Article

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Igniting Metastasis: CCR1 Promotes Lung Cancer Dissemination Through an ERK/c-FOS-RhoGDIβ Axis

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Background: Lung cancer is one of the common malignancies worldwide and is spotlighted due to high mortality. Evidence indicates that RhoGDIβ is a crucial driver of lung cancer metastasis. Therefore, this study aims to elucidate the upstream mechanisms underlying the role of RhoGDIβ in metastatic progression of lung cancer.
Methods: Potential RhoGDIβ-interacting proteins were identified using bioinformatic approaches. Functional assays were performed in lung cancer cell lines to assess migration, invasion, and epithelial-mesenchymal transition (EMT) following C-C chemokine receptor type 1 (CCR1) overexpression, extracellular signal-regulated kinase (ERK) pathway inhibition, or Proto-Oncogene C-Fos (c-FOS) induction. Moreover, signaling activity and RhoGDIβ expression were evaluated by immunoblotting. Additionally, a lung metastasis mouse model was used to validate metastatic nodule formation and pathway activation *in vivo*.
Results: CCR1 overexpression significantly enhanced the metastatic behaviors of lung cancer cells, including migration, invasion, and EMT, and increased lung nodule formation in mice ($p < 0.05$). CCR1 overexpression also activated the ERK pathway, elevated levels of phosphorylated ERK (p-ERK) and c-FOS, and suppressed RhoGDIβ expression both *in vitro* and *in vivo* ($p < 0.05$). These trends were abolished by treatment with an ERK pathway inhibitor. Importantly, c-FOS overexpression rescued the effect of ERK blockade on metastasis inhibitory and RhoGDIβ upregulation, confirming that c-FOS was a downstream factor of ERK ($p < 0.05$). *In vivo* experiments further substantiated that CCR1 promoted metastasis through ERK/c-FOS-mediated repression of RhoGDIβ ($p < 0.05$).
Conclusion: Our study unveils a novel CCR1-ERK-c-FOS signaling axis that promotes lung cancer metastasis and is associated with RhoGDIβ. Intervening in this axis represents a promising strategy for the migration of lung cancer metastasis.

Keywords: lung cancer; metastasis; RhoGDIβ; ERK pathway; C-C chemokine receptor 1

Introduction

Lung cancer (LC) is a common malignancy worldwide and is spotlighted due to high mortality, with an estimated 22 million new cases and 1.79 million deaths every year [1]. As an extremely invasive tumor, LC has a 5-year survival rate of only 10%–15% [2]. Clinically, it is divided into small cell lung cancer and non-small cell lung cancer (NSCLC), with NSCLC accounting for 85% of all cases [3–5]. Metastasis is one of the key determinants of prognosis. Specifically, when LC is detected at an early stage without metastasis, the 5-year survival rate can reach 50%, whereas it drops to 1%–2% in advanced metastatic cases [2]. The most common metastatic sites are bone, brain, lymph nodes, and liver [6]. Current therapeutic strategies for metastatic LC, such as surgery, radiotherapy, and chemotherapy, offer only limited efficacy [7,8]. Advances in molecular sciences have underscored targeted therapy as a promising approach for managing LC metastasis. Therefore, identifying novel therapeutic targets is critical to improve the survival rate in patients with metastatic LC.

RhoGDIβ is a member of the small guanine nucleotide dissociation inhibitor (GDI) family and is predominantly expressed in hematopoietic tissues, implying its expression in lung tissues with a rich vascular system [9]. RhoGDIβ exerts multiple biological functions, particularly by modulating cell morphology and metastasis through its effects on the regulation of actin and tubulin. The role of RhoGDIβ in cancer appears context-dependent, with differential expression observed across various human conditions, suggesting it may exert either tumor-promoting or tumor-suppressive effects [9]. Importantly, RhoGDIβ has been reported as a tumor-suppressor in LC [10]. However, given its multifaceted function, the underlying mechanisms of its role in LC progression require further investigation.

The predicted interacting proteins of RhoGDIβ included Proto-Oncogene C-Fos (c-FOS, also called FOS) and C-C chemokine receptor 1 (CCR1), and analysis using the STING database revealed that FOS and CCR1 are co-expressed. *C-FOS* is an essential gene involved in early cell proliferation and acts as a transcription factor that regulates tumor metastasis. It can induce epithelial-mesenchymal

transition (EMT) in tumor cells and has been proven to be up-regulated in LC [11]. CCR1 interacts with chemokines secreted by tumor cells and acts on interstitial cells to induce chemotaxis, thereby promoting tumor cell metastasis [12].

A previous study reported that CCR1 can induce neuropathic pain by activating extracellular signal-regulated kinase (ERK) pathway [13]. The activated ERK pathway can mediate up-regulation of c-FOS [14]. Interestingly, activation of the ERK pathway has been reported to induce cisplatin resistance in gastric cancer cells by downregulating RhoGDIβ levels, indicating that RhoGDIβ is regulated by ERK signaling [15]. Therefore, this study aims to investigate whether CCR1 promotes LC metastasis by inhibiting RhoGDIβ through the ERK/c-FOS signaling cascade.

Materials and Methods

Bioinformatics Analysis: The Protein-Protein Interaction

RhoGDIβ-interacting proteins were predicted using the HitPredict database (http://www.hitpredict.org/). Furthermore, interactions between c-FOS (UniProt ID: P01100) and CCR1 (UniProt ID: P32246) were assessed using the STRING database (https://cn.string-db.org/) under default parameters, applying a reliability threshold of 0.4.

Cell Culture

A549 (CL-0016) and H1299 (CL-0165), derived from human NSCLC of adenocarcinoma subtype, were obtained from Procell (China) and cultured in DMEM complete medium (DH1011, Yajimall, Shanghai, China). Cells were maintained at 37 °C in a humidified incubator (SR80W, hbzhan, Shanghai, China) with 5% CO_2. Cell lines were confirmed to be mycoplasma-free and identified by Short Tandem Repeat (STR) method.

Cell Transfection

Full-length coding sequences of CCR1 and c-FOS were cloned into the pcDNA3.1+ vector (VT1001, Youbio, Changsha, China) to generate CCR1 (oe-CCR1) and c-FOS (oe-c-FOS) overexpression plasmids, with the empty vector serving as the negative control (NC). Sequence information for CCR1, c-FOS, and NC is shown in **Supplementary File 1**.

For transfection, cells were seeded into 6-well plates at a density of 2×10^5 cells per well and cultured overnight until approximately 70–80% confluence. Overexpression plasmids (oe-CCR1, oe-c-FOS) or empty vector (NC) were transfected into cells using a lipofectamine-based transfection reagent (CBMD250, Abace-biology, Beijing, China). Briefly, for each well, 2.5 µg of plasmid DNA was diluted in 250 µL of serum-free Opt-MEM medium. In parallel, 5 µL of the lipofectamine reagent was diluted in 250 µL of Opt-MEM medium and incubated at room temperature for 5 minutes. The diluted DNA and lipofectamine reagent were then combined, gently mixed, and incubated for an additional 20 minutes at room temperature to allow for DNA-lipid complex formation. The resulting 500 µL transfection mixture was added dropwise to each well. After 6 hours, the culture medium was replaced with fresh complete medium. Cells were cultured for 48 hours at 37 °C, harvested, and the transfection efficiency was assessed by quantitative real-time polymerase chain reaction (qRT-PCR).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from cells using an RNA isolation kit (RE-03111, ForeGene, China) and subsequently underwent one-step reverse transcription and quantitative PCR using the Cell Direct RT-qPCR Kit–SYBR GREEN I (DRT-02011, ForeGene, Chengdu, China). Primer sequences are listed in Table 1. Relative expression levels of the target genes were determined using the $2^{-\Delta\Delta CT}$ method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as the internal control.

Cells Treatments

First, LC cells were transfected with oe-CCR1 or oe-c-FOS. To evaluate whether CCR1 promotes LC metastasis through the ERK/c-FOS signaling pathway, cells were co-transfected with oe-CCR1 and oe-c-FOS, and treated with the ERK inhibitor U0126 (10 µM; HY-12031A, MedChemExpress, Shanghai, China) for 24 hours to inhibit the ERK pathway [16].

Wound Healing Assay

The monolayer of LC cells was scratched using a pipette to establish a wound. Detached cells were eliminated by washing with phosphate-buffered saline (PBS, E607008, Sangon, Shanghai, China). The wound area was measured immediately after scratching (A) and again after 24 hours (B). The cell migration rate was calculated as (A–B)/A $\times$ 100%.

Table 1. The list of primers used in this study.

Gene (Human)	5′→3′
c-FOS (F)	GGAACAGTTATCTCCAGAAGAA
c-FOS (R)	GCATTTGGCTGCAGCCATCT
CCR1 (F)	AATGTAATGGTGGCCTGGGG
CCR1 (R)	TCCTCCCAACCCCCTATCAG
GAPDH (F)	GTCTCCTCTGACTTCAACAGCG
GAPDH (R)	ACCACCCTGTTGCTGTAGCCAA

Abbreviation: *c-FOS*, Proto-Oncogene C-Fos; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *CCR1*, C-C chemokine receptor 1; F, Forward; R, Reverse.

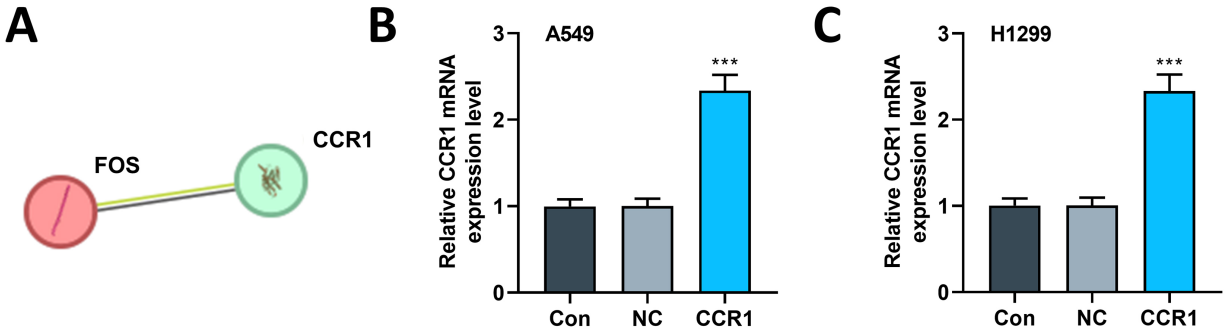

Fig. 1. Transfection efficiency of the CCR1 overexpression plasmid. (A) The STRING database was used to detect the interactions between FOS and CCR1. (B,C) The transfection efficiency of *CCR1* in A549 and H1299 cells (qRT-PCR; *GAPDH* as internal control). n = 3. ***$p < 0.001$ vs. NC. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; qRT-PCR, Quantitative real-time polymerase chain reaction; CCR1, C-C Chemokine receptor 1; FOS, Proto-Oncogene C-Fos.

Transwell Assay

Matrigel (354262, Corning, Corning, NY, USA) was pre-coated onto Transwell inserts to evaluate the invasive capability of LC cells. Serum-depleted LC cells were seeded into the upper chamber of the Transwell inserts, while the lower chamber was filled with medium containing 20% FBS to serve as a chemoattractant. Following a 24-h incubation, cells that had invaded through the Matrigel were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet (WKQ-0007202, Weikeqi-biotech, Chengdu, China). Finally, cells were evaluated and counted under an inverted microscope (N300M, Yongxin, Ningbo, China).

Experimental Animals and Development of a Lung Metastasis Model

Male BALB/c nude mice (n = 40; 6-week-old; 12–20 g) were obtained from Hangzhou Medical College, China, and were housed under controlled conditions (natural circadian rhythm, 50% humidity, and 21 °C). All experiments involving animals were approved by the Institutional Animal Care and Use Committee of the Zhejiang Center of Laboratory Animals (ZJCA) and performed in accordance with the China Council on Animal Care and Use guidelines (No. ZJCLA-ACUC-20010443).

Experimental mice were randomly divided into 4 groups (n = 10 per group): NC+NC, CCR1, CCR1+U0126, and CCR1+U0126+c-FOS group. A549 cells were transfected with NC, oe-CCR1, and/or oe-c-FOS as appropriate. After 48 hours, transfected cells (2×10^6 cells in 200 µL per mouse) were injected through the tail vein to establish a lung metastasis model [17]. Furthermore, mice in the relevant groups received intravenous U0126 treatment (5 mg/kg, twice weekly) through the tail vein for 4 weeks. After 8 weeks, mice were anesthetized with pentobarbital sodium (65 mg/kg, i.p., P3761, Sigma-Aldrich, Saint Louis, MO, USA) and euthanized by cervical dislocation. Lung tissues were collected following intratracheal perfusion with Bouin's fixative (HT101128, Sigma-Aldrich, Saint Louis, MO, USA) to fully inflate the lobes and facilitate visualization of metastatic lesions. Metastatic nodules on the lung surface were counted manually by two independent investigators who were blinded to group assignment.

Immunohistochemistry (IHC)

Paraffin-embedded lung tissue sections were deparaffinized in xylene and rehydrated through a gradient alcohol series, followed by antigen repair using microwave treatment. The slices were incubated with endogenous peroxidase, blocked with BSA (GC35542, GLPBIO, Shanghai, China). Tissue sections were then incubated with primary antibody against RhoGDIβ (ab181252, Abcam, Cambridge, UK), followed by treatment with HRP-conjugated secondary antibody (ab205718, Abcam, Cambridge, UK). DAB (D0202, yajimall, Shanghai, China) was used for coloring, and nuclei were counterstained with hematoxylin (DH0013, LEAGENE, Beijing, China). Slides were sealed (mounted) with neutral resin (IH0265, LEAGENE, Beijing, China) and examined under a microscope. Positive IHC staining was identified as yellow-brown coloration. Quantitative image analysis was conducted using ImageJ software (version 1.53, National Institutes of Health, Bethesda, MA, USA), and the rate of positive expression area was determined as: positive area/total tissue area × 100%.

Western Blot Analysis

Total protein was extracted using RIPA buffer (PK10020, Proteintech, Wuhan, China) and quantified with a Protein Quantitative Kit (PT0001, LEAGENE, Beijing, China). Equal amounts of protein were resolved by SDS-PAGE (PH1493, Phygene, Fuzhou, China) and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes (FFP24, Beyotime, Shanghai, China). The membranes were blocked with BSA and incubated overnight at 4 °C with primary antibodies (Table 2). The

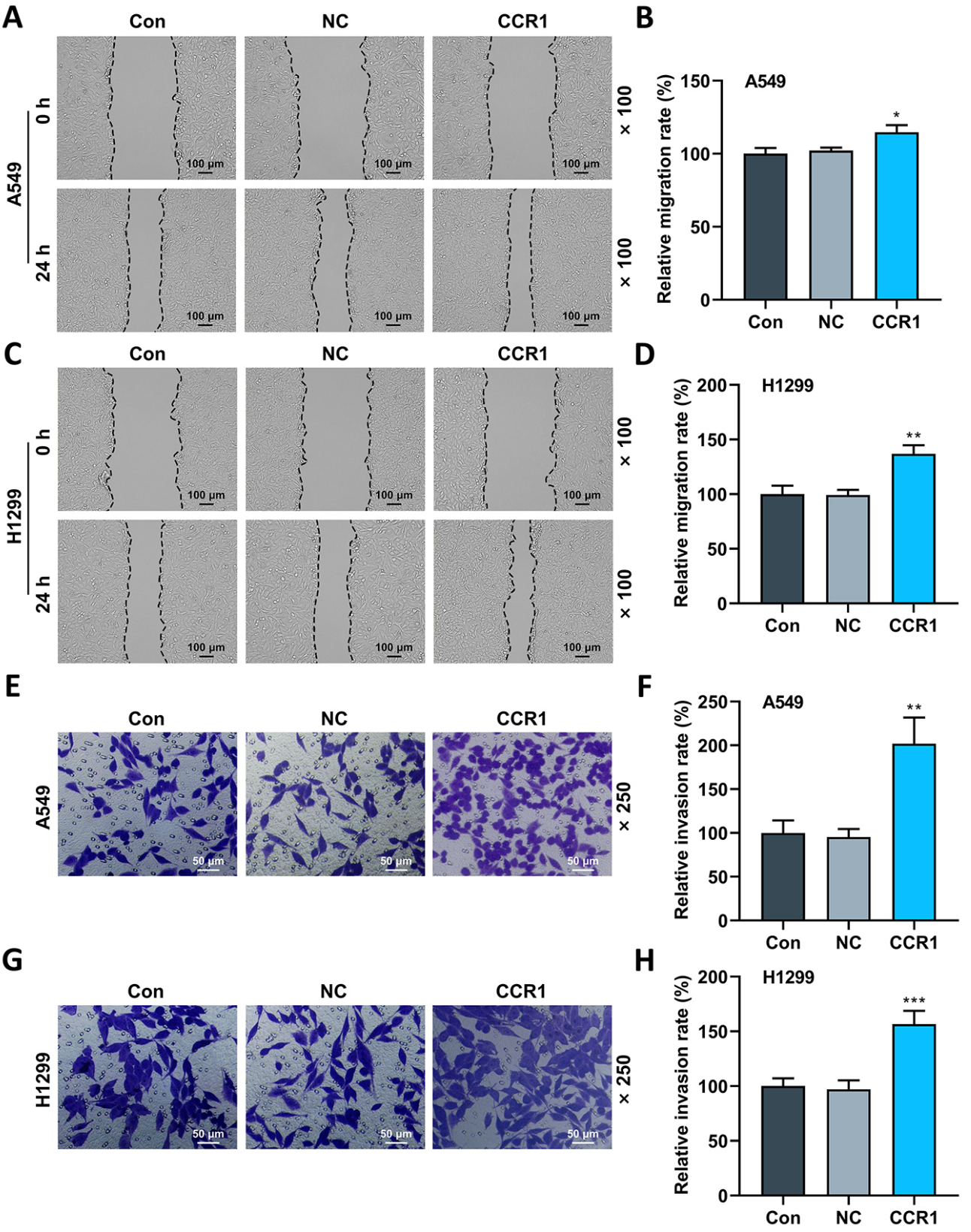

Fig. 2. CCR1 overexpression enhances the migration and invasion of lung cancer cells. (A–D) Migration of oe-CCR1-transfected A549 and H1299 cells (wound healing assay; 100×, scale bar = 100 μm). (E–H) Invasion of oe-CCR1-transfected A549 and H1299 cells (transwell assay; 250×, scale bar = 50 μm). n = 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. NC.

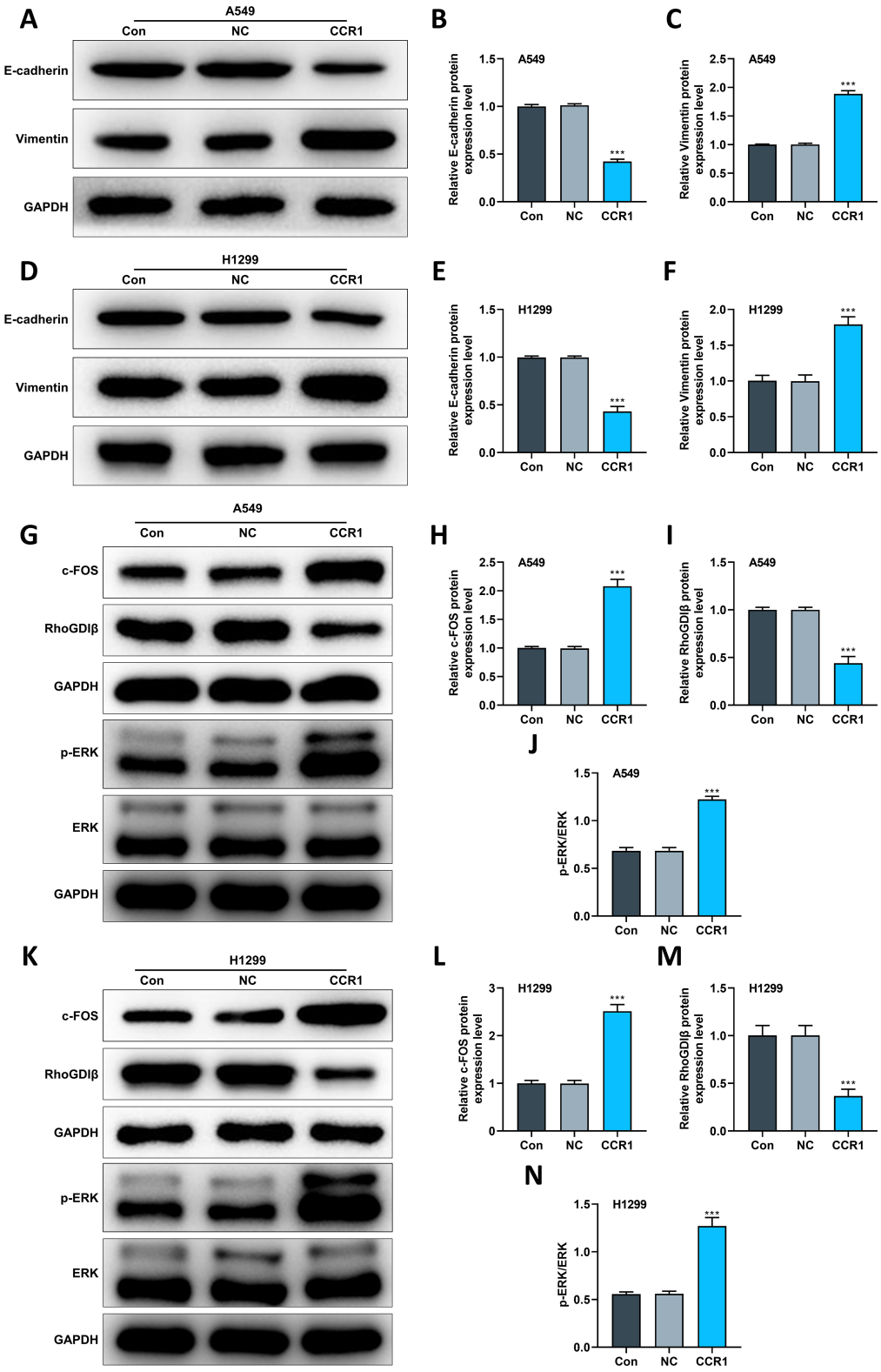

Fig. 3. CCR1 overexpression modulates EMT and ERK/c-FOS/RhoGDIβ signaling pathway in lung cancer cells. (A–F) The expression levels of EMT-related proteins (E-cadherin and vimentin) in oe-CCR1-transfected A549 and H1299 cells (Western blot analysis; GAPDH served as internal control). (G–N) The expression levels of ERK/c-FOS/RhoGDIβ signaling pathway-related proteins (p-ERK/ERK, c-FOS, and RhoGDIβ) in oe-CCR1-transfected A549 and H1299 cells (Western blot analysis; GAPDH served as internal control). n = 3. ***$p < 0.001$ vs. NC. EMT, Epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase.

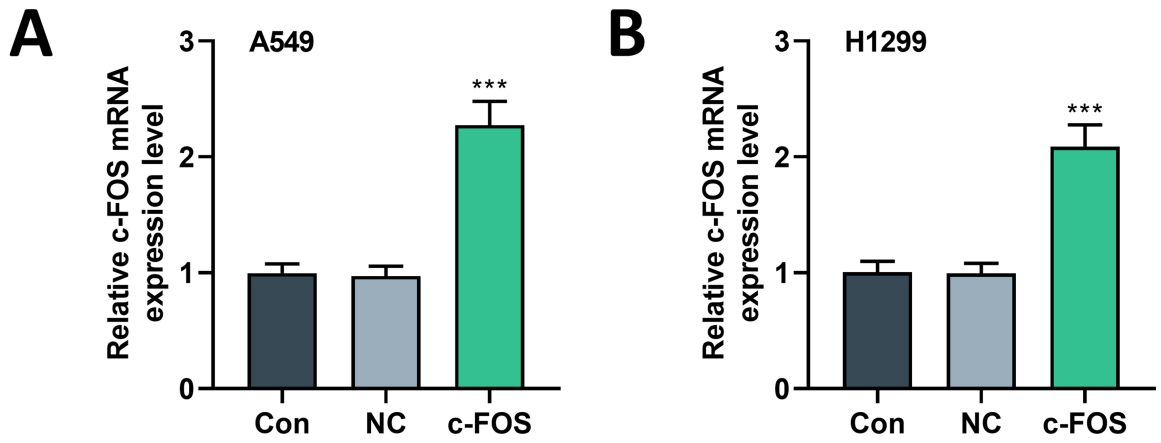

Fig. 4. The transfection efficiency of the c-FOS overexpression plasmid. (A,B) The transfection efficiency of the *c-FOS* construct in A549 and H1299 cells (qRT-PCR; *GAPDH* served as an internal control). n = 3. ***$p < 0.001$ vs. NC.

Table 2. Antibodies used in Western blot analysis.

Antibody	Catalog No.	Molecular weight	Dilution	Manufacturer (UK)
E-cadherin	ab231303	97 kDa	1/1000	Abcam
Vimentin	ab92547	54 kDa	1/500	Abcam
p-ERK	ab214036	43/41 kDa	1/1000	Abcam
c-FOS	ab208942	41 kDa	1/1000	Abcam
RhoGDIβ	ab250472	23 kDa	1/1000	Abcam
GAPDH	ab8245	37 kDa	1/1000	Abcam
goat anti rabbit	ab205718	—	1/2000	Abcam
goat anti mouse	ab205719	—	1/2000	Abcam

Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ERK, extracellular signal-regulated kinase.

next day, membranes were incubated with the corresponding secondary antibodies (Table 2) for 2 hours at room temperature. Protein bands were developed using ECL Western Blotting Substrate (MF078, mei5bio, Beijing, China) and visualized with an iBright FL1500 Imaging System (A44115, Invitrogen, Carlsbad, CA, USA). GAPDH was applied as the internal loading control. The grey values of protein bands were quantified by ImageJ software (version 1.53, National Institutes of Health, Bethesda, MA, USA) and relative protein levels were assessed as the ratio of target protein gray value to GAPDH gray value. Normalized values were then compared with the control group.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Prism 8.0; San Diego, CA, USA). Measurement data were expressed as mean ± standard deviation. Comparisons among multiple groups were conducted using a one-way ANOVA followed by Tukey's post-hoc test. A p-value of <0.05 was considered statistically significant.

Results

CCR1 Overexpression Enhances Metastasis, Promotes the ERK/c-FOS Signaling Pathway, and Suppresses RhoGDIβ Expression in LC Cells

The STRING database to investigate the potential functional association between FOS and CCR1 (Fig. 1A). This analysis revealed that c-FOS and CCR1 may participate in a common interaction network, although it did not establish direct physical interaction. To further explore their roles in LC, we constructed oe-CCR1 and oe-c-FOS overexpression constructs. As illustrated in Fig. 1B,C, successful transfection of oe-CCR1 was confirmed ($p < 0.05$).

Furthermore, we evaluated the impact of oe-CCR1 on LC cell metastasis. Functionally, oe-CCR1 facilitated LC cell migration (Fig. 2A–D, $p < 0.05$) and invasion (Fig. 2E–H, $p < 0.05$), and promoted EMT, as evidenced by downregulation of E-cadherin and upregulation of Vimentin protein levels (Fig. 3A–F, $p < 0.05$). Mechanistically, oe-CCR1 inhibited RhoGDIβ expression and activated the ERK/c-FOS signaling pathway, as evidenced by elevated p-ERK/ERK and c-FOS expression (Fig. 3G–N, $p < 0.05$).

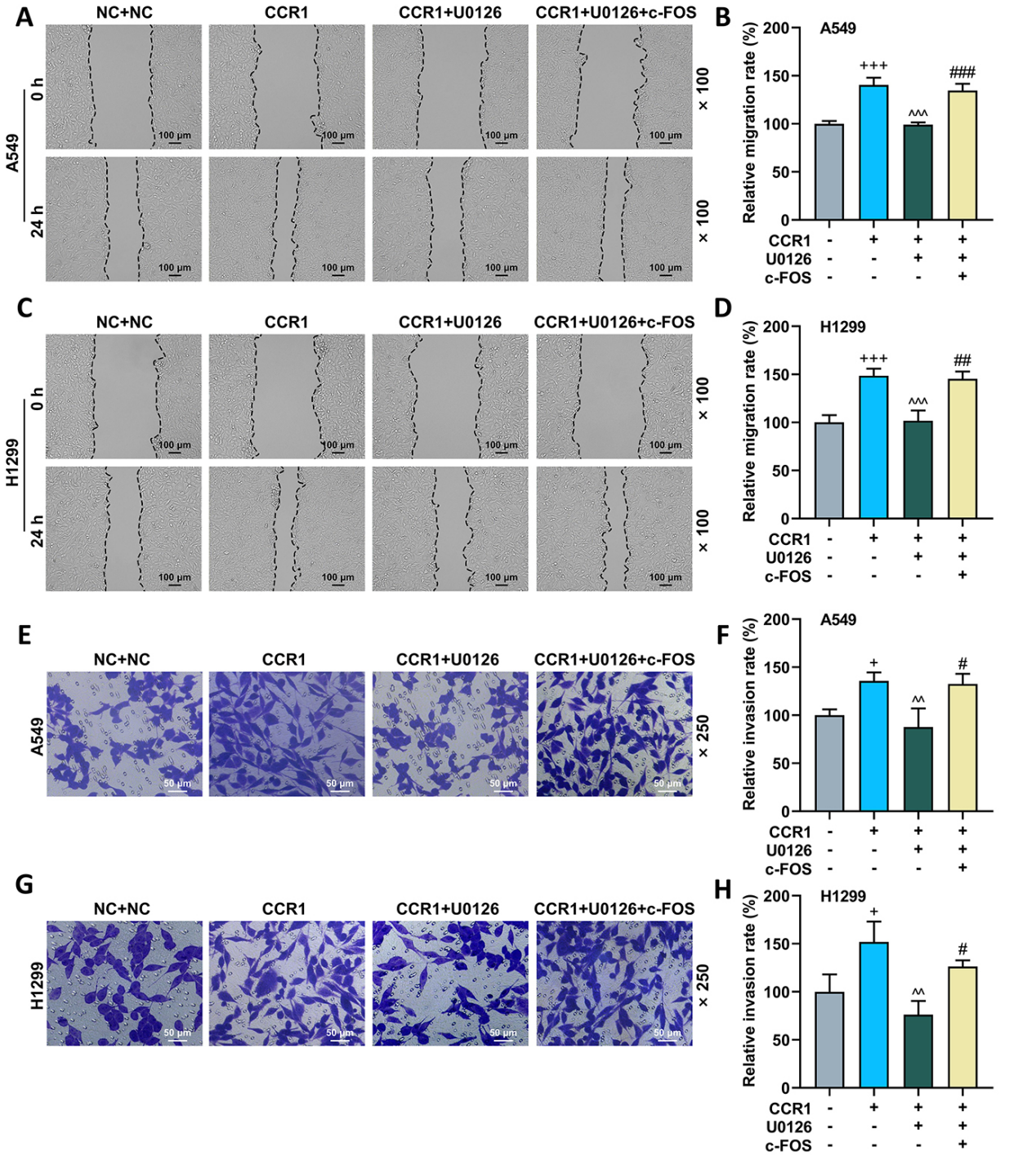

Fig. 5. The effects of CCR1, c-FOS, and ERK pathway inhibitor on the migration and invasion of lung cancer cells. Lung cancer cells were transfected with CCR1 or c-FOS plasmids and treated with the ERK pathway inhibitor U0126 (10 μM). (A–D) The migrations of A549 and H1299 cells (wound healing assay; 100×, scale bar = 100 μm). (E–H) The invasions of A549 and H1299 cells (transwell assay; 250×, scale bar = 50 μm). n = 3. $^{+}p < 0.05$, $^{+++}p < 0.001$ vs. NC+NC. $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. CCR1. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. CCR1+U0126.

CCR1 Enhances LC Metastasis by Inhibiting RhoGDIβ Expression and Activating the ERK/c-FOS Signaling Pathway

We evaluated whether CCR1 regulates LC metastasis through the ERK/c-FOS pathway using rescue experiments. The transfection efficiency of oe-c-FOS is shown in Fig. 4A,B ($p < 0.05$). Treatment with ERK inhibitor U0126, which inhibits ERK signaling, reversed the effects of oe-CCR1 on cell migration (Fig. 5A–D, $p < 0.05$), invasion (Fig. 5E–H, $p < 0.05$), EMT (Fig. 6A–F, $p < 0.05$),

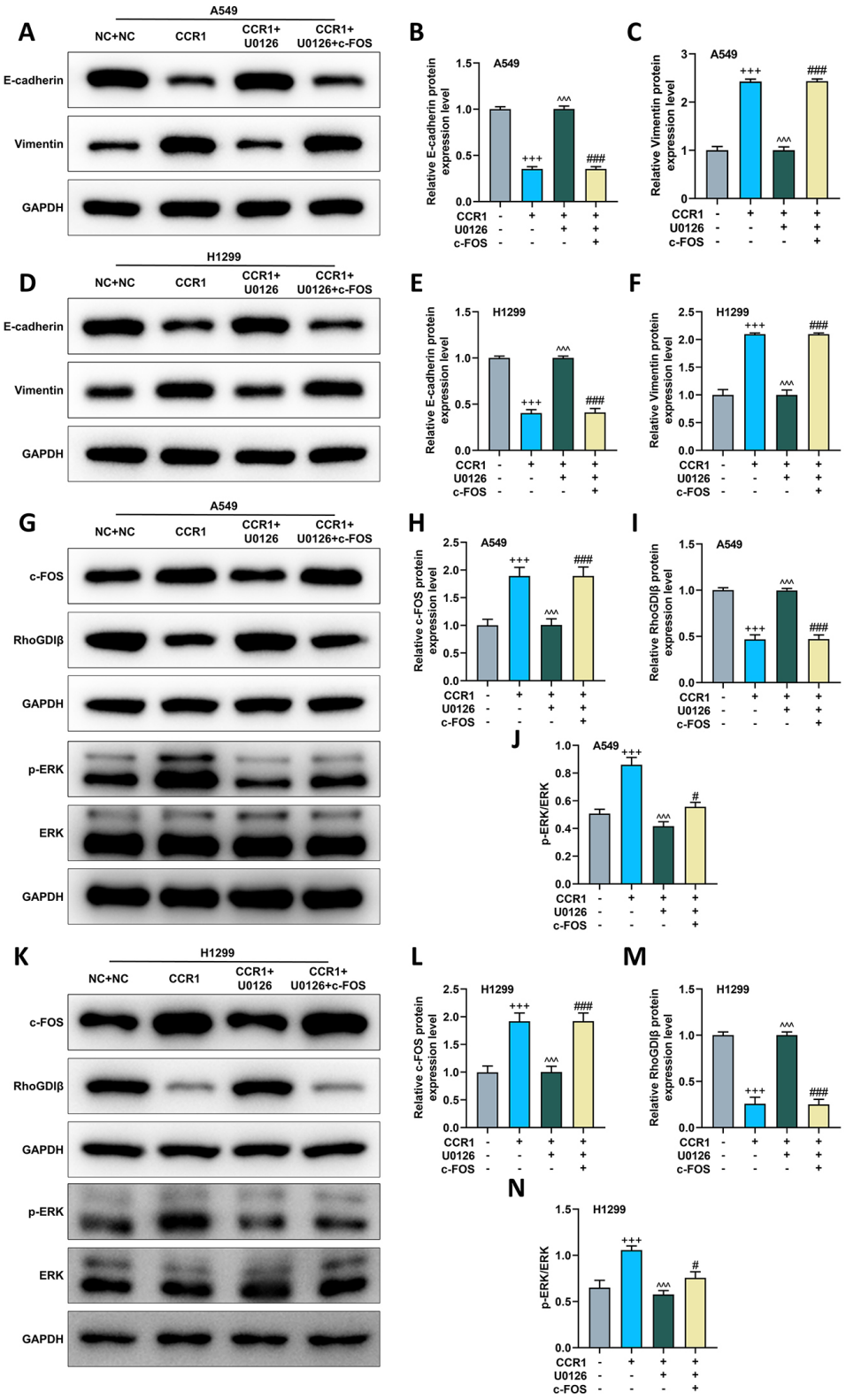

Fig. 6. The effects of CCR1, c-FOS, and ERK pathway inhibitor on EMT and ERK/c-FOS/RhoGDIβ pathway in lung cancer cells. Lung cancer cells were transfected with CCR1 or c-FOS plasmids and treated with the ERK pathway inhibitor U0126 (10 µM). (A–F) The expression levels of EMT-related proteins (E-cadherin and vimentin) in A549 and H1299 cells (Western blot analysis; GAPDH served as an internal control). (G–N) The expression levels of the ERK/c-FOS/RhoGDIβ pathway-related proteins (p-ERK/ERK, c-FOS and RhoGDIβ) in A549 and H1299 cells (Western blot analysis; GAPDH served as an internal control). n = 3. $^{+++}p < 0.001$ vs. NC+NC. $^{\wedge\wedge\wedge}p < 0.001$ vs. CCR1. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. CCR1+U0126.

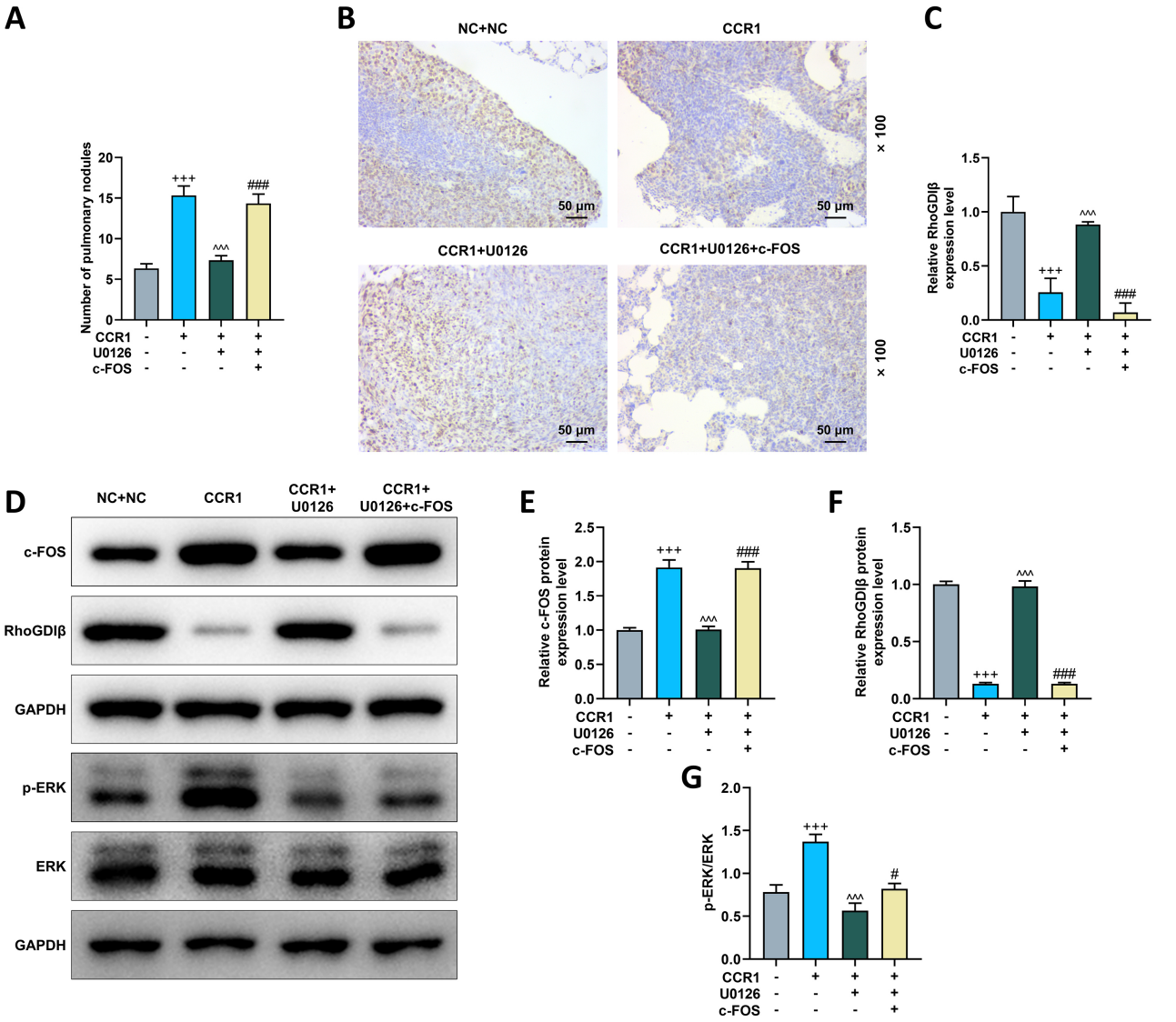

Fig. 7. The effects of CCR1, c-FOS, and ERK pathway inhibitor on ERK/c-FOS/RhoGDIβ pathway in lung metastatic mice. The treated A549 cells were intravenously injected into the tail vein of each mouse to establish a lung metastatic model. (A) The number of pulmonary nodules. (B,C) RhoGDIβ expression (immunohistochemistry; 100×, scale bar = 50 μm). (D–G) The expression levels of ERK/c-FOS/RhoGDIβ pathway-related proteins (p-ERK/ERK, c-FOS, and RhoGDIβ) in tumor tissues (Western blot analysis; GAPDH served as an internal control). n = 3. $^{+++}p < 0.001$ vs. NC+NC. $^{\wedge\wedge\wedge}p < 0.001$ vs. CCR1. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. CCR1+U0126.

and RhoGDIβ expression and activation of the ERK/c-FOS pathway (Fig. 6G–N, $p < 0.05$).

However, after oe-CCR1 and U0126 treatment, co-transfection with oe-c-FOS offset the regulation of U0126 on migration (Fig. 5A–D, $p < 0.05$), invasion (Fig. 5E–H, $p < 0.05$), EMT (Fig. 6A–F, $p < 0.05$), and on RhoGDIβ expression, and ERK/c-FOS pathway (Fig. 6G–N, $p < 0.05$).

CCR1 Facilitates LC Metastasis by Suppressing RhoGDIβ Expression and Activating the ERK/c-FOS Pathway in vivo

To further verify these findings, we conducted *in vivo* experiments using mouse models. Oe-CCR1 significantly increased the number of pulmonary nodules (Fig. 7A, $p < 0.05$), downregulated RhoGDIβ expression (Fig. 7B,C,G, $p < 0.05$), and increased p-ERK/ERK and c-FOS levels (Fig. 7D–F, $p < 0.05$). However, these effects were reversed by treatment with U0126 (Fig. 7A–G, $p < 0.05$). Consistent with the *in vitro* findings, the above-mentioned effects of U0126 were overturned by oe-c-FOS (Fig. 7A–D,F,G, $p < 0.05$).

Discussion

CCR1 is a member of the chemokine receptor family, expressed on neutrophils, monocytes, and T and B lymphocytes, where it mediates various reactions through ligand binding. Reportedly, CCR1 is highly expressed in several

cancer types, including multiple myeloma, prostate cancer, and colorectal cancer [18–20]. Previous studies have reported that CCR1 is significantly upregulated in LC with bone metastases [21]. Additionally, its overexpression promotes proliferation, metastasis, and EMT of NSCLC [22]. Consistently, our study demonstrated that oe-CCR1 promoted the migration, invasion, and EMT of LC cells and increased metastatic nodule formation in LC mice. CCR1 has been found to promote the invasion of prostate cancer cells and enhance the homing capability of glioma cells by activating the ERK pathway [23,24]. Herein, we provide the first evidence that oe-CCR1 not only activates the ERK pathway in LC cells but also increases c-FOS expression.

Furthermore, we conducted rescue experiments to assess whether CCR1 promotes LC metastasis through the ERK pathway activation and found that U0126 reversed the effects of oe-CCR1. Previous research has revealed the role of the RAS/RAF/MEK/ERK signaling cascade in cancer treatment (oncogenesis), with tumor cell growth highly dependent on ERK signaling [25]. Activation of the ERK pathway has also been found to promote the progression and metastasis of LC [26,27]. Moreover, the role of the ERK/c-FOS pathway in cancer development has been recorded. For example, Chang *et al*. [28] reported that activation of the Ras/ERK/c-FOS pathway promotes the progression of colorectal cancer, while Jiang *et al*. [29] proved that inhibition of the ERK/c-FOS signaling pathway alleviates bone cancer pain. Notably, in LC, activation of the ERK/c-FOS signaling pathway has been reported to enhance tumor progression by promoting paracrine of mesenchymal stem cells [30]. In our study, oe-c-FOS reversed the regulation of U0126 on oe-CCR1-induced LC cell metastasis. C-FOS, a member of the FOS family, forms AP-1 transcription factor through dimerization with Jun proteins [29]. C-FOS is frequently overexpressed in tumor cells and promotes tumor occurrence and metastasis by inducing EMT [31]. In NSCLC, inhibition of c-FOS has been reported to reverse EMT [32], highlighting its role in the metastasis mechanism [33]. Collectively, our evidence supports that CCR1 promotes LC metastasis by activating the ERK/c-FOS signaling pathway.

In this study, we observed that CCR1 inhibits RhoGDIβ expression by activating the ERK/c-FOS signaling pathway. RhoGDIβ is generally recognized as a metastasis suppressor and primarily functions by inhibiting the activation of Rho GTPase in the cytoplasm. Cell migration relies on the extension of lamellipodia and filopodia, whereas invasion needs degradation of the extracellular matrix, a process involving the formation of invadopodia and podosomes. Both mechanisms rely on Rho GTPase to form dynamic assembly and disassembly of the actin filament structure at different cellular positions [34]. Therefore, RhoGDIβ is closely linked to the malignant potential and metastatic behavior of LC [35]. Although the direct relationship between RhoGDIβ and c-FOS has not yet been reported, activation of the ERK pathway has been proven to reduce RhoGDIβ expression [15]. In contrast, inhibition of ERK phosphorylation has been observed in gastric cancer cells after RhoGDIβ knockdown, indicating that RhoGDIβ may also reverse the activation of ERK [36], although this reciprocal regulation was not directly verified in this study. Collectively, U0126 reversed the effect of oe-CCR1, whereas oe-c-FOS offset the impact of U0126 and oe-CCR1 on RhoGDIβ expression. Based on these observations, we speculate that CCR1 downregulates RhoGDIβ expression by activating the ERK/c-FOS pathway, a mechanism that requires further elucidation.

However, despite certain valuable findings, this study has several limitations. First, we did not evaluate the effects of U0126 on overall survival in mice injected with oe-CCR1-expressing lung cancer cells. Second, the results are derived from a specific lung adenocarcinoma cell line, and the generalizability of the findings to other cancer types and models should be interpreted with caution. Third, the proposed mechanism by which c-FOS mediates the transcriptional inhibition of RhoGDIβ is primarily inferred from the strong correlation between genetic intervention and phenotypic changes, without direct evidence of c-FOS binding to the RhoGDIβ promoter. Moreover, we did not directly confirm overexpression at the protein level (e.g., by Western blot analysis) for the constructs used.

Future studies should address these limitations by incorporating protein-level validation of transfection efficacy, conducting knockdown and promoter-binding assays to elucidate the mechanistic link between c-FOS and RhoGDIβ, and extending *in vivo* inhibitor testing across diverse lung cancer models. Furthermore, we plan to validate our results in patient-derived cohorts to better assess the clinical applicability of these findings.

Conclusion

In summary, our *in vitro* and *in vivo* observations prove that CCR1 promotes LC metastasis through downregulation of RhoGDIβ and activation of the ERK/c-FOS singling pathway. This study will provide new therapeutic targets and strategies for LC metastasis. However, the cell models used in this study were derived from human NSCLC, which may limit the generalizability of our findings. Therefore, additional investigations using diverse LC subtypes and real-world clinical samples are warranted to validate these findings.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

JY and JJR designed the research study; DFZ and JDL performed the research; SSH and HJL collected and analyzed the data. JY has been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors give final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

According to the China Council on Animal Care and Use rules, all experiments in this study was approved by the Institutional Animal Care and Use Committee, Zhejiang Center of Laboratory Animals (ZJCA) and performed in accordance with the China Council on Animal Care and Use guidelines (No. ZJCLA-ACUC-20010443).

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202537203.240.

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