

Received: 2020.04.10

Accepted: 2020.06.17

Available online: 2020.07.03

Published: 2020.09.03

Positive Cross-Talk Between CXC Chemokine Receptor 4 (CXCR4) and Epidermal Growth Factor Receptor (EGFR) Promotes Gastric Cancer Metastasis via the Nuclear Factor kappa B (NF- κ B)-Dependent Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEG 1,2,3

A 1,2,3

C 1,2,3

F 1,2,3

F 1,2,3

AG 1,2,3

AG 1,2,3

Yu Cheng
Xiaofang Che
Simeng Zhang
Tianshu Guo
Xin He
Yunpeng Liu
Xiujuan Qu

1 Department of Medical Oncology, The First Hospital of China Medical University, Shenyang, Liaoning, P.R. China
2 Key Laboratory of Anticancer Drugs and Biotherapy of Liaoning Province, The First Hospital of China Medical University, Shenyang, Liaoning, P.R. China
3 Liaoning Province Clinical Research Center for Cancer, Shenyang, Liaoning, P.R. China

Corresponding Authors:

Xiujuan Qu, e-mail: xiujuanqu@yahoo.com, Yunpeng Liu, e-mail: ypliu@cmu.edu.cn

Source of support:

The present study was supported by National Natural Science Foundation of China (No. 81803092, No. 81972331), China Postdoctoral Science Foundation (No. 2018M641747), Technological Special Project of Liaoning Province of China (2019020176-JH1/103), Science and Technology Plan Project of Liaoning Province (No. 2013225585), The Key Research and Development Program of Liaoning Province (2018225060), Science and Technology Plan Project of Shenyang city (19-112-4-099)

Background:

Previous studies have established cross-talk between CXC chemokine receptor 4 (CXCR4) and epidermal growth factor receptor (EGFR) in gastric cancer, however, the effect of dual CXCR4/EGFR tumor status on patient survival and mechanisms regulating expression has yet to be investigated.

Material/Methods:

A total of 56 gastric cancer patients were recruited to reveal the relationship between CXCR4 and EGFR expression, and the clinic-pathological features of samples were investigated by immunohistochemical staining. Two gastric cancer cell lines were treated with CXCL12 or EGF, and expression levels of CXCR4 and EGFR were detected by reverse-transcription-polymerase chain reaction and western blotting. Cells were treated with an NF- κ B pathway inhibitor to investigate its role in the regulation of CXCL12 or EGF-mediated CXCR4 and EGFR expression and migration ability.

Results:

The results show that CXCL12 upregulated CXCR4 and EGFR. Similarly, EGF could induce the expression of CXCR4 and contribute to gastric cancer cell metastasis. In addition, both CXCL12 and EGF could induce the activation of IKK α / β and P65. Conversely, suppression of the NF- κ B pathway remarkably decreased the expression of CXCR4/EGFR and migration ability induced by EGF or CXCL12. Furthermore, a significantly positive correlation between CXCR4 and EGFR expression was observed in gastric cancer patient tissues ($r=0.372$, $P=0.005$). Samples expressing both receptors had significantly poorer patient prognosis than other patient groups ($P=0.002$).

Conclusions:

Our results showed that the CXCL12/CXCR4 and EGF/EGFR axis can regulate the expression of each other through the NF- κ B pathway to promote metastasis. These data suggested that simultaneous inhibition of EGFR and CXCR4 may be a potential therapeutic strategy in gastric cancer.

MeSH Keywords:

Genes, erbB-1 • NF-kappa B • Receptors, CXCR4 • Stomach Neoplasms

Full-text PDF:

<https://www.medscimonit.com/abstract/index/idArt/925019>

 2477

 2

 6

 29



Background

Human gastric cancer is one of the most common causes of cancer related death throughout the world [1]. Despite recent advances in understanding the molecular networks of gastric cancer, the high mortality rate has not largely changed. The failure of comprehensive therapies and poor prognosis in gastric cancer are attributed to the high diversity of signaling outcomes and heterogeneity of the disease [2]. Therefore, there is an urgent need to demonstrate the molecular mechanisms of different types of gastric cancer and to identify novel molecular targets for treatment.

CXC chemokine receptor 4 (CXCR4), as the receptor of CXCL12, has been identified to be important in tumor proliferation, invasion, and metastasis [3]. Several studies have confirmed that G-protein coupled receptors (such as chemokine receptors, for example CXCR4) can cross-activate other receptors, which then stimulate signaling pathways involved in tumor progression [4–6]. In recent years, the cross-talk between CXCR4 and epidermal growth factor receptor (EGFR) was found to be essential for gastric, non-small cell lung, and ovarian cancer cell migration [7–9]. EGFR/CXCR4 co-expression identified a subset displaying worse prognosis in non-small cell lung and breast cancers [9,10]. However, the relationship between CXCR4 and EGFR in gastric cancer tissue samples, and the mechanism regulating their expressions remain unclear. EGFR was shown to increase the expression level of CXCR4 through the PI3K/AKT signaling pathway to enhance tumor growth in ovarian cancer cell lines [11]. Another study in ovarian cancer cells reported that EGFR activates CXCR4, resulting in the increased metastatic ability [8]. Similarly, activation of both EGFR and human epidermal growth factor receptor 2 (HER-2) was found to increase CXCR4 expression in triple-negative breast cancer cells [10]. However, the effects of EGF/EGFR on the upregulation of the expression of CXCR4 and CXCL12/CXCR4-induced EGFR upregulation in gastric cancer are unclear.

In the current study, we demonstrated that CXCL12/CXCR4 up-regulated CXCR4 and EGFR. Similarly, EGF/EGFR was able to induce the expression of CXCR4 and contribute to gastric cancer cell metastasis. In addition, the mechanisms of molecular regulation of CXCR4 and EGFR, and their associations with the clinic-pathological parameters and prognosis were determined from gastric cancer tissue specimens.

Material and Methods

Cells and cell culture

The human gastric cancer cell lines MGC-803, SGC-7901, BGC-823, N87, MKN45, and NUGC4 were purchased from the

Type Culture Collection of the Chinese Academy of Sciences (Beijing, China). All medium contained 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37°C in a humidified chamber that contained 5% CO₂.

Reagents and antibodies

Recombinant (CXCL12) SDF-1α was purchased from Pepro Tech (USA). The CXCR4 antagonist AMD3100 and NF-κB pathway inhibitor BAY117082 were obtained from Sigma (St. Louis, MO, USA), C225 (cetuximab) was obtained from Merck KGaA (Darmstadt, Germany). The EGFR antagonist was purchased from Cell Signaling Technology. Rabbit anti-CXCR4 antibodies were obtained from Abcam (Cambridge, UK). All the other antibodies were purchased from Santa Cruz Biotechnology (USA).

Reverse-transcription-polymerase chain reaction (RT-PCR)

Cells were cultured and harvested at the indicated times. Total cellular RNA was isolated with TRIzol reagent (Takara Bio, Kusatsu, Japan) according to the manufacturer's instructions. Referring to our previous method [12]. PCR conditions were 95°C for 30 seconds; 45 cycles at 95°C for 5 seconds and 58°C for 34 seconds. The primer sequences were as follows: EGFR, forward: 5'-GGCACTTTTGAAGATCATTCTC-3' EGFR, reverse: 5'-CTGTGTTGAGGGCAATGAG-3' CXCR4, forward: 5'-GGCCCTCAAGACCACAGTC-3' CXCR4, reverse: 5'-TTAGCTGGAGTGA AAACTTG-3' 18S, forward: 5'-CCCGGGGAGGTAGTGACGAAAAAT-3' 18S, reverse: 5'-CGCCCGCCGCTCCCAAGAT-3'

Cellular fractionation

After stimulation with CXCL12 or EGF for 1 hour, cells were washed twice in ice-cold phosphate-buffered saline (PBS). According to the Nuclear and Cytoplasmic Protein Extraction Kit by Keygen BioTECH. Cells were suspended in hypotonic lysis buffer containing protease and phosphatase inhibitors and incubated on ice for 30 minutes. The cytoplasmic proteins: the homogenate was centrifuged (3000 g for 10 minutes at 4°C), therefore separating the cytoplasmic extracts (supernatant) from the nuclear extracts (pellet). The nuclear proteins: the supernatants containing isolate nuclear protein extracts were resuspended in ice-cold hypertonic lysis buffer and incubated for 40 minutes, followed by centrifugation at (14 000 g for 30 minutes at 4°C). Nuclear and cytosolic NF-κB immunocontent were analyzed by western blot in relation to PARP and β-tubulin as controls.

Western blot

Western blot was performed as described previously [4]. Immunoblotting was performed on 30 µg of total protein from

the whole cells, transferred to a nitrocellulose membrane, and probed with specific antibodies. The blots were washed in Tween-Tris buffered saline (TTBS) before incubation with the secondary antibodies. The protein was visualized with the Electrophoresis Gel Imaging Analysis System.

Chemotaxis assay

The migration assay was performed as described previously [7]. Briefly, cells were seeded at 1.25×10^5 cells/mL in serum-free RPMI 1640, and the top chamber of the Transwell was loaded with 100 μ L of cell suspension. The bottom chamber was loaded with 0.5 mL 1640 containing 2.5% FBS with or without CXCL12 (100 ng/mL) or EGF (100 ng/mL) or CXCL12 (100 ng/mL) plus EGF (100 ng/mL). The migrated cells to the lower chambers were then washed, fixed, and stained. The number of migrating cells was counted in 3 high power fields (200 \times).

Patients and tissue samples

The files of 56 GC patients who underwent D2 and R0 surgical resection between 2006 and 2011 at the First Hospital of China Medical University, were collected in our study. No patient had received pre-operative chemotherapy or radiotherapy. Age, sex, tumor size, lymphovascular invasion, depth of invasion, lymph node metastasis, and tumor node metastasis (TNM) stage and Lauren grade were evaluated by reviewing medical charts and pathology records. This study was approved by the Human Ethics Review Committee of the First Hospital of China Medical University.

Immunohistochemistry

Immunohistochemical staining was performed as described previously [4,13]. Positivity (high expression) was defined as >10% of tumor cells showing staining in the sample. Final scores were assigned by three observers.

Statistical analysis

All the presented data were confirmed in at least 3 independent experiments and were expressed as the mean \pm SD. Differences between groups were compared using Student's *t*-test. Patient characteristics were tabulated by CXCR4 and EGFR, and differences between groups were compared using chi-square test. The correlation between CXCR4 and EGFR expression was assessed using Spearman rank correlation for continuous variables. The log-rank test and the Kaplan-Meier method were estimated for OS. SPSS 22.0 computer software was used for statistical analysis. Statistical significance was considered as *P*-values below 0.05.

Results

CXCL12 and EGF cooperatively promote GC cells migration

In order to identify the relationship between CXCR4 and EGFR, we screened gastric cancer cell lines that co-express CXCR4 and EGFR (Figure 1A). Serum-starved SGC-7901 and MGC-803 cells were stimulated with CXCL12 for 48 hours, and then CXCR4 and EGFR mRNA levels were evaluated by RT-PCR. The expression of CXCR4 was increased by 3 ± 0.5 -fold in SGC-7901 and 2.5 ± 0.3 -fold in MGC-803 cells. The expression of EGFR was increased by 3 ± 0.3 -fold in SGC-7901 and 3.5 ± 0.2 -fold in MGC-803 cells (Figure 1B, *P*<0.05). The protein levels of CXCR4 and EGFR were upregulated under CXCL12 stimulation (Figure 1C). Serum-starved SGC-7901 and MGC-803 cells were stimulated with EGF for 48 hours. CXCR4 and EGFR mRNA levels were then assessed by RT-PCR. The expression of CXCR4 was increased by 3.2 ± 0.25 -fold and 2.5 ± 0.3 -fold in SGC-7901 and MGC-803 cells, respectively (*P*<0.05), however, no significant change in EGFR expression was observed (Figure 1D). The protein level of CXCR4 was upregulated with EGF stimulation and no change was observed in EGFR (Figure 1E). Transwell migration assays indicated that the cell migration ability when treated with CXCL12 or EGF increased ($210 \pm 4\%$ and $141 \pm 9\%$ for SGC-7901, $395 \pm 14\%$ and $290 \pm 8\%$ for MGC-803, respectively, *P*<0.05). However, the migration ability of gastric cancer cells was remarkably increased after treatment with CXCL12 plus EGF ($331 \pm 6\%$ for SGC-7901, $505 \pm 14\%$ for MGC-803) (Figure 1F). These results indicated that CXCL12 and EGF cooperatively promote gastric cancer cell migration.

NF- κ B transcription factor contributes to CXCL12/CXCR4-mediated EGFR and CXCR4 upregulation

We previously found that CXCL12 affects the activation of EGFR via SRC [7]; however, the molecular mechanism through which CXCL12 could induce EGFR and CXCR4 upregulation in GC is unclear. To examine which pathways mediate these events, cells were treated with CXCL12. MGC-803 cells were stimulated with 100 ng/mL of CXCL12 for 15 minutes, the levels of p-p65/IKK α / β were upregulated (Figure 2A). The MGC-803 cells were incubated with 100 ng/mL CXCL12 for 1 hour, and then cytosolic and nuclear fractions were prepared to identify the translocation of P65/NF- κ B. CXCL12 provoked the cytosol-to-nucleus translocation of P65 (Figure 2B). To determine the contribution of the NF- κ B signaling axis, MGC-803 cells were pretreated with BAY117082 (15 μ M), an NF- κ B inhibitor, for 2 hours prior to stimulation with CXCL12. This resulted in marked inhibition of p65/IKK α / β phosphorylation and upregulation of EGFR/CXCR4 (Figure 2C). In addition, BAY117082 was shown to significantly suppress CXCL12-induced GC cell migration ($400 \pm 50\%$ versus $120 \pm 20\%$ for MGC-803, *P*<0.05) (Figure 2D). Inhibition of CXCR4 blocked CXCL12-induced activation of p65/IKK α / β and

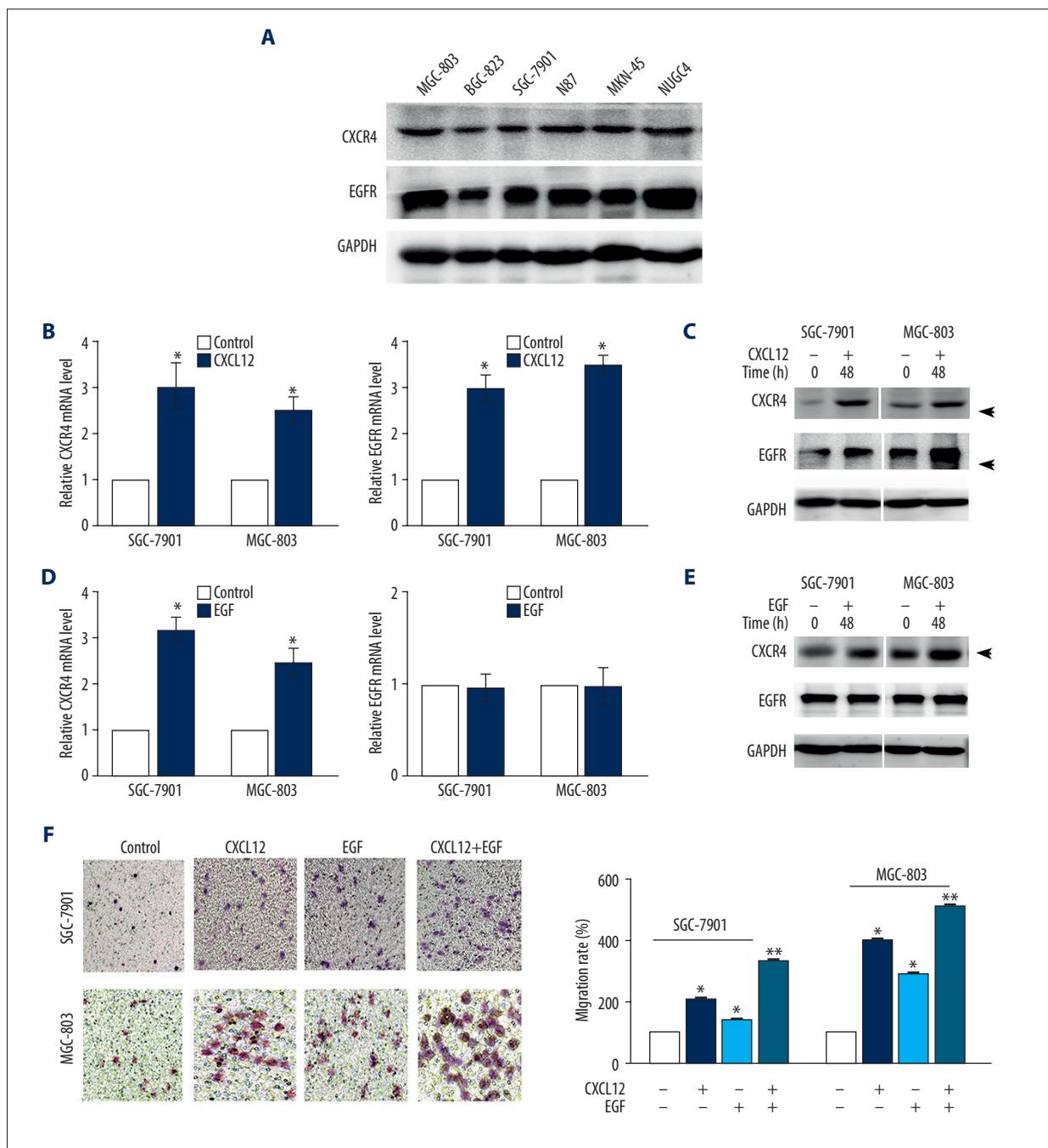


Figure 1. CXCL12 and EGF cooperatively promote gastric cancer cell migration. **(A)** Protein levels of EGFR and CXCR4 in gastric cancer cells. **(B, C)** Relative mRNA and protein levels of CXCR4 and EGFR were evaluated after treatment with CXCL12 (100 ng/mL). **(D, E)** Relative mRNA and protein levels of CXCR4 and EGFR were evaluated after treatment with EGF (100 ng/mL). **(F)** MGC-803 and SGC-7901 cells were treated with CXCL12 (100 ng/mL) or EGF (100 ng/mL) or CXCL12 (100 ng/mL) plus EGF (100 ng/mL), cells migration was performed using the Transwell assay. Values are represented as mean±SD in 3 independent experiments. (* $P<0.05$, ** $P<0.01$).

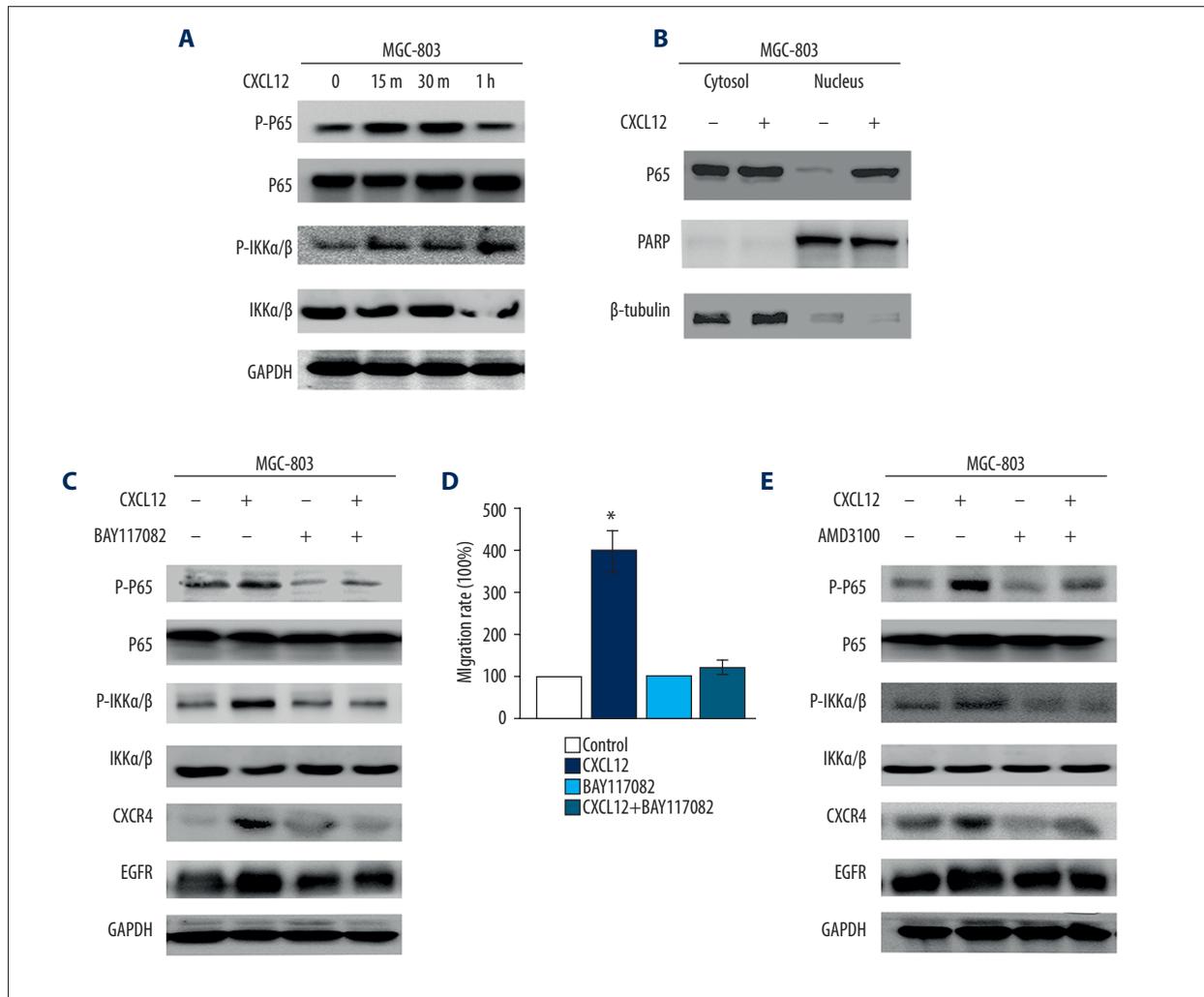


Figure 2. NF- κ B transcription factor contributes to CXCL12/CXCR4-mediated EGFR upregulation. **(A)** MGC-803 cells were treated with CXCL12 (100 ng/mL). Phosphor-p65/IKK α / β were determined by western blot assay (m, minute; h, hour). **(B)** MGC-803 cells were incubated with CXCL12 (100 ng/mL) for 1 h. Nuclear and cytoplasm cell lysate proteins were analyzed by western blot assay. **(C)** MGC-803 cells were treated with CXCL12 (100 ng/mL) for 48 h and pretreated with or without BAY117082 (15 μ M). Western blot analysis of EGFR and CXCR4. **(D)** MGC-803 cells were treated with CXCL12 (100 ng/mL) with or without BAY117082 (15 μ M). Cell migration was examined by Transwell assay. Values are represented as mean \pm standard deviation (SD) in 3 independent experiments (* P <0.05). **(E)** MGC-803 cells were treated with CXCL12 (100 ng/mL) for 48 h and pretreated with or without AMD3100 (10 μ g/mL). Western blot analysis of phosphor-p65/IKK α / β , EGFR, and CXCR4.

EGFR/CXCR4 upregulation (Figure 2E). The results showed that inhibition of the NF- κ B signaling axis reduced the upregulation of CXCR4/EGFR and migration ability induced by CXCL12.

NF- κ B transcription factor contributes to EGF/EGFR-mediated CXCR4 upregulation

To ascertain the essential role of NF- κ B signaling in EGF/EGFR-induced CXCR4 upregulation, cells were incubated with EGF. After treatment with 100 ng/mL EGF for 15 minutes, the level of p-p65/IKK α / β increased (Figure 3A). MGC-803 cells were incubated with 100 ng/mL EGF for 1 hour, and cytosolic and

nuclear fractions were prepared to determine translocation of P65/NF- κ B. EGF provoked cytosol-to-nucleus translocation of P65 (Figure 3B). MGC-803 cells were pretreated with BAY117082 (15 μ M) for 2 hours prior to stimulation with EGF, a marked inhibition of p65/IKK α / β phosphorylation and CXCR4 upregulation could be observed (Figure 3C). In addition, BAY117082, an NF- κ B inhibitor, significantly reduced EGF-induced gastric cancer cells migration (250 \pm 20% versus 110 \pm 10% for MGC-803, P <0.05) (Figure 3D). Inhibition of EGFR was shown to block EGF-induced activation of p65/IKK α / β and upregulation of CXCR4 (Figure 3E). The results indicated that EGF/EGFR could induce CXCR4 upregulation and migration ability via NF- κ B signaling.

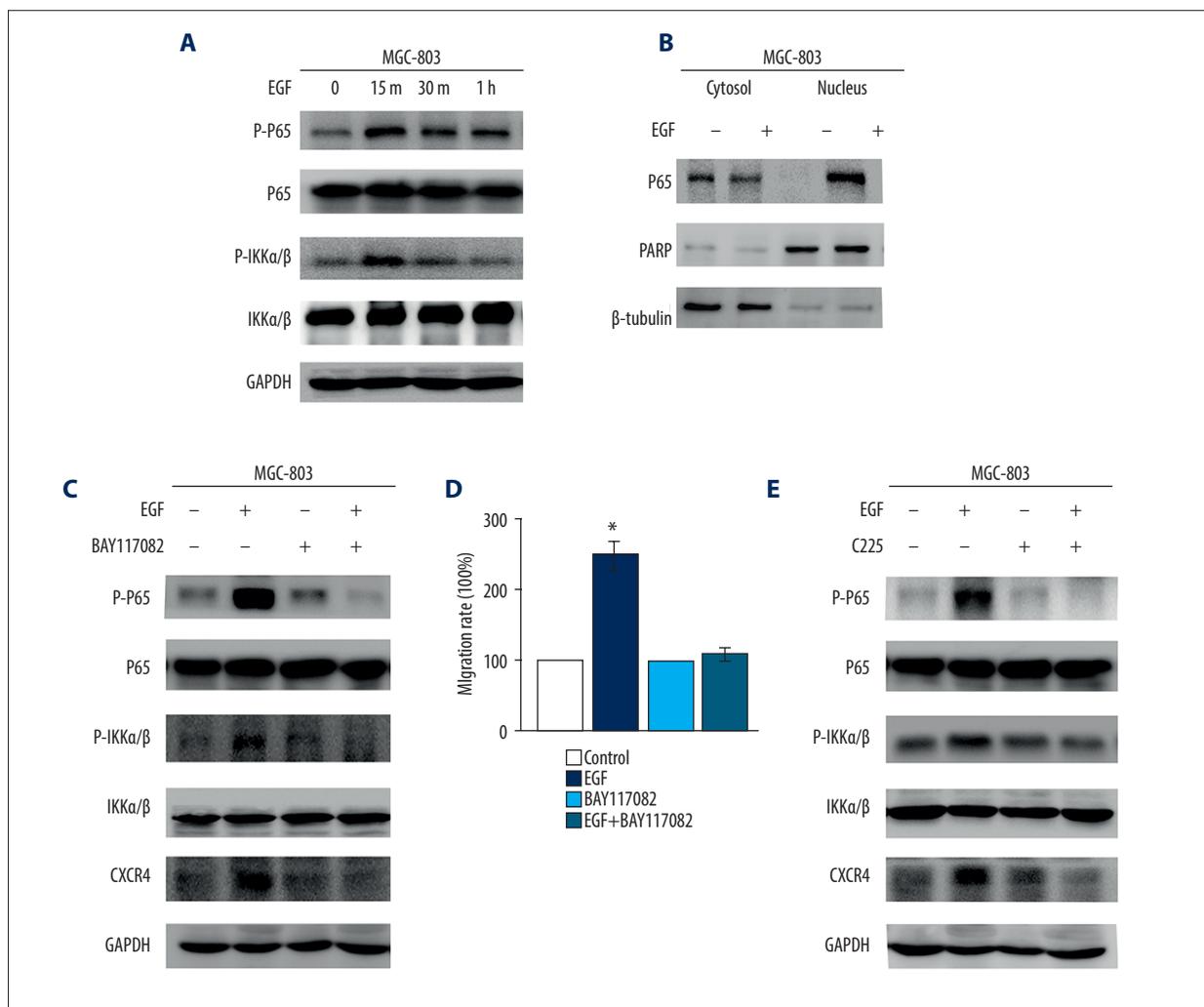


Figure 3. NF-κB transcription factor contributes to EGF/EGFR-mediated CXCR4 upregulation. **(A)** MGC-803 cells were treated with EGF (100 ng/mL). Phosphor-p65/IKKα/β were determined by western blot assay (m, minute; h, hour). **(B)** MGC-803 cells were incubated with EGF (100 ng/mL) for 1 h. Nuclear and cytoplasm cell lysate proteins were analyzed by western blot assay. **(C)** MGC-803 cells were treated with EGF (100 ng/mL) for 48 h and pretreated with or without BAY117082 (15 μM). Western blot analysis of CXCR4. **(D)** MGC-803 cells were treated with EGF (100 ng/mL) with or without BAY117082 (15 μM). Cell migration was examined by Transwell assay. Values are represented as mean±standard deviation (SD) in 3 independent experiments (* $P < 0.05$). **(E)** MGC-803 cells were treated with EGF (100 ng/mL) for 48 h and pretreated with or without C225 (10 μg/mL). Western blot analysis of phosphor-p65/IKKα/β and CXCR4.

CXCR4 and EGFR levels influence GC prognosis

A total of 56 histologically confirmed resected gastric cancer tissues were collected in this study. Of the 56 patient specimens, 34 (60.7%) showed high-level expression of CXCR4 (Figure 4) which was significantly associated with lymph node metastasis ($P=0.024$; Table 1). In 17 samples (30.4%), high-level expression of EGFR (Figure 4) was associated with depth of invasion and lymph node metastasis ($P=0.045$ and $P=0.022$, respectively; Table 1). The surgical outcomes of patients were poorer with EGFR and CXCR4 expressions. ($P=0.001$ and $P=0.011$, respectively; Figure 5A, 5B). Co-expression of CXCR4 and EGFR

showed worse prognosis compared to other patient groups ($P=0.002$; Figure 5C). Importantly, a significantly positive correlation was found between the expressions of CXCR4 and EGFR ($r=0.372$, $P=0.005$; Table 2).

Discussion

In the last few years, evidence from our laboratory and others, has demonstrated a key role of the tumor microenvironment in tumorigenesis, metastasis and chemoresistance. Furthermore, targeting tumor-stroma interactions is a major

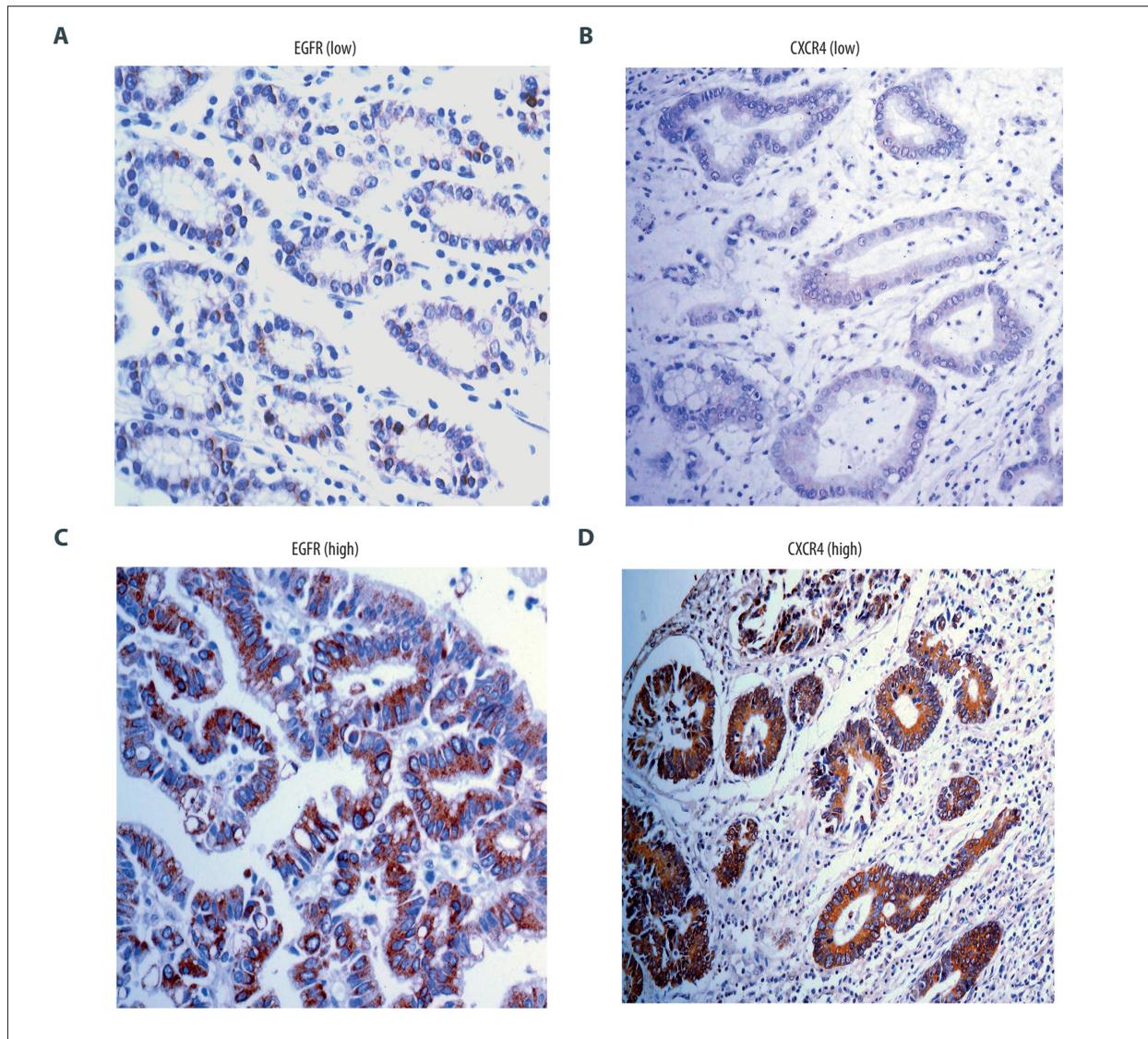


Figure 4. Representative images showing CXCR4 and EGFR immune-histochemical staining in gastric cancer tissues. Representative images showing CXCR4 and EGFR immunohistochemical staining in gastric cancer tissues. EGFR (A) and CXCR4 (B) low-staining levels; EGFR (C) and CXCR4 (D) high-staining levels (in brown), magnification 200 \times .

potential area for therapeutic development [4,14,15]. Growth factors and inflammatory cytokines are enriched in the tumor microenvironment and have a synergistic effect on the enhancement of tumor infiltration and metastasis [16,17]. Rø et al. reported that HGF/IGF-1 can cooperate with CXCL12 to promote migration of myeloma cells [18]. However, in this study cytokine receptor expression did not increase after cytokine stimulation. Kim et al. demonstrated that EGF and CXCL12 synergistically modulate breast cancer cell motility in 3D environments [19]. In our study, we found that CXCL12 and EGF cooperatively promote gastric cancer cells migration and potential synergy between them could be explained by upregulation of receptors. CXCR4 and EGFR have been considered as 2 predictive factors of prognosis and metastasis in several

malignancies [20–22]. Al Zobair et al. reported co-expressed CXCR4 and EGFR are associated with poorer prognosis in non-small cell lung cancer (NSCLC) [9]. More notably, similar observations have been found in inflammatory breast cancer and pancreatic ductal adenocarcinoma [10,23]. However, no study has focused on dual CXCR4/EGFR expression and its prognostic impact in gastric cancer

In our study, 56 gastric cancer specimens were collected. Our results suggested that patients with co-expression of CXCR4 and EGFR were more likely to have lymph node metastasis and poor prognosis, which is consistent with previous studies [4,13]. Furthermore, the impact of co-expression of CXCR4 and EGFR on prognosis in gastric cancer was investigated.

Table 1. Correlation between CXCR4/EGFR levels and clinicopathological factors in patients with primary GC.

Factors	All cases	EGFR		P value	CXCR4		P value
		Negative	Positive		Negative	Positive	
Gender							
Female	14	11	3		5	9	
Male	42	28	14	0.513	17	25	0.752
Age							
<60	28	20	8		10	18	
≥60	28	19	9	1.000	12	16	0.584
Depth of invasion							
T1+T2	9	9	0		5	4	
T3+T4	47	30	17	0.045*	17	30	0.294
Tumor size							
≥5 cm		18	7		10	19	
<5 cm		21	10	0.730	12	15	0.922
Lymphovascular invasion							
Yes		13	6		5	14	
No		26	11	0.887	17	20	0.154
LN metastasis							
N0	16	15	1		10	6	
N1–3	40	24	16	0.022*	12	28	0.024*
Lauren classification							
Intestinal	22	14	8		8	14	
Diffuse	21	16	5		8	13	
mixed	13	9	4	0.670	6	7	0.840

LN – lymph node. * P<0.05.

In the analysis of the combined status of the expressions of CXCR4 and EGFR, patients with EGFR⁺ and CXCR4⁺ had shorter overall survival compared to the other groups. We also found that CXCR4 expression was positively associated with EGFR expression. These observations may be useful in predicting the prognosis of patients with gastric cancer and offer a promising approach towards the development of targeted therapies.

Previous research has shown that GPCRs (such as chemokine receptors, for example CXCR4) can cross-activate other receptors, including EGFR [24]. Firstly, CXCL12/CXCR4-mediated ectodomain shedding of EGFR ligands has been shown as a potential mechanism for EGFR transactivation in gastric cancer [25]. Secondly, EGFR transactivation may occur through intracellular phosphorylation via SRC, which was observed in our previous study [7]. However, whether CXCL12/CXCR4 could up-regulate EGFR and the underlying molecular mechanisms remain largely unknown in gastric cancer.

CXCR4 activation promotes nuclear translocation of NF-κB. Activation of NF-κB stimulated by inflammatory cytokines was demonstrated in diverse progressions of cancer development, including epithelial-mesenchymal transition (EMT), motility, and invasion of tumor cells [26]. We hypothesized that NF-κB signaling pathway may participate in CXCL12/CXCR4-induced EGFR upregulation and gastric cancer migration. In the present study, following exposure to CXCL12, there was a gradual increase in the phosphorylation of p65/IKKα/β and upregulation of CXCR4/EGFR. Furthermore, inhibition of the NF-κB signaling pathway reversed CXCR4/EGFR upregulation, and enhanced the migration ability mediated by CXCL12. These results clearly indicated that CXCL12/CXCR4 induced EGFR upregulation and migration ability, at least partially, via the NF-κB signaling axis.

It is well known that EGF/EGFR signaling is crucial in the metastatic progression of many tumors [27]. Previous studies have

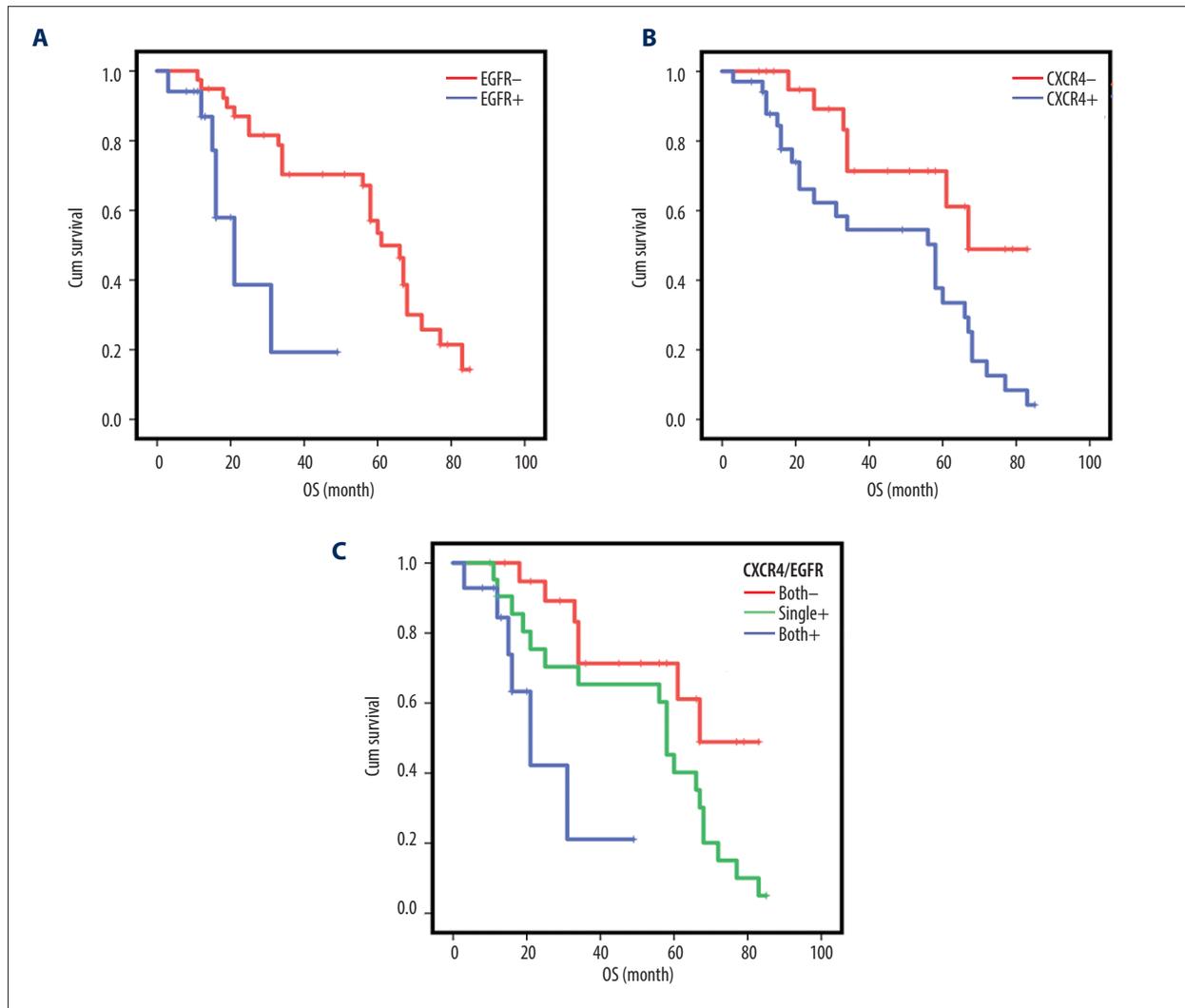


Figure 5. CXCR4 and EGFR levels influence the prognosis of patients with gastric cancer. CXCR4 and EGFR levels influence the prognosis of patients with gastric cancer. Kaplan-Meier analysis of overall survival (OS) for EGFR (A) and CXCR4 (B) expressions in all gastric cancer patients ($n=56$, $P=0.001$ and $P=0.011$). (C) Kaplan-Meier analysis of OS for patients with co-expression of CXCR4 and EGFR was undertaken ($P=0.002$).

Table 2. Correlations between CXCR4 expression and EGFR levels in patients with primary GC.

CXCR4 expression	Number	EGFR		R value	P value
		Negative (%)	Positive (%)		
Negative (%)	22	20 (35.7)	2 (3.5)	0.372	0.005*
Positive (%)	34	19 (34)	15 (26.8)		
Number (%)	56	39 (69.7%)	17 (30.3%)		

* $P < 0.05$.

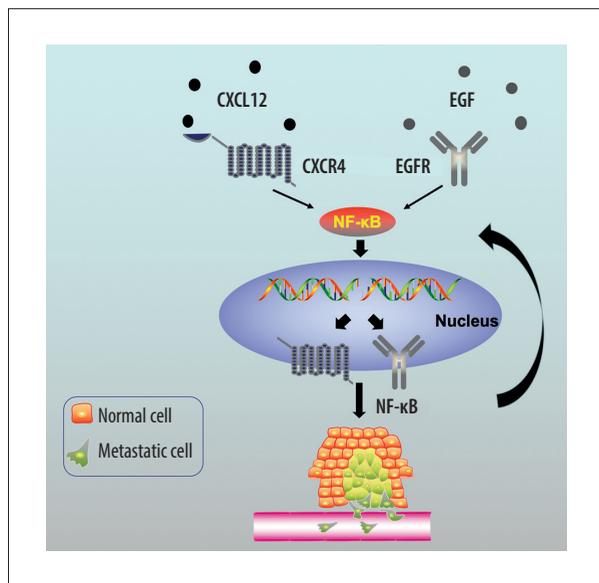


Figure 6. A working model for amplified cross-talk between CXCR4 and EGFR to promote gastric cancer metastasis. A positive feedback loop of CXCR4/EGFR-NF- κ B-CXCR4/EGFR exists in the gastric cancer signaling network, which ultimately increases gastric cancer cellular metastasis.

reported that EGF can upregulate expression of CXCR4 and migration capacity in ovarian and lung cancer cells [8,28]. NF- κ B signaling is a classical downstream pathway of EGF/EGFR [29]. The aforementioned studies suggest that NF- κ B signaling is also involved in CXCR4 upregulation stimulated by EGF in gastric cancer. We further studied the expression of CXCR4 under the stimulation of EGF. Fortunately, we found significantly higher levels in the phosphorylation of p53/IKK α / β and upregulation of CXCR4. BAY117082 inhibition of NF- κ B was found to suppress upregulation of CXCR4 and enhance migration ability mediated by EGF. These findings indicated that EGF induced CXCR4 upregulation and migration ability at least partially, via the NF- κ B signaling axis.

Conclusions

Collectively, our data indicated that co-expression of CXCR4 and EGFR in GC may represent a subpopulation of patients with unfavorable prognosis. Furthermore, *in vitro* experiments demonstrated that NF- κ B transcription factor contributes to CXCL12/CXCR4-mediated EGFR upregulation and EGF/EGFR-mediated CXCR4 upregulation (Figure 6). Synergy between CXCR4 and EGFR may support a rationale for potent combination therapy in GC.

References:

- Ajani JA, Lee J, Sano T et al: Gastric adenocarcinoma. *Nat Rev Dis Primers*, 2017; 3: 17036
- Waldum HL, Fossmark R: Types of gastric carcinomas. *Int J Mol Sci*, 2018; 19: 12
- Kawaguchi N, Zhang TT, Nakanishi T: Involvement of CXCR4 in normal and abnormal development. *Cells*, 2019; 8(2): 185
- Cheng Y, Song Y, Qu J et al: The chemokine receptor CXCR4 and c-MET cooperatively promote epithelial-mesenchymal transition in gastric cancer cells. *Transl Oncol*, 2018; 11: 487–97
- Sengupta S, Schiff R, Katzenellenbogen BS: Post-transcriptional regulation of chemokine receptor CXCR4 by estrogen in HER2 overexpressing, estrogen receptor-positive breast cancer cells. *Breast Cancer Res Treat*, 2009; 117: 243–51
- Zheng F, Zhang Z, Flamini V et al: The axis of CXCR4/SDF-1 plays a role in colon cancer cell adhesion through regulation of the AKT and IGF1R signalling pathways. *Anticancer Res*, 2017; 37: 4361–69
- Cheng Y, Qu J, Che X et al: CXCL12/SDF-1 α induces migration via SRC-mediated CXCR4-EGFR cross-talk in gastric cancer cells. *Oncol Lett*, 2017; 14: 2103–10
- Guo Z, Cai S, Fang R et al: The synergistic effects of CXCR4 and EGFR on promoting EGF-mediated metastasis in ovarian cancer cells. *Colloids Surf B Biointerfaces*, 2007; 60: 1–6
- Al Zobair AA, Al Obeidi BF, Yang L et al: Concomitant overexpression of EGFR and CXCR4 is associated with worse prognosis in a new molecular subtype of non-small cell lung cancer. *Oncol Rep*, 2013; 29: 1524–32
- Li RH, Huang WH, Wu JD et al: EGFR expression is associated with cytoplasmic staining of CXCR4 and predicts poor prognosis in triple-negative breast carcinomas. *Oncol Lett*, 2017; 13: 695–703
- Porcile C, Bajetto A, Barbieri F et al: Stromal cell-derived factor-1 α (SDF-1 α)/CXCL12 stimulates ovarian cancer cell growth through the EGF receptor transactivation. *Exp Cell Res*, 2005; 308: 241–53
- Zeng X, Qu X, Zhao C et al: FEN1 mediates miR-200a methylation and promotes breast cancer cell growth via MET and EGFR signaling. *FASEB J*, 2019; 33(10): 10717–30
- Zhang X, Song Y, Song N et al: RANKL/RANK pathway abrogates cetuximab sensitivity in gastric cancer cells via activation of EGFR and c-Src. *Oncotargets Ther*, 2017; 10: 73–83
- Wang X, Che X, Liu C et al: Cancer-associated fibroblasts-stimulated interleukin-11 promotes metastasis of gastric cancer cells mediated by upregulation of MUC1. *Exp Cell Res*, 2018; 368: 184–93
- Zhang C, Li Z, Xu L et al: CXCL9/10/11, a regulator of PD-L1 expression in gastric cancer. *BMC Cancer*, 2018; 18: 462
- Makowiecka A, Simiczyjew A, Nowak D, Mazur AJ: Varying effects of EGF, HGF and TGF β on formation of invadopodia and invasiveness of melanoma cell lines of different origin. *Eur J Histochem*, 2016; 60: 2728
- Holland JD, Gyorffy B, Vogel R et al: Combined Wnt/ β -catenin, Met, and CXCL12/CXCR4 signals characterize basal breast cancer and predict disease outcome. *Cell Rep*, 2013; 5: 1214–27
- Ro TB, Holien T, Fagerli UM et al: HGF and IGF-1 synergize with SDF-1 α in promoting migration of myeloma cells by cooperative activation of p21-activated kinase. *Exp Hematol*, 2013; 41: 646–55
- Kim BJ, Hannanta-anan P, Chau M et al: Cooperative roles of SDF-1 α and EGF gradients on tumor cell migration revealed by a robust 3D microfluidic model. *PLoS One*, 2013; 8: e68422
- Goto M, Yoshida T, Yamamoto Y et al: CXCR4 expression is associated with poor prognosis in patients with esophageal squamous cell carcinoma. *Ann Surg Oncol*, 2017; 24: 832–40
- Ema A, Waraya M, Yamashita K et al: Identification of EGFR expression status association with metastatic lymph node density (ND) by expression microarray analysis of advanced gastric cancer. *Cancer Med*, 2015; 4: 90–100
- Zuo J, Wen M, Li S et al: Overexpression of CXCR4 promotes invasion and migration of non-small cell lung cancer via EGFR and MMP-9. *Oncol Lett*, 2017; 14(6): 7513–21

23. Wu H, Zhu L, Zhang H et al: Coexpression of EGFR and CXCR4 predicts poor prognosis in resected pancreatic ductal adenocarcinoma. *PLoS One*, 2015; 10: e0116803
24. Bholra NE, Grandis JR: Cross-talk between G-protein-coupled receptors and epidermal growth factor receptor in cancer. *Front Biosci*, 2008; 13: 1857–65
25. Yasumoto K, Yamada T, Kawashima A et al: The EGFR ligands amphiregulin and heparin-binding EGF-like growth factor promote peritoneal carcinomatosis in CXCR4-expressing gastric cancer. *Clin Cancer Res*, 2011; 17: 3619–30
26. Xiang Z, Zhou ZJ, Xia GK et al: A positive cross-talk between CXCR4 and CXCR2 promotes gastric cancer metastasis. *Oncogene*, 2017; 36: 5122–33
27. Holbro T, Hynes NE: ErbB receptors: Directing key signaling networks throughout life. *Annu Rev Pharmacol Toxicol*, 2004; 44: 195–217
28. Phillips RJ, Mestas J, Gharaee-Kermani M et al: Epidermal growth factor and hypoxia-induced expression of CXCR4 chemokine receptor 4 on non-small cell lung cancer cells is regulated by the phosphatidylinositol 3-kinase/PTEN/AKT/mammalian target of rapamycin signaling pathway and activation of hypoxia inducible factor-1alpha. *J Biol Chem*, 2005; 280: 22473–81
29. Chung S, Jin Y, Han B et al: Identification of EGF-NF-kappaB-FOXO1 signaling axis in basal-like breast cancer. *Cell Commun Signal*, 2017; 15: 22