Upregulation of Fas in epithelial ovarian cancer reverses the development of resistance to Cisplatin

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This study was to investigate the role of Fas in the development of Cisplatin-resistant ovarian cancer. On the cellular level, Fas expression was significantly reduced in Cisplatin resistant A2780 (A2780/CP) cells compared with A2780 cells. Fas silence with siRNA would promote tumor cell lines proliferation, facilitate tumor cell cycle transition of G1/S, prevent cell apoptosis, and promote cell migration. Expression of drug resistance gene was negatively correlated to Fas. In nude mice metastasis model of human ovarian carcinoma by subcutaneous transplantation, after Ad-Fas injected intratumorly, we found that upregulation of Fas could inhibit transplantation tumor tissue growth and reduce the expression of drug resistance gene. Our results indicated that upregulation of Fas in epithelial ovarian cancer reverses the development of resistance to Cisplatin. In conclusion, our findings suggested that Fas might act as a promising therapeutic target for improvement of the sensibility to Cisplatin in ovarian cancer. [BMB Reports 2015; 48(1): 30-35]

INTRODUCTION

Ovarian cancer is common gynecologic malignant tumor around the world, and is one of the leading mortality causes in women (1). Platinum-based chemotherapeutic strategies are broadly required for patients with ovarian cancer, but Cisplatin-resistance has been a major barrier to the successful ovarian cancer treatment (2). Resistance to platinum has been reported to be associated with several mechanisms, including increased drug efflux (3), drug inactivation (4), alterations in drug target (5), processing of drug-induced damage (6) and evasion of apoptosis (7). However, the direct evidence of apoptosis evasion in ovarian cancer has not been clearly found out.

Fas is a prototypical death receptor consisting of a N-terminal region containing three cysteine-rich domains (CRDs), with ligand binding occurring predominantly at the second and third CRDs, a transmembrane domain and an intracellular region containing an less than 80 aminoacid domain called the “death domain” (DD) (8). It is ubiquitously expressed in the body, but is particularly abundant in liver, heart, brain, and colon tissues, and in activated mature lymphocytes (9). Fas was reported to be involved in many physiological processes such as proliferation, apoptosis, migration etc. In these years Fas has attracted more and more attention in cancer progression. It was reported that upregulation or hyperactivation of fatty acid synthase (FAS) has recently been found in most ovarian cancer and in most human solid tumors, where it is highly associated with high aggressiveness and poor patient survival (10-12). Inhibition of FAS activity is selectively cytotoxic to human cancer cells in vitro and in vivo including human ovarian cancer xenografts (13). However, the mechanisms linking the inhibition of Fas activity to cancer cell proliferation, apoptosis and migration need to be researched intensively. In this study, we also focused on the role of Fas regulation in the treatment of Cisplatin-resistant ovarian cancer.

RESULTS

Down regulation of Fas is associated with development of Cisplatin resistance

Western blot results showed that after treatment with various concentration of Cisplatin, the expression of Fas decreased in a concentration-dependent manner in A2780, SKOV3 and ES2 cells (P < 0.05, Fig. 1A-C). And, Cell viabilities of A2780, SKOV3 and 8910 were significantly lower than vehicle control (P < 0.05, Fig. 1D). Fas expression was remarkably decreased in Cisplatin-resistant A2780 cells when compared with parent A2780 cells (P < 0.01, Fig. 1E). Besides, the half maximum inhibitory concentration of Cisplatin to A2780 was significantly lower than A2780/CP (IC50:43.56 versus 65.75 μM, Fig. 1F). These re-
results indicated that decreased Fas might be associated with Cisplatin-resistance ovarian cancer.

Fas silence promotes cell proliferation and cell cycle transition

Small interfere RNA technology was taken to investigate the silence effect of Fas. We screened the effective Fas siRNA concentration in A2780, and the interfere efficiency reached to 73% (Fig. 2A). Next, we observed the effect of Fas siRNA on cell proliferation, and cell cycle transition. Fas was proven to be involved in cell proliferation, our results also demonstrated that Fas silence could promote A2780 and A2780/CP cells proliferation, and this effect was enlarged in A2780/CP cells (Fig. 2B). As shown in Fig. 2C, Fas silence decreased the percentage of cells in the G0/G1 phase from 87.36 ± 3.26% to 69.15 ± 3.41% and enhanced the percentage of cells in the S phase from 9.62 ± 4.13% to 19.32 ± 2.56% (P < 0.05; Fig. 2D). And this effect was enhanced in A2780/CP cells. Our data indicates that Fas silence promoted cell cycle transition from the G0/G1 phase to S phase in A2780 and thus promoted cell proliferation, and Cisplatin resistance had much significant effect on cell cycle transition.

Cisplatin-resistant A2780 cells was sensitive to Fas silence induced apoptosis

Effects of Fas silence on A2780 apoptosis was analyzed by Annexin V-FITC/PI flow cytomety. In Fig. 2, transfection with Fas siRNA could inhibit H2O2 induced apoptosis in A2780 an apoptotic rate of 24.36 ± 3.25%, which was much lower than H2O2 treated A2780 cells (P < 0.01; Fig. 2E), and transfection with Fas silence on Cisplatin-resistant A2780 cells induced an apoptotic rate of 4.54 ± 1.67%, which was also lower than H2O2 treated A2780/CP cells (P > 0.05; Fig. 2F). This result showed that Fas silence induced apoptosis was enhanced in A2780/CP cells.

Cisplatin promotes Fas silence induced cell migration and invasion

To detect whether knockdown of Fas affects the migration of A2780 and A2780/CP cells, transwell migration assay was performed to assess the proportion of cells which migrated through polycarbonate membranes after transfection for 48h. We found that Fas knockdown increased the number of invaded cells accounting for 32.2% rise in A2780 cells (Fig. 2G, H, P < 0.01), besides, A2780/CP cells also increased the number of invaded cells, Fas silence definitely boosterized this invasion. So Fas silence promotes the ability of migration and invasion of A2780 cells, while Cisplatin resistance could promote this ability of A2780 cells.

Fas silence influences Cisplatin induced the expression of MDR1, MRP2 and LRP

To further investigate the mechanism that how deos Fas impact Cisplatin resistance, we observed the effect of Fas silence on expression of drug-resistance gene MDR1, MRP2 and LRP. The results showed that A2780/CP cells had higher expression of MDR1, MRP2 and LRP than A2780 cells. Fas silence could reduce the expression of MDR1, MRP2 and LRP in A2780 cells, while Fas overexpression could reverse their expression in A2780/CP cells (Fig. 3).

Re-expression of Fas could reverse resistance in ovarian cancer in vivo

To study whether overexpression of Fas is effective in inhibiting drug-resistant ovarian cancer in vivo, we established transplantation tumor model by subcutaneous injection with A2780...
cells in nude mice. 12 days later, the nude mice were treated with Cisplatin alone or combined with Fas over expression vector. Fas expression was remarkably increased in tumor in combination group, suggesting that Ad-Fas was effective in vivo (Fig. 4A, P < 0.01). Then we found that the transplantation tumors in the combination group tended to grow more slowly than those in Cisplatin alone group. After 35 days in combination group, tumor size was 1842.16±325.64 mm³, smaller than 2957.13±29.16 mm³ in those given Cisplatin alone (Fig. 4B, C, P < 0.01). In line with results of our experiments in vitro, immunohistochemistry staining of tumor sections revealed a dramatic decrease in the expression of MDR1, MRP2 and LRP in tumors received combination therapy (Fig. 4D).

**DISCUSSION**

Many recent studies have proven that Fas played a critical role in pathogenesis of ovarian cancer, some evidences suggested that Fas might be connected to Cisplatin-resistance of cancer therapy (14). In our present study, we confirmed that ex-
Expression of Fas decreased in Cisplatin-resistant A2780 cells, which indicated Fas might played the protective role in Cisplatin resistance of ovarian cancer. Then we designed serious of experiments to explore the involving mechanism. Consistent with other reports, Fas inhibition promoted A2780/CP cells proliferation and migration, but inhibit A2780/CP cells apoptosis, which were consistent with results in A2780 cells and brought adverse effect for Cisplatin-resistance tumor therapy. Except for these in vitro evidences, we also got direct data from nude mice of transplanted-tumor model. We found that artificially elevated expression of Fas, played a good inhibitory effect in tumor treatment, such as minimize tumor progression.
size and reduce expression of drug resistance gene.

Fas was reported to upregulate in various of human solid tumor including breast, stomach, lung, ovary and so on (15-18), which suggested the functional role of Fas in the progression of malignant cancer. Inhibition of Fas activity could promote tumor cell growth by arresting cell cycle transition and inhibit apoptosis by affecting Bad pathway in ovarian cancer (19). So Fas was considered as a potential target for anticancer therapy. But as the drug-resistant problem in tumor therapy is becoming more and more serious, especially for platinum chemotherapy, whether Fas regulation could be useful in Cisplatin-resistant ovarian cancer deserved to be investigated furtherly.

Cell abnormal proliferation and apoptosis contributed to pathogenesis of tumor. Cancer growth was controlled by the balance between cell proliferation and apoptosis. According to this point, regulation of cell proliferation and apoptosis could become an effective pathway to cancer therapy. Consistent with previous reports, we confirmed that Fas inhibition really increased cell proliferation and prevented apoptosis in A2780 cells, and Fas inhibition could also promote proliferation and inhibit apoptosis of Cisplatin-resistant A2780 cells. Anyway, cell proliferation had been closely related to cell cycle. If cell cycle arrest, cell growth was bound to be affected. The results showed that Fas inhibition promoted transition of G0/G1 to G2/M.

Cell migration and invasion was important process involved in pathological processes (20). The high mortality rate of ovarian cancer patients was attributed to the highly invasive characteristic of the disease (21). Besides, malignant ovarian epithelial cells primarily spread to adjacent organs by local invasion. So inhibition of cell migration could be an important way to control ovarian cancer. In our study, Fas inhibition promoted cell migration in A2780 by transwell assay, and this ability was enlarged in A2780/CP cells.

Our studies demonstrated that down regulation of Fas was involved in Cisplatin resistance development in ovarian cancer cell lines. The in vitro study proved that Fas played an protective role in cancer development. There were reports that Fas gene transduction could reverse multidrug resistance of human drug resistant SCLC cell H446/CDDP, for which the enhanced cell sensitivity to apoptosis and decreased expression of GST-π and ERCC1 might be responsible (14). That means up-regulation of Fas reverse Cisplatin resistance of human small cell lung cancer cell. Our data also indicated that treatment with Fas overexpression combined with Cisplatin could significantly minimize the growth of tumors in vivo, suggesting higher Fas level might be required for more effective tumor growth inhibition. Fas regulation might be the target of solving Cisplatin resistance in ovarian cancer therapy.

**MATERIALS AND METHODS**

Materials and Methods are available as supplementary data on BMB Reports online.

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