Increased expression of GRP94 protein is associated with decreased sensitivity to adriamycin in ovarian carcinoma cell lines

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Summary

Purpose: This study examined the involvement of glucose regulated protein 94 (GRP94) in chemotherapy-resistance in human ovarian cancer cells. *Methods:* Three human ovarian cancer cells were examined for basal levels of GRP94 mRNA by RT-PCR and protein by Western blotting. Sensitivities to adriamycin of these cell lines were determined by means of MTT assay. The suppression of GRP94 expression was performed using specific siRNA in HO-8910PM cells, and cell apoptosis was assessed by flow cytometry. One-way ANOVA and the Student-Newman-Keuls test were used to determine which were significantly different. *Results:* HO-8910PM cells, with the highest basal levels of GRP94, exhibited the lowest sensitivity to adriamycin. In HO-8910PM cells, the sensitivity to adriamycin was increased when the GRP94 gene was disturbed by specific siRNA transfection. *Conclusions*: High GRP94 expression might be one of the molecular mechanisms causing resistance to adriamycin, and therefore GRP94 siRNA maybe useful in tumor-specific gene therapy in ovarian cancer.

Key words: Glucose regulated protein 94; Adriamycin-resistance; Human ovarian cancer cells; siRNA.

Introduction

Chemotherapy plays a very important role in the treatment of epithelial ovarian cancer. However drug-resistance seriously affects the effect of treatment and survival rate. Current approaches to the treatment of ovarian cancer are limited because of the development of the resistance to chemotherapy [1]. GRP94 is a possible candidate protein that contributes to development of drug resistance, which could be targeted in neoplastic cells [2]. Silencing of GRP94 by small interfering RNA (siRNA) could induce cell apoptosis [2]. Our study was designed to determine the role of GRP94 in regulating cellular apoptosis in ovarian cancer cells. Furthermore, silencing of GRP94 gene expression may prove to be a valuable therapeutic approach for chemoresistant ovarian cancer by increasing sensitivity of cancer cells to apoptosis.

The glucose regulated proteins (GRPs) were first described as a set of proteins whose synthesis was enhanced when mammalian cells were deprived of glucose [3]. These proteins are ubiquitously expressed and are located in the endoplasmic reticulum (ER). Because of their ability to assist in protein folding and assembly, the GRPs are referred to as molecular chaperones [4]. GRP94 has a molecular weight of 94 kDa and is one of the most common members of the GRP family [5, 6]. Its expression is up-regulated by so-called ER stress conditions, such as low glucose levels, low pH, viral infection, hypoxia and the expression of mutated proteins [7]. Elevated levels of GRPs appear to protect tissue culture cells against adverse physiological conditions [8]. Through multiple actions, many of which remain unidentified, GRPs contribute to tumor proliferation and confer resistance to anti-cancer treatment [9]. From these reports, we speculated that GRP94 might be involved in drug-resistance in ovarian cancer.

In this study we used ovarian cancer cell lines to determine the levels of GRP94 and their relationship to adriamycin-resistance. Furthermore, we also examined whether decrease in GRP94 protein level by the specific small-interfering RNA (siRNA) would lead to a reduction in adriamycin-resistance.

Materials and Methods

Cell line and culture

HO-8910PM, $SKOV_3$ and 3AO cell lines were obtained from Scientific Research Foundation of the Second Affiliated Hospital of Harbin Medical University. The three cell lines were epithelial ovarian cancer cell lines, in which HO-8910PM is a highly metastasizing human ovarian cancer cell line and malignant degree is higher than the other two cell lines; $SKOV_3$ is a serous cystadenocarcinoma cell line and 3AO is a mucinous cystadenocarcinoma cell line. These cell lines were maintained in DMEM (Gibco, Rockville, MD) supplemented with 10% fetal bovine serum containing 100 u/ml penicilin and 100 u/ml streptomycin.

RT-PCR

We detected the basal levels of GRP94 mRNA of the three cell lines by RT-PCR. Total RNA was isolated from the three

Revised manuscript accepted for publication May 2, 2008

cell lines, respectively. The mixture in a 20 ul volume, made up of 1 ug total RNA, 0.5 ug random primers, 2 ul reverse transcription 10 x buffer, 2 ul 10 mmol/l dNTP mixture, 15 u AMV reverse transcriptase, 4 ul 25 mmol/l $MgCl_2$, 0.5 ul recombinant RNAsin ribonuclease inhibitor, and nuclease-free water to a final volume of 20 ul. The mixture was incubated at 42° for 30 min and heated at 95° for 10 min. Polymerase chain reactions were performed in 100 ul PCR buffer containing 7.5 ul 25 mmol/l $MgCl_2$, 1.8 ul 10 mmol/l dNTP mixture, 2.5 u Taq DNA polymerase, 10-20 ul first-stand cDNA reaction, 50 pmol of each primer, 9.8 ul reverse transcription 10 x buffer, and nuclease-free water to a final volume of 100 ul. β-action DNA amplification was used as the internal PCR control. The sequences of GRP94 and β-action were as follows: GRP94: 5' ACTGTTGAGGAGCCCATGGAGG3' and 5'GCTGAAGAGTCTCGCGGGAAAC3' (496bp); β-actin: 5'CTGCAATCCGAAAGAAGCTG3' and 5'ATCTTCAAACCTCCATGATG 3' (320bp). The reactive mixture was subjected to 30 cycles of PCR amplification. Each cycle included denaturation at 94° for 30 sec, annealing at 60° for 30 sec, and primer extension at 72° for 30 sec. The last extension cycle was kept at 72° for 10 min. The test was repeated three times; 5 ul of each amplification mixture was subjected to electrophoresis on 1.2% agarose gel, and DNA was visualized by ethidium bromide staining.

Western blotting

We detected the basal levels of GRP94 protein by Western blotting assay in the three human ovarian cancer cell lines. Equal amounts of total protein (20 μg) were loaded into 10% SDS-PAGE gel and transferred to PVDF membrane. The membrane was rinsed in TBS-T solution and incubated in blocking buffer (5% skim milk in TBS-T) at room temperature for 2 hr. Then the filter was incubated with specific goat anti-human GRP94 antibody (Santa Cruz, C-19) at room temperature for 1 hr. The blot was then washed with TBS-T for 15 min and the wash was repeated three times, followed by incubation with a rabbit anti-goat IgG (Sigma) secondary antibody, and the immunoreactive bands were visualized using 3,30-diaminobenzidene-4 HCl (DAB). The test was repeated three times.

MTT assay

We detected the sensitivity to adriamycin of HO-8910PM, $SKOV_3$ and 3AO by MTT assay. The three cell lines were seeded into 96-well culture plates respectively and treated with different concentrations of adriamycin for 72 hr. The cells were then incubated in 200 μl of DMEM plus 20 μl of MTT (5 mg/ml) at 37° for 4 hr. The MTT formazan reaction product was then solubilized by adding acidic isopropanol to each well, and absorbance values for each sample were measured at 546 nm using a microplate reader (Bio-Rad Laboratories). The sensitivity to adriamycin of HO-8910PM cell, psiSTRIKE™/GRP94 HO-8910PM cell and psiSTRIKE™/NC-GRP94 HO-8910PM cell was also analyzed by methyl thiazolyl tetrazolium (MTT) assay. The method was described as above.

SiRNA transfection

The small interfering RNA GRP94 mRNA (siGRP94) was prepared according to the requirement of siRNA expression vector siSTRIKE™U6. The human GRP94 gene coding sequence was chosen in GeneBank as analyzed sequence. The sense (top) and antisense (bottom) sequences of the GRP94 siRNA duplex were as follows:

5'-ACCGAGGAAGAAGAAGAAGAAATTCAAGAGATTTCTTCTTCTTCTTCCTCTTTTTC -3'

5'-TGCAGAAAAAGAGGAAGAAGAAGAAGAAGAAATCTCTTGAATTTCTTCTTCTTCTTCCT -3'.

As a nonspecific control, a NC (nonspecific control) siRNA duplex with random sequences was designed as follows:

5'-ACCGGGAAAGAAGAAGAAGAAATTCAAGAGATTTCTTCTTCTTCTTCCTCTTTTTC-3'

5'-TGCAGAAAAAGGGAAAGAAGAAGAAGAAGAAATCTCTTGAATTTCTTCTTCTTCTTCCT-3'.

Then after oligonucleotide dilution and anneal, we connected oligonucleotide and psiSTRIKE™U6 vector to form the recombination vector according to the general method, because the high basal protein level of GRP94 was correlated with an obvious decrease in sensitivity to adriamycin in the ovarian cancer HO-8910PM cell line. Thus we chose the HO-8910PM cells for siRNA to estimate the relationship between the sensitivity to adriamycin and protein level of GRP94 further. We chose the HO-8910PM cells, which were trypsinized, suspended in fresh medium (2.5 x 10^6 cells/ml), and transferred to 6 well plates (2 ml per well). After 24 hours, 4.0 ug of the recombination vector was mixed with 10 ul of lipofectamine 2000, and applied to the cells. The cells were harvested at 24 hr, 48 hr, 72 hr, respectively, after transfection. We transfected siGRP94 to the test group and transfected the nonspecific siRNA to the comparison group. As to the blank group, nothing was added. Then, we detected the expression of GRP94 mRNA by RT-PCR and GRP94 protein by Western blotting assay in the different times, respectively, and cell apoptosis assessment and MTT analysis were also performed. The method was described as above.

Cell apoptosis assessment

Cell apoptosis assessment was performed using flow cytometry by the Annexin-V-FITC kit (BD Biosciences, USA). We detected the apoptosis assessment in the HO-8910PM cells, NC- HO-8910PM cells and siRNA-transfected HO-8910PM cells after transfection at 24 hr, 48 hr and 72 hr, respectively. Cells were trypsinized and regulated to 5 x 10^5-1 x 10^6/ml. The cells were washed with cold PBS and centrifuged with 1000 r/min at 4° for 10 min three times. Then 200 ul combined buffer was added to the suspended cells and mixed gently with 5 ul Annexin-V-FITC and 5 ul PI reacted at room temperature for 15 min without light. Afterwards, 300 ul combined buffer was added. The mixture was assessed by flow cytometry (BD Biosciences, USA) immediately.

The upper left of Figure 4 (A) represents systematic error; the upper right of the figure (B) represents late apoptotic cells or necrotic cells; the lower left of the figure (C) represents normal live cells and the lower right of the figure (D) represents early apoptotic cells.

Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical analysis was performed using SPSS 15.0 statistical package. One-way ANOVA was used to determine whether there were significant differences within the groups, followed by the Student-Newman-Keuls test to determine which groups were significantly different. The value of $p < 0.05$ was considered as statistical significance.

Results

Basal levels of GRP94 mRNA in human ovarian cancer cell lines

RT-PCR analysis showed that GRP94 mRNA in the three ovarian cancer cell lines was easily detectable.

Figure 1A shows that the expression of GRP94 mRNA in the HO-8910PM cell line was significantly higher compared to the other two cell lines ($SKOV_3$ and 3AO). The relative GRP94 mRNA level in the HO-8910PM cell line was 0.67 ± 0.019, while it were 0.33 ± 0.023 and 0.33 ± 0.026, respectively in $SKOV_3$ and 3AO cell lines. There was statistical significance between the HO-8910PM cell line and $SKOV_3$, 3AO cell lines ($p < 0.001$), while there was no statistically significant differences in $SKOV_3$ and 3AO cell lines ($p > 0.05$).

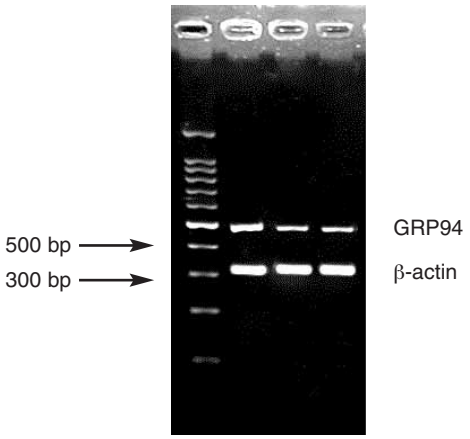

Figure 1A. — Basal levels of GRP94 mRNA in human ovarian cancer cell lines.
Lane 1: 100 bp DNA maker; Lane 2: HO-8910PM;
Lane 3: $SKOV_3$, Lane 4: 3AO

Basal levels of GRP94 protein in human ovarian cancer cell lines

The semi-quantified Western blot technique was used to detect the basal levels of GRP94 protein in three ovarian cancer cell lines. During the experiments, β-actin was found to be suitable as standard proteins for the normalization of total protein because the expression was steady in different cell lines (Figure 1B). Figure 1B shows that the expression of GRP94 protein in the HO-8910PM cell line was significantly higher than the other two cell lines ($SKOV_3$ and 3AO). The relative GRP94 protein levels of HO-8910PM, $SKOV_3$ and 3AO were 0.774 ± 0.108, 0.387 ± 0.085, 0.369 ± 0.120, respectively. From this, we can see that the expression of GRP94 in $SKOV_3$ and 3AO cell lines was nearly half compared with that of the HO-8910PM cell line.

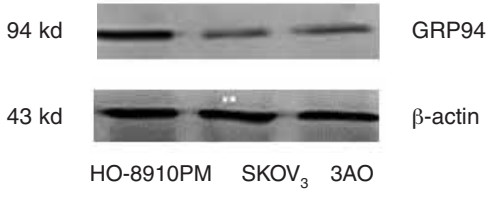

Figure 1B. — Basal levels of GRP94 protein in human ovarian cancer cell lines.

The sensitivity to adriamycin of HO-8910PM, $SKOV_3$ and 3AO

The sensitivity to adriamycin of HO-8910PM, $SKOV_3$ and 3AO was analyzed by methyl thiazolyl tetrazolium (MTT) assay. The results of MTT indicated that the survival rates of the three cell lines were gradually decreased with the gradually increased adriamycin concentration (Table 1). In the same adriamycin concentration, the survival rate of HO-8910PM cell line was significantly higher than $SKOV_3$ and 3AO ($p < 0.05$), which indicated HO-8910PM had lower sensitivity to adriamycin than the other two cell lines. From the above results, we can hypothesize that the low sensitivity to adriamycin is relative to the high expression of GRP94.

Table 1. — *Comparison of cell viability (%) among HO-8910PM, $SKOV_3$ and 3AO cells exposed to ADM till 72 hr.*

Group	Adriamycin concentration (ug/ml)						
	0	0.01	0.25	1.0	2.0	4.0	8.0
HO-8910PM	100	99.5*	92.4*	87.9*	82.4*	77.3*	71.8*
$SKOV_3$	100	75.4	70.9	66.3	61.8	58.4	52.9
3AO	100	73.8	69.6	66.5	62.9	56.8	50.3

Cell viability (%) = A_{546} (experimental group)/A_{546} (blank group) x 100%; * $p < 0.05$.

Recombination vector assessment

PsiSTRIKE™U6 vector contains a pst enzyme site and the recombination vector will form two pst enzyme sites. After the digestion of restriction pst incision enzyme, the successful recombination vector will form two DNA fragments, while the vector without oligonucleotide insertion will have only one linearity fragment in the agarose gel electrophoresis. In the agarose gel electrophoresis (Figure 2), two DNA fragments presented, which indicated the vector construction was successful.

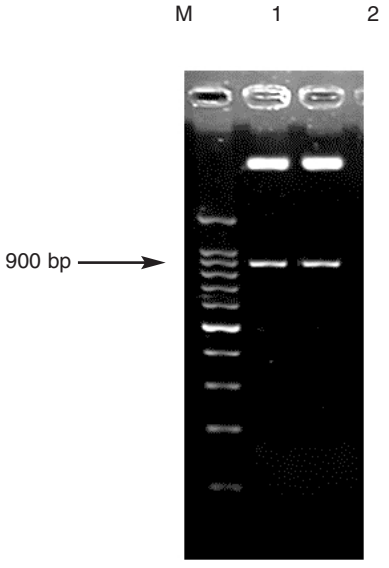

Figure 2. — GRP94 recombination vector assessment.
M: 100 bp marker; 1: psiSTRIKE™/GRP94 recombination vector; 2: psiSTRIKE™/NC-GRP94 recombination vector.

GRP94 mRNA expression in HO-8910PM cells and siRNA-transfected HO-8910PM cells

To deregulate the basal GRP94 mRNA expression, siRNA targeted to GRP94 was designed and transfected into HO-8910PM cells (psiSTRIKE™/GRP94) and non-specific siRNA was also designed (psiSTRIKE™/NC-GRP94) and used for observing the non-specific effect of siRNA transfection. As Figure 3A shows, we can see that GRP94 mRNA expression was significantly degraded after siRNA-transfection, especially at 48 hr and 72 hr, while the expression of GRP94 mRNA was not degraded significantly with the treatment of NC siRNA. The specific value is shown in Table 2. β-actin mRNA expression was invariable before and after siRNA-transfection.

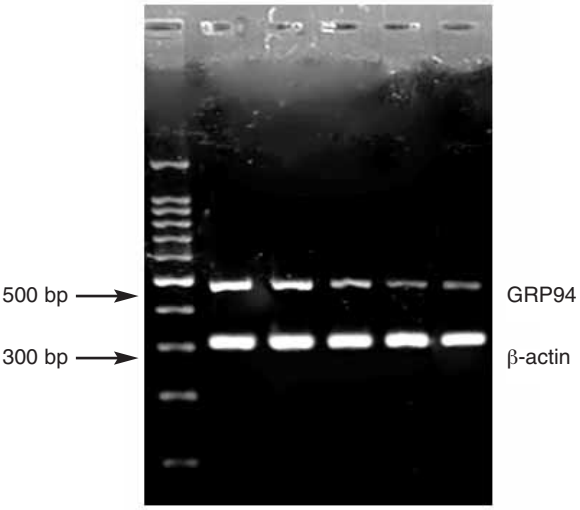

Figure 3A. — GRP94 mRNA expression in HO-8910PM cells and siRNA-transfected HO-8910PM cells.
Lane 1: 100bp DNA maker; Lane 2: HO-8910PM; Lane 3: NC siRNA-transfected HO-8910PM cells; Lane 4: siRNA-transfected HO-8910PM cells after transfection 24 hr; Lane 5: siRNA-transfected HO-8910PM cells after transfection 48 hr; Lane 6: siRNA-transfected HO-8910PM cells after transfection 72 hr.

Table 2. — *The value of GRP94 mRNA expression in HO-8910PM cells and siRNA-transfected HO-8910PM cells.*

Group		Comparative expression of mRNA	p
I	HO-8910PM cell	0.61 ± 0.16	[a]p < 0.001
II	NC- HO-8910PM cell	0.55 ± 0.40	[b]p > 0.05
III	siRNA-transfected 24 hr	0.27 ± 0.13	[c]p < 0.001
IV	siRNA-transfected 48 hr	0.13 ± 0.08	[d]p < 0.001
V	siRNA-transfected 72 hr	0.12 ± 0.03	[e]p > 0.05

a: total comparison; b: comparison between I and II; c: comparison between II and III; d: comparison between III and IV; e: comparison between IV and V.

GRP94 protein expression in HO-8910PM cells and siRNA-transfected HO-8910PM cells

The results of protein expression were similar to that of mRNA expression. We can see in Figure 3B that the expressions of HO-8910PM cells and psiSTRIKE™/NC-GRP94 HO-8910PM cells were high and the expression was gradually decreased at 24 hr, 48 hr and 72 hr after siRNA-transfection, while β-actin protein expression was invariable. The difference was significant (Table 3).

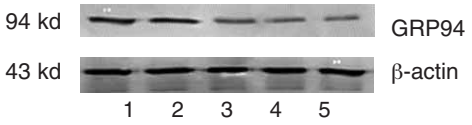

Figure 3B. — GRP94 protein expression in HO-8910PM cells and siRNA-transfected HO-8910PM cells
1: HO-8910PM cells; 2: NC siRNA-transfected HO-8910PM cells; 3: siRNA-transfected HO-8910PM cells after transfection 24 hr; 4: siRNA-transfected HO-8910PM cells after transfection 48 hr; 5: siRNA-transfected HO-8910PM cells after transfection 72 hr;

Table 3. — *The value of GRP94 protein expression in HO-8910PM cells and siRNA-transfected HO-8910PM cells.*

Group		Comparative expression of protein	p
I	HO-8910PM cell	0.765 ± 0.0151	[a]p < 0.001
II	NC- HO-8910PM cell	0.759 ± 0.0162	[b]p > 0.05
III	siRNA-transfected 24 hr	0.396 ± 0.094	[c]p < 0.001
IV	siRNA-transfected 48 hr	0.213 ± 0.0111	[d]p < 0.001
V	siRNA-transfected 72 hr	0.207 ± 0.090	[e]p > 0.05

a: total comparison; b: comparison between I and II; c: comparison between II and III; d: comparison between III and IV; e: comparison between IV and V.

Increased sensitivity to adriamycin in GRP94 of siRNA-transfected HO-8910PM cells

The sensitivities to adriamycin of HO-8910PM cells, psiSTRIKE™/GRP94 HO-8910PM cell and psiSTRIKE™/NC-GRP94 HO-8910PM cells were analyzed by methyl thiazolyl tetrazolium (MTT) assay. The results of MTT indicated that survival rates of the three groups were gradually decreased with the gradually increased adriamycin concentration, but the degree of decrease was different; the psiSTRIKE™/GRP94 HO-8910PM cells decreased significantly (Table 4). With the same adriamycin concentration, the survival rate of psiSTRIKE™/GRP94 HO-8910PM cells was significantly lower than HO-8910PM cells and psiSTRIKE™/NC-GRP94 HO-8910PM cells ($p < 0.05$). The results indicated that psiSTRIKE™/GRP94 HO-8910PM cells had higher sensitivity to adriamycin than the other two groups. The sensitivity to adriamycin of HO-8910PM cells before and after siRNA transfection was changed dramatically.

Table 4. — *Comparison of cell viability (%) among HO-8910PM, NC-HO-8910PM and Si-HO-8910PM cells exposed to ADM till 72 hr.*

Group	Adriamycin concentration (ug/ml)						
	0	0.01	0.25	1.0	2.0	4.0	8.0
HO-8910PM	100	99.4	93.1	87.4	81.9	75.6	71.3
NC-HO-8910PM	100	98.7	93.2	86.3	81.5	74.8	69.4
Si-HO-8910PM	100	60.6*	52.2*	43.9*	34.7*	27.5*	21.9*

Cell viability (%) = A_{546} (experimental group)/A_{546} (blank group) x 100%; *p < 0.05.

Cell apoptosis assess in GRP94 of siRNA-transfected HO-8910PM cells

In the psiSTRIKE™/GRP94 HO-8910PM cells, the apoptosis ratio was gradually increased with transfection and the apoptosis ratio at 72 hr after transfection was the highest reaching 22.72 ± 0.94. The change of apoptosis ratio in the psiSTRIKE™/NC-GRP94 group and blank group was not obvious. The difference between the psiSTRIKE™/GRP94 HO-8910PM group and psiSTRIKE™/NC-GRP94 group, and the blank group had statistical significance, respectively ($p < 0.01$), while the difference between the psiSTRIKE™/NC-GRP94 group and blank group had no statistical significance ($p > 0.05$). GRP94 siRNA can obviously induce apoptosis (Figure 4, Table 5).

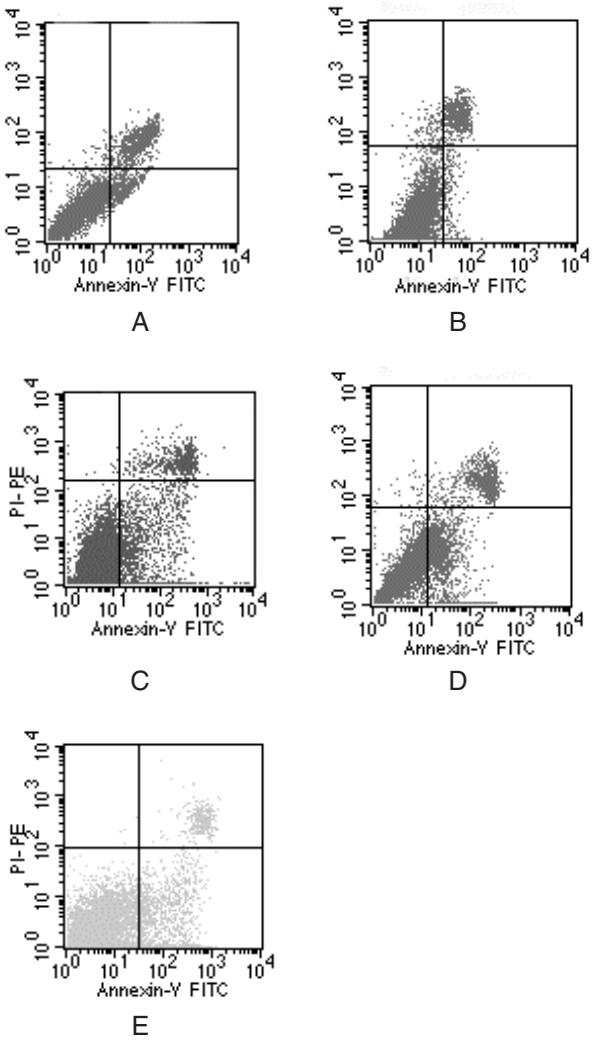

Figure 4. — Cell apoptosis.
A: HO-8910PM cells;
B: NC- HO-8910PM cells;
C: siRNA-transfected HO-8910PM cells after transfection 24 hr;
D: siRNA-transfected HO-8910PM cells after transfection 48 hr;
E: siRNA-transfected HO-8910PM cells after transfection 72 hr.

Table 5. — *Cell apoptosis ratio.*

Group		Cell apoptosis ratio	p
I	HO-8910PM cell	6.12 ± 0.095	[a]$p < 0.001$
II	NC- HO-8910PM cell	6.19 ± 0.085	[b]$p > 0.05$
III	siRNA-transfected 24 hr	13.59 ± 0.065	[c]$p < 0.001$
IV	siRNA-transfected 48 hr	21.77 ± 0.935	[d]$p < 0.001$
V	siRNA-transfected 72 hr	22.72 ± 0.94	[e]$p > 0.05$

a: total comparison; b: comparison between I and II; c: comparison between II and III; d: comparison between III and IV; e: comparison between IV and V.

Discussion

Mammalian stress response is an evolutionarily conserved mechanism, which can induce cells to respond to stimulates from adverse environmental or metabolic conditions [10]. This response is represented at the molecular level by the induced synthesis of specific sets of cellular proteins with protective functions. The most functional proteins are glucose-regulated proteins (GRPs) and the most important place in cells to produce such proteins is the endoplasmic reticulum (ER) [11]. GRP94 is one of the most important members of GRPs. In a variety of cancer cell lines, solid tumors and human cancer biopsies, the levels of GRP94 are elevated, correlating with malignancy.

The glucose-regulated protein (GRP) system in mammalian cells is induced by glucose deprivation, anoxia, calcium ionophore A23187, and 2-deoxyglucose [12]. Since a glucose-regulated response is produced by hypoxia and nutrient deprivation that occurs naturally in solid tumors, the resistance observed here can occur in some solid tumors and can be an obstacle to chemotherapy [13].

GRP94 expression is known to be up-regulated by viral infections and other ER stresses [14]. It has also been reported that GRP94 was an effective protein in vaccination against cancer cell proliferation [15, 16]. GRP94 has also been shown to be over-expressed in some cancer cell lines and human tumor samples, and to be correlated with malignancy [17-19]. In addition, GRP94 overexpression could be closely related to increased tumor cell survival resulting in resistance to cancer treatment. Some reports suggested that resistance to hyperoxgen - or thermotherapy and chemotherapy might be related to GRP94.

The induction of glucose-regulated proteins by a variety of specific inducers leads to an increase in resistance to adriamycin [12].

For example, GRP94 inhibited etoposide-induced apoptosis, which is dependent on Ca^{2+}, in a human T cell leukemia line, Jurkat, and a hamster fibroblast line, K12 [20], while the induction of GRP94 inhibited apoptosis of esophageal cancer cells induced by reactive oxygen stress [19].

According to recent researches, overexpression of GRP94 was associated with many malignant tumors, such as lung cancer, esophagus cancer and so on [21, 22]. Wang *et al's.* research indicated that GRP94 was related to the differentiation and progression of lung cancer, and the expression in mRNA and protein level may be valuable in evaluating the grade of differentiation and clinical stage of human lung cancer [23]. Another research also

showed that the majority of esophageal cancer expressed GRP94, which differed significantly from that in adjacent normal tissue [24]. A recent study suggests that higher GRP94 protein expression is one of the molecular mechanisms causing resistance to radiation, and therefore GRP94 siRNA might be useful in tumor-specific gene therapy by reversing radio-resistance prior to radiation in cervical cancer [25]. As to ovarian cancer, there is no systemic research on the expression of GRP94 and the relationship of drug-resistance thus far.

In our study, we have found that basal expression of GRP94 mRNA and protein existed in three human ovarian cell lines (HO-8910PM, $SKOV_3$, 3AO), and the expression in the HO-8910PM cell line was obviously higher than the other two cell lines. This result was associated with the malignant degree of cells. The malignant degree of HO-8910PM was higher than the other two cell lines and is a kind of high metastasis cell line. And high basal levels of GRP94 expression were related with an obvious decrease in sensitivity to adriamycin chemotherapy. In addition, when GRP94 expression was inhibited specifically by siRNA in the HO-8910PM cell line, sensitivity to adriamycin chemotherapy was increased dramatically. This result suggests a strong correlation between GRP94 expression levels and resistance to adriamycin-chemotherapy. This abnormal elevation could be attributed to glucose deprivation, oxidative stress, and hypoxia in ovarian cancer cells. Such situation may induce ER stress, and GRP94 may be produced.

As a therapy target, GRP94 has been used to treat many cancers in past years. In one study on breast carcinomas, GRP94 has been shown to associate with and stabilize p185/erbB2, which is commonly overexpressed and associated with a poor prognosis. Disrupting the GRP94-p185 complex by using an antiproliferative agent, called geldanamycin, enables degradation of p185 in breast cancer cells [24]. Antisense knockdown strategies could suppress the expression levels of GRP94, which results in enhanced sensitivity to etoposide-induced cell death [27, 28]. The enhanced sensitivity could trigger proteolytic cleavage of GRP94 by calpain, which also cleaves Bcl-xl during apoptosis, therefore turning an anti-apoptotic protein into a pro-apoptotic molecule [29]. GRP94 could also be used in immunotherapy [30]. In a recent study, Liu *et al's.* results indicated that combined GRP94-based immunotherapy and radiation therapy may be a potentially effective strategy for cancer treatment [31]. Our results showed that the siRNA of the GRP94 reversed chemotherapy sensitivity in vitro thus providing further evidence for the notion that GRP94 specific RNA interference might be a viable approach to ovarian cancer chemotherapy treatment. Moreover, ovarian cancers with higher levels of GRP94 mRNA and protein, treated with siRNA, could be treated with a lower chemotherapy dose to reduce the adverse reaction of drugs. We further suggest that it may be a possible therapeutic agent to improve clinical outcome of ovarian cancer. The success of such an approach, however, still awaits the development of an efficient delivery system that will affect most tumor cells in vitro.

Acknowledgements

This work was supported in part by grants from the Scientific Research Foundation, Second Affiliated Hospital of Harbin Medical University.

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