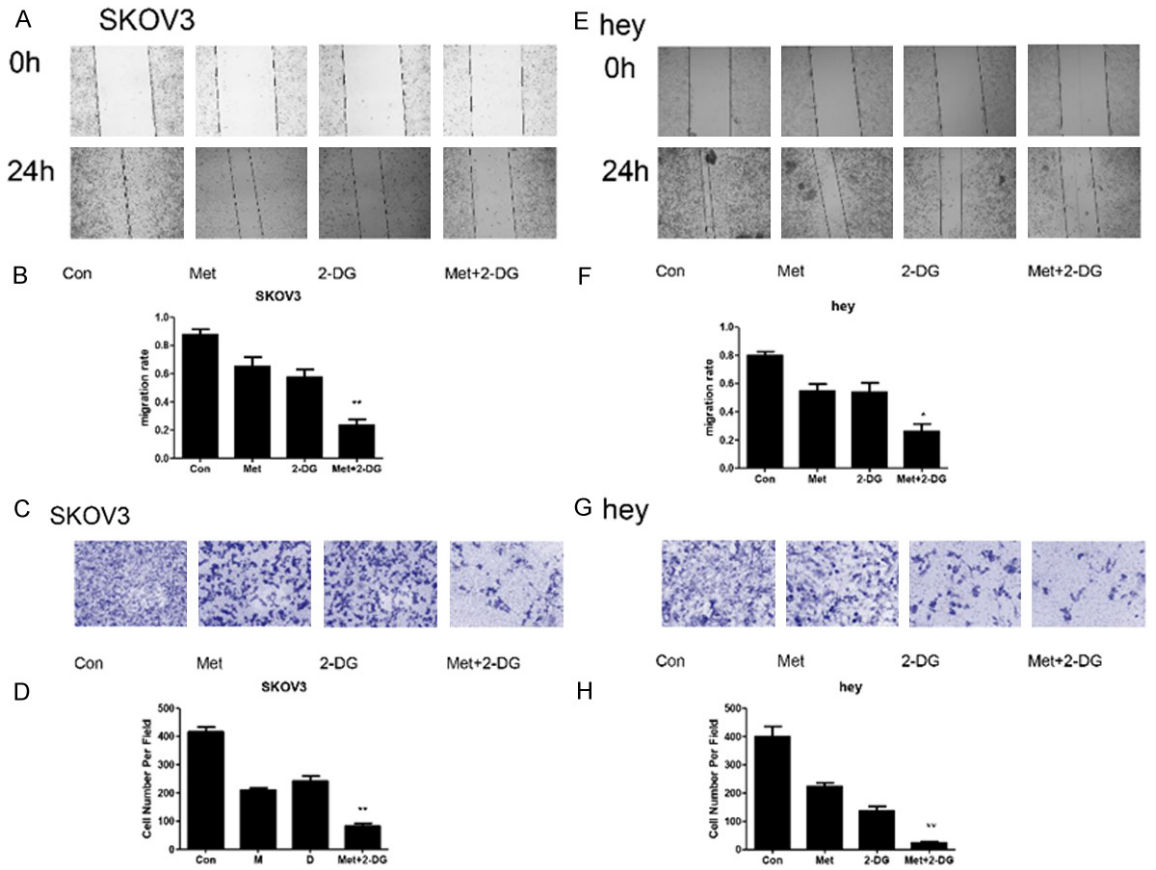

Figure 3. Effects of metformin and/or 2-DG on migration and invasion in SKOV3 and hey cell lines. Migration was evaluated with wound healing in SKOV3 and hey cells after treatment with metformin (10 mM) and/or 2-DG (10 mM) for 24 h. Invasion was evaluated with transwell assay in SKOV3 and hey cells after treatment with metformin (10 mM) and/or 2-DG (10 mM) for 48 h. A and E: Typical picture of migration in SKOV3 and hey cells. B and F: Migration rate of three independent experiments, *, $P < 0.05$ and **, $P < 0.01$ for comparisons between cells treated with the combined treatment and cells treated with the single agent. C and G: Typical picture of invasion in SKOV3 and hey cells. D and H: Cell numbers of each invasion field of three independent experiments, **, $P < 0.01$ for comparisons between cells treated with the combined treatment and cells treated with the single agent.

and/or cell cycle alterations, we examined cell cycle and apoptosis after treatment of metformin and 2-DG. According to the flow cytometric analysis, both metformin and 2-DG alone increased the number of apoptotic cells compared to that observed in the untreated cells; additionally, the combination of two agents significantly increased SKOV3 and hey cell apoptosis to 35.4% and 17.9%, respectively (**Figure 4A**, **4B**, **4D** and **4E**). In addition, these results were confirmed by the western blot analysis. Both metformin and 2-DG were able to increase the protein of cleaved caspase-3 while decrease the protein of Bcl-2 in both SKOV3 and hey cells, and the combination of metformin and 2-DG was accompanied by increased expression of cleaved caspase-3 and decreased expression of Bcl-2 (**Figure 4C** and **4F**). In the meantime, a significant increase in G0/G1-phase cells was observed after treatment with metformin and 2-DG combination comparing to any single treatment in SKOV3 and hey cells (**Figure 5A** and **5C**). Moreover, according to western blot analysis, the combination of metformin and 2-DG was accompanied by decreased expression of CDK-4 and cyclin D1 (**Figure 5B** and **5D**).

Metformin and 2DG combination activates p38 MAPK and JNK pathways

P38 MAPK and JNK are famous as stress activated protein kinases, as they can be activated by various stress stimuli. In addition, activation of p38 MAPK and JNK plays a critical role in natural compounds-induced apoptosis. As

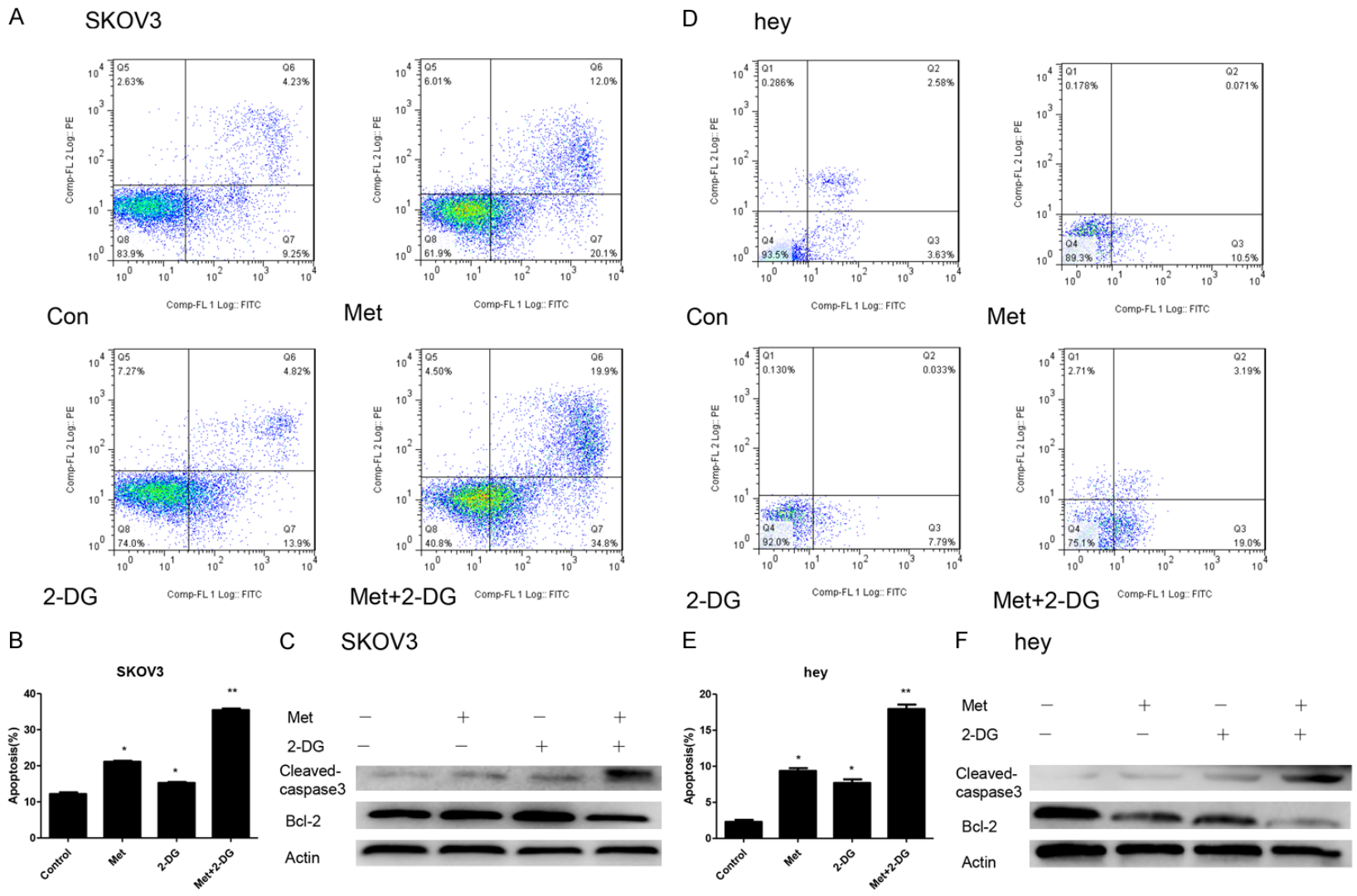

Figure 4. Effects of metformin and/or 2-DG on apoptosis in SKOV3 and hey cell lines. Apoptosis was evaluated with Annexin V staining in SKOV3 and hey cells after treatment with metformin (10 mM) and/or 2-DG (10 mM) for 48 h. A and D: Representative dot plots illustrating the data near the mean of the groups in B and E, *, $P < 0.05$ for comparisons between cells treated with the single treatment and untreated cells, **, $P < 0.01$ for comparisons between cells treated with the combined treatment and cells treated with the single agent. C and F: Western blotting analysis of cleaved-caspase 3 and Bcl-2. Western blotting of β-Actin was included as a loading control.

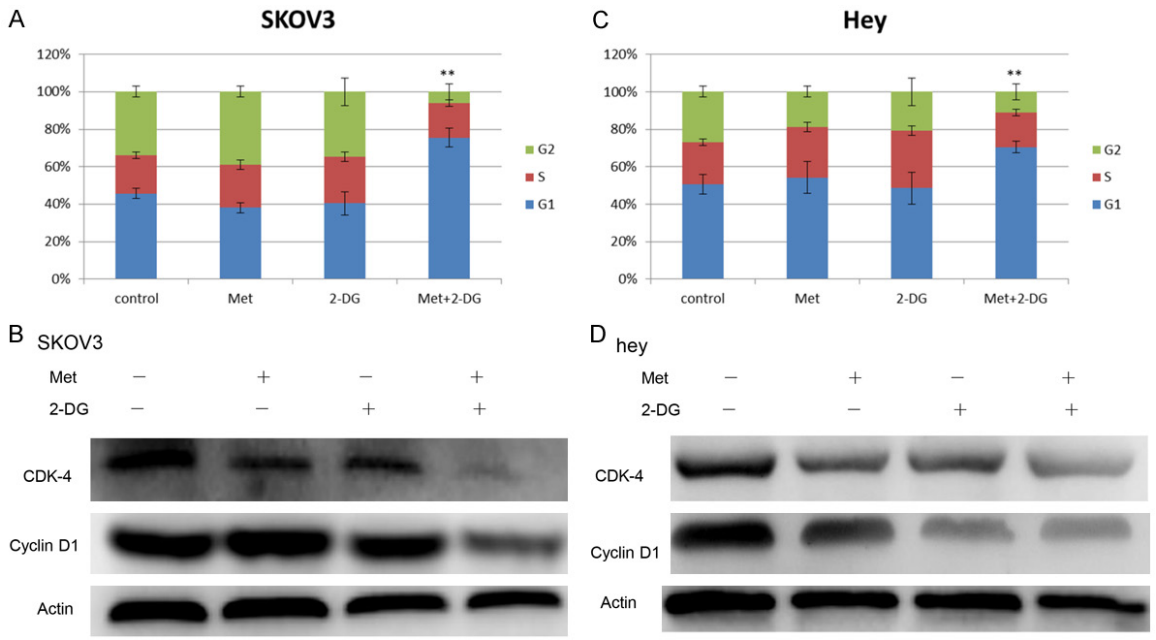

Figure 5. Effect of metformin and/or 2-DG on cell cycle in SKOV3 and hey cell lines. Cell cycle was assayed with propidium iodide of SKOV3 and hey cells after treatment with metformin (10 mM) and/or 2-DG (10 mM) for 48 h. A and C: Histogram reflected the cell cycle distribution after treatment; **, $P < 0.01$ for comparisons between cells treated with the combined treatment and cells treated with the single agent. B and D: Western blotting analysis of CDK-4 and Cyclin D1. Western blotting of β-Actin was included as a loading control.

reported, many anticancer compounds can activate MAPK signaling, and ultimately cause apoptosis in cancer cells. In our previous study, we have shown that metformin and 2-DG could cause apoptosis in ovarian cancer cells. In order to explore related mechanism, we next examined the phosphorylation status of the p38 MAPK and JNK proteins. SKOV3 and hey cells were exposed to 10 mM and/or 10 mM 2-DG for 24 h and the activations of the p38 MAPK and JNK pathways were evaluated by immunoblotting. As shown in **Figure 6**, metformin and 2-DG alone could increase the levels of phosphorylated p38 MAPK and JNK, and combination of two drugs was accompanied by increased phosphorylation of p38 MPAK and JNK proteins. Meanwhile, the levels of cleaved caspase-3 were elevated after treatment of metformin and 2-DG. These results suggested that the combination of metformin and 2-DG regulated apoptosis in ovarian cancer cells via activating p38 MAPK/JNK signaling pathway.

Afterwards, we examined whether activation of p38 MAPK and JNK pathways was necessary for metformin and 2-DG-induced apoptosis in ovarian cancer cells. SKOV3 and hey cells were first pretreated with p38 MAPK inhibitor SB203580 and JNK inhibitor SP600125, respectively, before they were treated with metformin and/or 2-DG for additional 24 h. As shown in **Figure 6**, SB203580 and SP600125 significantly reduced the levels of cleaved caspase-3 induced by metformin and 2-DG, indicating that SB203580 and SP600125 could protect ovarian cancer cells from apoptosis induced by metformin and 2-DG. These results suggested that apoptosis induced by metformin and 2-DG in ovarian cancer cells was mediated by p38 MAPK and JNK signaling pathways.

Discussions

Targeting metabolism is a new and promising strategy in cancer therapy [23]. This is a particularly potential target for aggressive tumors such as ovarian cancer, as they are in high demands for energy during development and progression [24]. In this study, we aimed to verify the concept of targeting tumor metabolism in ovarian cancer cell lines using metformin, a

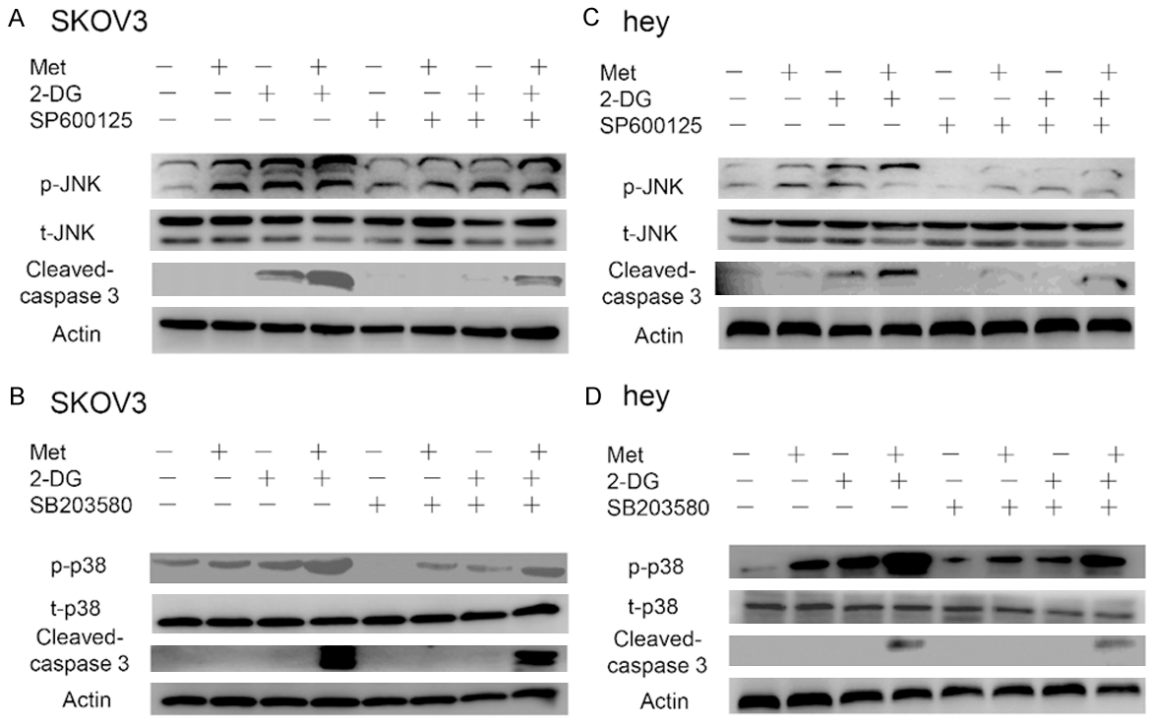

Figure 6. p38 MAPK/JNK pathways mediated metformin and 2-DG-induced apoptosis in ovarian cancer cells. Western blotting analysis of cleaved-caspase 3 and proteins related to p38 MAPK/JNK pathways. (A) and (B) SKOV3 cells were treated with metformin (10 mM) and/or 2-DG (10 mM) with or without 10 μM SP600125 (A) or 10 μM SB203580 (B) for 24 h. (C) and (D) hey cells were treated with metformin (10 mM) and/or 2-DG (10 mM) with or without 10 μM SP600125 (C) or 10 μM SB203580 (D) for 24 h. Western blotting of β-Actin was included as a loading control.

promising anti-cancer agent which can promote reprogramming of glucose metabolism [25], in combination with the glycolysis inhibitor 2-DG [26].

Metformin has been shown to act directly on mitochondria to inhibit complex I-mediated mitochondrial respiration and citric acid cycle functions [17]. In terms of impaired function of mitochondrial oxidation, cells increase aerobic glycolysis to improve bioenergetics as compensation for ATP depletion. As reported in other studies in prostate, colon carcinoma and pediatric glioma [13, 20, 21], here we show that ovarian cancer cells treated with metformin became increasingly dependent on glycolysis as metformin combined with 2-DG resulted in a greater cell inhibition than observed with 2-DG alone. As showed in **Figure 1**, ATP levels were decreased by metformin or 2-DG alone, suggesting that both oxidative phosphorylation and glycolysis are essential for cells to maintain energy levels. Under the conditions of two treatments, dysfunctions of both oxidative phosphorylation and glycolysis resulted in a substantial depletion of ATP. This phenomenon was in agreement with the anti-proliferative effect, as the combination of metformin and 2-DG had a much stronger deleterious effect than either agent (**Figure 2**). Peritoneal metastasis is a big problem in ovarian cancer, which is one of the main reasons that the overall survival of ovarian cancer is still very low [27]. We further examined the effects of metformin and 2-DG on the migration and invasion of SKOV3 and hey cells (**Figure 3**). Results showed that either metformin or 2-DG could inhibit cell migration and invasion in both SKOV3 and hey cells, while the combination of two agents exhibited more significant effect. In brief, these results suggested that targeting metabolic pathways using the combination of metformin and 2-DG may be effective in ovarian cancer cells. In addition, despite of the rare lactic acidosis caused by metformin [28], metformin and 2-DG have the clinical advantage of inducing little adverse side effects.

Cell cycle machinery could regulate a variety of molecular events and cell fate [29]. In this pres-

ent study, we found that the combination of metformin and 2-DG led to a strong accumulation of cells in G0/G1 in SKOV3 and hey cells. Additionally, G0/G1 arrest was in agreement with cyclin D1 degradation. Moreover, CDK4, expression pattern of cyclin D1 was also decreased by metformin and 2-DG. These results illustrated that the anti-proliferative effect of metformin and 2-DG was partially due to cell cycle arrest.

Metformin and 2-DG have been reported to induce apoptosis in prostate cancer cell lines which is p53-dependent [19]. In the present study, we also found metformin and 2-DG alone could induce apoptosis in SKOV3 and hey cells, while the combination of the two agents had more significant effect. In order to explore the mechanism how metformin and 2-DG induce apoptosis, we tested some signaling pathways related to apoptosis. P38 MAPK and JNK are famous as stress activated protein kinases, as they can be activated by various stress stimuli. In addition, activation of p38 MAPK and JNK plays a critical role in natural compounds-induced apoptosis. As reported, many anticancer compounds can activate MAPK signaling, and ultimately cause apoptosis in cancer cells [30]. In agreement with these reports, metformin and 2-DG alone could increase the levels of phosphorylated p38 MAPK and JNK, and the combination of two agents was accompanied by increased phosphorylation of p38 MPAK and JNK proteins. Meanwhile, the levels of cleaved caspase-3 were elevated after treatment of metformin and 2-DG. These results suggested that the combination of metformin and 2-DG regulated apoptosis in ovarian cancer cells via activating p38 MAPK/JNK signaling pathway. In addition, when we used SB203580 and SP600125 to inhibit p38 MAPK and JNK respectively, the expression of cleaved caspase-3 inducing by metformin and 2-DG was decreased. This further suggested that apoptosis induced by metformin and 2-DG in ovarian cancer cells was mediated by p38 MAPK and JNK signaling pathways.

Taken together, we showed for the first time that metformin in combination with 2-DG inhibited growth, migration, invasion and induced cell cycle arrest of ovarian cancer cells in vitro. Particularly, metformin and 2-DG could efficiently induce apoptosis in ovarian cancer cells, which was achieved by activating p38 MAPK and JNK pathways. Therefore, our findings suggested that targeting cell metabolism represents a promising strategy for the treatment of ovarian cancer. In the future, more in vivo experiments could be done to verify the effects of metformin and 2-DG before they could be applied in the clinical setting.

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Disclosure of conflict of interest

None.

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References

[1] White KL, Rider DN, Kalli KR, Knutson KL, Jarvik GP and Goode EL. Genomics of the NF-kappaB signaling pathway: hypothesized role in ovarian cancer. Cancer Causes Control 2011; 22: 785-801.

[2] Barnholtz-Sloan JS, Schwartz AG, Qureshi F, Jacques S, Malone J and Munkarah AR. Ovarian cancer: changes in patterns at diagnosis and relative survival over the last three decades. Am J Obstet Gynecol 2003; 189: 1120-1127.

[3] McGuire V, Jesser CA and Whittemore AS. Survival among U.S. women with invasive epithelial ovarian cancer. Gynecol Oncol 2002; 84: 399-403.

[4] Hoskins WJ, Bundy BN, Thigpen JT and Omura GA. The influence of cytoreductive surgery on recurrence-free interval and survival in small-volume stage III epithelial ovarian cancer: a Gynecologic Oncology Group study. Gynecol Oncol 1992; 47: 159-166.

[5] Otto AM. Warburg effect(s)-a biographical sketch of Otto Warburg and his impacts on tumor metabolism. Cancer Metab 2016; 4: 5.

[6] Warburg O, Wind F and Negelein E. The Metabolism of Tumors in the Body. J Gen Physiol 1927; 8: 519-530.

[7] Dang CV, Hamaker M, Sun P, Le A and Gao P. Therapeutic targeting of cancer cell metabolism. J Mol Med (Berl) 2011; 89: 205-212.

[8] Vander Heiden MG. Targeting cancer metabolism: a therapeutic window opens. Nat Rev Drug Discov 2011; 10: 671-684.
[9] Marin-Valencia I, Yang C, Mashimo T, Cho S, Baek H, Yang XL, Rajagopalan KN, Maddie M, Vemireddy V, Zhao Z, Cai L, Good L, Tu BP, Hatanpaa KJ, Mickey BE, Mates JM, Pascual JM, Maher EA, Malloy CR, Deberardinis RJ and Bachoo RM. Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain in vivo. Cell Metab 2012; 15: 827-837.
[10] Mischel PS. HOT models in flux: mitochondrial glucose oxidation fuels glioblastoma growth. Cell Metab 2012; 15: 789-790.
[11] Schneider MB, Matsuzaki H, Haorah J, Ulrich A, Standop J, Ding XZ, Adrian TE and Pour PM. Prevention of pancreatic cancer induction in hamsters by metformin. Gastroenterology 2001; 120: 1263-1270.
[12] Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR and Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005; 330: 1304-1305.
[13] Ben Sahra I, Le Marchand-Brustel Y, Tanti JF and Bost F. Metformin in cancer therapy: a new perspective for an old antidiabetic drug? Mol Cancer Ther 2010; 9: 1092-1099.
[14] Pollak M. Potential applications for biguanides in oncology. J Clin Invest 2013; 123: 3693-3700.
[15] Kim NY, Lee HY and Lee C. Metformin targets Axl and Tyro3 receptor tyrosine kinases to inhibit cell proliferation and overcome chemoresistance in ovarian cancer cells. Int J Oncol 2015; 47: 353-360.
[16] Bridges HR, Jones AJ, Pollak MN and Hirst J. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. Biochem J 2014; 462: 475-487.
[17] Andrzejewski S, Gravel SP, Pollak M and St-Pierre J. Metformin directly acts on mitochondria to alter cellular bioenergetics. Cancer Metab 2014; 2: 12.
[18] Ralser M, Wamelink MM, Struys EA, Joppich C, Krobitsch S, Jakobs C and Lehrach H. A catabolic block does not sufficiently explain how 2-deoxy-D-glucose inhibits cell growth. Proc Natl Acad Sci U S A 2008; 105: 17807-17811.
[19] Ben Sahra I, Laurent K, Giuliano S, Larbret F, Ponzio G, Gounon P, Le Marchand-Brustel Y, Giorgetti-Peraldi S, Cormont M, Bertolotto C, Deckert M, Auberger P, Tanti JF and Bost F. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. Cancer Res 2010; 70: 2465-2475.
[20] Cheong JH, Park ES, Liang J, Dennison JB, Tsavachidou D, Nguyen-Charles C, Wa Cheng K, Hall H, Zhang D, Lu Y, Ravoori M, Kundra V, Ajani J, Lee JS, Ki Hong W and Mills GB. 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: 2350-2362.
[21] Levesley J, Steele L, Taylor C, Sinha P and Lawler SE. ABT-263 enhances sensitivity to metformin and 2-deoxyglucose in pediatric glioma by promoting apoptotic cell death. PLoS One 2013; 8: e64051.
[22] Saito S, Furuno A, Sakurai J, Sakamoto A, Park HR, Shin-Ya K, Tsuruo T and Tomida A. Chemical genomics identifies the unfolded protein response as a target for selective cancer cell killing during glucose deprivation. Cancer Res 2009; 69: 4225-4234.
[23] Munoz-Pinedo C, El Mjiyad N and Ricci JE. Cancer metabolism: current perspectives and future directions. Cell Death Dis 2012; 3: e248.
[24] Wang Y, Yun Y, Wu B, Wen L, Wen M, Yang H, Zhao L, Liu W, Huang S, Wen N and Li Y. FOXM1 promotes reprogramming of glucose metabolism in epithelial ovarian cancer cells via activation of GLUT1 and HK2 transcription. Oncotarget 2016; [Epub ahead of print].
[25] Marini C, Ravera S, Buschiazzo A, Bianchi G, Orengo AM, Bruno S, Bottoni G, Emionite L, Pastorino F, Monteverde E, Garaboldi L, Martella R, Salani B, Maggi D, Ponzoni M, Fais F, Raffaghello L and Sambuceti G. Discovery of a novel glucose metabolism in cancer: The role of endoplasmic reticulum beyond glycolysis and pentose phosphate shunt. Sci Rep 2016; 6: 25092.
[26] Zhang D, Fei Q, Li J, Zhang C, Sun Y, Zhu C, Wang F and Sun Y. 2-Deoxyglucose Reverses the Promoting Effect of Insulin on Colorectal Cancer Cells In Vitro. PLoS One 2016; 11: e0151115.
[27] Bast RC Jr, Hennessy B and Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer 2009; 9: 415-428.
[28] Lalau JD and Race JM. Lactic acidosis in metformin therapy. Drugs 1999; 58 Suppl 1: 55-60; discussion 75-82.
[29] Hanahan D and Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.
[30] Zikaki K, Aggeli IK, Gaitanaki C and Beis I. Curcumin induces the apoptotic intrinsic pathway via upregulation of reactive oxygen species and JNKs in H9c2 cardiac myoblasts. Apoptosis 2014; 19: 958-974.