

GFI1 promotes the proliferation and migration of esophageal squamous cell carcinoma cells through the inhibition of SOCS1 expression

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Received October 21, 2020; Accepted July 6, 2021

DOI: 10.3892/ijmm.2021.5017

Abstract. Growth factor-independent 1 (GFI1) has been reported to serve a vital role in hematopoietic development. However, the function and molecular mechanism of GFI1 in esophageal squamous cell carcinoma (ESCC) remains unknown. In the present study, the biological functions and the molecular mechanism of the effects of GFI1 in ESCC were analyzed. The results demonstrated that GFI1 expression levels were significantly upregulated in ESCC compared with those in normal esophageal tissues. Knockdown of GFI1 using small interfering RNA suppressed ESCC cell proliferation and migration. Furthermore, GFI1 enhanced STAT3 and NF- κ B signaling by inhibiting the expression of suppressor of cytokine signaling 1 (SOCS1) in ESCC cells. Taken together, the results of the present study demonstrated that GFI1 promoted the proliferation and migration of ESCC cells via inhibition of SOCS1 expression. These results suggested that GFI1 may be a valuable target for ESCC therapy.

Introduction

Esophageal cancer (ESCA) is the 6th leading cause of cancer-associated mortality and one of the most common gastrointestinal tumors worldwide (1). ESCA includes

esophagus squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) (2,3). EAC is the most common type of ESCA in Western countries, whereas ESCC is the most common type in China, where it accounts for >70% of cases of ESCA (4,5). Although research into treatments for patients with ESCC has achieved significant progress, the 5-year survival rate remains unfavorable due to the high rates of recurrence and metastasis (4,6,7). Therefore, there is an urgent need to elucidate the molecular mechanism underlying ESCC progression.

As a zinc-finger transcriptional repressor, growth factor-independent 1 (GFI1) can bind histone deacetylases and inhibit transcription; its function was initially discovered due to its ability to serve as a cellular proto-oncogene in T cell lymphomas, where it promotes IL-2-independent growth (8). GFI1 has been reported to be involved in several types of cancer, including pancreatic ductal adenocarcinoma, cervical carcinoma and acute myeloid leukemia (AML), and it has also been found that GFI1 can control the transcription of suppressors of cytokine signaling (SOCS)1 (9-12). SOCS inhibit the JAK/STAT and NF- κ B pathways, among others (13,14). Among the SOCS family of proteins, SOCS1 is the most potent inhibitor of proinflammatory cytokine signaling (15). In an ESCC xenograft mouse model, SOCS1 overexpression has been demonstrated to exhibit a potent antitumor effect against ESCC (16). Similarly, in a murine xenograft model, ectopic SOCS1 expression has been reported to improve radiosensitivity by inducing apoptosis and enhancing DNA damage following radiotherapy (17). Thus, SOCS1 serves an antitumor role in ESCC. It has been reported that GFI1 can bind directly to the SOCS1 promoter and suppress SOCS1 transcription in AML cells (12). Therefore, determining whether GFI1 is associated with ESCC through enhancing STAT3 and NF- κ B signaling activity via inhibiting SOCS1 may improve the current understanding of ESCC development.

The present study aimed to determine the expression of GFI1 in patients with ESCC and ESCC cells, as well as the functions of GFI1 in ESCC cells. Furthermore, the molecular mechanism underlying GFI1 activation in ESCC was analyzed.

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Key words: growth factor-independent 1, esophageal squamous cell carcinoma, suppressor of cytokine signaling 1, STAT3 and NF- κ B signaling

Materials and methods

Bioinformatics analysis. The mRNA expression levels of GFI1 from The Cancer Genome Atlas (TCGA; <https://portal.gdc.cancer.gov/>) were analyzed using UALCAN (<http://ualcan.path.uab.edu>) and OncoLnc (www.oncoLnc.org) databases. The databases from UALCAN contains the RNA-seq data (from TCGA), which includes the GFI1 expression levels from 184 tumor and 11 adjacent normal esophageal tissues. The survival curves based on GFI1 mRNA expression from OncoLnc were used to determine the effects of GFI1 expression levels on the survival of patients with ESCC. Kaplan-Meier analysis with the log-rank test was used for the survival analysis.

Clinical tissue samples. A total of 40 pairs of ESCC and adjacent normal esophageal tissues from patients with ESCC, including 22 women and 18 men (mean age, 60 years; aged from 46-75 years old), who received surgical treatment between March and December 2019, were collected at The First Affiliated Hospital of Anhui Medical University (Hefei, China) and were frozen in liquid nitrogen until required for RNA and protein extraction. The tumors were staged using the 8th edition of the American Joint Committee on Cancer Tumor-Node-Metastasis staging system (18). Patients were recruited if they had not received any radiotherapy or chemotherapy prior to surgery and had provided signed informed consent prior to inclusion. The present study was approved by the Ethics Committee of Anhui Medical University (approval no. 20190356). In addition, after radical esophagectomy, tumor tissues did not contain any necrotic area, and adjacent normal tissues were resected at >5 cm from cancer tissue. The clinicopathological characteristics of the patients are summarized in Table I.

Cell culture. Normal esophageal epithelial cells (SHEE10) were obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences, and four human ESCC cell lines (KYSE30, KYSE150, KYSE450 and KYSE510) were obtained from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH. All cells were cultured in RPMI-1640 medium containing 10% FBS (both Thermo Fisher Scientific, Inc.), streptomycin (100 µg/ml) and penicillin (100 IU/ml) at 37°C with 5% CO₂.

Cell transfection. KYSE30 and KYSE150 cells (3x10⁵ cells/well) were cultured in 6-well plates. The cells were transfected with small interfering (si)RNA (30 nM) or control siRNA (30 nM) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After incubation at 37°C for 6 h, the culture medium was changed. After transfection for 48 h, the knockdown efficiency was assessed using reverse transcription-quantitative (RT-q) PCR and western blotting. siRNA targeting GFI1 (siGFI1) and SOCS1 (siSOCS1) were synthesized by GENERAL BIOL. The sequences of the siRNA were as follows: GFI1-siRNA, 5'-GCUCGGAGU UUGAGGACUU-3'; SOCS1-siRNA, 5'-GCAUCCGCGUGC ACUUUCAUU-3'; and control siRNA, 5'-UUCUCCGAA CGUGUCACGUTT-3'.

Table I. Summary of patient clinicopathological characteristics.

Patient characteristics	n
Sex	
Female	22
Male	18
Age, year	
<60	16
≥60	24
Differentiation	
Well/moderate	29
Poor	11
Invasion	
Absent	14
Present	26
Clinical stage ^a	
Early (I-II)	25
Advanced (III-IV)	15
Lymph node metastasis	
Absent	21
Present	19

^aAmerican Joint Committee on Cancer/The Union for International Cancer Control Tumor-Node-Metastasis staging system (18).

Cell counting kit-8 (CCK-8) and colony formation assays. Cell proliferation was examined using CCK-8 and colony formation assays. The CCK-8 assay was purchased from Dojindo Molecular Technologies, Inc. KYSE30 and KYSE150 cells (1x10³ cells/well) were seeded into 96-well plates following transfection with siGFI1 or control siRNA for 48 h. CCK-8 solution (10 µl) was added to each well at 24, 48 or 72 h, and the cells were further incubated at 37°C for 1.5 h. Subsequently, the absorbance was measured at 450 nm using a microplate spectrometer (Thermo Fisher Scientific, Inc.).

For the colony formation assay, KYSE30 and KYSE150 cells (2x10³ cells/well) were seeded in 6-well plates, incubated overnight and subsequently transfected with siGFI1. Following 14-day culture, the cells were fixed with 4% formaldehyde at room temperature, washed three times with PBS and stained with trypan blue for 10 min at room temperature. The number of colonies (containing ≥50 cells) were counted using a light microscope (magnification, x200; Olympus Corporation).

Cell migration and wound healing assay. For the cell migration assay, suspensions of transfected KYSE30 and KYSE150 cells (1x10⁴ cells/well) were re-suspended in FBS-free RPMI-1640 medium and added to the upper chamber of a Transwell chamber (8-µm pore size; Corning, Inc.). RPMI-1640 medium supplemented with 10% FBS (600 µl) was added to the lower chamber. Following culturing for 24 h at 37°C, the adherent cells on the upper surface of the insert membrane were removed using cotton swabs, and the migrated cells were fixed with 4% formaldehyde at room temperature, washed three times with PBS and stained with trypan blue as

Table II. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Direction	Sequence (5'-3')
GFI1	Forward	GCAAGGCATTTCAGCCAGAG
	Reverse	AAGGCAAAGGAGGAGCAA
SOCS1	Forward	TTCGCCCTTAGCGTGAAGAT
	Reverse	GCTCGAAGAGGCAGTCGAA
Cyclin D1	Forward	TGACCCCGCACGATTTCAIT
	Reverse	CAGAGGGCAACGAAGGTCTG
Survivin	Forward	TTTTGATTCCCGGGCTTACCA
	Reverse	ACATTCCTGTGGAAGGCTCT
N-cadherin	Forward	CCTGAGGGATCAAAGCCTGG
	Reverse	ACATGTTGGGTGAAGGGGTG
E-cadherin	Forward	AATTCCTGCCATTCTGGGGA
	Reverse	GGGCAGTAAGGGCTCTTTGA
Vimentin	Forward	GTTTCCAAGCCTGACCTCAC
	Reverse	GTCATTGTTCCGGTTGGCAG
SOCS1	Forward	TTCGCCCTTAGCGTGAAGATGG
	Reverse	TAGTGCTCCAGCAGCTCGAAGA
GAPDH	Forward	GTCTCCTCTGACTTCAACAGCG
	Reverse	ACCACCCTGTTGCTGTAGCCAA

GFI1, growth factor-independent 1; SOCS1, suppressor of cytokine signaling 1.

mentioned. The migratory cells were imaged and counted under a light microscope (magnification, x200; Olympus Corporation).

For the wound healing assay, 1×10^6 transfected cells were seeded in 6-well plates for 48 h at 37°C. The cell monolayer was scraped using a 200- μ l pipette tip when they reached 80-90% confluence, washed three times with PBS, and 2 ml RPMI-1640 medium without FBS was added. The wound width was measured by capturing images under a microscope (magnification, x200; Olympus Corporation) at 0 and 24 h.

RT-qPCR. Total RNA was isolated from clinical samples and cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse-transcribed into cDNA using a HiScript III 1st Strand cDNA Synthesis kit (Vazyme Biotech Co., Ltd.) according to the manufacturer's protocol. qPCR was performed using LightCycler® FastStart DNA Master SYBR® Green I (Roche Diagnostics GmbH) on a light Cycler 96 (Roche Diagnostics GmbH), according to the manufacturer's instructions. The thermocycling conditions were as follows: Initial denaturation at 95°C for 30 sec, following by 40 cycles of denaturation at 95°C for 10 sec, annealing and elongation 60°C for 60 sec. GAPDH served as an internal control for gene expression, and the relative expression level was calculated using the $2^{-\Delta\Delta C_q}$ method (19). The sequences of the primers used are listed in Table II.

Western blotting assay. Total protein was extracted from cells or tissues using RIPA lysis buffer (Beyotime Institute of Biotechnology), and the protein concentration was determined using a BCA protein assay kit (Beyotime Institute

of Biotechnology). Total protein (50 μ g) was resolved using 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc.). The membranes were blocked with 5% non-fat milk for 1.5 h at room temperature, incubated with primary antibodies at 4°C overnight. The membranes were washed three times with PBST and then incubated with HRP-conjugated goat anti-mouse IgG (1:2,000; cat. no. SA00001-1; ProteinTech Group, Inc.) and goat anti-rabbit IgG (1:2,000; cat. no. 511203; Chengdu Zen Bioscience Co., Ltd.) secondary antibodies for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence detection system (Beyotime Institute of Biotechnology), and densitometry analysis was performed using ImageJ (v1.8.0, National Institutes of Health). Rabbit polyclonal antibodies against P65 (1:1,000; cat. no. 10745-1-AP) and mouse monoclonal antibodies against GAPDH (1:1,000; cat. no. 60004-1-Ig) were purchased from ProteinTech Group, Inc. Rabbit polyclonal antibodies against SOCS1 (1:1,000; cat. no. ab62584) and GFI1 (1:1,000; cat. no. ab21061) were obtained from Abcam. Rabbit polyclonal antibodies against phosphorylated (p)-p65 (1:1,000; cat. no. 310012) were obtained from Chengdu Zen Bioscience Co., Ltd. Rabbit monoclonal antibodies against STAT3 (1:1,000; cat. no. 4904S) and p-STAT3 (1:1,000; cat. no. 4113S) were purchased from Cell Signaling Technology, Inc.

Immunohistochemistry (IHC). ESCC and paired normal tissues were embedded in paraffin, fixed in 4% paraformaldehyde at room temperature for 2 days and cut into 5- μ m sections for IHC analysis. IHC staining was performed by Wuhan Servicebio Technology Co., Ltd. The sections were examined,

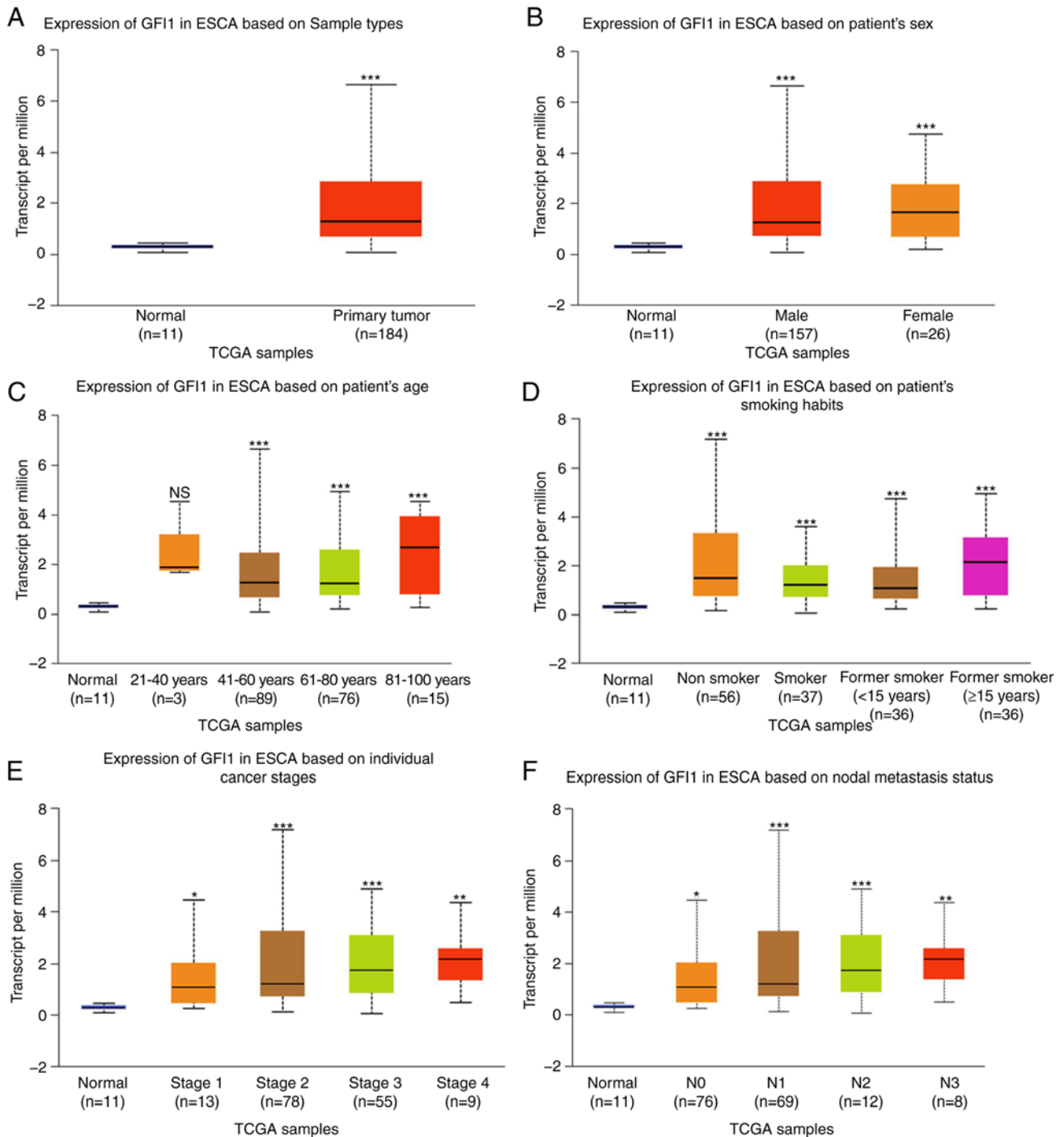


Figure 1. GFI1 mRNA expression is upregulated in ESCA tissues in TCGA. (A) GFI1 expression levels were significantly higher in ESCA tissues compared with those in normal tissues. (B-F) GFI1 expression levels in patients grouped by (B) sex, (C) age, (D) smoking status, (E) cancer stage and (F) nodal metastasis status of ESCA. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. normal. GFI1, growth factor-independent 1; TCGA, The Cancer Genome Atlas; ESCA, esophageal cancer; N, node.

and images were captured using a microscope (magnification, $\times 200$; Olympus Corporation) in five random fields of view per sample.

Luciferase reporter assay. KYSE30 and KYSE150 cells (2×10^5 /well) were seeded and cultured in 12-well plates overnight and co-transfected with an NF- κ B reporter plasmid ($1 \mu\text{g}$ /well; cat. no. D2206; Beyotime Institute of Biotechnology) and siGFI1 (30 nM), siSOCS1 (30 nM) or siCtrl (30 nM) using Lipofectamine[®] 2000 according to the

manufacturer's instructions. After incubation at 37°C for 6 h, the culture medium was changed. At 48 h post-transfection, cell samples were lysed using Firefly Luciferase Reporter Gene Assay Cell Lysis Buffer (cat. no. RG126S; Beyotime Institute of Biotechnology). After brief centrifugation at $13,778 \times g$ for 20 min, $10 \mu\text{l}$ aliquot of the supernatant was assayed using a Luciferase Assay kit (Promega Corporation), and the same amount of supernatant was used to measure the concentration of total protein using a BCA protein assay (cat. no. P0012S; Beyotime Institute of Biotechnology). The

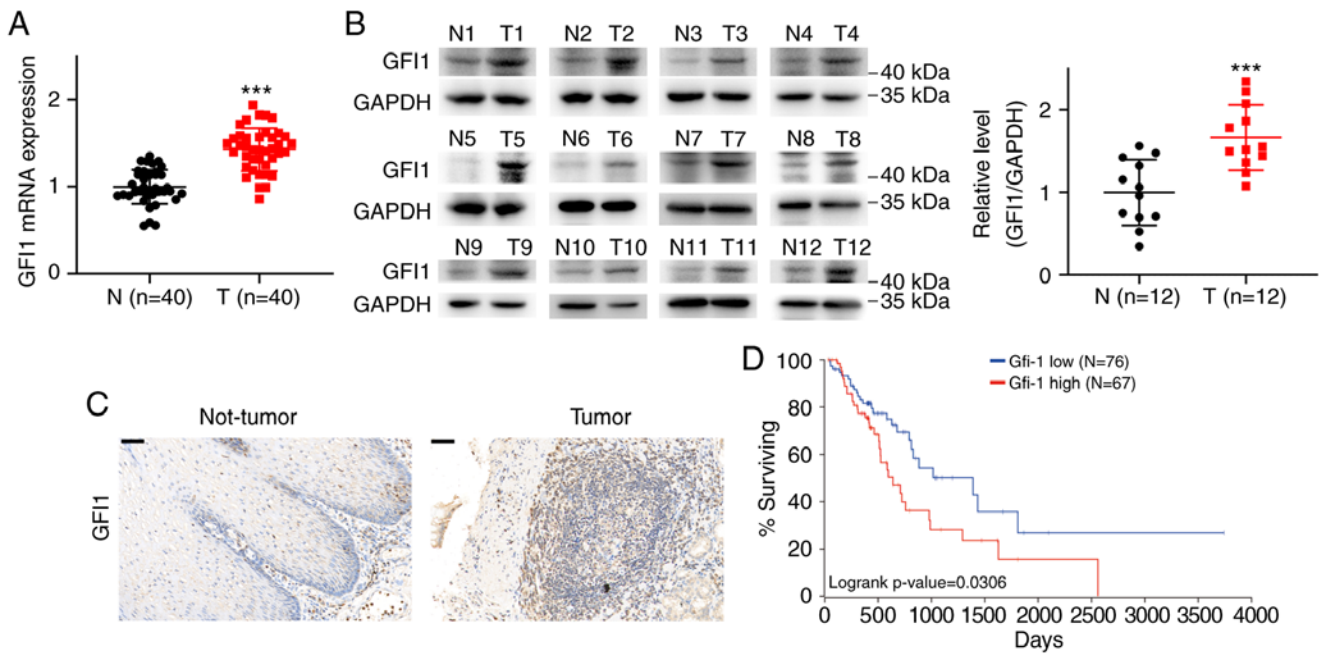


Figure 2. GF11 expression is upregulated in ESCC tissues. (A) mRNA and (B) protein expression levels of GF11 in tissues of patients with ESCC were examined using reverse transcription-quantitative PCR (n=40) and western blotting (n=12). (C) Immunohistochemical staining of GF11 in ESCC and normal tissues. Scale bar, 50 μ m. (D) Kaplan-Meier overall survival analysis of patients with esophageal cancer from the OncoLnc database stratified by median GF11 mRNA expression. ***P<0.001 vs. N. GF11, growth factor-independent 1; ESCC, esophageal squamous cell carcinoma; N, normal; T, tumor.

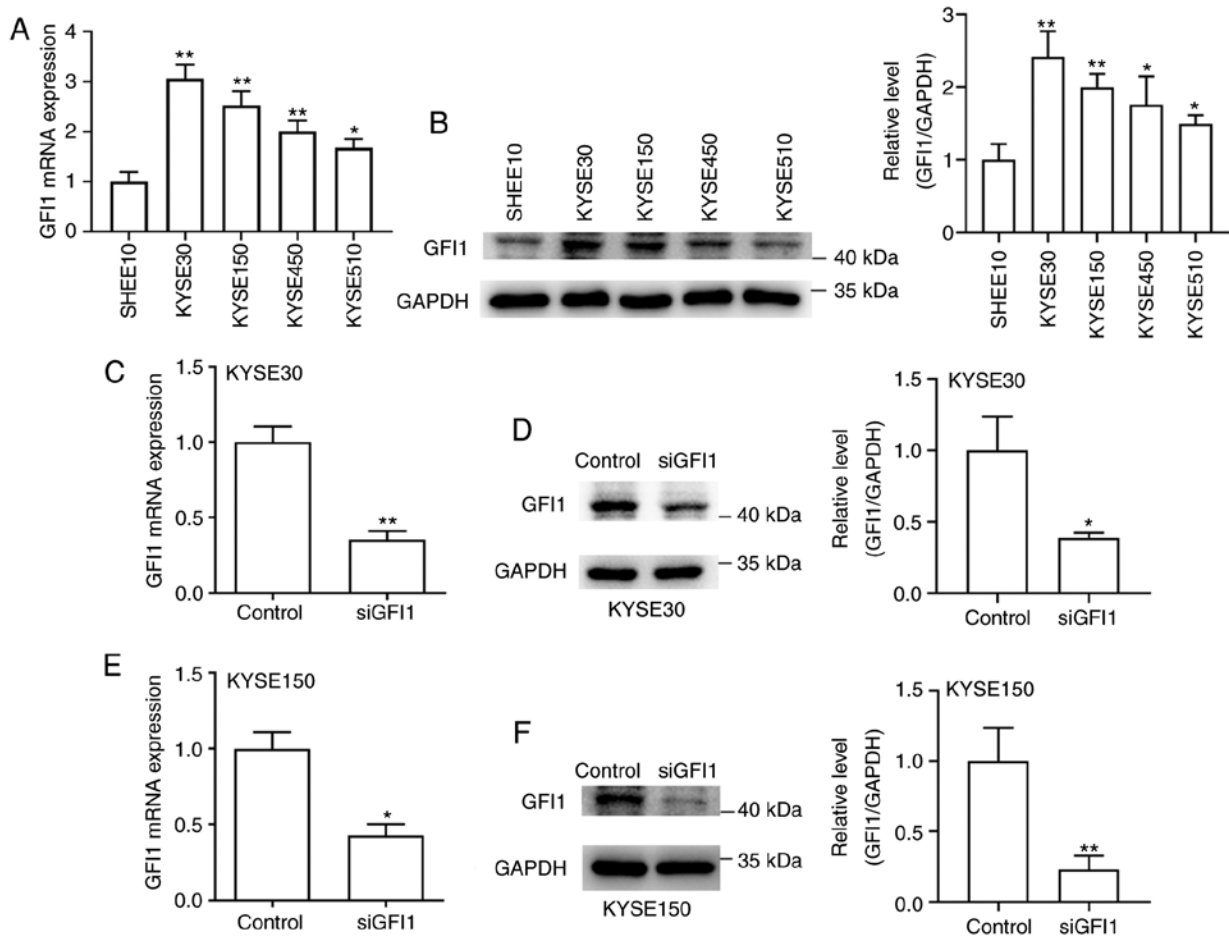


Figure 3. Efficiency of GF11 knockdown in ESCC cells using siRNA. (A) mRNA and (B) protein expression levels of GF11 in the SHEE10 normal esophageal epithelial cells and four ESCC cell lines: KYSE30, KYSE150, KYSE450 and KYSE510. (C-F) GF11-siRNA was transfected into the KYSE30 and KYSE150 ESCC cell lines. The mRNA and protein expression levels of GF11 in ESCC cell lines were detected using reverse transcription-quantitative PCR and western blotting. *P<0.05 and **P<0.01 vs. SHEE10 or control. siRNA/si, small interfering RNA; GF11, growth factor-independent 1; ESCC, esophageal squamous cell carcinoma.

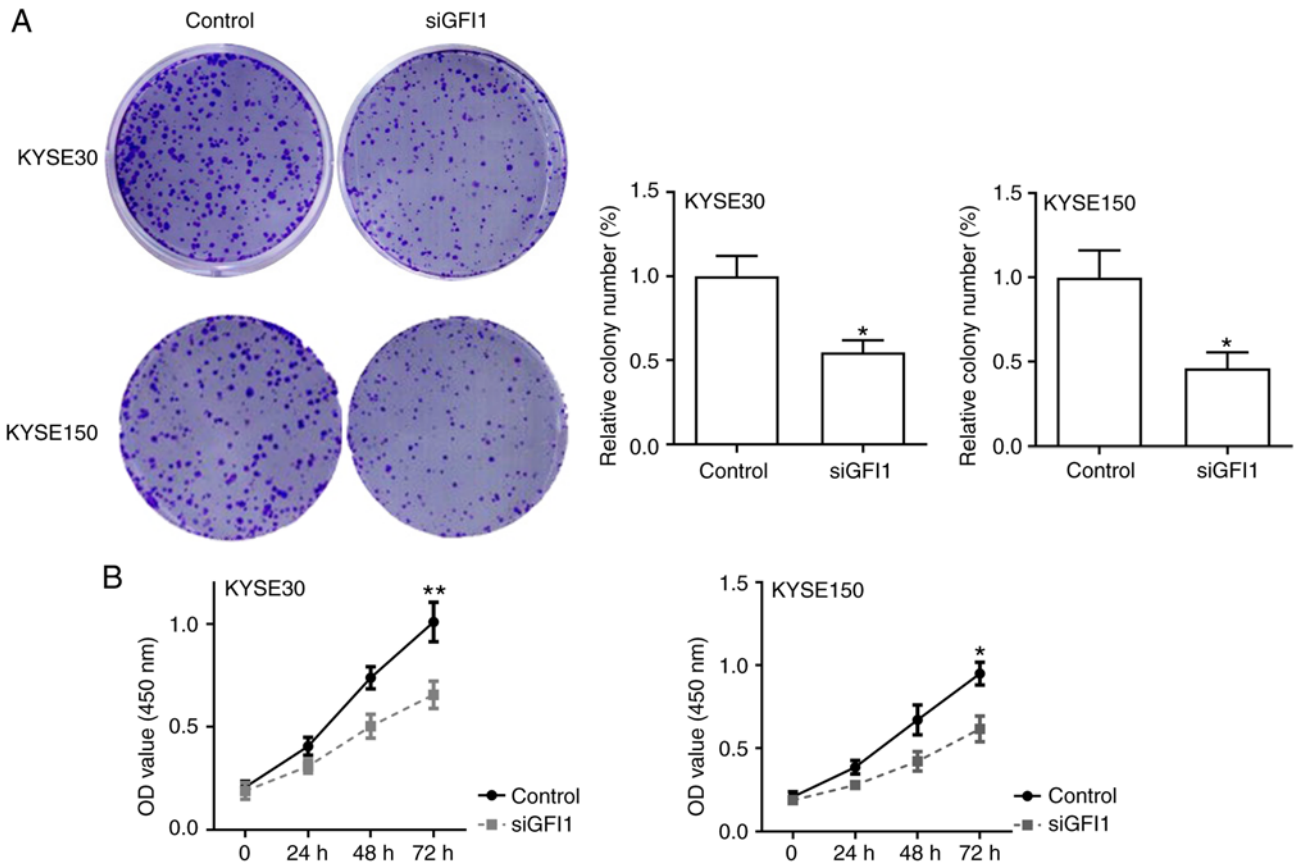


Figure 4. GFII knockdown decreases the proliferation of ESCC cell lines. (A) Colony formation assays were performed using the siGFII-transfected ESCC cells. (B) Cell proliferation was assessed in the siGFII-transfected ESCC cell lines (KYSE30 and KYSE150) using a Cell Counting Kit-8 assay. * $P < 0.05$ and ** $P < 0.01$ vs. control. si, small interfering RNA; GFII, growth factor-independent 1; ESCC, esophageal squamous cell carcinoma; OD, optical density.

luciferase activity was normalized against the concentration of total protein.

Statistical analysis. All experiments were performed in triplicate. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, Inc.), and data are presented as the mean \pm SEM. Statistical differences between groups were compared using a paired or unpaired Student's t-test as appropriate, a one-way ANOVA with Tukey's post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

GFII expression is upregulated in ESCC tissues. The expression levels of GFII in ESCA were analyzed using data obtained from TCGA using the UALCAN platform. As presented in Fig. 1A, GFII expression levels were significantly upregulated in ESCA tissues compared with those in normal tissues. Furthermore, a statistically significant association between the mRNA levels of GFII and the clinicopathological features of ESCA were observed based on the UALCAN platform. GFII mRNA expression levels were increased in ESCA regardless of sex, patient age (20-40, 41-60, 61-80 and 81-100 years old), smoking status [non-smoker, smoker, short-term former smoker (<15 years) and long-term former smoker (≥ 15 years)], cancer stages (S1-4) and nodal metastasis status (N0-4)

compared with those in normal tissues (Fig. 1B-F). These results suggested that GFII was upregulated in ESCA and may affect ESCA progression.

Next, the potential functions of GFII in ESCC were explored in the present study. The mRNA expression levels of GFII in 40 pairs of ESCC and adjacent normal tissues were assessed using RT-qPCR. The results demonstrated that the mRNA levels of GFII were increased in ESCC tissues compared with those in the normal adjacent tissues (Fig. 2A). Western blotting also revealed that GFII protein levels were significantly upregulated in ESCC compared with those in normal tissues (Fig. 2B). Similar to the results of western blotting, IHC analysis of samples from ESCC and matched adjacent tissues demonstrated that high GFII expression was observed in ESCC tissues (Fig. 2C). Kaplan-Meier survival analysis using the OncoLnc online tool suggested that patients with ESCA that exhibited higher than the median expression levels of GFII had shorter overall survival times compared with those observed in patients with low GFII levels (Fig. 2D).

Together, these results indicated that GFII was upregulated in ESCC tissues and associated with a poor prognosis, suggesting that GFII may promote the progression of ESCC.

GFII is highly expressed in ESCC cell lines and is knocked down by siRNA. The expression levels of GFII in SHEE10 and four ESCC cell lines (KYSE30, KYSE150, KYSE450 and KYSE510) were assessed, and the results demonstrated

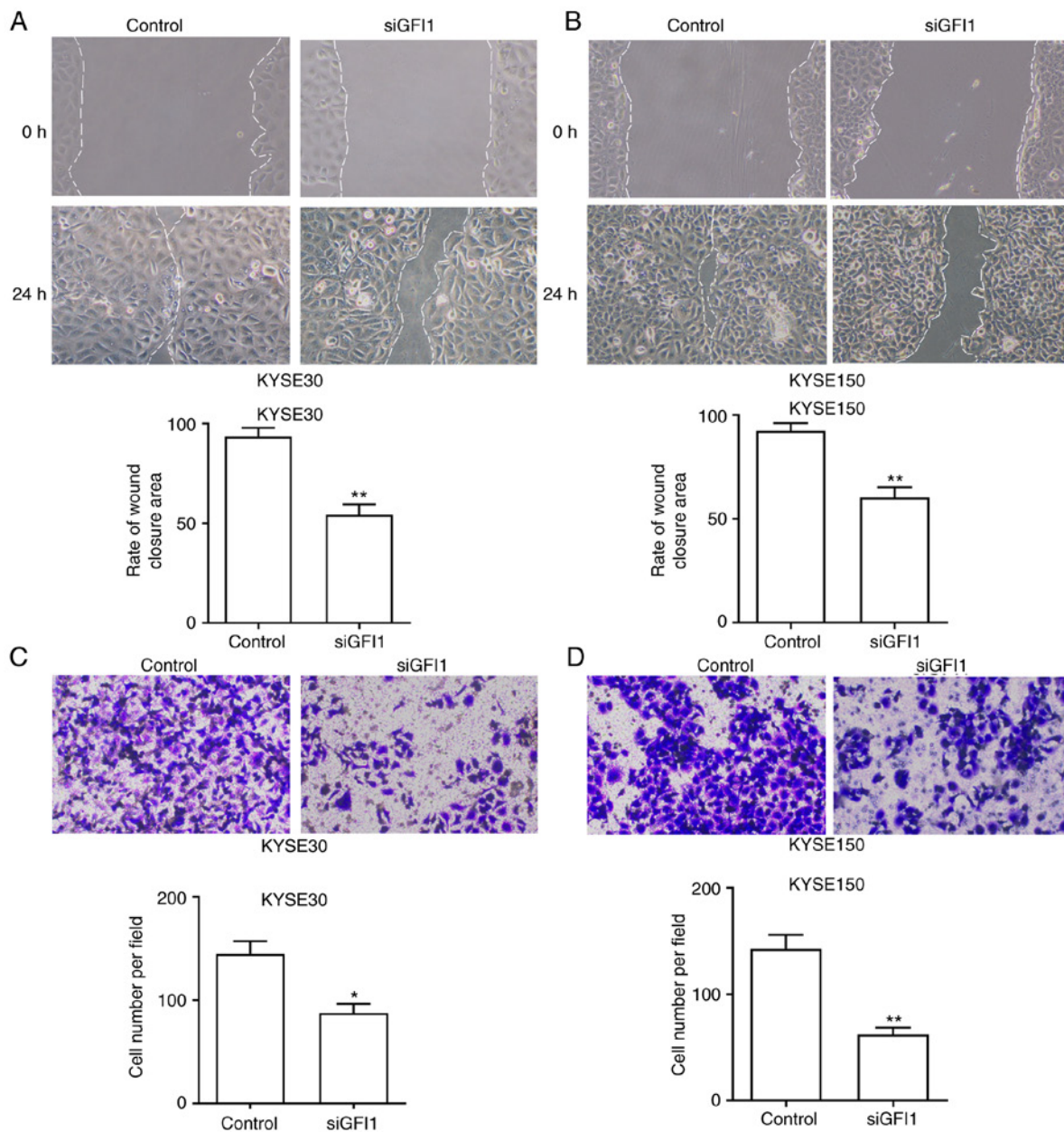


Figure 5. GFII knockdown reduces the migration of esophageal squamous cell carcinoma cells. (A and B) Wound healing and (C and D) Transwell assays were used to determine the effects of GFII knockdown on KYSE30 and KYSE150 cell migration. Magnification, x200. * $P < 0.05$ and ** $P < 0.01$ vs. control. si, small interfering RNA; GFII, growth factor-independent 1.

that the mRNA and protein levels of GFII were significantly increased in ESCC cells compared with those in the normal esophageal cell line (Fig. 3A and B). Subsequently, KYSE30 and KYSE150 cells, which exhibited the highest levels of endogenous GFII expression, were transfected with siGFII. As presented in Fig. 3C-F, both cell lines transfected with siGFII exhibited lower GFII mRNA and protein expression levels compared with those in the control group. Thus, KYSE30 and KYSE150 cells were both successfully transfected.

GFII knockdown suppresses the proliferation of ESCC cells. The effects of GFII on cell proliferation *in vitro* were next assessed. The results of colony formation and CCK-8 assays demonstrated that GFII knockdown significantly reduced the proliferative rates of KYSE30 and KYSE150 cells compared with those of the control cells (Fig. 4A and B). In addition, the

expression levels of the cell cycle-associated genes cyclin D1 and survivin were assessed using RT-qPCR. As presented in Fig. S1A, knockdown of GFII significantly downregulated the levels of cyclin D1 and survivin in both cell lines compared with those in the respective control groups. These results suggested that GFII promoted ESCC cell proliferation.

GFII knockdown reduces the migration of ESCC cells. To investigate the function of GFII on ESCC cell migratory ability, wound healing and Transwell assays were performed. The wound healing assay results demonstrated that the tumor cell migration was significantly reduced in both cell lines transfected with siGFII compared with that in the control cells (Fig. 5A and B). In the Transwell assays, the number of cells that had migrated through the membrane was reduced in KYSE30 and KYSE150 cells transfected with siGFII compared with

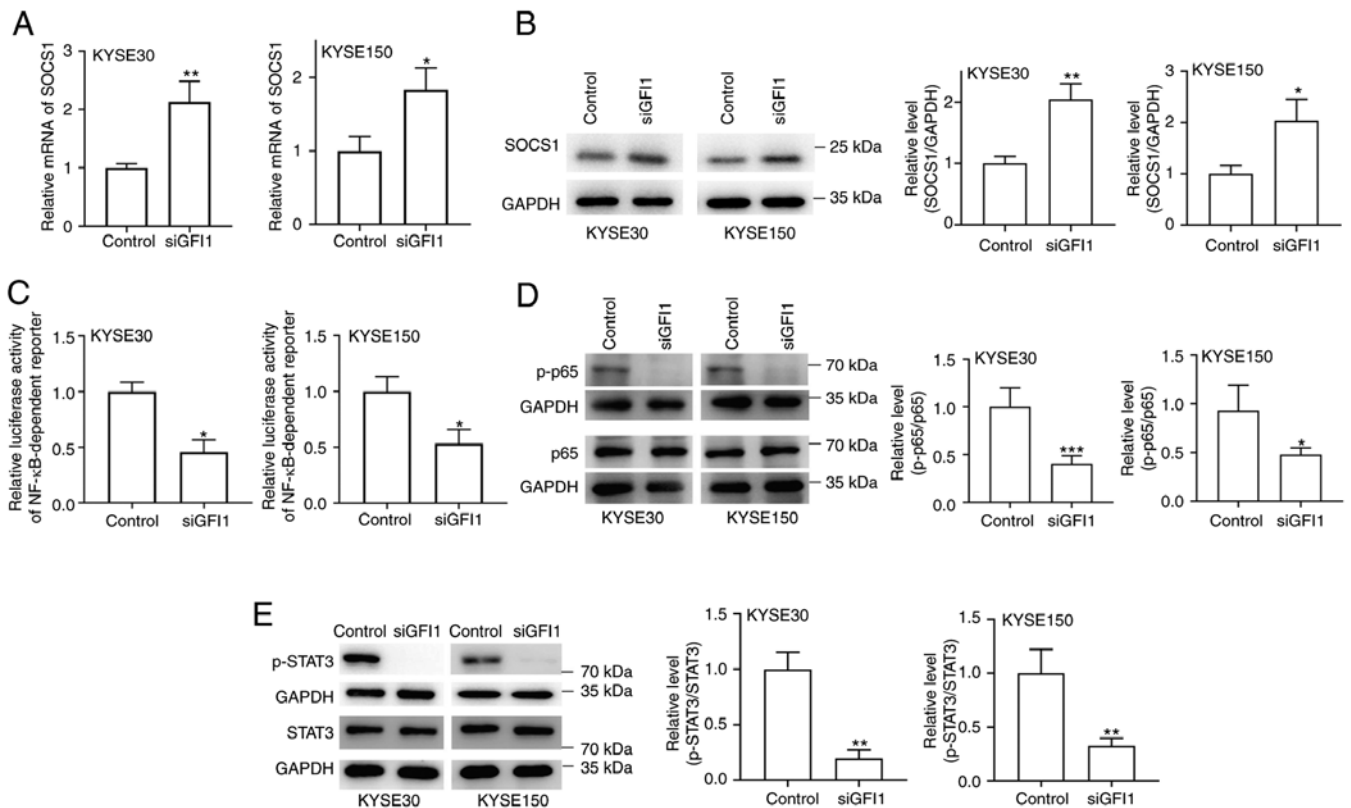


Figure 6. GFII enhances NF- κ B and STAT3 activity by inhibiting SOCS1 expression in esophageal squamous cell carcinoma cell lines. (A and B) siGF11 was transfected into KYSE30 and KYSE150 cells. (A) mRNA and (B) protein expression levels of SOCS1 were examined using reverse transcription-quantitative PCR and western blotting, respectively. (C) NF- κ B activity, (D) p-p65 and (E) p-STAT3 protein levels were analyzed in KYSE30/KYSE150 cells following transfection with siGF11. * P <0.05, ** P <0.01 and *** P <0.001 vs. control. si, small interfering RNA; GFII, growth factor-independent 1; SOCS1, suppressor of cytokine signaling 1; p-, phosphorylated.

those in the respective control groups (Fig. 5C and D). In addition, the expression levels of epithelial and mesenchymal markers E-cadherin, N-cadherin and vimentin in KYSE30 and KYSE150 cell lines treated with siGF11 were determined. As demonstrated in Fig. S1B, the mRNA expression levels of E-cadherin were increased, and the levels of N-cadherin and Vimentin were reduced compared with those in the control cells. Together, these results indicated that GFII promoted ESCC cell migration and may induce the EMT progression.

GFII knockdown inhibits STAT3 and NF- κ B signaling via upregulation of SOCS1 expression. Whether GFII regulated the STAT3 and NF- κ B signaling pathways by inhibiting SOCS1 expression in ESCC cell lines was determined in the present study. As demonstrated in Fig. 6A and B, GFII knockdown in KYSE30 and KYSE150 cells increased SOCS1 mRNA and protein levels compared with those in the control cells. Additionally, NF- κ B activity was reduced in both cell lines following knockdown of GFII compared with that in the control groups (Fig. 6C). When the NF- κ B signaling pathway is activated, p65 is rapidly phosphorylated and translocates to the nucleus (20); when GFII expression was knocked down in KYSE30 and KYSE150 cells, the levels of p-p65 were notably decreased compared with those in the control cells (Fig. 6D). In addition, as presented in Fig. 6E, knockdown of GFII inhibited STAT3 phosphorylation in both cell lines.

To analyze whether SOCS1 inhibition was required for the GFII-mediated increases in the proliferative and migratory

capacities of ESCC cells, the present study transfected siSOCS1 into KYSE30 and KYSE150 cells. The transfection efficiency was confirmed by RT-qPCR and Western blotting analyses, and the results demonstrated that both cell lines transfected with siSOCS1 exhibited lower SOCS1 mRNA and protein expression levels compared with those in the control groups (Fig. S2A-D). Subsequently, SOCS1 expression was knocked down in ESCC cells following GFII knockdown. Colony formation and Transwell migration assay results revealed that knockdown of SOCS1 compensated for the loss of proliferation and migration induced by GFII knockdown (Fig. 7A and B). In addition, SOCS1 knockdown attenuated the reduction in NF- κ B activity and the reduction in p-p65 and p-STAT3 levels (Fig. 7C-H). Taken together, these results demonstrated that GFII may promote ESCC cell proliferation via inhibiting SOCS1 expression and enhancing NF- κ B and STAT5 activity (Fig. 8).

Discussion

Although a number of improvements have been achieved in the treatment of ESCC, surgical resection remains the most effective treatment for patients with ESCC (21). However, the 5-year overall survival rates remains poor at an estimated 15-20%, and 43~53% of patients who undergo surgery experience local recurrence and/or distant metastasis (22,23). Therefore, elucidating the molecular mechanisms underlying the development and progression of ESCC, and identifying novel therapeutic targets may improve ESCC treatment.

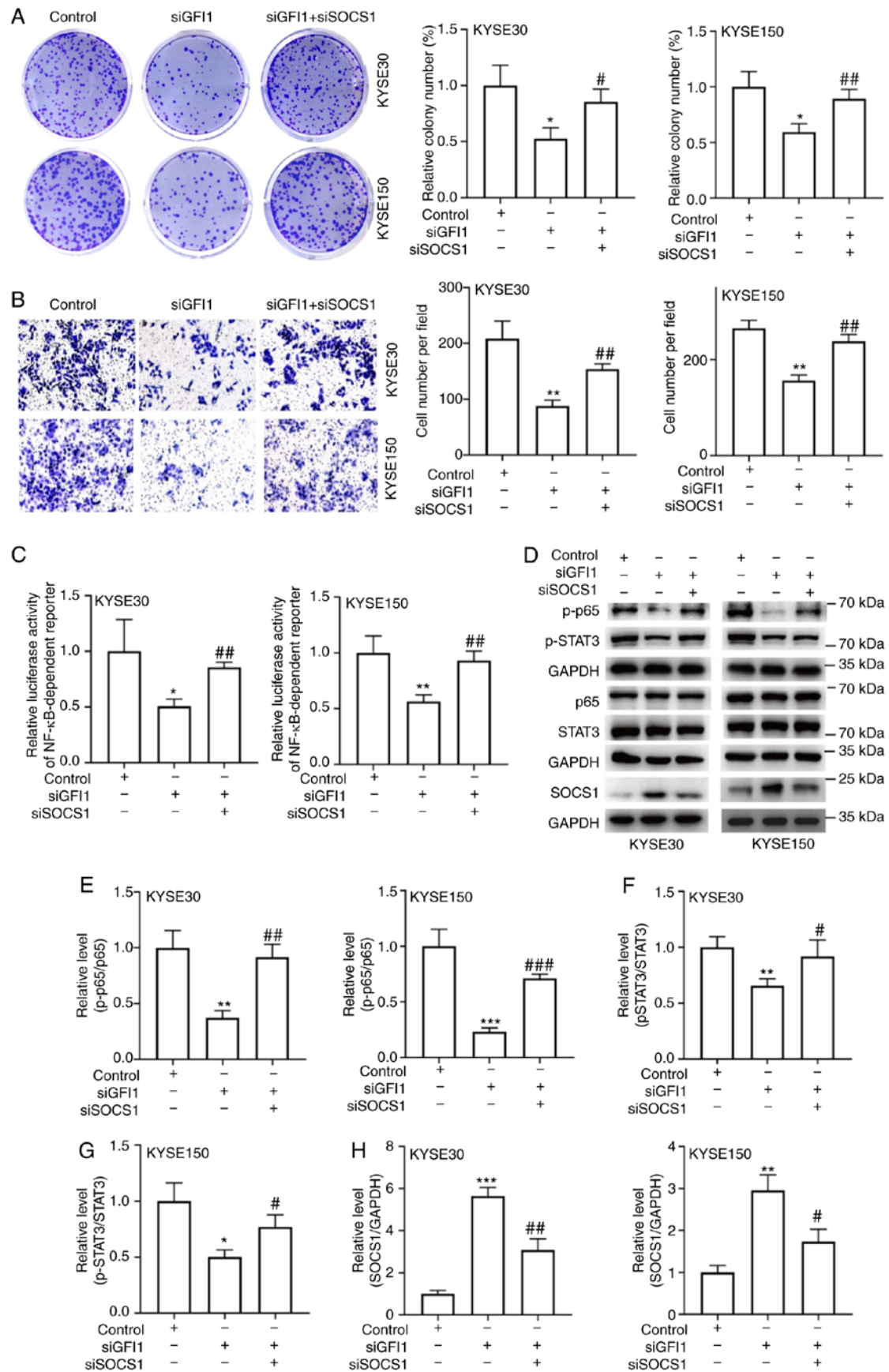


Figure 7. SOCS1 inhibits the increase in esophageal squamous cell carcinoma cell proliferation and migration induced by GFI1. (A-H) siSOCS1 was transfected into the GFI1-knockdown KYSE30 and KYSE150 cells. (A) Colony formation assays were performed on the double transfected cells. (B) Cell migration was determined using Transwell assays. (C) NF- κ B activity, (D) p-p65, p-STAT3 and SOCS1 protein levels were analyzed. (E-G) Ratio of p-p65 to p65 and STAT3 to p-STAT3, as well as (H) the semi-quantification of SOCS1 protein expression were determined using ImageJ. Magnification, $\times 200$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control; # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$ vs. siGFI1. SOCS1, suppressor of cytokine signaling 1; si, small interfering RNA; GFI1, growth factor-independent 1; p-, phosphorylated.

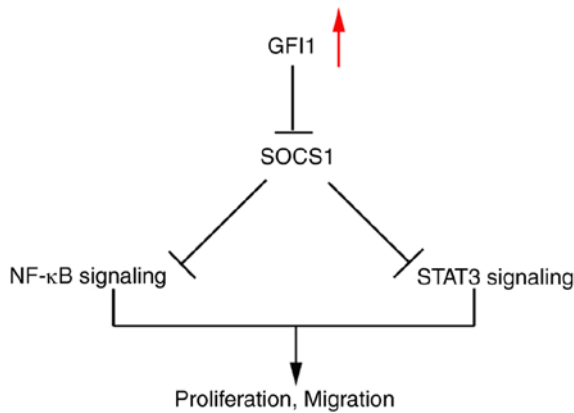


Figure 8. Regulatory model of the role of GFII in ESCC progression. The results of the present study demonstrated that the upregulation of GFII promoted ESCC proliferation and migration via inhibition of SOCS1 expression, leading to the activation of the NF- κ B and STAT3 pathways. GFII, growth factor-independent 1; ESCC, esophageal squamous cell carcinoma; SOCS1, suppressor of cytokine signaling 1.

Previous studies have demonstrated that GFII not only regulates the development of multiple hematopoietic lineages, including macrophages, dendritic cells, granulocytes, as well as T and B cells, but it also participates in the self-renewal and survival of hematopoietic stem cells (24,25). GFII-knockout mice exhibit defects in B cell, T cell and neutrophil differentiation, as well as in the response of mature B and T cells to antigens (26). In addition, GFII inhibits T cell death induced by cultivation of IL-2-dependent T cell lines in IL-2-deficient media by repressing Bax expression (27). Although GFII has been reported to be involved in the development and progression of cervical cancer and lymphomas, its functions in ESCC remain unclear. In the present study, GFII expression levels were markedly upregulated in ESCC tissues compared with those in adjacent normal tissues, and GFII knockdown reduced cell proliferation and migration of ESCC cells.

As an inhibitor of DNA binding, GFII targets a range of proteins in various types of cells, including STAT5B, MCL1 apoptosis regulator, BCL2 family member, RUNX family transcription factor 2, suppressor of cytokine signaling 1 and F-box and WD repeat domain-containing 7 (9,12,28,29). SOCS1 was originally identified as a suppressor of cytokine signaling, and belongs to the SOCS family of proteins (30). SOCS1 inhibits proliferation signals modulated by various oncogenes (CDK2, CyclinD1 and STATs) in cancer development (31). Previous studies have reported that aberrant downregulation of SOCS1 is involved in the clinical progression of a number of types of cancer, including hepatocellular carcinoma, bladder and triple-negative breast cancer (32-34). In the present study, the results demonstrated that GFII knockdown increased the levels of SOCS1 expression compared with those in the control cells. An increasing number of studies have reported that SOCS1 targets several signaling pathways, including the JAK/STAT, MAPK and NF- κ B pathways (15,35,36). The STAT3 and NF- κ B signaling pathways are two vital intracellular pathways that serve key roles in modulating cell differentiation, proliferation, migration and metabolism (37,38). These pathways are frequently overactivated in various types of cancer, including colorectal, non-small lung and breast cancer, and have been

reported to contribute to tumor progression and drug resistance (39-41). Previous studies have demonstrated that the STAT3 and NF- κ B pathways are aberrantly activated during ESCC development (17,42,43). However, whether both pathways are associated with the role of GFII in ESCC remains unknown. The results of the present study demonstrated that knocking down GFII expression significantly reduced SOCS1 expression levels and further reduced the levels of p-STAT3 as well as NF- κ B activity.

The major limitation of the present study was that the data were obtained from both EAC and ESCC, since hypothesized from the EAC results that GFII may also be relevant in ESCC.

In conclusion, the results of the present study demonstrated that GFII was significantly upregulated in ESCC tissues and cells compared with those in normal esophageal tissues and cells. Knockdown of GFII reduced ESCC cell proliferation and migration by decreasing SOCS1 expression levels, which occurred via the regulation of the STAT3 and NF- κ B signaling pathways. Thus, GFII may serve as a potential therapeutic target for the treatment of ESCC.

Acknowledgements

Not applicable.

Funding

This study was supported by the National Science Foundation of China Grants (grant no. 82003048), the Anhui Provincial Natural Science Foundation (grant no. 1908085QC131) and Grants for Scientific Research of BSKY from Anhui Medical University (grant no. XJ201726).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WH and RZ designed the study. YH, RR and YF performed the experiments. KW, LY and RR analyzed the data. WH and RZ wrote the manuscript. All authors read and approved the final manuscript. WH and RZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Anhui Medical University (Hefei, China; approval no. 20190356). All patients provided signed informed consent prior to the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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