AZD1480 Can Inhibit the Biological Behavior of Ovarian Cancer SKOV3 Cells in Vitro

Zhao-Ling Sun, Ya-Juan Tang, Wei-Guang Wu, Jun Xing, Yan-Fang He, De-Mei Xin, Yan-Li Yu, Yang Yang, Ping Han*

Abstract

Objective: To study the mechanism of effects of AZD1480 on the SKOV3 ovarian cancer cell line. Methods: The MTT method was used to assess cellular proliferation, flow cytometry for cellular apoptosis, the scratch test to determine migration, transwell chamber assays to detect cellular invasion, plate clone experiments to detect the clone forming ability and Western blotting to determine p-STAT3 protein levels. Results: The proliferation rate, migration ability, invasiveness and the clone forming ability of SKOV3 cells were reduced after treatment with AZD1480, while apoptosis rate and chemotherapeutic susceptibility were increased. After treatment with AZD1480 plus cisplatin, the apoptosis rate increased significantly while the expression level of p-STAT3 protein was decreased. Conclusion: AZD1480 can inhibit the proliferation, invasion, metastasis and clone formation of SKOV3 cells, induce cellulsar apoptosis, increase the chemotherapeutic sensitivity and reduce the expression level of p-STAT3 protein.

Keywords: SKOV3 cells - JAK inhibitor - AZD1480 - biological behavior - p-STAT3

Introduction

Ovarian cancer is one of the malignant tumors of female, epithelial ovarian cancer (EOC) is relatively common one. It has the features of occult, easy to metastasis and invasion, the chemotherapy drug tolerance and poor prognostic. Treatment of EOC was mainly by operation, combined with chemotherapy. With the research of cell signal transduction pathways, blocking the cell signal transduction pathway may be a new direction for therapy of malignant tumor.

JAK2 is a kind of cytoplasmic protein tyrosine kinase (PTK). JAK plays an important role in signal transduction of a variety of cytokines. The signal transducer and activator of transcription (STATs) in signal transduction pathways have multiple family members, in which STAT3 is very important. It was detected to increase abnormally in many solid tumors and participated in malignant tumor cell proliferation, differentiation, invasion, migration and immune escape process (Subramaniam, et al., 2013; Swiatek-Machado, et al., 2013). It was proved in vitro and in vivo that the sustained activation of JAK/STAT3 signal transduction pathway in tissues of head and neck cancer, prostate cancer, breast cancer, colon cancer and other malignant tumors affected the evolution process of biological behavior of tumor cells (Jing et al., 2004; Shin et al., 2009; Lai et al., 2010; Shodeinde et al., 2012). A number of studies have found that there was overexpression of STAT3 gene in ovarian cancer, persisting of its active form p-STAT3 was closely related with the regulation of proliferation, apoptosis, migration and invasion of ovarian cancer cells (Lai et al., 2010; Kandala et al., 2012).

AZD1480 is a specific antagonist of JAK2 and can block the activation of STAT3 protein, then regulate the transcription of downstream target gene. It shows the antitumor effects in vivo and in vitro experiments (Guo et al., 2010; Bid et al., 2012; Couto et al., 2012; Ptak et al., 2012; Yang et al., 2012). However, there was a few data about the effect of AZD1480 on the biological behavior of ovarian cancer cells, so we explored it in this study.

Materials and Methods

Cell culture and morphological observation

SKOV3 cells were cultured with RPMI1640 (GIBCO) medium containing 10% fetal bovine serum, under 37℃, 5% CO₂ and saturated humidity conditions. The logarithmic growth phase cells within 10 generation were used to do experiments. SKOV3 cells were treated with 5 μmol/L AZD1480 (the selleck company, USA), the morphological changes of the cells were observed under microscope after 24 h.

Proliferation of cells was tested by MTT method

The cells were divided into control group (Group...
Transwell chamber (Corning) was covered with a layer of Matrigel matrix. Cells in the logarithmic growth phase of each group were cultured with serum free RPMI1640 medium for 24 h, then they were digested and the single cell suspension was diluted with serum free medium and the concentration of cell was 2.5 × 10^4/ml, each well was 200 μl. The lower chamber was added 800 μl RPMI1640 medium containing 30% fetal bovine serum medium and cultured for 24 h under 37°C, 5% CO_2, and saturated humidity conditions. The membranes were took out and the Matrigel and ventricular cells were wiped with a cotton swab. After formalin fixing and toluidine blue staining, we counted the cells through membrane under microscope and selected 9 different view randomly. The experiment was repeated for 3 times.

The clone forming ability was detected by plate clone experiment

Cells in the logarithmic growth phase of each group were digested and the single cell suspension was inoculated into 6 well plates, each well was 2ml, the concentration of cell was 5 × 10^5/ml. They were cultured at 37°C with 5% CO_2, and saturated humidity conditions for 10 days. When visible clone cell clusters with naked eye appeared in culture dish, we terminated culture and discarded supernatant. They were washed with PBS carefully for 2 times. Each well was added into 800 μl methanol and fixed for 30 minutes. Then the fixed solution was discarded and 1ml toluidine blue staining solution was added and stained for 30 minutes. They were washed with water slowly and dried in air. They were counted with image analysis software and calculated the rate of cloning efficiency.

p-STAT3 protein was detected using Western blot method.

The cells were divided into 5 groups (medium control and MDSO control group, 1μmol/L AZD1480, 5 μmol/L AZD1480 and 10 μmol/L AZD1480 group). Cells were collected after treatment for 4h and washed with cold PBS for 3 times. Then the cellular lysis buffer was added and incubated on ice for 30min. The total proteins were extracted after centrifugation. Quantitative protein determination was done with BCA kit in accordance with the kit manual.

Statistical analysis

All statistical tests were analyzed by SPSS16.0 statistical software and p < 0.05 was considered statistically significant. Differences among groups of single drug treatment were analyzed with single factor analysis of variance. Differences among groups of combined drug treatment were analyzed with double factor variance analysis.

Results

Morphological observation of cells treated with AZD1480

The cellular morphology was meniscus shrinkage, clear boundary, pseudopodia reducing, cytoplasm aggregating to be particles on the cellular inside membrane and particles partly suspended in culture medium after the treatment of AZD1480.
Cell survival rate in different groups

There was no significant difference between control group and DMSO group about the cell survival rate ($P > 0.05$). AZD1480 had significant inhibition effect on the growth of SKOV3 cells and the inhibition effect enhanced with the increase of concentration ($P < 0.05$) (Table 1).

In the AZD1480 combined with cisplatin treatment groups (Table 2), cisplatin could inhibit the proliferation of SKOV3 cells with dose dependent ($P < 0.05$). There was no significant difference between 1 μmol/L AZD1480 and control group ($P > 0.05$) but was statistically significant difference 5 μmol/L and 10 μmol/L AZD1480 groups compared with the control group ($P < 0.05$). The Coefficient of drug interaction (CDI) values were (0.902, 0.914, 0.95, 0.893, 0.848, 0.974, 0.923, 0.767, 0.372) < 1, which confirmed that these two drugs were synergistic. CDI value was 0.372 when the concentration was 80 μg/ml cisplatin + 10 μmol/L AZD1480, which showed that their synergistic effects were very significant.

The apoptosis rate in different groups

There was no significant difference between control group and DMSO group about the cell apoptosis rate ($P > 0.05$). The cell apoptosis rate increased in the AZD1480 groups and the apoptosis rate increased with the increase of concentration ($P < 0.05$) (Table 1).

In the AZD1480 combined with cisplatin treatment groups (Table 3), cisplatin could inhibit the apoptosis rate of SKOV3 cells and was dose-independent ($P > 0.05$). There was no significant difference between 1 μmol/L AZD1480 and control group ($P > 0.05$) but there was statistically significant difference between 5 μmol/L and 10 μmol/L AZD1480 groups and the control group ($P < 0.05$).

The cellular migration ability

There was no significant difference between control group and DMSO group about the cellular migration ability ($P > 0.05$). AZD1480 had significant inhibition effect on the migration ability of SKOV3 cells, the migration ability decreased with the increase of concentration ($P < 0.05$). (Table 1)

The cellular invasion ability

There was no significant difference between control group and DMSO group about the clone number ($P > 0.05$). AZD1480 had significant inhibition effect on the invasion ability of SKOV3 cells, the invasion ability decreased with the increase of concentration ($P < 0.05$). (Table 4)

The clone forming ability

There was no significant difference between control group and DMSO group about the clone number ($P > 0.05$). AZD1480 had significant inhibition effect on the clone forming ability of SKOV3 cells, the clone forming ability decreased with the increase of concentration ($P < 0.05$) (Table 4).

The p-STAT3 protein expression

There was no significant difference between control group and DMSO group about the expression level of p-STAT3 gene. AZD1480 decreased the expression level of p-STAT3 gene.

Discussion

The treatment of EOC was mainly on the basis of cytoreductive surgery combined with cisplatin and paclitaxel chemotherapy, the clinical symptoms of most patients were relieved (Piccart et al., 2003; Alberts et al., 2006), but the tumor of most complete remission patients was prone to recurrent (Thigpen et al., 1993), some patients developed resistance. In addition, chemotherapy drugs had toxic effect on normal cells, which limited their clinical application (Monk et al., 2007). At present, Gene therapy for cancer has been the focus of cancer therapy study. The studies have confirmed that ECO was caused by gene expression imbalance and was related to a variety of genes (Son et al., 2012; Shirali et al., 2013; Skirnisdottir et al., 2013). Regulating the expression of genes related with ECO to the dynamic balance will become the future development goal for the treatment of ECO.

In this study, we found that JAK2 inhibitor AZD1480 could inhibit the proliferation, apoptosis, invasion and...
migration of SKOV3 cells. AZD1480 could increase the cellular sensitivity to cisplatin and these two drugs were synergistic. These provided the experimental basis for reducing dosage and side effects of cisplatin and improving its efficacy in clinical trials.

It was found in many tumors that the excessive activation of STAT3 could affect the growth, apoptosis, invasion and metastasis of tumor (Bromberg et al., 1999; Leong et al., 2003). Many studies confirmed that microdose AZD1480 could inhibit the phosphorylation of JAK2-STAT3 in multiple tumors, the expression of its function protein p-STAT3 decreased obviously, thereby regulate downstream genes, such as cyclinD1, cyclinB, cmyc, Bcl-2, MCL1, survivin, which could promote the apoptosis of tumor cells, inhibit the cellular proliferation, migration and invasion and increase the sensitivity to platinum-based chemotherapy drugs (Hedvat et al., 2009; McFarland et al., 2011; Scuto et al., 2011; de et al., 2012; Loveless et al., 2012).

The results of this study showed that 1μm/L AZD1480 could reduce the p-STAT3 protein in SKOV3 cells significantly. However, other cellular biology behavior changes need much higher doses. The possibilities were that the proliferation of SKOV3 cells may also be regulated by other signal pathways, or the inhibition time of small-dose AZD1480 was short and p-STAT3 could fast recovery. The cellular invasion and migration may be closely related to the JAK-STAT3 pathway which may be the main regulation. The biological behavior of cells were inhibited severely and could not recovery in short-term even if the p-STAT3 recovery quickly.

In a conclusion, AZD1480 could inhibit JAK/STAT3 signal pathway in SKOV3 cells effectively and decrease the expression of p-STAT3 protein, which could change some malignant behavior of tumor cells. It gave us inspiration for the treatment of EOC. However, there are many problems need to be further explored. Such as: Only one drug-resistant cell line of ovarian cancer was used in this experiment without comparison with other ovarian cancer cell lines. So we cannot judge the effect of AZD1480 on ovarian cancer cell comprehensively. In addition, in vivo experiments should be done due to the complex metabolic processes in vivo, the real effect of drugs need to be further confirmed.

References


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