

Original Article

Interaction between CXCR4 and EGFR and downstream PI3K/AKT pathway in lung adenocarcinoma A549 cells and transplanted tumor in nude mice

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Abstract: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality all over the world, particularly in China. Metastasis is the main factor resulting in the poor prognosis of patients with NSCLC. CXCR4 and EGFR have been widely studied due to their critical role in tumor metastasis, but it remains more elusive than the relationship between CXCR4 and EGFR. Studies have demonstrated that many tumors have been found the existence of the “cross-talk” between EGFR and CXCR4 signaling pathways. In this context, we explored the relationship between EGFR and CXCR4 signaling pathways in lung cancer invasion and metastasis by both *in vitro* and *in vivo* experiments.

Keywords: Lung adenocarcinoma, epidermal growth factor receptor, chemokine receptor 4, PI3K/AKT signaling pathway, invasion

Introduction

CXC chemokine receptor 4 (CXCR4), a member of the G-protein-coupled receptor family, has received considerable attention since it has been demonstrated to be important in tumor metastasis [1]. CXC chemokine ligand 12, first called as stromal cell derived factor-1 (SDF-1), belongs to the chemokine family, which binds to its receptor CXCR4, exerting its effects in inflammation, organ vascularization, hematopoiesis, immune cell homing and trafficking, and tumorigenesis [2]. Besides their critical role in the regulation of leukocyte recruitment in the immune system, chemokines and their receptors are also implicated in tumor invasion and metastasis following a mechanism similar to that of inflammatory cell invasion [3]. CXCR4 overexpression has been observed in a various variety of human tumor tissue types, including breast cancer, ovarian cancer, melanoma, and prostate cancer [4-6]. Previous studies have shown that high CXCR4 expression had a significant association with distant metastasis that has and could be used as an independent

prognostic biomarker for indicating poor prognosis in NSCLC [7].

Epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases, and is frequently highly expressed in many epithelial cancers, including lung, breast, colon, and prostate cancers [8]. The augmented or aberrant EGFR is associated with NSCLC and promoted cell proliferation, survival, and migration. Several EGFR targeted therapies, including gefitinib, lapatinib and cetuximab, have been used in the clinic. In ovarian cancer, EGFR has been shown to enhance the expression of CXCR4 in ovarian cancer cell lines through the activation of Src kinase that enhances tumor growth [9]. Another study showed that EGFR activates not only CXCR4 but also MMP9, leading to the increased metastatic potential of tumors [10].

Similarly, concerning breast cancer, the activation of both EGFR and ErbB2 has been shown to increase CXCR4 expression in breast cancer cells [11]. Furthermore, it was found that

The interaction between CXCR4 and EGFR

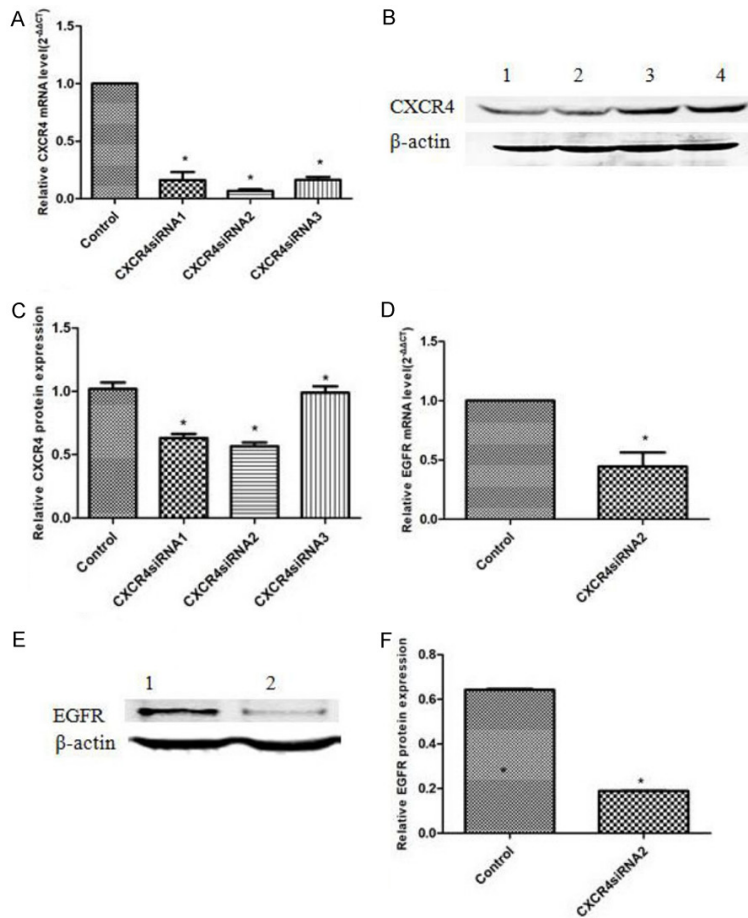


Figure 1. The expression of EGFR is suppressed by EGFR siRNA in A549 cells. A. The expression levels of CXCR4 mRNA in different groups of A549 cells. * vs control group: $P < 0.05$; Control: CXCR4 siRNA NC group. B. The expression of CXCR4 protein detected by western-blot in different groups of A549 cells. 1: CXCR4 siRNA-1 group; 2: CXCR4 siRNA-2 group; 3: CXCR4 siRNA-3 group; 4: CXCR4 siRNA-NC group. C. The expression of CXCR4 protein detected by western-blot in different groups of A549 cells. * vs control: $P < 0.05$; Control: CXCR4 siRNA NC group. D. The expression of EGFR mRNA in different groups of A549 cells. * vs control group: $P < 0.05$; Control: CXCR4 siRNA-NC group. E. The expression of EGFR protein in different groups of A549 cells. 1: CXCR4 siRNA-NC group; 2: CXCR4 si-RNA2 group. F. The expression of EGFR protein in different groups of A549 cells; * vs control group: $P < 0.05$; Control: CXCR4 siRNA-NC group.

Table 1. The sequence of three different CXCR4 siRNA

Si-RNA	Sequence
siRNA1	5'-GAAGCATGACGGACAAGTA-3'
siRNA2	5'-GCACATCATGTTGGCCTT-3'
siRNA3	5'-CTGCCTGCTATTGCATTA-3'

patients with tumors co-expressing CXCR4 and EGFR had a high incidence of inflammatory breast cancer-related death and a lower overall survival rate [12]. Regarding colon cancer,

co-expression of both EGFR and CXCR4 has been shown to be positively correlated with lymph node metastasis and distant metastasis, when compared with high expression of each molecule alone [12]. However dual expression of EGFR and CXCR4 and its relationship with prognosis has not been previously investigated in NSCLC [7].

We herein explored the relationship between EGFR and CXCR4 signaling pathways in lung cancer invasion and metastasis by both *in vitro* and *in vivo* experiments. In the present study, we showed that knockdown of CXCR4 gene blocked the expression of EGFR and the addition of CXCL12 increased the expression of EGFR. Moreover, the use of inhibition of PI3K (LY-294002) decreased the expression of CXCR4 and partially prevented the ability of migration induced by EGFR, which indicated that EGFR signaling is located downstream of CXCR4.

Materials and methods

Cell lines, culture conditions, and reagents

Human lung adenocarcinoma A549 cell lines were obtained from pathology laboratory of Hebei medical university (Shijiazhuang, China). Cells were cultured in RPMI-1640 medium (GIBCO) containing with 10% fetal bovine serum (CLARK) and 1% penicillin-streptomycin (BI) in a humidified atmosphere of 95% air and 5% CO₂ at 37°C with medium changed every two days.

Transfections with siRNA

The A549 cells were seeded at a density of 2×10^5 cells/well on 6-well plates and incubated overnight at 37°C. The cells were transfected

The interaction between CXCR4 and EGFR

Table 2. The expression of CXCR4 mRNA in A549 cells in different groups (by real time-PCR)

Group	Expression of CXCR4 mRNA
Control group	1.0000 ± 0.0000
siRNA1 group	0.1618 ± 0.0698 ^a
siRNA2 group	0.0685 ± 0.0133 ^a
siRNA3 group	0.1633 ± 0.0261 ^a

Note: Values are expressed as mean ± SD; ^aP<0.05 vs control group.

Table 3. The expression of CXCR4 protein in A549 cells in different groups (by western-blot)

Group	Expression of CXCR4 protein
Control group	1.008 ± 0.036
siRNA1 group	0.736 ± 0.015 ^a
siRNA2 group	0.684 ± 0.015 ^a
siRNA3 group	0.980 ± 0.037 ^a

Note: Values are expressed as mean ± SD; ^avs control group: P<0.05.

Table 4. The expression of EGFR mRNA in A549 cells in two CXCR4 si-RNAs groups (by qRT-PCR)

Group	Expression of EGFR mRNA
Control group	1.0000 ± 1.0000
CXCR4 siRNA2 group	0.4450 ± 0.1184 ^a

Note: Values are expressed as mean ± SD; ^avs control group: P<0.05.

Table 5. The expression of EGFR protein in A549 cells in two CXCR4si-RNA2 groups (by western-blot)

Group	Expression of EGFR protein
Control group	0.6424 ± 0.0049
CXCR4 siRNA2 group	0.1891 ± 0.0014 ^a

Note: Values are expressed as mean ± SD; ^avs control group: P<0.05.

ed with siRNAs using Lipofectamine® 2000 (Invitrogen) according to the manufacturer's protocol. The siRNA sequence (Genepharma, Inc., Sunnyvale, CA, USA) for CXCR4 was as follows: 5'-GAAGCATGACGGACAAGTA-3', 5'-GC-ACATCATGGTTGGCCTT-3', 5'-CTGTCCTGCTATTGCATTA-3', and the control sequence was non-silencing siRNA. After 24 h of transient transfection at 37°C, the cells were analyzed using qRT-PCR and western blotting to examine the effect of CXCR4 siRNA.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cells after treatment at an indicated time point and the cDNA was amplified using Total RNAPure and cDNA

reagent. The cDNAs were subjected to RT-PCR analysis. The assay was performed using qPCR master mix. PCR conditions were 94°C for 15 s, 55-60°C for 30 s, and 72° for 30 s for 40 cycles. All samples were run in triplicates and normalized using β-ACTIN expression values. Quantification of relative expression was calculated using the comparative threshold cycle (CT) and 2^{-ΔΔCT} relative quantification method.

Western blot analysis

Total cell extracts were prepared with the NP-40 lysis buffer. The lysate was centrifuged at 14000 RPM at 4°C and supernatants reserved. The total cell lysate (75 mg) was resolved by SDS PAGE using 10% gels and transferred to NC membrane, blocked with 5% BSA and probed with appropriate antibodies. After washing, the membrane was detected using ImageJ software.

Invasive assay

The Matrigel was coated to the upper 24-well chemotaxis chamber which was coagulate

into Matrigel basement membrane after 3 h at 37°C. The cells (5×10⁴) were then suspended in serum-free RPMI-1640 medium, and 200 μl cell suspension was added into the upper chamber. The bottom chamber was added with 600 μl RPMI-1640 supplemented with 10% FBS. Cells were incubated at 37°C with 5% CO₂ for 24 h, and then the cells were fixed with 4% paraformaldehyde for 20 min and stained with crystal violet for 30 min at room temperature. Non-migrated cells on the upper side of the membranes were removed and the migrated cells on the underside of the membranes were observed under an inverted fluorescence microscope in five randomized fields.

The interaction between CXCR4 and EGFR

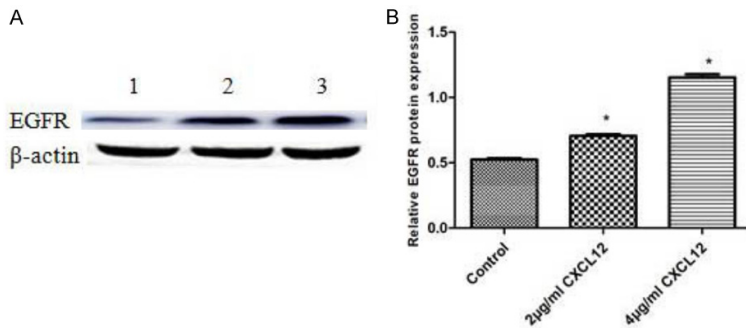


Figure 2. CXCL12 promoted the expression of EGFR in A549 cells. A. The expression of EGFR protein in different groups of A549 cells; 1: control group; 2: 2 µg/ml CXCL12 group; 3: 4 µg/ml CXCL12 group. B. The expression of EGFR protein in different groups of A549 cells. * vs control group: $P < 0.05$.

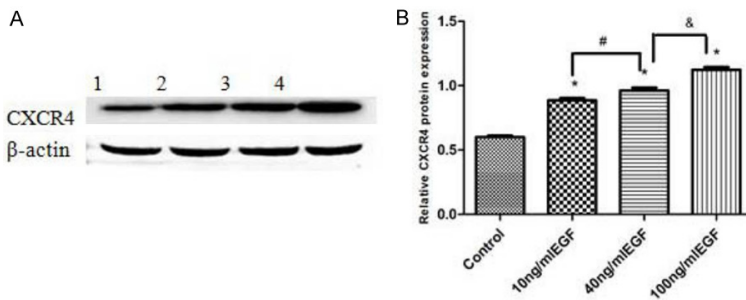


Figure 3. EGF up-regulate the expression of CXCR4. A. The expression of CXCR4 protein detected by western-blot in different groups of A549 cells. 1: control group; 2: 10 ng/ml EGF group; 3: 40 ng/ml EGF group; 4: 100 ng/ml EGF group. B. The expression of CXCR4 protein detected by western-blot in different groups of A549 cells. * vs control group: $P < 0.05$; # vs 10 ng/ml EGF group: $P < 0.05$; & vs 40 ng/ml EGF group: $P < 0.05$.

Table 6. The Expression of CXCR4 protein in A549 cells in different EGF groups (by western-blot)

Group	Expression of CXCR4 protein
Control group	0.6009 ± 0.0115
10 ng/ml EGF group	0.8861 ± 0.0170 ^a
40 ng/ml EGF group	0.9629 ± 0.0215 ^{a,b}
100 ng/ml EGF group	1.1230 ± 0.0212 ^{a,b,c}

Note: Values are expressed as mean ± SD; ^avs control group: $P < 0.05$, ^{a,b}vs 10 ng/ml EGF: $P < 0.05$, ^{a,b,c}vs 40 ng/ml EGF: $P < 0.05$.

Tumor xenografts

4 week-old male nude mice (n = 16; weights 16-18 g) were purchased for the tumor xenografts. Tumor cells were inoculated into nude mice by subcutaneous injection of 0.2 ml 5×10^6 /ml A549 cells into the right armpit using 1 ml syringe. Mice started drug treatment 1 week after tumor inoculation. Mice were evaluated daily, and tumor measurements were taken three times per week using Vernier cali-

pers. Tumor volumes were calculated using the formula: tumor volume = (length × width²)/2, where the length was the longest dimension, and the width was the dimension perpendicular to length. Mice were divided into four groups (n = 4 mice/group): Control group (saline+5% trehalose), EGF group (0.1 µg/ml EGF+5% trehalose), LY294002 group (saline+25 mg/kg LY294002) EGF+LY294002 group (0.1 µg/ml EGF+25 mg/kg LY294002). EGF and 5% trehalose (100 µl) were injected into the tumour side. LY294002 and saline (200 µl) were injected intraperitoneally. Samples were collected 15 days later, and then the tumors were separated in situ, fixed with 10% formalin, embedded with paraffin, sectioned into 5 µm-thicks, and subjected to H&E staining to observe metastasis. Principles of laboratory animal care were followed and all procedures were conducted according to the guidelines established by the National Institutes of Health, and every effort was made to minimize

suffering. This study was approved by the Animal Experiment Committee of the fourth hospital of Hebei Medical University.

Statistical analysis

All the experimental data are expressed as the mean ± standard deviation, and the mean values were calculated from >3 independent experiments. SPSS software (version 18; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Statistical comparisons were made with Student's two-tailed t-tests. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

CXCR4 suppression inhibits the expression of EGFR in A549 cells

To elucidate the potential role of CXCR4 in EGFR expression in A549, we sought to com-

The interaction between CXCR4 and EGFR

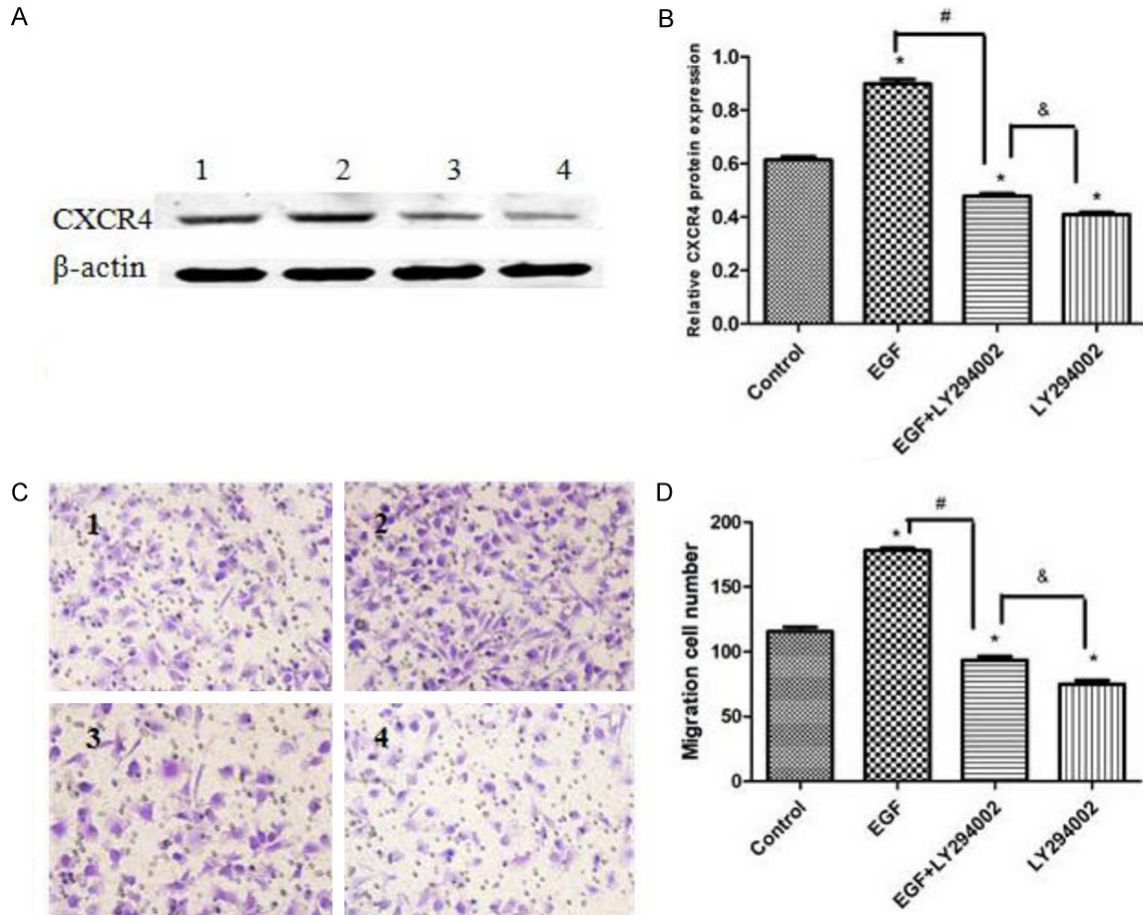


Figure 4. PI3K/AKT suppression reduced the expression of CXCR4. A. The expression of CXCR4 protein detected by western-blot in different groups of A549 cells. 1: EGF group; 2: control group; 3: EGF+LY294002 group; 4: LY294002 group. B. The expression of CXCR4 protein detected by western-blot in different groups of A549 cells. * vs control group: $P < 0.05$; # vs EGF group: $P < 0.05$; & vs EGF+LY294002 group: $P < 0.05$. C. The migration of A549 cells in transwell chamber. 1. control group; 2. EGF group; 3. E+LY294002 group; 4. LY294002 group. D. The migration number of A549 cells in transwell chamber. Data are presented as the mean \pm standard deviation of 3 independent experiments. * vs control group: $P < 0.05$; # vs EGF group: $P < 0.05$; & vs EGF+LY294002 group: $P < 0.05$.

Table 7. The Expression of CXCR4 protein in A549 cells in different group (by western-blot)

Group	Expression of CXCR4 protein
Control group	0.6140 \pm 0.0118
EGF group	0.8989 \pm 0.0172 ^a
EGF+LY294002 group	0.4779 \pm 0.0092 ^{a,b}
LY294002 group	0.4067 \pm 0.0078 ^{a,b,c}

Note: Values are expressed as mean \pm SD; ^avs control group: $P < 0.05$, ^{a,b}vs EGF group: $P < 0.05$; ^{a,b,c}vs EGF+LY294002 group: $P < 0.05$.

pare the transcriptome of cells transfected with CXCR4-specific siRNA (1, 2, 3) and control siRNA. Compared with control cells, all CXCR4 siRNAs knockdown the expression of CXCR4, one of which, CXCR4 si-RNA2, was most efficient. We chose the CXCR4 siRNA-2 for the

EGFR expression experiment due to our finding noted above that this siRNA displayed more pronounced CXCR4 knockdown compared to the others. We identified that the expression of EGFR was significantly differential in control and siRNA-2 groups, based on a $P < 0.05$ threshold (**Figure 1**) (**Tables 1-5**). This analysis demonstrated that the expression of EGFR were down-regulated by CXCR4 knockdown in A549 cells.

CXCL12/CXCR4 effects the expression of EGFR

To further assess whether CXCR4 modulation the expression of EGFR, A549 cells were stimulated with CXCL12, a highly specific chemokine receptor CXCR4 agonist. After induction with

The interaction between CXCR4 and EGFR

Table 8. The migration numbers of A549 cells in transwell chamber

Group	Migration numbers
Control group	135.2 ± 4.97
EGF group	178.2 ± 3.96 ^a
EGF+LY294002 group	100.4 ± 3.36 ^{a,b}
LY294002 group	78.4 ± 4.28 ^{a,b,c}

Note: Values are expressed as mean ± SD; ^avs control group: P<0.05, ^{a,b}vs EGF group: P<0.05; ^{a,b,c}vs EGF+LY294002 group: P<0.05.

Table 9. Average nude mice body weight of the transplanted model before and after treatment

Group	Body Weight (g)	
	Pre-treatment (g)	Post-treatment (g)
Control group	19.01 ± 0.26	23.61 ± 0.44
EGF group	19.47 ± 0.25	23.76 ± 0.38
EGF+LY294002 group	18.53 ± 0.84	23.35 ± 0.37
LY294002 group	18.32 ± 0.29	23.22 ± 0.39

Note: Values are expressed as mean ± SD; Pairwise comparison between different groups, P>0.05.

Table 10. Average tumor volume of the transplanted mode before and after treatment (X ± S)

Group	Tumor volume (cm ³)	
	Pre-treatment	Post-treatment
Control group	0.0210 ± 0.002	0.3250 ± 0.0249
EGF group	0.0212 ± 0.0069	0.5220 ± 0.0093
EGF+LY294002 group	0.0234 ± 0.0058	0.2073 ± 0.0085
LY294002 group	0.0234 ± 0.0049	0.1353 ± 0.0104

Note: Values are expressed as mean ± SD.

different concentrations of SDF-1 α , the protein levels of EGFR were detected respectively (**Figure 2**). The results demonstrated that the activation of CXCR4 increased the expression of EGFR stimulated by CXCL12.

EGF up-regulate the expression of CXCR4

In NSCLC, EGF binds to the tyrosine phosphorylated residues in EGFR and further triggers downstream signaling. To elucidate the interaction between CXCR4 and EGFR, CXCR4 was detected following EGF treatment in various concentrations. The protein levels of CXCR4 was enhanced by EGF stimulation in a concentration dependent manner (P<0.05) (**Figure 3**) (**Table 6**). Hence, the interaction between EGF and EGFR facilitate CXCR4 expression.

PI3K/AKT suppression reduced the expression of CXCR4

Suppression of PI3K/AKT reverses EGF-induced CXCR4 expression.

Given that the phosphorylation of EGFR is important for CXCR4 expression and PI3K/AKT signal pathway is known to be downstream of EGFR, we determined if PI3K/AKT altered the expression of CXCR4 in A549 cells. According to the drug treatment, A549 cells were divided into 4 groups: control, EGF, LY294002 (PI3K/AKT antagonist), EGF+LY294002 (**Figure 4**) (**Table 7**).

The changes of CXCR4 expression induced by EGF or PI3K/AKT suppression effected the invasion of lung cancer cells

After stimulated by EGF, the numbers of migrating A549 cells were (178.2 ± 3.96). The invasive abilities of A549 cells was significantly exceeded compared with the control group (135.2 ± 4.97). However, the capacities of EGF group significantly reduced after addition of LY294002 (78.4 ± 4.28).

After addition of PI3k/AKT antagonist, the numbers of invasive A549 cells was (100.4 ± 3.36). The invasion capacities decreased significantly (P<0.05) (**Figure 4**) (**Table 8**).

The effect of EGF and LY294002 on lung adenocarcinoma cell proliferation in vivo experimental

In the in vivo experiment, the tumorigenic rates of sixteen mice inoculated with A549 cells was 100%. There was no significant difference in body weight change before and after treatment (P>0.05) (**Table 9**). After the completion of drug treatment, the xenograft volume of nude mice in each group (blank group, EGF group, EGF+LY294002 group, LY294002 group) was shown in **Table 10**. The xenograft tumor of nude mice in the EGF group was significantly greater than that in the blank group, EGF+LY294002 group and LY294002 group, while the xenograft volume of nude mice in the LY294002 group was significantly lower than that in the other three groups.

The interaction between CXCR4 and EGFR

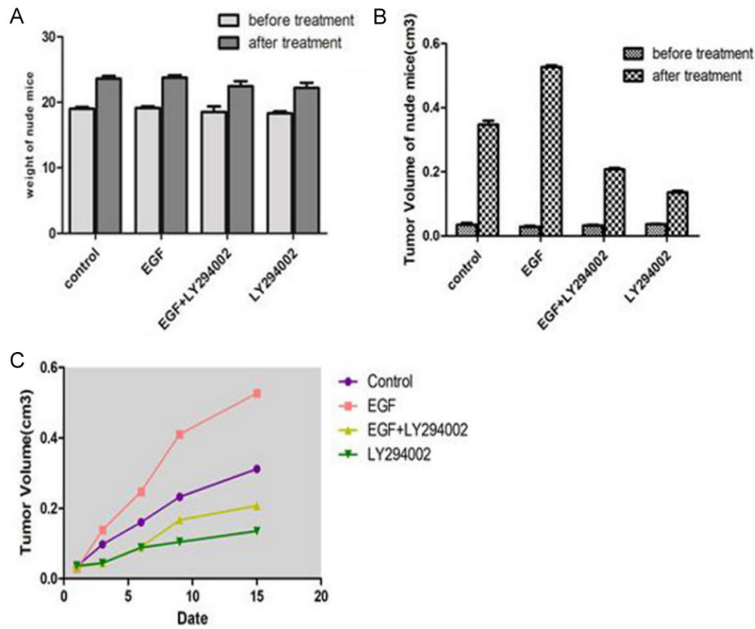


Figure 5. Effects of LY294002 and EGF on in vivo proliferation of lung cancer cells. A. The change of nude mice body weight before and after treatment. B. The change of nude mice tumor volume before and after treatment. C. Gross sample of tumor volume in different dates EGF (0.1 $\mu\text{g/ml}$); EGF+LY (0.1 $\mu\text{g/ml}$ +25 mg/kg); LY (25 mg/kg).

ups. Tumor volume was 0.3250 \pm 0.0249, 0.5220 \pm 0.0093, 0.2073 \pm 0.0085, 0.1353 \pm 0.0104, respectively, which was statistically significant compared with the blank group ($P < 0.05$) (Figure 5) (Tables 9, 10).

The expression of CXCR4 protein in xenograft tumor of nude mice was changed after drug injection

In vivo experiments, as shown in Figure 1, CXCR4 protein expression in xenograft tumor of nude mice was strongest in LY294002, and EGF+LY294002 group was stronger than that of LY294002 group, which was consistent with the results of in vitro experiments (Figure 6) (Tables 11, 12).

Discussion

CXCL12/CXCR4 axis which has been demonstrated plays a significant role in the tumor metastasis. CXCR4 overexpression has been observed in many human tumor tissue types.

One study indicated that CXCL12 induced a dose-dependent proliferation linking to the interaction between CXCL12 and CXCR4 through the activation of ERK1/2 and Akt in ovarian cancer [13].

Another study showed that epidermis growth factor (EGF) up-regulated the expression of CXCR4 and MMP-9, which promoted EGF-mediated metastasis in ovarian cancer cells [5]. Similarly, the over-expression of CXCR4 induced by EGF was regulated by the PI3K/PTEN/AKT/mTOR signal transduction pathway, activation of hypoxia inducible factor (HIF)-1 α in non-small cell lung cancer cells [14]. Furthermore, it was found that the CXCR4 activation induces EGFR phosphorylation that in turn was related with the downstream intracellular pathways such as Akt and ERK1/2 leading to proliferative effects in ovarian cancer [13].

Recent studies have shown that EGFR and its downstream PI3K/AKT signaling pathway

form a complex process in tumor cells. EGF binds to EGFR, activates downstream PI3K/AKT signaling pathway, and participates in the growth and proliferation of tumor cells. Guo Z et al. showed that EGF combined with EGFR could up-regulate the expression of CXCR4 and enhance the invasiveness of ovarian cancer SKOV3 cells. LY294002, an inhibitor of PI3K, inhibited the up-regulation of CXCR4 by EGF, that is, EGF promoted the expression of CXCR4 through PI3K/AKT signaling pathway [15]. Studies have shown that the co-expression of EGFR and CXCR4 is associated with poor prognosis in breast and lung cancer [16, 17]. In order to investigate whether EGF also regulates the expression of CXCR4 and the invasiveness of cells through PI3K/AKT signaling pathway in lung cancer, we studied the relationship between CXCR4 and EGFR in A549 cells of lung adenocarcinoma and its subcutaneous transplanted tumor in nude mice. The expression of CXCR4 was detected by EGF (40 ng/ml) [18] combined with (single) PI3K inhibitor LY294002 [18] in vitro. In vivo, EGF (100 ng/0.1 ml) [19] was used to detect the growth changes and the expression of CXCR4 in transplanted tumors by combined (single) PI3K inhibitor LY294002 (25 mg/kg) [20]. PI3K inhibitor LY294002 is a spe-

The interaction between CXCR4 and EGFR

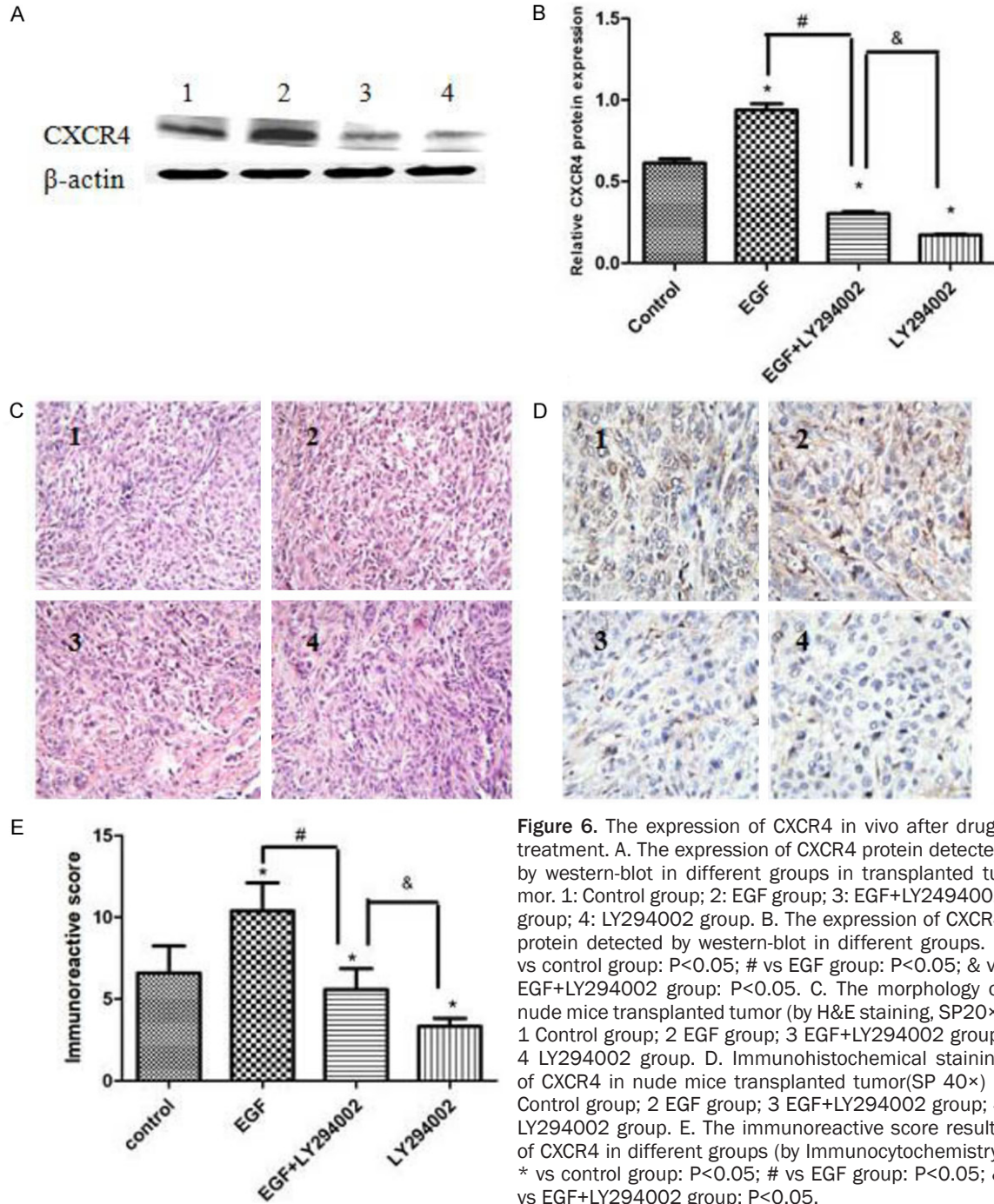


Figure 6. The expression of CXCR4 in vivo after drugs treatment. A. The expression of CXCR4 protein detected by western-blot in different groups in transplanted tumor. 1: Control group; 2: EGF group; 3: EGF+LY294002 group; 4: LY294002 group. B. The expression of CXCR4 protein detected by western-blot in different groups. * vs control group: $P < 0.05$; # vs EGF group: $P < 0.05$; & vs EGF+LY294002 group: $P < 0.05$. C. The morphology of nude mice transplanted tumor (by H&E staining, SP20 \times) 1 Control group; 2 EGF group; 3 EGF+LY294002 group; 4 LY294002 group. D. Immunohistochemical staining of CXCR4 in nude mice transplanted tumor (SP 40 \times) 1 Control group; 2 EGF group; 3 EGF+LY294002 group; 4 LY294002 group. E. The immunoreactive score results of CXCR4 in different groups (by Immunocytochemistry) * vs control group: $P < 0.05$; # vs EGF group: $P < 0.05$; & vs EGF+LY294002 group: $P < 0.05$.

cific inhibitor of PI3K $\alpha/\delta/\beta$. Flavonoids-based synthetic compounds that inhibit phosphatidylinositol 3-kinase (PI3K) can inhibit phosphorylation of downstream AKT in vitro and in vivo. The same conclusion was obtained in this study. In lung adenocarcinoma A549 cells and subcutaneous transplanted tumor in nude mice, EGF binds to EGFR and activates its downstream PI3K/AKT signaling pathway to up-

regulate the expression of CXCR4 and enhance the invasion ability of A549 cells. It also affected the growth of xenograft tumor in nude mice. In conclusion, we can see that CXCL12/CXCR4 enhances the expression of EGFR and EGF activates PI3K/AKT signaling pathway to enhance the expression of CXCR4. The interaction between CXCR4 and EGFR (cross-talk) and the PI3K/AKT signaling pathway related to the

The interaction between CXCR4 and EGFR

Table 11. The expression of CXCR4 protein in different groups in transplanted tumor (by western-blot)

Group	Expression of CXCR4 protein
Control group	0.6133 ± 0.0253
EGF group	0.9378 ± 0.0387 ^a
EGF+LY294002 group	0.3033 ± 0.0125 ^{a,b}
LY294002 group	0.1713 ± 0.0071 ^{a,b,c}

Note: Values are expressed as mean ± SD; ^avs control group: P<0.05; ^{a,b}vs EGF group: P<0.05; ^{a,b,c}vs EGF+LY294002 group: P<0.05.

Table 12. The immunoreactive score of CXCR4 in different groups (by immunohistochemistry)

Group	Immunoreactive score
	CXCR4
Control group	6.60 ± 1.65
EGF group	10.40 ± 1.71 ^a
EGF+LY294002 group	5.30 ± 1.16 ^{a,b}
LY294002 group	3.40 ± 0.52 ^{a,b,c}

Note: Values are expressed as mean ± SD; ^avs control group: P<0.05; ^{a,b}vs EGF group: P<0.05; ^{a,b,c}vs EGF+LY294002 group: P<0.05.

expression of CXCR4 and EGFR will provide a new method for the treatment of lung cancer.

In summary, our findings demonstrate that the EGF upregulates the expression of CXCR4 and enhances cell invasion ability through the PI3K/AKT signaling pathway in A549 cells and nude mouse transplanted tumors, and then affects the growth function of transplanted tumor.

Disclosure of conflict of interest

None.

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The interaction between CXCR4 and EGFR

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