

ORIGINAL ARTICLE

RUNX3 reverses cisplatin resistance in esophageal squamous cell carcinoma via suppression of the protein kinase B pathway

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Keywords

Akt1; cisplatin-resistance esophageal squamous cell carcinoma; RUNX3.

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Received: 28 February 2016;

Accepted: 2 May 2016.

doi: 10.1111/1759-7714.12370

Thoracic Cancer 7 (2016) 570–580

Abstract

Background: Preoperative chemoradiation combined with surgery has been of focus recently in order to improve prognosis in esophageal squamous cell carcinoma (ESCC) patients. Finding biological markers that may assist in predicting the therapeutic effect of chemoradiation may benefit the treatment effect. In this study, the role of RUNX3 in the formation of cisplatin resistance in ESCC was examined.

Methods: The study enrolled 103 stage IIa–IIIb ESCC patients who had undergone esophagectomy. RUNX3 expression in ESCC tissue was detected.

Results: A higher expression of RUNX3 in ESCC patients correlated with a more sensitive response to cisplatin-based chemotherapy. A consistently lower expression of RUNX3 was found in the ESCC tissues of patients who agreed to perioperative chemotherapy compared with patients who had undergone no preoperative treatment. A lower RUNX3 expression in cisplatin-resistant ESCC cell lines, Eca109 and TE-1, was observed compared with parental cell lines. Heterologous RUNX3 expression significantly suppressed cisplatin resistance in Eca109 and TE-1, both in vitro and vivo. Meanwhile, heterologous RUNX3 expression could inhibit growth and induce apoptosis in cisplatin resistant Eca109 and TE-1 cell lines in vitro. Remarkable inhibition of the Akt pathway was observed in heterologous RUNX3 expression in Eca109 and TE-1. Silencing Akt1 could reverse cisplatin resistance in Eca109 and TE-1.

Conclusion: Our results confirmed that a loss of RUNX3 in ESCC may contribute to cisplatin-resistance. RUNX3 could reverse cisplatin resistance via suppression of the Akt pathway in ESCC patients.

Introduction

Esophageal carcinoma (EC) is one of the most common digestive tumors worldwide and its incidence rate remains high. Esophageal squamous cell carcinoma (ESCC) is the most common EC in China.¹ Because patients in early stage have no obvious symptoms, most patients with ESCC are diagnosed at advanced stage. Surgery still remains the first choice for resectable ESCC; however, the therapeutic effect is not satisfactory: the five-year survival rate ranges from 30–50%. Our former study demonstrated that 50% of patients experience recurrence 2–3 years after esophagectomy, with main

recurrence patterns of regional lymphatic and hematogenous metastases.^{2,3}

Preoperative chemoradiation combined with surgery was recommended by the 2014 National Comprehensive Cancer Network guidelines for ESCC patients with local advanced stage. Several clinical trials have shown that a response to preoperative chemoradiation is crucial for the prognosis of patients after surgery: an obvious improvement to survival was observed in 25–30% of patients, with pathological complete response after surgery.^{4–6} Thus, to avoid ineffective treatment, finding biological markers that could predict the response to chemoradiation would

benefit clinical treatment. Several biological markers, such as p53, excision repair cross-complementation group 1 (ERCC1), proliferating cell nuclear antigen (PCNA), bcl-2-like protein 4 (Bax), and epidermal growth factor receptor (EGFR) have been confirmed to be correlated with the therapeutic effect of chemoradiation in EC.^{7–11}

RUNX3, as a member of Runt-related transcription factor family, could directly bind with the promoter of certain target genes through the common “runt” motif to regulate transcription. A number of studies have revealed the important role of RUNX3 in cell differentiation and tissue development, such as gastric epithelial growth, neurogenesis of dorsal root ganglia, and T-cell differentiation.¹² Interestingly, recent research has reported a loss of RUNX3 in various cancers, including gastric, colon, breast, and lung cancers.¹³ Subsequent studies have confirmed that RUNX3 is involved in several important signal pathways ways to inhibit carcinogenesis. RUNX3 could: form a complex with receptor-regulated (R)-Smads and P300 to achieve the tumor suppressor function of the transforming growth factor (TGF)- β pathway; attenuate the WNT signaling pathway via suppression of the DNA binding ability of β -catenin/transcription factor 4 (TCF4) complex; and suppress the Notch and protein kinase B (Akt) signal pathways through direct binding to the promoter of Jagged-1 and Akt1 to suppress transcription.^{14–17} Sakakura *et al.* proved a relationship between RUNX3 and radioresistance in ESCC.¹⁸ Meanwhile, recent studies have shown that a loss of RUNX3 in pancreatic, gastric, and lung cancers and hepatocellular carcinoma could increase multiple drug resistance.^{19–22} However, a correlation between RUNX3 expression and drug resistance in ESCC has not yet been reported. In this study, we investigate the effect of RUNX3 on drug resistance and reveal underlying molecular mechanisms.

Methods

Patients

The study enrolled 103 stage IIa–IIIb ESCC patients who had undergone esophagectomy in the Department of Thoracic Surgery at the Provincial Hospital Affiliated to Shandong University from January 2009 to January 2012. Preoperative chemotherapy (30 mg/m² cisplatin, days 1–3; 175 mg/m² paclitaxel, day 1, 30 mg/m² cisplatin, days 2–4; 1000 mg/m² gemcitabine, days 1 and 8 for 2 cycles) was administered to 38 patients; 33 patients underwent esophagectomy; five patients were treated with chemoradiation; and 65 patients refused preoperative treatment. Tumor node metastasis (TNM) staging was determined using 2009 International Union Against Cancer (UICC) criteria and

lymph node dissection was undertaken according to the lymph node mapping system for EC established by the American Joint Committee on Cancer (AJCC) in 1997. Written informed consent was obtained from all patients and the Medical Ethics Committee of Shandong Provincial Hospital approved the study protocol.

Cell lines and culture

Human ESCC cell lines Eca109 and TE-1 were obtained from the Cancer Institute and Hospital, Chinese Academy of Medical Sciences, China. The cells were cultured in RPMI 1640 enriched with 1% penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and 10% fetal bovine serum (Hyclone, Logan, UT, USA). Cell culture plates were maintained in humidified incubators with 5% CO₂ at 37°C.

Real-time reverse transcription-polymerase chain reaction

The extraction of total cell ribonucleic acid (RNA) was performed using RNAiso Plus (TaKaRa, Dalian, China) according to the manufacturer's instructions. The OD260/280 value was measured to identify the purity of RNA by ultraviolet spectrophotometer. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed using a LightCycler 480 II Real-Time PCR System (Roche, Basel, Switzerland) with SYBR Premix Ex Taq (TaKaRa, China) according to the manufacturer's instructions. The cycling conditions were as follows: five minutes at 95°C for preincubation, 45 amplification cycles of 10 seconds at 95°C, 10 seconds at 60°C, and 10 seconds at 72°C. PCR primers were as follows: RUNX3, 5'-TTC CAG TGA GGA CAG GCC AGA-3' (forward) and 5'-AGG GGC CGG ACT CGT CAT ACT-3' (reverse); GAPDH, 5'-AGA AGGCTG GGG CTC ATT TG-3' (forward) and 5'-AGG GGC CAT CCA CAG TCT TC-3' (reverse). PCR was performed at least three times in all samples. The expression level of RUNX3 messenger (m)RNA was calculated using a ratio of RUNX3 mRNA against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA.

Immunohistochemistry

The formalin-fixed, paraffin-embedded tissues were cut into 4 μ m sections and then deparaffinized and incubated with 3% hydrogen peroxide. Specific mouse polyclonal anti-RUNX3 antibody (Abnova, Taipei, Taiwan) and specific mouse polyclonal anti-AKT1 antibody (Abcam, Cambridge, UK) were used at a dilution of 1:100 and incubated at 4°C overnight. For the negative control, the primary antibody was replaced by phosphate buffered saline (PBS). Subsequently, the secondary biotinylated

antibody kit instructions were followed (Zhongshan Biotech, Beijing, China). Two independent pathologists blinded to the clinical data examined all sections. The immunohistochemical score (IHS) was calculated by combining the proportion score (percentage of positive stained cells) with the staining intensity score. The proportion score ranged from 0–4, as follows: 0 (< 5%), 1 (5–24%), 2 (25–49%), 3 (50–74%), and 4 (\geq 75%). The staining intensity was scored as follows: 0 (negative), 1 (weak), and 2 (strong).

Stable transfection by lentivirus

The lentiviral vector containing specific small interfering (si)RNA targeting Akt1 (siAkt1, specific sequence targeting Akt1: 5'-GGA GAU CAU GCA GCA UCG C-3'), full-length cDNA of RUNX3 (-R3) or a dummy sequence which served as a scrambled negative control, were obtained from GeneChem (Shanghai, China). The ESCC cell lines were transfected by the lentivirus according to the manufacturer's instructions, with a multiplicity of infection of 50:1. Twenty-four hours later, the medium containing the lentivirus was replaced with complete medium containing 1 μ g/mL puromycin to pick out the stable transfected cells.

Immunoblotting

Immunoblotting was performed as previously described.²³ Antibodies against RUNX3 were obtained from Abcam; antibodies against Akt1, phosphorylated (p)Akt1, B-cell lymphoma 2 (bcl2), bcl-xl, Bax, cyclinD1, and p21 were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibodies against β -actin were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA).

Cell Counting Kit-8 assay

Initially, 3×10^4 cells were seeded in 96-well plates (3 wells per group). Cell counting kit-8 (CCK-8) was added to the wells (10 μ L per well) during the experimental period. After two hours of incubation at 37°C in 5% CO₂, optical density (OD) was determined by a Tecan Infinite M200 Multimode microplate reader (Tecan, Basel, Switzerland) at the absorbance of each well at 450 nm wavelengths. Triplicate wells were used for each data point. All experiments were performed in triplicate, and the average of the results was calculated.

Flow cytometry analysis

Cells were harvested at the 70% confluent stage and fixed in 70% ethanol at -20°C. After washing with PBS, the cells

were treated with PBS containing 100 mg/mL ribonuclease A at 37°C for 30 minutes. After centrifugation, the cells were resuspended in PBS containing 50 μ g/mL propidium iodide and stained at room temperature for 30 minutes. An Annexin V-PE/7AAD Apoptosis Detection Kit (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used to detect apoptosis, according to the manufacturer's instructions. DNA content and apoptosis were evaluated by a FACSaria III cytometer and BD FACSDiva 7.0 software (Becton, Dickinson and Company, USA).

Hoechst staining

The cells were fixed in 4% paraformaldehyde for 20 minutes. After washing with PBS three times, the cells were stained with Hoechst 33 258 (Beyotime, Beijing, China) for five minutes. The cells were then washed with PBS another three times and coverslips were sealed with a drop of Prolong Gold antifade reagent (Invitrogen, Carlsbad, CA, USA). Images were acquired by Eclipse Ti laser scanning microscopy and analyzed by NIS-Elements D 3.2 (Nikon, Tokyo, Japan). Five random visual fields were counted to calculate the apoptosis rate.

Xenograft transplantation

BALB/c-nu mice (4–5 weeks old, 16–18 g) were purchased from the Experimental Animal Center of Shandong University. The Institutional Animal Care and Use Committee of Shandong University approved all experimental procedures. The BALB/c nude mice were randomly divided into two groups ($n = 6$ /group). The Eca109-PR cells (1×10^7) transfected with the lentivirus containing full-length cDNA of RUNX3 or the dummy sequence were inoculated into subcutaneous tissue. Principles of Laboratory Animal Care were followed and the Medical Ethics Committee of Shandong Provincial Hospital approved the animal study protocol.

Results

RUNX3 expression correlated with adjuvant chemotherapy sensitivity in esophageal squamous cell carcinoma (ESCC) patients

Thirty-eight patients with local advanced ESCC agreed to two cycles of adjuvant chemotherapy, based on cisplatin and paclitaxel/gemcitabine. In the 17 patients who responded to adjuvant chemotherapy (the transverse diameter of the tumor was reduced at least 30%), the RUNX3 IHS was 4.647 ± 0.500 , while in patients who experienced no response to adjuvant chemotherapy (the transverse diameter of the tumor was reduced less than 30%), the

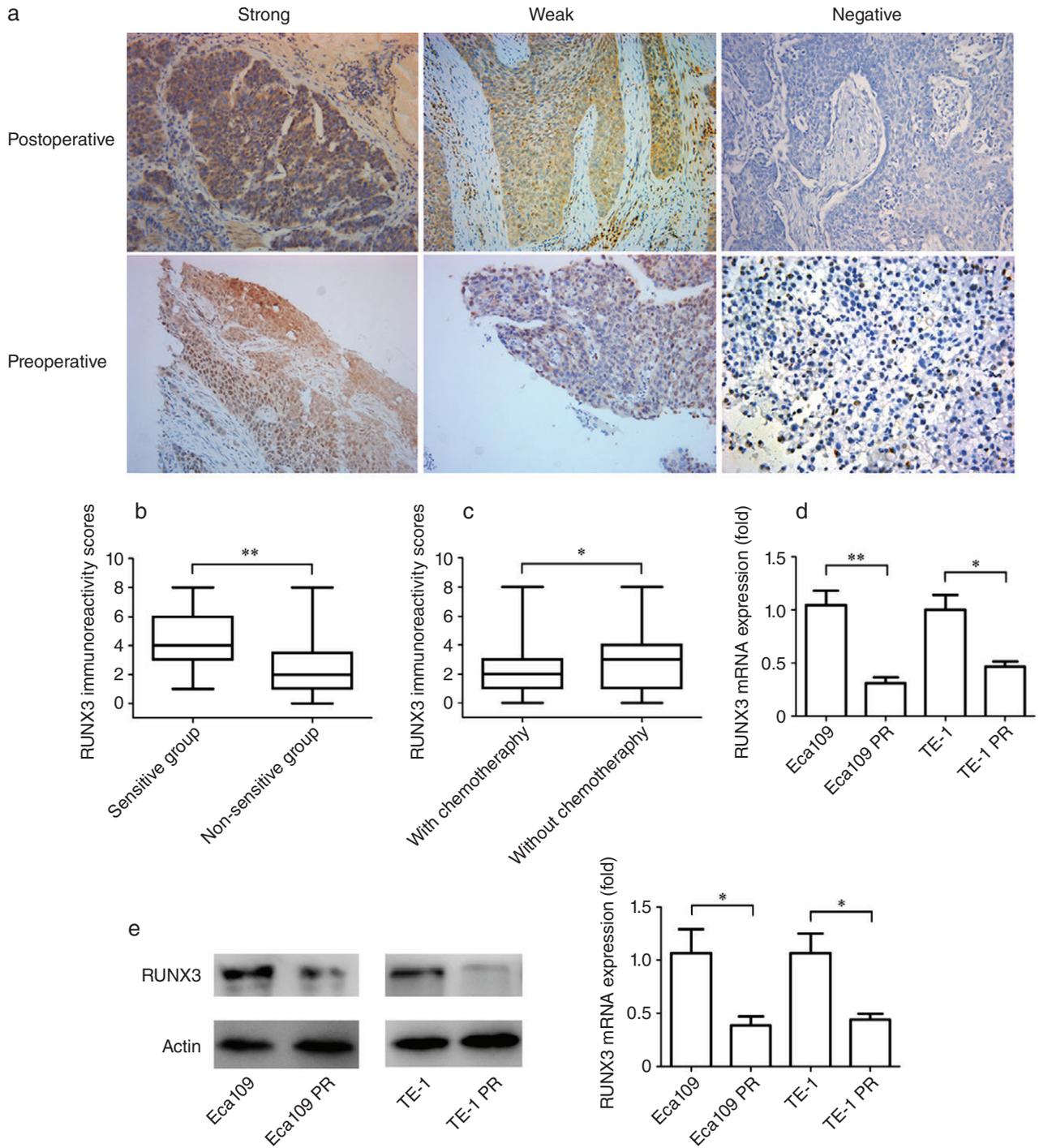


Figure 1 Expression of RUNX3 in esophageal squamous cell carcinoma (ESCC): **(a)** RUNX3 expression in ESCC derived from preoperative biopsy and postoperative pathology. **(b)** The RUNX3 immunohistochemical score (IHS) in a group of ESCC patients sensitive to preoperative chemotherapy was significantly higher than in the non-sensitive group. **(c)** The RUNX3 IHS in ESCC patients that received preoperative chemotherapy was significantly lower than those who did not receive preoperative chemotherapy. **(d, e)** The decreased RUNX3 expression in cisplatin-resistant Eca109 and TE-1 compared with parental Eca109 and TE-1, in both messenger ribonucleic acid (mRNA) and protein level (* $P < 0.05$, ** $P < 0.01$).

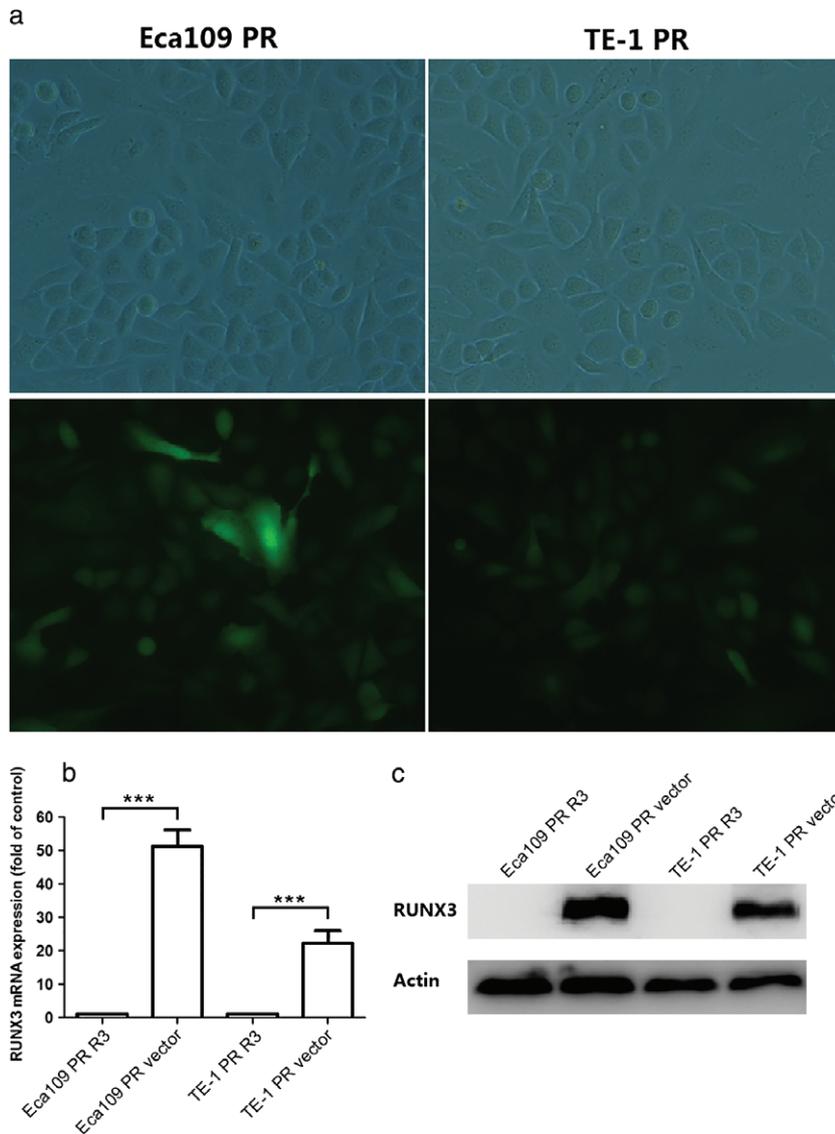


Figure 2 Heterologous expression of RUNX3 in esophageal squamous cell carcinoma (ESCC) cell lines. (a) Representative graphs of Eca109 infected with indicated lentivirus. (b) RUNX3 protein expression levels after stable transfection. (c) RUNX3 messenger ribonucleic acid (mRNA) expression level after stable transfection (***P* < 0.001).

RUNX3 IHS was 2.381 ± 0.672 . RUNX3 expression was significantly higher in the group that responded to treatment (*P* < 0.01, Fig 1a,b).

Downregulation of RUNX3 in ESCC tissue of patients treated with adjuvant chemotherapy

To further confirm the relationship between RUNX3 and chemotherapy sensitivity, we detected RUNX3 expression in another 65 tissue samples derived from ESCC patients who did not undergo any preoperative treatment. The RUNX3 expression level in ESCC patients who were treated with adjuvant chemotherapy was significantly lower than in the patients not treated with chemotherapy (IHS 1.972 ± 0.321 vs. 3.062 ± 0.304 ; *P* = 0.026, Fig 1c).

Downregulation of RUNX3 in ESCC cisplatin-resistant cells

Considering that there is no standard chemotherapy regimen for ESCC and that cisplatin-based chemotherapy is the common regimen applied in clinics, we explored the effect of RUNX3 on cisplatin resistance in ESCC cells. We treated ESCC cell lines Eca109 and TE-1 with cisplatin in gradually increasing concentrations to establish cisplatin resistance (Eca109PR and TE-1PR). The cisplatin-resistant cell lines were cultured in complete medium with cisplatin at 1ug/ml. We then detected RUNX3 expression in parental and cisplatin-resistant cell lines, with the results confirming RUNX3 downregulation, both on mRNA and protein level in the cisplatin-resistant cell lines (Fig 1d,e).

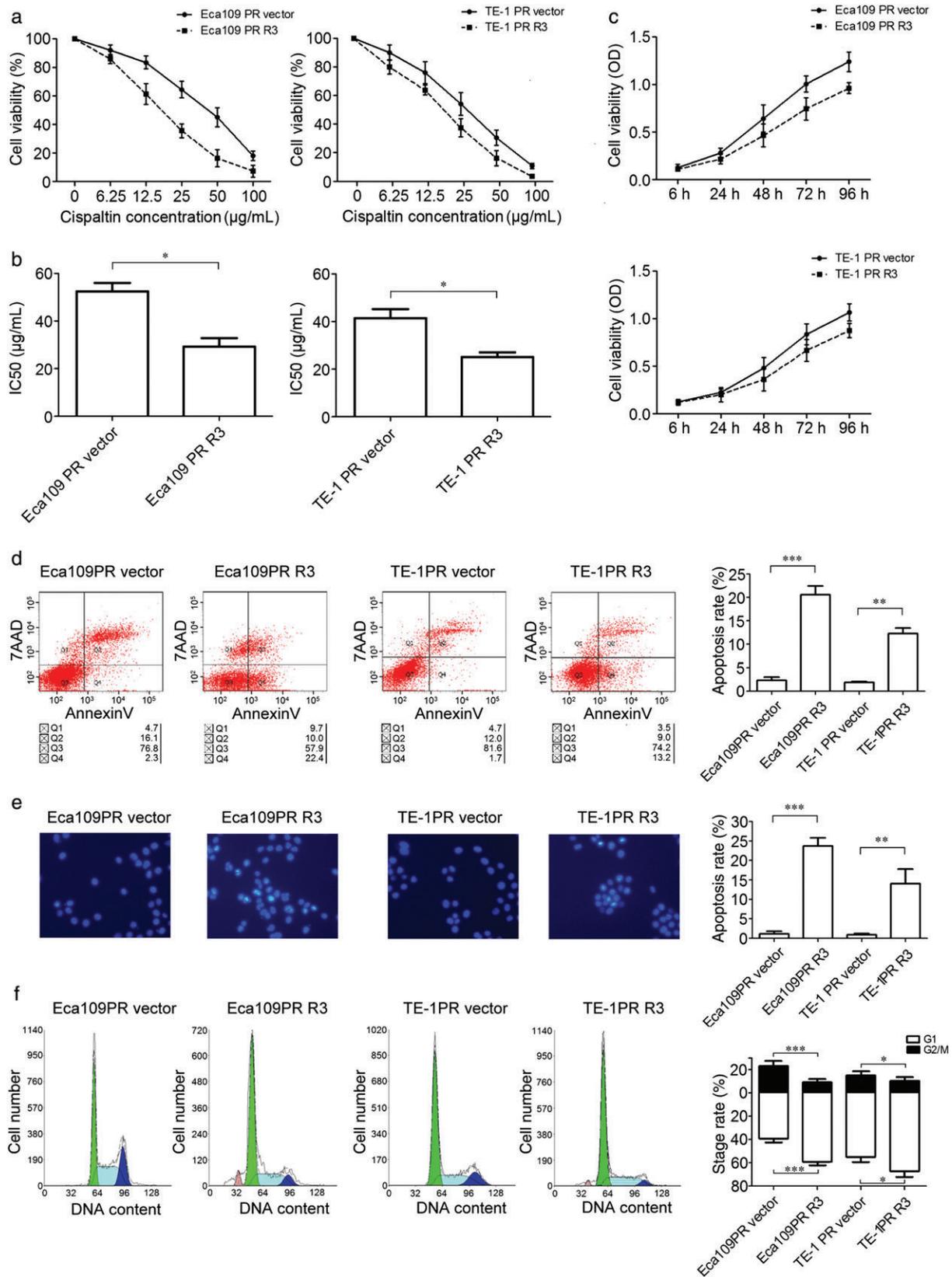


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Heterologous RUNX3 reversed cisplatin resistance in ESCC cell lines

The IHC results showed significant RUNX3 downregulation in patients treated with adjuvant cisplatin-based chemotherapy. The cisplatin-resistant cell lines also showed lower RUNX3 expression compared with the parental cell lines. To detect the effect of RUNX3 on the response to cisplatin, we transfected the cisplatin-resistant cell lines with lentivirus containing the full length of RUNX3 cDNA or the lentivirus with the dummy sequence as the control. Quantitative RT-PCR and Western blot confirmed RUNX3 upregulation in the cisplatin-resistant cell lines (Fig 2). The cisplatin inhibitory concentration (IC)₅₀ value was significantly reduced by heterologous RUNX3 (Fig 3a,b).

Heterologous RUNX3 suppressed growth and promoted apoptosis in ESCC cisplatin-resistant cell lines in vitro

We attempted to identify the role of RUNX3 on the growth of cisplatin-resistant ESCC cell lines. CCK-8 assay confirmed that heterologous RUNX3 remarkably suppressed the growth ability of cisplatin-resistant ESCC cell lines (Fig 3c). We then used cell flow cytometry and Hoechst staining to detect the role of RUNX3 on the apoptosis and cell cycle of ESCC cell lines, and the results showed that heterologous RUNX3 could promote apoptosis, induce G1 arrest, and decrease G2/M compared with the control (Fig 3d,e,f). Therefore, RUNX3 could inhibit ESCC cell growth via the regulation of cell cycles and induce apoptosis.

RUNX3 reversed cisplatin resistance via suppression of the protein kinase B pathway

Considering the important role the Akt pathway plays in regulating the cell cycle and inhibiting apoptosis in tumor cells, and the function of RUNX3 on the attenuating Akt pathway, we attempted to identify the effect of RUNX3 on Akt pathway activation in ESCC cell lines. Western blot showed a decreasing level of Akt1 and phospho (p)-Akt in Eca109PR-R3 and TE-1PR-R3 compared with the control group, and a consistent change of downstream genes in the

Akt pathway, which are involved in the cell cycle and apoptosis; bcl-2, bcl-xl, cyclinD1 and p21 were also observed (Fig 4a). Further, we detected RUNX3 and Akt1 expression in 65 ESCC samples by IHC, and a negative correlated expression was confirmed (Fig 4b,c). To identify whether the activated Akt pathway was responsible for cisplatin resistance in ESCC cells, Akt1-silencing was established on Eca109PR and TE-1PR lines. The IC₅₀ cisplatin value was significantly decreased in Eca109PR-small interfering (si)Akt1 and TE-1PR-siAkt1 compared with the control group. We also observed a consistent increase in apoptosis, G1 arrest, and a decrease in the G2/M phase in the siAkt1 ESCC cell lines compared with the controls (Fig 5a–c). Furthermore, a decrease in bcl-2, bcl-xl, cyclinD1 and an increase in p21 and Bax were detected in the in Eca109PR-siAkt1 and TE-1PR-siAkt1 compared with the controls (Fig 4a).

Heterologous RUNX3 reversed cisplatin resistance in ESCC cells in vivo

To further investigate the role of RUNX3 on cisplatin resistance in ESCC cell lines in vivo, the ESCC cells expressing RUNX3 and corresponding NC cells were subcutaneously inoculated into nude mice. When the average tumor size reached 50 mm³, cisplatin was administered via intraperitoneal injection at a dose of 2.0 mg/kg (one dose every other day, for a total of 2 weeks). Tumors derived from Eca109PR-RUNX3 cells grew more slowly when compared with NC-transfected cells. All mice were sacrificed two weeks after initial cisplatin treatment. The average size of tumors derived from Eca109PR-R3 cells was significantly reduced compared with the NC-transfected cells (1730 ± 289 mm³ vs. 401 ± 111 mm³; *P* < 0.01, Fig 6).

Discussion

Because the therapeutic effect of preoperative chemoradiation has gradually been realized, it has been applied in the treatment of ESCC patients. The therapeutic response directly determines the outcome for patients after surgical treatment. Rizk *et al.* reported that 25–30% of ESCC patients could achieve a pathological complete response and, thus, these patients had a remarkably better

Figure 3 Heterologous RUNX3 expression reversed cisplatin resistance, inhibited growth, and induced apoptosis and G1 arrest in esophageal squamous cell carcinoma (ESCC) cells. (a) The cells were treated with medium containing cisplatin at concentrations of 0, 6.25, 12.5, 25, 50, and 100 µg/mL for 24 hours, then cell counting kit-8 assay was performed to detect cell viability. (b) Inhibitory concentration (IC)₅₀ in the heterologous RUNX3 group was significantly reduced compared with the control. (c) The growth curves of Eca109PR R3, Eca109 PR vector, TE-1PR R3, and TE-1PR vector. Cell proliferation was determined at six, 24, 48, 72, and 96 hours, respectively. (d) Apoptosis detected by flow cytometry. Higher early apoptosis rates were detected in Eca109PR R3 and TE-1PR R3 compared with the control. (e) Apoptosis of Eca109PR R3, TE-1PR R3 and corresponding control were detected by Hoechst staining. (f) Cell cycle detected by cytometry; higher G1 arrest was observed in Eca109PR R3 and TE-1PR R3 compared with the control (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

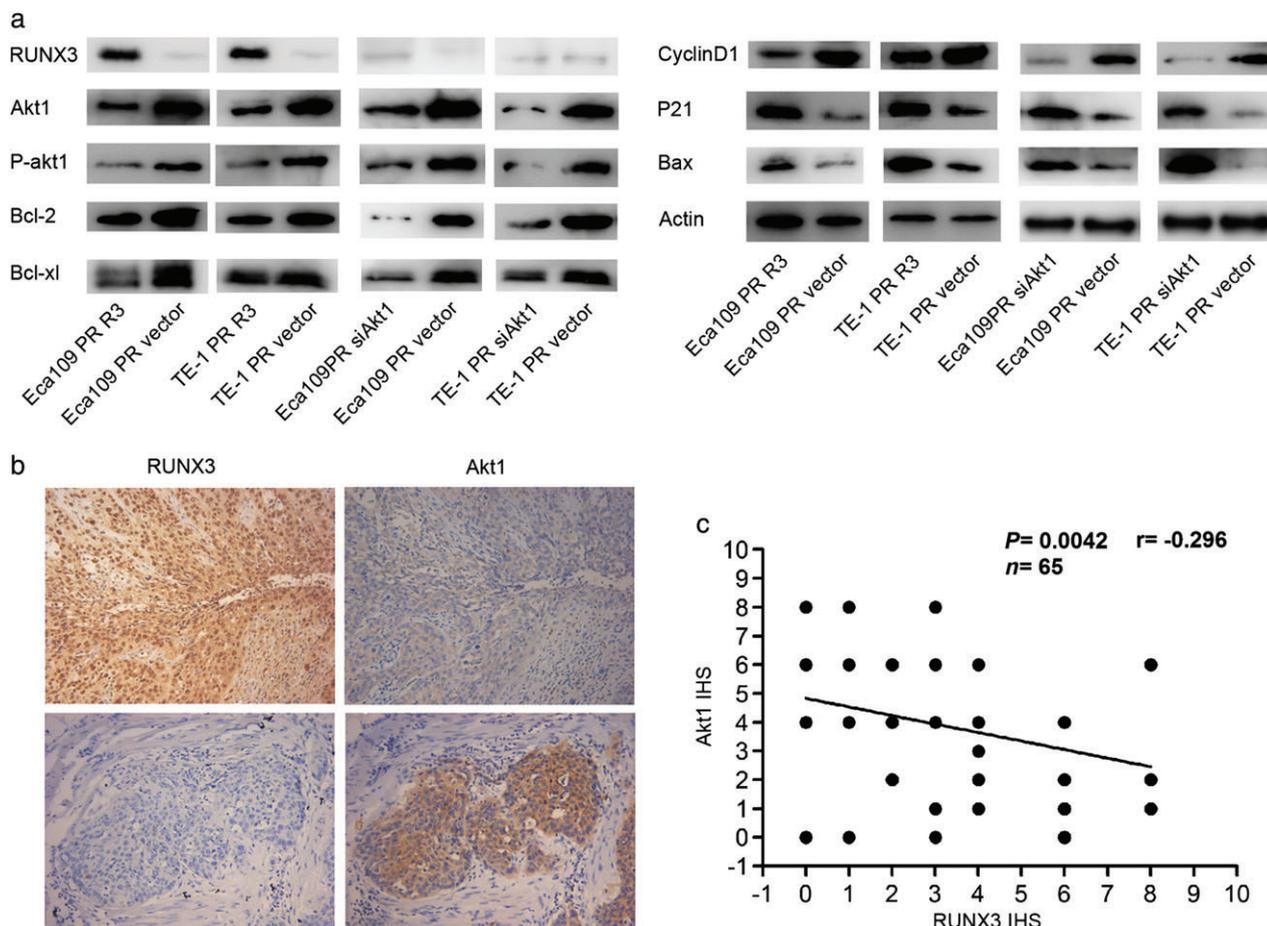


Figure 4 RUNX3 attenuated protein kinase B (Akt) pathway in esophageal squamous cell carcinoma (ESCC) (a) Western blot showed heterologous RUNX3 suppressed the Akt pathway in Eca109PR R3 and TE-1PR R3 cell lines. (b) RUNX3 and Akt1 expression in ESCC tissue samples detected by immunohistochemistry (IHC) (c) RUNX3 expression negatively correlated with the Akt1 expression in ESCC tissue samples ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

prognosis.²⁴ Reynolds *et al.* found that pN0 staging after preoperative chemoradiation was an independent prognostic factor in ESCC patients.²⁵ Therefore, finding biological markers that could predict the preoperative adjuvant therapeutic effect would benefit the effectiveness of ESCC treatment.

RUNX3 loss has been reported in various cancers, including ESCC. Our former study also confirmed that constant inactivation of RUNX3 correlated with poor prognosis in ESCC patients and that heterologous RUNX3 expression could reverse the malignant phenotype in ESCC cell lines.^{26,27} Recently, several studies have demonstrated a correlation between RUNX3 expression and multiple drug resistance. Horiguchi *et al.* reported that a loss of RUNX3 induced gemcitabine resistance in pancreatic cancer and that heterologous RUNX3 could reverse gemcitabine-resistance.¹⁹ Guo *et al.* reported that RUNX3 loss correlated with multiple drug resistance in gastric cancer cells.²⁰ Xu,

et al. found that RUNX3 could reverse cisplatin resistance in hepatocellular carcinoma cells.²¹ Zheng *et al.* revealed the mechanism of paclitaxel-resistance as a result of the frequent silencing of RUNX3 in non-small cell lung cancer (NSCLC).²² In this study, IHC showed that RUNX3 expression correlated with a more sensitive response to cisplatin-based chemotherapy and a consistently lower expression of RUNX3 in the ESCC tissue of patients treated with perioperative chemotherapy. These results indicate that RUNX3 may correlate to cisplatin resistance. Furthermore, we observed lower RUNX3 expression in cisplatin-resistant ESCC cell lines compared with parental cell lines, and RUNX3 heterologous expression significantly reversed cisplatin resistance in ESCC cell lines. All of these results confirm that RUNX3 improves cisplatin sensitivity in ESCC patients.

Obviously, the proliferation and anti-apoptosis abilities of tumor cells determine the response to chemotherapy.

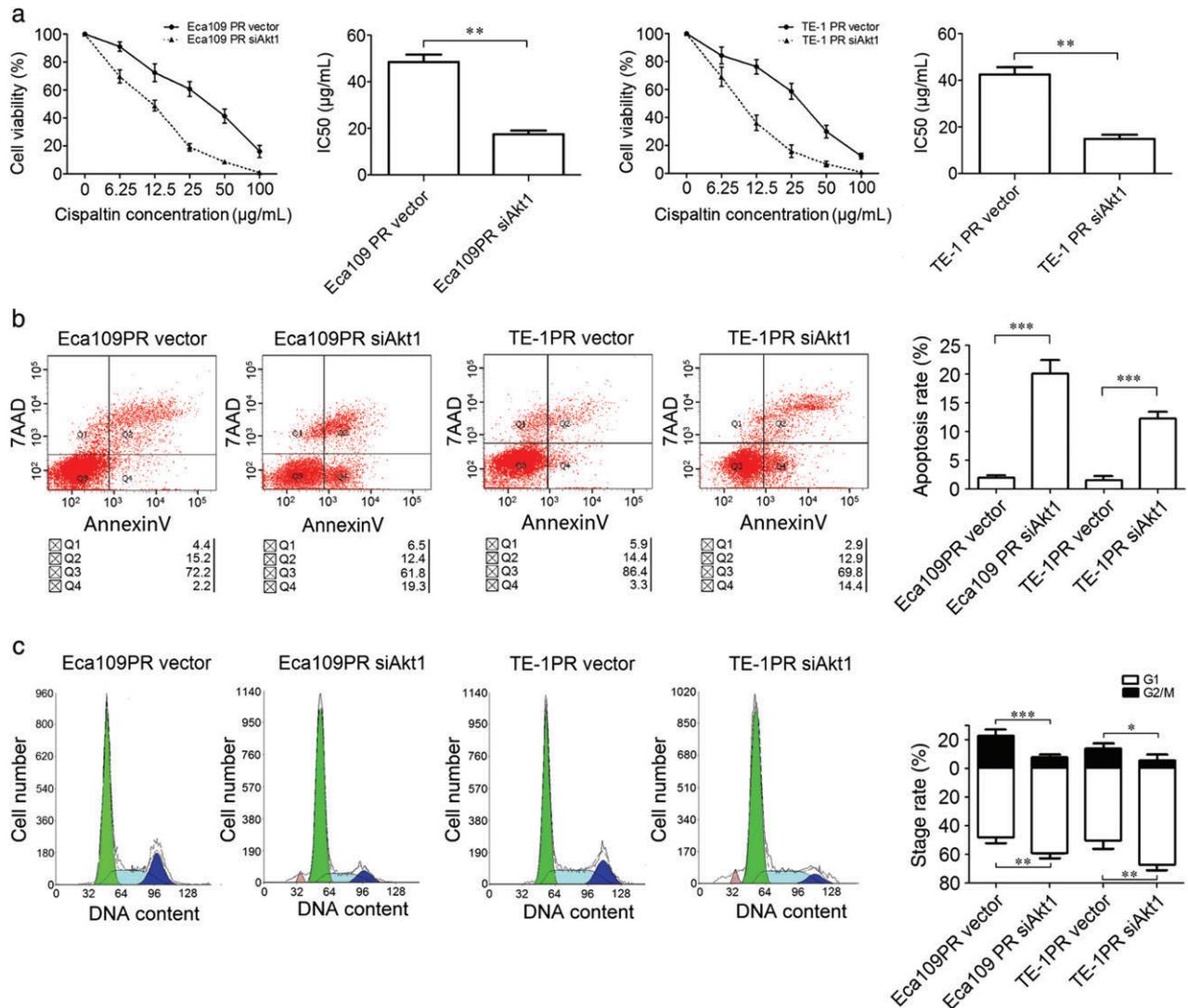


Figure 5 Silencing protein kinase B (Akt) reversed cisplatin resistance in esophageal squamous cell carcinoma (ESCC) cell lines. (a) Silencing Akt1 reversed cisplatin resistance in Eca109PR and TE-1PR. (b) Apoptosis detected by flow cytometry; higher early apoptosis rates were detected in small interfering (si)-Akt1 Eca109PR and si-Akt1 TE-1PR compared with the control. (c) Cell cycle detected by cytometry; higher G1 arrest was observed in si-Akt1 Eca109PR and si-Akt1 TE-1PR compared with the control.

Some previous reports have indicated that RUNX3 suppresses the proliferation and induction of apoptosis in various cancers, such as esophageal adenocarcinoma, and gastric and colorectal cancers.^{28–30} To investigate the role of RUNX3 in ESCC cisplatin resistance, we detected the effect of RUNX3 on the growth, cell cycle, and apoptosis of cisplatin-resistant ESCC cell lines. Restored RUNX3 could significantly inhibit ESCC cell growth by regulating the cell cycle and promoting apoptosis. A loss of RUNX3 in ESCC patients may result in cisplatin resistance.

The excellent work by Lin *et al.* proved that RUNX3 could suppress Akt1 expression by binding to the

promoter. Zheng *et al.* yielded consistent results for NSCLC, confirming that activation of the Akt pathway as a result of a loss of RUNX3 correlated with paclitaxel-resistance in NSCLC.^{16,22} We also found remarkable down-regulation in Akt1 expression and a consistently lower p-Akt protein level in ESCC cells with heterologous RUNX3. The alternation of downstream genes in the Akt pathway, which is involved in the cell cycle and apoptosis (bcl-2, bcl-xl, bax, cyclinD1, and p21), was also confirmed. A negatively correlated relationship was identified between RUNX3 and Akt1 in ESCC tissue samples. The aberrant activation of the Akt pathway and its relationship with cisplatin resistance has previously been confirmed in many

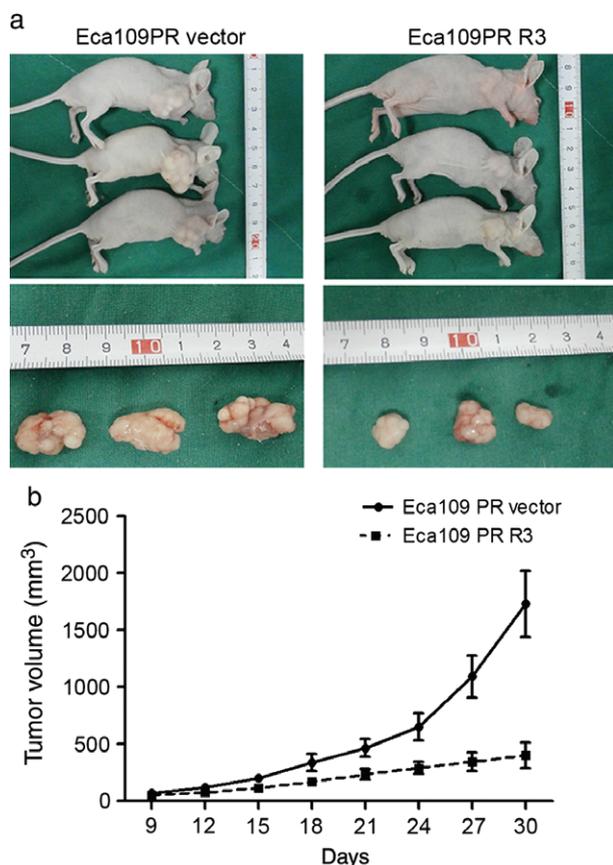


Figure 6 Heterologous RUNX3 reversed cisplatin resistance in vivo. (a) Representative photographs of tumors formed four weeks after subcutaneous transplantation. (b) The growth curve of tumors derived from Eca109PR R3 and Eca109PR vector cells. Each point represents the mean \pm standard error of six mice.

cancers, including ESCC. Yoshioka *et al.* reported that Akt activation predicted a poor prognosis in ESCC patients treated with preoperative chemotherapy.³¹ Liu *et al.* confirmed that an activated Akt pathway could inhibit apoptosis induced by cisplatin.³² Chen found that suppression of the phosphoinositide 3-kinase/Akt and signal transducer and activator of transcription 3 pathways could enhance cisplatin sensitivity in ESCC cells.³³ In our study, silencing Akt1 significantly reversed cisplatin resistance in two cell lines. These findings confirmed that activation of the Akt pathway induced cisplatin resistance in ESCC, and that RUNX3 may improve cisplatin sensitivity via suppression of the Akt pathway. However, considering that RUNX3 can also inhibit growth, induce apoptosis, and reverse drug-resistance via regulation of TGF- β , WNT and Notch pathways or directly suppresses the expression of multi-drug resistance proteins, these molecular mechanisms could also be involved in RUNX3-reversed cisplatin resistance in ESCC.

In conclusion, our findings confirmed that RUNX3 expression correlated with chemotherapeutic effect in ESCC patients, and heterologous RUNX3 could reverse cisplatin resistance in ESCC cell lines via suppression of the Akt pathway. Therefore, RUNX3 could be an indicator for chemotherapy and a therapeutic target for ESCC patients.

Acknowledgment

This work was supported by grants from the National Natural Science Foundation of China (No. 8117216).

Disclosure

No authors report any conflict of interest.

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