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Inhibitory effect and mechanism of metformin on human ovarian cancer cells SKOV-3 and A2780

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Abstract. – OBJECTIVE: Ovarian cancer is the most common malignant tumor in female reproductive system. Metformin is an orally taken hypoglycemic agent, which is extensively applied in the clinic. Clinical trials find that there may be a certain degree of action of the metformin in inhibiting malignant tumors. This paper aims to investigate the inhibitory effect and mechanism of metformin on human ovarian cancer cells.

MATERIALS AND METHODS: Through *in vitro* cell experiment, the influences of metformin on the proliferation, colony formation and apoptosis of ovarian carcinoma cells were studied. Ovarian cancer cells SKOV-3 and A2780 in logarithmic growth phase were selected and cell proliferation was measured by MTT method. The metformin was processed for 48 h to calculate the survival rate of cells. Also, metformin was processed for 24 h and two weeks or stained with crystal violet, after which Quantity One (Bio-Rad, Hercules, CA, USA) method was used to quantitatively analyze the cell clone formation, meanwhile, the FCM (flow cytometry) was used for the detection and analysis.

RESULTS: Intervened by metformin with different concentrations for 48 h, the cell viabilities of SKOV-3 and A2780 cells were respectively reduced by 19.49 ± 2.92%, 45.41 ± 7.95%, 53.84 ± 5.53%, 64.04 ± 4.36% and 11.45 ± 3.12%, 35.42 ± 7.55%, 43.77 ± 5.77%, 53.05 ± 5.55% as compared with that in the control group with statistical significances. After processed by metformin with different concentrations for two weeks, the cells clone numbers of SKOV-3 and A2780 were significantly reduced. Treatment of metformin on SKOV-3 and A2780 cells of human ovarian cancer showed significant apoptosis.

CONCLUSIONS: The metformin has the inhibitory effect on the cells of human ovarian cancer, which may be through inducing ovarian cancer cell apoptosis.

Key Words:
Metformin, Ovarian cancer, Proliferation, Clone formation, Cell apoptosis.

Introduction

The ovarian cancer is the most common malignant tumor of the female reproductive system[1]. So far, resection and chemotherapy are the main treatment means for ovarian cancer[2]. The resection, however, is facing the issue of high recurrence after surgery[3,4]. At present, chemotherapy drugs of ovarian cancer mainly include paclitaxel, camptothecin derivatives, retinoids compounds, etc. However, these drugs are generally having serious problems, for example, easy to cause side effects and drug resistance. Metformin is an orally taken hypoglycemic agent, which is widely used in the clinic. Since the 1950s, it has been widely used for the treatment of type II diabetes[5]. In recent years, a large number of clinical trials show that metformin may inhibit malignant tumor to a certain extent[4,6]. This paper aims to explore the influences of metformin on the proliferation, clone formation and apoptosis of ovarian cancer cells *in vitro*.

Materials and Methods

Experimental Material

Cell Strain

Cell strains SKOV-3 and A2780 of human ovarian cancer were bought from ATCC (Manassas VA, USA).

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Main Experimental Apparatuses

The cell workbench was bought from Nanjing Water Purification Equipment (Co., Ltd, Jiangsu, China). The ultra-clean water purifier was bought from Beijing Liuyi Instrument Plant (Beijing, China), phase contrast microscope from Olympus (Shinjuku, Tokyo, Japan), -80°C refrigerator from Shandong Haier (Shandong, China). The FCM (flow cytometer) and microplate reader were both from the BD Company (San Jose, CA, USA).

Main Reagents and Materials

Culture medium RPMI-1640 and DMEM were both bought from the BD Company (San Jose, CA, USA), fetal calf serum from Hangzhou Sijiqing Biological Engineering (Co., Ltd, Hangzhou, China), trypsin, metformin, MTT and EDTA all from and the Annexin V-FITC/PI detection kit from the BD Company (San Jose, CA, USA).

Experimental Method

Detecting the Influence of Metformin on the Proliferation of Ovarian Cancer Cell In Vitro with MTT Method

When they were in logarithmic phase, the ovarian cancer cells SKOV-3 and A2780 were digested, centrifuged and resuspended to generate single cell suspension. 5×10^3 cells/well was inoculated in the 96-well plate and kept overnight in an incubator with 37°C and 5% CO_2. The medium was changed and 100 μL of metformin culture solution with different concentration were added to each well and cultivated for 72 h. 20 μL of 5 mg/mL MTT were added to each well, the supernatant was sucked after 4 h, 150 μL of DMSO were added and shocked for 10 min at the condition away from the light. Finally, the 96-well tissue culture plate was put in the microplate reader.

Clone Formation Assay

When they were in logarithmic phase, the ovarian cancer cells SKOV-3 and A2780 were digested, centrifuged and re-suspended to generate single cell suspension. 1×10^3 cells/well was inoculated in the 6-well plate and kept overnight in an incubator with 37°C and 5% CO_2. The medium was changed and 100 μL of metformin culture solution with different concentration were added to each well and cultivated for two weeks. The solution was changed once every 3 days. The supernatant was sucked; 4% of DMSO was fixed for 15 min, and stained with 0.1% crystal purple for 5 min and recorded with the digital camera. For calculating more accurately, the Quantity One was used for cell clone.

FCM was Used to Detect the Cell Apoptosis

The ovarian cancer cells SKOV-3 and A2780 were conventional digested, centrifuged and resuspended to generate single cell suspension. 2×10^3 cells/well was inoculated in the 96-well plate and kept overnight in an incubator with 37°C and 5% CO_2. The culture medium was sucked in wells, then the medium was changed and 100 μL of metformin culture solution were added with different concentration into each well, and the cultivation continued for 24 h and collected by digesting and centrifuging. 250 μL of assembly buffer solution were added for resuspending and regulate the cell concentration to about 1×10^6 cells/mL. 100 μL of cell suspension were added to 5 μL of Annexin V-FITC and 10 μL of PI, incubated for 15 min and put in the flow cytometry (FCM) for assay.

Statistical Analysis

The experiment was repeated with each group for 3 times and the data were represented as mean ± standard deviation (SD). According to the experimental design, the one-way analysis of variance was applied to compare. *t*-test was used between groups for comparison. $p < 0.05$ was statistically significant.

Results

Inhibitory Effect of Metformin on the Proliferation of Ovarian Cancer Cells

The MTT method was used in this research to detect the influence of metformin on the proliferation of ovarian cancer cells SKOV-3 and A2780. Figure 1 showed that, intervened by metformin with different concentrations (2, 5, 10, 20 mM) for 48 h, the cell viabilities of cell SKOV-3 were respectively reduced by 19.49 ± 2.92%, 45.41 ± 7.95%, 53.84 ± 5.53%, 64.04 ± 4.36% as compared with that in the control group with showed significance. Figure 2 showed that, intervened by metformin for 48 h, the cell viabilities of cell A2780 were respectively reduced by 11.45 ± 3.12%, 35.42 ± 7.55%, 43.77 ± 5.77%, 53.05 ± 5.55% as compared with that in the control

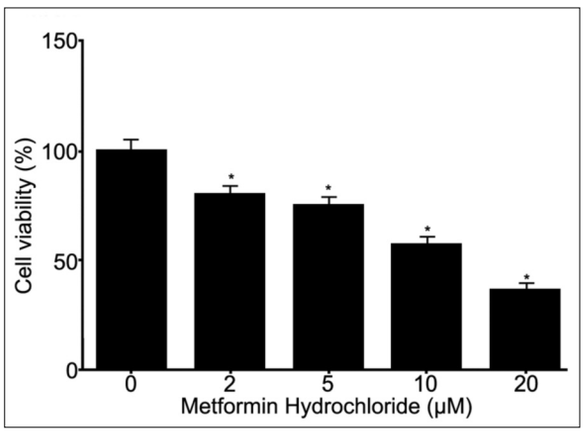

Figure 1. Influence of metformin on ovarian cancer cell SKOV-3, *$p < 0.05$.

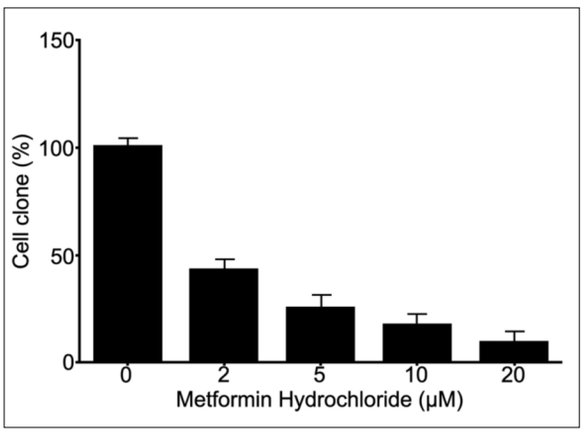

Figure 3. Influence of metformin on the clone formation ability of ovarian cancer cell SKOV-3, *$p < 0.05$.

group, which showed statistical significance. Taken together, these data showed that metformin could significantly inhibit the viability of ovarian cancer cells in a dose-dependent manner.

Inhibitory Effect of Metformin on the Clone Formation of Ovarian Cancer Cells

This study has further explored the influence of metformin on the clone formation ability of ovarian cancer cells. Figure 3 showed that interfered by metformin with different concentrations (2, 5, 10, 20 mM) for two weeks, the cell clone number of cell SKOV-3 were respectively reduced by 57.11 ± 11.55%, 75.45 ± 7.95%, 83.84 ± 5.77%, 91.01 ± 3.55% as compared with that in the control group. When the concentration of metformin was increased, the cell clone formation number was decreased, showing a significant dose-dependence. Figure 4 showed that interfered by metformin with different concentrations (2, 5, 10, 20 mM) for two weeks, the cell clone number of cell A2780 was respectively reduced by 55.15 ± 10.55%, 61.47 ± 8.15%, 75.45 ± 5.46%, 90.15 ± 7.45% as compared with that in the control group. Similar to the SKOV-3 cell, the cell clone formation number of A2780 also showed a significant dose-dependence. The experimental results showed that the metformin had influences on the clone formation ability of ovarian cancer cells.

Ovarian Cancer Cells Apoptosis Induced by Metformin

At present, there is still a controversy whether the metformin can induce the apoptosis of tumor cells. This paper further detected the apoptosis of

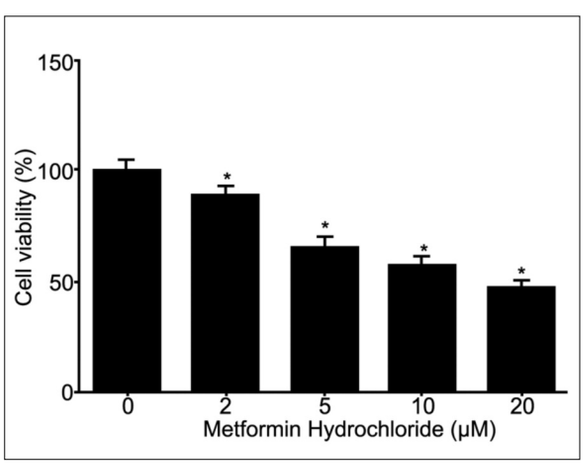

Figure 2. Influence of metformin on ovarian cancer cell A2780, *$p < 0.05$.

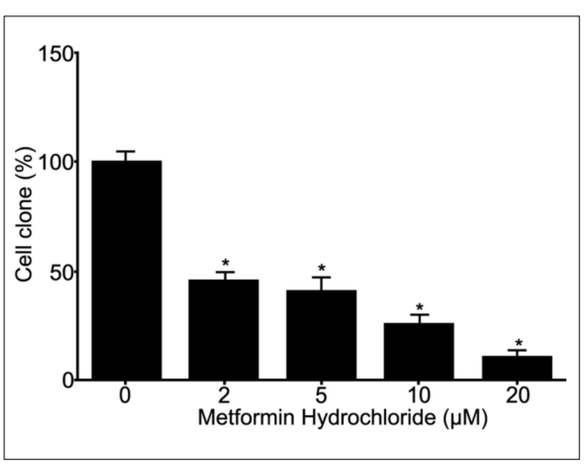

Figure 4. Influence of metformin on the clone formation ability of ovarian cancer cell A2780, *$p < 0.05$.

the ovarian cancer cells induced by metformin. Figure 5 and 6 showed that metformin could induce apoptosis of ovarian cancer cells SKOV-3 and A2780. After interfered by metformin with different concentration (2, 5, 10, 20 mM) for two weeks, the cell apoptosis rate of SKOV-3 was 11.67 ± 1.17%, 32.34 ± 5.57%, 41.45 ± 5.15%, 59.45 ± 7.34%, which all showed statistical significance. After interfered by metformin with different concentration (2, 5, 10, 20 mM) for two weeks, the cell apoptosis rate of A2780 was 17.54 ± 1.83%, 30.45 ± 5.34%, 48.34 ± 5.11%, 58.19 ± 9.18%, which all showed statistical significance. The above results showed that the metformin could induce the apoptosis of ovarian cancer cells.

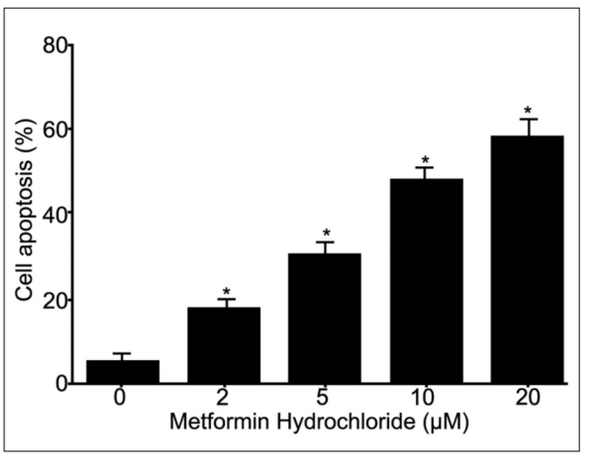

Figure 6. Influence of metformin on cell A2780 apoptosis of ovarian cancer, *$p < 0.05$.

Discussion

As a kind of oral hypoglycemic agent, which is widely applied in the clinic, metformin has the advantages of significant and rapid curative hyperglycemic effects and minor side effects[7]. Metformin can inhibit the malignant cells proliferation, such as kidney cancer, etc.[8,9]. The latest clinical trial results show that patients with bladder cancer and type 2 diabetes were treated with metformin orally. At the same time of lowering blood glucose, also it could significantly decrease the tumor recurrence risk. The experimental results indicate that metformin may have certain of inhibitory effects on bladder cancer[10,11]. Also, because the insulin and other oral hypoglycemic drugs may raise the risk to suffer from bladder cancer, therapeutic and protective effects of metformin on patients with bladder cancer complicated with type II diabetes have raised an increasing and extensive attention in medical field[12,13]. However, the inhibitory effect and the mechanism of metformin on ovarian cancer cells remain poorly understood. Rattan et al[12] have reported that metformin can exert the effects on the proliferation and metastasis of ovarian cancer cells by increasing the cytotoxicity of cisplatin. This paper aims to explore the direct actions of metformin on proliferation, clone formation and apoptosis of ovarian cancer cells.

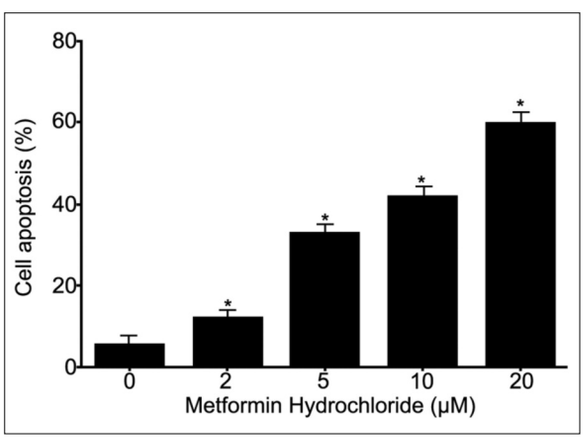

Figure 5. Influence of metformin on cell SKOV-3 apoptosis of ovarian cancer, *$p < 0.05$.

SKOV-3 and A2780 were selected in the research as the cell models for *in vitro* cell experiments. The results showed that processed and interfered by metformin with different concentrations for 48 h, the cell viabilities of SKOV-3 and A2780 were significantly decreased. The experimental results showed that metformin could dose-dependently inhibit the proliferation of these two cells, which was consistent with the research results showing metformin's effects on other cancer cells[14,15]. Processed by metformin with different concentrations for two weeks, cell clone numbers of ovarian cancer cells SKOV-3 and A2780 were significantly decreased as compared with that in control group. Even though the action concentration of metformin was low (2 mM), the clone formation number of cell SKOV-3 was still reduced by 57.11 ± 11.55% ($p < 0.05$). With the increasing of drug concentrations, the clone formation number of cell A2780 was still reduced, which showed significant dose-dependence. Results show that the metformin could inhibit the clone formation ability of ovarian cancer cells.

In the research finding of prostate cancer, metformin inhibits the proliferation of tumor cells mainly by inducing the cell apoptosis. In this study, FCM was used to detect the influence of metformin on ovarian cancer cells apoptosis and the results showed that the percent of apoptotic cells were significantly increased after interfered by metformin. Metformin could induce the significant apoptosis of ovarian cancer cells SKOV-3 and A2780. Even though the action concentration of metformin was low (2 mM), the apoptosis induction rates of metformin on cells SKOV-3 and A2780 were still kept at 11.67 ± 1.17% and 17.54 ± 1.83%. These results indicated that the induction of cell apoptosis might be the critical mechanism of metformin on the ovarian cancer cells proliferation. The apoptosis of cells SKOV-3 and A278 induced by metformin indicated that the inhibition on ovarian cancer cells might be through induction of cell apoptosis[15,16]. The induced cell apoptosis was known as the critical mechanism of metformin on the ovarian cancer cells proliferation, which had been confirmed in various malignant tumor cells, such as prostate cancer, kidney cancer, gastric cancer, breast cancer, etc.[9,10,17].

Conclusions

The metformin has the inhibitory effects on the cells of human ovarian cancer, which may be through inducing ovarian cancer cell apoptosis.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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