

Functional Role of RRS1 in the Chemosensitivity of Drug Resistant Breast Cancer Cell to Cisplatin

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Abstract

RRS1(human regulator of ribosome synthesis 1), a critical nuclear protein participated in ribosome biogenesis, plays important roles in the genesis and development of breast cancer. Here, we reported that RRS1 was highly expressed in cisplatin resistant breast cancer cell MCF-7/DDP than that in parent MCF-7. RRS1 silencing increased the sensitivity of MCF-7/DDP cells to cisplatin, inhibited proliferation, affected cell cycle distribution and promoted apoptosis. Furthermore, in nude mice xenograft study, the content of RRS1 in cisplatin treatment group was significantly higher than that in saline treatment group. In addition, we found that RRS1 could bind to AEG-1 and subsequently strengthened AEG-1 abundance in breast cancer cells. Although AEG-1 did not affect AEG-1 gene transcription, it inhibited ubiquitination and subsequent proteasome-mediated degradation of AEG-1 protein. Our research in current study documented for the first time that RRS1 participated in the sensitivity of breast cancer cells to cisplatin through binding to AEG-1, indicating that RRS1 may be a promising target for the therapy of breast cancer.

1 Introduction

Breast cancer overtook lung cancer to become the most leading diagnosed cancer worldwide for the first time in 2020, accounting for nearly 12% of new cases occur every year^[1]. Preoperative chemotherapy, combining with surgical treatment are widely used in clinical treatment for breast cancer. Because of its own characteristics, surgical treatment affects the life quality of patients to a certain extent; Therefore, chemotherapy is very important in the treatment of early breast cancer. Cisplatin, a broad-spectrum chemotherapeutic agent, was used to treat various human malignant tumors, including breast carcinoma^[2]. However, the clinical usage of this drug was limited because of intrinsic and acquired resistance^[3, 4]. Therefore, it is particularly important to explore the molecular mechanism of chemoresistance and find a key molecular target regulating breast cancer chemotherapy resistance.

RRS1 was a novel ribosomal synthesis regulatory protein discovered by Tsuno *et al*/in yeast in 1999. It was mainly involved in the maturation of 25s rRNA and the assembly of 60S ribosomal large subunit in the process of ribosomal biosynthesis^[5]. Recent studies have shown that RRS1 had abnormal expression characteristics in the breast cancer^[6-8], cervical cancer^[9], colorectal cancer^[10], hepatocellular carcinoma^[11] and thyroid cancer^[12], and was closely related to the proliferation and apoptosis of cancer cells^[7]. Meanwhile, apoptosis resistance was considered to be one of the causes of chemoresistance^[13, 14]. Therefore, exploring the relationship between RRS1 and chemosensitivity of breast cancer cells will provide an important target for the treatment of breast cancer.

AEG-1 was initially found in primary human fetal brain glial cells, and was highly expressed in a variety of malignant tumors^[15-18]. Chemoresistance was one of the influencing factors of AEG-1 high expression, for example, the activation of AEG-1 could promote drug resistance and metastasis of breast cancer cells^[19]; AEG-1 regulated chemosensitivity of hepatoma cell to adriamycin though interfering

ubiquitination and proteasomal degradation of MDR1 protein^[20]. AEG-1 could also activate a series of survival signaling pathways, including MEK/ERK, AKT, NF- κ B and WNT signaling pathways^[21]. As a transmembrane protein, AEG-1 was mainly located in the endoplasmic reticulum and perinuclear space^[22, 23]; while RRS1 was mainly situated in the endoplasmic reticulum and nucleolus^[24]. Considering the co-localization of them on endoplasmic reticulum, we speculate that RRS1 may interact with AEG-1 to mediate cisplatin resistance in breast cancer cells.

In the current study, we evaluated the difference in RRS1 expression between the resistant cell MCF-7/DDP and its parent cell MCF-7. We used lentiviral system to silence RRS1 gene in the resistant cell, examined their proliferation, cell cycle distribution, apoptosis, sensitivity to chemotherapeutic, and mechanism involving in chemoresistance; the proliferation tumor model of nude mice in vivo further verified the correlation between RRS1 and cisplatin chemosensitivity, to investigate the possibility of using the RRS1 gene as a therapeutic target in breast cancer.

2 Materials And Methods

2.1 Cell line authentication

The breast cancer cisplatin resistant cell line MCF-7/DDP was purchased from Chuanqiu biology in October, 2019, Shanghai, China. The cell line was tested and authenticated by STR and the last time the cell line was tested was in June, 2018. The cell was test by extracting DNA from MCF-7/DDP, then it was amplified by PowerplexTM16 ID System STR compound amplification kit. ABI 3130xl genetic analyzer was used to detect the STR locus and sex gene amelogenin, and the map and genotype were analyzed. The MCF-7 human breast cancer cell line was obtained from the library of affiliated hospital of Qingdao University in October, 2016. The cell line was tested and authenticated by STR and the last time the cell line was tested was in

August, 2020. Test method: took an appropriate amount of samples, extracted DNA with Microread Genomic DNA kit, amplified 20 STR sites and gender identification sites with MicroreaderTM21 ID Systemsystem, detected PCR products with ABI 3730xl genetic analyzer, analyzed the detected products with GeneMapperID-X software(Applied Biosystems), and compared them with ATCC and DSMZ databases.

2.2 Cell culture and chemical reagents

MCF-7 was cultured in DMEM (HyClone, USA) supplemented with 10% fetal bovine serum (ExCell Bio,China). The breast cancer cisplatin resistant cell line MCF-7/DDP was cultured in RPMI-1640 (HyClone, USA) supplemented with 10% fetal bovine serum (ExCell Bio,China) and 0.5uM DDP (Solarbio, China) at 37°C in a tissue culture incubator with 5% CO₂. Total RNA and protein were extracted from the cells in logarithmic phase. Real-time PCR and Western Blotting were used to detect mRNA and protein levels. Puromycin (Yeasen, China) was used at a concentration of 1 ug/ml. Protein synthesis inhibitor

cycloheximide (CHX) was used at a concentration of 200 ug/ml. Proteasome inhibitor MG132 (Selleck, China) was used at a concentration of 10 μ M.

2.3 Lentivirus transfection

Logarithmic growth cells with confluence of 80-90% were prepared single cell suspension with complete medium, and inoculate 2×10^5 cells per well into 6-well plate. RRS1-targeting sh-RRS1, RRS1-targeting OE-RRS1 and a non-targeting control were expressed via pSuper constitutive expression constructs (Genecard, China). The amount of lentivirus was accurately calculated according to the number of cells (The number of virus=the number of cells \times MOI/the titer of virus, and the MOI of MCF-7 was 20). The solution was changed after transfection for 12h. Its efficiency was observed under fluorescence microscope after transfection for 48-72 h, and the total protein and mRNA were extracted for detection of RRS1.

2.4 Quantitative PCR

Total RNA was extracted from cells or tumors using a Trizol RNA isolation kit (Vazyme, China) and detected by RT-qPCR according to the manufacturer's protocol (Vazyme, China). All samples were analyzed in triplicate. Primers for GAPDH were forward 5'-AGAAGGCTGGGGCTCATTTG-3' and reverse 5'-AGGGGCCATCCACAGTCTTC-3'. The primers for RRS1 were forward 5'-CCCTACCGGACACCAGAGTAA-3' and reverse 5'-CCGAAAAGGGGTTGAACTTCC-3'. The primers for AEG-1 were forward 5'-CGAGAAGCCCAAACCAAATG-3' and 5'-TGGTGGCTGCTTTGCTGTT-3'.

2.5 Sensitivity of Resistant Cell MCF-7/DDP to cisplatin

Cells were prepared into single cell suspension by trypsinization and seeded into 96-well plates at 3×10^3 cells per well. When cells grow to the logarithmic growth stage, DDP were added to final concentrations of 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.5, 2.5, 3.5, 4.5, 6 μ g/ml. Incubated under normal conditions for 24 h, then the viabilities were examined using CCK-8 (Solarbio, China) at 10 ul/well. The absorbance values were used to calculate the inhibition ratios and IC50 at 24 h.

2.6 Cell proliferation

The cells, treated according to the experimental group requirements, were inoculated into 96-well plates at 3×10^3 cells per well, added CCK-8 (10ul/well) (Solarbio, China) in different time periods, the cells were maintained at 37°C and 5% CO₂ for 2 h, and then absorbance (optic density, OD) at 450 nm was measured.

2.7 Cell cycle and apoptosis

Cell cycle analysis was conducted with FlowJo software following staining with propidium iodide 25 ug (Solarbio, China) and flow cytometric (Cytoflex S, Beckman). Apoptosis was also evaluated by flow cytometric analyses of Annexin V-APC binding (Elabscience, China).

2.8 Co-Immunoprecipitation

Co-IP experiment was performed using a protein A/G kit (Epizyme, China). Beads were activated though incubating overnight with 25 ul lysis buffer. MCF-7/DDP cells were lysed in cell lysis buffer at 4°C for 30 minutes. Protein concentration was measured with BCA assay (Solarbio, China). The equal resulting lysate, about 500 ul with antibody against AEG-1 (1 ug, Abcam, ab227981) or IgG (1 ug, Bioss, bs-0297R), was incubated overnight with activated beads for immunoprecipitants, immunoprecipitants were separated by SDS-PAGE after washing with lysis buffer and were analysed by immunoblotting with the indicated antibodies.

2.9 Protein stability assay

OE-CON and OE-RRS1 cells of 80% confluence were treated with 200 ug/ml cycloheximide (CHX), and protein was collected at the indicated time and analyzed with Western Blot.

2.10 Nude mice xenograft study

Four-week-old male nude (BALB/c) mice (vitalriver, China) were used in the experiments. MCF-7 cells (5×10^6 cells/0.2 ml liquid/mouse, medium/matrix=1) were injected into the mice subcutaneously to generate the mouse models. Xenografts were allowed to grow to approximately 100 mm³ about 1 week and randomly divided into two groups (n=5 in each group) as follows: control group (100 ul, saline solution) and DDP group (15 mg/kg). Cisplatin and saline were administered by intraperitoneal injection every one week for four weeks. The longest diameter (a) and the shortest diameter (b) of the tumor were measured using digital calipers, and the tumor volume ($V = a \times b \times b / 2$) was calculated. At the end of the experiments, tumor tissues were harvested from these mice for Q-PCR or Western Blot analysis.

h2>2.11 Western Blot

Cells were lysed in RIPA buffer (Sparkjade, China) with protease inhibitor cocktail (Servicebio, China) and phosphatase inhibitor A and B on ice for 30 minutes and then centrifuged for 15 min at 12,000 rpm. After being resolved on 10% SDS-PAGE gels, proteins were transferred to a PVDF membrane and blocked with 5% BSA in TBST for 2 h at room temperature. The membranes were subsequently incubated overnight at 4 °C with the corresponding antibody GAPDH (Abclonal, AC002), RRS1 (Abcam, AB188161), AEG-1 (Abcam, ab227981), ABCG2 (ZenBio, R26465), MDR1 (Proteintech, 22336-1-AP), ERK (ZenBio, 340373), p-ERK (ZenBio, 340767), BAX (CST, #89477), Bcl-2 (CST, #3498), BAD (Abcam, ab32445), p-BAD (Abcam, ab129192), Ubiquitin (Proteintech, 10201-2-AP). After being washed with TBST three times, the blots were incubated with 1:1000 dilution of the HRP-conjugated secondary antibody (Bioss, bs-0295G-HRP), and washed again three times with TBST. The transferred proteins were visualized by ECL (MDBio,China).

2.12 Statistical analysis

The data was analysed by statistical software SPSS13. All data was expressed as mean \pm standard deviation (SD), and student's t test was used to compare the difference between two groups. $P < 0.05$ was considered as statistical significant.

3 Results

3.1 RRS1 was highly expressed in cisplatin resistant breast cancer cell MCF-7/DDP

For 24 h exposure, the half maximal (50%) inhibitory concentration (IC₅₀) values of cisplatin (DDP) for MCF-7 cells was 1.501 ± 0.026 ug/ml, and that for MCF-7/DDP cells was 3.602 ± 0.011 ug/ml, increased 2.4-fold. There were significant differences between these two groups ($P < 0.001$) (Fig. 1a). Both the results of RRS1 protein and gene detection showed significant increases of RRS1 in MCF-7/DDP (protein level: $P < 0.0001$, mRNA level: $P < 0.01$), in comparison with the parent MCF-7 cell (Fig. 1b and 1c). P-glycoprotein /MDR1/ABCB1 and breast cancer resistance protein (BCRP/ABCG2) were the marker proteins of the ABC transporter family. They had a conserved catalytic domain of ATP hydrolysis and were used to pump intracellular drugs out of cells in ATP dependent manners. These two efflux transporters were initially identified as multidrug resistant proteins in cancer cells^[25]. Combined with the resistant protein MDR-1 and ABCG2 in those two cells, the results indicated that RRS1 was closely related to the sensitivity of breast cancer cells to cisplatin (ABCG2: $P < 0.001$,MDR1: $P < 0.01$) (Fig. 1c).

3.2 RRS1 participated in the sensitivity of MCF-7 cells to cisplatin *in vivo*

To further validate the role of RRS1 in the regulation of drug resistance *in vivo*, MCF-7 cells were injected subcutaneously into athymic nude mice to generate the mouse proliferation model. As shown in Fig. 2a, comparing with control group, a significant decrease in tumor size was observed in the cisplatin-treated groups ($P < 0.01$). Next, we detected the protein and mRNA levels of RRS1 in these two groups by Western blot and q-PCR. Results showed that the content of RRS1 in cisplatin treatment group was significantly higher than that in control group ($P < 0.05$) (Fig. 2b and 2c). These results further confirmed that RRS1 was related to the chemoresistance of cisplatin *in vivo*, which was consistent with the results of cell experiment *in vitro*.

3.3 Effects of RRS1 gene silencing on proliferation, cell cycle distribution, and apoptosis of MCF-7/DDP cells

The lentiviral system was employed in the study, since it can knock down the RRS1 gene in MCF-7/DDP cells in an efficient, permanent and stable way. As is exhibited in green fluorescent protein (GFP) expression, the gene delivery efficiency had overtook 70% (Fig. 3a). RRS1 mRNA and protein detection in the cells demonstrated that this sequence could inhibit RRS1 expression (Fig. 3b and 3c). By comparing the growth curves of MCF-7/DDP before and after RRS1 gene knockdown, RRS1 interfering seemed to restrain proliferation in the drug-resistant cell lines. The cell numbers at 3 days in gene knockdown groups were significantly lower than the control groups ($P < 0.001$), and there was no obvious difference between the sh-CON group and the blank cell group ($P \geq 0.05$) (Fig. 3d). The results of cell cycle detection

indicated that RRS1 blocked cells at G1 phase in the drug-resistant cell lines (Fig. 3e), in addition, knockdown of RRS1 could significantly increase the apoptosis rate (Fig. 3f), suggesting that RRS1 has a direct impact on the proliferative activity of breast cancer.

3.4 Effects of RRS1 interference on sensitivity and relevant proteins of resistant cells to cisplatin

Disturbing RRS1 in the resistant cells significantly increased sensitivity to cisplatin. For MCF-7/DDP cells, IC_{50} values of cisplatin were reduced from 3.602 ± 0.011 ug/ml to 1.654 ± 0.02 ug/ml ($P < 0.001$), while there was no significant difference detected between the sh-CON and blank group (Fig. 4a). By comparing the influence of RRS1 silencing on proliferation, apoptosis, and drug-resistance related proteins in MCF-7/DDP, we found that p-ERK, p-BAD and Bcl-2 had evidently decreased after RRS1 silencing, while the specific value of Bcl-2/BAX, one protein promoting apoptosis, had increased obviously, consistent with the results of proliferation and cell cycle detection. The expression levels of ABCG2 and MDR1 in MCF-7/DDP cells were significantly higher than those in MCF-7, coincident with the production of drug resistance, while RRS1 disturbing had significantly inhibited their expression (Fig. 4b, 4c and 4d). All these results indicated that RRS1 was associated with chemosensitivity of breast cancer cells to cisplatin.

3.5 RRS1 regulated chemosensitivity of MCF-7/DDP cells to cisplatin was associated with AEG-1

In order to find new proteins in RRS1 related signaling pathway, we tried to find candidate proteins interacting with RRS1. AEG-1 conferred resistance to broad-spectrum chemotherapeutics, including adriamycin, cisplatin, paclitaxel, hydrogen peroxide and 4-hydroxycyclophosphamide^[26]. Studies have showed that AEG-1 interacted with MDR1, promoting the MDR1 translation and interfering MDR1 ubiquitination and proteasomal degradation without affecting the transcription level of MDR1^[20]. In this study, we found that the expression of RRS1 and AEG-1 in MCF-7/DDP was significantly higher than that in MCF-7 (Fig. 5a). the interaction between RRS1 and AEG-1, AEG-1 and MDR1 was confirmed by CO-IP experiment (Fig. 5b), however, we did not confirm the interaction between RRS1 and MDR1, which need further exploring. There was no evident change at mRNA level before and after RRS1 silencing (Fig. 5c), but changed obviously at protein contents (Fig. 5d). These data eliminated the possibility that RRS1 increased AEG-1 level through promoting the transcriptional activation of AEG-1. Then, we investigated whether RRS1 increased AEG-1 content in breast cancer cells by regulating its protein stability. The stability of AEG-1 protein was detected by protein synthesis inhibitor CHX. Considering a large number of cells are needed in the later experiment, we chose to construct RRS1 stable expression cell strain (Fig. 5e). As was presented in figure 5F, overexpression of RRS1 remarkably extended the half time of AEG-1 protein. In addition, we also found that proteasome inhibitor MG132 treatment significantly promoted the increase of AEG-1 caused by RRS1 overexpression (Fig. 5f). Therefore, to detect whether RRS1 regulated ubiquitination of AEG-1 protein, we detected the total level of ubiquitinated AEG-1 in OE-CON and OE-RRS1 cells. The cells were treated with proteasome inhibitor MG132, immunoprecipitated with anti-AEG-1

antibody, and then immunoblotted with anti-ubiquitination antibody. Compared with the OE-CON group, the ubiquitination level in the OE-RRS1 group decreased significantly ($P < 0.05$) (Fig. 5h). These results indicated that RRS1 enhanced the level of AEG-1 in breast cancer resistant cells by interfering with ubiquitination and proteasome degradation of AEG-1.

4 Discussion

Breast cancer, a cancer occurring in the breast epithelium, was the most common cancer in women worldwide with an incidence rate of 24.5%^[1]. So far, chemotherapy is still the main mean to treat this disease. However, the use of chemotherapy is usually limited by drug resistance. Acquired drug resistance was a major cause of clinical treatment failure and cancer mortality, which was often accompanied by the enhance of cell viability and metastatic potential^[27]. Therefore, it is urgent to investigate the molecular mechanism of chemoresistance.

Studies have shown that the increase of ribosomal biogenesis may promote the transformation of cells into tumor cells. Both the oncogenes overexpression and the suppressor genes expression inhibition will lead to the abnormality of ribosomal biosynthesis^[28]. RRS1 is one of approximately 70 proteins connected with rRNA in the large and small subunits of ribosome. In the process of ribosome biosynthesis, RRS1, combining with ribosomal product factor 2 (RPF2), recruited RPL5, RPL11 and 5S rRNA into the pre-90s ribosome, and then processed to form a mature 60S ribosomal subunit^[29-31]. More and more evidence have showed that ribosomal proteins possesses other functions except from joining in ribosome^[32]. Several of these examples suggested a role for ribosomal proteins in tumor multidrug resistance. For instance, when comparing the differentially expressed gene profiles between HNSC cell line UMSCC10b and its cisplatin-resistant cell UMSCC10b/Pt-S15, ribosomal protein S28 and elongation factor 1 α were identified as two upregulated genes in association with acquired cisplatin resistance. After trastuzumab treatment, the prognosis of patients with phosphorylated RPS6 highly expression was better, if there was no change, it may indicate drug resistance^[33]. Our study had confirmed that RRS1 promoted proliferation and apoptosis resistance in breast cancer cells^[6], while apoptosis resistance was one of the mechanisms of tumor chemoresistance^[13, 14]. Therefore, we explored the effect of RRS1 in the chemosensitivity of breast cancer cell to cisplatin.

Firstly, we found that cisplatin treatment induced the expression of RRS1 protein, the content of RRS1 in cisplatin treatment group was significantly higher than that in control group *in vivo*. Subsequent silencing of RRS1 reversed the drug resistance in MCF-7/DDP, indicating that RRS1 participated in mediating cisplatin resistance in MCF-7 cells. Meanwhile, the results of proteins contents showed that RRS1 effected the proliferation, cell cycle distribution and apoptosis through influencing ERK signal pathway and Bcl-2/BAX value.

The activation of AEG-1 could promote drug resistance and metastasis of breast cancer cells^[19]. Studies have showed that AEG-1 interacted with MDR1, promoting the MDR1 translation and interfering MDR1

ubiquitination and proteasomal degradation without affecting the transcription level of MDR1^[20]. Our study firstly found RRS1 and AEG-1 were highly expressed in MCF-7/DDP than those in MCF-7, and confirmed the interaction between RRS1 and AEG-1. In addition, AEG-1 decreased when RRS1 disturbing. To identify the mechanism by which RRS1 raise AEG-1 abundance in breast cancer cells, we performed q-PCR and found RRS1 knockdown didn't affected the mRNA level of AEG-1, suggesting that RRS1 couldn't increase AEG-1 level by a transcriptional mechanism. Then, we further investigated whether RRS1 affected AEG-1 protein stability in breast cancer cells. The data presented that RRS1 overexpression significantly extended the half-time of AEG-1 in MCF-7/DDP. In addition, the overexpression of RRS1 increased AEG-1 protein abundant through inhabiting AEG-1 ubiquitination after blocking the proteasomal degradation with MG132. These data demonstrated that RRS1 is critical for inhabiting the proteasomal degradation of AEG-1 and thus increasing the stability of AEG-1.

In conclusion, our study confirmed that RRS1 is associated with cisplatin resistance in breast cancer cells for the first time. It can regulate the abundance of AEG-1 through enhancing protein stability and reducing proteasome degradation, thereby affecting the chemosensitivity of drug resistant breast cancer cell to cisplatin. Considering that independent, non-coordinate changes in expression of an individual ribosomal protein gene, or of a subset of ribosomal protein genes, can occur under various cellular conditions, and have no direct association or correction with protein synthetic activities per se^[34]. Naoro found that inducing exogenous ribosomal protein S3a expression lonely in NIH 3T3 cells did not increase protein synthetic activity^[35]. Therefore, it was reasonable that the effect of RRS1 on drug resistance in breast cancer cells was not associated with ribosome function. Our findings highlighted the role of RRS1 in the chemoresistance of breast cancer cells to cisplatin and provided the possibility of RRS1/AEG-1 axis as a therapeutic target for breast cancer.

Declarations

Author Contribution Statement

FS and LH provided the idea and designed the article. CP analyzed the data, edited the figures and wrote the manuscript. RW and JS contributed to data curation. YH, QW, LD, LZ, and YC provided the methods. FS and LH reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Ethics approval

The animal study was approved by Qingdao university ethics committee (No.20201204BALB/C-nu1420210107050) and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests.

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<https://doi.org/10.1083/jcb.141.3.741>

Figures

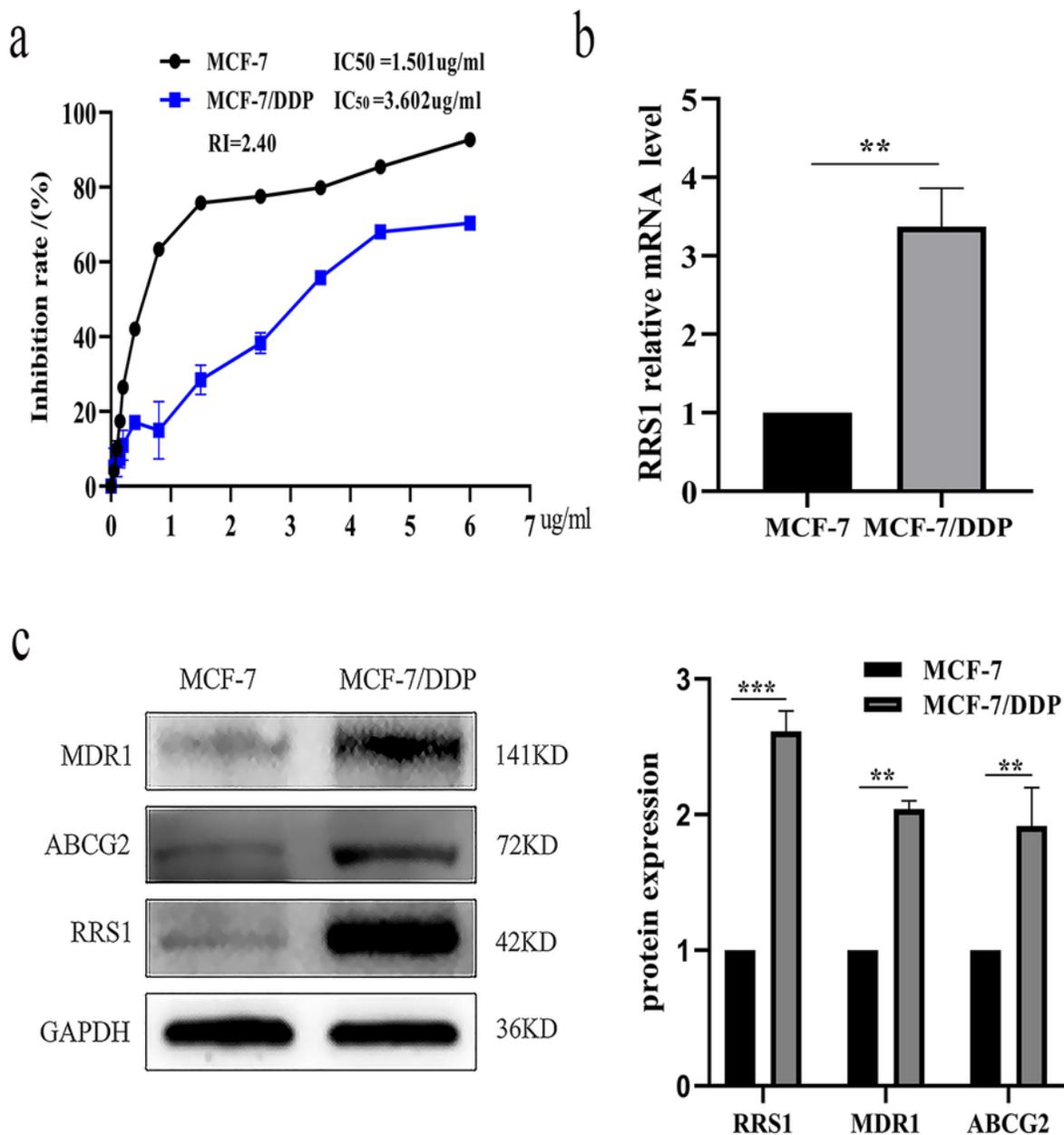


Figure 1

Identification of the drug resistant cell (MCF-7/DDP) line and its expression of RRS1. (a) IC₅₀ of DDP for MCF-7 and MCF-7/DDP cells; (b) mRNA levels of RRS1 were assessed by q-PCR in the indicated cell lines; and (c) Protein levels of RRS1, ABCG2, MDR1 were assessed by Western Blotting in the indicated cell lines. Data are expressed as mean \pm SD at least three independent experiments. ** P < 0.01 and *** P < 0.001, when compared to the corresponding MCF-7 group.

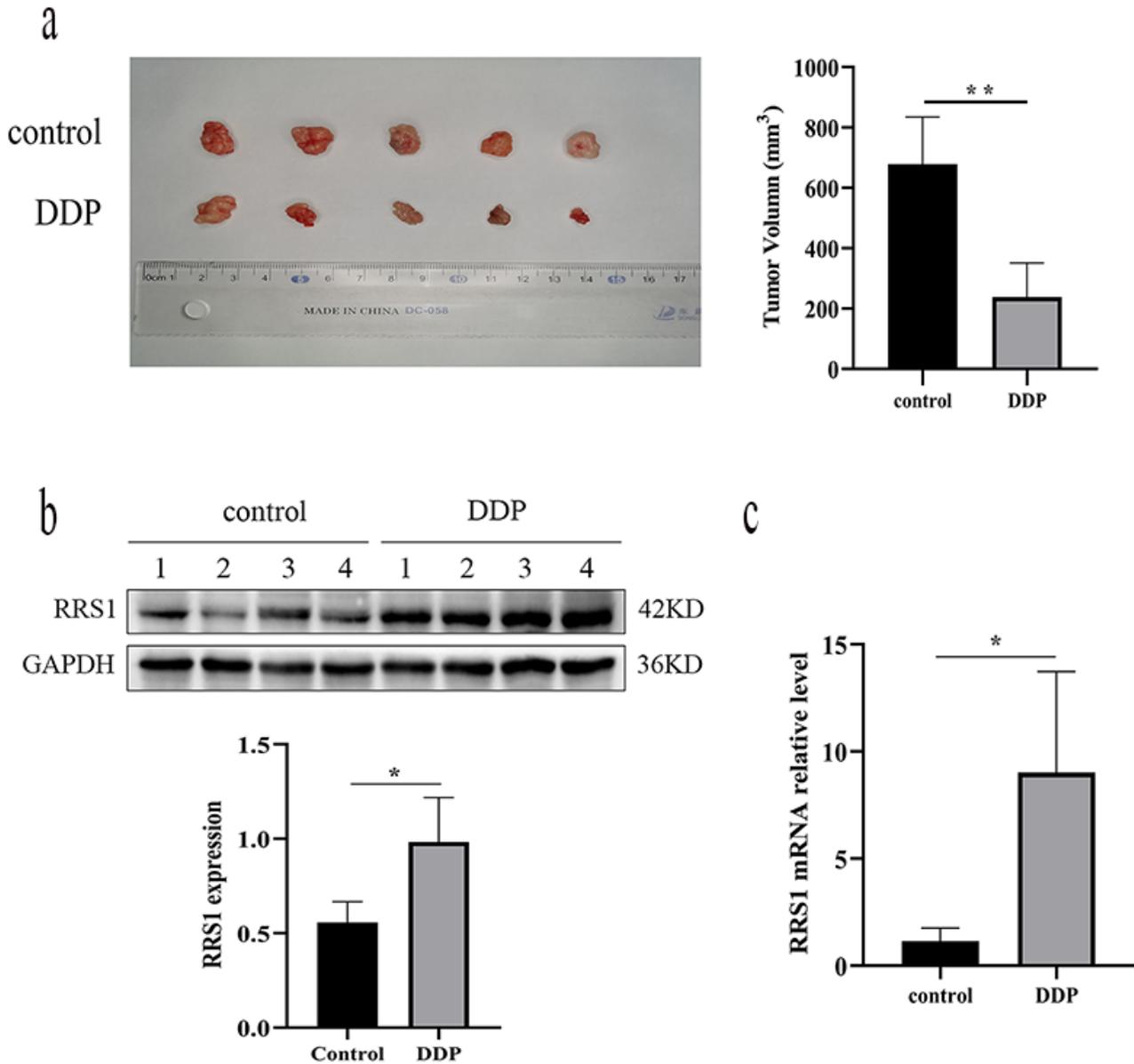


Figure 2

RRS1 participated in the sensitivity of MCF-7 cells to cisplatin in vivo. (a) MCF-7 cells were injected into nude mice, which were treated with DDP 1 time/week for 4 weeks at a dose of 15 mg/kg. Tumors were removed from nude mice and measured using a Vernier caliper at the end of the study; (b) Proteins were extracted from tumor tissues of two groups and detected by Western blot; (c) RNA was extracted from tumor tissues of two groups and detected by q-PCR. Data are expressed as mean \pm SD at least three independent experiments. * $P < 0.05$ and ** $P < 0.01$, when compared to the normal saline treatment group.

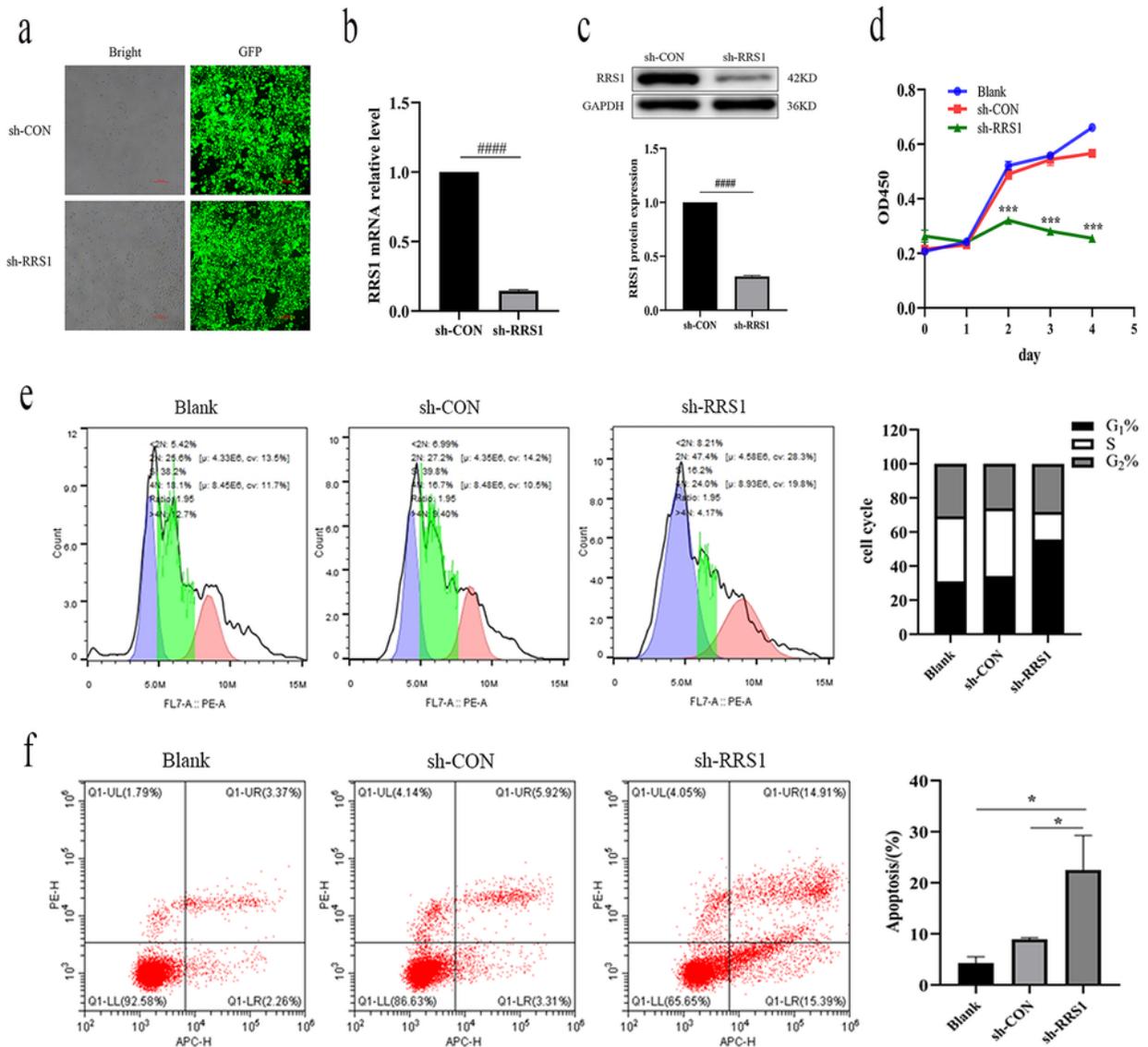


Figure 3

RRS1 knockdown inhibited proliferation, changed cell cycle distributions and promote cell apoptosis in MCF-7/DDP cell. (a) MCF-7/DDP cells were infected with lentiviral virus and GFP was observed under fluorescence microscopy after 72h, scale bars = 100 um; (b) MCF-7/DDP cells were infected with Lv-Con or Lv-shRNA and mRNA levels of RRS1 were assessed by q-PCR; (c) MCF-7/DDP cells were infected with Lv-Con or Lv-shRNA and protein levels of RRS1 were detected by Western Blot; (d) Growth curves of MCF-

7/DDP before and after RRS1 gene knockdown; (e) Cell cycle distribution. Data were obtained by flow cytometry and FlowJo, presented as mean (n = 3). Purple bars represented the percentages of G1 fractions, green bars represented the percentage of G2 fraction, and the pink bars represented the percentage of S fraction; (f) Apoptosis rates before and after RRS1 knockdown. Data are expressed as mean \pm SD of at least three independent experiments. ####P < 0.0001, when compared with sh-CON group; *P < 0.05 and ***P < 0.001, when compared to the blank cells.

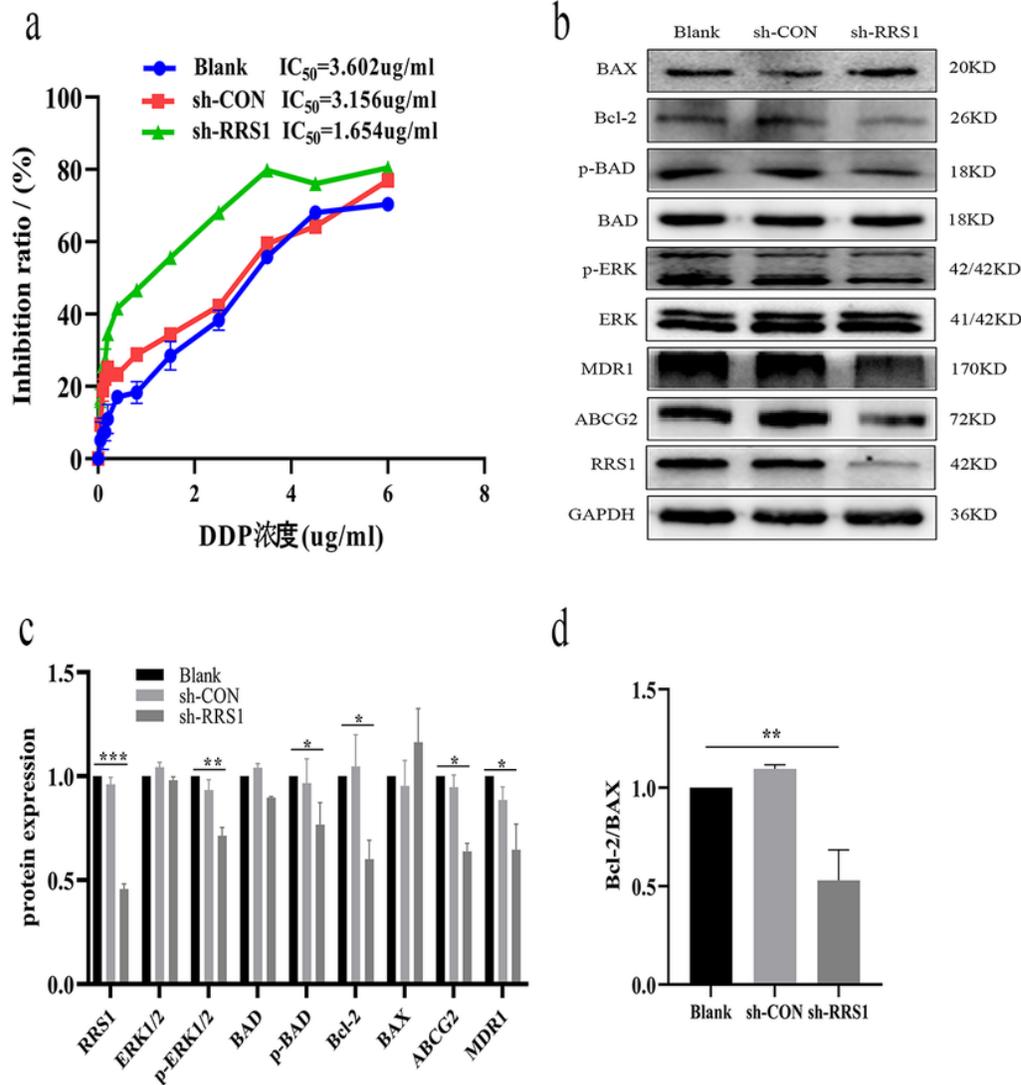


Figure 4

Effects of RRS1 silence on sensitivity and relevant proteins of resistant cells to cisplatin. (a) IC50 of DDP for MCF-7/DDP cells before and after RRS1 silencing; (b) Effects of RRS1 knockdown on relevant proteins; (c) Quantitative results of related proteins; (d) Changes of Bcl-2/BAX value. Data are expressed as mean \pm SD of at least three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001, when compared to the blank cells.

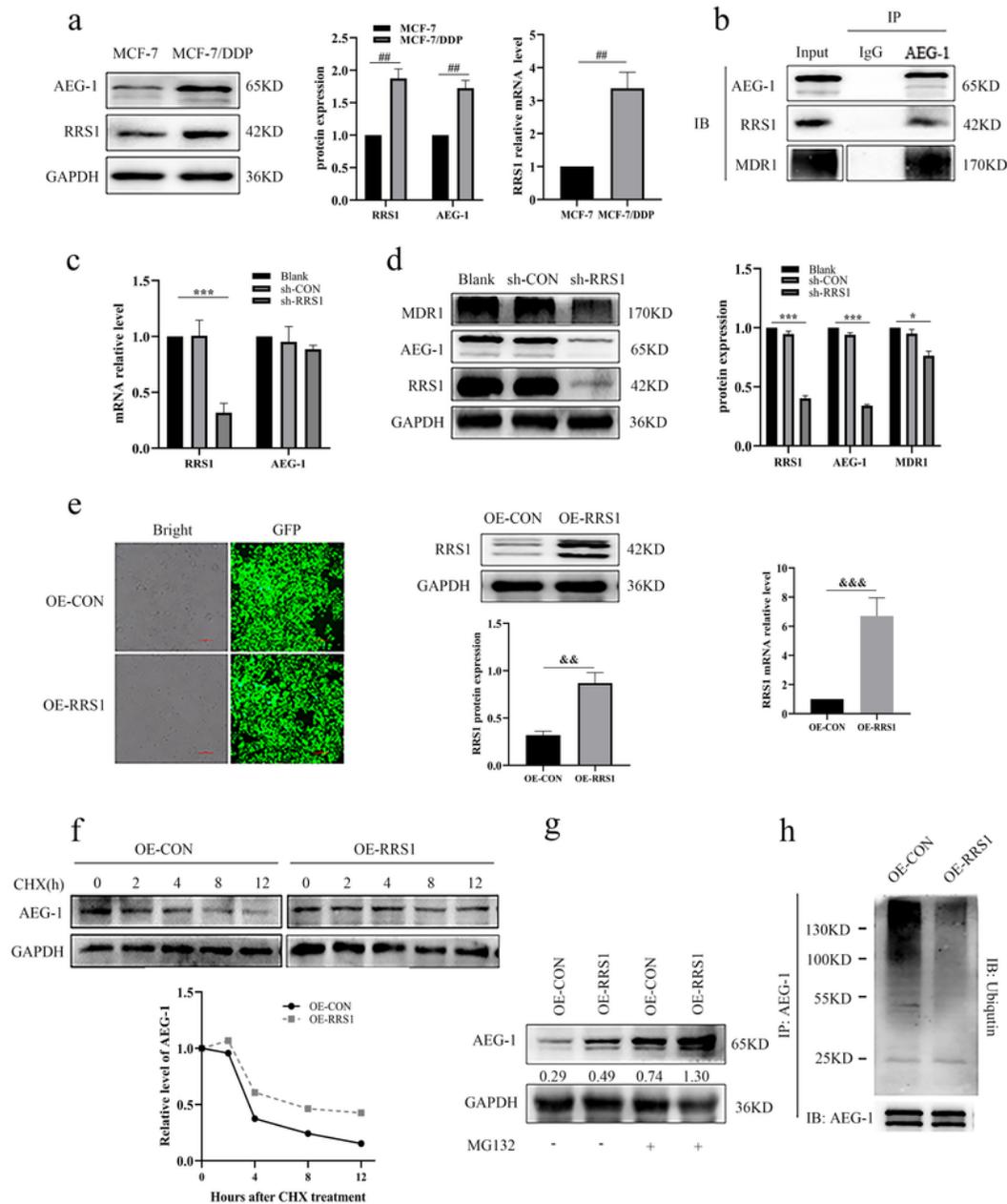


Figure 5

RRS1 regulated chemosensitivity of MCF-7/DDP cells to cisplatin was associated with AEG-1; (a) The expression of RRS1 and AEG-1 in MCF-7 and MCF-7/DDP. (b) Co-IP verified whether AEG-1, RRS1 and MDR1 could form protein complexes in MCF-7/DDP cells; (c) the mRNA level change before and after RRS1 knockdown; (d) the protein contents before and after RRS1 silencing; (e) Stable transfection strain with stably and highly expressed RRS1 was constructed. After 72 h transfection, the cells were screened with puromycin of 1ug/ml for 1 week, and the working solution concentration was 5 mg/ml; (f) The protein half-life of AEG-1 was analyzed following treatment with CHX (200 ug/ul); (g) Protein expression after MG132 (10 μ M) treating for 12 h; (h) Cells were treated with MG132 (10 μ M) for 12 h, the cell lysates were subjected to immunoprecipitation by anti-AEG-1 antibody, then the immunoprecipitation were subjected to Western blot analysis using anti-Ubiquitin antibody. Data are expressed as mean \pm SD of at least three independent experiments. ##P < 0.01, when compared with MCF-7 group; *P < 0.05, **P < 0.01 and ***P < 0.001, when compared to the blank cells; &&P < 0.01 and &&&P < 0.001, when compared with OE-CON group.