Original Research

SREBP2 contributes to cisplatin resistance in ovarian cancer cells

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Impact statement

Transcriptome of cisplatin resistant and sensitive A2780 epithelial ovarian cancer cells was obtained from GSE15372 and TCGA. Twelve transcription factors and their targets were involved in cisplatin resistant. Among these factors, the targets of EZH2 and SREBP2 revealed by Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining were also enriched in differentially expressed genes. Their targets were enriched mainly in cell cycle and cholesterol metabolic process. Three targets of SREBP2, namely LDLR, FDFT1, and HMGCR were increased in A2780-resistant cell lines and were found elevated in live cells after cisplatin treatment via qPCR. RNAi of SREBP2 in A2780 cell line resulted in a decrease of cell viability after cisplatin treatment. SREBP2 played important roles in cisplatin resistance and might be a novel target for cancer therapy.

Abstract

This study is to investigate transcription factors involved in cisplatin resistance in ovarian cancer cells. The transcriptome of cisplatin resistant and sensitive A2780 epithelial ovarian cancer cells was obtained from GSE15372. Ovarian transcriptome data GSE62944 was downloaded from TCGA and applied for transcription regulatory network analysis. The analysis results were confirmed using quantitative polymerase chain reaction. The roles of SREBP2 in cisplatin-resistant cells were investigated by RNA inference and cell viability analysis. Transcription regulatory network analysis found that 12 transcription factors and their targets were involved in cisplatin resistant in A2780 cells. Among these factors, the targets of EZH2 and SREBP2 revealed by Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining were also enriched in differentially expressed genes between cisplatin resistant and cisplatin sensitive cells. Their targets were enriched mainly in cell cycle and cholesterol metabolic process, respectively. Bioinformatic analysis illustrated three known targets of SREBP2, namely LDLR, FDFT1, and HMGCR were increased in A2780-resistant cell lines. Additionally, the three genes and SREBP2 were also elevated in live cells after cisplatin treatment via quantitative polymerase chain reaction. Importantly, RNA inference of SREBP2 in A2780 cell line resulted in a decrease of cell viability after cisplatin treatment. SREBP2 played important roles in cisplatin resistance and cholesterol metabolic process might be a novel target for cancer therapy.

Keywords: Cisplatin resistance, transcription factors, differentially expressed genes, transcription regulatory inference, sterol regulatory element binding protein 2, cholesterol metabolic process

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Introduction

Ovarian malignancy is one of the common malignancies in female reproductive system, and its mortality ranks first in gynecological tumors.[1] According to the cancer statistics report released by American Cancer Society in 2016, the United States will add 22,280 cases of ovarian cancer patients, and the number of new ovarian cancer deaths will reach 14,240.[2] China's cancer statistics report discovers that the new ovarian cancer cases in 2015 are 52,100 cases and up to 22,500 deaths.[3] The vast majority of advanced ovarian cancer patients are treated with surgery followed by adjuvant chemotherapy, and cisplatin (cis-diamminedichloroplatinum) is the first line chemotherapeutic agent. Unfortunately, some cancer cells could acquire cisplatin resistance, hence leading to cisplatin-based treatment failure. In fact, up to 75% treated patients will relapse within 18 months due to drug resistance.[4] Importantly, there is no practical alternative medicine for the treatment of drug-resistant patients. Eventually, 90% ovarian cancer deaths can be caused by drug resistance.[5]

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Recent studies have indicated that cisplatin resistance is multifactorial and the mechanisms might contain insufficient DNA binding, increased DNA repair, changes in metabolism, and alterations in apoptotic pathways. For example, knockdown of glycolysis metabolic enzymes regulator HIF-1α hypoxia-inducible factor-1 (HIF-1) could elevate the response of cisplatin-resistant ovarian cancer cells to cisplatin.[6] Another study showed that oxidative metabolism could induce inflammation-induced platinum resistance in human ovarian cancer.[7] Overall, these studies indicate that metabolism may be involved in drug resistance and eventually leading to new approaches for overcoming the resistance. However, the exact pathways that are significant for drug resistance occurrence in ovarian cancer are not yet known. As a result, it is possible to reverse or circumvent drug resistance if we know the molecular mechanisms of drug resistance.[5,8]

Sterol regulatory element binding protein (SREBP) family could regulate the expression of genes encoding the major enzymes related to *de novo* lipid synthesis.[9] It is well documented that SREBP2 functions as a key transcriptional regulator of metabolic genes and is enrolled in tumor progression.[10] SREBP2 induces the expression of oncogenic isocitrate dehydrogenase 1 (IDH1) and influences 2-hydroxyglutarate (2-HG) production from glucose.[11] At low pH, SREBP2 increases cholesterol biosynthetic genes, which in turn provides a growth advantage to cancer cells, leading to reduced overall survival of cancer patients.[10] SREBP2 emerges as major drivers of lipid synthesis cancers like glioblastoma, placental choriocarcinoma, colonic cancer, and ovarian cancer.[12–15]

To date, no study has focused on the involvement of SREBP2 in cisplatin resistance. In this study, public data from GSE15372 and GSE62944 were downloaded and applied for the analysis of transcription regulatory network and differentially expressed genes (DEGs). The analyzing results were then confirmed by using quantitative polymerase chain reaction (qPCR), RNA inference (RNAi), and MTS assay in A2780 cells. We intended to identify molecules that play important roles in the process of cisplatin resistance.

Materials and methods

Bioinformatic analysis

The transcriptome data of ovarian cancer samples including, 1119 tumors were downloaded and extracted from GSE62944.[16] Bioinformatic analysis then was undertaken and all processes were performed using the R programming language (https://www.r-project.org/) together with its packages. Transcription regulatory network analysis (TRNA) was conducted with the RTN package.[17] The cisplatin-resistant cells transcriptome data of GSE15372 were used for DEGs analysis (adj. $P < 0.01$, see Supplementary Table 1) between cisplatin-resistant cells and sensitive cells using limma package.[18] DEGs were then used for RTN package to identify TFs associated with cisplatin resistance. Online tool STRING (www.string-db.org) was applied for protein–protein interaction (PPI) network prediction and gene function enrichment.[19]

Drug preparation

Cis-diamminedichloroplatinum (II) (cisplatin) was obtained from Sigma–Aldrich, (St. Louis, MO, USA) and dissolved in 0.9% NaCl at a final concentration of 1.5 g/L. Aliquots were stored at −20°C and thawed immediately before use. The drug was adjusted to different final concentrations (0, 2, 4, 6, 8 and 10 μM) during usage.

q-PCR

A2780 cell line was provided by the Cell Bank of the Shanghai Institute of Biochemistry & Cell Biology (http://www.cellbank.org.cn), and was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). After treating with different concentrations of cisplatin for 48 h, q-PCR was performed. For q-PCR, briefly, total RNA was extracted from cells using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Total RNA was then reverse transcribed into cDNA in a final volume of 20 μl using random primers under the standard conditions of PrimeScript RT Master Mix (Takara, Dalian, China). The qPCR was then undertaken with 2 × SYBR Premix Ex Taq (TaKaRa, Dalian, China) using ABI ViiA7 System (Applied Biosystems, Foster City, CA, USA). The program was as follows: 95°C for 30 s, 95°C for 5 s, and 58°C for 30 s for 40 cycles. The relative mRNA levels were analyzed by the $2(-\Delta\Delta Ct)$ method with β-actin gene as an internal control and represented as the ratio of the assessed genes to β-actin.[20] The primers were available upon request.

RNAi

For RNAi, A2780 cells were seeded on a 6-well culture plate before 24 h of the transfection. The medium was 2 ml without antibiotics and the cell fusion rate was about 40% at the time of transfection. The solid double-stranded siRNA was prepared with DEPC water at a concentration of 20 μM of the siRNA. The siRNA liquor was stored at −20°C until completely dissolved. The transfection method was performed according to the Lipofectamine RNAiMA's (Thermo Fisher) instructions. Briefly, 5 μl of RNAiMAX was added to 100 μl of Opti-MEM (Invitrogen, Carlsbad, CA, USA) or serum-free medium, and gently mixed with 5 μl of the interfering sequence (20 μM) to 100 μl of Opti-MEM or serum-free medium. The diluted siRNA was added to the diluted RNAiMAX, mixed, and incubated at room temperature for 10–15 min to form siRNA and RNAiMAX complex. The mixture of siRNA and RNAiMAX was added to the cultured cells, gently mixed, and continued to culture. After transfection, cells were cultured for 24 h and then treated with different concentrations of cisplatin. Cells were harvested after cultured for another 48 h and applied for RNA extraction.

3–(4,5-Dimethylthiazol-2-yl)-5–(3-carboxymethoxyphenyl)-2–(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay

Cells were seeded in 96-well plates with medium of 100 μL/well, containing 3000 cells and treated with cisplatin. Cells were cultured under the conditions of 37°C and 5% CO2. After treating with different concentrations of cisplatin for 48 h, 20 μl MTS reagent was added to each well and was incubated for 1 h. The absorbance was measured at 490 nm using a BLX808 microplate reader (BioTek, Winooski, VT).

Statistical analysis

Statistical analysis was performed using the R programming language (https://www.r-project.org/). Data were presented as the mean ± SD. Student's *t*-test or one-way analysis of variance (ANOVA) were used to assess the significance among groups, while hypergeometric test was applied for enrichment analysis. Graphpad/Prism6 was used for plotting graphs and statistical analysis. $P < 0.05$ was considered to be a significant difference (*$P < 0.05$).

Results

EZH2 and SREBP2 are involved in cisplatin resistance

In order to screen the transcription factors that are involved in cisplatin resistance in ovarian, previously reported TCGA RNA-sequencing data GSE62944 were obtained firstly. Transcription network was then inferenced using the ovarian tumor data. DEGs between cisplatin resistant and sensitive cells were analyzed with GSE15372 (Supplementary Table 1). Finally, 12 TFs, whose targets were revealed by TRNA, were enriched in DEGs with gene set enrichment analysis and considered to be associated with cisplatin resistance (Figure 1). To confirm our inferenced transcription network, data from Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST, a manually curated database of human transcriptional regulatory network) were also analyzed. And the result demonstrated that targets of 26 TFs were enriched in DEGs (Supplementary Table 2). Additionally, we found two TFs, namely EZH2 and SREBP2, were overlapped between TRRUST and TRNA (Figure 1). These results suggested that EZH2 and SREBP2 might be involved in cisplatin resistance.

Targets of SREBP2 enriched in cholesterol metabolic process

The pathways of EZH2 and SREBP2 together with their targets were then analyzed to investigate the molecular mechanism. A total of 35 targets of EZH2 and 43 targets of SREBP2 were included in the pathways (Figure 2(a) and (b)). In accordance with the known functions of EZH2 and SREBP2, their targets were mainly enriched in cell cycle and cholesterol metabolic process, respectively (Figure 2(c) and (d)).

SREBP2-dependent genes were increased in cisplatin resistance cells

It is well documented that EZH2 could lead to cisplatin resistance in ovarian cells; however, the roles that SREBP2 plays in cisplatin resistance have not been investigated so far.[21] We then examined the functions of SREBP2 and its targets in A2780 cell lines. The targets of SREBP2 contained 43 genes, and there were 19 genes differentially expressed in cisplatin-resistant cell compared with cisplatin-sensitive cells. The expressions of the 19 differentially expressed targets were mostly increased in cisplatin-resistant cells

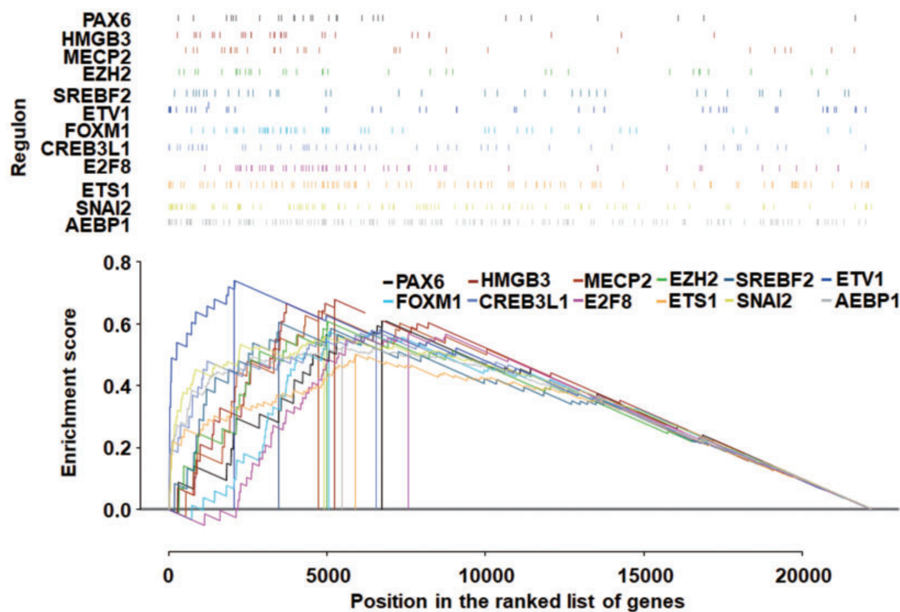

Figure 1. Identification of transcription factors involved in cisplatin resistance. TCGA ovarian transcriptome data from GSE62944 were applied for transcription regulatory network analysis. Differentially expressed genes between cisplatin resistant and sensitive cells were analyzed from GSE15372. The targets of 12 TFs were enriched in DEGs (adj. $P < 0.05$). (A color version of this figure is available in the online journal.)

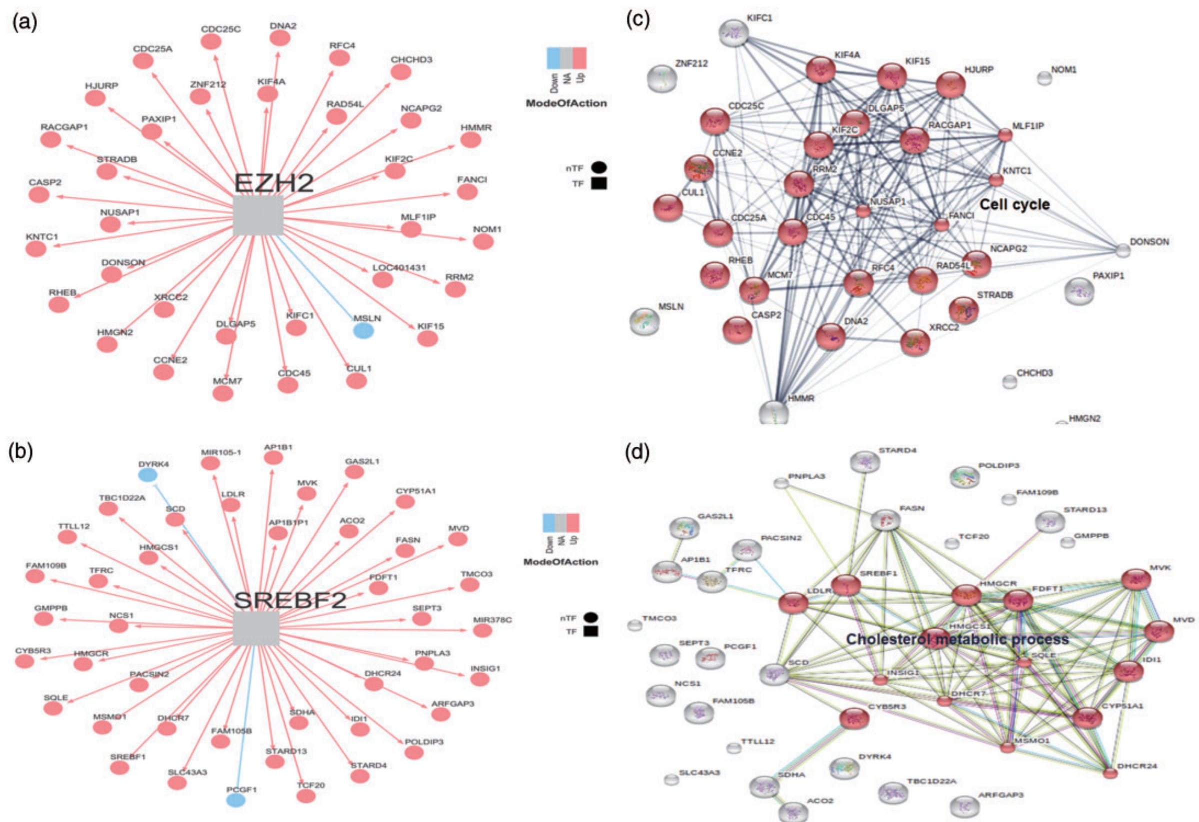

Figure 2. Targets of EZH2 and SREBP2 and their functions. The targets of EZH2 (a). The targets of SREBP2 (b). The protein–protein interaction (PPI) network of the targets of EZH2. Red nodes showed genes included in cell cycle biological process (c). The PPI network of the targets of SREBP2. Red nodes showed genes included in cholesterol metabolic process biological process. (A color version of this figure is available in the online journal.)

except STARD13 (Figure 3(a)). Among these targets, LDLR, FDFT1, and HMGCR were also found to be controlled by SREBP2 in TRRUST database and their expressions were all increased (Figure 3(b) to (d)). These results illustrated that SREBP2 regulating genes were altered in cisplatin resistance cells and suggested that SREBP2 might played important roles in cisplatin resistance.

SREBP2-dependent genes were induced by cisplatin treatment

In order to identify whether SREBP2 and its targets could be induced by cisplatin treatment, A2780 cells were treated with cisplatin and qPCR was performed. Our results demonstrated that the expressions of SREBP2, LDLR, and FDFT1 in 8 μM or 10 μM cisplatin-treated cells were significantly up-regulated compared with the untreated cells, while HMGCR gene in 2 μM or more concentration cisplatin-treated cells was up-regulated (Figure 4). These results suggested that cisplatin could induce the expressions of SREBP2-dependent genes.

Blocking SREBP2 pathway increased cisplatin sensitivity

To further investigate the roles of SREBP2 in cisplatin resistance, we knocked down SREBP2 and FDFT1 in A2780 cell lines (Figure 5(a) and (b)) and measured the MTS of the deserved cells. Inferencing SREBP2 or both SREBP2 and FDFT1 could delay cell proliferation (Figure 5(c)) compared with siNC or siFDFT1 (all $P < 0.01$). Interestingly, suppressing FDFT1 could result in a little but statistically significant increase of cell viability ($P < 0.01$, compared with siNC). Further analysis found that cell lines transfected with SREBP2 siRNA or both SREBP2 and FDFT1 siRNAs could lead to the suppression of A2780 cells exposing to 8 μM or 10 μM cisplatin according to an MTS assay (Figure 5(d)). Collectively, these results illustrated that SREBP2 contributed to cisplatin resistance.

Discussion

In this study, online transcriptome of cisplatin resistant and sensitive cell line of epithelial ovarian cancer A2780 was applied for DEGs identification. EZH2 and SREBP2 were overlapped between TRRUST and TRNA among the TFs investigated. Pathway analysis demonstrated that the targets of SREBP2 were enriched in cholesterol metabolic process. Further study showed that SREBP2-dependent genes LDLR, FDFT1, and HMGCR were increased in cisplatin resistance cells. qPCR argued that the mRNA expression of SREBP2, LDLR, FDFT1, and HMGCR increased after cisplatin treatment. Blocking SREBP2 pathway could increase cisplatin sensitivity in A2780 cells.

SREBPs are a family of transcription factors regulating lipid homeostasis and expression of a series of enzymes involved in triacylglycerol, fatty acid, endogenous

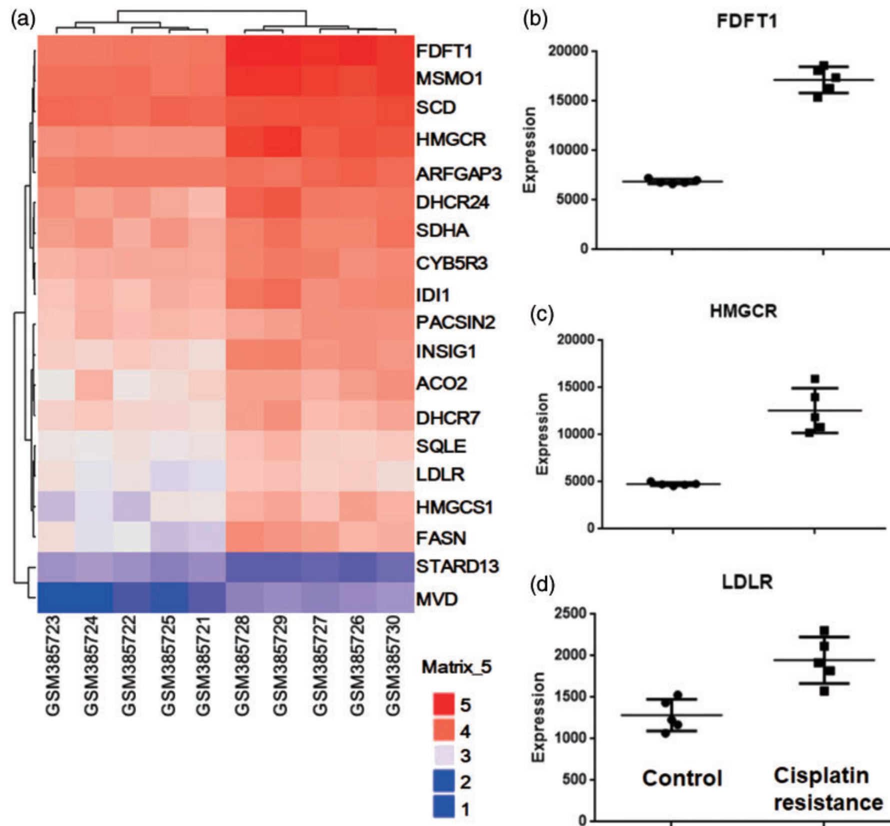

Figure 3. Bioinformatic analysis showed the expressions of most differentially expressed targets of SREBP2 were increased. The 19 targets of SREBP2 were differentially expressed between cisplatin resistant and sensitive cells, and their expressions were elevated except STARD13 (a). The expressions of FDFT1 (b), HMGCR (c) and LDLR (d) were all increased in cisplatin-resistant cells. $^*P < 0.05$, compared with cisplatin sensitivity. (A color version of this figure is available in the online journal.)

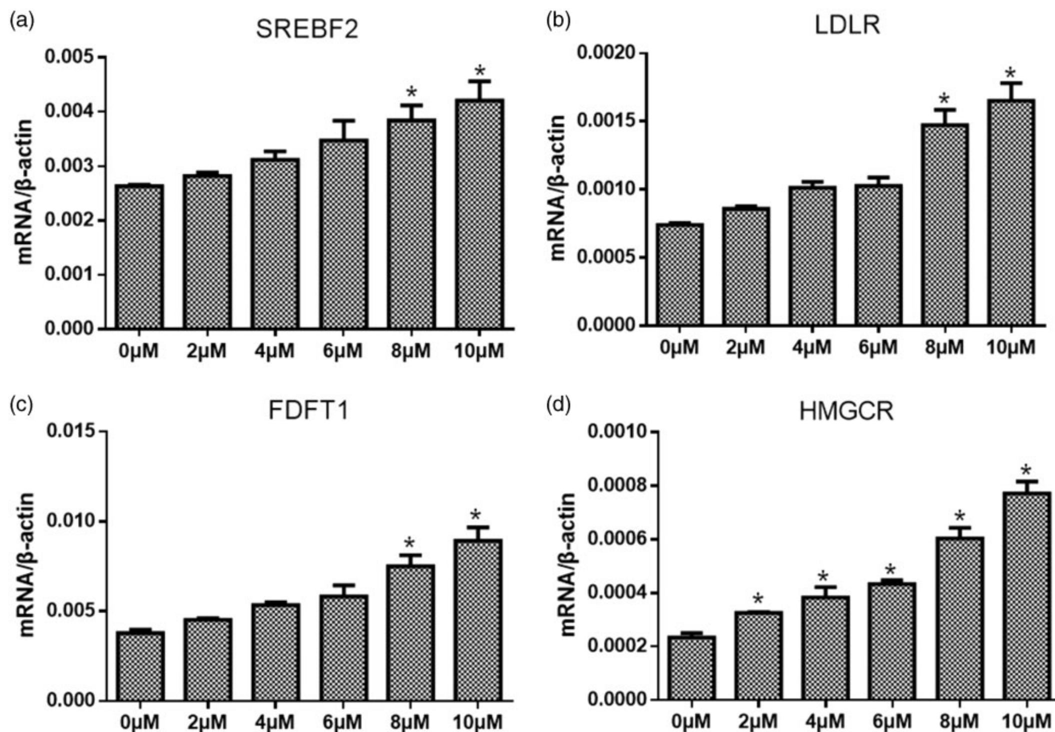

Figure 4. SREBP2 and its dependent genes were increased in A2780 cells treated with cisplatin. The expressions of SREBP2 (a), LDLR (b), and FDFT1 (c) were significantly up-regulated in 8 μM or 10 μM cisplatin-treated cells compared with the untreated cells, while HMGCR (d) gene was increased in 2 μM or more cisplatin-treated cells. $^*P < 0.05$ compared with the untreated cells.

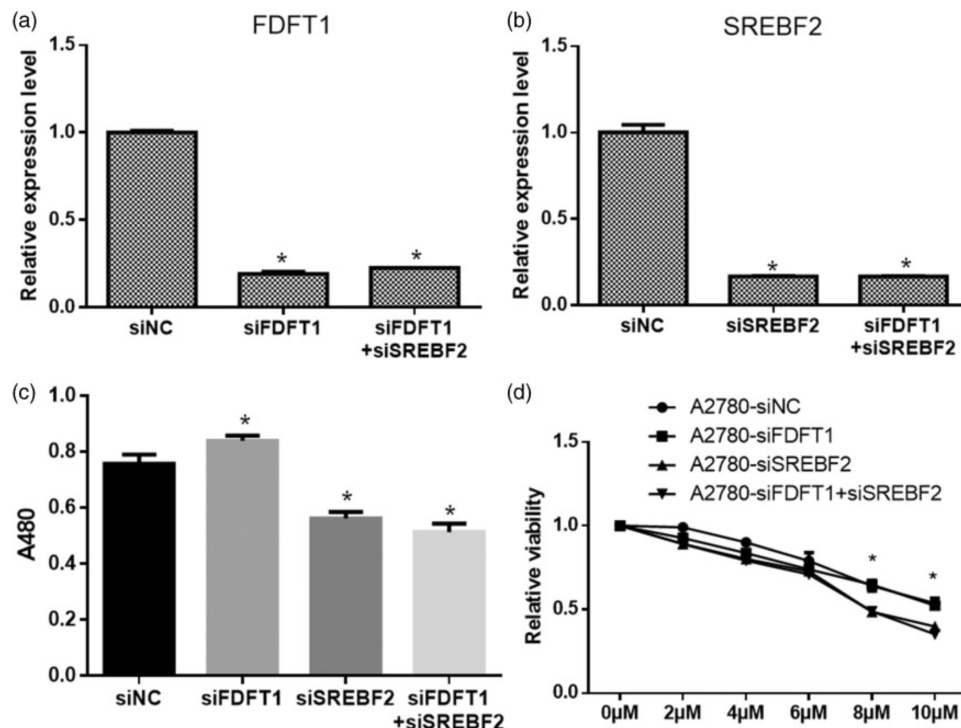

Figure 5. Knock down of SREBP2 increased cisplatin sensitivity in A2780 cells. RNAi decreased the expressions of SREBP2 (a) and FDFT1 (b) in A2780 cells (*$P < 0.05$, compared to the control siNC). SREBP2 RNAi decreased cell viability (c), while FDFT1 alone RNAi increased that (*$P < 0.05$, compared to the control siNC). SREBP2 siRNA (d) transfection resulted in a decrease of cell viability (*$P < 0.05$, compared with the control of 0 μM).

cholesterol, and phospholipid synthesis is regulated by SREBPs.[22] SREBP2, which is relatively specific to cholesterol synthesis and metabolic process, plays an important role in cancer invasion.[22–24] In this study, SREBP2 and its targets were mainly enriched in cell cycle and cholesterol metabolic process, which is consistence with the previous studies. Notably, SREBP2 and its three targets of LDLR, FDFT1, and HMGCR were found to be increased in cisplatin-treated cells, indicating that they might be involved in cisplatin resistance.

SREBP2 and its regulated cholesterol levels are documented to be associated with cancer development, such as colorectal cancer.[15,25] A previous study has shown that low-density lipoprotein (LDL) could inhibit the proliferative capacity of colonic adenocarcinomas, which possesses a high cholesterol synthesis rate associated with fibroblasts.[15] An enzyme named CYP27A1, which is involved in SREBP2 inhibition and LDLR downregulation, is highly linked to higher tumor grade and shorter disease-free survival.[26] Gene expression profiles in prostate cancer using LNCaP xenograft tumor model at several time points also show that SREBP2 is the key gene increased, which is consistently confirmed by Northern blots and Western blot.[27] Inhibition of SREBP function could block the lipid biosynthesis and impair cell survival under hypoxia in hypoxic cancer cells.[28] These reports have suggested the key roles of SREBP2 in cancer cells and are consistent with our results. Additionally, the similar functions of SREBP2 found in various cancer cells suggested that it might be a general regulator for cancer development.

Cisplatin, a chemotherapy drug that contains platinum, has been widely used for treating patients with ovarian cancer in clinic. Unfortunately, its clinical effectiveness is markedly decreased due to the occurrence of cisplatin resistance, either acquired or intrinsic. As a result, it is of great importance to find out the molecules playing roles in limiting the therapeutic efficacy of cisplatin. Metabolic reprogramming is documented to be a hallmark of cancer, and several metabolism pathways have been linked to cisplatin resistance.[6,29–31] In this study, our results suggested the change of cholesterol metabolic process contributed to cisplatin resistance and the inhibition of SREBP2 sensitized cells to cisplatin. Our study illustrated a new mechanism of cisplatin resistance.

Decreased SREBP2 expression could down-regulate mevalonate pathway and inhibit the active statin-induced sterol feedback, leading to enhanced statin toxicity.[32] Xiang *et al*. found that miR-185 represses SREBP2 expression through targeting SREBP2 mRNA 3′UTR. Additionally, they argue that by inhibiting proliferation and promoting apoptosis, miR-185 increases cisplatin sensitivity in both SKOV3/DDP and A2780/DDP cells.[33] Our results also found a decrease of proliferation by knocking down SREBP2, which proposed a possibility that increased cisplatin sensitivity of SREBP2 inhibition was associated with its roles in proliferation. These data suggested that there might be a pathway, containing miRNA-185, SREBP2, and molecules involved in cell proliferation, regulating cisplatin resistance. However, more experiments were needed to represent the molecular mechanisms of

SREBP2 in cisplatin sensitivity and discover the possible pathway.

The cytotoxicity of cisplatin alone or in the combination of other drugs in a panel of human cancer cell lines was widely investigated.[10,11,34,35] The concentration of cisplatin applied is up to 64 μg/mL (~200 μM).[35] It is reported that 3 μM cisplatin is possible to maintain cisplatin resistance and 7 μM was high enough for high-grade serous ovarian cancer, which was not tolerated by the sensitive cells.[34,36] As a result, cisplatin concentrations of 0, 2, 4, 6, 8, and 10 μM were undertaken in this study so as to avoid excessive cell death caused by high cisplatin concentrations. The cells showed different response to cisplatin, indicating that the concentrations were reasonable; however, it is possible to assume that higher concentrations might be tolerant. In conclusion, our data demonstrated an important role of SREBP2 in cisplatin response of ovarian cancer cell line. SREBP2 and its targets LDLR, FDFT1, and HMGCR were increased in cisplatin-resistant cells and induced by cisplatin. Importantly, inhibition of SREBP2 sensitized A2780 cells to cisplatin. Our results suggested that cholesterol metabolic process may contribute to cisplatin resistance, and thus targeting SREBP2 might be a promising strategy for overcoming ovarian cancer cisplatin resistance.

Authors' contributions: Zheng L and Li L collected the information, performed the experiment, and drafted the initial manuscript. Lu Y participated in the discussion of the manuscript. Jiang F and Yang XA conceived and designed the study, performed data analysis, and wrote the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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