Original Article
miRNA-574-3p inhibits metastasis and chemoresistance of epithelial ovarian cancer (EOC) by negatively regulating epidermal growth factor receptor (EGFR)

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Abstract: Background: This current study explored the role of miRNA-574-3p and the related molecular mechanisms in epithelial ovarian cancer (EOC). Methods: Tissues of ovarian cancer patients were applied to explore the correlation between miRNA-574-3p and EOC. The role of miRNA-574-3p in migration, invasion and chemoresistance of EOC cells was evaluated by overexpression and suppression of miRNA-574-3p in SKOV3 and CAOV3 cells. For the sake of exploring how miRNA-574-3p regulated tumor migration, invasion and chemoresistance of EOC cells, we detected several related molecular expressions and activities of signaling pathways. Results: Overexpression of epidermal growth factor receptor (EGFR) was correlated with downregulation of miR-574-3p in EOC tissues. Overexpression of miRNA-574-3p in EOC cells led to inhibition of cell migration as well as invasion, and it significantly promoted the sensitivities of EOC cells to paclitaxel and cisplatin. Molecular experiments showed miR-574-3p inhibited activation of AKT, FAK and c-Src, as well as MMP-9 expression via targeting EGFR. Conclusion: Taken together, these data demonstrated that miRNA-574-3p inhibits both tumor metastasis and chemoresistance in EOC via targeting EGFR. Thus, targeting miRNA-574-3p may become a potential molecular method for EOC.

Keywords: microRNA, miR-574-3p, EGFR, epithelial ovarian cancer

Introduction

Ovarian cancer is the most fatal kind among all gynaecological cancers and ranks the fifth fatal tumors among women worldwide [1, 2], and ovarian cancer is a highly invasive form of cancer leading to almost 140,000 deaths yearly [3, 4]. The high mortality of ovarian cancer is mainly because of the high percentage (> 70%) of late-stage diagnosis of patients, resulting in substantially poor prognosis. Despite the efforts that have been made in the last several decades towards detection and cytotoxic therapies for ovarian cancer, patient prognosis has only modestly promoted [1]. Among all pathological types of ovarian cancer, epithelial malignant tumor accounts for the vast majority.

Over the last decade, improvement of expression profiling technologies has enabled the simultaneous detection of numerous genes and proteins or other molecules, allowing identification of dominant pathways which may cause the occurrence of cancer. The application of these new technologies has identified few molecular mechanisms related to the occurrence and development of EOC [5]. Several publications have shown the power of these technologies in identifying common pathways and mutations in EOC so far [6, 7].

Recent evidence has indicated a novel kind of regulatory RNAs, namely microRNAs (miRNAs), which are up-regulated or mutated in cancers, suggesting they may play important parts in cancer initiation and development [8]. MiRNAs are an abundant class of endogenous small, non-coding RNA molecules that contain 20-25 nucleotides and are processed from hairpin pre-miRNAs of 70-100 nucleotide [9]. DNA

Table 1. Clinicopathologic features of 73 patients with EOC

Clinicopathologic features	Number of Patient
Age (year)	
> 50	32
≤ 50	41
Tumor histology	
Serous	49
Mucinous	11
Endometroid	5
Clear cell	6
Mixed epithelial	2
FIGO stage*	
I	12
II	8
III	44
IV	9
Tumor grade	
1	29
2	11
3	33

*FIGO represents International Federation of Gynecology and Obstetrics.

methylation or histone modification may affect gene expression without changes of DNA sequence, but miRNAs present another epigenetic regulatory programme. MiRNAs may mediate posttranscriptional gene expression primarily via base-pairing to the 3'-UTR of the target mRNA, resulting in translational repression, mRNA cleavage, or destabilization through formation of RNA-induced silencing complexes [10]. There is strong evidence that miRNAs involved in the onset and progression of diverse malignant tumors. A large amount of miRNAs are found abnormally expressed in EOC, and many studies have begun to elucidate the biological significance of some of these miRNAs [11].

EGFR, situated at chromosome 7p12, is frequently aberrantly overexpressed in ovarian cancer and this abnormal expression can be found in every pathological type [12, 13]. In addition, aberrant EGFR expression is found in patients with high tumor grade, high cell proliferation index, aberrant P53 expression, and poor prognosis [14, 15]. This present study investigated miR-574-3p, a possible target miRNA of EGFR in EOC, using a series of experiments to elucidate the changes in miRNA

expression and to identify potential association with EGFR.

Materials and methods

Patient samples

Two sample sets, namely, a normal set (n = 15) and an EOC set (n = 73, **Table 1**), were studied. Medical records, as well as clinicopathologic information were extracted from institutional and laboratory databases. Cases were selected from the Obstetrics and Gynaecology Hospital of Fudan University, and cases were classified based on the International Federation of Gynaecology and Obstetrics (FIGO) criterion. Samples were collected after being approved by the ethics committee of the hospital. In addition, sample collection began after receiving written informed consent of patients. Furthermore, patients did not receive any chemotherapy before operation. Tissues were used for mRNA or protein extraction to measure the expressions of miR-574-3p and EGFR on the basis of the manufacturer's protocol.

Cell culture

SKOV3 and CAOV3 cells were obtained from American Type Culture Collection (ATCC). Cells were cultured in RPMI-1640 with 10% foetal bovine serum (HyClone, UT), 2 mM penicillin and streptomycin (GIBCO Invitrogen by Life Technologies Corp., Carlsbad, CA). HEK293T cells were grown in DMEM with 10% foetal bovine serum (HyClone, UT), 2 mM penicillin and streptomycin (GIBCO Invitrogen by Life Technologies Corp., Carlsbad, CA).

Vector constructs

The 3'-UTR of mutant EGFR, including the putative miR-574-3p-binding region, was cloned downstream of a cytomegalovirus (CMV) promoter-driven firefly luciferase cassette in a pcDNA3.0 vector.

Luciferase reporter assay

Cells were put in 96-well plates before they were transfected with a mixture of 50 ng of pcDNA-luc-UTR, 5 ng of pRL-CMV and 5 pmol miR-574-3p or negative control (Promega, Madison, WI). After 48 h, luciferase activity was detected using the Dual Luciferase Reporter Assay System (Promega, Madison, WI) based

on manufacturer's protocols. The luciferase activity of each sample was normalized to Renilla luciferase activity.

Transfection and transduction

Lentivirus with vectors that upregulated or downregulated miR-574-3p were constructed by Gene-Chem Co. Ltd. (Shanghai, China). Cells were cultured in 60-mm plates (1×10^6/plate). Cells were transfected with negative control lentivirus (NC), miR-573-3p lentivirus or anti-miR-574-3p lentivirus. After 72-96 h, cells were treated with puromycin to select for stable expression. Gene-specific siRNAs and non-targeting siRNA were obtained from Shanghai GenePharma (China). The EGFR siRNA sequences were 5'-CCUAUGCCUUAGCAGUCUUTT-3', 5'-GCAGUGACUUUCUCAGCAATT-3' and 5'-GC-AAAGUGUGUAACGGAAUTT-3'. The sequence of the non-targeting control siRNA was 5'-UU-CUCCGAACGUGUCACGUTT-3'. Small interfering RNAs were transfected into cells via Lipofectamine® 2000 (Invitrogen, NY, USA).

Western blot

Western blot was performed as we reported before [4]. Proteins from total cell lysates were separated using an 8-12% SDS-PAGE gel and then transferred to PVDF membranes (Millipore, Billerica, MA). Membranes were blocked, washed, and incubated with specific primary antibodies followed by incubation with HRP-conjugated secondary antibodies. Bands were detected with an enhanced chemiluminescence assay (Millipore, Billerica, MA). Antibodies against EGFR, p-AKT, AKT, p-Stat3, Stat3, p-ERK, ERK, p-FAK (Tyr397), FAK, Src, p-Src (Tyr416), MMP-9 and c-Jun were obtained from Cell Signaling Technology (Danvers, MA), and antibody against GAPDH was obtained from Kangchen Bio Co. (Shanghai, China).

Quantitative real-time PCR (qRT-PCR)

Total cellular RNA of tissues or cells was extracted and then synthesized to cDNA using relevant kits (Tiangen, Beijing, China). qRT-PCR was performed to measure the relative expression levels of miR-574-3p, and the primers were obtained from Promega (Madison, WI, USA). The quantitative PCRs included 2 µL of cDNA, 10 µL of SYBR Green Master Mix (TaKaRa Bio Inc., Dalian, China) and primers. The reactions were monitored via the ABI PRISM 7500

sequence system (Applied Biosystems Co., Foster City, CA) as described in the manufacturer's protocol. U6 served as an internal control for gene expression normalization.

Cell proliferation assay

Cell proliferation and drug sensitivity assay were measured by CCK-8 Kit (Dojindo Laboratories, Rockville, MD) as previously reported [16]. Three independent experiments were performed.

Transwell migration and invasion assay

In vitro cell migration and invasion assays were performed as we reported before [4]. In vitro cell invasion assays using SKOV3 and CAOV3 cells transfected with miR-574-3p or anti-miR-574-3p were performed using transwell plates (Millipore, Billerica, MA). Briefly, membranes were pretreated with 30 µg of Matrigel (BD Biosciences, San Jose, CA) on the upper surface at 37°C for 30 min, which formed a reconstituted basement membrane. Cells (1×10^5 cells per well in 200 µL of RPMI-1640 without FBS) were seeded onto the upper well of the chamber, and the lower well was filled with 600 µL of RPMI-1640 containing 10% FBS as a chemoattractant. After incubation for 48 h, cells were fixed for 30 min in 4% formaldehyde and stained for 15 min with haematoxylin. Non-migrating cells were then carefully removed from the upper surface (inside) of the transwell with a wet cotton swab. Cells that migrated or invaded to the bottom surface of the filter were counted. Migration was determined by counting the cell number with a microscope at × 200 magnification. Five visual fields were randomly selected for each assay. The average number of migrating cells in 5 fields was considered as the cell migration number of the group. Three independent experiments were examined, and the groups represented the mean of the 3 separate experiments. For the migration assay, transwell chambers were not pretreated with Matrigel, but the other procedural steps were performed as described for the invasion assay. Three independent experiments were examined, and the groups represented the mean of the three separate experiments.

Data analysis

Statistical analysis was performed using R and SPSS v.15 (SPSS Inc., Chicago, IL). Continuous data was expressed as the mean ± SD, and

analyzed by independent t-test between two groups. Among multiple groups, one-way AN-OVA was applied, and Turkey test was applied as a post hoc test. Values of $P < 0.05$ were regarded as significant.

Results

Overexpression of EGFR is correlated with downregulation of miR-574 in EOC tissues

EGFR has been frequently reported in EOC and is correlated with an unfavourable patient prognosis. To better understand if the expression of EGFR is associated with miR-574-3p, the protein level of EGFR and the mRNA level of miR-574-3p were detected in normal ovarian tissues and EOC tissues via western blot and qRT-PCR, respectively. The EOC tissues had a miR-574-3p mean expression level that was 2.58-fold lower than that of normal ovarian tissues ($P < 0.001$) (**Figure 1A**). On the contrary, the EOC had an EGFR mean expression level that was 6.17-fold higher than that in normal ovarian tissues ($P < 0.001$) (**Figure 1B**). Furthermore, EGFR protein level in stage III-IV tissues (1.30 ± 0.43) was higher than those in stage I-II tissues (0.70 ± 0.21), whereas miR-574-3p level was lower in stage III-IV tissues (4.25 ± 2.76) than those in stage I-II tissues (7.78 ± 4.30) (**Figure 1A** and **1B**). The upregulation of EGFR protein was negatively associated with the downregulation of miR-574-3p in EOC tissues (**Figure 1C**).

EGFR 3'-UTR contains a specific target sequence for miR-574-3p

Firstly, the efficiency of upregulation and downregulation of miR-574-3p in SKOV3 and CAOV3 cells was detected. According to qRT-PCR, miR-574-3p lentivirus increased miR-574-3p expression in SKOV3 and CAOV3 cells, and anti-miR-574-3p lentivirus decreased miR-574-3p expression significantly in SKOV3 and CAOV3 cells (**Figure 2A** and **2B**). TargetScan identified a putative miR-574-3p-binding site, which complements the seed region (nt 2-8) of human miR-574-3p (hsa-miR-574-3p). Next, in order to verify this hypothesis, we built a wild-type EGFR 3'-UTR luciferase reporter vector and a mutant-type EGFR 3'-UTR luciferase reporter vector to explore the interaction between miR-574-3p and its predicted EGFR 3'-UTR target site (**Figure 2C**). In HEK293T cells, miR-574-3p inhibited the activity of the luciferase reporter gene fused to wild-type EGFR 3'UTR significant-

ly ($P < 0.001$). When the luciferase reporter contained a mutant EGFR 3'UTR with substitution of seven nucleotides within the miR-574-3p-binding site, there was no significant reduction (**Figure 2D**). The above experiments were repeated in both SKOV3 and CAOV3 cells, and similar results were achieved (**Figure 2D**).

To further investigate if miR-574-3p regulates endogenous EGFR expression, SKOV3 and CAOV3 cells were transfected with miR-574-3p lentivirus and anti-miR-574-3p lentivirus. Based on western blot analyses, EGFR expression was reduced significantly in miR-574-3p-overexpressed SKOV3 and CAOV3 cells compared to NC cells (**Figure 2E**). Furthermore, anti-miR-574-3p lentivirus dramatically increased the endogenous expression of EGFR protein in SKOV3 and CAOV3 cells compared to NC cells (**Figure 2E**). In summary, the above data illuminated that EGFR may be a specific target of miR-574-3p.

MiR-574-3p inhibits migration and invasion of EOC cells in vitro

As the above data demonstrated that miR-574-3p inhibited EGFR expression in EOC cells, a hypothesis was put forward that miR-574-3p might affect proliferation, migration or invasion of EOC cells. Strikingly, miR-574-3p upregulation inhibited the migration and invasion of SKOV3 as well as CAOV3 cells. In contrast, miR-574-3p downregulation promoted the migration as well as invasion of SKOV3 and CAOV3 cells (**Figure 3A** and **3B**). However, miR-574-3p levels did not affect cell viability (**Figure 3C** and **3D**). Therefore, these data demonstrated that miR-574-3p suppresses the metastasis capacity of ovarian cancer cells in vitro.

Mir-574-3p decreases the activation of AKT, FAK, Src and MMP-9 in EOC cells

Phosphorylation of AKT, FAK and Src was strongly inhibited after miR-574 upregulation and was strongly increased after miR-574-3p downregulation in SKOV3 and CAOV3 cells. Moreover, the expression of MMP-9, which is related to tumor invasion, showed the same trend with the above phosphorylated signaling pathway molecules. In contrast, miR-574-3p did not significantly affect p-Stat3, p-ERK or c-Jun (**Figure 4**).

To further verify that miR-574-3p regulates p-AKT, p-FAK, p-Src and MMP-9 via targeting EGFR, EGFR siRNA was transfected into miRNA-
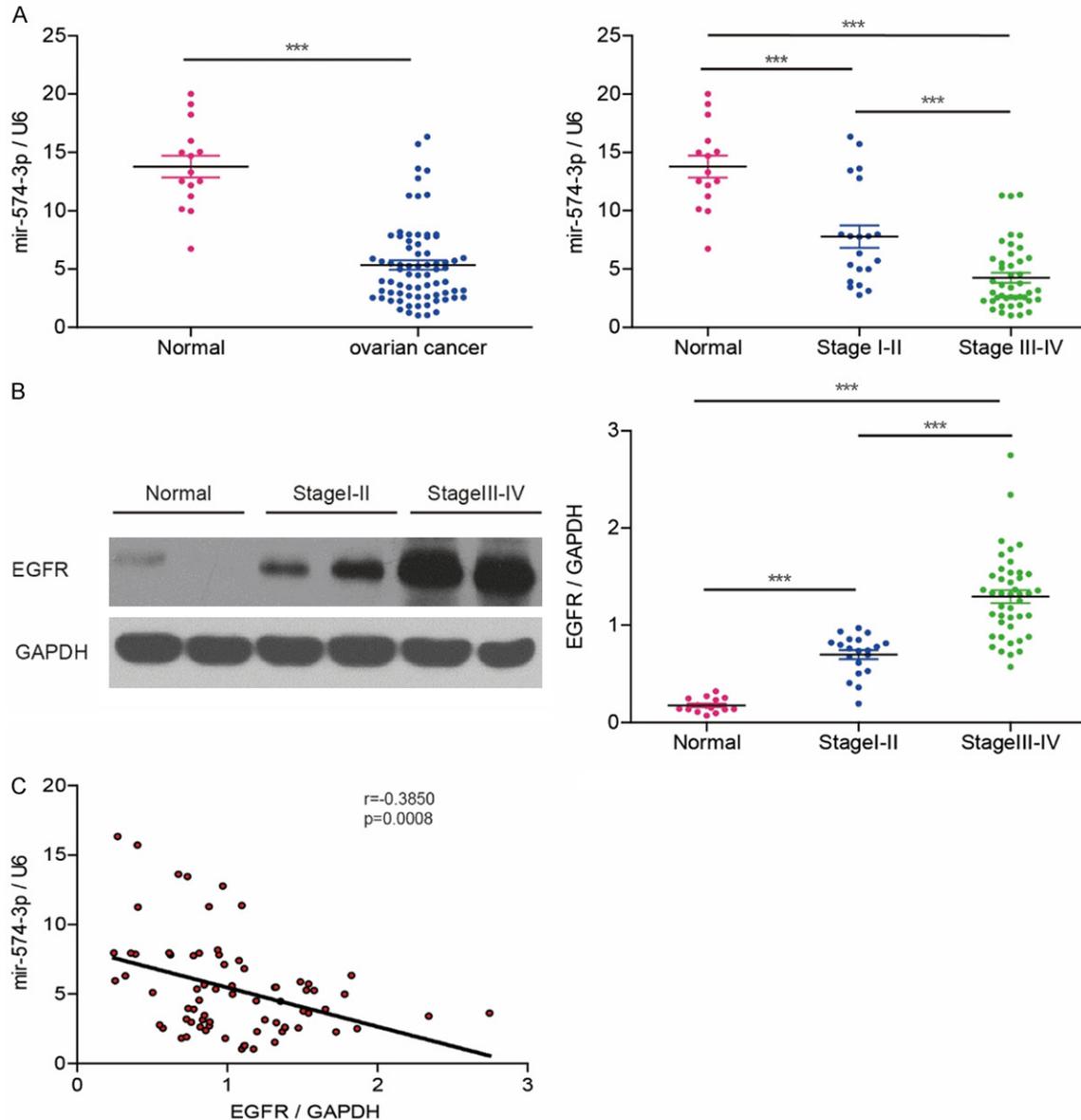
Figure 1. MiR-574-3p expression is significantly correlated with EGFR in EOC. EGFR and miR-574-3p expression in normal ovarian tissues (n = 15) and EOC tissues (n = 73). Normal ovarian tissues and EOC tissues were detected for miRNA expression via qRT-PCR and EGFR expression via western blot normalized to GAPDH. A. miR-574-3p was downregulated in EOC tissues compared to normal ovarian tissues (P < 0.001). MiR-574-3p expression was significantly decreased in stage III-IV (distant metastasis, n = 53) compared to normal tissues (n = 15, P < 0.001) and stage I-II (localized to peritoneum; n = 20, P < 0.001). B. EGFR was upregulated in EOC tissues compared to normal ovarian tissues (P < 0.001). EGFR expression was significantly increased in stage III-IV compared to normal tissues (*P* < 0.001) and stage I-II (*P* < 0.001). C. The expression of miR-574-3p was significantly correlated with EGFR expression in normal ovarian tissues and EOC tumours via Pearson's correlation analysis (r = 0.3850, *P* = 0.0008). ***, P < 0.001.

574-3p-deficient cells. EGFR siRNA decreased p-AKT, p-FAK, p-Src and MMP 9 levels in miR-574-3p-deficient cells (**Figure 5**). Thus, these findings suggested that miR-574-3p affects various signaling pathways via targeting EGFR to reduce the metastasis of EOC cells.

Furthermore, cell migration as well as invasion regulated by miR-574-3p via EGFR was further verified (**Figure 6**). In miR-574-3p-deficient cells, the migration as well as invasion abilities were reversed after EGFR knockdown by siRNA, suggesting that miR-574-3p suppresses cell
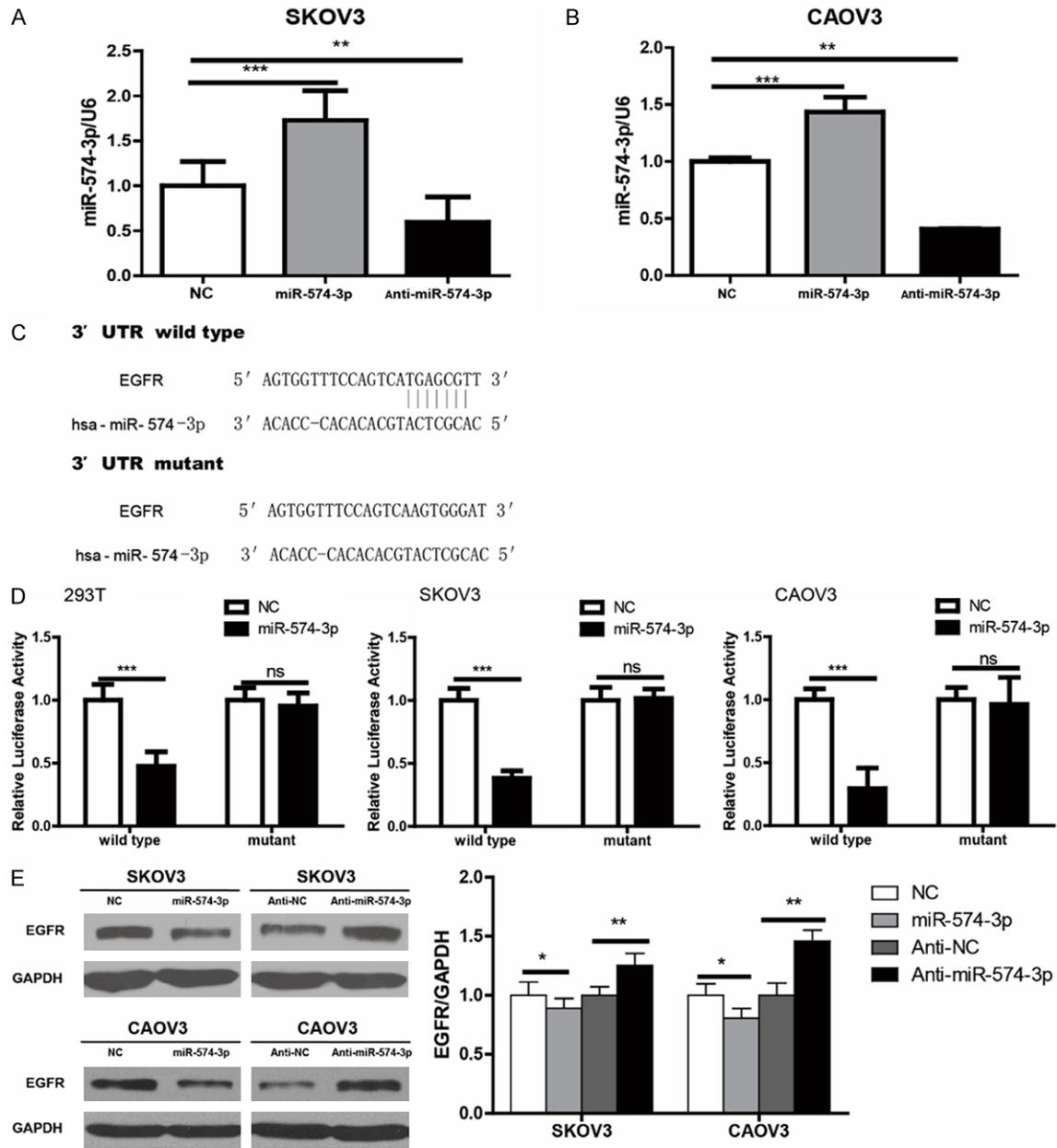
Figure 2. MiR-574-3p decreases EGFR expression by directly targeting its 3'-UTR in EOC cells. According to the qRT-PCR results, miR-574-3p and anti-miR-574-3p lentiviruses significantly increased and decreased, respectively, miR-574-3p expression in both SKOV3 (A) and CAOV3 cells (B). C. Putative miR-574-3p-binding site in the EGFR 3'-UTR nucleotides. D. Luciferase activity assays with wild-type or mutant EGFR 3'-UTR were performed after co-transfection with miR-574-3p or negative control (NC) lentivirus in 293T, SKOV3 and CAOV3 cells. E. EGFR protein levels were determined by western blot analyses after transfection with miR-574-3p, anti-miR-574-3p or negative control lentivirus in SKOV3 and CAOV3 cells. GAPDH served as an internal control. Ns, none significant, *, P < 0.05, **, P < 0.01 and ***, P < 0.001.

migration as well as invasion by downregulating EGFR expressionin EOC cells.

Mir-574-3p increases the drug sensitivity of EOC cells via targeting EGFR

Previous reports have showed that abnormal miRNA expression may be related to chemore-sistance of human cancers. However, it is not completely understood if miR-574-3p expression affects the sensitivity of EOC cells. Therefore, we further investigated the sensitivity of EOC cells to paclitaxel and cisplatin, which are first-line drugs for EOC. Transfected SKOV3 cells (miR-NC, miR-574-3p and anti-miR-574-3p) were treated with different concentrations
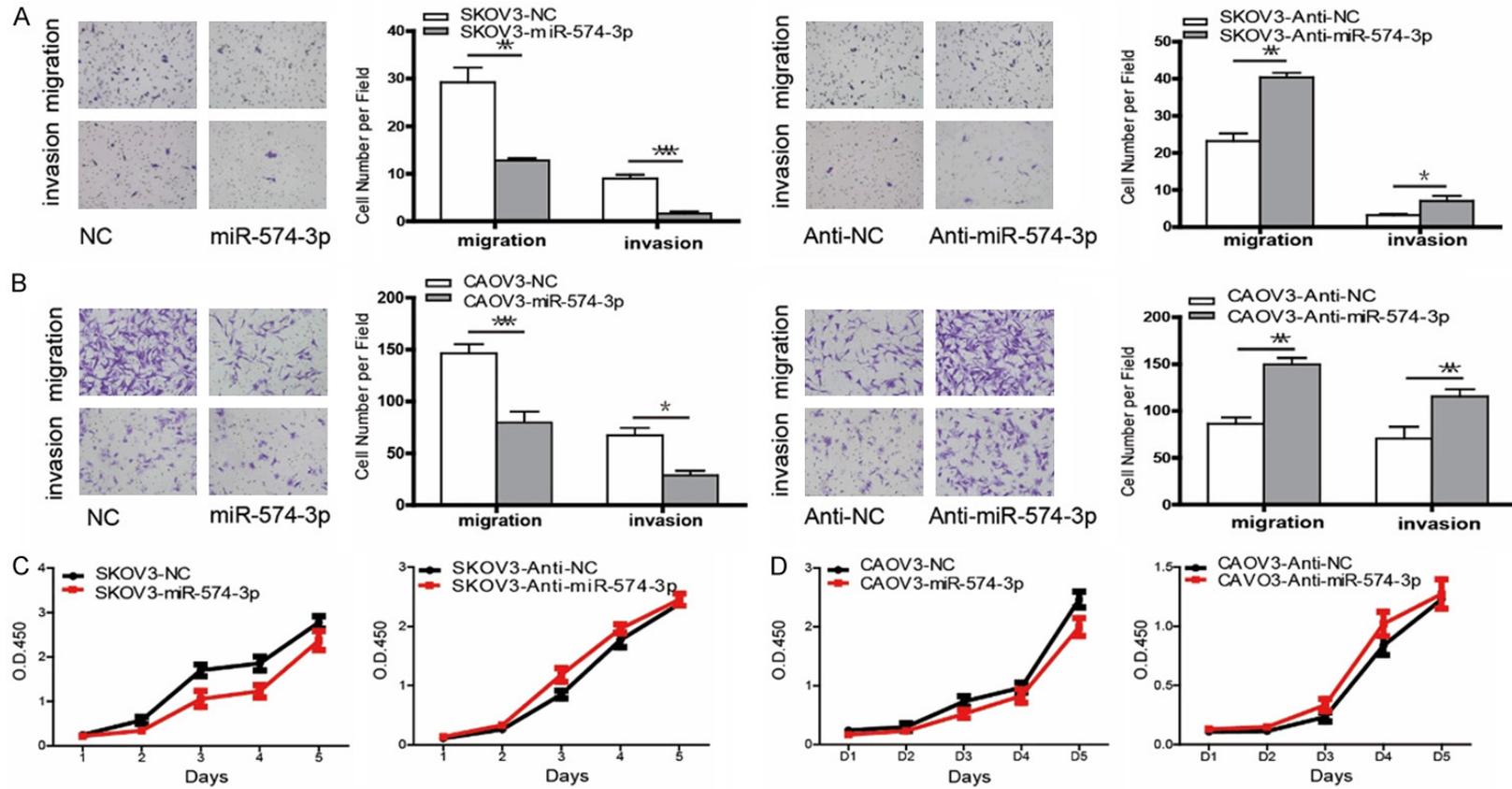
Figure 3. MiR-574-3p decreases EOC cell migration and invasion but does not affect cell viability. MiR-574-3p and anti-miR-574-3p decreased and promoted, respectively, cell migration and invasion in vitro in both SKOV3 (A) and CAOV3 cells (B). MiR-574-3p or anti-miR-574-3p had no effect on cell viability in both SKOV3 (C) and CAOV3 cells (D). *, P < 0.05, **, P < 0.01 and ***, P < 0.001.
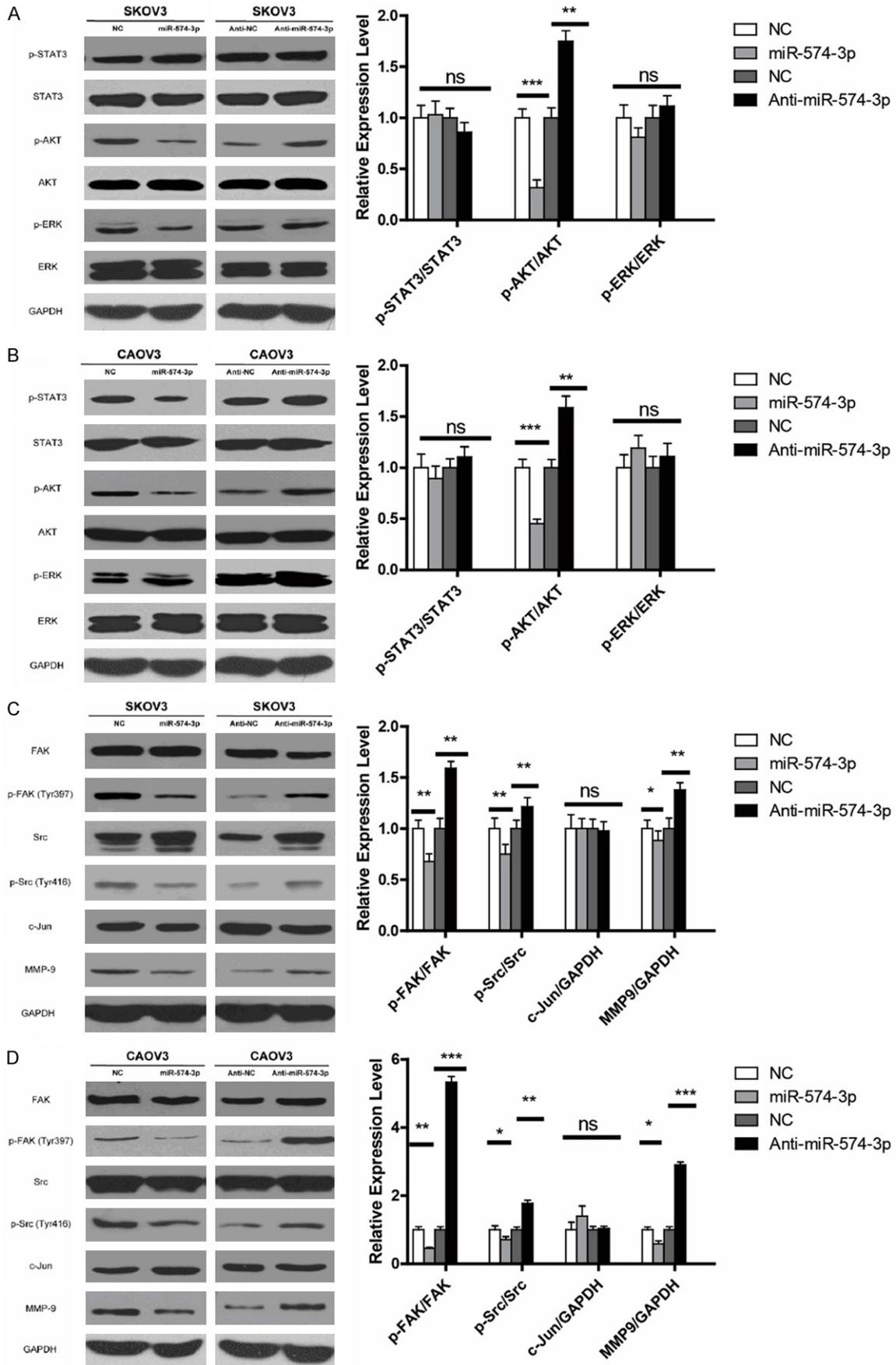
Figure 4. MiR-574-3p inhibits multiple signaling pathways. A. Protein levels of Stat3, p-Stat3, AKT, p-AKT, ERK and p-ERK in transfected SKOV3 cells (NC, miR-574-3p, anti-NC or anti-miR-574-3p) cells. GAPDH was used as a loading control for western blotting. B. Protein levels of Stat3, p-Stat3, AKT, p-AKT, ERK and p-ERK in transfected CAOV3 cells (NC, miR-574-3p or anti-miR-574-3p) cells. GAPDH was used as a loading control for western blotting. C. Protein levels of FAK, p-FAK, Src, p-Src, c-Jun and MMP-9 in transfected SKOV3 cells (NC, miR-574-3p, anti-NC or anti-miR-574-3p) cells. GAPDH was used as a loading control for western blotting. D. Protein levels of FAK, p-FAK, Src, p-Src, c-Jun and MMP-9 in transfected CAOV3 cells (NC, miR-574-3p, anti-NC or anti-miR-574-3p) cells. GAPDH was used as a loading control for western blotting. Ns, none significant, *, P < 0.05, **, P < 0.01 and ***, P < 0.001.
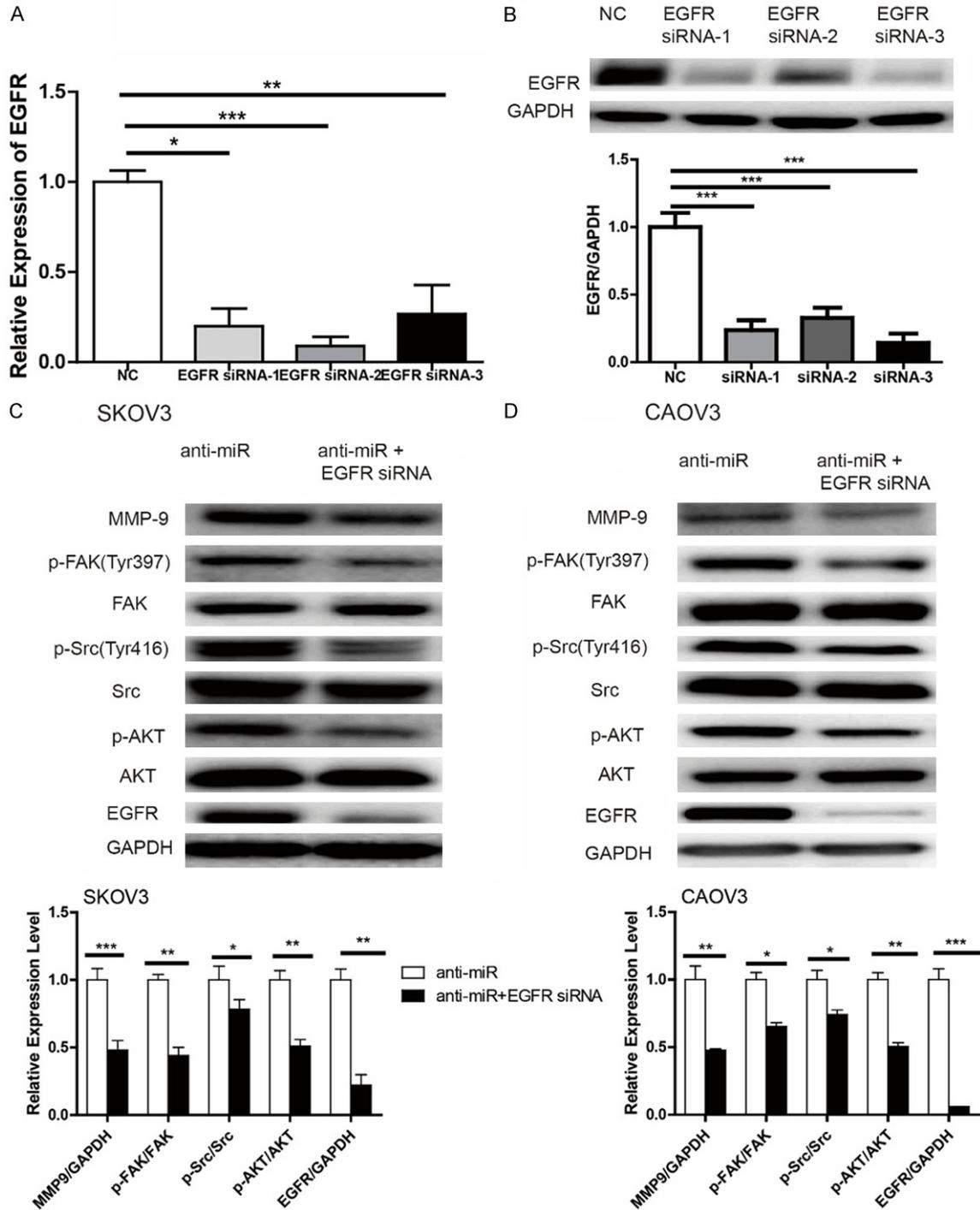
Figure 5. MiR-574-3p inhibits signaling pathways via targeting EGFR. A. EGFR siRNAs inhibited EGFR mRNA levels as confirmed by qRT-PCR. U6 was used as an internal control. B. EGFR siRNAs inhibited EGFR protein levels as confirmed by western blot analysis. GAPDH was used as an internal control. C. Protein levels of MMP-9, FAK, p-FAK, Src, p-Src, AKT, p-AKT and EGFR in anti-miR-574-3p-transfected SKOV3 cells with or without EGFR siRNA-3 co-transfection. GAPDH was used as a loading control for western blotting. D. Protein levels of MMP-9, FAK, p-FAK, Src, p-Src, AKT, p-AKT and EGFR in anti-miR-574-3p-transfected CAOV3 cells with or without EGFR siRNA-3 co-transfection. GAPDH was used as a loading control for western blotting. *, P < 0.05, **, P < 0.01 and ***, P < 0.001.

(0.0001, 0.0003 and 0.001 μg/ml) of paclitaxel or different concentrations (2.5, 5 and 10 μM) of cisplatin for 48 hours. The CCK-8 assay suggested that upregulation of miR-574-3p dramatically augmented the cytotoxic effect of paclitaxel and cisplatin in a dose-dependent manner. Furthermore, downregulation of miR-574-3p inhibited the chemoresistance of paclitaxel as well as cisplatin in a dose-dependent manner. Results demonstrated that miR-574-3p may effectively enhance the sensitivity of EOC cells to paclitaxel and cisplatin (**Figure 7A** and **7B**).

Moreover, in miR-574-3p-deficient cells, the viabilities inhibited by paclitaxel and cisplatin were partially reversed after EGFR knockdown by siRNA (**Figure 7C** and **7D**). In summary, the above data leads to a conclusion that miR-574-3p increases the drug sensitivity of EOC cells via targeting EGFR.

Discussion

MiRNAs and other small non-coding RNAs have recently been reported to play a significant role in gene expression, and abnormal miRNA expression has been discovered in various diseases, including human cancers. Although more than several hundred candidate miRNAs harbour EGFR-binding sites, not every putative site is a functional miRNA target in ovarian cancer. To further explore miRNAs targeting EGFR mRNA in the present study, five databases were searched, which identified six predicted microRNAs. Among them, miR-200 [17], miR-101 [18], miR-214 [19], miR-125a [20], and miR-34 [21] have well established ovarian cancer associations. Because reports showed that miR-574 may serve as a key factor in the occurrence as well as development of other cancers, such as gastric cancer [22], head and neck cancer [23], prostate cancer [24], and bladder cancer [25], miR-574-3p was further investigated as a potential oncogene in EOC in the present study.

The present data showed a reverse correlation of miR-574-3p expression with clinical stage of EOC, and this trend was in accordance with EGFR. The investigation of the aberrant expression of miR-574-3p and EGFR in clinical samples led to the identification of EGFR as a target of miR-574-3p in EOC. However, the correlation of miR-574-3p expression with EOC patient survival was not determined in the present study, so it is necessary to complete the follow-up of patients in the future.

The current study demonstrated that transfection of miR-574-3p inhibited the expression of EGFR, causing a dramatic decrease in EOC cell migration as well as invasion. The luciferase reporter assays suggested that miR-574 associated with the 3'-UTR of EGFR mRNA. The EGFR 3'-UTR was fused to a luciferase reporter constructed, which indicated a decrease in luciferase activity after up-regulation of miR-574-3p. Such activity required specific complementarity with target sequences, and fluorescent activity was not detected in the vector containing mutated miR-574-3p-binding sites, which was consistent with the predicted results generated using computational algorithms from the databases.

Although EGFR has been reported to induce cell migration through multiple signaling pathways, the correlation between miR-574-3p and the occurrence or development of EOC is still unknown. EGFR can activate several signaling pathways like the MAPK/ERK and PI3K/AKT pathways [26], and these signaling ways can affect genetic transcription, promoting cellular proliferation, migration, invasion, angiogenesis and inhibiting apoptosis. Thus, the functional regulation between EGFR and signaling pathways in response to miR-574-3p were investigated in this research. Overexpression of miR-574-3p inhibited cell phosphorylation of downstream molecules, such as c-Src, AKT and FAK. AKT promotes growth factor-mediated cell survival and regulates cell cycle. c-Src controls proliferation, cell adhesion as well as cell migra-
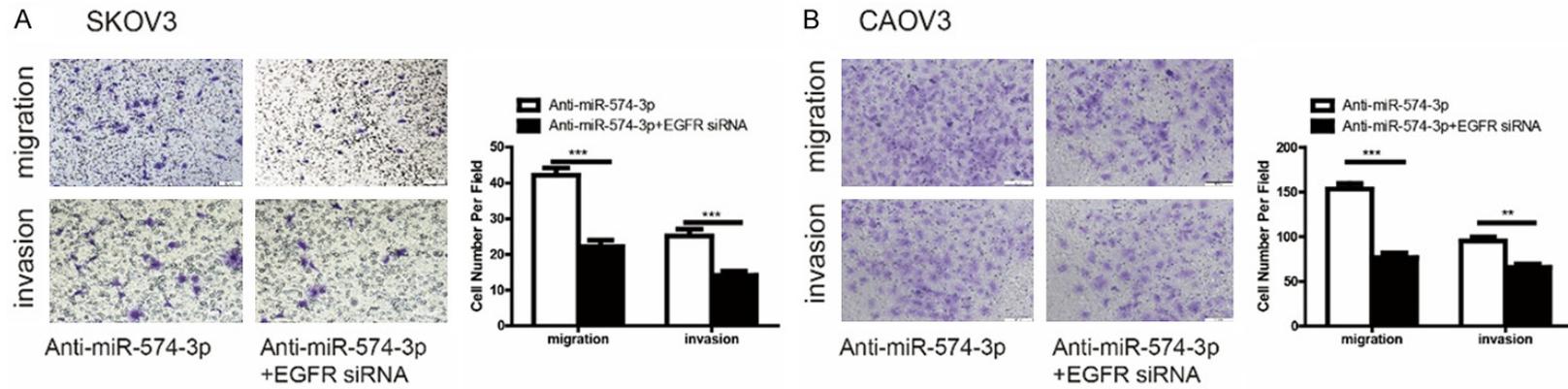
Figure 6. EGFR knockdown reverses the promoting effect of anti-miR-574-3p on migration and invasion of EOC cells. In miR-574-3p-deficient SKOV3 (A) and CAOV3 (B) cells, the migration and invasion abilities were reversed after EGFR knockdown by siRNA. **, P < 0.01 and ***, P < 0.001.
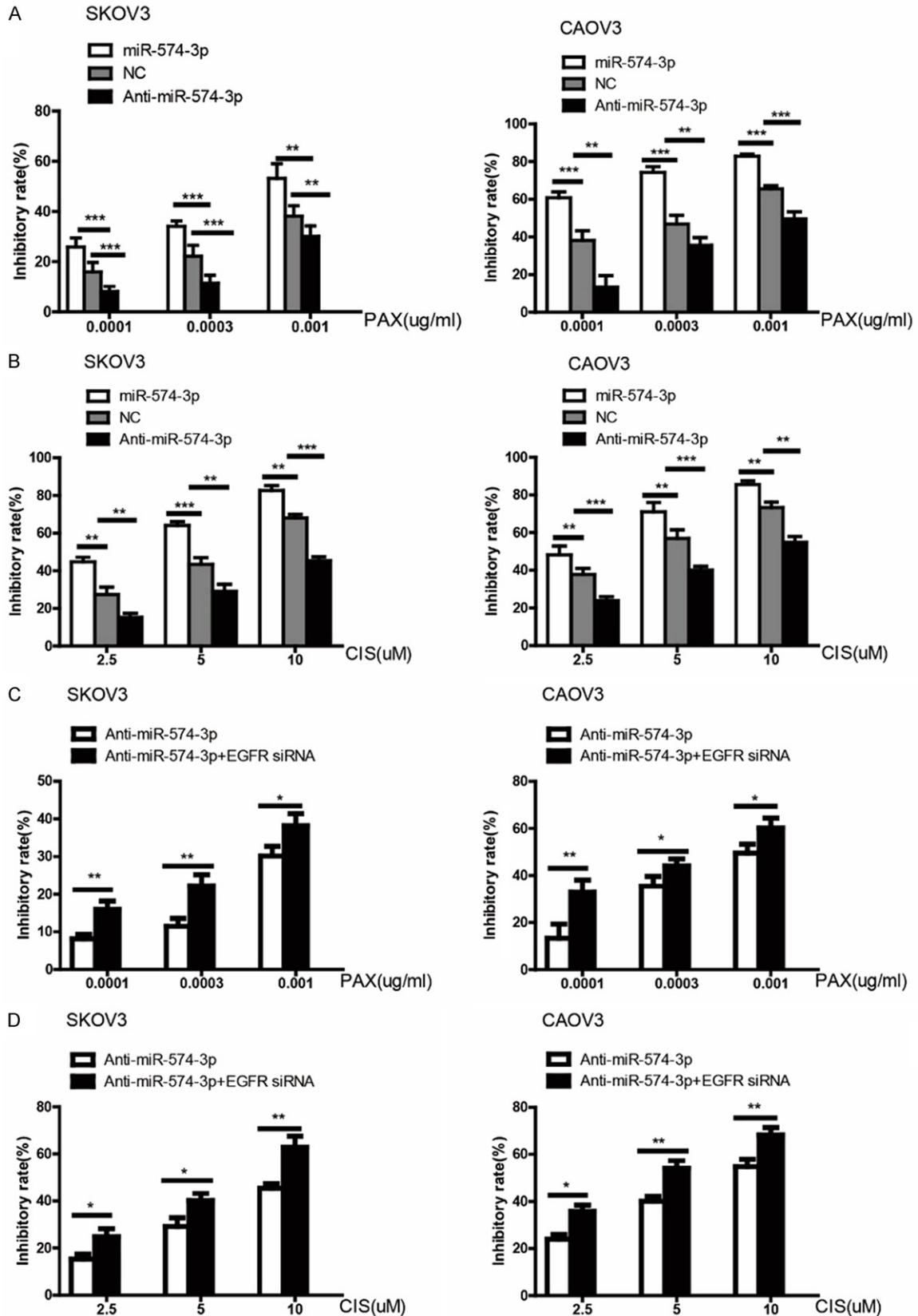
Figure 7. Mir-574-3p enhances the drug sensitivity of EOC cells. A. SKOV3 and CAOV3 cells transfected with miR-574-3p, NC or anti-miR-574-3p lentivirus were treated with different concentrations of paclitaxel (0.0001, 0.0003,

and 0.001 µg/ml). The cell inhibitory rate was quantified by the CCK-8 assay. B. SKOV3 and CAOV3 cells transfected with miR-574-3p, NC or anti-miR-574-3p lentivirus were treated with different concentrations of cisplatin (2.5, 5, and 10 µM). The cell inhibitory rate was quantified by the CCK-8 assay. C. MiR-574-3p-deficient SKOV3 and CAOV3 cells were transfected with or without EGFR siRNA and were treated with different concentrations of paclitaxel (0.0001, 0.0003, and 0.001 µg/ml). The cell inhibitory rate was quantified by the CCK-8 assay. D. MiR-574-3p-deficient SKOV3 and CAOV3 cells were transfected with or without EGFR siRNA and were treated with different concentrations of cisplatin (2.5, 5, and 10 µM). The cell inhibitory rate was quantified by the CCK-8 assay. *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$.

tion. FAK influences the cytoskeleton, cell adhesion site structures and membrane protrusions to regulate cell mortality and migration [27]. Activation of EGFR/AKT, EGFR/c-Src and EGFR/FAK cascades by loss of miR-574-3p resulted in EOC cell migration and invasion. Because ERK and Stat3 play a vital part in many cellular processes (such as cell growth, cell apoptosis, mitosis and postmitotic functions in differentiated cells), and because disruption of the ERK or Stat3 pathway is common in cancers, the present study investigated if EGFR/ERK and EGFR/Stat3 cascades were involved; however, negative results were achieved.

In addition, MMP-9, which is a member the matrix metalloproteinase (MMP) family and serves as a key effector of extracellular matrix (ECM) remodelling that promotes the progress of migration, was also expressed at low levels in response to miR-574-3p upregulation, which agreed with the transwell migration and invasion assay.

Although much progress in surgery and chemotherapy for EOC has been made in recent years, drug resistance remains a great challenge for treating EOC. Thus, new strategies are of great significance to improve drug resistance. The research demonstrated that miR-574-3p increased the drug sensitivity of EOC cells via targeting EGFR, suggesting that drug sensitivity of first-line chemotherapy for EOC may be improved via targeting miR-574-3p.

In conclusion, these data show that decreased miR-574-3p expression in EOC may serve as a key factor in its development to advanced stages. MiR-574-3p serves as a key factor in EOC as an inhibitor of migration, invasion and chemoresistance. Therefore, miR-574-3p may be an attractive candidate for therapeutic approaches of EOC.

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Disclosure of conflict of interest

None.

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