Biochemical and Biophysical Research Communications 435 (2013) 188–194

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Silencing of the STAT3 signaling pathway reverses the inherent and induced chemoresistance of human ovarian cancer cells

Zhiqiang Han [a,1], Jing Feng [a,1], Zhenya Hong [a], Lijuan Chen [b], Wei Li [a], Shujie Liao [a], Xiaoli Wang [a], Teng Ji [a], Shixuan Wang [a], Ding Ma [a], Gang Chen [a,*], Qinglei Gao [a,*]

[a] Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, PR China
[b] Department of Gynecology and Obstetrics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, PR China

ARTICLE INFO

Article history:
Received 24 April 2013
Available online 7 May 2013

Keywords:
STAT3
Signaling pathway
Chemoresistance
Ovarian cancer

ABSTRACT

Ovarian cancer is the leading cause of gynecologic cancer deaths among women. Although platinum-based chemotherapy is the first-line treatment for human ovarian cancer, chemoresistance remains a major obstacle to successful treatment, and there are currently no approved molecularly targeted therapies. Recent evidence indicates that signal transducer and activator of transcription-3 (STAT3) is a determinant of chemoresistance and is related to tumor recurrence in a large number of solid malignancies. In this study, we demonstrated that high levels of pSTAT3 were associated with chemoresistance in human ovarian cancer cells. Targeting STAT3 by siRNA technology markedly enhanced cisplatin-induced apoptosis in cisplatin-resistant ovarian cancer cells that expressed a high level of pSTAT3. Interleukin-6 (IL-6) could induce STAT3 activation in cisplatin-sensitive ovarian cancer cells and led to protection against cisplatin. The STAT3 siRNA treatment also blocked IL-6-induced STAT3 phosphorylation, resulting in the attenuation of the anti-apoptotic activity of IL-6. We found that the combination of cisplatin and STAT3 siRNA resulted in the collapse of the mitochondrial membrane potential, attenuated the expression of Bcl-xL and Bcl-2, and increased the release of cytochrome C and expression of Bax. Taken together, these results suggest that the pharmacological inhibition of STAT3 may be a promising therapeutic strategy for the management of chemoresistance in ovarian cancer.

1. Background

Ovarian cancer is the fourth most lethal cancer among women and the leading cause of gynecological cancer deaths worldwide. Despite improvement in 5-year survival, long-term survival for advanced-stage ovarian cancer has not changed and is still no more than 20–30% [1]. Patients usually respond to the initial therapy, which includes surgical debulking and chemotherapy with platinum-based drugs and taxanes. Most survivors of ovarian cancer eventually experience recurrent disease and develop resistance to multiple types of chemotherapy. Progression of a multidrug-resistant tumor ultimately leads to a significant morbidity and eventual mortality. Indeed, drug resistance remains one of the most difficult barriers to overcome in patients with ovarian cancer. Moreover, there are currently no approved molecularly targeted therapies.

Signal transducer and activator of transcription-3 (STAT3) is one member of a family of transcription factors that participate in relaying signals from cytokines and growth factors. Upon activation by a wide variety of cell surface receptors, tyrosine-phosphorylated STAT3 (pSTAT3) dimerizes and translocates to the nucleus to modulate the expression of the target genes that are involved in various physiologic functions, including cell development, differentiation, proliferation and survival [2]. In normal cells, the activation of the STAT3 protein is a reversible and tightly controlled process that typically lasts for a limited duration [2], and studies have shown that the constitutive activation or dysregulation of STAT3 is associated with a number of human tumors and cancer cell lines [2]. Using gene expression profiling and comparative genomic hybridization, Meinhold-Heerlein and colleagues [3] demonstrated that high-grade ovarian cancers were characterized by the expression of genes associated with STAT3-induced transcription. Studies have also shown that pSTAT3 is correlated with aggressive clinical behavior in ovarian carcinoma specimens [4,5].

Several upstream pathways have been identified in drug resistance, including the interleukin (IL)-6, Src family kinase, and phosphatidylinositol-3-kinase (PI3K)/Akt pathways. The above pathways have been shown to be elevated in ovarian cancer, leading to the activation of STAT3 [6–8]. Such studies have shown that STAT3 prevents tumor cell cycle arrest and cell death through multiple mechanisms that were previously linked to drug

* Corresponding authors. Fax: +86 27 83662681.
E-mail addresses: qlgao@tjh.tjmu.edu.cn (Q. Gao), gumpc@126.com (G. Chen).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.04.087

resistance. These mechanisms include the increased expression of the oncogenes encoding cyclin D and c-Myc and the anti-apoptotic proteins MCL-1 (myeloid cell leukemia-1), survivin, and Bcl-xL [9].

In the present study, we hypothesized that the STAT3 signaling pathway was instrumental in human ovarian cancer chemoresistance and that the targeted disruption of this pathway could attenuate the inherent and induced chemoresistance of human ovarian cancer cells. We used siRNA (small interfering RNA) to downregulate STAT3 expression in human ovarian cancer cells, and our results confirmed the critical role of STAT3 in the resistance to cisplatin, which provided direct evidence that targeting STAT3 signaling could reverse inherent and induced cisplatin resistance.

2. Methods

2.1. Cell culture

The human ovarian cancer cell lines SKOV-3 and A2780 were purchased from American Type Culture Collection (Rockville, MD, USA), and OV2008 and C13K were gifts from Dr. Rakesh Goel (Ottawa Regional Cancer Center, Ottawa, Canada). The cells were cultured in RPMI 1640 (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Human umbilical vein endothelial cells (HUVECs) were isolated as previously described [10].

2.2. Cell transfection

Exponential-phase ovarian cancer cells were plated into 6-well plates and allowed to adhere for 24 h. When the cell density reached approximately 70%, the cells were transfected with 50 nM STAT3 siRNA using Lipofectamine™ 2000 (Life Technologies, Rockville, MD, USA) following manufacturer's instructions. siRNA against human STAT3 (5′-GGA GCA GCA CCU UCA GGA UTT-3′) was synthesized according to the published sequence [11], and the non-targeting control siRNA (5′-UAC CGA CUA AAG ACA UCA AUU-3′) was purified by high-performance liquid chromatography (HPLC). The RNA oligonucleotides were obtained from Invitrogen (Shanghai, PR China).

2.3. Real-time PCR

Total RNA was isolated from the cells and treated with DNase I for 30 min at 37 °C, and RT-PCR was performed using the SYBR Premix Ex Taq Kit (TaKaRa Biotechnology Co., Ltd, Dalian, China). STAT3 expression in the experimental and control groups was analyzed using the $2^{-\Delta\Delta CT}$ method [12]. The primers used for real-time PCR are as follows: STAT3 – forward 5′-ACCTGCAGCAATACCATTGAC-3′ and reverse 5′-AAGGTGAGGGACTCAAACTGC-3′ and GAPDH – forward 5′-CCACTCCTCCACCTTTGAC-3′ and reverse 5′-ACCCTGTTGCTGTAGCCA-3′.

2.4. Western blot analysis

A standard western blot analysis was performed using anti-STAT3, anti-phospho-STAT3 (Tyr-705), anti-Bcl-2, anti-Bcl-xL, anti-Bax (Cell Signaling Technology, Inc., Beverly, MA), anti-β-actin, and anti-cytochrome C (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. The probe proteins were detected using enhanced chemiluminescence (Pierce Biotechnology/Thermo Fisher Scientific, Rockford, IL).

2.5. Drug sensitivity assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to analyze cell viability, as previously described [13].

2.6. Apoptosis assay

Annexin V-FITC and PI staining (BD Biosciences Pharmingen, San Diego, CA, USA) were used to determine apoptosis according to our previously described methods [14].

2.7. Measurement of $\Delta\Psi m$

Mitochondrial membrane depolarization was determined using the fluorescent probe TMRE [15]. Briefly, cells (1×10^6/ml) were incubated with TMRE (37 °C for 10 min) in the dark and analyzed by flow cytometry. Additionally, $\Delta\Psi m$ was observed by fluorescence microscopy, as previously described [16].

2.8. Statistical analysis

All the values represent the means ± SD of at least three independent experiments. The significance of the differences between the groups was assessed by Student's *t* test; statistical significance was defined as $P < 0.05$.

3. Results

To examine the cisplatin antitumor activity in different ovarian cancer cells, four human ovarian cancer cell lines (C13K, SKOV-3, A2780, and OV2008) were exposed to different concentrations of cisplatin (0–80 μM); a cell viability assay was performed after 48 h of treatment. No significant cell viability changes were found in the C13K or SKOV-3 lines treated with 10 μM cisplatin, whereas approximately 20% and 40% decreases, respectively, were observed when these cells were treated with 40 μM cisplatin (Supplementary Fig. 1A). In contrast, cell viability decreased by approximately 40% and 90% in the OV2008 lines and by 30% and 85% in the A2780 lines when treated with 10 and 40 μM cisplatin, respectively (Supplementary Fig. 1A).

We also observed that cisplatin (20 μM) treatment decreased the viability of all the cells in a time-dependent manner. With increasing time, the viability of OV2008 and A2780 was significantly decreased when compared to C13K and SKOV-3 (Supplementary Fig. 1B). These results demonstrated that the C13K and SKOV-3 cells were more resistant to cisplatin than the OV2008 and A2780 cells.

Increased anti-apoptotic activity is one of the major mechanisms of cisplatin resistance [17]. On the basis of the critical role of STAT3 in cancer cell proliferation and survival [2], we compared the expression profiles of endogenous STAT3 and pSTAT3 in cisplatin-resistant ovarian cancer cells with those of cisplatin-sensitive ovarian cancer cells and normal human umbilical vein endothelial cells (HUVECs). A western blot analysis showed that high levels of STAT3 were expressed in all of the ovarian cancer cell lines, with no significant differences in the levels of total STAT3 protein. However, the level of pSTAT3 in the C13K and SKOV-3 cells was notably higher than in the OV2008, A2780, and HUVEC lines (Supplementary Fig. 1C).

Based on the above results, we hypothesized that the STAT3 signaling pathway plays a vital role in the chemoresistance of ovarian cancer cells and that the inhibition of this pathway would lower the apoptotic threshold and increase chemotherapy sensitivity. To test this hypothesis, we first performed STAT3-targeted

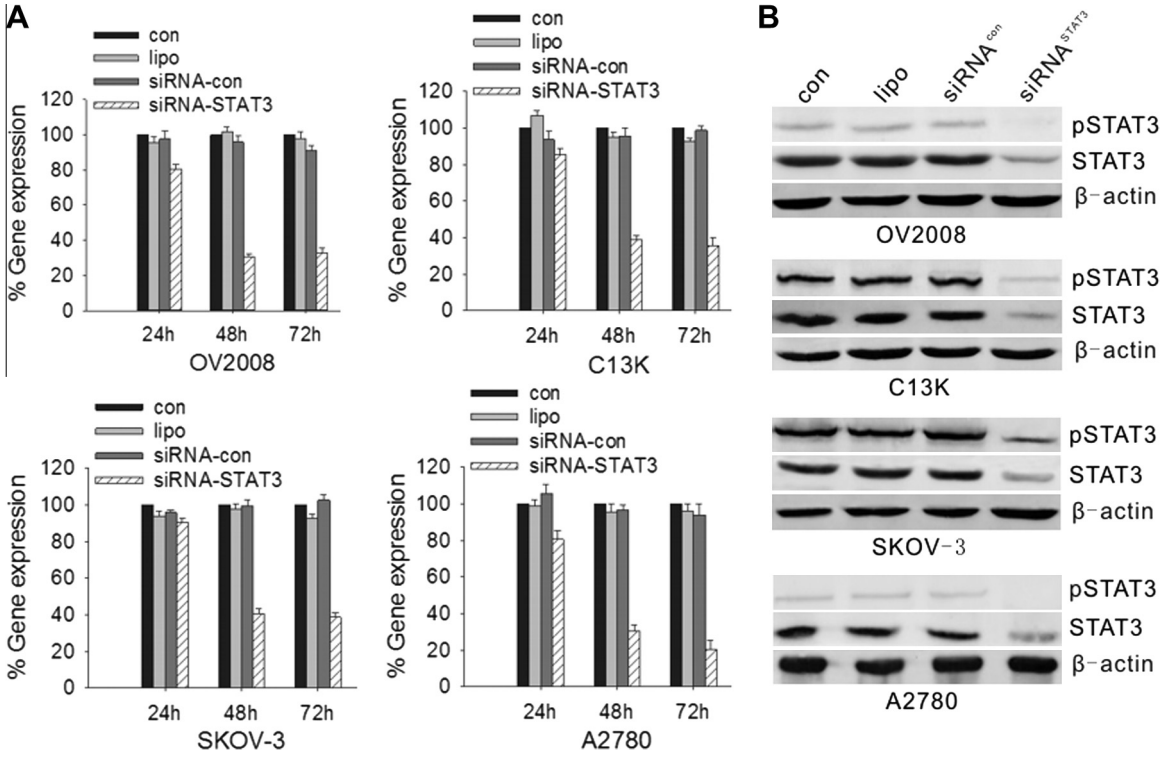

Fig. 1. Real-time PCR and western blot analysis of STAT3 and pSTAT3 expression in ovarian cancer cells transfected with STAT3 siRNA. (A) Real-time PCR examination at the indicated times of STAT3 in OV2008, C13K, SKOV-3, and A2780 cells transfected with STAT3 siRNA. The columns and error bars represent the means and SD. (B) Western blot analysis of STAT3 and pSTAT3 in OV2008, C13K, SKOV-3, and A2780 cells transfected with STAT3 siRNA ($siRNA^{STAT3}$) and a non-targeting control siRNA ($siRNA^{con}$) after 72 h.

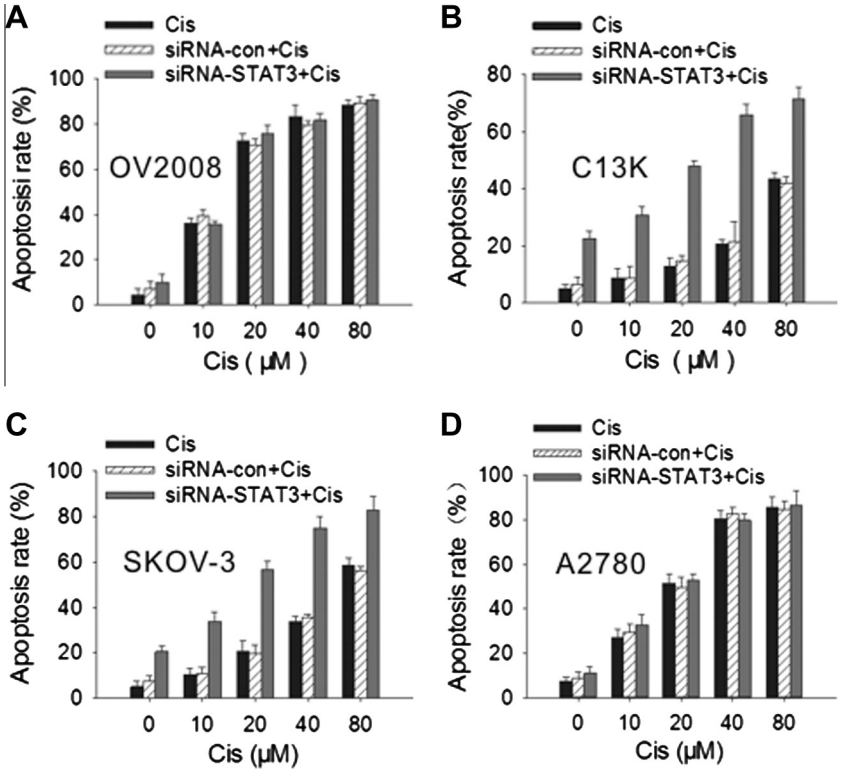

Fig. 2. Silencing the STAT3 signaling pathway enhanced the sensitivity of cisplatin-resistant ovarian cancer cells to cisplatin. OV2008 (A), C13K (B), SKOV-3 (C), and A2780 (D) cells were transfected with STAT3 siRNA for 24 h and then treated with different doses of cisplatin (0–80 μM) for 48 h. The rate of apoptosis was measured by FITC-annexin V and PI staining. The columns and error bars represent the means and SD. Cis, cisplatin.

Table 1
Effect of STAT3 siRNA on cisplatin-induced cytotoxicity in Ov2008, Caov-3, C13K, SKOV-3, or A2780 cells.

	IC50 (μM)		
	Cisplatin	$siRNA^{con}$ + cisplatin	$siRNA^{STAT3}$ + ciplatin
OV2008	14 ± 2	12 ± 3	13 ± 1
C13K	80 ± 9	76 ± 5	23 ± 3
SKOV-3	58 ± 5	56 ± 3	18 ± 2
A2780	19 ± 3	20 ± 2	17 ± 2

silencing using siRNA, and our real-time PCR analysis showed significant reductions in the levels of STAT3 mRNA expression in all the ovarian cancer cells (Fig. 1A). Furthermore, our western blot analysis showed that the STAT3 and pSTAT3 (try-705) proteins were also efficiently inhibited (Fig. 1B). To minimize off-target effects of siRNA, we used three non-overlapping siRNAs targeting STAT3 (Supplementary Fig. 2). These results indicated that STAT3 siRNA could effectively inhibit the expression of STAT3 and pSTAT3 in these ovarian cancer cells.

We next examined whether blocking the STAT3 signaling pathway could enhance the sensitivity of ovarian cancer cells to cisplatin. The cells were first treated with STAT3 siRNA and, after a 24-h transfection period, with different doses of cisplatin for 48 h. We then analyzed the effect of STAT3 siRNA on cisplatin-induced apoptosis and found that the combination of cisplatin with STAT3 siRNA resulted in significantly greater cell death compared with cisplatin or STAT3 siRNA alone in C13K and SKOV-3 cells (Fig. 2). To investigate if a small molecule STAT3 inhibitor would increases cisplatin sensitivity, we chose 10 μM of Stattic to treat A2780 and C13K cells for 48 h. The results demonstrated cisplatin induced more apoptosis in Stattic-treated C13K cells than in control cells (Supplementary Fig. 3). Next, we assessed the changes in cisplatin sensitivity. STAT3 siRNA treatment combined with cisplatin in C13K and SKOV-3 cells resulted in IC_{50} values (23 ± 3 μM and 18 ± 2 μM, respectively) that were significantly lower than the IC_{50} values for cisplatin alone (80 ± 9 μM and 58 ± 5 μM, respectively, $P < 0.05$) (Table 1). However, this sensitivity to cisplatin was not observed in the OV2008 and A2780 cells (Fig. 2, Table 1). These data suggested that the enhanced response to cisplatin was associated with pSTAT3 depletion in the ovarian cancer cells.

Interleukin-6 (IL-6), which is secreted by a variety of cells, including the tumor cells, participates in cancer cell proliferation and differentiation [18]. IL-6 was found to be elevated in the serum and peritoneal fluid collected from patients with ovarian cancer [19,20]. Furthermore, high levels of IL-6 in body fluids were previously associated with a poor prognosis and resistance to chemotherapy [21]. Studies have proven that IL-6 activates STAT3 and regulates the behavior of cancer cells through autocrine and paracrine pathways [22]. To evaluate the role of the IL-6/STAT3 signaling pathway in ovarian cancer cell chemoresistance, we first explored whether IL-6 could induce STAT3 phosphorylation in cells

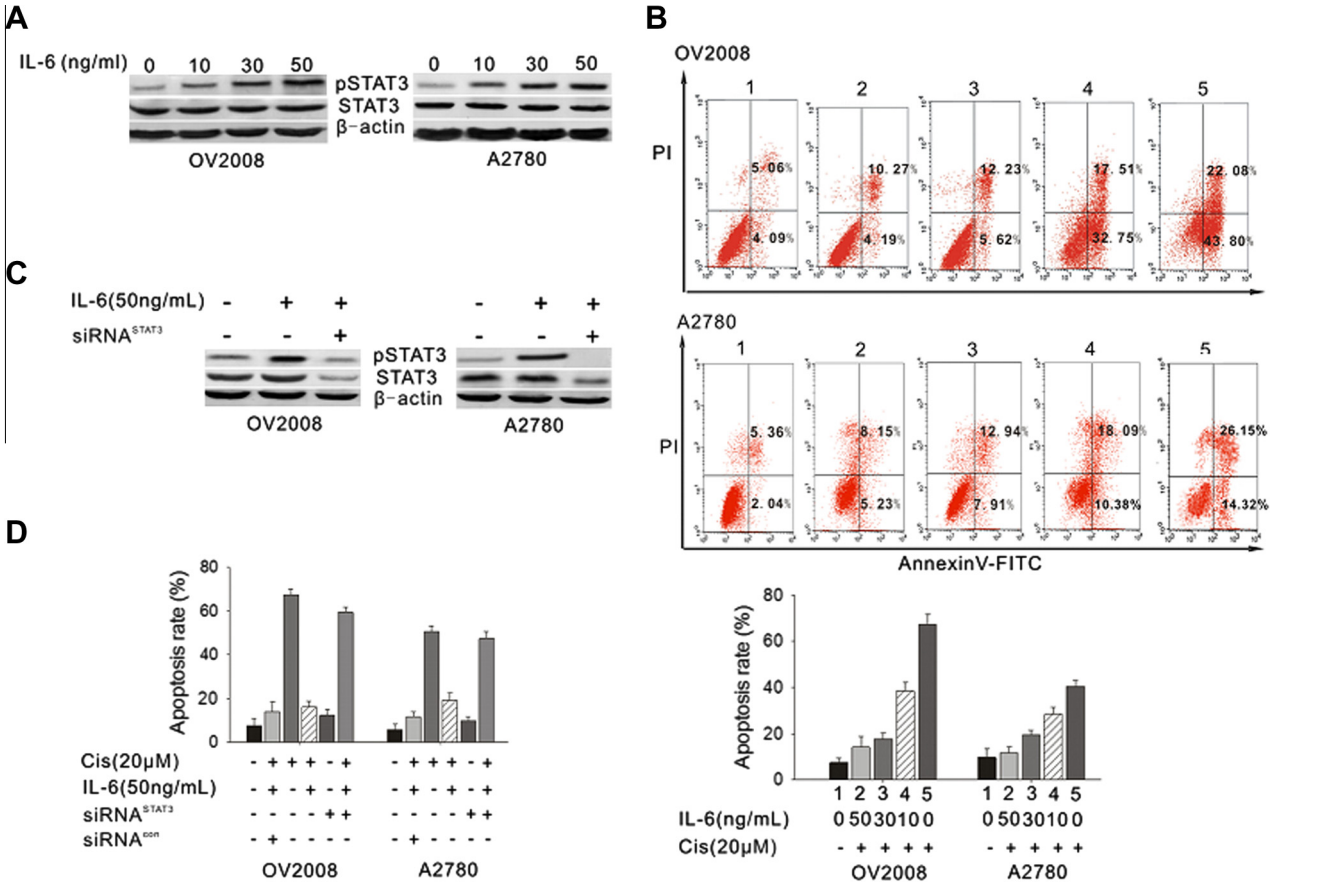

Fig. 3. STAT3 siRNA reversed the IL-6-induced resistance to cisplatin in ovarian cancer cells. (A) Western blot analysis of STAT3 and pSTAT3 in cisplatin-sensitive OV2008 and A2780 cells treated with different concentrations of IL-6 (0–50 ng/ml) for 1 h. (B) OV2008 and A2780 cells were pretreated with various concentrations of IL-6 (0–50 ng/ml) for 1 h. After the pretreatment, the cells were treated with 20 μM cisplatin for 24 h, and a cell apoptosis assay was performed. The graphs represent typical cell apoptosis results. The columns and error bars represent the means and SD. (C) Western blot analysis of STAT3 and pSTAT3 in OV2008 and A2780 cells pretreated with STAT3 siRNA for 6 h, followed by incubation with IL-6 (50 ng/ml) for 48 h. (D) OV2008 and A2780 cells were pretreated with IL-6 at 50 ng/ml for 1 h and then transfected with STAT3 siRNA (50 nM) for 48 h, followed by incubation with cisplatin (20 μM) for 24 h. At the end of the treatment period, cell apoptosis was examined. The columns and error bars represent the means and SD. Cis, cisplatin.

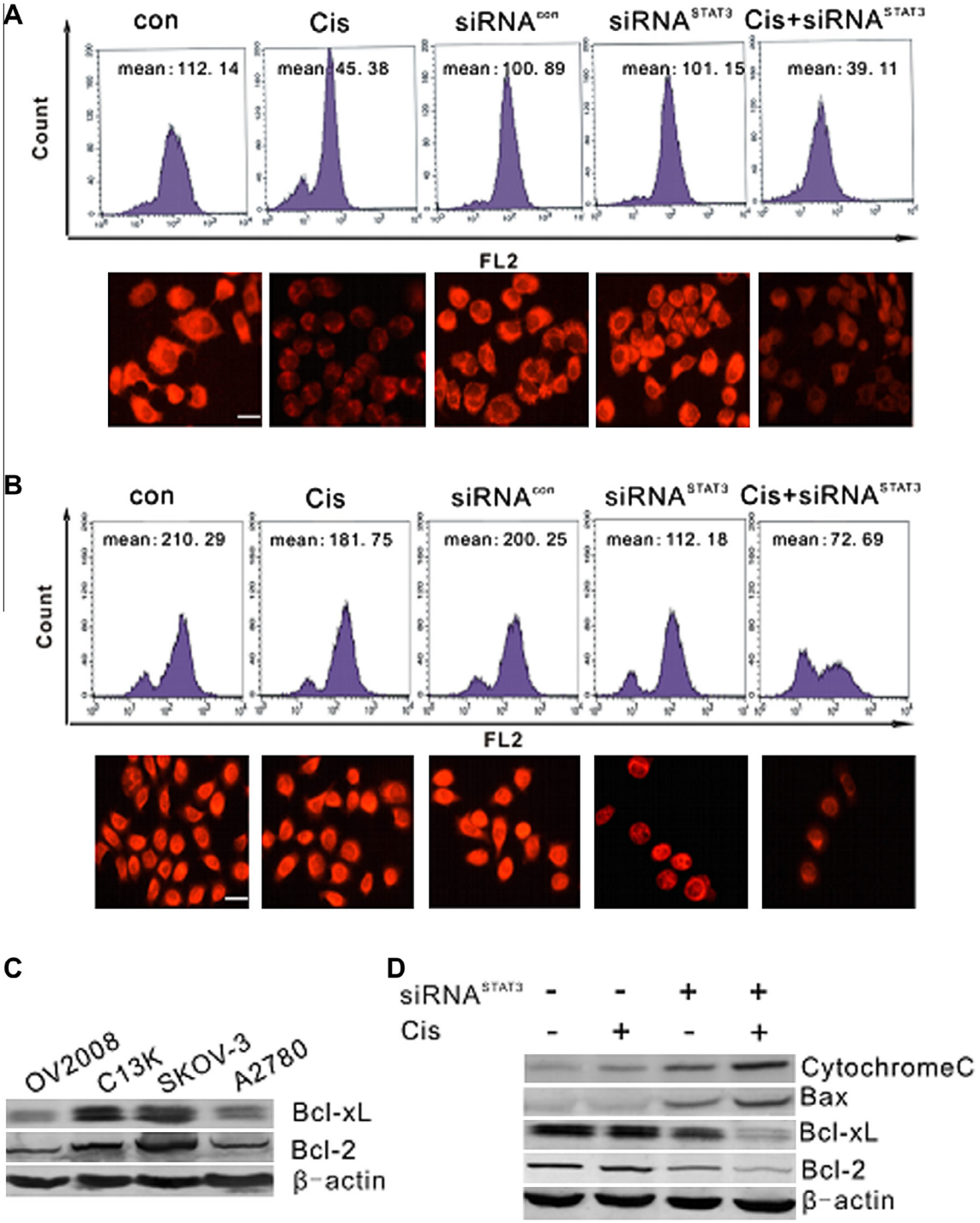

Fig. 4. STAT3 siRNA promoted cisplatin-induced mitochondrial-related apoptosis in ovarian cancer cells. OV2008 (A) and C13K (B) cells were transfected with STAT3 siRNA for 24 h, followed by incubation with 20 μM cisplatin for 24 h. After the cisplatin treatment, the loss of the mitochondrial membrane potential was measured using TMRE staining. Scale = 20 μm. (C) Western blot analysis of Bcl-2 and Bcl-xL in OV2008, C13K, SKOV-3, and A2780 cells. (D) Western blot analysis of cytoplasmic cytochrome C, Bax, Bcl-xL, and Bcl-2 in C13K cells treated with STAT3 siRNA for 24 h, followed by incubation with 20 μM cisplatin for 24 h.

with lower endogenous levels of pSTAT3. The cisplatin-sensitive OV2008 and A2780 cells were treated with different concentrations of IL-6 (0–50 ng/ml), and the results showed that IL-6 activated STAT3 in a dose-dependent manner but did not alter the expression of total STAT3 (Fig. 3A). Next, the OV2008 and A2780 cells were pretreated with IL-6 (0–50 ng/ml) for 1 h, followed by 20 μM cisplatin treatment; the apoptosis analysis revealed that pretreatment with IL-6 rescued the cells from the apoptosis caused by cisplatin. Furthermore, pretreatment with 50 ng/ml IL-6 reduced cisplatin-induced (20 μM) apoptosis by approximately 3-fold and 4-fold, respectively, in the A2780 and OV2008 cells (Fig. 3B). These results indicated that exogenous IL-6 could induce STAT3 phosphorylation in ovarian cancer cells and increase the resistance to cisplatin treatment. Lastly, to investigate whether the knockdown of STAT3 could counteract the effects of IL-6 in ovarian cancer cell chemoresistance, OV2008 and A2780 cells were

pretreated with IL-6 at 50 ng/ml for 1 h and then transfected with STAT3 siRNA (50 nM) for 48 h, followed by incubation with cisplatin (20 μM) for 24 h. Fig. 3C and D demonstrates that the STAT3 siRNA markedly blocked the activation of STAT3 and reversed the IL-6-induced resistance to cisplatin.

Recent studies have shown that mitochondria play a critical role in the chemoresistance process of cancer cells [23,24]. To further examine the role of STAT3 in the mitochondria-related apoptosis of cisplatin-resistant ovarian cancer cells, OV2008 and C13K cells were treated with cisplatin, STAT3 siRNA, or both, and the mitochondrial transmembrane potential ($\Delta\Psi m$) was assessed using TMRE staining. The results showed that cisplatin or cisplatin combined with STAT3 siRNA significantly lowered the fluorescence intensity of the cells, but there was no significant difference between the two groups for the OV2008 cells (Fig. 4A). In the C13K cells, STAT3 siRNA alone significantly lowered the fluorescence intensity, and treatment with both STAT3 siRNA and cisplatin dramatically reduced the fluorescence intensity (Fig. 4B). We next examined the levels of four important factors in mitochondria-related apoptosis: Bcl-2, Bcl-xL, Bax, and cytoplasmic cytochrome C. As shown in Fig. 4C, the levels of the Bcl-2 and Bcl-xL proteins in the C13K and SKOV-3 cells were higher than in the OV2008 and A2780 cells. In the C13K cells, the levels of cytoplasmic cytochrome C and Bax were higher in the STAT3 siRNA and STAT3 siRNA combined with cisplatin groups than in the control and cisplatin groups, but the levels of Bcl-2 and Bcl-xL were significantly decreased in the former two groups (Fig. 4D).

4. Discussion

Ovarian cancer sustains a poor prognosis among female gynecological malignancies. The standard treatment is cytoreductive surgery, followed by adjuvant chemotherapy and re-treatment with platinum-based chemotherapy at the time of relapse. This treatment course has shown beneficial effects in a high percentage of cases, but *de novo* and acquired resistance has restricted the success of this potent chemotherapeutic agent. Thus, the development of a more effective chemotherapy treatment through the identification of genes involved in modulating both the intrinsic and acquired mechanisms of drug resistance would be instrumental in the ability to fight this disease.

The development of chemoresistance is associated with many events. Although several studies have been performed to identify the gene signatures associated with the resistance to chemotherapy [25,26], no consistent profile has emerged. STAT3 mediates the expression of a variety of genes in response to cell stimuli and, thus, plays a key role in many cellular processes, including cell growth and apoptosis. As a consequence of anti-apoptotic and proliferative lesions, we propose that this oncogenic pathway is also involved in intrinsic and acquired drug resistance; indeed, STAT3-expressing tumors are resistant to chemotherapeutic agents.

The present study showed that OV2008 and A2780 cells treated with the first-line agents used in the clinical treatment of ovarian cancer (cisplatin) suffered a concentration-dependent decrease in cell viability. The C13K and SKOV3 cells treated with cisplatin also underwent a concentration-dependent decrease in cell viability but to a much lesser degree, indicating resistance to cisplatin-induced apoptosis. A western blot analysis indicated that higher levels of pSTAT3 were present in the cisplatin-resistant C13K and SKOV-3 cells than in the cisplatin-sensitive OV2008 and A2780 cells, whereas HUVECs did not contain obvious levels of pSTAT3. However, there were no significant differences in the levels of total STAT3 protein in any of these cells. These results indicated that elevated pSTAT3 might be positively correlated with resistance to cisplatin in ovarian cancer cells. Molecularly targeted therapy provides a unique method for inhibiting the activation of specific proteins involved in cancer cell chemotherapy resistance. To gain a deeper understanding of the role of STAT3 as a mediator of resistance to cisplatin resistance at the molecular level, we evaluated the effect of blocking the activity of this protein in ovarian cancer cells with poor responses to cisplatin using STAT3 siRNA. The results showed that STAT3 siRNA could effectively knock down the STAT3 and pSTAT3 proteins, with the sensitivity to cisplatin being significantly enhanced in the intrinsically cisplatin-resistant SKOV-3 and C13K cells. Our *in vitro* analysis suggests that constitutively activated STAT3 is not only a biomarker of cisplatin resistance but is also functionally linked to a poor response to cisplatin.

Recent studies have shown that IL-6 and its soluble receptor (sIL-6R) are significantly elevated in the peritoneal fluid and cancer tissue collected from patients with ovarian cancer, and elevated levels of IL-6 and sIL-6R are closely associated with a poor prognosis and multidrug resistance [27,28]. IL-6 could regulate the biological behavior of cancer and normal cells by activating STAT3 in autocrine and paracrine pathways [29]. It has been demonstrated that cisplatin-sensitive ovarian cancer lines that do not secrete IL-6, but express the IL-6 receptor, are sensitive to IL-6 [30]. Based on these results, we hypothesized that IL-6 would promote the survival of ovarian cancer cells via STAT3 upon drug treatment. Our results showed that IL-6 activated STAT3 in a dose-dependent manner in cisplatin-sensitive OV2008 and A2780 cells. Interestingly, the exogenous addition of IL-6 protected the cancer cells from cisplatin-induced apoptosis. To explore whether the IL-6-promoted cell survival with cisplatin treatment was caused by activated STAT3, we targeted STAT3 using siRNA and found that the STAT3 knockdown cells showed higher level of apoptosis. The above data indicated that the blockage of the STAT3 signaling pathway may reduce IL-6-induced anti-apoptosis.

Abnormal mitochondrial functions have been closely correlated to chemoresistance in ovarian cancer [31]. STAT3 plays an important role in mitochondria and is involved in cell respiration, nutrient metabolization, and apoptosis [32,33]. Here, we showed that STAT3 siRNA depolarized the mitochondrial membrane potentials in the cisplatin-resistant C13K cells. Furthermore, STAT3 siRNA markedly reduced the mitochondrial membrane potential of the C13K cells when used in combination with cisplatin. Our western blot analysis showed that STAT3 siRNA increased the release of cytochrome C in C13K cells and that the expression of Bax, which is related to the apoptosis pathway of mitochondria, was increased, whereas Bcl-2 and Bcl-xL expression decreased. These results indicated that the inhibition of the STAT3 signaling pathway could activate the mitochondrial apoptosis pathway in cisplatin-resistant ovarian cancer cells, suggesting a mechanism to enhance the sensitivity of cancer cells to cisplatin.

In conclusion, the STAT3 pathway can promote ovarian cancer cell survival upon drug treatment. The blockage of this pathway may be able to render those ovarian cancer cells with constitutively or exogenously activated STAT3 more sensitive to chemotherapeutic agent-induced apoptosis, thus providing a potential therapeutic strategy for the treatment of ovarian cancer.

Acknowledgments

National Science Foundation of China (81101963, 81200379, 81272859, 81072135, 81000257, 81001152, 30901586), Fundamental Research Funds for the Central Universities (HUST, 2011JC037; HUST, 2012TS058), Nature and Science Foundation of Hubei Province (2011CBD542), and the "973" Program of China (No. 2009CB521808).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.04.087.

References

[1] R.C. Bast Jr, Molecular approaches to personalizing management of ovarian cancer, Ann. Oncol. 22 (Suppl. 8) (2011) 85–815.
[2] H. Yu, R. Jove, The STATs of cancer: new molecular targets come of age, Nat. Rev. Cancer 4 (2004) 97–105.
[3] I. Meinhold-Heerlein, D. Bauerschlag, F. Hilpert, et al., Molecular and prognostic distinction between serous ovarian carcinomas of varying grade and malignant potential, Oncogene 24 (2005) 1053–1065.
[4] Z. Duan, R. Foster, D.A. Bell, et al., Signal transducers and activators of transcription 3 pathway activation in drugresistant ovarian cancer, Clin. Cancer Res. 12 (2006) 5055–5063.
[5] D.G. Rosen, I. Mercado-Uribe, G. Yang, et al., The role of constitutively active signal transducer and activator of transcription 3 in ovarian tumorigenesis and prognosis, Cancer 107 (2006) 2730–2740.
[6] Y. Guo, F. Xu, T. Lu, et al., Interleukin-6 signaling pathway in targeted therapy for cancer, Cancer Treat. Rev. 38 (2012) 904–910.
[7] J.A. George, T. Chen, C.C. Taylor, SRC tyrosine kinase and multidrug resistance protein-1inhibitions act independently but cooperatively to restore paclitaxel sensitivity to paclitaxel-resistant ovarian cancer cells, Cancer Res. 65 (2005) 10381–10388.
[8] S. Lee, E.J. Choi, C. Jin, et al., Activation of PI3K/Akt pathway by PTEN reduction and PIK3CA mRNA amplification contributes to cisplatin resistance in an ovarian cancer cell line, Gynecol. Oncol. 97 (2005) 26–34.
[9] B. Barré, A. Vigneron, N. Perkins, et al., The STAT3 oncogene as a predictive marker of drug resistance, Trends Mol. Med. 13 (2007) 4–11.
[10] Z. Han, Z. Hong, C. Chen, et al., A novel oncolytic adenovirus selectively silences the expression of tumor-associated STAT3 and exhibits potent antitumoral activity, Carcinogenesis 30 (2009) 2014–2022.
[11] W. Zhou, J.R. Grandis, A. Wells, STAT3 is required but not sufficient for EGF receptor-mediated migration and invasion of human prostate carcinoma cell lines, Br. J. Cancer 95 (2006) 164–171.
[12] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, Methods 25 (2001) 402–408.
[13] X. Yan, M. Fraser, Q. Qiu, et al., Overexpression of PTEN sensitizes human ovarian cancer cells to cisplatin-induced apoptosis in a p53-dependent manner, Gynecol. Oncol. 102 (2006) 348–355.
[14] Z. Hong, M. Xiao, Y. Yang, et al., Arsenic disulfide synergizes with the phosphoinositide 3-kinase inhibitor PI-103 to eradicate acute myeloid leukemia stem cells by inducing differentiation, Carcinogenesis 32 (2011) 1550–1558.
[15] C. Carlo-Stella, M. Di Nicola, M.C. Turco, et al., The anti-human leukocyte antigen-DR monoclonal antibody 1D09C3 activates the mitochondrial cell death pathway and exerts a potent antitumor activity in lymphoma-bearing nonobese diabetic/severe combined immunodeficient mice, Cancer Res. 66 (2006) 1799–1808.
[16] B. Chazotte, Labeling mitochondria with TMRM or TMRE, Cold Spring Harb. Protoc. 2011 (2011) 895–897.
[17] J.P. Guo, S.K. Shu, L. He, et al., Deregulation of IKBKE is associated with tumor progression, poor prognosis, and cisplatin resistance in ovarian cancer, Am. J. Pathol. 175 (2009) 324–333.
[18] M.H. Lee, S.J. Kim, S.N. Park, Development of porous cellulose-hydrogel system for enhanced transdermal delivery of quercetin and rutin, Korea Polym. J. 37 (3) (2013) 347–355.
[19] B. Nolen, A. Marrangoni, L. Velikokhatnaya, et al., A serum based analysis of ovarian epithelial tumorigenesis, Gynecol. Oncol. 112 (2009) 47–54.
[20] M.M. Moradi, L.F. Carson, B. Weinberg, et al., Serum and ascitic fluid levels of interleukin-1, interleukin-6, and tumor necrosis factor-alpha in patients with ovarian epithelia cancer, Cancer 72 (1993) 2433–2440.
[21] R.T. Penson, K. Kronish, Z. Duan, et al., Cytokines IL-1beta, IL-2, IL-6, IL-8, MCP-1, GM-CSF and TNFalpha in patients with epithelial ovarian cancer and their relationship to treatment with paclitaxel, Int. J. Gynecol. Cancer 10 (2000) 33–41.
[22] S. Li, N. Wang, P. Brodt, Metastatic cells can escape the proapoptotic effects of TNF-α through increased autocrine IL-6/STAT3 signaling, Cancer Res. 72 (2012) 865–875.
[23] C.E. Griguer, C.R. Oliv, Bioenergetics pathways and therapeutic resistance in gliomas: emerging role of mitochondria, Curr. Pharm. Des. 17 (2011) 2421–2427.
[24] D.H. Suh, M.K. Kim, J.H. No, et al., Metabolic approaches to overcoming chemoresistance in ovarian cancer, Ann. N. Y. Acad. Sci. 1229 (2011) 53–60.
[25] S. Arora, K.M. Bisanz, L.A. Peralta, et al., RNAi screening of the kinome identifies modulators of cisplatin response in ovarian cancer cells, Gynecol. Oncol. 118 (2010) 220–227.
[26] J. Kang, A.D. D'Andrea, D. Kozono, A DNA repair pathway-focused score for prediction of outcomes in ovarian cancer treated with platinum-based chemotherapy, J. Natl. Cancer Inst. 104 (2012) 670–681.
[27] C.W. Lo, M.W. Chen, M. Hsiao, et al., Malignant ascites in ovarian cancer IL-6 trans-signaling in formation and progression of malignant ascites in ovarian cancer, Cancer Res. 71 (2011) 424–434.
[28] Y. Guo, J. Nemeth, C. O'Brien, et al., Effects of siltuximab on the IL-6-induced signaling pathway in ovarian cancer, Clin. Cancer Res. 16 (2010) 5759–5769.
[29] S. Grivennikov, E. Karin, J. Terzic, et al., IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer, Cancer Cell 15 (2009) 103–113.
[30] J.G. Asschert, E. Vellenga, M.H. Ruiters, et al., Regulation of spontaneous and TNF/IFN-induced IL-6 expression in two human ovarian-carcinoma cell lines, Int. J. Cancer 82 (1999) 244–249.
[31] M. Montopoli, M. Bellanda, F. Lonardoni, et al., "Metabolic reprogramming" in ovarian cancer cells resistant to cisplatin, Curr. Cancer Drug Targets 11 (2011) 226–235.
[32] J. Liu, X. Xu, X. Feng, et al., Adenovirus-mediated delivery of bFGF small interfering RNA reduces STAT3 phosphorylation and induces the depolarization of mitochondria and apoptosis in glioma cells U251, J. Exp. Clin. Cancer Res. 30 (2011) 80.
[33] J. Wegrzyn, R. Potla, Y.J. Chwae, et al., Function of mitochondrial Stat3 in cellular respiration, Science 323 (2009) 793–797.