Original Article
Long noncoding RNA FER1L4 suppresses proliferation, invasion, migration and lymphatic metastasis of gastric cancer cells through inhibiting the Hippo-YAP signaling pathway

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Abstract: Gastric cancer (GC) is one of the most malignant tumors in the world. Growing evidence has highlighted the crucial role of long noncoding RNAs (lncRNAs) in the tumorigenesis of GC. The aim of the research was to elucidate the effects of lncRNA Fer-1-like family member 4 (FER1L4) in GC and identify the potential mechanisms. The present study investigated FER1L4 controlling cell survival and migration of SGC-7901 cells. Results indicated that the expression level of FER1L4 was distinctly decreased in GC cells, as evidenced by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. By using cell proliferation assay, Transwell assay, wound healing assay and western blotting, we found out that overexpression of FER1L4 in SGC-7901 cells hindered the capacities of cell proliferation, invasion, migration and lymphatic metastasis. Furthermore, results of the western blotting and immunofluorescence assay unveiled that overexpression of FER1L4 led to a notable reduction in the expression of C-X-C chemokine receptor type 4 (CXCR4) and C-X-C motif chemokine 12 (CXCL12) in SGC-7901 cells. Besides, activation of Hippo pathway by upregulating Yes-associated protein (YAP) expression or treatment of CXCR4 inhibitor WZ811 reversed the inhibitory effects of FER1L4 on proliferation and metastasis of SGC-7901 cells. Moreover, co-transfection with YAP and FER1L4 overexpression plasmids abrogated the repressive effects of FER1L4 overexpression on proliferation and metastasis. Taken together, these results demonstrated that lncRNA FER1L4 suppressed cell proliferation, invasion, migration and lymphatic metastasis of GC cells by inactivation of the Hippo-YAP pathway, providing novel insights into regulatory mechanism under GC and new strategies for clinical practice.

Keywords: Gastric cancer, proliferation, invasion, migration, lymphatic metastasis, FER1L4

Introduction

Gastric cancer (GC) is a frequent malignancy causing deaths, with the second highest incidence and mortality rate among all carcinomas. Owing to the lack of identification of typical early symptoms for GC patients, the prognosis of GC is always poor [1]. Recently, the incidence of GC is gradually increasing in young people [2]. Patients with GC exhibit high incidence, metastasis and mortality rates, but low rate of early diagnosis, radical resection rate, and 5-year survival rate [3]. Although many researches have studied the potential mechanisms of GC, finding a novel treatment target is still a challenge [4-6]. Significant advances

have been made in explaining the molecular mechanisms involved in GC metastasis, however, the overall view of the mechanism map is limited and ambiguous [7, 8]. Therefore, an urgent demand currently exists for excavating possible diagnostic and therapeutic targets to improve the survival rates of GC patients.

The Hippo signaling pathway is dysregulated in a variety of human cancers and plays vital roles in tumorigenesis and development [9]. And it is essential to the malignant transformation process, standing out as a novel pathway that warrants further investigation [10]. The Hippo pathway has been acknowledged as the central part in regulating cell proliferation, cell fate and tis-

sue size [11], further stimulating the speculation that many members of this pathway are involved in GC carcinogenesis [12]. In mammalian systems, phosphorylated yes-associated protein (YAP) is displaced in the cytoplasm to encourage degradation, when Hippo signaling is activated. Inactivation of Hippo pathway pushes unphosphorylated YAP into the nucleus and therefore induces the transcriptional activity of genes associated with cell growth [13]. Though the Hippo pathway develops a considerable role in various cancers, but only few studies have reported the association of lncRNAs with the Hippo pathway. Hence, clarifying the relationship between lncRNAs and the regulation of the Hippo pathway appears to be crucial in human cancers, especially in GC.

Long noncoding RNAs (lncRNAs) are RNAs whose transcripts are longer than 200 nt in length but possess no ability to encode proteins [14]. An increasing number of studies prove that lncRNAs partake in multiple cellular processes, such as signal transduction regulation, genomic imprinting, transcription activation and so on [12, 15]. Although most functions of lncRNAs remain unclear, the ability of lncRNAs in cancer development has attracted researcher's attention. For example, lncTCF7 affected the invasion and metastasis of hepatocellular carcinoma through EMT [16], and ZNFX1 antisense RNA 1 exhibited an oncogenic role in glioma progression by regulating epithelial-mesenchymal transition and the Notch signaling pathway [17]. Among these lncRNAs, lncRNA Fer-1-like family member 4 (FER1L4) was found to regulate paclitaxel resistance in ovarian cells [18]. Emerging evidence supports the notion that FER1L4 could inhibit the proliferation, invasion and migration of hepatocellular carcinoma cells by regulating PI3K/AKT signaling pathway [19]. These findings imply that FER1L4 may represent important therapeutic potentials in tumors.

Since the previous data have shown that the expression of FER1L4 was downregulated in patients with GC [20], the main goal of this work was to address FER1L4 effects on GC. In this study, we investigated the role of FER1L4 in GC and identified the possible underlying mechanisms. These results might provide a novel insight of GC treatment in the future.

Materials and methods

Cell culture and transfection

GES-1, AGS, MKN-45, MGC-803, SGC-7901 and FU97 cells were cultivated in RPMI1640 media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37°C in a humidified incubator with 5% CO_2. For the transfection, SGC-7901 cells (2×10^5) were seeded in 6-well plates. When reaching to 90% confluent, cells were transfected with FER1L4 overexpression plasmid (pcDNA-FER-1L4), pcDNA-C-X-C chemokine receptor type 4 (CXCR4), pcDNA-YAP or the empty negative control (NC) plasmid (pcDNA-NC), respectively, using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Above plasmids were all the products of Shanghai GenePharma co., ltd (Shanghai, China). At 48 h after transfection, cells were collected and the transfection efficiency was evaluated using RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis or western blotting.

Cell counting kit-8 (CCK-8) assay

Cellular proliferation was detected using the CCK8 kit (Beyotime, Beijing). Briefly, SGC-7901 cells (2000 cells/well) were seeded into 96-well culture dishes and cultured at 37°C in a humidified incubator with 5% CO_2. After incubation for 24, 48, 72 h, 10 μL CCK-8 solution was supplemented into each well. Absorbance was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA).

Cell cycle analysis

Cells were washed twice with ice-cold PBS before isolation, resuspended in PBS and fixed by adding 70% ethanol, and left at -20°C for 48 h. Following incubation with 1 mg/ml RNase A for 30 min at 37°C, the cells were stained utilizing PI (50 μg/ml; Becton-Dickinson, San Jose, CA) in PBS with 0.5% Tween-20, and analyzed using a Becton-Dickinson BD Fascine flow cytometry (Becton Dickinson, Franklin Lakes, NJ).

Transwell assay

The Transwell assay was performed using Transwell (pore size, 8.0 μm; Corning Inc.) coated

with Matrigel (BD Biosciences). 500 µl serum-free media containing 2×10^5 cells were placed into the upper chamber, and the lower chamber was filled with media containing 10% FBS. 500 µl serum-free media containing 2×10^5 cells were seeded into the upper chamber, and the lower chamber was filled with complete cell culture medium containing 10% FBS. After 48 h of incubation, cells were fixed and stained with crystal violet for 10 min. Representative images were photographed under a microscope (Olympus Corporation) and the relative number of invasive cells was counted using ImageJ software.

Wound healing assay

The ability of cell migration was assessed by wound healing assay. Briefly, cells were seeded in a six-well plate under culture at 37°C until 80% confluence. Confluent cells were scraped with a pipette tip across a 24-well plate. Following wounding, culture medium was replaced with fresh medium at the indicated time. It should be noted that the wound healing assay requires confluent cells to provide the necessary cell layer for creating wounding by scraping. The average distance of cells migrated into wound surface was detected after 48 h of wounding under an inverted microscope (Olympus Corporation).

Immunofluorescence assay

For immunostaining, cells were grown on glass cover slips after transfection. SGC-7901 cells were immobilized with 4% paraformaldehyde for 15 min. After being permeabilized with 0.1% Triton X-100, cells were incubated with 5% normal goat serum for 1 h at room temperature. Afterward, SGC-7901 cells were exposed to primary antibody at 4°C overnight. Following rinse for three times with PBS, cells were incubated with DyLight™ 488-conjugated secondary antibody (Thermo Scientific) for 1.5 h. Finally, after being stained with 4', 6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, MO, USA) for 5 min, the samples were imaged using a fluorescence microscope (Olympus Corporation).

RT-qPCR

To determine transcript levels, total RNAs were obtained by TRIzol reagent (Invitrogen). RNAs were reverse-transcribed into complementary DNA using the PrimeScript® RT reagent kit (Takara Bio, Inc.). qPCR was performed on an ABI PRISM 7500 Sequence Detector System (Applied Biosystems) using gene-specific primers for FER1L4, CXCR4 and C-X-C motif chemokine 12 (CXCL12) and SYBR Green Master Mix (Applied Biosystems). GAPDH was employed as internal control. Relative mRNA expression was determined using the $2^{-\Delta\Delta Cq}$ method [21].

Western blotting

Cells were washed twice with ice-cold PBS and suspended in ice-cold lysis buffer (50 mM Tris/HCl, 1% Triton X-100 pH 7.4, 1% sodium deoxycholate, 0.1% SDS, 0.15% NaCl, 1 mM EDTA, 1 mM sodium orthovanadate) containing a protease inhibitor cocktail (Sigma-Aldrich, MO, USA). The protein concentration was determined using a BCA Protein Assay Kit (Beyotime, Shanghai). Protein were solubilized in sample buffer at 95°C for 5 min and resolved by 10% SDS-PAGE. Immunoblotting proteins were electro-transferred onto a PVDF membrane, together with block with 5% non-fat milk in TBS-0.10% Tween 20 at room temperature for another 1 h. Then, the membrane was incubated with primary antibodies at 4°C overnight. Afterward, these blots were incubated with secondary antibody (Cell Signaling Technology, Boston, MA, USA) for 1 h at room temperature. The blots were visualized using the Odyssey Infrared Imaging System (LI-COR Biosciences). Anti-Cyclin-dependent kinase 2 (CDK2), anti-cyclin D1, anti-p21, anti-Matrix metalloproteinase 2 (MMP2), anti-MMP9, anti-Vascular endothelial growth factor C (VEGFC), anti-Podoplanin, anti-vascular endothelial growth factor receptor (VEGFR)-3, anti-CXCL12, anti-phospho-YAP (p-YAP), anti-YAP, anti-connective tissue growth factor (CTGF) and anti-GAPDH antibodies were obtained from Cell Signaling Technology (Boston, MA, USA). Anti-CXCR4 and anti-baculoviral IAP repeat containing 5 (BIRC5) antibodies were purchased from Santa Cruz Biotechnology (CA, USA). GAPDH was used as the loading control.

Statistical analysis

Data are provided as means ± standard deviation (SD). Data was tested for significance using unpaired student's t-test or ANOVA as appropriate. Differences were considered statistically significant when *p*-values were less than 0.05.

Statistical analysis was performed using GraphPad Prism 6 (GraphPad, San Diego, CA, USA).

Results

The expression of FER1L4 in GC cell lines was notably downregulated

To study the role of FER1L4 in GC, we detected its expression in several human GC cell lines and a normal gastric epithelial cell line by using RT-qPCR. Results showed that FER1L4 level was remarkably decreased in GC cell lines relative to the normal epithelial cells (**Figure 1A**), indicating that downregulation of FER1L4 is likely to be involved in the pathogenesis of GC. Among the six cell lines, SGC-7901 cells exhibited the lowest expression level of FER1L4. Therefore, SGC-7901 cell line was selected to perform the following experiments.

FER1L4 overexpression inhibited proliferation of GC cells

To explore the biological roles of FER1L4 in GC, we constructed a FER1L4 overexpression plasmid, pcDNA-FER1L4. RT-qPCR assay revealed that the expression level of FER1L4 was dramatically increased in pcDNA-FER1L4 group compared with the pcDNA-NC group (**Figure 1B**), affirming the successful overexpression of FER1L4 in SGC-7901 cells. To investigate whether FER1L4 affected proliferation of SGC-7901 cells, CCK-8 assay was used. As exhibited in **Figure 1C**, SGC-7901 cells transfected with pcDNA-FER1L4 showed the weakest cell proliferation. Additionally, flow cytometry analysis was carried out to determine the possible alterations in cell cycle after FER1L4 overexpression. Results of **Figure 1D** and **1E** revealed that, compared with the pcDNA-NC group, the percentages of SGC-7901 cells in the G0/G1 phase were significantly enhanced after transfection with pcDNA-FER1L4, whereas that in the S phase were dramatically reduced, which suggested that FER1L4 overexpression suppressed cell cycle progression from G1 into S phase.

As cyclin D1 and CDK2 are the major cyclin members controlling the G1/S checkpoints, and p21 is recognized as a potent cyclin-dependent kinase inhibitor [22], we tested their expression levels in SGC-7901 cells treated with pcDNA-FER1L4. Western blot analysis discovered that FER1L4 overexpression led to obvious decrease in the expression levels of cyclin D1 and CDK2 and a remarkable increase in that of p21 (**Figure 1F**). These findings hinted that FER1L4 overexpression could effectively hamper the proliferation ability of SGC-7901 cells.

FER1L4 overexpression suppressed invasion, migration and lymphatic metastasis of GC cells

Invasion and migration are two key phases involved in GC metastasis. A Transwell assay and a scratch wound healing assay were conducted to explore the effects of FER1L4 overexpression on the invasion and migration of GC cells. As displayed in **Figure 2A** and **2B**, the invasive capability of SGC-7901 cells under transfection with pcDNA-FER1L4 was dramatically attenuated relative to the negative control group. Consistently, migration of SGC-7901 cells (**Figure 2C** and **2D**) presented the same tendency with invasion, accompanied by significant downregulation of MMP2 and MMP9 expression (**Figure 2E**), two key proteins implicated in cancer metastasis [23, 24]. Lymphatic metastasis is another reason of cancer death. Malignant tumors release lymphangiogenic growth factors such as VEGF-C and their specific receptor like VEGFR-3 to induce lymphangiogenesis in primary tumors and in draining sentinel lymph nodes, thus promoting lymph node metastasis [25]. Podoplanin, a small mucin-like type-1 transmembrane protein, was reported to promote lymphangiogenesis and lymphoinvasion in a variety of cancers [26]. Herein, the expression of lymphatic metastasis-associated proteins was examined using western blotting. Results of **Figure 2F** indicated that FER1L4 overexpression dramatically reduced the expression of VEGFC, VEGFR-3 and Podoplanin in SGC-7901 cells. Through the above findings we proved that FER1L4 promotion suppressed invasion, migration and lymphatic metastasis of GC cells.

FER1L4 overexpression inhibited the Hippo-YAP pathway through CXCR4/CXCL12 axis in GC cells

Recently, increasing studies have demonstrated that CXCR4/CXCL12 is involved in cell invasion and lymphatic metastasis of GC [25, 27, 28]. Whether FER1L4 developed its function by CXCR4/CXCL12 axis in SGC-7901 cells was not
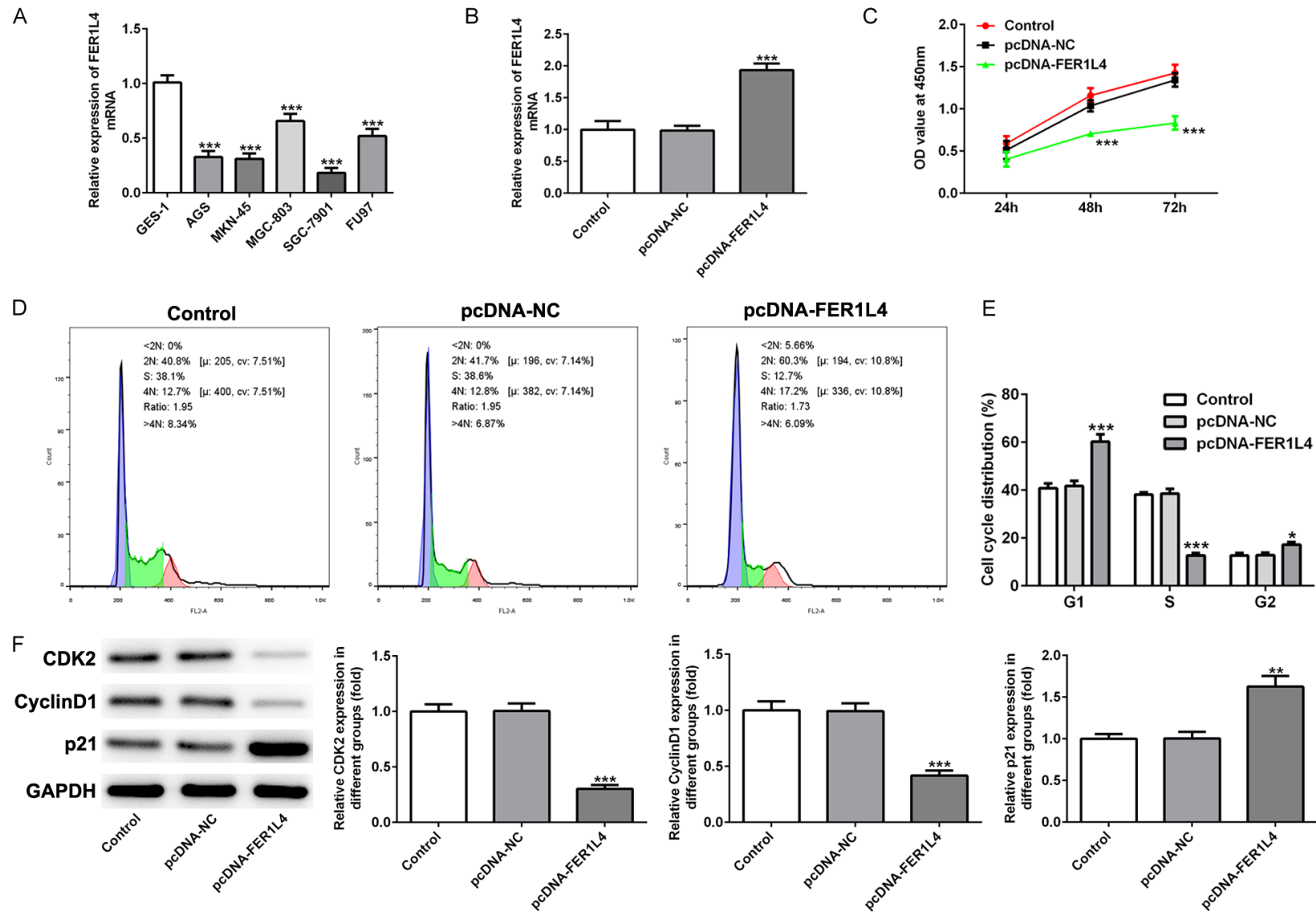
Figure 1. Expression level of FER1L4 was downregulated in GC cells and FER1L4 overexpression inhibited proliferation of SGC-7901 cells. A. RT-qPCR analysis was performed to examine the level of FER1L4 in different GC cell lines and normal gastric epithelial GES-1 cells. ***P<0.001 vs. GES-1. B. The expression of FER1L4 was tested using RT-qPCR after transfection with FER1L4 overexpression plasmid. C. CCK8 assay was utilized to measure proliferation property of SGC-7901 cells following transfection of FER1L4 overexpression plasmid. D. Cell Cycle was determined using flow cytometry assay after transfection. E. The cell cycle distribution was quantified. F. Western blot analysis depicted the protein expression levels of CDK2, cyclin D1 and p21 in SGC-7901 cells transfected with the FER1L4 overex-

pression plasmid. *P<0.05, **P<0.01, ***P<0.001 vs. pcDNA-NC. FER1L4, Fer-1-like family member 4; GC, gastric cancer; CCK8, Cell counting kit-8; CDK2, Cyclin-dependent kinase 2.
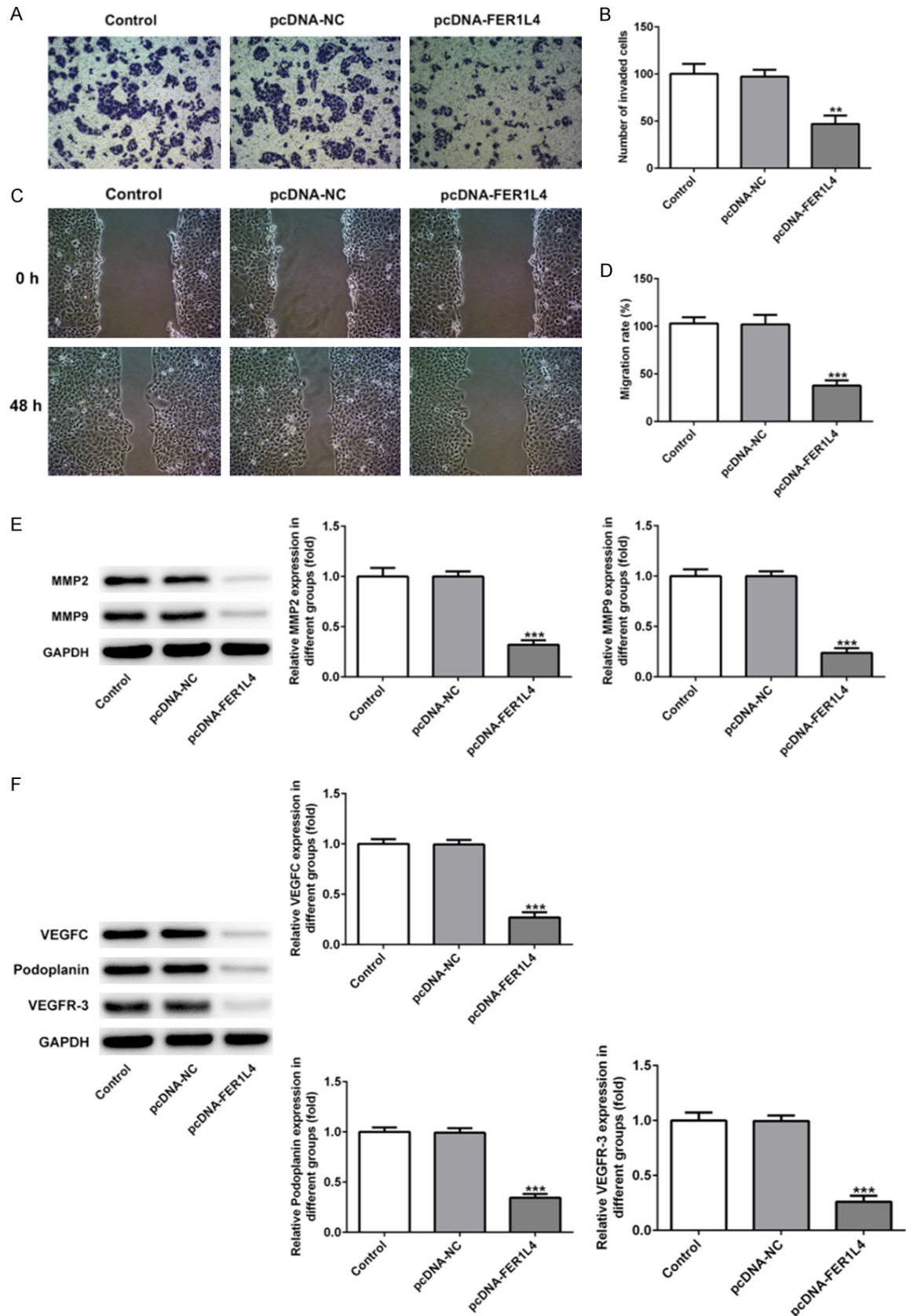
Figure 2. Overexpression of FER1L4 suppressed invasion, migration and lymphatic metastasis of SGC-7901 cells. (A) Representative images and (B) relative quantification of cell invasion, as measured using Transwell assay. (C) Representative images of the wound healing assay. (D) Quantification of wound healing assay data. Western blot analysis was employed to examine the expression of (E) migration-related proteins and (F) lymphatic metastasis-related proteins in SGC-7901. $^{**}P<0.01$, $^{***}P<0.001$ vs. pcDNA-NC. FER1L4, Fer-1-like family member 4; GC, gastric cancer; MMP, matrix metalloproteinase; VEGFC, Vascular endothelial growth factor C; VEGFR-3, vascular endothelial growth factor receptor (VEGFR)-3.
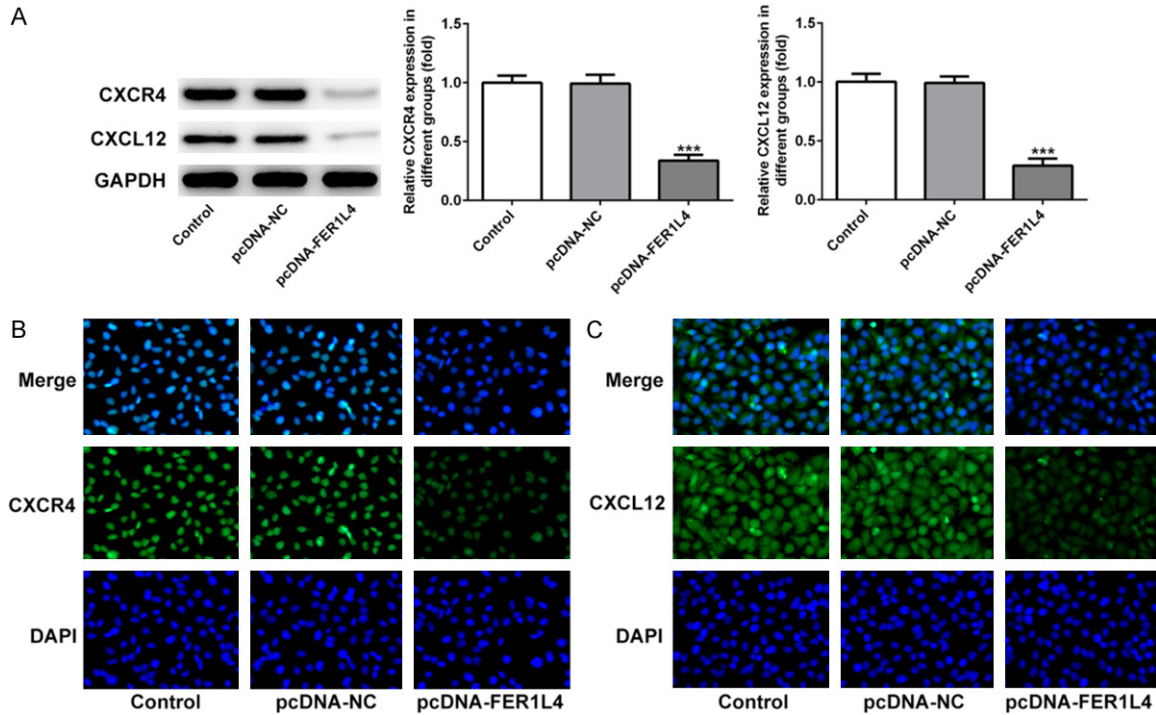

Figure 3. Overexpression of FER1L4 reduced the expression of CXCR4 and CXCL12 in SGC-7901 cells. (A) Western blot analysis and (B and C) immunofluorescence assay were conducted to evaluate the levels of CXCR4 and CXCL12 in SGC-7901 cells. Magnification, × 200. $^{***}P<0.001$ vs. pcDNA-NC. FER1L4, Fer-1-like family member 4; GC, gastric cancer; CXCR4, C-X-C chemokine receptor type 4; CXCL12, C-X-C motif chemokine 12.

known, which was investigated in this study. As shown in **Figure 3A**, there was a significant downregulation in expression of CXCR4 and CXCL12 in SGC7901 cells after pcDNA-FER1L4 transfection. Consistently, results of immuno-fluorescence assay revealed that CXCR4 and CXCL12 were clearly diminished in FER1L4-overexpressed SGC-7901 cells compared with negative control cells (**Figure 3B** and **3C**). Compelling evidence indicates that inhibition of CXCR4 could decrease invasion and migration of non-small cell lung cancer cells through the Hippo signaling pathway [29]. We further addressed whether FER1L4 influenced Hippo signaling pathway by inhibition of CXCR4/CXCL12 axis. An overexpression plasmid of CXCR4 was constructed and transfected into SGC7901 cells. A significant increase in CXCR4

expression was observed following transfection (**Figure 4A** and **4B**). As CXCR4 is an alpha-chemokine receptor specific for CXCL12 [30], CXCL12 expression was also clearly higher in CXCR4 overexpression cells than the empty vector-transfected cells (**Figure 4C** and **4D**). Additionally, in FER1L4-overexpressed SGC-7901 cells, the expression of phosphorylation of YAP, as well as Hippo signaling downstream genes CTGF and BIRC5 were significantly decreased, the results of which were similar to treatment with WZ811, a CXCR4 inhibitor (**Figure 4E**). Interestingly, CXCR4 addition could rescue the inhibition of FER1L4 overexpression on Hippo-YAP pathway (**Figure 4E**). To sum up, these results implicated that FER41L overexpression inactivated Hippo pathway through CXCR4/CXCL12 axis in GC cells.
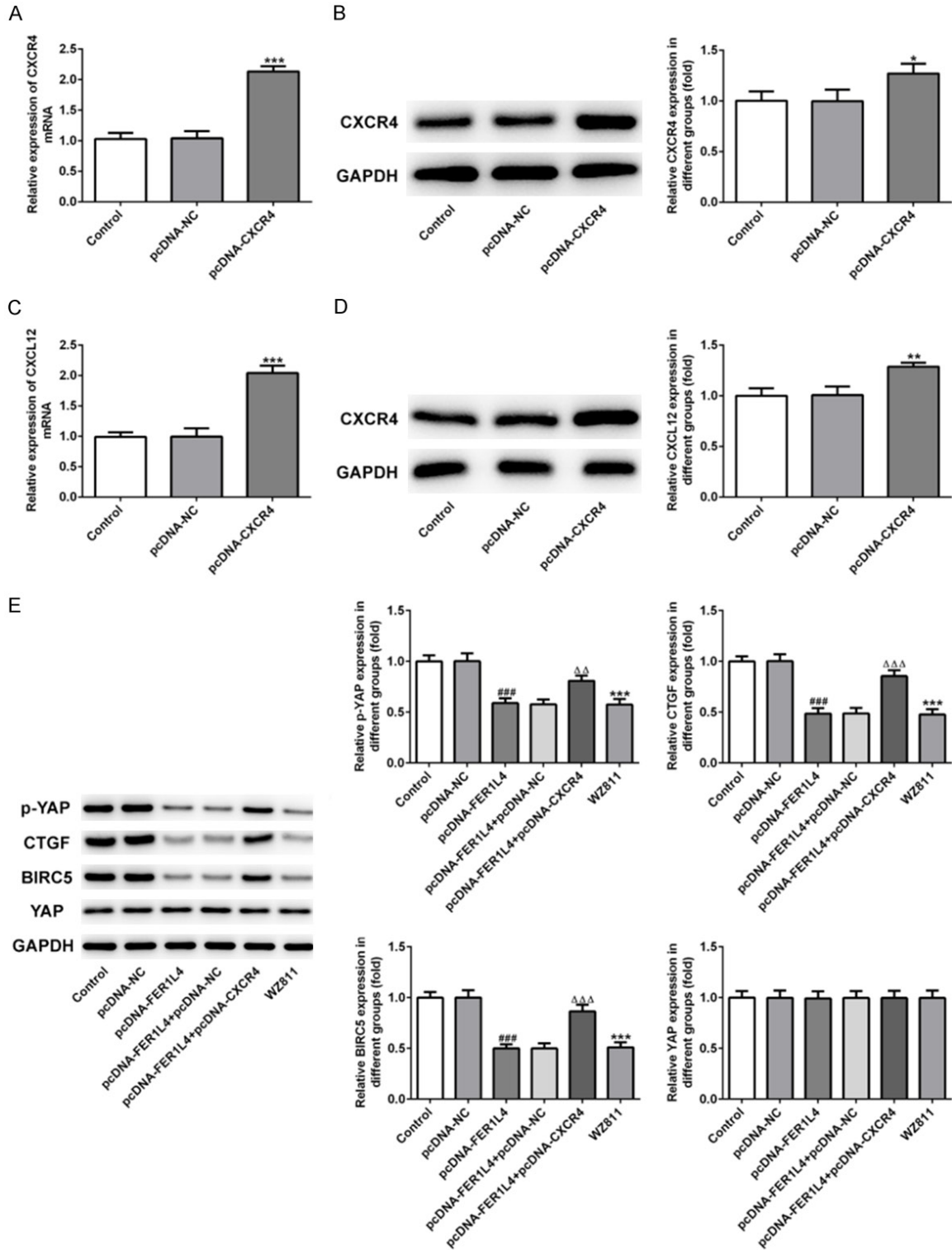
Figure 4. FER1L4 overexpression suppressed the Hippo pathway via CXCR4/CXCL12 axis in SGC-7901 cells. The expression of CXCR4 was tested by (A) RT-qPCR and (B) western blot analysis after transfection with CXCR4 overexpression plasmid. CXCL12 expression was analyzed by (C) RT-qPCR and (D) western blotting after CXCR4 overexpression. *P<0.05, **P<0.01, ***P<0.001 vs. pcDNA-NC. (E) Western blot analysis depicted the protein expression levels of p-YAP, CTGF, BIRC5 and YAP in SGC-7901 cells transfected with the FER1L4 overexpression plasmid, treated with CXCR4 inhibitor WZ811 or co-transfected with FER1L4 and CXCR4 overexpression plasmids. ###P<0.001 vs. pcDNA-NC; △△P<0.01, △△△P<0.001 vs. pcDNA-FER1L4+pcDNA-NC; ***P<0.001 vs. control. FER1L4, Fer-1-like family

member 4; GC, gastric cancer; CXCR4, C-X-C chemokine receptor type 4; CXCL12, C-X-C motif chemokine 12; YAP, Yes-associated protein; p-YAP, phospho-YAP; CTGF, connective tissue growth factor; BIRC5, baculoviral IAP repeat containing 5.

FER1L4 overexpression restrained proliferation of GC cells through inhibiting Hippo-YAP pathway

To elucidate whether FER1L4 regulated proliferation of SGC7901 cells via Hippo-YAP pathway, YAP was overexpressed by transfection with YAP overexpression plasmid. A remarkable upregulation of YAP expression was observed after transfection in SGC-7901 cells (**Figure 5A**). As presented in **Figure 5B**, co-transfection with pcDNA-FER1L4 and pcDNA-YAP enhanced the cell proliferation relative to cells with transfection of pcDNA-FER1L4 alone. And YAP overexpression partially rescued the repression of FER1L4 overexpression on cell cycle, with promotion on G1/S transition (**Figure 5C** and **5D**). Moreover, co-overexpression of FER1L4 and YAP reversed the decreased levels of cyclin D1 and CDK2 as well as the augmented level of p21 caused by FER1L4 overexpression (**Figure 5E**). Collectively, these findings provided a clue that FER1L4 suppressed proliferation of GC cells by downregulating the Hippo-YAP signaling pathway.

FER1L4 overexpression hampered invasion, migration and lymphatic metastasis of GC cells through inhibiting Hippo-YAP pathway

We wonder whether the effects of FER1L4 on cell metastasis were upon regulation of the Hippo-YAP pathway. Results of both wound healing assay and Transwell assay identified that co-transfection with pcDNA-YAP attenuated the inhibitory effects of FER1L4 overexpression on the invasive and migrated abilities of SGC7901 cells (**Figure 6A-D**). Moreover, the protein levels of MMP2 and MMP9 were restored after upregulation of FER1L4 and YAP in contrast to single treatment of FER1L4 elevation (**Figure 6E**). Furthermore, as exhibited in **Figure 6F**, YAP overexpression alleviated the suppression of pcDNA-FER1L4 on the expression levels of VEGFC, VEGFR-3 and Podoplanin. These results proved that FER1L4 suppressed invasion, migration and lymphatic metastasis in GC cells through repressing Hippo-YAP pathway.

Discussion

GC is the most frequently happened cancer worldwide [31]. Plenty of GC patients are in advanced stage at the first time of diagnosis, presenting rapid progression and poor prognosis. Therefore, identification of the novel biomarkers for the early diagnosis with the best response to therapy is urgently needed. Due to the characteristics of lncRNAs containing high specificity, easy accessibility in a noninvasive manner, as well as their aberrant expression under different pathological and physiological conditions, lncRNAs receive a great attention, using as potential diagnostic and predictive biomarkers recently. In this study, we proved that FER1L4 was a novel factor of GC. Overexpression of FER1L4 markedly blocked proliferation, invasion, migration and lymphatic metastasis in GC cells. These results supported that FER1L4 could serve as a novel target for treatment of GC.

LncRNA FER1L4 has been identified to play an important role in many cancers. For instance, in colon cancer, FER1L4 inhibits oncogenesis and acts as a prognostic marker [16]. Also, FER1L4 suppresses proliferation and migration of hepatocellular carcinoma and lung cancer via regulating the PI3K/AKT signal pathway [19, 32]. Moreover, FER1L4 can suppress proliferation of endometrial carcinoma cells [33]. These studies reported the function diversity of FER1L4 in various cancer entities, but the mechanism of FER1L4 in solid tumors remains unclarified. In 2013, FER1L4 was firstly identified to be downregulated in lung tissues of patients with GC [20]. After that, FER1L4 was demonstrated to suppress tumorigenesis by acting as a ceRNA and downregulating PTEN in GC [34]. However, the function role of FER1L4 was still not fully understand in GC. In the present study, we revealed that the function of FER1L4 was closely associated with more GC physiological parameters, including proliferation, invasion, migration and lymph node metastasis and distant metastasis. These data verified that overexpression of FER1L4 exerted suppressive effects in proliferation, invasion, migration and lymphatic metastasis in GC cells.
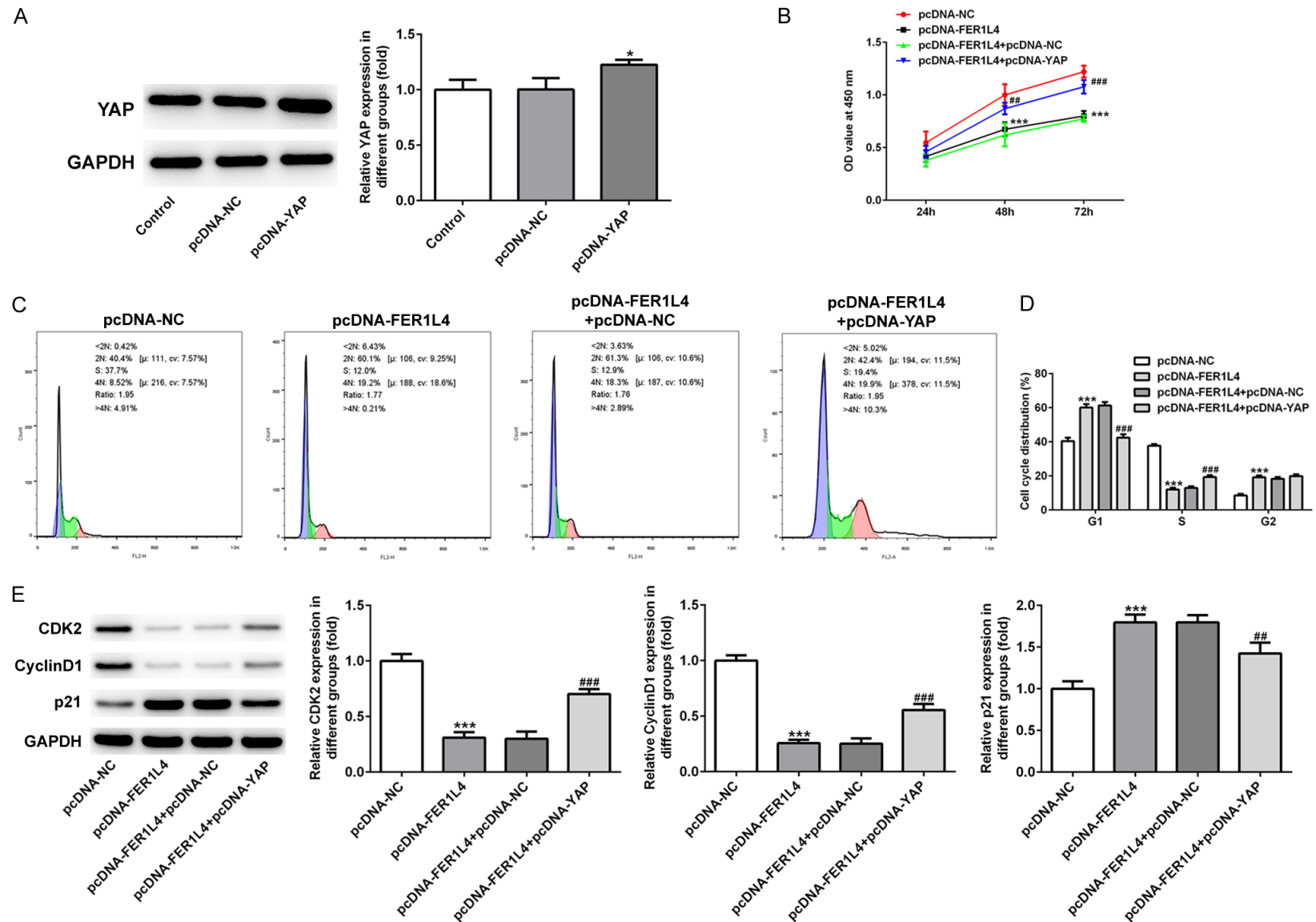
Figure 5. YAP overexpression attenuated the suppression of FER1L4 overexpression on proliferation of SGC-7901 cells. A. The transfection efficiency of YAP over-expression plasmid was tested using western blot analysis. *P<0.05 vs. pcDNA-NC. B. CCK-8 assay was performed to estimate proliferation of SGC-7901 cells. C. Cell Cycle was assessed using flow cytometry assay. D. The cell cycle distribution was quantified. E. Western blot analysis depicted the protein expression levels of CDK, cyclin D1 and p21 in SGC-7901 cells. ***P<0.001 vs. pcDNA-NC; ##P<0.01, ###P<0.001 vs. pcDNA-FER1L4+pcDNA-NC. FER1L4, Fer-1-like family member 4; GC, gastric cancer; YAP, Yes-associated protein; CDK2, Cyclin-dependent kinase 2.
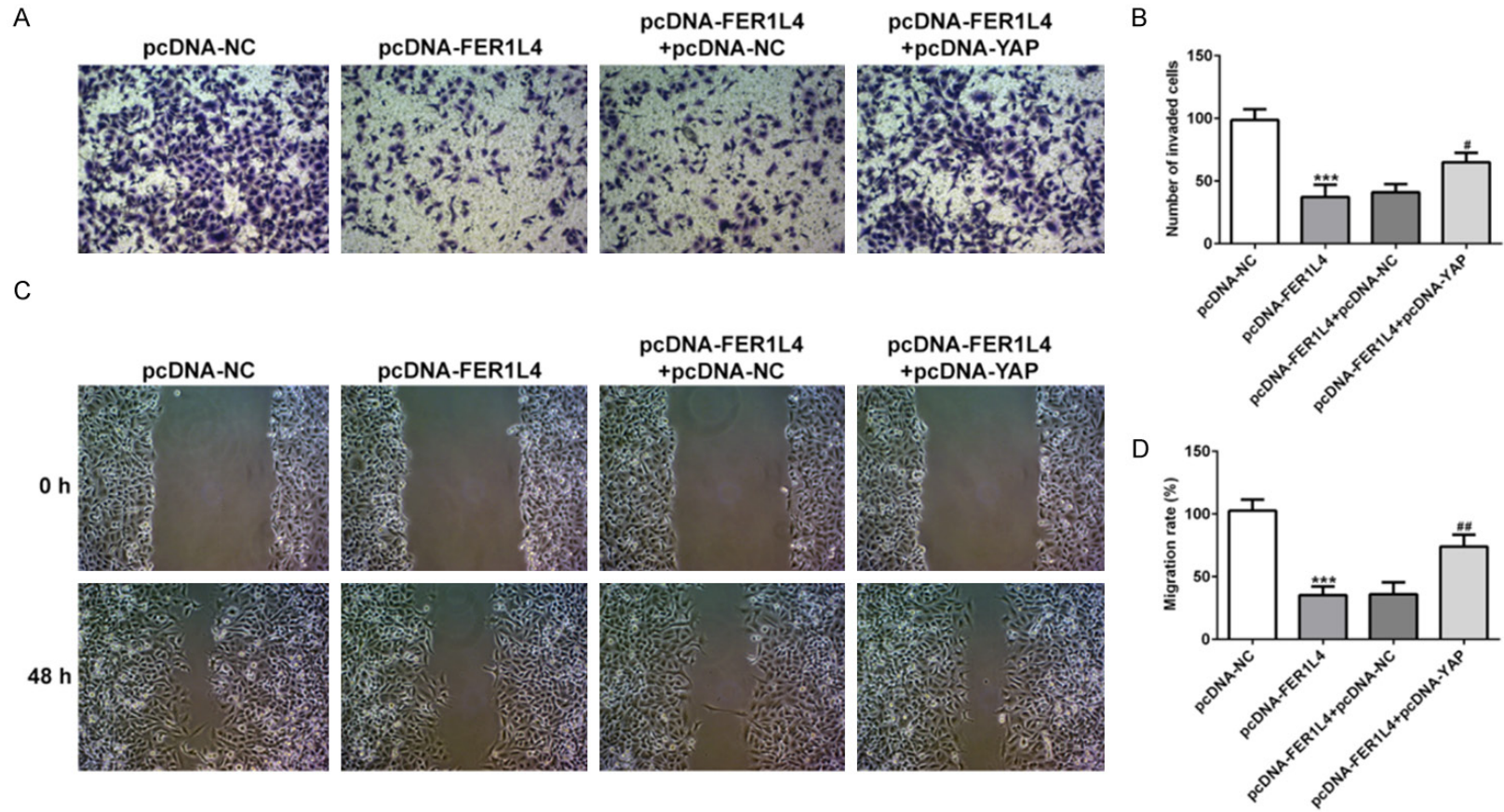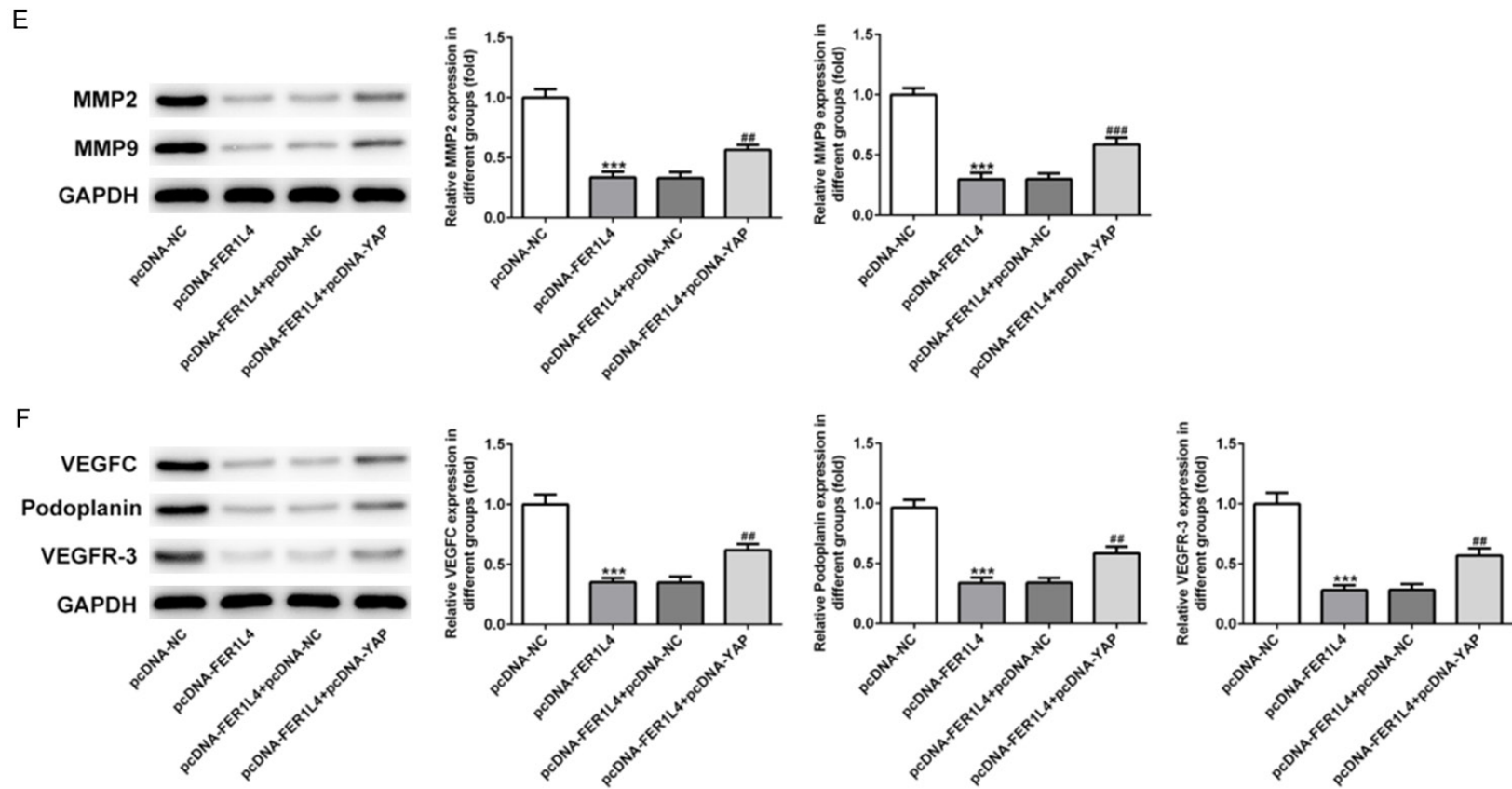
Figure 6. YAP overexpression reversed the inhibitory effects of FER1L4 overexpression on invasion, migration and lymphatic metastasis of SGC-7901 cells. (A) Representative images of the Transwell assay. (B) Quantification of Transwell assay data. (C) Representative images and (D) relative quantification of cell migration, as measured using wound healing assay. Western blot analysis was employed to evaluate the expression of (E) migration-related proteins and (F) lymphatic metastasis-related proteins in SGC-7901 cells. ***P<0.001 vs. pcDNA-NC; #P<0.05, ##P<0.01, ###P<0.001 vs. pcDNA-FER1L4+pcDNA-NC. FER1L4, Fer-1-like family member 4; GC, gastric cancer; MMP, matrix metalloproteinase; VEGFC, Vascular endothelial growth factor C; VEGFR-3, vascular endothelial growth factor receptor (VEGFR)-3.

It's widely accepted that Hippo pathway is closely related to the tumorigenesis and development in various human cancers, such as hepatocellular carcinoma, breast cancer, pancreatic cancer and GC [35-38]. The major target gene of the Hippo pathway is the mammalian transcriptional activator YAP [39]. Activation of Hippo pathway leads to YAP phosphorylation and its translocation into the nucleus, and induces the expression of a wide range of genes implicated in cell proliferation, survival and migration [35, 39, 40]. Interfering with Hippo-YAP signaling could regulate cell migration in GC [41]. Many studies have already explained the relationship between Hippo pathway and small RNA in GC [35]. For example, miRNA-93-5p promotes the biological progression of GC cells via regulating Hippo signaling pathway [42]. In addition, miR-664a-3p is proved to modulate the development of GC by targeting Hippo pathway [43]. Herein, our results illustrated that lncRNA FER1L4 could inhibit Hippo-YAP pathway, in accordance with the previous study [44]. Furthermore, CXCR is associated with the Hippo-YAP pathway, thus implicating in the occurrence and progression of the disease [30]. Compelling evidence indicates that CXCR4 participates in the migration of non-small-cell lung cancer cells via the Hippo-YAP pathway [29]. The current study uncovered that FER1L4 overexpression decreased the expression of CXCR4 and CXCL12 in SGC7901 cells. And the inhibitory effects of FER1L4 on Hippo-YAP pathway could be rescued by co-transfection with pcDNA-CXCR4. These data implied that FER1L4 inhibited Hippo-YAP pathway through CXCR4/CXCL12 axis. And the results of the rescue experiments confirmed that FER1L4 overexpression blocked proliferation, invasion, migration and lymphatic metastasis of GC cells through inhibition of Hippo-YAP pathway.

Conclusion

In conclusion, these investigations delineated that FER1L4 overexpression could block proliferation, invasion, migration and lymphatic metastasis of GC cells through repression of the Hippo-YAP pathway, which could provide experimental and scientific evidence for the potential of FER1L4 using in guiding clinical diagnosis and treatment of GC. However, the use of a single GC cell line is a limitation of this study and therefore, a comprehensive analysis of more cell lines is required in the future.

Disclosure of conflict of interest

None.

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