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# IFN-γ Induces Gastric Cancer Cell Proliferation and Metastasis Through Upregulation of Integrin β3-Mediated NF-κB Signaling

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## Abstract

Interferon γ (IFN-γ), a multifunctional cytokine, was upregulated in the resected gastric cancer tissue. However, whether IFN-γ is involved in the regulation of gastric cancer has not been well elucidated. Herein, we aimed to investigate the effects and mechanism of IFN-γ on gastric cancer. In this study, we found a vital role of IFN-γ in enhancing proliferation, inhibiting apoptosis, and promoting cell migration and invasion in gastric cancer cells SGC-7901 and MGC-803. Additionally, IFN-γ activated nuclear factor κB (NF-κB) signaling pathway by upregulating the phosphorylation expression of p65 and IκBα, and induced the expression of integrin β3 *in vitro*. Therefore, to further investigate the relationship between IFN-γ and integrin β3, SGC-7901 cells were transfected with integrin β3 siRNA. And then cells expressed lower cell viability, migration, and invasion rates, while cell apoptosis was significantly enhanced. Meanwhile, expression of integrin β3, MMP-2, MMP-9, and NF-κB, including p65 and IκBα, and the nuclear translocation of NF-κB/p65 were dramatically repressed, whereas IFN-γ significantly improved the effects. Moreover, *in vivo*, the experiment of xenograft model and pulmonary metastasis model also retarded in integrin β3 siRNA group. And the expression of integrin β3, MMP-2, MMP-9, and NF-κB was repressed. However, the treatment with IFN-γ improved tumor volume, lung/total weight, tumor nodules, and the protein expression described above compared with integrin β3 siRNA group. Overall, the results indicated that IFN-γ induces gastric cancer cell proliferation and metastasis partially through the upregulation of integrin β3-mediated NF-κB signaling. Hence, the inhibition of IFN-γ or integrin β3 may be the key for the treatment of gastric cancer.

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## Introduction

Gastric cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death [1]. The development of gastric cancer is a multiple-steps process, starting with premalignant *in situ* lesions and, lastly, tumor cell migration into a near cavity or a distant organ, which is responsible for most gastric cancer deaths [2,3]. In addition, the dysregulated production of cytokines in inflammatory microenvironment stimulates the expression of genes associated with cancer development and modifies structural features of microenvironment to accelerate cancer initiation and progression [4–6]. However, the mechanism of some cytokines in inflammatory microenvironment, such as interferon γ and interleukin-13, on gastric cancer initiation and progression remains largely mysterious.

Integrins, a family of 24 heterodimeric, multifunctional glycoproteins, mediate cell-to-cell and cell-to-extracellular-matrix interactions and are involved in a great variety of physiological and pathological processes [7]. Integrins are important regulators of differentiation,

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tumor growth, survival, migration and invasion, and they are involved in several processes that characterize the tumor phenotype in malignant tumors [8]. Recently, integrins, particularly αvβ3, have been recognized as putative targets for the treatment of several cancers including lung cancers, which has spurred research on integrins in cancer biology [9–12]. However, little is known about integrins αvβ3 in gastric cancer.

Interferon γ (IFN-γ), a multifunctional cytokine, is produced mainly by T helper cells, cytotoxic T cells, natural killer cells, and macrophages during the onset of the infection [13]. IFN-γ is involved in wide range of remarkably distinct cellular programs including regulation of class II MHC molecules, synthesis of inducible nitric oxide, and cancer surveillance [14]. IFN-γ could be enhanced by human natural killer cells through upregulation of TLR-mediated nuclear factor κB (NF-κB) signaling [15]. Furthermore, IFN-γ and TNF-α could induce inflammatory condition through activating related transcription factors, such as NF-κB and STAT in keratinocytes [16]. In addition, IFN-γ, secreted by CD8-positive lymphocytes, could upregulate PD-L1 on ovarian cancer cells and promote tumor growth [17]. Besides, the secretion of IFN-γ and TNF-α was suppressed by regulatory B cells, which played an immunosuppressive role in gastric cancer [18]. And IFN-γ could be upregulated in the resected gastric cancer tissue compared to matched adjacent noncancerous tissue [19]. However, whether IFN-γ is involved in the regulation of gastric cancer is not well elucidated. Herein, this study was designed to investigate the effect and mechanism of IFN-γ on gastric cancer.

## Materials and Methods

### *Chemicals and Reagents*

Fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM)/F12, and trypsin were from the United States GIBCO company. IFN-γ and Matrigel were purchased from BD Transduction Laboratories (Lexington, KY). Antibodies against p65, phospho (p)-p65, p-IκBα, IκBα, and GAPDH were purchased from Cell Signaling Technology (Danvers, MA). Secondary antibodies for goat anti-rabbit immunoglobulin G and donkey anti-rabbit IgG-labeled were from Abcam (Cambridge, MA). 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) and DMSO were from the Sigma Company (St. Louis, MO).

### *Cell Culture*

The human gastric epithelial cell lines SGC-7901 and MGC-803 were purchased from the American Type Tissue Culture Collection (Manassas, VA). The cells were cultured in DMEM/F12, supplemented with 10% fetal bovine serum and 100 U/ml penicillin and streptomycin (Sigma-Aldrich, St. Louis, MO), in a humidified atmosphere containing 5% $CO_2$ at 37°C.

### *Cell Proliferation Assay*

Cell proliferation was assessed by the Cell Counting Kit8 (CCK-8). Briefly, cells were seeded on 96-well microplate at a density of $1 \times 10^4$ cells per well. After culturing for 4 hours, cells were harvested 24 hours after incubation with 5, 10, and 20 ng/ml IFN-γ. Then, 10 μl of CCK-8 solution was added to each well and incubated at 37°C for 3 hours. Optical density was determined at a wavelength of 450 nm.

### *Apoptosis Analysis*

The effect of IFN-γ on the apoptosis of SGC-7901 and MGC-803 cells was evaluated by flow cytometry using the Annexin V PE Apoptosis kit (BD Pharmingen, USA). Firstly, SGC-7901 and MGC-803 cells were incubated with 5, 10, and 20 ng/mL IFN-γ or treated with EDTA-free trypsin for 24 hours. Afterwards, cells were washed by PBS (4°C) followed by resuspending the cell pellet with 300 μl of 1× binding buffer. Next, 5 μl of Annexin V-PE was added to the cell suspension for 15 minutes in the dark at room temperature, according to the manufacturer's instructions. Five microliters of 7-AAD solution was added in the cell suspension at 5 minutes, and then 200 μl of 1× binding buffer was added for flow cytometry analysis. The percentage of apoptotic cells was evaluated by FACS Calibur (BD Biosciences, USA).

### *Wound-Healing Assay*

SGC-7901 and MGC-803 cells were seeded in a six-well plate and cultured in medium with or without the indicated doses of IFN-γ. When cells were grown to 80% confluency, the cell monolayers were wounded by yellow pipette tips and washed by serum-free medium to remove floating cells. Wounded monolayers were then incubated in fresh complete medium for 24 hours. Cell migration into the wound area was observed and counted under an inverted microscopy.

### *Cell Invasion Assay*

The cell invasion assay was carried out using Transwell chambers (8-μm pore size, Corning Costar, Cambridge, MA) coated with Matrigel. SGC-7901 and MGC-803 cells ($1.5 \times 10^5$ cells/chamber) were seeded in the upper chamber with or without the indicated doses of IFN-γ and incubated for 24 hours at 37°C, 5% $CO_2$. In the lower chambers, the medium with 20% FBS was placed acting as a chemoattractant. After incubation, all of the noninvaded cells on the upper surface were removed with a cotton swab; the invaded cells on the lower surface were fixed with 4% methanol and then stained with 1% crystal violet. The invaded cells were counted with a microscope, and six randomly chosen fields were counted for each assay.

### *Western Blot Analysis*

The total protein of cells was extracted according to the manufacturer's recommended protocol (Vazyme, USA). The protein concentrations were determined using the BCA Protein Assay Kit (Vazyme, USA). Samples with equal amounts of protein (50 μg) were fractionated on 10% SDS polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and blocked in 5% skim milk in TBST for 1.5 hour at 25°C ± 1°C. The membranes were then incubated at 4°C overnight with 1:1000 dilutions (v/v) of the primary antibodies. After washing the membranes with TBST, incubations with 1:1000 dilutions (v/v) of the secondary antibodies were conducted for 2 hours at 25°C ± 1°C. Protein expression was detected using an Enhanced Chemiluminescence Detection System. GAPDH were used as a loading control.

### *Immunofluorescence*

SGC-7901 and MGC-803 cells were cultured in medium with or without IFN-γ (5, 10, 20 ng/ml) for 24 hours. And then cells were fixed in formaldehyde for 1 hour at room temperature. After washing with PBS, cells were permeabilized with 0.05% Triton X-100 in PBS for 15 minutes and incubated with preblock buffer (3% BSA, 0.02% Triton X-100 in PBS) for 15 minutes before being probed with primary antibody. Then, cells were stained with primary antibody p65 (anti-rabbit, 1:100) at 4°C overnight, followed by donkey anti-rabbit IgG-labeled secondary antibody for 30 minutes at room temperature. Nuclei were stained with DAPI (1:1000). Images were obtained using a fluorescence microscope.

### *RNA Isolation and Quantitative Real-Time PCR (qRT-PCR)*

Total RNA was isolated using TRIzol according to the manufacturer's protocol. Equal amounts of RNA were transcribed into cDNA using RNeasy plus micro kit. The total cDNA was used as

starting material for real-time PCR with FastStart Universal SYBR Green Master (Roche Applied Science, Mannheim, Germany) on the StepOne real-time PCR System (Life Technologies Corp). The Primer Premier software (PREMIER Biosoft International, USA) was used to design specific primers for integrin β3 and GAPDH based on known sequences. The primers for integrin β3 were F: 5′-CCATG ATCGGAAGGAGTTTGCT-3′; R: 5′-AAGGTGGATG TGGCCTCTTT ATAC-3′. The expression levels of each target gene were normalized to corresponding GAPDH threshold cycle (CT) values using the $2^{-\Delta\Delta CT}$ comparative method.

### *Animal Model*

Female nude mice (6 weeks old, 18-22 g) were obtained from Shanghai Jiesijie Experimental Animal Company. Mice were given free access to water and standard rodent chow and were housed in pathogen-free cages. The animals were acclimated for a week before the experiments. Animal welfare and experimental procedures complied with national guidelines and were approved by the Animal Experimental Ethical Committee of Nanjing Medical University.

Mice were randomly divided into four groups: control (PBS), control-siRNA (nonspecific control siRNA), integrin β3-siRNA (integrin β3-siRNA), and IFN-γ (integrin β3-siRNA + IFN-γ 20 ng/ml).

*Xenograft Model.* After being anesthetized by inhalation, mice were inoculated the cells (100 μl of $1 \times 10^6$ cells) into the right axilla of the mice. And then mice were sacrificed on day 28; the subcutaneous tumors were removed, and the tumor volume was calculated.

*Pulmonary Metastasis Model.* Mice were injected 100 μl of $1 \times 10^5$ cells into the median tail vein. Then mice were sacrificed on day 21, and the lungs were removed and weighed.

### *TUNEL Staining*

The apoptosis of paraffin-embedded tumor sections was detected using a TUNEL assay kit according to the manufacturer's manual (Roche, USA). In brief, fixed and paraffin-embedded sections were dewaxed and then permeabilized with proteinase K for 15 minutes at room temperature. Sections were treated with 3% $H_2O_2$ to block endogenous peroxidases and incubated with equilibration buffer and terminal deoxynucleotidyl transferase enzyme. Finally, sections were incubated with antidigoxigenin-peroxidase conjugate. Tissue peroxidase activity was evaluated through 3, 3′-diaminobenzidine (DAB) application. Sections were examined under a light microscope.

### *Immunohistochemistry Assay*

Mice lung samples were freshly isolated and fixed in 10% neutral buffered formalin and then embedded in paraffin wax. Lung sections with a thickness of 4 μm were mounted onto slides. Slides were deparaffinized with xylene, rehydrated with ethanol, and incubated with $H_2O_2$ at 37°C for 10 minutes. Following blocking using 1.5% normal goat serum (Shanghai Yeasen Biotechnology Co., Ltd.) at 37°C for 20 minutes, sections were incubated overnight with Ki-67 or NF-κB p65 monoclonal antibody (1:1000 dilutions). The sections were incubated with biotin-conjugated goat anti-rabbit immunoglobulin G secondary antibody (diluted with 3% bovine serum albumin/PBS) at 37°C for 30 minutes and then incubated with horseradish peroxidase–conjugated streptavidin at 37°C for 30 minutes. DAB was used as chromogenic agent. Images were obtained using a fluorescence microscope (FSX100; Olympus, Southend-on-Sea, UK).

### *Histological Assay*

The lung tissues were obtained and fixed in 10% formalin and then stained by hematoxylin-eosin staining. Ten random areas of interest were examined in each section and were identified by computer-generated field identification. At least six different sections of lung tissues were examined for each animal in groups. Images were obtained using a fluorescence microscope.

### *Statistical Analysis*

GraphPad Prism 5 software was used to carry out all statistical analyses. One-way analysis of variance was used for multiple-group comparison; when only two groups were compared, Student's *t* test was performed. *P* values of $<.05$ were considered statistically significant.

## Results

### *IFN-γ Enhances Proliferation and Inhibits Apoptosis in Gastric Cells*

To evaluate the effect of IFN-γ on gastric cancer cell viability, the CCK-8 assay was used on SGC-7901 and MGC-803 cells. It showed that IFN-γ dramatically improved the cell viability in both SGC-7901 and MGC-803 cells with a dose-dependent manner (Figure 1*A*).

Furthermore, flow cytometry analysis was performed to determine the cell apoptosis. The cells in the upper-right (UR, Q2) and lower-right (LR, Q3) quadrants of the FACS histogram represent apoptotic cells. As shown in Figure 1*B*, after the treatment of IFN-γ, the apoptosis rates of SGC-7901 and MGC-803 cells were dose-dependently inhibited compared with the control.

### *IFN-γ Promotes Gastric Cell Migration and Invasion*

The wound-healing assay and Transwell assay were performed in SGC-7901 and MGC-803 cells to detect the cell migration and invasion, respectively. The rate of migration cells was obviously increased with the treatment of IFN-γ in a dose-dependent manner, which was similar to the rate of the invasion cells (Figure 2, *A* and *B*).

Matrix metalloproteinases (MMPs), the zinc-dependent proteolytic enzymes of the extracellular matrix, are widely used by cells during invasion and migration [20,21]. MMP2 and MMP9 have been strongly correlated with the invasiveness of many types of cancer cells [22,23]. Thus, the protein expression of MMP-2 and MMP-9 was detected to evaluate the effect of IFN-γ on the invasiveness of gastric cells. And it showed that IFN-γ significantly heightened the protein expression of MMP-2 and MMP-9 (Figure 2*C*).

### *IFN-γ Activates NF-κB Signaling Pathway*

NF-κB has a pivotal role in many cellular processes, including the inflammatory and immune responses [24]. Aberrant NF-κB activation, a consequence of underlying inflammation in tumor microenvironment that can promote cancer invasion and metastasis, has been observed in many tumors, including gastric cancer [25]. Herein, to further investigate the mechanism of IFN-γ on gastric cancer, the expression of NF-κB, including p65 and IκBα, was examined, and the immunohistochemistry assay was used to determine the protein level of NF-κB/p65. The results showed that IFN-γ significantly enhanced the phosphorylation expression of p65 and IκBα compared with the control (Figure 3*A*). Meanwhile, IFN-γ could dramatically block NF-κB/p65 nuclear translocation (Figure 3*B*). These data suggested that IFN-γ could activate NF-κB signaling pathway.

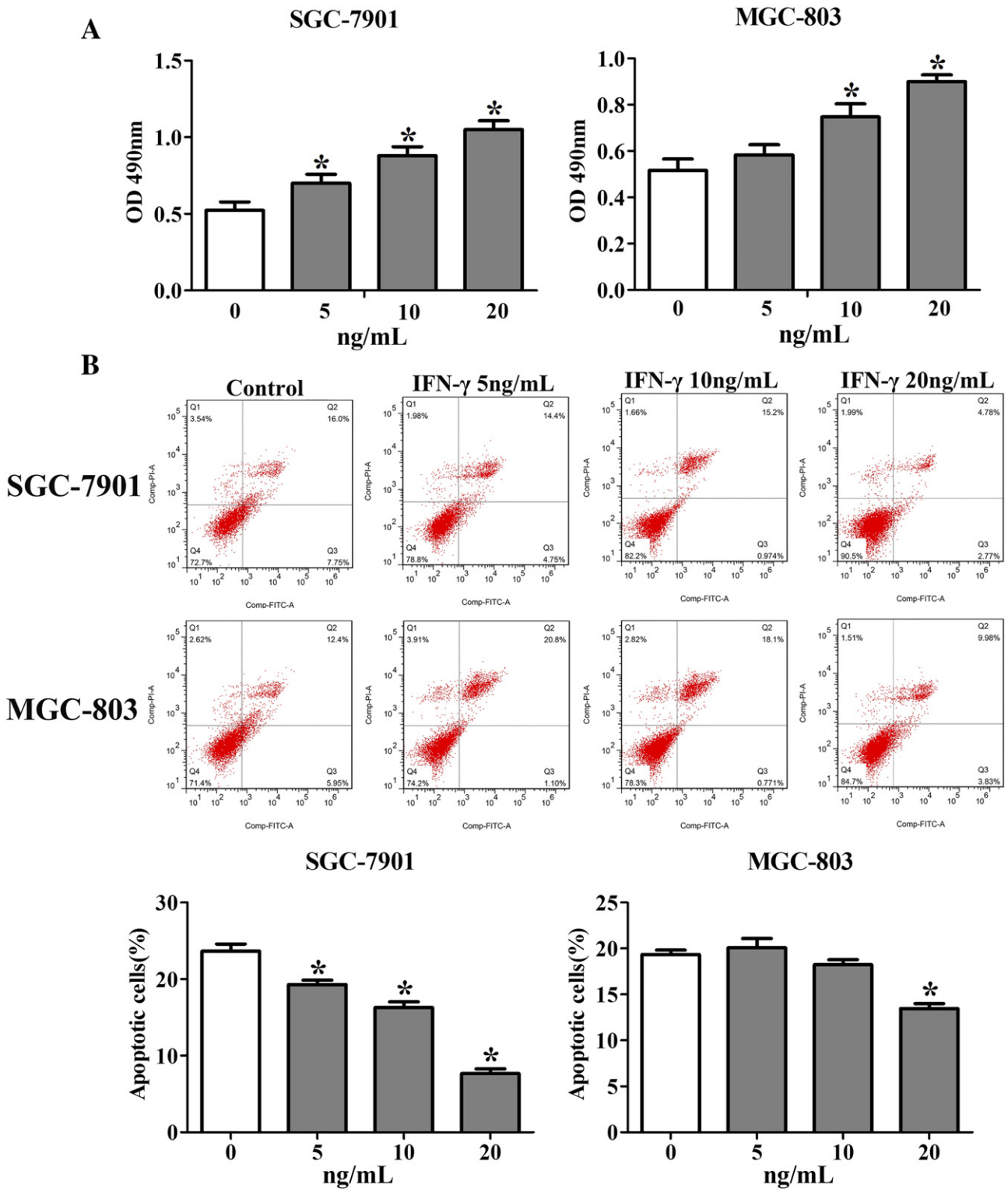


**Figure 1.** IFN-γ enhances proliferation and inhibits apoptosis in gastric cells. (A) SGC-7901 and MGC-803 cells were treated with different concentrations of IFN-γ for 24 hours and then assayed by CCK-8. (B) SGC-7901 and MGC-803 cell apoptosis was determined by flow cytometry analysis. Quantitative results for apoptosis cell rates are shown. Bars indicate the mean ± SEM, $P < .05$ versus control group.

### *IFN-γ Enhances Proliferation, Migration, and Invasion and Inhibits Apoptosis Partially Through the Upregulation of Integrin β3*

After the treatment of IFN-γ, the expression of integrin β3 was dose-dependently increased after the treatment of IFN-γ, indicating that there is a potential relationship between IFN-γ and integrin β3 (Figure 3*C*). Therefore, to further investigate the relationship between IFN-γ and integrin β3, the SGC-7901 cells were transfected with integrin β3 siRNA or nonspecific control siRNA.

After the transfection with integrin β3 siRNA, the mRNA and protein expressions of integrin β3 in SGC-7901 cells were

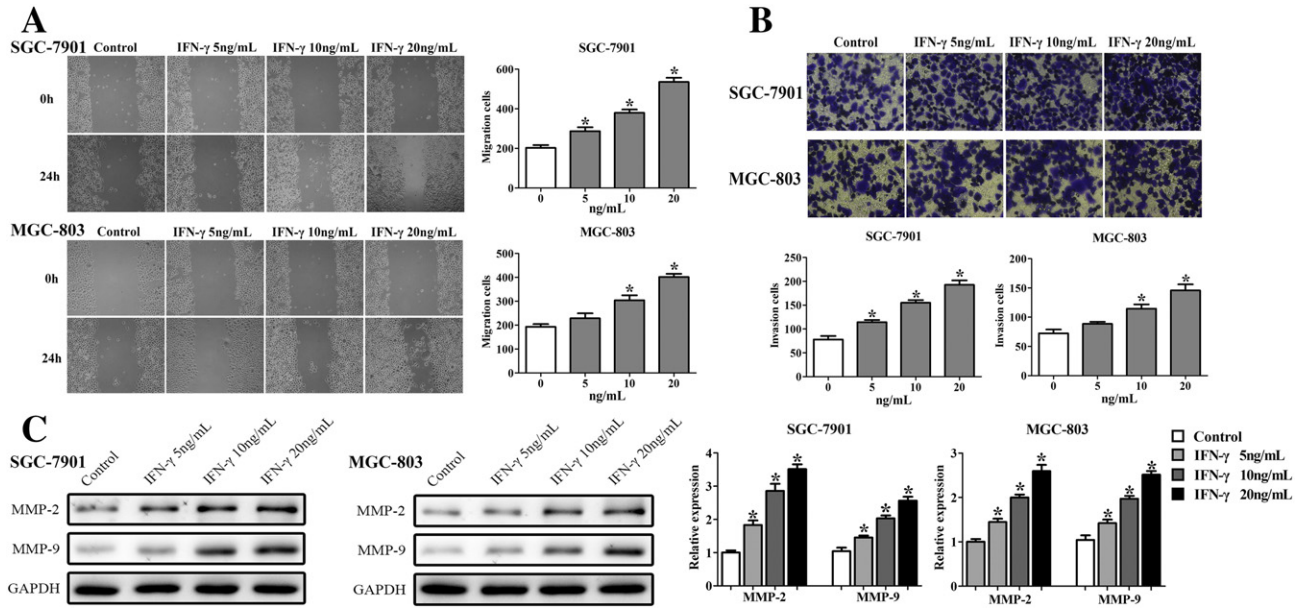


**Figure 2.** IFN-γ promotes gastric cell migration and invasion. (A) Migrated cells were counted in nine random fields from each treatment. The photographs were taken at the magnification of ×40. Quantitative results for wound healing data are shown. (B) The invasion ability of cells was quantified by counting the number of cells that invaded the underside of the porous polycarbonate membrane. The photographs were taken at the magnification of ×200. Quantitative results for invasion data are shown. (C) Western blots were performed to detect protein levels of MMP-2 and MMP-9. GAPDH was used as a control. Bars indicate the mean ± SEM, $P < .05$ versus control group.

dramatically reduced, as well as the cell viability, compared with the controls. However, the treatment of IFN-γ obviously improved the mRNA and protein expression of integrin β3, together with cell viability (Figure 4, *A*, *B*, and *F*). Analogously, the apoptosis rate of SGC-7901 cells dramatically improved in the integrin β3 siRNA group compared with the controls, whereas it was effectively inhibited by IFN-γ (Figure 4*C*). Besides, the rates of migration cells and invasion cells obviously decreased in the integrin β3 siRNA group compared with the controls. After the treatment of IFN-γ, the rates of both migration cells and invasion cells were significantly heightened compared with the integrin β3 siRNA group (Figure 4, *D* and *E*).

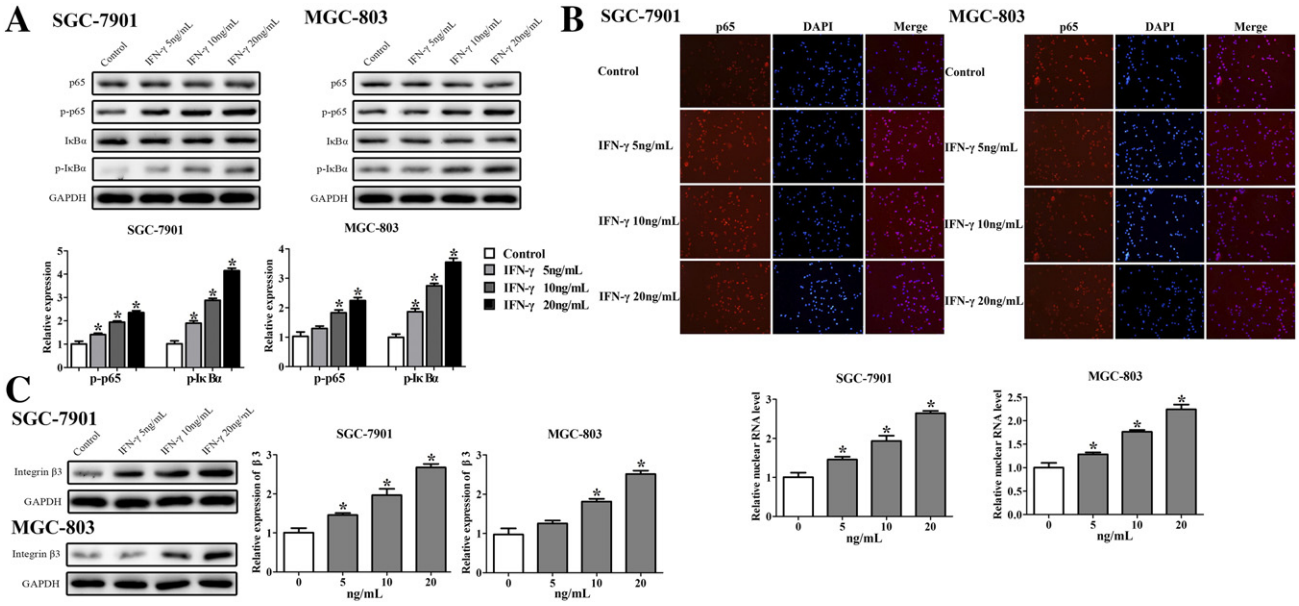


**Figure 3.** IFN-γ activates NF-κB signaling pathway and dose-dependently upregulates integrin β3. (A) Western blots were performed to detect protein levels of NF-κB along with their phosphorylation. GAPDH was used as a control. (B) Immunofluorescence assay was used to detect the translocation of NF-κB/ p65. The photographs were taken at the magnification of ×200. Quantitative results for relative nuclear mRNA level are shown. (C) The expression of integrin β3 was detected by Western blots. GAPDH was used as a control. Bars indicate the mean ± SEM, $P < .05$ versus control group.

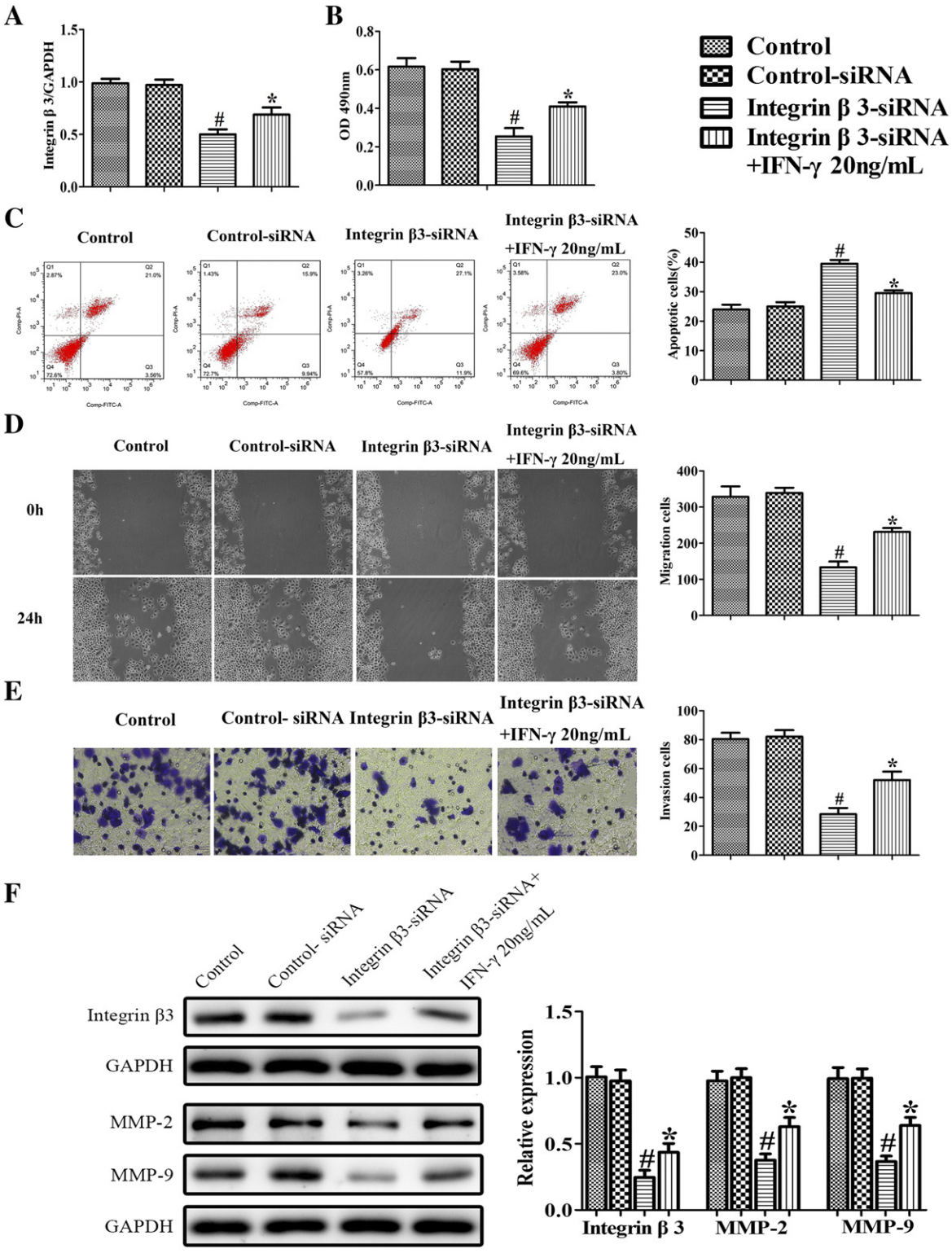


**Figure 4.** IFN-γ enhances proliferation, migration, and invasion and inhibits apoptosis partially through the upregulation of integrin β3. SGC-7901 cells were transfected with integrin β3 siRNA or nonspecific control siRNA. (A) The mRNA level of integrin β3 was experimented by qRT-PCR. GAPDH was used as a control. (B) CCK-8 assay was performed to determine cell viability. (C) Cell apoptosis was determined by flow cytometry analysis. Quantitative results for apoptosis cell rates are shown. (D) Migrated cells were counted in nine random fields from each treatment. The photographs were taken at the magnification of ×40. Quantitative results for wound healing data are shown. (E) The invasion ability of cells was quantified by counting the number of cells that invaded the underside of the porous polycarbonate membrane. The photographs were taken at the magnification of ×200. Quantitative results for invasion data are shown. (F) Western blots were performed to detect protein levels of integrin β3, MMP-2, and MMP-9. GAPDH was used as a control. Bars indicate the mean ± SEM, $^{\#}P < .05$ versus control group, $^{*}P < .05$ versus integrin β3 siRNA group.

Additionally, the protein expression of MMP-2 and MMP-9 was dramatically repressed in the integrin β3 siRNA group, while the expression was increased after the treatment with IFN-γ (Figure 4*F*). Therefore, the results revealed that IFN-γ could enhance proliferation, inhibit apoptosis, and promote SGC-7901 cell migration and invasion possibly by upregulating integrin β3.

### *IFN-γ Activates NF-κB Signaling Pathway Partially Through the Upregulation of Integrin β3*

To further investigate the mechanism of IFN-γ related to integrin β3, the expression of NF-κB, including p65 and IκBα, was examined, and the immunohistochemistry assay was used to determine the protein level of NF-κB/p65. The results showed that integrin β3 siRNA significantly inhibited the phosphorylation expression of p65 and IκBα compared with the controls. In contrast, after the treatment with IFN-γ, the phosphorylation expression of p65 and IκBα was recovered (Figure 5*A*). Furthermore, the nuclear entry of NF-κB/p65 was dramatically decreased by integrin β3 siRNA with the opposite result in IFN-γ group (Figure 5*B*). It suggested that IFN-γ activated NF-κB signaling pathway partially through the upregulation of integrin β3.

### *IFN-γ Increases Tumor Proliferation and Inhibits Cell Apoptosis in Mice Through the Upregulation of Integrin β3*

To further confirm the relationship between IFN-γ and integrin β3, different groups of SGC-7901 cells were inoculated subcutaneously into the right axilla of nude mice. All mice were sacrificed on day 28, and the tumors were obtained and measured the tumor volume. It revealed that integrin β3 siRNA significantly suppressed the tumor volume compared with the controls, while the treatment of IFN-γ dramatically increased the tumor volume (Figure 6*A*). The TUNEL assay indicated that, compared with the controls, the integrin β3 siRNA clearly decreased tumor cell apoptosis, whereas IFN-γ obviously enhanced the apoptosis rate, which was similar to the result of Ki-67 (Figure 6*B*).

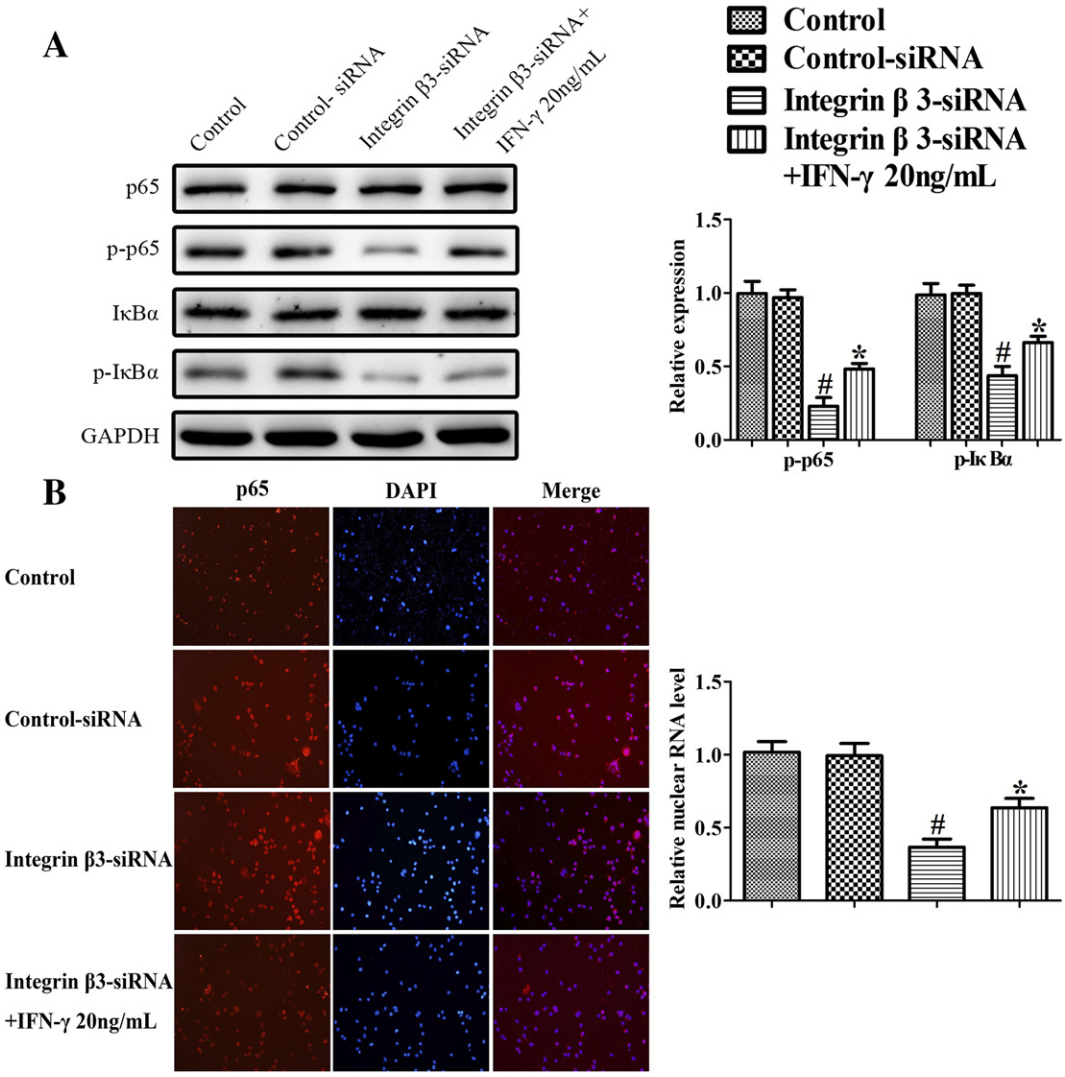


**Figure 5.** IFN-γ activates NF-κB signaling pathway partially through the upregulation of integrin β3. SGC-7901 cells were transfected with integrin β3 siRNA or nonspecific control siRNA. (A) Western blots were performed to detect protein levels of NF-κB along with their phosphorylation. GAPDH was used as a control. (B) Immunofluorescence assay was used to detect the translocation of NF-κB/ p65. The photographs were taken at the magnification of ×200. Quantitative results for relative nuclear mRNA level are shown. Bars indicate the mean ± SEM, $^{\#}P < .05$ versus control group, $P < .05$ versus integrin β3 siRNA group.

As shown in Figure 6*C*, integrin β3 siRNA group exhibited obviously lower expression of integrin β3 compared with the controls. Similarly, the phosphorylation expression of p65 and IκBα was significantly repressed in integrin β3 siRNA group. However, in IFN-γ group, the expression of integrin β3 along with the phosphorylation expression of p65 and IκBα was drastically strengthened compared with the integrin β3 siRNA group. Thus, the results revealed that IFN-γ could promote proliferation and inhibit apoptosis *in vivo* via the upregulation of integrin β3.

### *IFN-γ Promotes Tumor Metastasis in Mice Through the Upregulation of Integrin β3*

The pulmonary metastasis model was performed to further determine the relationship of IFN-γ and integrin β3. Cells were injected from tail vein, and then mice were sacrificed on day 21; the lung tissues were removed and weighed. The tumor nodules were counted, and then the lung tissues underwent histological assay. Afterwards, the total protein was collected from lung tissues, and an immunohistochemistry assay was used to detect the expression of NF-κB/ p65. The lung/total weight and tumor

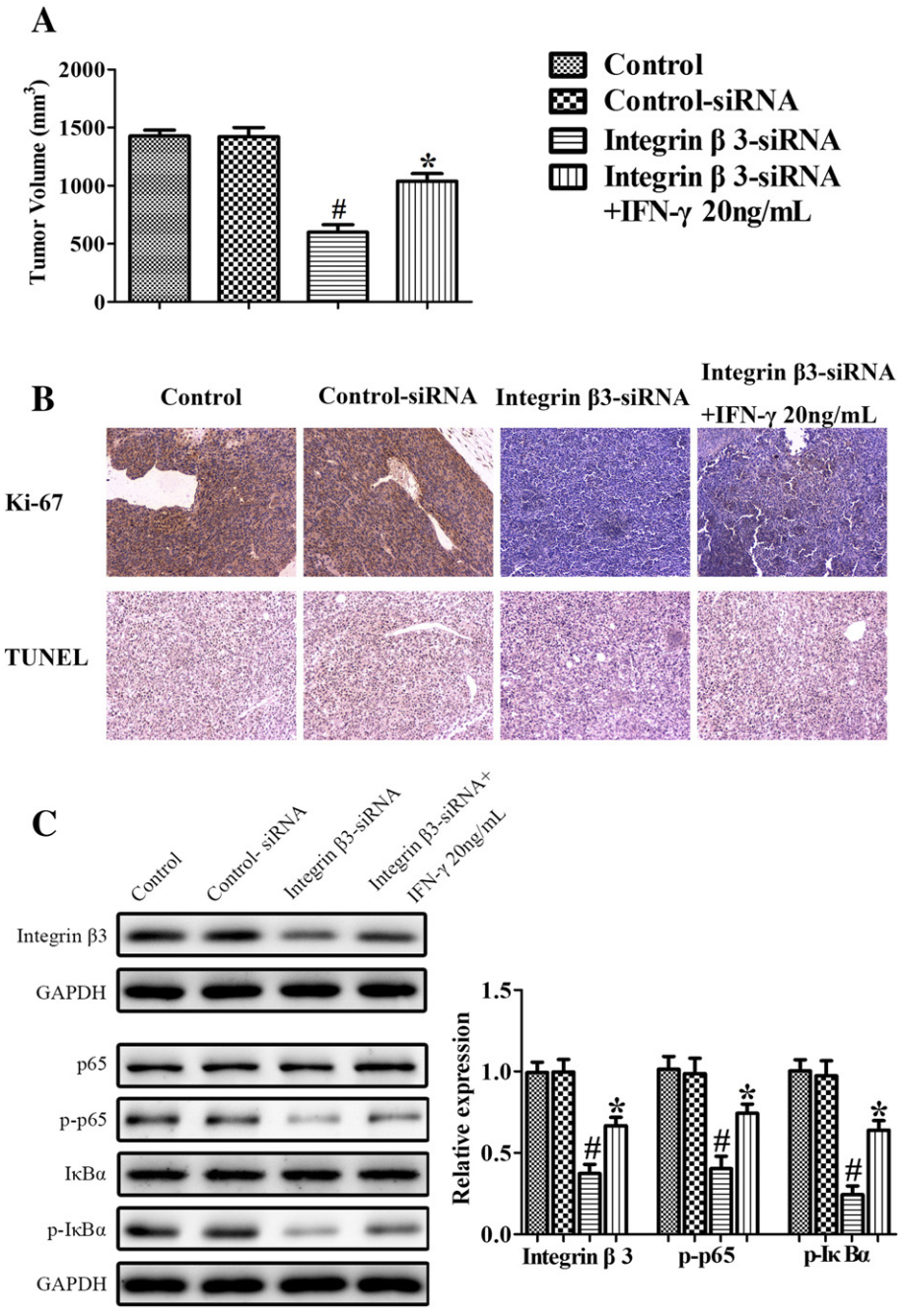


**Figure 6.** IFN-γ increases tumor proliferation and inhibits cell apoptosis in mice through the upregulation of integrin β3. After the transfection with integrin β3 siRNA, different groups of SGC-7901 cells were inoculated subcutaneously into the right axilla of nude mice. All mice were sacrificed on day 28, and the tumors were obtained. (A) The tumor volume was calculated. (B) The expression of Ki-67 was determined by immunohistochemistry assay, and the TUNEL assay in tumor issue was performed. The photographs were taken at the magnification of ×200. (C) Western blots were performed to detect protein levels of integrin β3, p65, p-p65, IκBα, and p-IκBα. GAPDH was used as a control. Bars indicate the mean ± SEM, $^{\#}P < .05$ versus control group, $P < .05$ versus integrin β3 siRNA group.

nodules were obviously reduced in the integrin β3 siRNA group, while they were significantly elevated in the IFN-γ group compared with the integrin β3 siRNA group (Figure 7*A*). Additionally, the lung metastases lesions were visualized by histological examination (Figure 7*B*). It indicated that mice in the IFN-γ group showed more metastases in the lungs than those in the integrin β3 siRNA group. Furthermore, the integrin β3 siRNA group showed obviously lower expression of integrin β3, as well as MMP-2 and MMP-9, and lower phosphorylation expression of p65 and IκBα compared with controls. However, after the treatment with IFN-γ, the expression was significantly improved (Figure 7*C*). Collectively, these results provide the direct evidence to support our hypothesis that IFN-γ increased tumor metastasis in mice through the upregulation of integrin β3.

## Discussion

Worldwide, gastric cancer is a major malignancy and the second leading cause of cancer-related death. Local invasion of the host tissue and metastasis are hallmarks of cancer progression. Focal adhesions are not only structural links between the extracellular matrix (ECM) and actin cytoskeleton but also comprise important sites of signal transduction pathways leading to various physiological and pathological processes, including cancer [26]. At the molecular level, focal adhesions are mainly mediated by integrins, which have been shown to play a critical role in the invasion and metastasis of cancer [27].

Integrin β3 mediates cellular adhesion to ECM substrates, including fibrinogen, and is an attractive therapeutic target for metastatic cancers [28]. Studies employing αv or β3 inhibitors

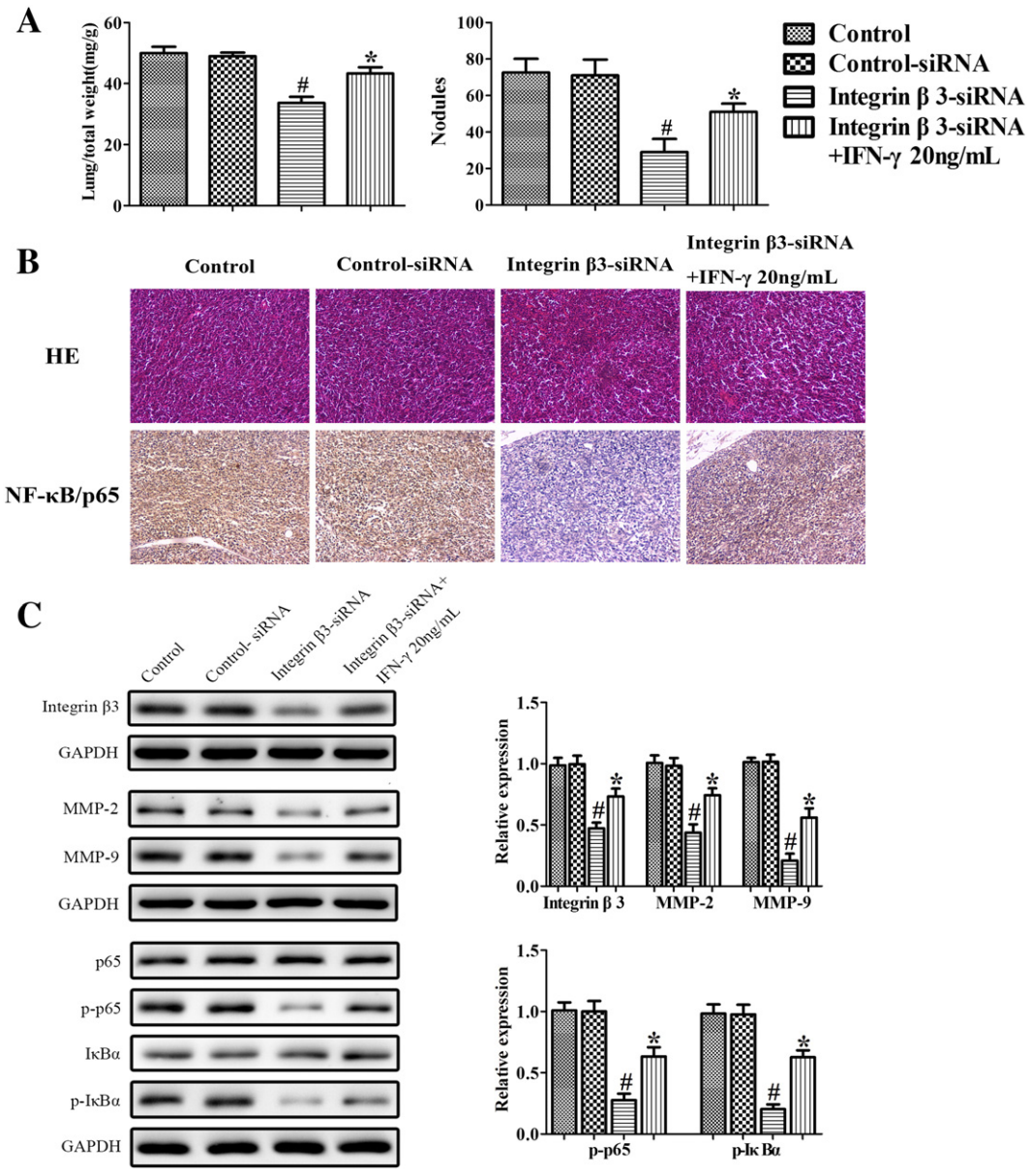


**Figure 7.** IFN-γ promotes tumor metastasis in mice through the upregulation of integrin β3. After the transfection with integrin β3 siRNA, different groups of SGC-7901 cells were injected from tail vein; mice were sacrificed on day 21, and the lung tissues were removed and weighed. (A) The lung/total weight and tumor nodules were detected. (B) A representative histological view of the liver sections was photographed, and the expression of NF-κB/ p65 was determined by immunohistochemistry assay. The photographs were taken at the magnification of ×200. (C) Western blots were performed to detect protein levels of integrin β3, MMP-2, MMP-9, and NF-κB. GAPDH was used as a control. Bars indicate the mean ± SEM, $^{\#}P < .05$ versus control group, $P < .05$ versus integrin β3 siRNA group.

demonstrated that αvβ3 integrin regulated multiple cellular responses required for metastasis, including cell survival, migration, and invasion, through the ECM and angiogenesis [29].

IFN-γ, a cytokine that is critical for innate and adaptive immunity, is secreted by activated effector T cells [30]. It showed that IFN-γ, which is secreted by CD8-positive lymphocytes, upregulated PD-L1 on ovarian cancer cells and promoted tumor growth [17]. Besides, the secretion of IFN-γ and TNF-α was suppressed by regulatory B cells, which played a immunosuppressive role in gastric cancer [18]. And IFN-γ was upregulated in the resected gastric cancer tissue compared to matched adjacent noncancerous tissue [19]. Nevertheless, whether IFN-γ is involved in the regulation of gastric cancer is not well elucidated. Herein, this study aimed to investigate the effect and mechanism of IFN-γ on gastric cancer.

3NF-κB is an important transcription factor in chronic inflammatory diseases and can regulate numerous inflammatory responses [31]. NF-κB proteins are a group of related transcription factors, which in mammals consists of five members, including Rel, RelA (p65), RelB, NF-κB1 (p50), and NF-κB2 (p52) [32]. NF-κB is activated by many divergent stimuli, including bacteria, lipopolysaccharide, and viruses, and proinflammatory cytokines, such as TNF-α and IL-1 [33]. It had been clearly established that constitutive activation of NF-κB may play an important role in gastric cancer initiation and progression [34–38].

In this study, the results revealed that IFN-γ enhanced proliferation, inhibited apoptosis, and promoted cell migration and invasion in human gastric epithelial cell lines SGC-7901 and MGC-803. Besides, IFN-γ activated NF-κB signaling pathway by upregulating the phosphorylation expression of p65 and IκBα. Furthermore, IFN-γ could dose-dependently upregulate integrin β3 *in vitro*. Therefore, to further investigate the relationship between IFN-γ and integrin β3, the SGC-7901 cells were transfected with integrin β3 siRNA, and the transfected cells were experimented *in vitro and* in vivo. Data showed that after the transfection of integrin β3 siRNA, SGC-7901 cells had expressed lower cell viability, migration, and invasion rates, while the cell apoptosis rate was enhanced. Moreover, NF-κB signaling pathway was blocked. *In vivo,* tumor proliferation and metastasis in mice were also inhibited via NF-κB signaling pathway. However, the treatment with IFN-γ obviously improved these effects. It suggested that IFN-γ could increase tumor proliferation and metastasis partially through the upregulation of integrin β3.

In conclusion, this study indicated that IFN-γ could induce gastric cancer cell proliferation and metastasis partially through the upregulation of integrin β3-mediated NF-κB signaling. Hence, the inhibition of IFN-γ and integrin β3 may be the key for the treatment of gastric cancer.

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## Conflict of Interests

The authors declare no conflict of interests.

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