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CDK8 Promotes Cell Proliferation, Migration and Invasion in Esophageal Squamous Cell Carcinoma Through JAK/ STAT3/EMT Pathway*

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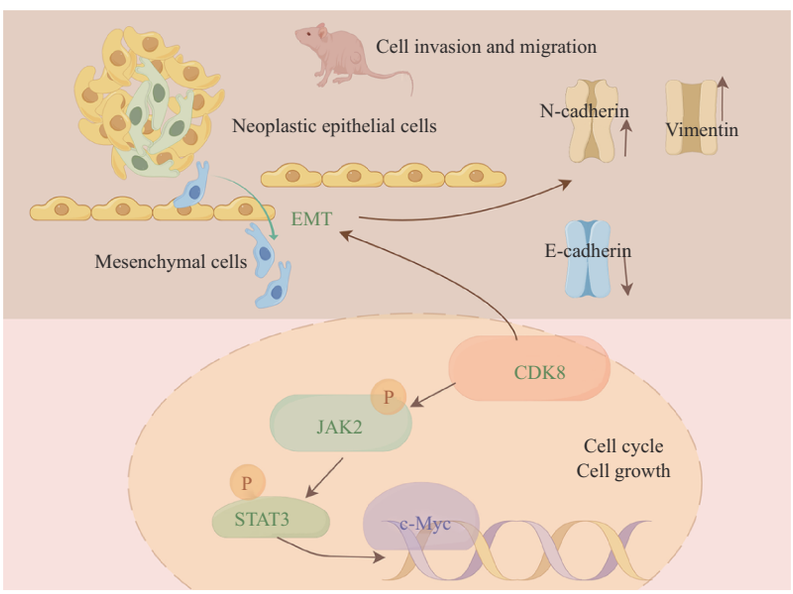
Graphical abstract



Abstract **Objective** To investigate the expression of cyclin-dependent kinase 8 (CDK8) in esophageal squamous cell carcinoma (ESCC) and its effect on ESCC cells, and to explore its potential molecular mechanism. **Methods** The expression level of CDK8 mRNA was analyzed using UALCAN database, and then the expression level of CDK8 protein in tumor tissues of ESCC patients was detected by immunohistochemistry (IHC). Esophageal cancer cell lines Kyse-30 and Kyse-150 were stably transfected with lentivirus to achieve knockdown and overexpression of CDK8. EdU proliferation assay, cell colony formation assay, cell cycle assay, cell scratch assay and invasion assay were used to explore the effect of CDK8 protein expression level on the phenotype of ESCC cells. Subsequently, the effect of CDK8 on the growth of esophageal cancer xenografts *in vitro* was observed by subcutaneous tumor formation assay in mice. Finally, the expression of proliferation and metastasis related proteins was detected by Western blot. **Results** CDK8 showed high transcription and protein expression levels in ESCC tissues compared with normal esophageal tissues. Knockdown of CDK8 expression significantly inhibited the proliferation, migration and invasion of ESCC cells. In addition,

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inhibition of CDK8 expression significantly affected the JAK2/STAT3 pathway and the expression of E-cadherin/N-cadherin, while overexpression of CDK8 reversed these effects. Inhibition of STAT3 pathway reversed the promoting effect of CDK8 overexpression on ESCC cell phenotype. **Conclusion** CDK8 is a cancer-promoting factor of ESCC, which mediates the phosphorylation of JAK2/STAT3 and epithelial-mesenchymal transition (EMT).

Key words CDK8, ESCC, JAK2/STAT3, EMT
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Esophageal cancer (EC) is one of the most common cancers worldwide. According to GLOBOCAN data, the global incidence of EC accounts for 3.2% of all cancer diagnoses, and the mortality rate accounts for 5.3% of all cancer deaths[1-2]. Among them, 9% of the world's ES cases occur in China, and it is the fourth leading cause of cancer death in China, with a five-year survival rate of only 20%−30%[3-4]. The high mortality rate of EC is related to its difficulty in detecting before metastasis, leading to delayed diagnosis and poor prognosis, and more than 90% of EC patients are esophageal squamous cell carcinoma (ESCC) [5-6]. Endoscopy is currently the gold standard for clinical diagnosis of ESCC precancerous squamous lesions. On the one hand, the cost-benefit study results of ESCC endoscopic screening indicate that microscopic screening is cost-effective only in areas with high incidence of ESCC, on the other hand, microscopy has the risk of perforation, bleeding, and infection[7]. Therefore, it is necessary to deeply study the etiology and tumor occurrence mechanism of ESCC, develop cheaper and safer screening strategies and therapeutic biomarkers, and use them in combination with existing technologies to improve patient survival rate.

Uncontrolled cell growth and proliferation are characteristic of tumor cells[8]. Cyclin-dependent kinases (CDKs) belong to the serine/threonine (Ser/Thr) family of protein kinases and play a key role in the regulation of cell cycle. Studies have confirmed that abnormal activation of CDKs can lead to disorders in the cell cycle process, which leads to abnormal proliferation of tumor cells. Cyclin-dependent kinase 8 (CDK8) is located on chromosome 13 and belongs to a member of the CDK protein family[9-10]. As a new therapeutic target, CDK8 is considered as a potential prognostic marker for colorectal cancer (CRC) patients[11]. In terms of treatment, inhibitors TSN084 that target CDK8/19, which are closely related to tumor proliferation and immune evasion, have shown significant tumor suppressive effects and are expected to become new hopes for tumor targeted therapy. In breast cancer, CRC, melanoma, and endometrial cancer, CDK8 promotes cancer cell growth by regulating the cell cycle[12-13]. In addition, CDK8 can also drive tumor progression by inducing invasiveness and metastasis of pancreatic cancer and CRC tumor cells through epithelial-mesenchymal transition (EMT) and invasion[14-15]. In summary, CDK8 plays a crucial role in cell cycle regulation and tumorigenesis. Currently, the CDK8-STAT3 signaling pathway has been shown to play an important role in the progression of triple-negative breast cancer and inflammation-related gastric cancer (GC)[16-17]. However, the biological role and mechanism of CDK8 in ESCC remains unclear.

In this study, we analyzed the expression of CDK8 in tumor tissues and adjacent tissues of ESCC patients, explored the effects of CDK8 on proliferation, migration and invasion of ESCC cells, and explored the possible mechanisms and signaling pathways by which CDK8 regulates ESCC progression.

1 Materials and methods

1.1 Public databases

UALCAN (https://ualcan.path.uab.edu/index.html) based on TCGA (The Cancer Genome Atlas) database, the integration of a variety of Cancer types of gene expression, methylation, clinical data and other information. We queried the expression level of CDK8 in esophageal cancer and compared it with normal tissues.

1.2 Immunohistochemical analysis

A total of 196 ESCC patients diagnosed at Taizhou Hospital of Zhejiang Province affliated to Wenzhou Medical University from 2004 to 2018 were collected. Formalin-fixed paraffin sections were subjected to immunohistochemical (IHC) staining. Tissue sections were first deparaffinized in xylene,

graded ethanol hydration, and high-pressure antigen repair, then inactivated endogenous peroxidase by treatment with 3% hydrogen peroxide, blocked with fetal bovine serum (FBS), and incubated with primary antibody for 4℃ overnight. Sections were washed and incubated with secondary antibodies for 2 h. After washing, the sections were developed with diaminobenzidine (DAB) and nucleated with hematoxylin. CDK8 expression levels were scored by intensity: 0 (negative), 1 (weak), 2 (medium), 3 (strong). This study was approved by the Ethics Committee of Taizhou Hospital (No. K20210618).

1.3 Cell culture and transfection

The ESCC cell lines Kyse-30 and Kyse-150 were purchased from National Collection of Authenticated Cell Cultures (Shanghai, China). The cells were incubated in 1640 medium (meilunbio) containing 10% FBS (Gibco) and cultured in a 37℃, 5% CO_2. pLVshRNA, pSPAX2, and pMD2.G at a ratio of 2∶1∶1 or pCDH, pLP1, pLP2, and pLP-VSVG at a ratio of 2∶1∶1∶1 were incubated in opti-MEM (Gibco) with Lipo3000 (Invitrogen, cat. no. L3000015) were transfected into 293T cells to prepare lentivirus. Kyse-30 and Kyse-150 cells were transduced with green fluorescent labeled recombinant lentiviral mixed polybrene (5 g/L) at a multiplicity of infection (MOI) of 5, and stable knockdown or overexpression cells were obtained by 3 mg/L and puromycin selection. Subsequently, the expression of CDK8 in cells was identified by quantitative PCR (qPCR) and Western blot. The shRNA were as follows: shRNA-1, GCACTTATGGTCACGTCTACA; shRNA-2, GCAGGGCAATAACCACACTAA; shRNA-3, GCTTACCATGGACCCAATAAA.

1.4 RNA isolation and reverse transcription PCR（RT-PCR） analysis

Total RNA was isolated by TRIzol reagent (Thermo Fisher, cat.no.15596018CN), and cDNA was obtained by reverse transcription using PrimeScript TM RT prex kit (Takara, cat. no. RR037A). The TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) kit (Takara, cat. no. RR820A) was used for quantitative analysis. The primers are as follows: 18 S forward 5'-TTTCTCGATTCCGTGGGTGG-3' and reverse 5'-AGCATGCCAGAGTCTCGTTC-3'; CDK8 forward 5'-ACCTGTTTGAATACGAGGGCT-3' and reverse 5'-TGCCGACATAGAGATCCCAGT-3'. Relative transcript amounts were determined using the $2^{-\Delta\Delta C_t}$ method and normalized with 18 S as the endogenous reference gene, and all reactions were performed in triplicate.

1.5 Western blot

Kyse-30 and Kyse-150 cells were harvested and total proteins were extracted using RIPA lysis buffer (Solarbio, cat. no. R0020), and 4 μg of total proteins were separated by SDS-PAGE and transferred to PVDF membranes. After blocking for 2 h in blocking buffer with 5% skim milk and incubation with primary antibodies overnight at 4℃, the primary antibodies used for Western blot assays are listed: CDK8 (1∶1 000, GeneTex, cat. no. GTX110495); E-cadherin (1∶1 000, GeneTex, cat. no. GTX100443); N-cadherin (1∶1 000, CST, cat. no. 14215); Vimentin (1∶1 000, CST, cat. no. 5741); JAK (1∶1 000, CST, cat. no. 3771); p-JAK2 (1∶1 000, CST, cat. no. 3771); STAT3 (1∶1 000, CST, cat. no. 9139); pSTAT3 (1∶1 000, CST, cat. no. 9145); c-Myc (1∶1 000, CST, cat. no. 9402); GAPDH (1∶10 000, Immunoway, cat. no. YM3029). After washing three times with TBST, anti-rabbit or anti-mouse secondary antibodies (1∶10 000) were incubated, the membranes were detected using an enhanced chemiluminescence system (Bio-Rad), and the protein was analyzed semi-quantified using Image J software.

1.6 CCK8 and EDU assays

Using Cell Counting Kit-8 (Beyotime, cat. no. C0038) to analyze cell proliferation. Kyse-30 and Kyse-150 cells were seeded into 96-well plates at a density of 3×10^3/well, and cells were detected at 24, 48, and 72 h by Cell Counting Kit-8. EDU method: EDU staining was performed to measure cell proliferation using the BeyoClick EDU cell proliferation kit (Biyuntian, cat. no. C00788L) according to the manufacturer's instructions. Cells were incubated with EDU (10 μmol/L) for 3 h and then fixed with a fixed solution for 15 min at room temperature. Cells were washed with PBS for 5 min and incubated with the permeabilized solution for 15 min at room temperature. After washing with PBS for 5 min, the cells were incubated with Click's solution for 30 min at room temperature in the dark. The percentage of EDU-positive cells was determined by flow cytometry (Beckman Coulter).

1.7 Clone formation assay

The esophageal squamous carcinoma cells were seeded into 35 mm dishes (1 000 cells/dish) and

cultured in a cell incubator for 7 d. They were fixed with 4% paraformaldehyde for 15 min, then stained with 0.2% crystal violet for 15 min, washed and dried and photographed to calculate the clone formation rate.

1.8 Wound healing assay

Kyse-30 and Kyse-150 cells were seeded in 6-well plates at a density of 5×10^5/well. When cells grew to confluence, the cells were treated with serum-free medium for 24 h. The monolayer was then scratched with a sterile pipette tip. After washing once with sterile PBS, serum-free medium was added. The cells were placed in a cell incubator to culture, and the wound healing was observed by photographing.

1.9 Cell invasion assay

Matrigel was diluted on ice with serum-free cell medium, and 50 μl/well of matrigel was added to the upper chamber of Transwell plates (BD Biosciences) and then incubated at 37℃ for 2 h until the gel solidified. 600 μl of 1640 medium containing 10% FBS was added to the lower chamber of Transwell plates, after which Kyse-30 and Kyse-150 cells were resuspended by trypsin digestion and counted. Cells seeded in the upper chamber at a density of 2×10^4/well, then they were placed in the incubator for 48 h. After erasure of the upper chamber cells with a cotton swab, the cells were fixed with 4% paraformaldehyde in methanol for 15 min at room temperature, followed by crystal violet staining for 15 min, microscopic photographs were taken and counted.

1.10 Subcutaneous xenografts

Four-week old male nude mice were randomly divided into 4 groups: knockdown control virus transfected cell group, overexpression control virus transfected cell group, CDK8 knockdown cell group and overexpressed cell group. Cells were harvested and resuspended in medium containing 50% Matrigel and subcutaneously injected (100 μl per injection) into the flank of athymic BALB/c nude mice (n=5/group, 5×10^6 cells per injection site). After six weeks, the mice were sacrificed. The tumor mass was excised, its weight measured, and part of the mass saved in formalin for subsequent pathological staining analysis. All animal experiments were conducted in accordance with the standards of the Guidelines for Ethical Review of Laboratory Animal Welfare issued by the People's Republic of China, and were approved by the Animal Ethics Committee of Taizhou Hospital (No.tyz-2022133).

1.11 HE staining and Ki67 expression analysis

The tumor tissue cut from nude mice was put into the pre-prepared fixative (10% formalin) to denature the protein and solidify, then dehydrated, transparent, wax embedded and sectioned. Subsequently, we processed the tumor tissue sections for hematoxylin-eosin (HE) staining and IHC staining for Ki67. HE staining indicated the arrangement and atypia of tumor cells, and the changes of nucleus and cytoplasm structure. Nuclear antigen Ki67 (MKI 67) was the most widely studied IHC marker of tumor proliferation, which was expressed in all stages of the cell cycle. Nuclear staining of Ki67 is considered a positive result. The sites with the highest Ki67 expression in the tissues were selected and photographed to evaluate the percentage of Ki67 immunopositive cells in the tumor cells.

1.12 Statistical analysis

Data are summarized as the mean±SD of three independent experiments. Statistical data were analyzed by SPSS19.0 software and t-test analysis. Two-tailed P values<0.05 were considered statistically significant, with significant group differences ($^*P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001).

2 Results

2.1 High expression of CDK8 in ESCC

First we analyzed CDK8 expression in tumor tissues of ESCC patients using the UALCAN database. The expression of CDK8 was significantly higher in ESCC tissues than in normal esophageal tissue (Figure 1a). Subsequently, we performed IHC staining of the tumor tissue from 196 ESCC patients. The results showed that CDK8 was expressed in the cytoplasm and nucleus of ESCC cells, and the expression level of CDK8 was significantly up-regulated in ESCC cells compared with adjacent cells (Figure 1b, c).

2.2 Establishment and verification of stable cell lines

To define the potential role of CDK8 in ESCC cells, we first constructed shRNA targeting CDK8, transduced ESCC cells to obtain Kyse-30-shCDK8 and Kyse-150-shCDK8 cells that inhibited CDK8 expression. and determined the lentiviral transfection efficiency by GFP expression. RT-PCR and Western blot results showed that the expression of CDK8 in

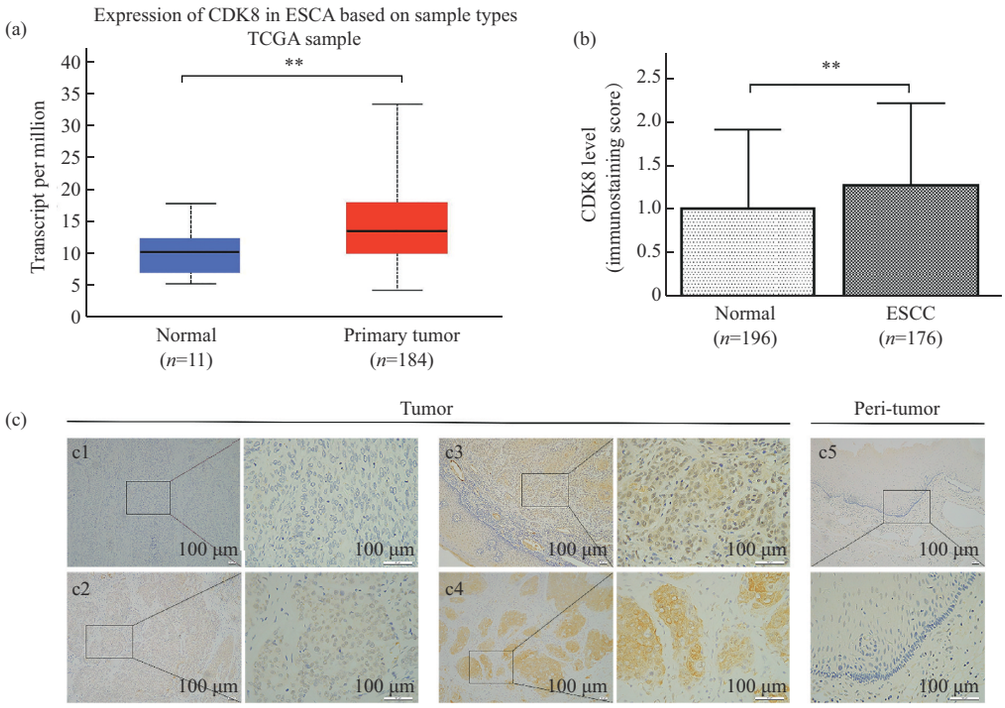

Fig. 1　Expression of CDK8 in tumor tissues of patients with ESCC

(a) UALCAN database analysis of CDK8 expression in cancer tissues of ESCA patients. (b) IHC results of CDK8 protein levels between cancer cells and pericarcinomatous tissue cells. (c) Representative pictures of the IHC. c1: CDK8 score 0; c2: CDK8 score 1; c3: CDK8 score 2; c4: CDK8 score 3; c5: pericarcinomatous tissue. ***P*<0.01.

the three interference groups was significantly lower than that in the control group (*P*<0.05), and the interference effect of sh-2 and sh-1 was good. Therefore, these two groups were selected for subsequent cell experiments (Figure 2a, b).

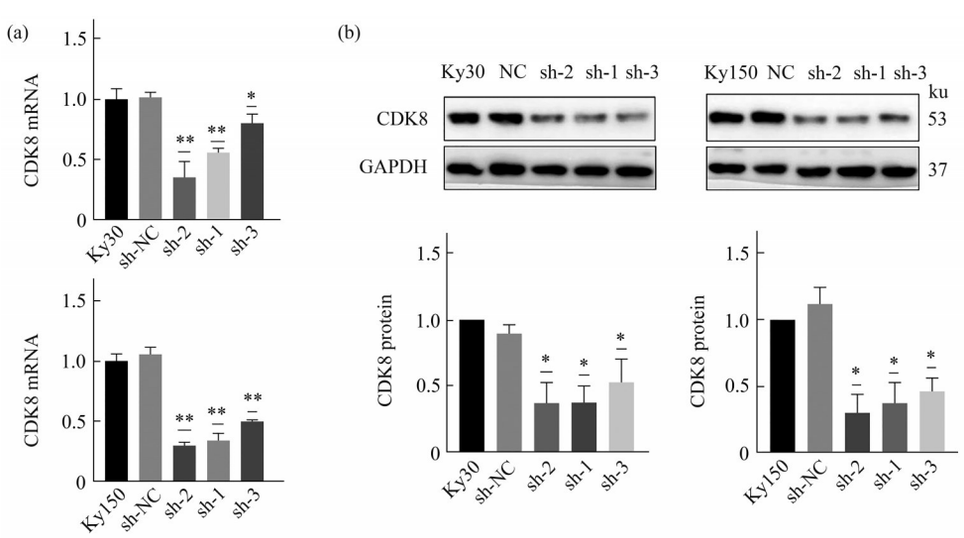

Fig. 2　CDK8 expression and knockdown screening in ESCC cell lines

The knockdown effect of shRNA-CDK8 in ESCC cells was confirmed by RT-PCR (a) and Western blot (b). Ky30, untransduced Kyse-30 cells; Ky150,untransduced Kyse-150 cells; sh-NC, knockdown control cells; sh-1/2/3, CDK8-knockdown cells. All the experiments were repeated at least three times.**P*<0.05; ***P*<0.01.

2.3 Knock−down of CDK8 inhibits the malignant phenotype of ESCC cells

To determine whether CDK8 regulates the growth of ESCC cells, we first constructed stable CDK8 knock-down cell lines, followed by CCK-8, colony formation and EDU experiments, and found that the cell proliferation capacity was reduced after silencing CDK8 in ESCC cells (Figure 3a−c). Cell cycle results showed that CDK8 inhibition induced a significant G1 phase arrest and increased the percentage of cells in the G0/G1 phase (Figure 3d). Wound-healing assays indicated that the control group filled the wound faster than the CDK8 knockdown cells (Figure 3e). Transwell assay further showed that the migration ability of ESCC cells was weakened after CDK8 knockdown (Figure 3f). These results suggest that CDK8 can promote the malignant phenotype of ESCC.

2.4 Overexpression of CDK8 promotes the malignant behavior of ESCC cells

To further confirm whether the effect of CDK8 inhibition on ESCC cell phenotype was caused by the increase of CDK8, we performed cell growth, migration, and invasion assays by constructing stable CDK8 overexpressing cells (Figure 4a). Consistent with the above results, overexpression of CDK8 promoted the proliferation, migration and invasion of EC cells, and reduced the number of cells in G0/G1 phase (Figure 4b−f).

2.5 Effect of CDK8 expression on the growth of ESCC cell xenograft tumors

In view of the *in vitro* demonstration that CDK8 promotes the growth and migration of ESCC cells, we further clarified the effect of CDK8 on ESCC by *in vivo* xenograft assays. Subcutaneous xenograft experiments were performed in nude mice using control knockdown (sh-NC) , control overexpression (o-NC), CDK8 knockdown (sh-CDK8) and CDK8 overexpression (o-CDK8) Kyse-30 cells. As shown in Figure 5a, sh-CDK8 xenograft tumors that were significantly smaller than those generated by sh-NC, while tumors in the o-CDK8 group were larger than those in the o-NC group. To demonstrate whether the difference in tumor size is caused by the reduced proliferation of ESCC cells, we further examined the expression of the proliferation marker Ki-67 in the tumor cells. The results showed that the tumors were less Ki-67 positive than the control cells, while the opposite was true in the overexpression group (Figure 5b). At the same time, we also performed HE staining of the sample tissue, and no significant differences in tumor structure were observed under the microscope (Figure 5c).

2.6 Effect of CDK8 expression on ESCC cell−related protein expression

The expression levels of related proteins were investigated in Kyse-30 cells. Compared with the control group, CDK8 knockdown (sh-CDK8) Kyse-30 cells decreased the protein expression levels of p-Jak2, p-Stat3, c-Myc, N-cadherin and Vimentin, and increased the expression level of E-cadherin (Figure 6a, c). In contrast, CDK8 overexpression (o-CDK8) Kyse-30 cells showed increased protein expression of p-JAK2, p-STAT3, c-Myc, N-cadherin and Vimentin, but decreased protein expression of E-cadherin (Figure 6b, d).

2.7 STAT3 inhibitors inhibited c−Myc expression and prevented phenotypic changes induced by CDK8 overexpression

To further determine the involvement of CDK8 in CDK8 function in ESCC cells by activating the JAK2/STAT3 signaling pathway to promote c-Myc protein expression, we treated Kyse-30 cells overexpressing CDK8 with Stattic, a STAT3 pathway inhibitor. Treatment of cells with Stattic (5 μmol/L) not only significantly reduced the expression level of p-STAT3, but also downregulated the expression of c-Myc (Figure 7a), indicating that c-Myc is downstream of the STAT3 pathway. In addition, we observed that Stattic treatment all significantly inhibited the promotion of CDK8 overexpression on Kyse-30 cell proliferation, migration, and invasion (Figure 7b−d).

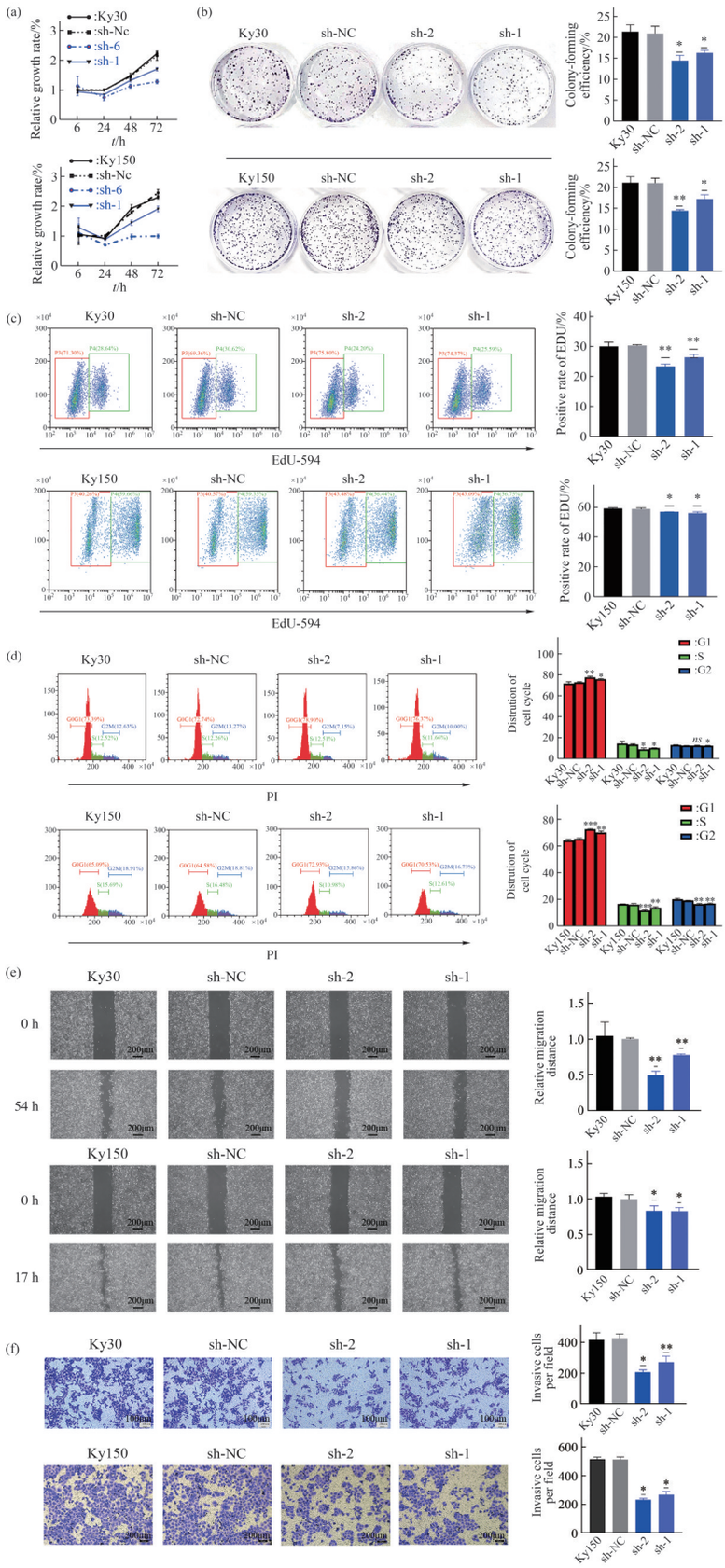

Fig. 3　Effects of CDK8 knockdown on ESCC cell proliferation, migration, and invasion

(a−c) CCK-8, cell cloning and EDU experiments verified the proliferation of ESCC cells. (d) Flow cytometry analysis of cell cycle. (e) Wound healing experiment demonstrated the migration ability of ESCC cells. (f) The invasive ability of ESCC cells was determined by the Transwell method. All the experiments were repeated at least three times. **P*<0.05; ***P*<0.01; *ns*: no significance.

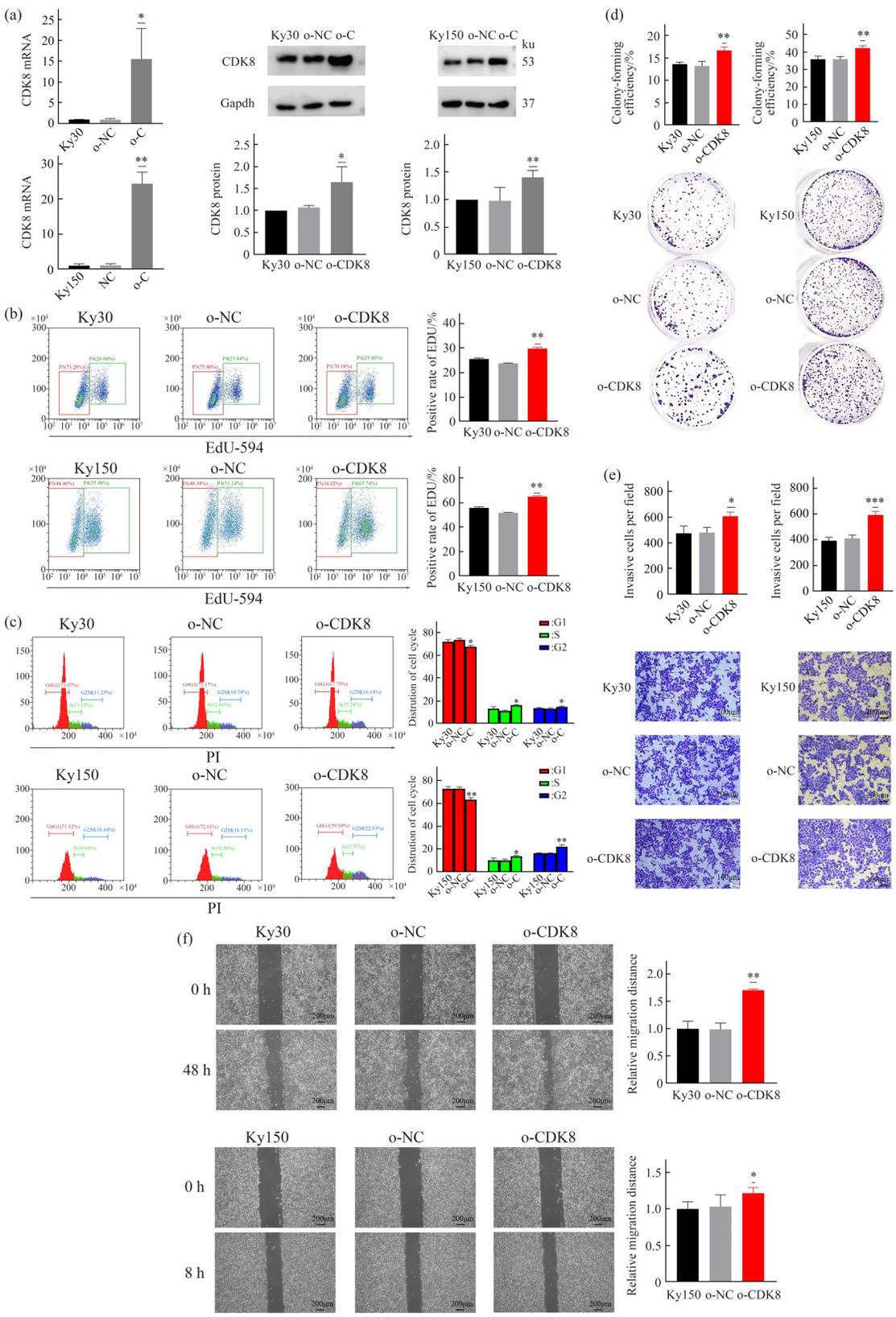

Fig. 4　Overexpression of CDK8 significantly promotes ESCC cell proliferation, migration, and invasion

(a) The CDK8 expression was confirmed by qPCR and Western blot analysis. (b, d) The influence of CDK8 overexpression in the proliferation of ESCC cells were evaluated by EDU assay (b) and cell clones formation assay (d). (c) Flow cytometric analysis of the o-CDK8 cell cycle. (e, f) Transwell and wound healing test for cell migration and cell invasion. o-NC, control overexpressed cells; o-CDK8,CDK8 overexpression cells. All the experiments were repeated at least three times. **P*<0.05; ***P*<0.01; ****P*<0.001.

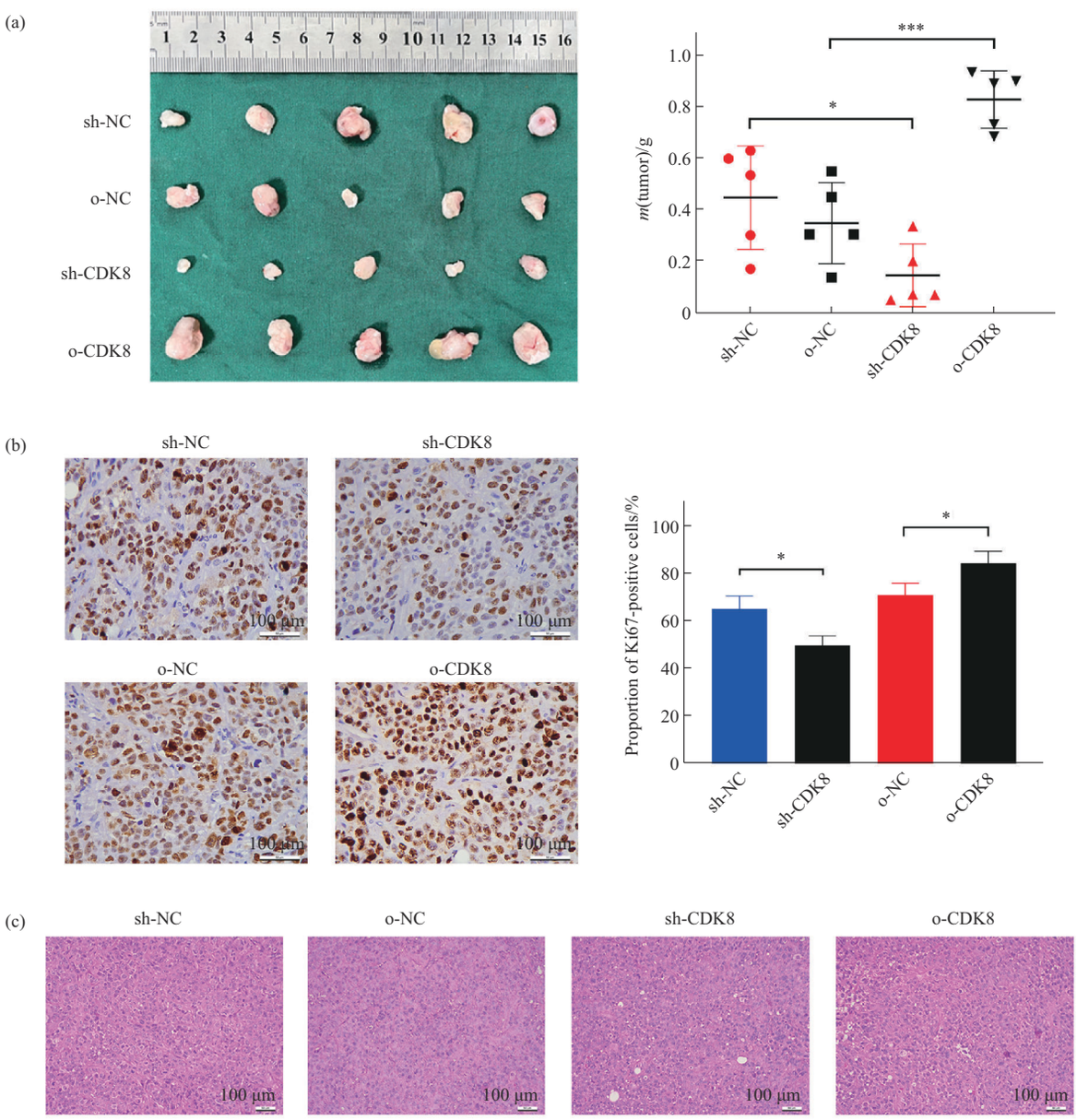

Fig. 5　Effect of CDK8 expression on the growth of xenograft tumors of ESCC cells

Overexpression of CDK8 significantly increased the tumor growth and the proportion of tumor Ki-67-positive cells in nude mice. (a) The results of tumor transplantation assay showed that overexpression of CDK8 promoted the growth of transplanted tumors. (b) IHC staining of Ki-67 showed that overexpression of CDK8 increased the proportion of Ki-67-positive cells in the tumor. (c)The HE staining results showed no structural differences between the tumor tissues. All the experiments were repeated at least three times. **P*<0.05; ****P*<0.001.

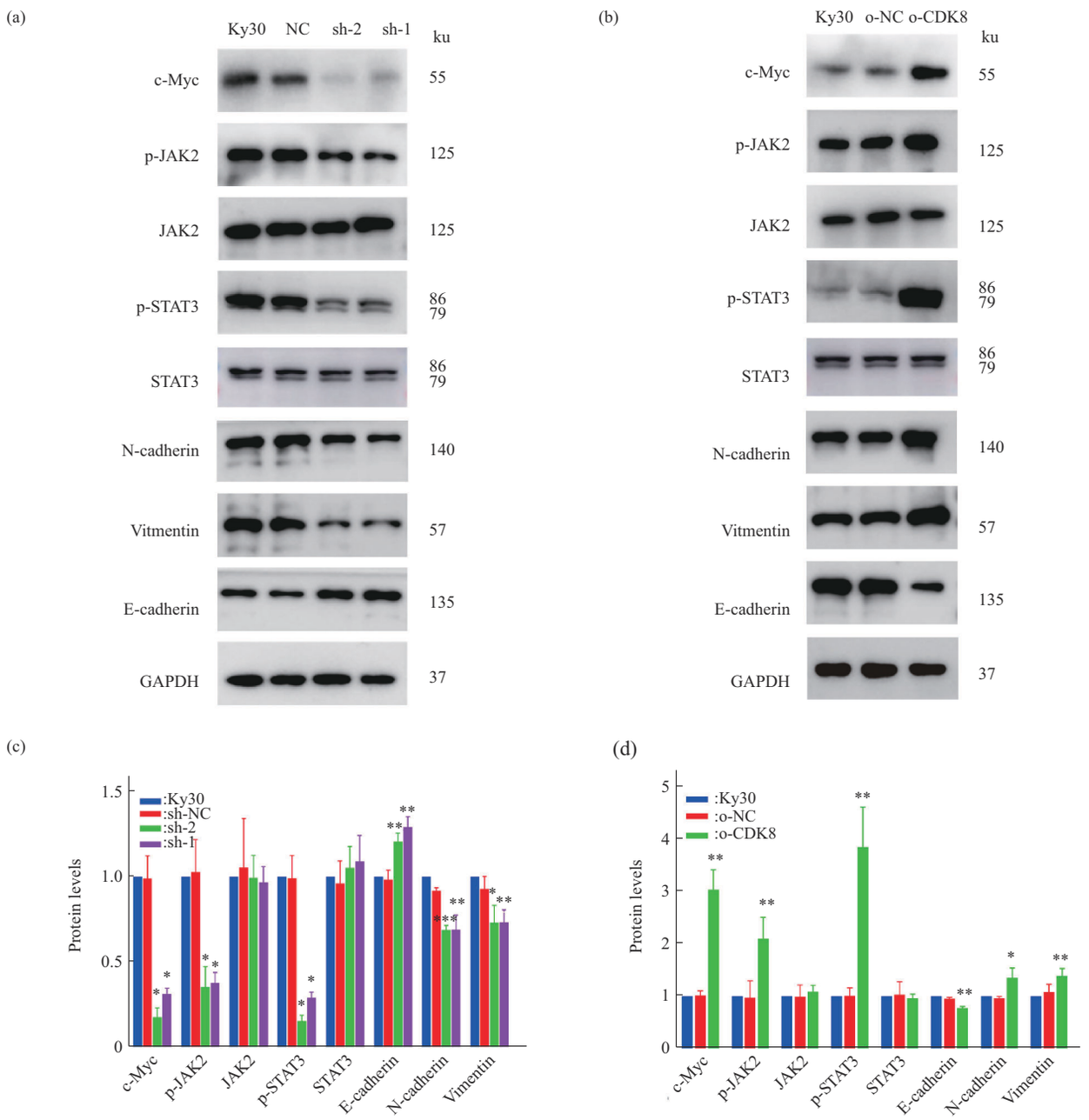

Fig. 6　Effect of CDK8 expression on the expression of ESCC cell−associated signaling pathways

(a,c) The expression levels of p-JAK2, p-STAT3, c-Myc, N-cadherin, E-cadherin and Vimentin after CDK8 knockdown in Kyse-30 cells. (b,d) The expression levels of p-JAK2, p-STAT3, c-Myc, N-cadherin, E-cadherinand Vimentin in CDK8 overexpression Kyse-30 cells. All the experiments were repeated at least three times. **P*<0.05; ***P*<0.01; ****P*<0.001.

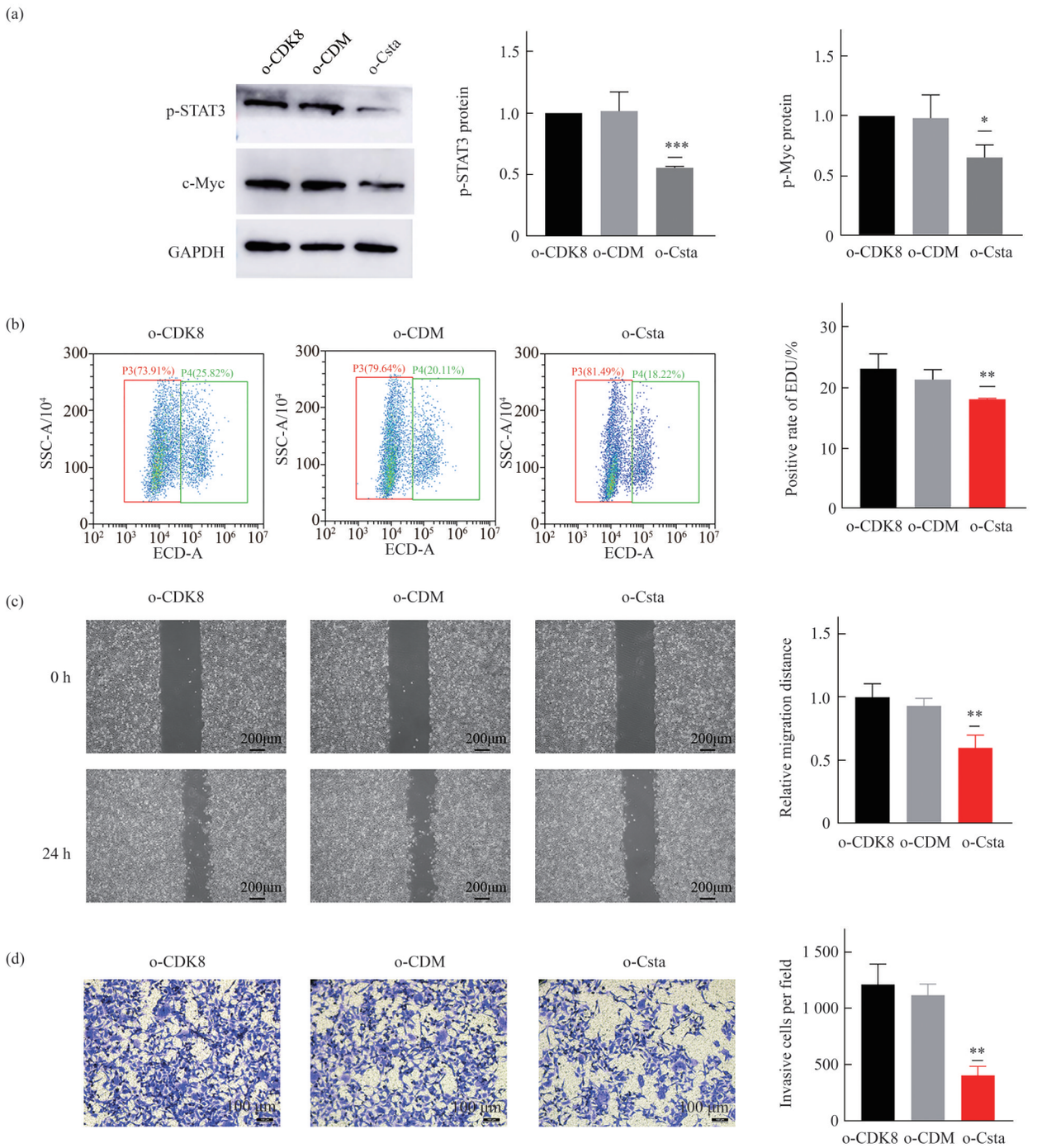

Fig. 7　STAT3 inhibitor inhibited CDK8−induced c−Myc expression and phenotypic changes

(a) Stattic treatment significantly reduced the levels of p-STAT3 and c-Myc in Kyse-30 cells overexpressing CDK8. (b) EDU test results. (c) Analysis results of wound healing capacity. (d) Results of cell invasion ability analysis. o-CDK8: Kyse-30 cells overexpressing CDK8; o-CDM: solvent (the same volume of DMSO) treated Kyse-30 cells; o-Csta: Stattic (5 μmol/L) treated Kyse-30 cells. All the experiments were repeated at least three times. **P*<0.05; ***P*<0.01; ****P*<0.001.

3 Discussion

In recent years, several studies have linked the dysregulation of CDK8 to different types of cancer, and found that the abnormal expression of CDK8 is related to tumor proliferation, invasion and metastasis[18-19]. As a transcriptional cyclin-dependent kinase, targeting CDK8 for the development of anti-tumor drugs holds great promise; therefore, it is of great interest to investigate the function of CDK8 to evaluate the efficacy of this approach and avoid potential side effects. In addition, CDK8, as a key transcriptional regulator, regulates transcription mainly by binding to the mediator complex and directly phosphorylating transcription factors, participating in Wnt/β-catenin, E2F1, STAT1 and SMAD signaling pathways[20]. This mechanism is important in many biological processes such as cell cycle control and tumorigenesis. However, little is known about the relationship between CDK8 and the development of EC. Studies have confirmed that conditionally induced CDK8 deletion does not inhibit normal cell growth and severe abnormalities in normal adult mice, which provides important theoretical support for CDK8 as a clinical therapeutic target for ESCC[21-22].

In this study, we first analyzed the expression of CDK8 in tumor tissues of ESCC patients through the UALCAN database as well as clinical patient pathology samples, and found that the protein expression level of CDK8 was upregulated in tumor tissues of ESCC patients. Firstly, knockdown of CDK8 in ESCC cells inhibited cell proliferation by CCK8, colony formation and EDU assays. Flow cytometry analysis showed that CDK8 knockdown induced G1 phase arrest in ESCC cells. In addition, wound healing and Transwell assays demonstrated that CDK8 knockdown attenuated ESCC cell migration and invasion, while CDK8 overexpression in ESCC cells reversed these effects. Subsequently, we conducted *in vivo* animal experiments and found that CDK8 knockdown ESCC cells generated smaller tumors, and the tumors in the CDK8 overexpression group were significantly larger. Moreover, compared with the tumors in the control group, the tumor cell proliferation marker Ki-67 positive cells were decreased in the tumor tissues of CDK8 knockdown group, while the overexpression group was increased. All these results suggest that CDK8 plays a cancer-promoting role in EC.

Studies have shown that CDK8 can regulate transcription and cell cycle progression by phosphorylating related transcription factors. The interaction between CDK8 and STAT3 is mainly through the interaction between CDK8 and STAT3 in the nucleus, regulating the phosphorylation state of STAT3, and directly participating in the regulation of signal transcription[23-25]. The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, as a classical oncogenic signaling pathway, is involved in the growth, migration, differentiation and apoptosis of cancer cells[26]. As an important downstream target gene of STAT3, *c-Myc* regulates 15%−20% of human cell growth and transformation related genes[27-28]. Consistent with multiple studies supporting CDK8 as an oncogene, downregulation of CDK8 in ESCC cells in the present study significantly inhibited the phosphorylation of JAK2/STAT3 and decreased the expression of the downstream oncogene c-Myc, which was reversed by CDK8 overexpression. We observed that treatment of cells with STAT3 specific inhibitors not only reduced the activation level of STAT3 pathway, such as the reduction of p-STAT3 level, but also significantly reduced the expression and activation level of c-Myc, which could prove that CDK8 regulates c-Myc expression through STAT3 pathway. Moreover, in diseases such as triple-negative breast cancer (TNBC) and pancreatic cancer, CDK8 participates in the EMT process through multiple mechanisms[29]. EMT refers to the phenotypic transition from epithelial cells to mesenchymal cells. In cancer, EMT promotes the invasion and metastasis of tumor cells and is a key step in the malignant progression of tumors. Examples include a decrease in E-cadherin and an increase in mesenchymal cell markers such as N-cadherin and Vimentin[30-31].Although there is no direct evidence for how CDK8 promotes EMT, it can be speculated that CDK8 may indirectly contribute to the EMT process through mechanisms such as influencing the cell cycle, participating in signaling pathways, or regulating specific gene expression. Therefore, we examined the EMT epithelial marker E-cadherin and the mesenchymal markers N-cadherin and Vimentin, demonstrating that CDK8 affects ESCC metastasis by participating in the EMT process.

Our study has certain limitations. Firstly, the

information of the clinical sample investigated in this study is not complete, especially in the part related to clinical prognosis. Secondly, CDK8 has been shown to be involved in the regulation of Wnt/β-catenin, NF-κB, transforming growth factor β (TGF-β) and Notch signaling pathways[32-33]. Therefore, other pathways in ESCC progression regulated by CDK8 remain to be investigated.

4 Conclusion

This study reported for the first time that CDK8 expression is up-regulated in ESCC cells and promotes the proliferation, migration and invasion of ESCC cells, which may be achieved by mediating the phosphorylation of JAK/STAT3, up-regulating the expression of c-Myc protein and participating in the EMT process. These findings may provide new ideas for the development of targeted therapy strategies for ESCC.

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CDK8通过JAK2/STAT3/EMT途径促进食管鳞状细胞癌的细胞增殖、迁移和侵袭*

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摘要　目的　本研究旨在探究细胞周期蛋白依赖性激酶8（cyclin-dependent kinase 8，CDK8）在食管鳞状细胞癌（esophageal squamous cell carcinoma，ESCC）中的表达及其对ESCC细胞的影响，并探讨潜在的分子机制。**方法**　首先通过UALCAN数据库分析了CDK8 mRNA的表达水平，然后利用免疫组织化学技术检测了CDK8蛋白在ESCC患者肿瘤组织中的表达水平。利用慢病毒稳定转染食管癌Kyse-30及Kyse-150细胞株以敲低或过表达CDK8，通过EdU增殖实验、细胞集落形成实验、细胞周期实验、细胞划痕实验和侵袭实验等多种方法探究CDK8蛋白表达水平的改变对食管癌细胞表型的影响。随后，通过小鼠皮下成瘤试验观察CDK8对体外食管癌移植瘤生长的影响。最后通过蛋白质印迹法检测增殖和转移相关蛋白质的表达情况。**结果**　与正常食管组织相比，CDK8在ESCC组织中转录和蛋白质表达水平均较高。敲低CDK8的表达能够显著抑制ESCC细胞的增殖、迁移和侵袭；此外，抑制CDK8表达可显著影响JAK2/STAT3通路以及E-cadherin/N-cadherin/Vimentin的表达，过表达CDK8则可以逆转上述结果，抑制STAT3通路逆转了CDK8过表达对ESCC细胞表型的促进作用。**结论**　CDK8是ESCC的促癌因子，可介导JAK2/STAT3磷酸化水平以及上皮-间充质转化（epithelial-mesenchymal transition，EMT）的过程。

关键词　CDK8，食管鳞状细胞癌，JAK2/STAT3，上皮-间充质转化
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