Life Sciences 232 (2019) 116679

Contents lists available at ScienceDirect

Life Sciences

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

ALC1 knockdown enhances cisplatin cytotoxicity of esophageal squamous cell carcinoma cells by inhibition of glycolysis through PI3K/Akt pathway

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ARTICLE INFO

Keywords:
ALC1
Cisplatin cytotoxicity
Esophageal squamous cell carcinoma
Glycolysis
PI3K/Akt pathway

ABSTRACT

Aims: Amplified in liver cancer 1 gene (ALC1), a recently identified oncogene, was reported to be overexpressed in esophageal cancer cell lines and identified as a target oncogene in esophageal cancer pathogenesis. However, little literature is available to illustrate its significance in cisplatin resistance of esophageal squamous cell carcinoma (ESCC) cells. The aim of the current study was to investigate the effect of ALC1 on cisplatin cytotoxicity of ESCC cells and to study the potential mechanisms.

Main methods: ALC1 at mRNA and protein levels were detected by qRT-PCR and western blot, respectively. Cell viability was evaluated using CCK-8 assay. Apoptosis was assessed using caspase-3/7 activity assay and flow cytometry analysis. Glycolysis level was evaluated by measuring glucose consumption and lactate production. The protein levels of p-protein kinase B (Akt) and Akt were determined by western blot.

Key findings: ALC1 was highly expressed in ESCC cells compared with human normal esophageal epithelial Het-1A cells. ALC1 knockdown suppressed the viability, induced apoptosis and enhanced cisplatin cytotoxicity in ESCC cells. In addition, ALC1 knockdown inhibited glycolysis and inactivated the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in ESCC cells. Mechanistically, activation of the PI3K/Akt pathway by 740Y-P blocked the effects of ALC1 knockdown on cisplatin cytotoxicity and glycolysis in ESCC cells. In contrast, inhibition of the PI3K/Akt pathway by LY294002 or glycolysis by 2-deoxyglucose resisted the effect of ALC1 overexpression on cisplatin cytotoxicity in ESCC cells.

Significance: ALC1 knockdown enhanced cisplatin cytotoxicity of ESCC cells by inhibition of glycolysis through inactivation of the PI3K/Akt pathway.

1. Introduction

Esophageal cancer is one of the most aggressive and frequently diagnosed malignances with a high mortality rate, ranking as the sixth leading cause of cancer-associated mortality worldwide [1]. To date, esophageal squamous cell carcinoma (ESCC) is the major histological subtype of esophageal cancer in eastern Asia, especially in China, accounting for > 90% of esophageal cancer cases [2]. Despite tremendous improvements in therapeutic interventions, the prognosis of ESCC patients still remains unfavorable and the 5-year survival rate of patients with ESCC is only 17% [3]. In recent decades, cytotoxic chemotherapy is recognized as a well-established therapeutic approach for ESCC patients [4]. Cisplatin is one of the most effective and commonly utilized anticancer drugs for the treatment of ESCC [5]. Unfortunately, most of ESCC patients ultimately relapse due to the development of drug resistance, which is a major challenge for successful treatment [6]. Hence, it is imperative to elucidate the molecular mechanisms responsible for drug resistance to improve the therapeutic efficacy of ESCC patients.

The amplified in liver cancer 1 gene (ALC1), also known as the chromodomain helicase/ATPase DNA binding protein 1-like gene (CHD1L), was recently identified as an oncogene which is isolated from the chromosome 1q21 amplicon in hepatocellular carcinoma (HCC) [7]. ALC1 belongs to the sucrose nonfermenting 2 (SNF2) superfamily of ATPases, which are ATP-dependent chromatin remodeling enzymes [8]. It is widely believed that ALC1 is highly expressed and involved in cell proliferation, migration, invasion, metastasis and tumorigenesis in

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https://doi.org/10.1016/j.lfs.2019.116679
Received 8 May 2019; Received in revised form 12 July 2019; Accepted 20 July 2019
Available online 21 July 2019
0024-3205/

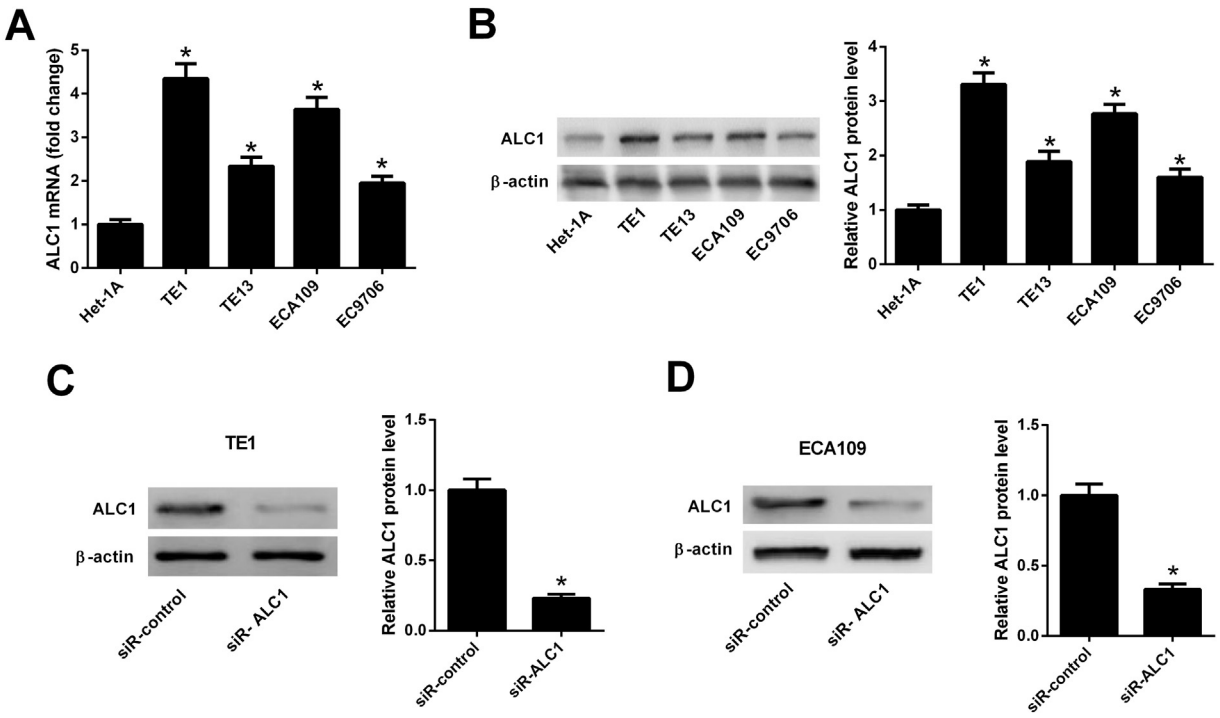

Fig. 1. Expression profile of ALC1 in ESCC cells. (A) qRT-PCR analysis of ALC1 mRNA expression in ESCC cells (TE1, TE13, ECA109, and EC9706) and human esophageal epithelial Het-1A cells. (B) Western blot analysis of ALC1 protein level in ESCC cells (TE1, TE13, ECA109, and EC9706) and Het-1A cells. Western blot analysis of ALC1 protein level in TE1 (C) and ECA109 (D) cells 48 h after transfection with siR-ALC1 or siR-control. *$P < 0.05$ (n = 3).

several types of tumor [9,10]. Therefore, substantive studies have proved that ALC1 is a novel predictive biomarker for the prediction of prognosis and cancer patient survival in human malignancies such as breast [11], gastric [12] and colorectal cancer (CRC) [13]. Notably, it was previously reported that ALC1 was overexpressed in esophageal cancer cell lines and identified as a target oncogene in esophageal cancer pathogenesis [14]. However, little literature is available to illustrate its significance in cisplatin resistance of ESCC cells.

In the present study, we investigated the biological role of ALC1 in cisplatin cytotoxicity in ESCC cells and further elaborated its molecular mechanism. We found that ALC1 was overexpressed in ESCC cells and ALC1 knockdown impeded cell growth and enhanced cisplatin cytotoxicity in ESCC cells by inhibition of glycolysis through inactivation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway.

2. Materials and methods

2.1. Cell culture and transfection

The ESCC cell lines (TE1, TE13, ECA109, and EC9706) and human esophageal epithelial cell line (Het-1A) were obtained from the Cell Bank Type Culture Collection of Chinese Academy of Science (Shanghai, China). These cells were maintained in RPMI-1640 medium (GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 100 μg/mL of penicillin (Invitrogen, Carlsbad, CA, USA), and 100 U/mL of streptomycin (Invitrogen) and kept in a 5% CO_2 incubator at 37 °C. siRNA targeting ALC1 (siR-ALC1) and its scrambled control (siR-control), pcDNA-ALC1 (ALC1) and pcDNA empty vector (Vector) were purchased from GenePharma (Shanghai, China). TE1 and ECA109 cells were seeded into plates and incubated overnight before transfection. Then, transient transfection with siR-ALC1 or siR-control was conducted using Lipofectamine 2000 (Invitrogen).

2.2. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from ESCC cells using RNAiso Plus (TaKaRa, Dalian, China) and reversely transcribed into cDNA using Primescript RT Reagent with gDNA Eraser (TaKaRa). ALC1 mRNA expression was then detected using SYBR® Green Real-Time PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) on the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The primer sequences were as below: β-actin, forward 5′-AGAG CTAC GAGC TGCC TGAC-3′ and reverse 5′-AGCA CTGT GTTG GCGT ACAG-3′; ALC1, forward 5′-GGTG GAGT TGGC ATGA ACTT-3′ and reverse 5′-CACT CAAC TGGA GGTC AGCA-3′. The relative ALC1 mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.3. Western blot analysis

Protein was extracted from treated ESCC cells using RIPA lysis buffer (Beyotime, Shanghai, China) and protein concentrations were measured using the BCA protein assay kit (Pierce, Rockford, IL, USA). Afterwards, protein samples (50 μg/lane) were subjected to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) prior to transfer onto nitrocellulose membranes (Millipore, Billerica, MA, USA). Following being blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 2 h, the membrane was incubated with primary antibodies against ALC1 (Abcam, Cambridge, MA, USA), Akt (Abcam), phosphorylated Akt (p-Akt; Abcam) and β-actin (Abcam) at 4 °C overnight, and then incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Abcam) at room temperature for 1 h. The protein signals were visualized using EasyBlot ECL Kit (Sangon Biotech, Shanghai, China).

2.4. Cell counting kit-8 (CCK-8) assay

Cell viability was evaluated by CCK-8 assay. In brief, TE1 and ECA109 cells were seed into 96-well plates at a density of 5×10^3 cells

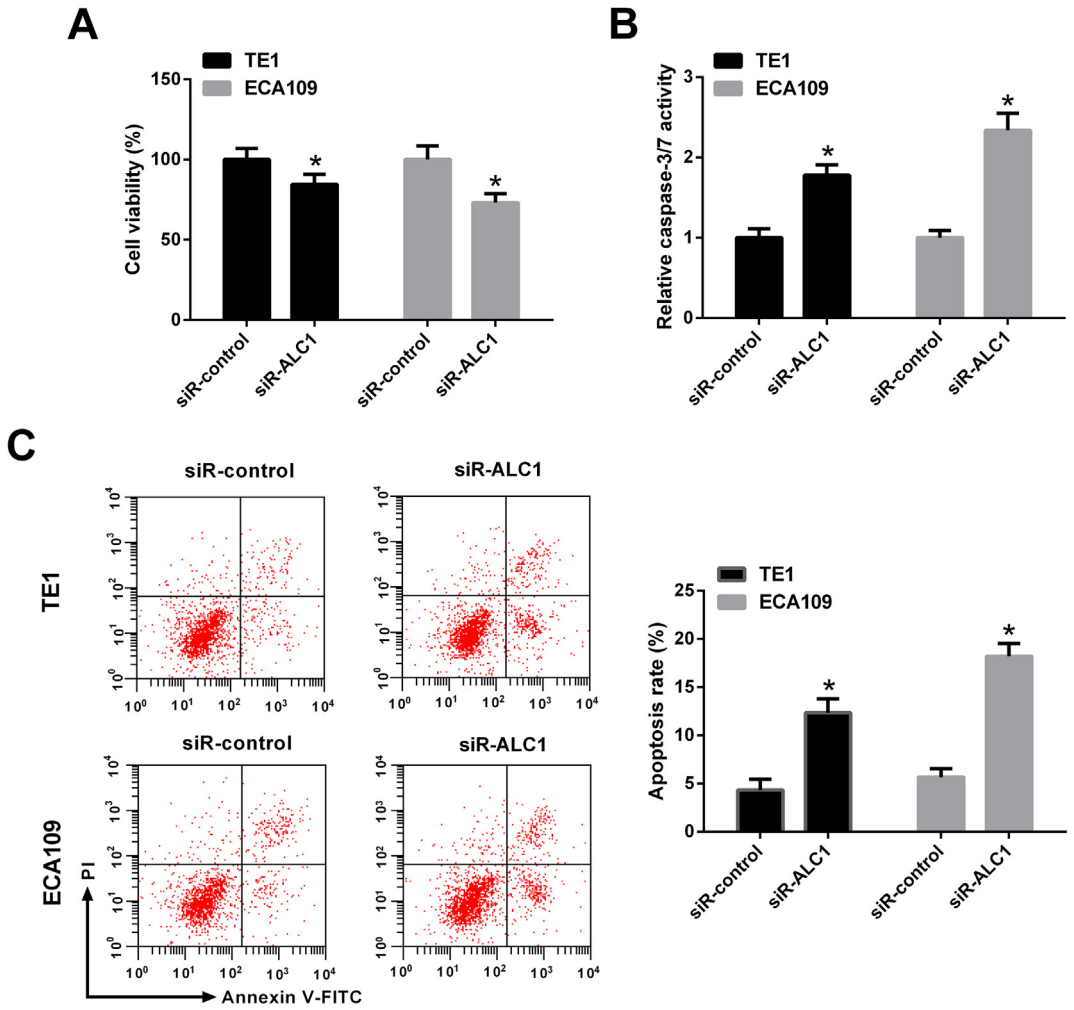

Fig. 2. Effects of ALC1 knockdown on the viability and apoptosis of ECSS cells. (A) CCK-8 assay was performed to evaluate the viability in TE1 and ECA109 cells transfected with siR-ALC1 or siR-control for 48 h. (B) Caspase-3/7 activity was measured using caspase-3/7 activity assay in TE1 and ECA109 cells 48 h after transfection with siR-ALC1 or siR-control. (C) Apoptosis was analyzed by flow cytometry analysis in TE1 and ECA109 cells 48 h after transfection with siR-ALC1 or siR-control. $^*P < 0.05$ (n = 3).

per well and incubated overnight. Then, TE1 and ECA109 cells were exposed to a series of concentrations of cisplatin (0, 0.25, 0.5, 1, 2, 4, and 8 μg/mL) for 48 h. In addition, TE1 and ECA109 cells transfected with siR-ALC1 or siR-control were administrated with 2 and 1 μg/mL cisplatin in the absence or presence of 10 μM 740Y-P (an activator of the PI3K/Akt pathway) (Sigma-Aldrich, St. Louis, MO, USA) for 48 h. TE1 and ECA109 cells transfected with ALC1 or Vector were treated with 2 and 1 μg/mL cisplatin in the absence or presence of 5 μM LY294002 (LY, an inhibitor of the PI3K/Akt pathway) (Sigma-Aldrich) or 5 mM 2-deoxyglucose (2-DG, a glycolytic inhibitor) (Sigma-Aldrich) for 48 h. After the indicated treatments, 10 μL of CCK-8 solution (Dojindo, Kumamoto, Japan) was added to each well, followed by incubation for 2 h at 37 °C. The optical density at 450 nm was measured by a microplate reader (Bio-Rad).

2.5. Flow cytometry analysis for apoptosis

Apoptosis of treated TE1 and ECA109 cells was assessed using flow cytometry with Annexin Vfluorescein isothiocyanate (FITC) Apoptosis Detection Kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer's recommendations. The percentage of apoptotic cells was detected by a FACScan Flow Cytometer (BD Biosciences).

2.6. Caspase-3/7 activity assay

The caspase-3/7 activity was measured using the Caspase-Glo 3/7 Assay kit (Promega, Madison, WI, USA). Following treatments as above, the treated TE1 and ECA109 cells were seeded into 96-well plates at a density of 5×10^3 cells/well and 100 μL of caspase-Glo 3/7 reagent was added to each well, followed by incubation for 2 h. The luminescence was measured using the GloMax-96 Microplate Luminometer (Promega).

2.7. Measurement of glucose consumption and lactate production

Following treatments as above, the culture medium of TE1 and ECA109 cells were collected for the determination of glucose consumption and lactate production. Glucose and lactate levels were measured using a Glucose Assay Kit (Sigma-Aldrich) and a Lactic Acid Assay Kit (Biovision, Milpitas, CA, USA), respectively.

2.8. Statistical analysis

Results are expressed as mean ± standard deviation (SD) and analyzed with GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA). Statistical differences between 2 groups or > 2 groups were determined using Student's *t*-test or one-way analysis of

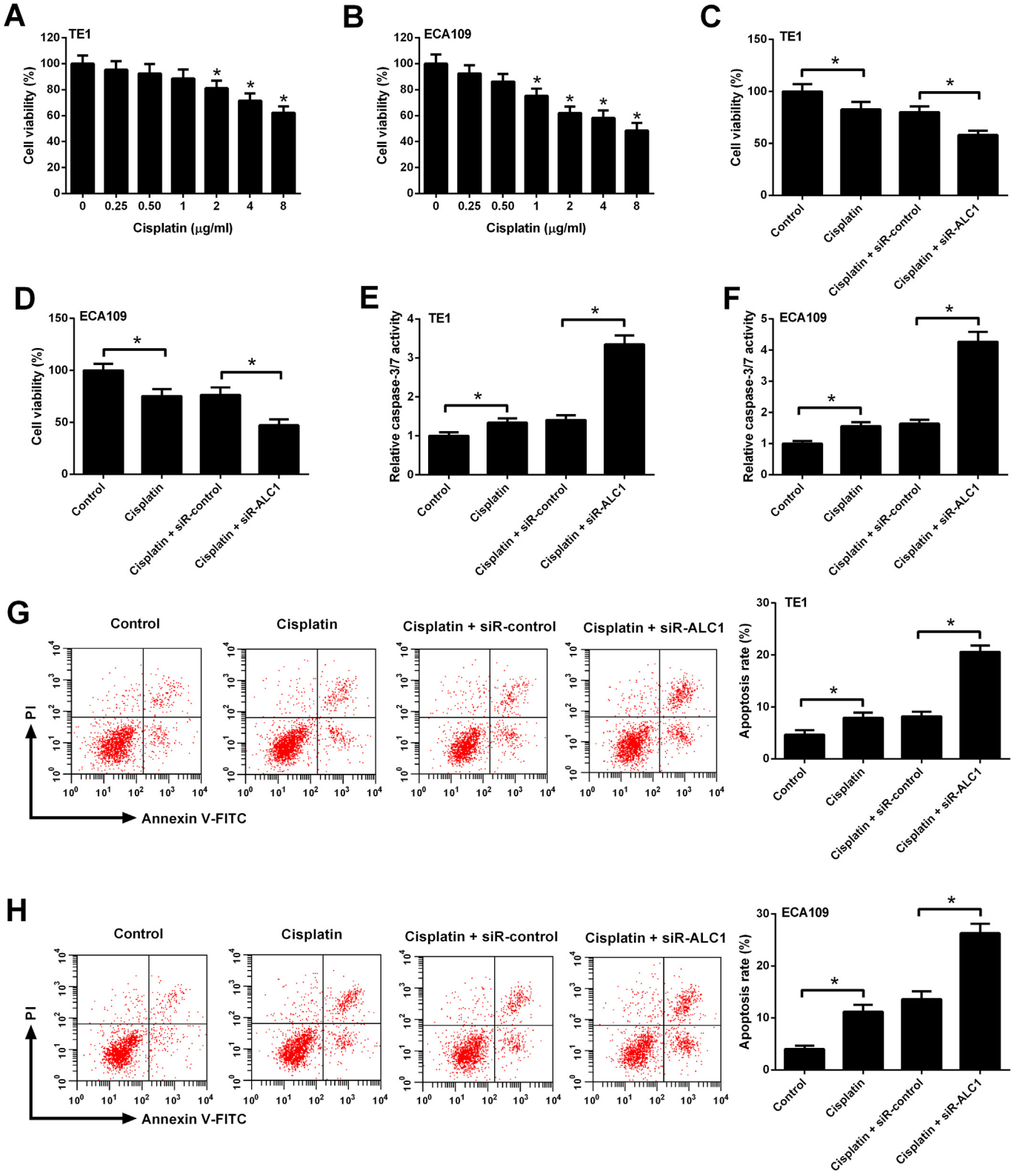

Fig. 3. Effect of ALC1 knockdown on cisplatin cytotoxicity in ESCC cells. (A and B) CCK-8 assay was used to examine cisplatin cytotoxicity in TE1 and ECA109 cells after treatment with various concentrations of cisplatin (0, 0.25, 0.5, 1, 2, 4, and 8 μg/mL) for 48 h. TE1 and ECA109 cells were transfected with siR-ALC1 or siR-control, followed by exposure to 2 and 1 μg/mL cisplatin, respectively, for 48 h. Then, cell viability in the treated TE1 (C) and ECA109 (D) cells was determined by CCK-8 assay. Caspase-3/7 activity in the treated TE1 (E) and ECA109 (F) cells was measured by caspase-3/7 activity assay. Apoptosis in the treated TE1 (G) and ECA109 (H) cells was evaluated by flow cytometry analysis. *$P < 0.05$ (n = 3).

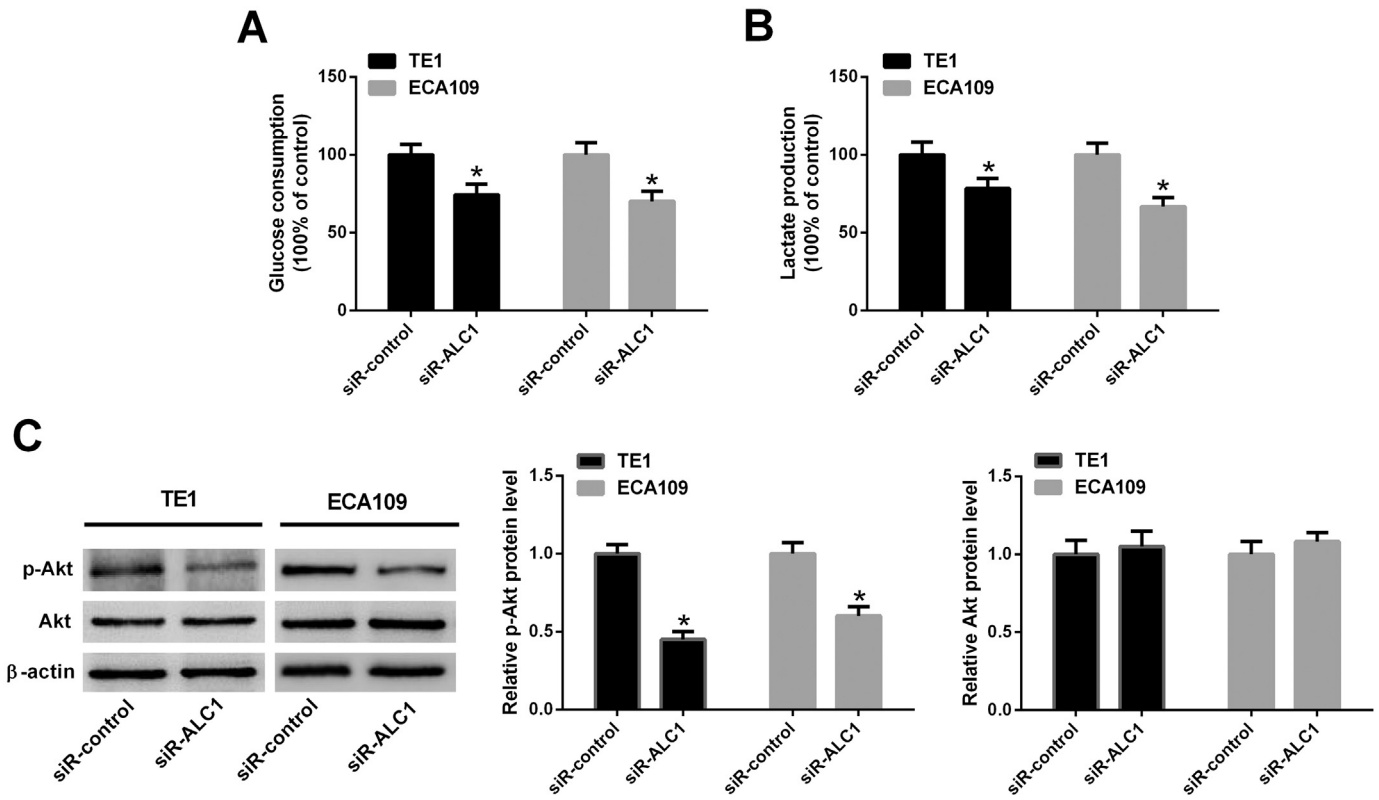

Fig. 4. Effects of ALC1 knockdown on the glycolysis and PI3K/Akt pathway in ESCC cells. (A and B) Glucose consumption and lactate production were measured in TE1 and ECA109 cells 48 h after transfection with siR-ALC1 or siR-control. (C) Western blot analysis was applied to detect the protein levels of p-Akt and Akt in TE1 and ECA109 cells 48 h after transfection with siR-ALC1 or siR-control. *$P < 0.05$ (n = 3).

variance (ANOVA). $P < 0.05$ was considered as statistically significant.

3. Results

3.1. ALC1 was highly expressed in ESCC cells

The expression of ALC1 in ESCC cells was detected by qRT-PCR and western blot. As shown in Fig. 1A and B, the mRNA and protein levels of ALC1 were significantly upregulated in ESCC cells (TE1, TE13, ECA109, and EC9706) compared with those in human esophageal epithelial Het-1A cells, particularly in TE1 and ECA109 cells. TE1 and ECA109 cells were chosen for further analyses. To further clarify the potential biological function of ALC1 in ESCC, loss-of-function experiments in TE1 and ECA109 cells were conducted by introducing with siR-ALC1. Western blot analysis manifested that ALC1 protein expression was decreased following delivery of siR-ALC1 in TE1 (Fig. 1C) and ECA109 (Fig. 1D) cells.

3.2. ALC1 knockdown suppressed the viability and induced apoptosis of ESCC cells

The effect of ALC1 knockdown on the viability of ESCC cells was explored by CCK-8 assay. As compared with siR-control group, ALC1 depletion effectively repressed the viability of TE1 and ECA109 cells (Fig. 2A). Meanwhile, caspase-3/7 activity assay and flow cytometry analysis were conducted to investigate the influence of ALC1 silencing on apoptosis in ESCC cells. Caspase-3/7 activity assay unveiled that there was a significant enhancement of caspase-3/7 activity in TE1 and ECA109 cells transfected with siR-ALC1 when compared to siR-control group (Fig. 2B). Flow cytometry analysis demonstrated that ALC1 knockdown led to a significant increase in apoptotic rate in TE1 and ECA109 cells relative to control group (Fig. 2C). These data revealed that ALC1 knockdown suppressed the viability and induced apoptosis of ESCC cells.

3.3. ALC1 knockdown enhanced cisplatin cytotoxicity in ESCC cells

CCK-8 assay was used to assess cisplatin cytotoxicity in ESCC cells and the results demonstrated that cisplatin conspicuously inhibited cell viability in TE1 cells (Fig. 3A) at 2, 4, and 8 μg/mL and in ECA109 cells (Fig. 3B) at 1, 2, 4, and 8 μg/mL in a dose-dependent manner, suggesting the cisplatin cytotoxicity in ESCC cells. To determine the role of ALC in cisplatin cytotoxicity in ESCC cells, TE1 and ECA109 cells were transfected with siR-ALC1 or siR-control, followed by exposure to 2 and 1 μg/mL cisplatin, respectively. CCK-8 assay uncovered that delivery of siR-ALC1 exacerbated cisplatin-induced viability reduction in TE1 (Fig. 3C) and ECA109 (Fig. 3D) cells versus siR-control group. Caspase-3/7 activity assay revealed that caspase-3/7 activity was elevated in response to cisplatin in TE1 (Fig. 3E) and ECA109 (Fig. 3F) cells, while depletion of ALC1 by siR-ALC1 reinforced this effect. As demonstrated by flow cytometry analysis, cisplatin treatment resulted in a higher ratio of apoptosis than control group in TE1 (Fig. 3G) and ECA109 (Fig. 3H) cells, which was notably strengthened by introduction with siR-ALC1. Collectively, these results suggested that ALC1 knockdown enhanced cisplatin cytotoxicity in ESCC cells.

3.4. ALC1 knockdown inhibited glycolysis and inactivated the PI3K/Akt pathway in ESCC cells

To gain insight into the mechanism by which ALC1 knockdown enhanced cisplatin cytotoxicity in ESCC cells, we investigated the effect of ALC1 knockdown on glycolysis in ESCC cells by measuring the glucose consumption and lactate production. As presented in Fig. 4A and

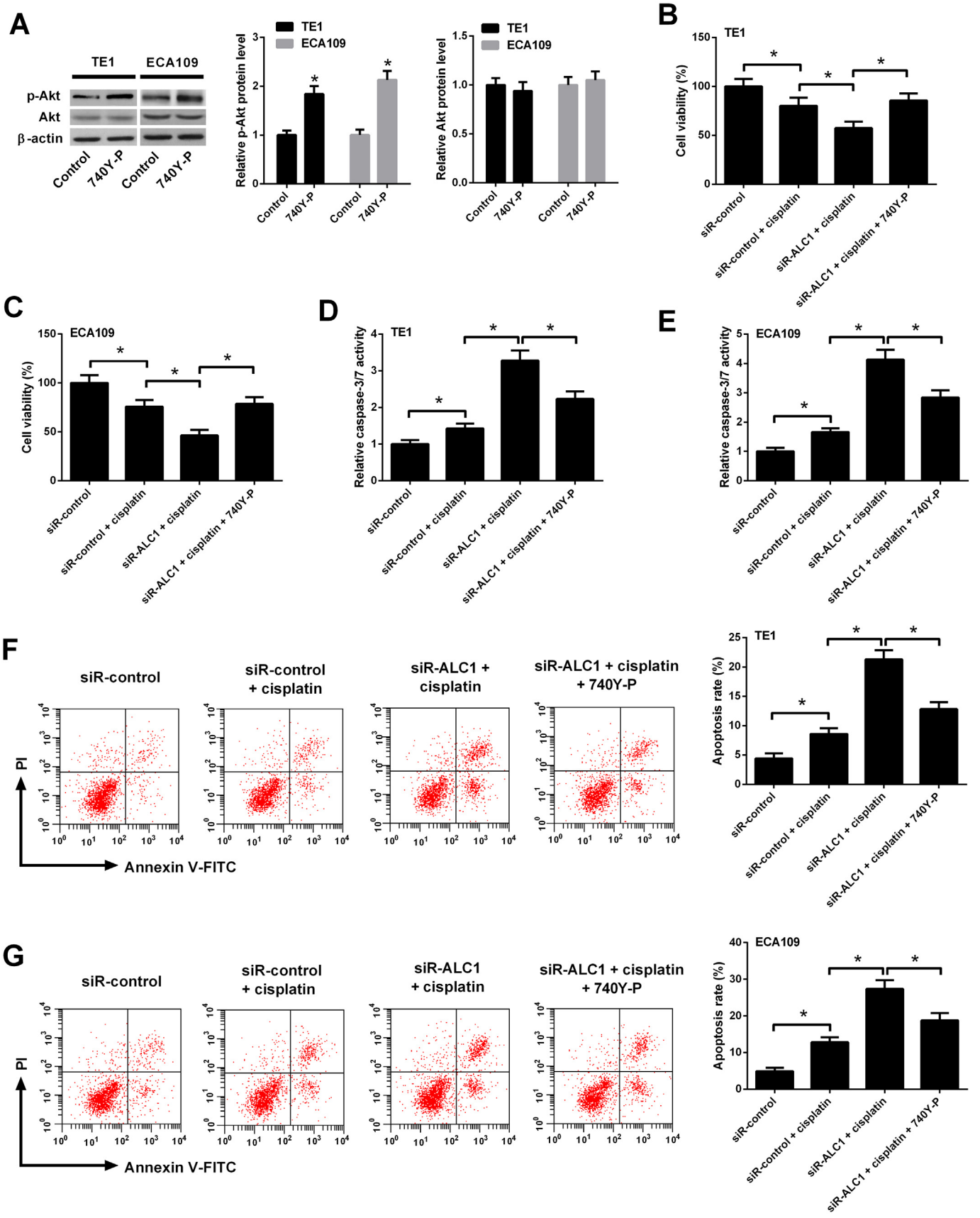

Fig. 5. Effects of activation of the PI3K/Akt pathway on ALC1 knockdown-induced enhancement of cisplatin cytotoxicity in ESCC cells. (A) TE1 and ECA109 cells were treated with 10 μM 740Y-P for 48 h and then the protein levels of p-Akt and Akt were determined by western blot. TE1 and ECA109 cells transfected with siR-ALC1 or siR-control were exposed to 2 and 1 μg/mL cisplatin, respectively, in the absence or presence of 10 μM 740Y-P for 48 h. CCK-8 assay was used to examine cell viability in the treated TE1 (B) and ECA109 cells (C). Caspase-3/7 activity in the treated TE1 (D) and ECA109 cells (E) was measured by caspase-3/7 activity assay. Apoptosis of treated TE1 (F) and ECA109 cells (G) was analyzed by flow cytometry analysis. *$P < 0.05$ (n = 3).

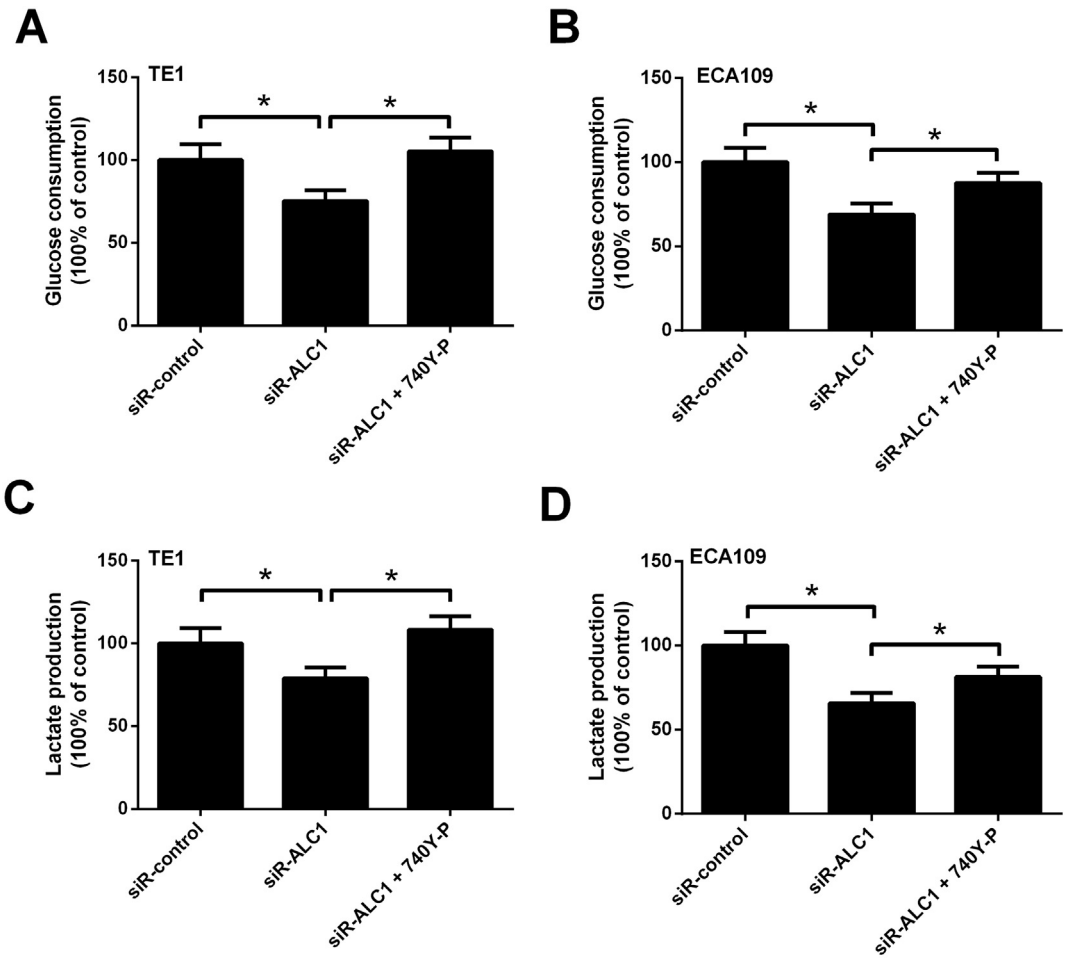

Fig. 6. Effect of activation of the PI3K/Akt pathway on the inhibitory effect of ALC1 knockdown on glycolysis in ESCC cells. TE1 and ECA109 cells were transfected with siR-ALC1 or siR-control and then treated with or without 10 μM 740Y-P for 48 h, followed by measurement of glucose consumption and lactate production in TE1 (A and C) and ECA109 (B and D) cells. *$P < 0.05$ (n = 3).

B, ALC1 knockdown repressed glycolysis level in TE1 and ECA109 cells relative to control group, as evidenced by the decreased glucose consumption and lactate production. Meanwhile, we also explored the effect of ALC1 knockdown on the PI3K/Akt pathway in TE1 and ECA109 cells. ALC1 silencing reduced p-Akt protein level but did not affect the total Akt level in TE1 and ECA109 cells (Fig. 4C), suggesting that ALC1 knockdown inhibited the PI3K/Akt pathway in ESCC cells.

3.5. Activation of the PI3K/Akt pathway blocked the effect of ALC1 knockdown on cisplatin cytotoxicity and glycolysis in ESCC cells

TE1 and ECA109 cells were treated with 10 μM 740Y-P, an activator of the PI3K/Akt pathway, for 48 h. Western blot confirmed that 740Y-P obviously increased the phosphorylation of Akt in TE1 and ECA109 cells, suggesting the activation of PI3K/Akt pathway (Fig. 5A). To determine whether activation of the PI3K/Akt pathway can reverse the biological function of ALC1 knockdown in cisplatin cytotoxicity in ESCC cells, TE1 and ECA109 cells transfected with siR-ALC1 or siR-control were exposed to 2 and 1 μg/mL cisplatin in the absence or presence of 10 μM 740Y-P for 48 h, respectively. As demonstrated by CCK-8 assay, the promotive effect of ALC1 silencing on cisplatin-induced viability reduction in TE1 and ECA109 cells was overturned following the addition of 740Y-P (Fig. 5B and C). Caspase-3/7 activity assay showed that ALC1 knockdown-induced increase of caspase-3/7 activity in TE1 and ECA109 cells in the presence of cisplatin was distinctly attenuated by 740Y-P (Fig. 5D and E). Furthermore, flow cytometry analysis revealed that 740Y-P strikingly receded the effect of ALC1 knockdown on cisplatin-induced apoptosis in TE1 and ECA109 cells (Fig. 5F and G). These results demonstrated that activation of the PI3K/Akt pathway blocked the effect of ALC1 knockdown on cisplatin cytotoxicity in ESCC cells. In addition, we found that the inhibitory effect of ALC1 knockdown on glucose consumption and lactate production in TE1 and ECA109 cells was ameliorated after treatment with 740Y-P (Fig. 6A–D), indicating that activation of the PI3K/Akt pathway blocked the effect of ALC1 knockdown on glycolysis in ESCC cells.

3.6. Inhibition of the PI3K/Akt pathway or glycolysis resisted the effect of ALC1 overexpression on cisplatin cytotoxicity in ESCC cells

To determine the influence of inhibition of the PI3K/Akt pathway or glycolysis on the effect of ALC1 overexpression on cisplatin cytotoxicity in ESCC cells, TE1 and ECA109 cells transfected with ALC1 or Vector were treated with 2 and 1 μg/mL cisplatin in the absence or presence of 5 μM LY, an inhibitor of the PI3K/Akt pathway, or 5 mM 2-DG, a glycolytic inhibitor, for 48 h. CCK-8 assay exhibited that ectopic expression of ALC1 significantly reverted cisplatin-induced viability reduction in TE1 (Fig. 7A) and ECA109 (Fig. 7B) cells, while this effect was abolished following the addition of LY or 2-DG. Caspase-3/7 activity assay disclosed that ALC1 overexpression dramatically weakened cisplatin-induced increase of caspase-3/7 activity in TE1 (Fig. 7C) and ECA109 (Fig. 7D) cells, while LY or 2-DG evidently reversed the suppressive effect of ALC1 overexpression on cisplatin-induced increase of caspase-3/7 activity. Furthermore, flow cytometry analysis hinted that the inhibitory effect of ALC1 overexpression on cisplatin-induced apoptosis in

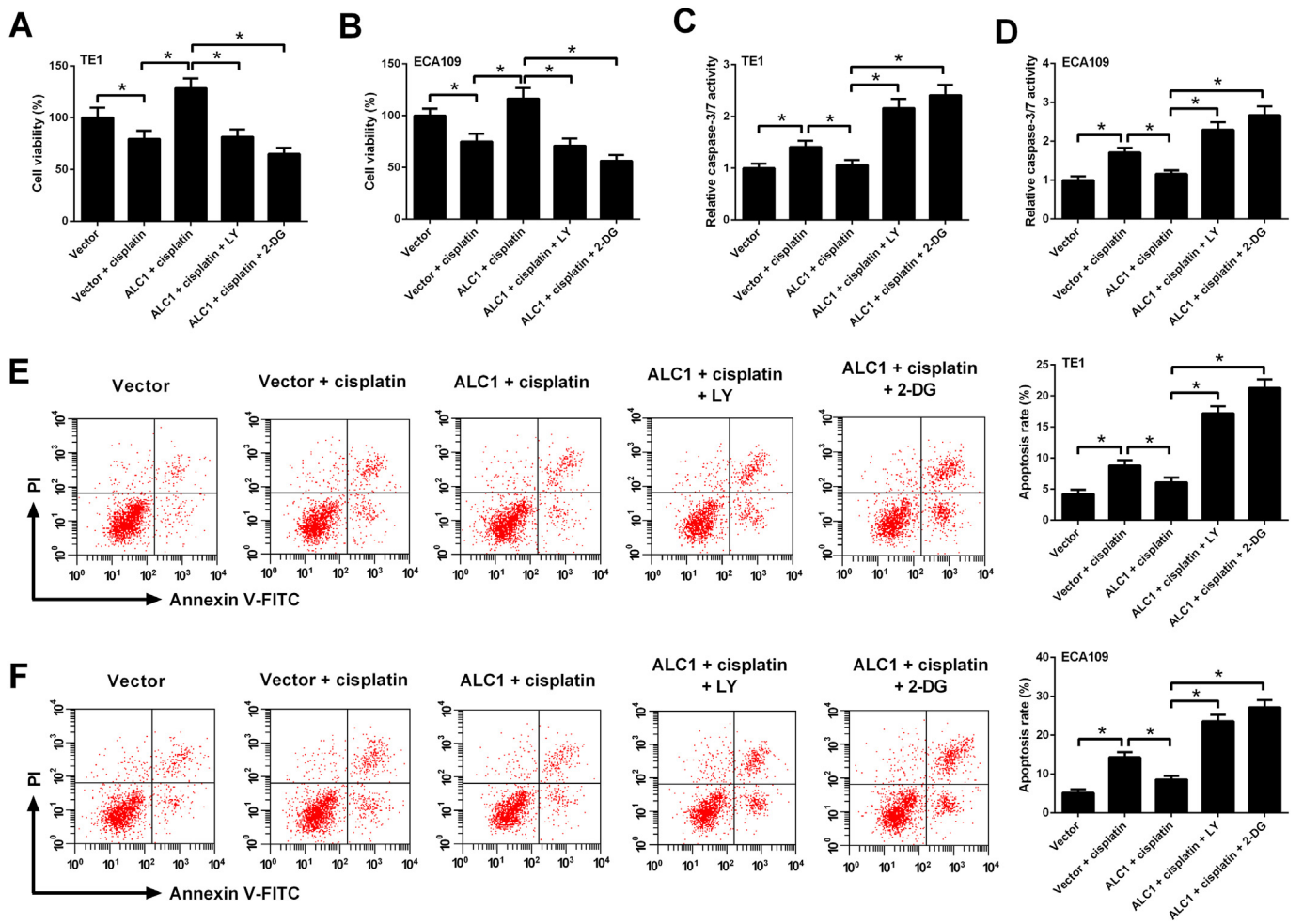

Fig. 7. Effects of inhibition of the PI3K/Akt pathway or glycolysis on the influence of ALC1 overexpression on cisplatin cytotoxicity in ESCC cells. TE1 and ECA109 cells transfected with ALC1 or Vector were treated with 2 and 1 μg/mL cisplatin in the absence or presence of 5 μM LY or 5 mM 2-DG. Then, cell viability in the treated TE1 (A) and ECA109 (B) cells was detected by CCK-8 assay. Caspase-3/7 activity in the treated TE1 (C) and ECA109 (D) cells was measured by caspase-3/7 activity assay. Apoptosis of treated TE1 (E) and ECA109 (F) cells was evaluated by flow cytometry analysis. $^*P < 0.05$ (n = 3).

TE1 (Fig. 7E) and ECA109 (Fig. 7F) cells was recuperated by administration with LY or 2-DG. Therefore, we concluded that inhibition of the PI3K/Akt pathway or glycolysis resisted the effect of ALC1 overexpression on cisplatin cytotoxicity in ESCC cells.

3.7. ALC1 knockdown did not affect the viability, apoptosis, glycolysis, and the PI3K/Akt pathway in human normal esophageal epithelial Het-1A cells

To determine the biological effects of ALC1 knockdown on the human normal esophageal epithelial line, Het-1A cells were transfected with siR-control or siR-ALC1. After 48 h of transfection, ALC1 level was significantly decreased (Fig. 8A). Knockdown of ALC1 did not affect the viability and apoptosis of Het-1A cells (Fig. 8B and C). Glucose consumption and lactate production was unchanged in Het-1A cells after transfection with siR-ALC1 (Fig. 8D and E), suggesting that knockdown of ALC1 did not affect glycolysis in Het-1A cells. Knockdown of ALC1 did not change the expression levels of p-Akt and Akt in Het-1A cells (Fig. 8F), indicating that knockdown of ALC1 did not affect the PI3K/Akt pathway in human normal esophageal epithelial cells.

4. Discussion

Currently, the development of drug resistance has become a huge obstacle that significantly diminishes the effectiveness of chemotherapeutic drugs, and remains a major cause of treatment failure in patients with ESCC [15]. Therefore, it is indispensable to develop new therapeutic strategies for combating drug resistance in ESCC. Herein, this is the first time, to our knowledge, that ALC1 was closely associated with cisplatin cytotoxicity in ESCC cells. We proved that ALC1 was highly expressed in ESCC cells, and ALC1 knockdown suppressed the viability, induced apoptosis and enhanced cisplatin cytotoxicity in ESCC cells. Mechanistically, the biological function of ALC1 in cisplatin cytotoxicity in ESCC cells was mediated by inhibition of glycolysis through inactivation of the PI3K/Akt pathway.

In recent years, accumulating evidence has shown that abnormally highly expressed ALC1 is promulgated as an oncogene contributing to the carcinogenesis of several types of tumors [16]. For example, ALC1 protein expression was higher in pancreatic cancer patients, which acted as an independent predictive prognostic biomarker in pancreatic cancer, and promoted pancreatic cancer proliferation via activation of the Wnt/β-catenin/TCF signaling pathway [17]. Overexpression of ALC1 promoted the invasion and metastasis of breast cancer cells via the PI3K/Akt/ARK5/mTOR/MMP signaling pathway [18]. More interestingly, it was previously reported that ALC1 expression was increased after cisplatin treatment in non-small-cell lung cancer (NSCLC) cells and increased expression of ALC1 contributed to cisplatin resistance in human NSCLC via c-Jun-ABCB1-NF-κB axis [19]. Accordingly, we hypothesized whether ALC1 was involved in cisplatin cytotoxicity in ESCC cells. In the present study, we confirmed that ALC1 expression at mRNA and protein levels was both upregulated in ESCC cells, and ALC1

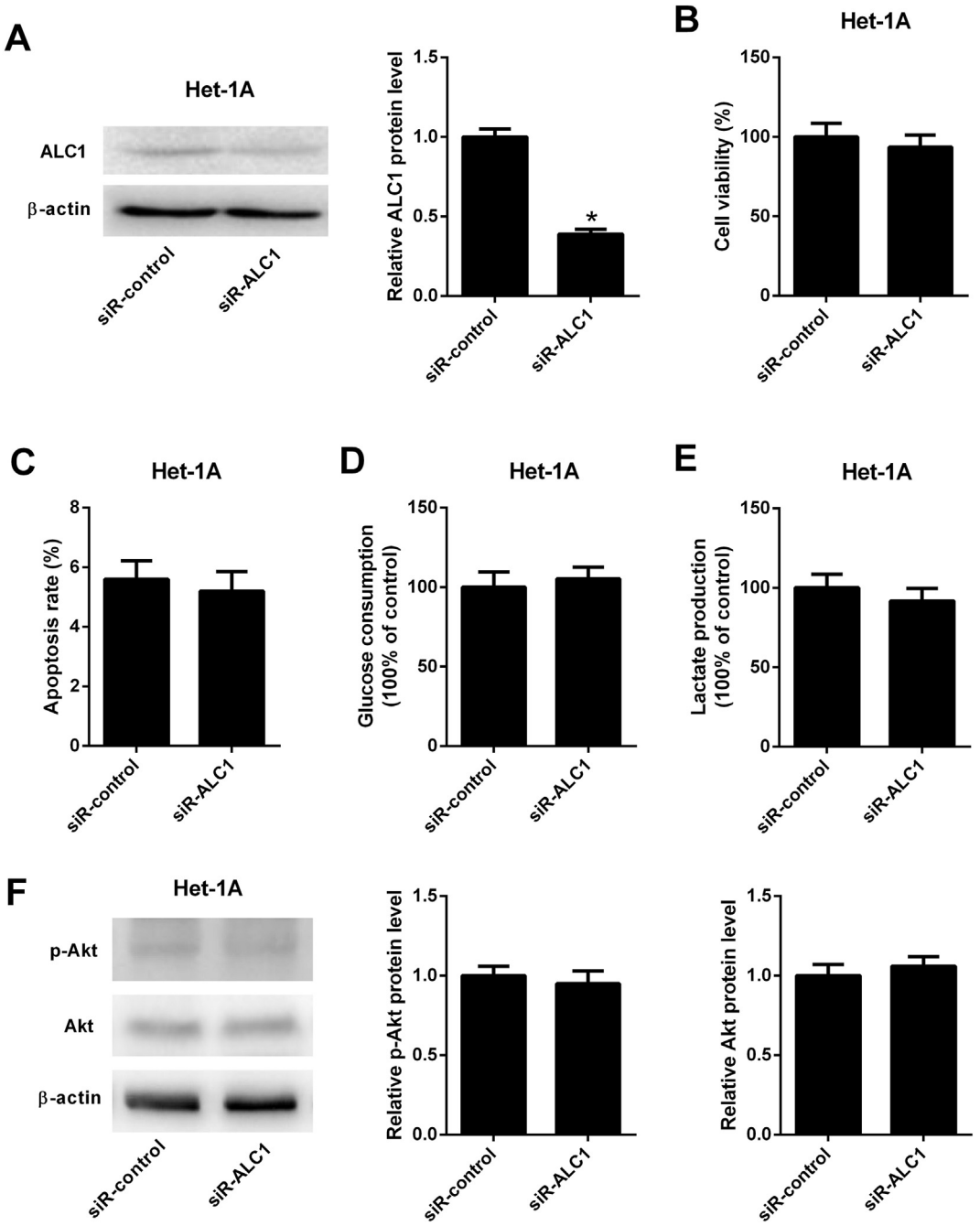

Fig. 8. Effects of ALC1 knockdown on the viability, apoptosis, glycolysis, and the PI3K/Akt pathway in human normal esophageal epithelial Het-1A cells. Het-1A cells were transfected with siR-ALC1 or siR-control for 48 h. (A) ALC1 level was determined by western blot analysis. (B and C) The viability and apoptosis of Het-1A cells were evaluated by CCK-8 assay and flow cytometry analysis, respectively. (D and E) Glucose consumption and lactate production were measured in Het-1A cells. (F) Western blot analysis was applied to detect the protein levels of p-Akt and Akt in Het-1A cells 48 h after transfection. $^*P < 0.05$ (n = 3).

knockdown suppressed the viability and induced apoptosis of ESCC cells, which were in line with the previous study [14]. More notably, our study further found that ALC1 knockdown also exacerbated cisplatin-induced viability reduction and increase of caspase-3/7 activity and apoptotic rate in ESCC cells, suggesting that ALC1 knockdown enhanced cisplatin cytotoxicity in ESCC cells.

In most cases, cancer cells preferred aerobic glycolysis (also called Warburg effect) to metabolize glucose into lactate for their high-energy demands, even in the presence of abundant oxygen [20]. Therefore, the level of glycolysis can be detected by measuring glucose consumption and lactate production [21]. A growing body of evidence has demonstrated that aerobic glycolysis is frequently elevated in many tumors, which is recognized as one of the major hallmarks of cancer cells [22]. It has been proposed that deranged metabolism, particularly increased aerobic glycolysis, contributes to tumorigenesis as well as the development of drug resistance in various malignancies [23,24]. Therefore, targeting glycolysis may be a novel promising therapeutic strategy for overcoming drug resistance in various cancers [25]. In our study, we demonstrated that ALC1 knockdown reduced glucose consumption and lactate production in ESCC cells, suggesting that ALC1 knockdown inhibited glycolysis in ESCC cells. Moreover, we found that inhibition of glycolysis by 2-DG resisted the effects of ALC1 overexpression on cisplatin cytotoxicity in ESCC cells. These results demonstrated that ALC1 overexpression exerted its role in cisplatin cytotoxicity in ESCC cells

through activation of glycolysis.

The PI3K/Akt signaling pathway is a crucial intracellular signaling pathway that plays an important role in the regulation of various oncogenic processes, such as cell growth, apoptosis, differentiation, and metastasis [26]. It is widely recognized that the PI3K/Akt signaling pathway is frequently activated in multiple human cancers including ESCC, and is involved in cancer progression [27,28]. Moreover, recent studies have demonstrated that activation of the PI3K/Akt signaling pathway contributes to the development of drug resistance in cancer cells [29]. Abnormal activation of the PI3K/Akt signaling pathway has been shown to be related to cisplatin resistance in ESCC cells [30]. Hence, inhibition of the PI3K/Akt signaling pathway may be an effective therapeutic approach to improve the antitumor effects of chemotherapeutic agents in ESCC. Herein, we proved that ALC1 knockdown inactivated the PI3K/Akt pathway in ESCC cells. Moreover, activation of the PI3K/Akt pathway by 740Y-P blocked the effects of ALC1 knockdown on cisplatin cytotoxicity and glycolysis in ESCC cells. In contrast, inhibition of the PI3K/Akt pathway by LY abolished the effect of ALC1 overexpression on cisplatin cytotoxicity in ESCC cells. Collectively, these results revealed that ALC1 knockdown enhanced cisplatin cytotoxicity in ESCC cells by inhibition of glycolysis through inactivation of the PI3K/Akt pathway.

We found that ALC1 knockdown significantly reduced ALC1 expression level, but did not significantly affect the viability, apoptosis, glycolysis, and the PI3K/Akt pathway in human normal esophageal epithelial Het-1A cells. There are some possible reasons for this: (1) ALC1 baseline expression level is very low in normal cell line Het-1A, so ALC1 knockdown did not significantly affect the viability and apoptosis of Het-1A cells; (2) Glycolysis is frequently elevated in many tumors, which is recognized as one of the major hallmarks of cancer cells, but not normal cells, so ALC1 knockdown did not significantly affect glucose consumption and lactate production in normal cell line Het-1A; (3) The PI3K/Akt signaling pathway is frequently activated in multiple human cancer cells, but not in normal cells, therefore ALC1 knockdown did not significantly affect the PI3K/Akt signaling pathway in Het-1A cells.

5. Conclusion

In summary, we provided the first evidence that ALC1 knockdown enhanced cisplatin cytotoxicity in ESCC cells by inhibition of glycolysis through inactivation of the PI3K/Akt pathway. Based on these results, targeting ALC1 might serve as a novel promising therapeutic regimen to improve cisplatin cytotoxicity in ESCC.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgement

This study was supported by Natural Science Foundation of Henan Province (Grant No. 182300410376).

References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108.
[2] S. Ekman, M. Dreilich, J. Lennartsson, B. Wallner, D. Brattstrom, M. Sundbom, M. Bergqvist, Esophageal cancer: current and emerging therapy modalities, Expert. Rev. Anticancer. Ther. 8 (2008) 1433–1448.
[3] T. Sudo, T. Iwaya, N. Nishida, G. Sawada, Y. Takahashi, M. Ishibashi, K. Shibata, H. Fujita, K. Shirouzu, M. Mori, K. Mimori, Expression of mesenchymal markers vimentin and fibronectin: the clinical significance in esophageal squamous cell carcinoma, Ann. Surg. Oncol. 20 (2013) S324–S335.
[4] L. Hong, Y. Han, H. Zhang, D. Fan, Prognostic markers in esophageal cancer: from basic research to clinical use, Expert Rev. Gastroenterol. Hepatol. 9 (2015) 887–889.
[5] H. Toshimitsu, K. Hashimoto, A. Tangoku, N. Iizuka, K. Yamamoto, S. Kawauchi, A. Oga, T. Furuya, M. Oka, K. Sasaki, Molecular signature linked to acquired resistance to cisplatin in esophageal cancer cells, Cancer Lett. 211 (2004) 69–78.
[6] A.K. Rustgi, H.B. El-Serag, Esophageal carcinoma, N. Engl. J. Med. 371 (2014) 2499–2509.
[7] N.F. Ma, L. Hu, J.M. Fung, D. Xie, B.J. Zheng, L. Chen, D.J. Tang, L. Fu, Z. Wu, M. Chen, Y. Fang, X.Y. Guan, Isolation and characterization of a novel oncogene, amplified in liver cancer 1, within a commonly amplified region at 1q21 in hepatocellular carcinoma, Hepatology 47 (2008) 503–510.
[8] T. Woodage, M.A. Basrai, A.D. Baxevanis, P. Hieter, F.S. Collins, Characterization of the CHD family of proteins, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11472–11477.
[9] L.R. He, N.F. Ma, J.W. Chen, B.K. Li, X.Y. Guan, M.Z. Liu, D. Xie, Overexpression of CHD1L is positively associated with metastasis of lung adenocarcinoma and predicts patients poor survival, Oncotarget 6 (2015) 31181–31190.
[10] X. Xu, Y. He, X. Miao, Y. Wu, J. Han, Q. Wang, J. Liu, F. Zhong, Y. Ou, Y. Wang, S. He, Cell adhesion induces overexpression of chromodomain helicase/ATPase DNA binding protein 1-like gene (CHD1L) and contributes to cell adhesion-mediated drug resistance (CAM-DR) in multiple myeloma cells, Leuk. Res. 47 (2016) 54–62.
[11] J. Wu, Y. Zong, X. Fei, X. Chen, O. Huang, J. He, W. Chen, Y. Li, K. Shen, L. Zhu, Presence of CHD1L over-expression is associated with aggressive tumor biology and is a novel prognostic biomarker for patient survival in human breast cancer, PLoS One 9 (2014) e98673.
[12] Z. Su, J. Zhao, G. Xian, W. Geng, Z. Rong, Y. Wu, C. Qin, CHD1L is a novel independent prognostic factor for gastric cancer, Clin. Transl. oncol. 16 (2014) 702–707.
[13] X. Ji, J. Li, L. Zhu, J. Cai, J. Zhang, Y. Qu, H. Zhang, B. Liu, R. Zhao, Z. Zhu, CHD1L promotes tumor progression and predicts survival in colorectal carcinoma, J. Surg. Res. 185 (2013) 84–91.
[14] Z.H. Liu, Q. Zhang, Y.J. Ding, Y.H. Ren, H.P. Yang, Q. Xi, Y.N. Cheng, G.L. Miao, H. K. Liu, C.X. Li, W.Q. Yan, Y. Li, Z. Xue, L. Zhang, X.Y. Li, C.L. Zhao, Y. Da, X.Z. Wu, J.Q. Chen, R. Zhang, Z.G. Li, Overexpression of CHD1L is associated with poor survival and aggressive tumor biology in esophageal carcinoma, Oncotarget 8 (2017) 74178–74187.
[15] S. Pasquali, G. Yim, R.S. Vohra, S. Mocellin, D. Nyanhongo, P. Marriott, J.I. Geh, E.A. Griffiths, Survival after neoadjuvant and adjuvant treatments compared to surgery alone for resectable esophageal carcinoma: a network meta-analysis, Ann. Surg. 265 (2017) 481–491.
[16] W. Cheng, Y. Su, F. Xu, CHD1L: a novel oncogene, Mol. Cancer 12 (2013) 170.
[17] C. Liu, X. Fu, Z. Zhong, J. Zhang, H. Mou, Q. Wu, T. Sheng, B. Huang, Y. Zou, CHD1L expression increases tumor progression and acts as a predictive biomarker for poor prognosis in pancreatic cancer, Dig. Dis. Sci. 62 (2017) 2376–2385.
[18] Q.J. Mu, H.L. Li, Y. Yao, S.C. Liu, C.G. Yin, X.Z. Ma, Chromodomain helicase/ATPase DNA-binding protein 1-like gene (CHD1L) expression and implications for invasion and metastasis of breast cancer, PLoS One 10 (2015) e0143030.
[19] Y. Li, L.R. He, Y. Gao, N.N. Zhou, Y. Liu, X.K. Zhou, J.F. Liu, X.Y. Guan, N.F. Ma, D. Xie, CHD1L contributes to cisplatin resistance by upregulating the ABCB1-NF-kappaB axis in human non-small-cell lung cancer, Cell Death Dis. 10 (2019) 99.
[20] J.W. Kim, C.V. Dang, Cancer's molecular sweet tooth and the Warburg effect, Cancer Res. 66 (2006) 8927–8930.
[21] J.J. Mou, J. Peng, Y.Y. Shi, N. Li, Y. Wang, Y. Ke, Y.F. Zhou, F.X. Zhou, Mitochondrial DNA content reduction induces aerobic glycolysis and reversible resistance to drug-induced apoptosis in SW480 colorectal cancer cells, Biomed. Pharmacother. 103 (2018) 729–737.
[22] B. Bhattacharya, M.F. Mohd Omar, R. Soong, The Warburg effect and drug resistance, Br. J. Pharmacol. 173 (2016) 970–979.
[23] T. Yamamoto, N. Takano, K. Ishiwata, M. Ohmura, Y. Nagahata, T. Matsuura, A. Kamata, K. Sakamoto, T. Nakanishi, A. Kubo, T. Hishiki, M. Suematsu, Reduced methylation of PFKFB3 in cancer cells shunts glucose towards the pentose phosphate pathway, Nat. Commun. 5 (2014) 3480.
[24] Y. Zhou, F. Tozzi, J. Chen, F. Fan, L. Xia, J. Wang, G. Gao, A. Zhang, X. Xia, H. Brasher, W. Widger, L.M. Ellis, Z. Weihua, Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells, Cancer Res. 72 (2012) 304–314.
[25] R.H. Xu, H. Pelicano, Y. Zhou, J.S. Carew, L. Feng, K.N. Bhalla, M.J. Keating, P. Huang, Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia, Cancer Res. 65 (2005) 613–621.
[26] B.T. Hennessy, D.L. Smith, P.T. Ram, Y. Lu, G.B. Mills, Exploiting the PI3K/AKT pathway for cancer drug discovery, Nat. Rev. Drug Discov. 4 (2005) 988–1004.
[27] J. Zhu, M. Wang, M. Zhu, J. He, J.C. Wang, L. Jin, X.F. Wang, J.Q. Xiang, Q. Wei, Associations of PI3KR1 and mTOR polymorphisms with esophageal squamous cell carcinoma risk and gene-environment interactions in Eastern Chinese populations, Sci. Rep. 5 (2015) 8250.
[28] J.A. Fresno Vara, E. Casado, J. de Castro, P. Cejas, C. Belda-Iniesta, M. Gonzalez-Baron, PI3K/Akt signalling pathway and cancer, Cancer Treat. Rev. 30 (2004) 193–204.
[29] H.A. Burris III, Overcoming acquired resistance to anticancer therapy: focus on the PI3K/AKT/mTOR pathway, Cancer Chemother. Pharmacol. 71 (2013) 829–842.
[30] J. Xu, Z. Hu, Y-box-binding protein 1 promotes tumor progression and inhibits cisplatin chemosensitivity in esophageal squamous cell carcinoma, Biomed. Pharmacother. 79 (2016) 17–22.