Influence of tamoxifen on cisplatin-sensitivity and estrogen receptors expression in ovarian carcinoma cell lines

Wpływ tamoksifenu na platynowrażliwość i ekspresję receptorów estrogenowych linii komórkowych raka jajnika

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Abstract

Background: *Tamoxifen, used in breast cancer treatment, competitively inhibits estrogen receptor (ER) and also demonstrates direct antiproliferative effect on cancer cells even in ER lacking cancer tissue. However, its molecular mechanism of action is still unclear.*

Material and methods: *We exposed on tamoxifen 11 ovarian cancer cell lines, including well-documented platinum-sensitive and platinum-resistant ones, and studied tamoxifen-, cisplatin-sensitivity and expression of ERα and ß.*

Results: *We observed: no correlation between TAM-sensitivity and ERα and ERß expressions, no correlation between TAM influence on cisplatin-sensitivity and ERα and ERß expressions, increase of ERß expression after TAM-exposure in 3 cell lines; decrease in the 1 line, no TAM-exposure influence on ERα expression and increase of IC50 for cisplatin after TAM-exposure in 5 (45%) cell lines. These results show ovarian cancer cells being affected by TAM have different platinum sensitivity.*

Conclusions: *Our data suggests that ovarian cancer cells platinum-sensitivity are not linked with ER expressions. We claim the necessity of seeking some TAM predicting factors, using DNA microarrays.*

Key words: **ovarian carcinoma** / **cisplatin** / **tamoxifen** / **estrogen receptor alpha** / / **estrogen receptor beta** /

Streszczenie

Cel pracy: *Tamoksifen (TAM) kompetecyjne blokuje receptor estrogenowy (ER) i jest lekiem powszechnie używanym w terapii raka sutka. Wykazuje także bezpośredni efekt antyproliferacyjny w komórkach nowotworowych pozbawionych ER, jednak mechanizm tego działania nie jest znany. Celem pracy było zbadanie, w jaki sposób ekspozycja na tamoksyfen wpływa na platynowrażliwość linii komórkowych raka jajnika oraz określenie ekspresji ER w tych reakcjach.*

Materiał i metoda: *Użyto 11 linii komórkowych raka jajnika, zarówno o udokumentowanej wrażliwości na platynę jak również niewrażliwych na działanie cytostatyku. Inkubowano je z cisplatyną oraz 0,0 (kontrola) oraz 0,5 i 1,0 µMol tamoksyfenu. Wrażliwość na platynę oceniono wyznaczając IC50 dla poszczególnych hodowli. Metodą immunohistochemiczną określono ekspresję i lokalizację ERα i ERβ.*

Wyniki: *Zaobserwowano brak korelacji pomiędzy wrażliwością na tamoksyfen i ekspresją ERα i ERβ, choć wykazano różną lokalizację ERα i ERβ (jadrową i cytoplazmatyczną). Nie wykazano wpływu tamoksyfenu na platynowrażliwość linii komórkowych w powiązaniu z ekspresją ERα i ERβ. Po ekspozycji na tamoksyfen w 3 liniach zaobserwowano wzrost ekspresji ERβ a spadek w jednej linii. W 5 liniach nie odnotowano wpływu tamoksyfenu na ich platynowrażliwość przy braku wpływu na ekspresję ERα. W 6 liniach zaobserwowano wzrost IC50 dla platyny przy inkubacji z 0,5 (4 linie) i 1,0 µMol (3 linie) tamoksyfenu.*

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Wnioski: *Wyniki sugerują, że tamoksyfen modyfikuje w różny sposób platynowrażliwość linii komórkowych raka jajnika i nie jest ona powiązana z ekspresją ER. Należy poszukiwać innych czynników predykcyjnych wrażliwości na tamoksyfen u chorych na raka jajnika.*

Słowa kluczowe: **rak jajnika** / **cisplatyna** / **tamoksifen** / **receptor estrogenowy α** / / **receptor estrogenowy β** /

Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death among gynecological malignancies in Western countries. About 190,000 new cases and 114,000 deaths are estimated to occur annually due to ovarian cancer with the highest rates reported in Scandinavia, Eastern Europe, the USA and Canada [1].

Only about 35% of women will survive for 5 years without any symptoms of the disease. Treatment results have not improved significantly in the course of the last 30 years in spite of new chemotherapeutical agents and surgery protocol development. The unsatisfactory outcome is related to the ovarian tumor localization (deep in the pelvis) and, consequently, late clinical symptoms appearance, which does not facilitate earlier diagnosis. Over 75% of cases are diagnosed in stage FIGO III and IV, with only 20% surviving the period of 5 years [2].

Surgery is the first step of the medical procedures and the optimal cytoreduction remains the most predictive value of the treatment results and patient outcome [3]. Chemotherapy, administered in the vast majority of cases, constitutes the necessary next step. Non-radical surgical performance, together with the phenomenon of chemoresistance (MDR – *Multidrug Resistance*), are mostly responsible for ovarian cancer treatment failure and that is why ovarian cancer appears to be a persistent disease for most women. Thus, search for new solutions increasing chemosensitivity, remains an essential research area. Recently, certain authors have redirected their attention to tamoxifen (TAM), selective estrogen receptor modulator, and its ability to inhibit the ovarian cancer tissue growth. What is more, it has been proven that, contrary to breast cancer, this mechanism is independent of ERα expression [4, 5].

Moreover, TAM may enhance the cytostatic chemosensitivity [6]. In case of patients with advanced ovarian cancer, clinical TAM administration results, together with platinum-based agents, have not been homogenous. Markman et al. have not managed to prove the positive influence of TAM on chemotherapy efficacy, whereas Benedetti-Panici et al. have indeed demonstrated that patients, both resistant and sensitive to platinum-based chemotherapy, treated with platinum and TAM (80mg/day or 40mg/day) have shown the same treatment response and similar overall survival rate [7, 8].

Due to lack of coherent data, we have made an attempt to check the influence of TAM on various ovarian cancer cell lines, what may help to determine its usefulness in clinical practice.

The aim of our work was to investigate the influence of TAM exposure on cisplatin sensitivity in 11 cell lines, including well-documented platinum-sensitive and platinum–resistant ones. We have also assed the connection between the TAM effect and expressions of ERα and ERß on investigated cell-lines.

Material and methods

Cell culture

Human carcinoma cells were grown in Leibovitz L-15 medium (Biowhittaker, Walkersville, MD) supplemented with 10% fetal calf serum (FCS) (GIBCO/BRL, Grand Island, NY), 1 mM L-glutamine, 6.25 mg/l fetuin, 80 IE/l insulin, 2.5 mg/ml transferrin, 0.5 g/l glucose, 1.1 g/l $NaHCO_3$, 1% minimal essential vitamins and 20,000 kIE/l trasylol in a humidified atmosphere of 5% CO_2 at 37°C as described previously [9, 10, 11, 12]. The cisplatin-resistant cell line, A2780RCIS, was derived from the ovarian carcinoma cell line, A2780 [9].

The human ovarian carcinoma cell lines CAOV-3, EFO 21, EFO 27, ES-2, Mdah 2774, OAW 42, OVCAR-3, PA-1, and SKOV-3 were kindly provided by Dr. Carsten Denkert (Institute of Pathology, Charite´, Berlin, Germany). In order to ensure maintenance of cisplatin-resistant phenotype of A2780RCIS cells, the medium was supplemented with 10 Ag/mL of cisplatin (33.3µmol/L; GRY-Pharm, Kirchzarten, Germany).

Cell proliferation assay

Chemoresistance was tested with the use of the proliferation assay, based on sulphorhodamine B (SRB) staining as described previously [13]. Briefly, 800 cells per well were seeded in 96-well plates in triplicates. After 24-hour attachment, cisplatin or TAM (GRY-Pharm, Kirchzarten, Germany) were added in dilution series for a 5-day incubation, before SRB staining was performed. Incubation with studied substances was terminated by replacing the medium with 10% trichloroacetic acid, followed by incubation at 4°C for 1h. Subsequently, the plates were washed five times with water and stained by adding 100 µl 0.4% SRB (Sigma, St. Louis, MO,USA) in 1% acetic acid for 10 min at room temperature. Washing the plates five times with 1% acetic acid eliminated unbound dye. After air-drying and re-solubization of the proteinbound dye in 10 mM Tris-HCl (pH=8.0), absorbance was read at 562 nm in an Elisa-Reader (EL 340 Microplate Bio Kinetics Reader, BIO-TEK Instruments, Winooski, VT, USA). The measurements were performed in triplicates in three independent experiments. IC_{50}-values were calculated from three independent experiments for each cell line.

Exposure on TAM treatment

4-OH TAM was purchased from Sigma (Deisenhofen, Germany). In our first step we have determined TAM IC_{50} of the investigated cell lines. Next, the cell lines were cultured for two weeks with addition of 0.0 µM (control), and 0.5 µM, and 1 µM of TAM. The choice of such, TAM concentrations, nontoxic for ovarian cancer cells, was imposed to avoid direct TAM toxicity on the results of the assessment platinum sensitivity. Similar, non toxic for culture cells, TAM concentrations were described by Zhou et al [14]. Scambia et al. have proven that TAM concentrations starting from 0.1 µM increase the ovarian cancer cell platinum sensitivity [15].

Immunocytochemistry

In order to detect estrogen receptors α and ß proteins by immunocytochemistry, cells were seeded on slides, fixed 10 min in a methanol: acetone (1:1) mixture at -20^{0}C, and air-dried. Afterwards, the slides were incubated 5 min in 3% H_2O_2 to block endogenous peroxidase. Immunocytochemical reactions were performed using of following antibodies: mouse mAb 1D5 (DakoCytomation, Glostrup, Denmark) directed against ERα (optimally prediluted, 1 h at 20°C) and mouse mAb PPG5/10 (DakoCytomation) directed against ERß 1 (dilution 1:50 in Antibody Diluent, Background Reducing (DakoCytomation), 1 h at 20°C). Each reaction was accompanied by a negative control using Primary Mouse Negative Control (DakoCytomation). The antigens were visualized using biotinylated antibodies (15 min at room temperature), streptavidin–peroxidase complex (15 min at room temperature), LSAB+, HRP (DakoCytomation), and DAB (10 min at room temperature) (DakoCytomation). Preparations were counterstained with Mayer's hematoxylin (DakoCytomation), dehydrated, and mounted as described previously [11, 12].

Evaluation of reaction intensity

In case of immunostained cancer cell lines, specific staining reactions were localized by two experienced histologists. Immunostaining reactions were evaluated using a simplified scale, providing merely a score for the intensities of the reactions (0, total absence of staining; 1, only faint staining; 2, moderate staining; and 3, strong, intense staining) as described previously [12].

Statistical analysis

Statistical analyses of the results have been performed with the help of Statistica 98 PL software (Statsoft, Krakow, Poland). $P < 0.05$ indicated a significant relationship.

Results

TAM-sensitivity of investigated cells

The TAM IC_{50} values of the investigated cells are enclosed in table I.

TAM influence on ERα and ERβ expressions in investigated cell lines

In individual cell lines of the ovarian cancer, the performed immunocytochemical reactions showed nuclear or cytoplasmic localization and a variable intensity. Only cells A2780P have shown intense nuclear expression of ERα (score 3). (Figure 1).

Investigated cell lines exposure to TAM did not alter ERα expression. The results of immunocytochemical reactions of ERß expression are shown in Table II.

In cells not subjected to TAM the nuclear expression of ERß has only been observed in EFO 21 cell line (Figure 2A1). In these cells the down-regulation of the ERß expression was noted (Figure 2A2-3). Cell lines CAOV-3, ES-2 and PA-1 after two-week incubation with 0.5 µM TAM have caused up-regulation of nuclear expressions ERß. (Figure 2B). Nuclear expressions of ERß have not been observed after two-week incubation with 1µM of TAM. Cell lines A2780P, A2780RCIS, EFO27, Mdah 2774, OAW 42, OVCAR-3 and SKOV-3 have not demonstrated the nuclear expression of ERß. (Figure 2C). In some cases cytoplasmatic expression of ERß were traced. (Table II, Figure 2A2).

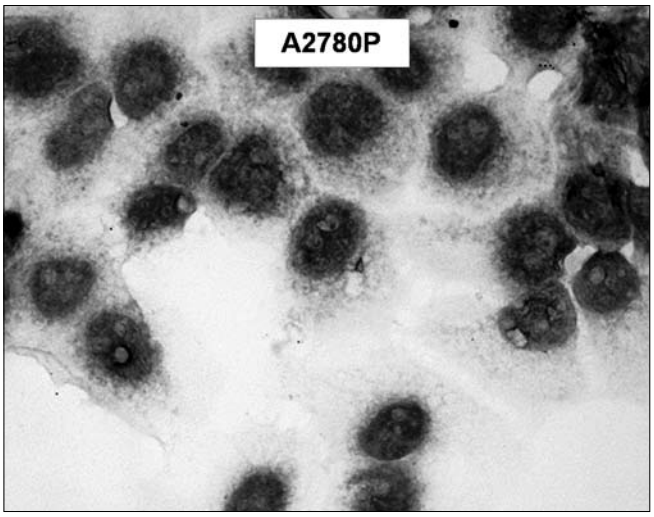

Figure 1.

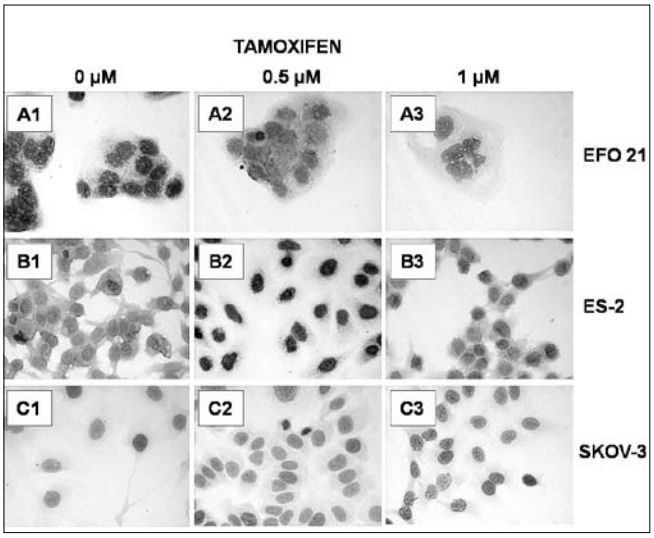

Figure 2.

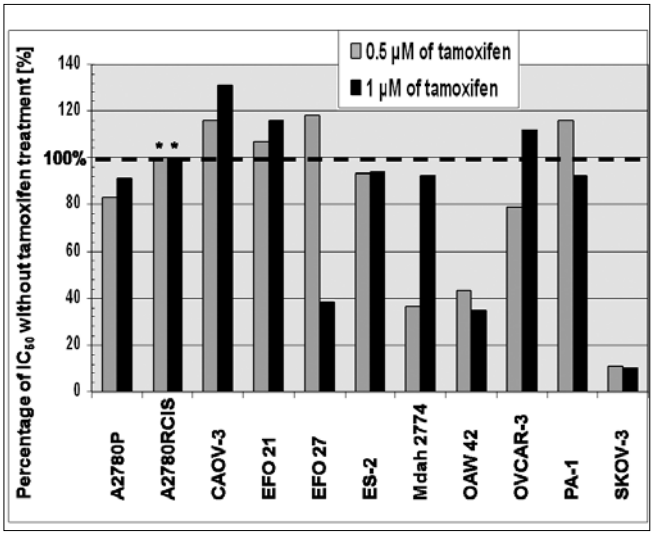

Figure 3.

Table I. TAM IC_{50} of investigated cell lines.

Cell line	TAM IC_{50} [μM]
A2780P	**46.4**
A2780RCIS	**215.4**
CAOV-3	**244.8**
EFO 21	**264.4**
EFO 27	**359.4**
ES-2	**316.2**
Mdah 2774	**77.4**
OAW 42	**35.9**
OVCAR-3	**35.9**
PA-1	**359.4**
SKOV-3	**408.4**

TAM influence on cisplatin sensitivity

The IC_{50} results in the investigated cell lines, both exposed to 0.5 μM and to 1 μM of TAM for two weeks and not exposed at all (the control group) are presented in table III and in Figure 3.

In the most platinum resistant cell line, A2780RCIS, platinum IC 50 was independent of TAM exposure (ANOVA rank test of Kruskal-Wallis, $P > 0.05$). Cell lines CAOV-3, EFO21, EFO27 and PA-1 incubations with 0.5 μM TAM have increased their IC_{50} for platinum (ANOVA rank test of Kruskal-Wallis, $P < 0.05$). Similarly, cell lines CAOV-3, EFO21 and OVCAR-3 incubations with 1 μM of TAM have increased their platinum IC_{50} (ANOVA rank test of Kruskal-Wallis, $P < 0.05$). In the remaining cell lines the ovarian cancer cells have demonstrated a certain sensibilization to platinum (ANOVA rank test of Kruskal-Wallis, $P < 0.05$).

ERs expressions and TAM sensitivity

The ANOVA rank test of Kruskal-Wallis has not demonstrated correlations between ERα and ERß expressions and TAM IC_{50} of the investigated cells ($P > 0.05$).

ER expression and TAM influence to cisplatin-sensitivity

ANOVA rank test of Kruskal-Wallis have not demonstrated correlation between ERα and ERß expression and modification of IC_{50} for the investigated cells as a result of TAM incubation ($P > 0.05$).

Discussion

The underlining principle of TAM action is the competitive inhibition of ERα activity. At present, TAM is routinely used only as hormonal drug in breast cancer treatment, where it is administered to women showing ERα expression in cancer tissue [4].

TAM toxicity is generally low and adverse events are noticed rarely [16]. Both types of ER (α and ß) are found in normal ovarian epithelium, benign as well as border-line tumors and in ovarian cancer (61% to 79%), especially in its serous and endometrial histological subtypes [17]. However, the ratio of ERα to ERß is different in cancer tissue comparing to normal ovarian epithelium [17, 18, 19]. ERα expression does not change but ERß expression is considerably lower in cancer cells, probably due to its selective down-regulation in carcinogenesis process, consequently promoting the mitogenic activity of ERα [20].

Two notable phenomena are directly responsible for TAM testing in ovarian cancer. The above mentioned expressions of ERα and ERß might be observed in most cases of ovarian cancers, therefore, theoretically, application of selective estrogen modulators should modify EOC development, growth and spread. However, clinical trials have shown only 7 to 18% clinical responses to treatment with TAM in case of women suffering from EOC [21, 22].

Currently, insufficient data prevents us from interpreting those results correctly. Other observations from clinical trials suggest that TAM, irrespective of its direct involvement in ER inhibitions, may increase platinum susceptibility in recurrent ovarian cancer, especially in platinum sensitive diseases [8, 23]. The nature of this platinum – TAM action has not been fully comprehended.

Table II. ERß in the investigated ovarian cancer cell lines: 0 μM of TAM (control group); after two-week incubations with 0.5 μM of TAM; after two-week incubations with 1 μM of TAM. The positive staining was observed in nuclei and in cytoplasm.

Cell line	0 μM of TAM ERβ expression		0.5 μM of TAM ERβ expression		1 μM of TAM ERβ expression	
	nuclear	cytoplasmic	nuclear	cytoplasmic	nuclear	cytoplasmic
A2780P	0	0	0	1	0	0
A2780RCIS	0	0	0	0	0	0
CAOV-3	0	0	3	0	0	0
EFO 21	3	0	0	1	0	0
EFO 27	0	0	0	0	0	1
ES-2	0	1	3	0	0	0
Mdah 2774	0	0	0	0	0	0
OAW 42	0	0	0	1	0	0
OVCAR-3	0	2	0	0	0	0
PA-1	0	0	3	0	0	0
SKOV-3	0	0	0	0	0	0

The expected advantages might result from their synergistic effect in cancer cells and the possibility of increasing ovarian cancer cells platinum sensibility. In ovarian cancer management we attempt to use TAM in partial platinum-sensitive tumor in order to prolong the platinum free interval, what may be effective in tumor cells platinum sensibilization; however the molecular mechanism of this action remains unclear [24, 25]. Clinical trials involving tamoxifen in case of ovarian cancer patients have not given unambiguous results so far, and *in vitro* investigations have been conducted in small groups of cells. We have shown, for the first time, the TAM influence on platinum sensitivity in a large group of cell lines (11 cell lines).

The present results demonstrate different platinum sensitivity of the investigated cell lines being affected by TAM. We have shown that TAM might influence cancer cells platinum sensitivity in different ways. In the cell line CAOV-3, EFO21, EFO27 and PA-1 well-defined decreasing platinum sensitivity was observed upon 0.5 μM of TAM but in the cell lines OVCAR-3 the effect was noted upon 1 μM of TAM. In the cisplatin-resistant A2780RCIS cell line, TAM has not affected platinum sensitivity. Similarly to other authors, we observed that inhibition of proliferations in cancer cells and their platinum sensitivity does not depend on ERα or ERß expression. Mabuchi et al. have proven that TAM inhibits ovarian cancer cells proliferation by means of mitogen-activated protein kinase cascades and this mechanism is mediated independently of ER [5]. Tavassoli et al. have reported the dual effect of proliferation prohibition by TAM action, irrespective of ER. TAM has induced p53-independent cell-cycles arrest at the G1 phase but has caused up-regulation of cyclin dependent kinase inhibitors p15/INK4b, p27/Kip-1, p21/WAF-1 activity and RB hypophosphorylation. P53 independent apoptosis induction might, potentially, be an important anticancer mechanism since it is cisplatin which induces p53 independent apoptosis processes [26]. Zhou et al. have reported ERß expression down-regulation on the BG-1 (ATCC) cell line in the course of TAM exposure [14].

The present results demonstrated that cancer cell incubation with TAM changes ERß expression. Our data shows that upon exposure to the 0.5 mM of TAM in cell line EFO 21 ERß down-regulation was observed and in the cell line CAOV-3, ES-2 and PA-1 its up-regulation were noticed. We have not observed ERα expression in the course of TAM exposure in the investigated cell lines. Bearing in mind considerable TAM differences in IC_{50} and related to them various cisplatin sensitivity, not to mention, the absence of correlation between ERα and ERß expression after cell lines TAM exposure results, we claim the necessity of seeking some TAM predicting factors, using DNA microarrays. It might allow us to distinguish the group of ovarian cancer patients who may benefit from TAM therapy

References

1. Greenlee R, Murray T, Bolden S, [et al.]. Cancer statistics, 2000. *Ca Cancer J Clin.* 2000, 50, 7-33.
2. Hensley M. Epithelial ovarian cancer. *Curr Treat Options Oncol.* 2002, 3, 131-141.
3. Cannistra S Cancer of the ovary. *N Engl J Med.* 2004, 351, 2519-2529.
4. Surowiak P, Materna V, Györffy B, Matkowski R, [et al.]. Multivariate analysis of oestrogen receptor alpha, pS2, metallothionein and CD24 expression in invasive breast cancers. *Br J Cancer.* 2006, 95, 339-346.
5. Mabuchi S, Ohmichi M, Kimura A, [et al.]. Tamoxifen inhibits cell proliferation via miogen-activated protein kinase cascades in human ovarian cancer cell lines in a manner not dependent on the expression of estrogen receptor or the sensitivity to cisplatin. *Endocrinology.* 2004, 145, 1302-1313.
6. Shen L-Z, Hua Y-B, Yu X-M, [et al.]. Tamoxifen can reverse multidrug resistance of colorectal carcinoma in vivo. *World J Gastroenterol.* 2005, 11, 1060-1064.
7. Markman M, Webster K, Zanotti K, [et al.]. Phase 2 trial of carboplatin plus tamoxifen in platinum-resistant ovarian cancer and primary carcinoma of the peritoneum. *Gynecol Oncol.* 2004, 94, 404-408.
8. Benedetti Panici P, Greggi S, Amoroso M, [et al.]. A combination of platinum and tamoxifen in advanced ovarian cancer failing platinum-based chemotherapy: results of a Phase II study. *Int J Gynecol Cancer.* 2001, 11, 438-444.
9. Materna V, Liedert B, Homale J, [et al.]. Protection of platinum-DNA adduct formation and reversal of cisplatin resistance by anti-MRP2 hammerhead ribozymes in human cancer cells. *Int J Cancer.* 2005, 115, 393-402.
10. Materna V, Stege A, Surowiak P, [et al.]. RNA interference-triggered reversal of ABCC2-dependent cisplatin resistance in human cancer cells. *Biochem Biophys Res Commun.* 2006, 348, 153-157.
11. Kowalski P, Surowiak P, Lage H. Reversal of different drug-resistant phenotypes by an autocatalytic multitarget multiribozyme directed against the transcripts of the ABC transporters MDR1/P-gp, MRP2, and BCRP. *Mol Ther.* 2005, 11, 508-522.
12. Surowiak P, Materna V, Kaplenko I, [et al.]. ABCC2 (MRP2, cMOAT) can be localized in the nuclear membrane of ovarian carcinomas and correlates with resistance to cisplatin and clinical outcome. *Clin Cancer Res.* 2006, 12, 7149-7158.
13. Györffy B, Surowiak P, Kiesslich O, [et al.]. Gene expression profiling of 30 cancer cell lines predicts resistance towards 11 anticancer drugs at clinically adviered concentrations. *Int J Cancer.* 2006, 118, 1699-1712.
14. Zhou R, Treeck O, Horn F, [et al.]. Effects of prolonged tamoxifen treatment on receptor expression and apoptosis of ovarian cancer cells. *Gynecol Oncol.* 2005, 96, 678-683.
15. Scambia G, Ranelletti F, Benedetti-Panici P, [et al.]. Synergistic antiproliferative activity of tamoxifen and cisplatin on primary ovarian tumors. *Eur J Cancer.* 1992, 28A, 1885-1889.
16. Ahlgren J, Ellison N, Gottlieb R, [et al.]. Hormonal palliation of chemoresistant ovarian cancer: three consecutive phase II trials of the Mid-Atlantic Oncology Program. *J Clin Oncol.* 1993, 11, 1957-1968.
17. Glavind K, Grove A. Estrogen and progesterone receptors in epithelial ovarian tumors. *APIMS.* 1990, 98 ,916-920.
18. Brandenberger A, Tee M, Jaffe R. Estrogen receptor alpha (ER-alpha) and beta (ER-beta) mRNAs in normal ovary, ovarian serous cystadenocarcinoma and ovarian cancer cell lines: down-regulation of ER-beta in neoplastic tissues. *J Clin Endocrinol Metab.* 1998, 83, 1025-1028.
19. Li A, Baldwin R, Karlan B. Estrogen and progesterone receptor subtype expression in normal and malignant ovarian epithelial cell cultures. *Am J Obstet Gynecol.* 2003, 189, 22-27.
20. Lazennec G, Bresson D, Lucas A, [et al.]. ER beta inhibits proliferation and invasion of breast cancer cells. *Endocrinology.* 2001, 142, 4120-4130.
21. Williams C. Tamoxifen for relapse of ovarian cancer. *Cohrane Database Syst Rev.* 2001, CD001034.
22. Hatch K, Beecham J, Bleesing J, [et al.]. Responsiveness of patients with advanced ovarian carcinoma to tamoxifen. A Gynecologic Oncology Group Study of second-line therapy in 105 patients. *Cancer.* 1991, 68, 269-271.
23. Schwartz P, Chambers J, Kohorn E, [et al.]. Tamoxifen in combination with cytotoxic chemotherapy in advanced epithelial ovarian cancer. A prospective randomized trial. *Cancer.* 1989, 63, 1074-1078.
24. Colombo N, Gore M. Treatment of recurrent ovarian cancer relapsing 6-12 months post platinum-based chemotherapy. *Crit Rev Oncol Hematol.* 2007, 64, 129-138.
25. Markman M, Webster K, Zanotti K, [et al.]. Use of tamoxifen in asymptomatic patients with recurrent small-volume ovarian cancer. *Gynecol Oncol.* 2004, 93, 390-393.
26. Tavassoli M, Soltaninia J, Rudnicka J, [et al.]. Tamoxifen inhibits the growth of head and neck cancer cells and sensitizes these cells to cisplatin induced-apoptosis: role of TGF-beta1. *Carcinogenesis.* 2002, 23, 1569-1575.

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