Effect of RNA Interference Inhibiting the Expression of the FUBP1 Gene on Biological Function of Gastric Cancer Cell Line SGC7901

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ABSTRACT

Background: *The research aimed to observe the effect of gene silencing on the proliferation, migration, cell cycle, apoptosis, and other biological functions of human gastric cancer cells with RNA interference inhibiting the expression of the far upstream element-binding protein 1 (FUBP1) in the gastric cancer cell line SGC7901.*

Methods: *The shRNA lentivirus vector of the target gene FUBP1 was constructed to transfect the gastric cancer cell line SGC7901. The qRT-PCR and western blot assays were used to detect the expression levels of FUBP1 mRNA and protein in the gastric cancer cells. The CCK-8 assay was used to detect the proliferation of gastric cancer cells. The cell scratch assay and the transwell assays were used to detect the migration of gastric cancer cells. Flow cytometry was used to detect cell cycle distribution and apoptosis.*

Results: *The shRNA lentiviral vector of FUBP1 was successfully transfected into the gastric cancer cell line SGC7901, and could effectively reduce the expression of mRNA and protein of FUBP1. The silencing of FUBP1 could inhibit the gastric cancer cell proliferation and affect the distribution of the cell cycle, resulting in S-phase arrest and cell growth inhibition. However, FUBP1 silencing has no significant effect on cell apoptosis and migration.*

Conclusions: *The expression of FUBP1 can be inhibited specifically and effectively by RNA interference technology, which can significantly affect the biological function of the gastric cancer cell line SGC7901.*

Keywords: *RNA interference, lentiviral transfection, FUBP1, gastric cancer, gene therapy*

INTRODUCTION

Malignant gastric tumor is a common type of gastrointestinal cancer around the world, especially in the developing countries, characterized by high malignancy, rapid development, and poor prognosis. Its incidence ranks fourth among the common cancers around the world and its rate of mortality ranks third.[1] The 2012 global cancer statistics show that the number of new cases of gastric cancer is nearly 1 000 000, of which over 700 000 result in mortality. More than half of these deaths occur in China.[2] In recent years, with the continuous improvement of technology in the early diagnosis and treatment of gastric cancer, the rates of incidence and mortality of patients with gastric cancer have decreased, but the prognosis of patients in an advanced stage is still relatively poor, with the 5-year survival rate still relatively low. The traditional treatment methods cannot meet the need of the current situation in the development of therapy for gastric cancer. Therefore, finding a method of gene therapy for gastric cancer has become a hot topic in the medical field.

RNA interference is a kind of gene-silencing phenomenon with high conservation and specificity in eukaryotic cells. Guo and Kemphues first found and determined that antisense RNA and sense RNA could inhibit the expression of the *par-1* gene of *Caenorhabditis elegans* when they used antisense RNA to inhibit the gene expression of *C. elegans*, but the cause of this phenomenon has not been explained.[3] In 1998, Fire found that double-stranded RNA (dsRNA) could recognize specific sequences of endogenous mRNA to induce the degradation of mRNA to inhibit the expression of specific genes, which is stronger than

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the inhibiting effect of single-stranded antisense RNA or sense RNA on gene expression.[4] This phenomenon is known as RNA interference (RNAi). In the last decade, with the continuous development of molecular biology and genetics, RNA interference has been widely used in gastric cancer studies as a kind of strong and effective gene-silencing technology, particularly to inhibit the expression of tumor-related oncogenes.

Far upstream element-binding proteins (FUBPs), consisting of FUBP1, FUBP2, and FUBP3, belong to the DNA-binding protein family.[5] In 1990, Avigan found that in undifferentiated leukemia cells, the far upstream element-binding protein 1 (FUBP1) could regulate the transcription of the oncogene *c-myc* through binding to the distal upstream regulatory element (FUSE), firstly determining that FUBP1 is a kind of DNA-binding protein.[6] Some subsequent studies have found that FUBP1 binds to FUSE, the FUBP-interference inhibitor (FIR), and the transcription repair system (TFIIH) to form the FUSE/FUBP/FIR/TFIIH complex,[7] which participates in the regulation of transcription expression of the oncogene *c-myc* and affects *c-myc* gene-mediated cell growth, proliferation, differentiation, and apoptosis, resulting in the development and progression of multiple malignant tumors.[8] This study aimed to apply lentiviral vector-mediated RNA interference to inhibit the expression of the *FUBP1* gene and to preliminarily explore the value of application of RNA interference on the gene therapy for gastric cancer, by observing the effect of *FUBP1*-silencing on the biological functions of the human gastric cancer cell line SGC7901 through in vitro cell experiments.

MATERIALS AND METHODS

Materials

The RNA interference lentiviral vector of the targeted gene *FUBP1* was provided by Shanghai Jikai Gene Science and Technology Ltd; the human gastric adenocarcinoma cell line SGC7901 was provided by Basic Experimental Center of Shandong Cancer Hospital; the RPMI1640 basal culture medium was purchased from the Corning Company (NY, USA); fetal bovine serum (FBS) was purchased from Ausbian Company (Australia); DMSO was purchased from Gibco (USA) ; Trizol reagent was purchased from Invitrogen Life Technologies; rabbit-anti-human FUBP1 monoclonal antibody was purchased from Abcam (Cambridge, UK); reverse transcription kit and SYBR real-time PCR kit were provided by TaKaRa (Shiga, Japan); CCK-8 reagent and PI (propodium iodide) were purchased from Sigma; the apoptosis assay kit was purchased from eBioscience Company; the transwell reagent kit was purchased from Corning (NY, USA).

Since this study was carried out on cells, ethics committee approval and informed consent were not required.

Construction and Packaging of FUBP1-RNA Lentiviral Vector

The design, construction, packaging, and sequencing of the RNA interference lentiviral vector for the target gene *FUBP1* were assisted by Shanghai Genechem Co., LTD. The lentiviral vector was named as GV248, with a sequence element of hU6-MCS-Ubiquitin-EGFP-IRES-puromycin. A total of 3 kinds of lentiviral vectors with different RNA interference sequences were designed, including FUBP1-RNAi-KD1, with the sequence of 5′-TGAGTATTATAGACAACAA-3′; FUBP1-RNAi-KD2, with the sequence of 5′-CGAAAGGATAGCACAAATA-3′; and FUBP1-RNAi-KD3, with the sequence of 5′-TGCTTATTACGCTCACTAT-3′. The designed negative-RNAi was 5′-TTCTCCGAACGTGTCACGT-3′.

Cell Culture

The human gastric cancer cell line SGC7901 was cultured in an RPMI1640 culture medium containing 15% FBS. The cell culture was performed at 37°C in a humidified atmosphere containing 5% CO_2. The cell medium was changed every 2 days. Cell passage culture and cell freezing were performed when the cell confluence reached 90%.

Cell Transfection

On the day before lentiviral transfection, the cells in logarithmic growth phase were seeded in different cell culture plates (6-well plate, 12-well plate, 24-well plate, and 96-well plate) to achieve 50-60% cell confluence. On the following day, according to the instructions for lentiviral transfection and with the appropriate multiplicity of infection (MOI), the corresponding amounts of virus and polybrene diluent were added into these wells and mixed for continuing cell culture. In 16 hours after cell transfection, the original medium was changed with 1640 complete medium for continuing cell culture. In 72 hours after cell transfection, the inverted fluorescence microscope was used for determining whether the transfection efficiency was more than 80% and the cell state was good. Then, the further experiments were performed.

Selection of Reasonable MOI

The experiment was divided into 4 groups: MOI= 0, MOI = 20, MOI = 30, and MOI=40. According to different values of MOI, negative-RNAi lentiviral vector was used

for transfection of the gastric cancer cells. After 72 hours, the expression of green fluorescent protein in each group was observed with an inverted fluorescence microscope, and flow cytometry was used to detect the percentage of cells containing green fluorescent proteins and calculate the transfection efficiency of each group. According to the obtained results, the group with a reasonable MOI value was chosen for the further experiments.

Total RNA Extraction and qRT-PCR

The experiment was divided into 5 groups: the FUBP1-RNAi-KD1 group (labeled as KD1), the FUBP1-RNAi-KD2 group (labeled as KD2), the FUBP1-RNAi-KD3 group (labeled as KD3), Negative-RNAi negative control group (labeled as NC), and the blank control group (CON), coupled with 3 duplications of each group. After 72 hours of transfection, the gastric cancer cells of the SGC7901 cell line were collected. Trizol reagent was used for RNA extraction in each group, and NANODROP 2000 spectrophotometer was used for determining the purity and concentration of RNA. According to the instructions of the reverse transcription kit, RNA was reversely transcribed to cDNA. The SYBR real-time PCR was used for PCR amplification and the detection of the expression level of the gastric cancer cell line *FUBP1* mRNA to screen out the RNA interference lentiviral vector with the highest inhibiting efficiency compared with the NC group as the effective target for the further experiments. The primers of the *FUBP1* gene and the reference gene were as follows: the size of the *FUBP1* fragment was about 121bp, coupled with upstream primer 5′-CTGGTGTTCGCATTCAGTT-3′ and downstream primer 5′-CCAGTTGCCTTGACCTCTAC-3′; the size of GAPDH was 191 bp, coupled with upstream primer 5′-TGACTTCAACAGCGACACCCA-3′ and downstream primer 5′-CACCCTGTTGCTGTAGCCAAA-3′. PCR reaction condition: 95°C, degeneration for 30 s, one cycle; PCR reaction: 95°C for 5 s, 60°C for 30 s, 40 cycles; fusion: 95°C for 5 s, 60°C for 1 min, one cycle; cooling: 50°C for 30 s, one cycle. The $2^{-\Delta\Delta Ct}$ method was used for the data analysis of the experimental results.

Total Protein Extraction and Western Blot

NP40 protein lysate was added into the gastric cancer cell line SGC7901 after 72 hours' transfection for extraction of total cell protein in each group. The protein concentration was measured in accordance with BCA quantification kit. SDS-PAGE electrophoresis was used for separating the objective protein and transferring it to the PVDF membrane. Blocking buffer (TBST solution containing 5% skim milk) was used for blocking the PVDF membrane for 1 hour at room temperature or 4°C overnight, followed by antibody hybridization. The first antibody reaction solution of rabbit-anti-human *FUBP1* monoclonal antibody (1:2000) was used for blocking the PVDF membrane, overnight at 4°C. Goat-anti-rabbit IgG secondary antibody reaction solution (1:5000) was used for blocking the PVDF membrane for 1.5 hours at room temperature. The image was developed using an ECL-PLUS kit.

CCK8-Detection of Cell Proliferation

The gastric cancer cells were inoculated into 96-well plates (3000/well), which were grouped and labeled, coupled with each group of 5 duplications. During the 5 days after transfection, one plate was taken out at the same time every day, and 10 μl of CCK-8 reagent was added into each well for 4 hours' culture. The value of the OD450 wave was measured by the microplate reader provided by Thermo Fisher. The experimental results were collected for data analysis.

Cell Cycle Examination

The gastric cancer cells were inoculated into 6-well plates, which were grouped and labeled, coupled with each group of 3 duplications. Cell passage culture was performed on the third day after transfection. The cells were collected on the fifth day and the number of cells in each sample was adjusted to more than 10^6. Pre-cooled PBS was used to wash the cell precipitation once, followed by centrifugation to remove the supernatant and then by the addition of 900 μL pre-cooled 75% ethanol. Finally, the cell sample was fixed at 4°C for one night. PBS was used to wash the cell precipitation once, and 500μl staining solution (50 ×PI (1 mg/mL): 50×RNaseA (2.5mg/ml): 1×PBS = 40: 20: 940) was added. After staining in a dark place in 4°C for 30mins, flow cytometry was used for detection and analysis of the results.

Cell Apoptosis Detection

The gastric cancer cells were inoculated into 6-well plate, which were grouped and labeled, coupled with each group of 3 duplications. Cell passage culture was performed on the third day after transfection. The cells were collected on the fifth day and the number of cells in each cell sample was adjusted to more than 10^6. Pre-cooled PBS and 1×blinding buffer were used to wash the cell precipitation once, and then centrifuged to remove the supernatant, followed by the addition of 400 μL 1×blinding buffer and 10 μL Annexin V-APC staining solution. After staining in a dark place at room temperature for 15 min, flow cytometry was used for the detection and analysis of results.

Cell Wound Scratch Assay

The gastric cancer cells were inoculated into 96-well plate (3×10^4/well), which were grouped and labeled, coupled with each group of 5 duplications. After transfection for 72 hours, the scratch tester was pushed up along the central part of the bottom of the 96-well plate to form a scratch. Serum-free 1640 medium was used to wash the 96-well plate 3 times, and then the medium with a low concentration of serum (0.5% FBS) was added. Images were obtained at 0, 8, and 24 hours after forming the scratch to calculate the migration distance and migration rate.

Transwell Assay

The transwell was inserted into the 24-well plate, grouped and labeled, coupled with 3 duplications. Trypsin was used to digest the gastric cancer cells after transfection for 72 hours, and serum-free 1640 medium was added to form a cell suspension. Then, 100μl of the cell suspension was added into the upper chamber (10^5/well) and 600 μL 1640 medium (30% FBS) was added into the lower chamber as chemokines. The cells were placed in an incubator to culture for 18 hours. A cotton swab was used to gently wipe the non-migratory cells on the upper surface, followed by the addition of 2-3 drops of Giemsa to stain the migrator cells on the lower surface for 5 min, which was then washed out with PBS and observed under the microscope. Images were obtained after drying. Four 100-fold pictures and 9200-fold pictures were obtained of for each transwell chamber. According to the 200-fold picture, the migratory cells per field of each group were calculated.

Statistical Analysis

The data of each experiment were represented as mean ± standard deviation. The homogeneity test of variances and the *t*-test were performed using SPSS 21.0 when any 2 groups were compared, and showed statistically significant difference ($P < .05$).

RESULTS

Selection of Multiplicity of Infection (MOI = 30)

Negative-RNAi lentiviral vectors were used to transfect the gastric cancer cell line SGC7901 in accordance with MOI = 0, MOI = 20, MOI = 30, and MOI = 40. After 72 hours, flow cytometry was used to detect the transfection efficiency, which was 1%, 60.6%, 85.2%, and 93.5% respectively. An inverted fluorescence microscope was used to observe the cell transfection (Figure 1). When

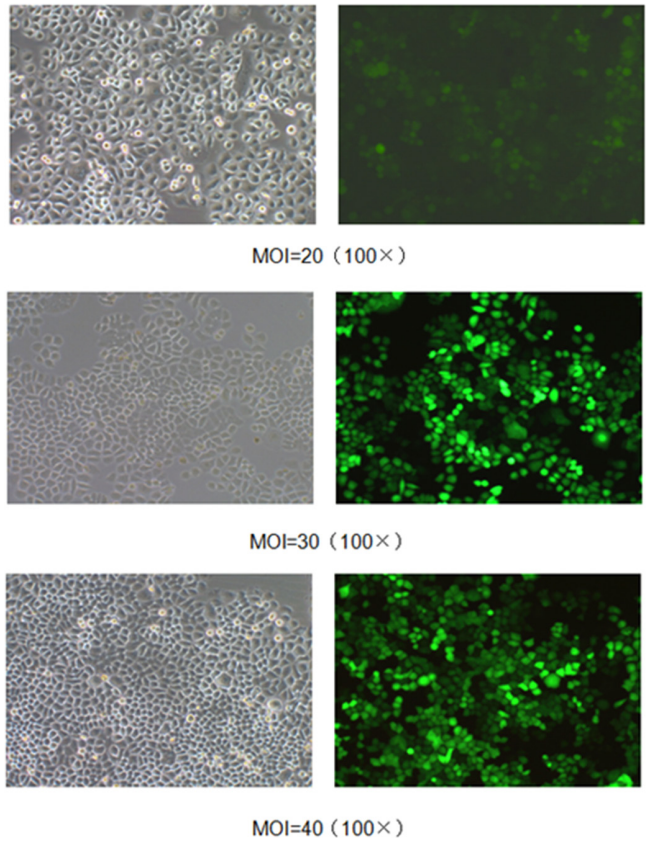

Figure 1. Lentiviral transfection photographs of the gastric cancer cell SGC7901 transfected with lentiviral vector. (magnification, 100x). (Left, white light; Right, green fluorescence)

MOI = 20, the transfection efficiency was about 50%, and when MOI = 30 or MOI = 40, the transfection efficiency was higher than 80%, and the cells were in good condition. Thus, MOI = 30 was elected as the best MOI, which could not only reduce experiment cost but obtain higher transfection efficiency, reflecting the rationality of the experimental design.

Lentiviral Vector-Mediated RNA Interference Inhibiting the Expression of FUBP1 *mRNA*

The results of the qRT-PCR (Table 1) show that the expressions of *FUBP1* mRNA of the CON group and NC group were 1.004 ± 0.066 and 1.001 ± 0.000, respectively. Compared with that of the NC group, the expression of *FUBP1* mRNA of the KD1 group was significantly down-regulated (0.343 ± 0.085, $P < .01$), coupled with knock-down efficiency of 65.7%. The expression of *FUBP1* mRNA in the KD2 group was significantly down-regulated (0.343 ± 0.085, $P < .01$), coupled with knock-down

Table 1. Effect of Different shRNA Sequences on the Expression of *FUBP1* mRNA ($\chi \pm S$)

Group	$2^{-\Delta\Delta Ct}$	Inhibition Ratio
CON	1.004 ± 0.066	−0.3%
NC	1.001 ± 0.000	0
KD1	0.343 ± 0.085*	65.7%*
KD2	0.296 ± 0.067*	70.4%*
KD3	1.054 ± 0.096	−5.4%

*$P < .05$.

efficiency of 70.4%. The expression of *FUBP1* mRNA of the KD3 group was not changed (1.054 ± 0.096, $P > .05$), and was without knock-down efficiency. The data above indicate that RNA interference could inhibit the expression of *FUBP1* mRNA of gastric cancer cells. The KD2 group had the highest knock-down efficiency. The FUBP1-RNAi-KD2 lentiviral vector was selected for the following experiments.

Lentiviral Vector-Mediated RNA Interference Inhibiting the Expression of FUBP1 Protein

The results of the western blot assay showed that compared to the NC group and the CON group, the expression of FUBP1 protein in the KD2 group was significantly reduced (Figure 2). The results could be obtained by the densitometry analysis of WB. Compared with that of the NC group, the relative expression of the FUBP1 protein of the KD2 group was 25.6%, decreasing by 74.4%, which was consistent with the result of the RT-PCR. It indicated that the lentiviral vector FUBP1-RNAi-KD2-mediated RNA interference could effectively inhibit the *FUBP1* expression of the gastric cancer cell line SGC7901.

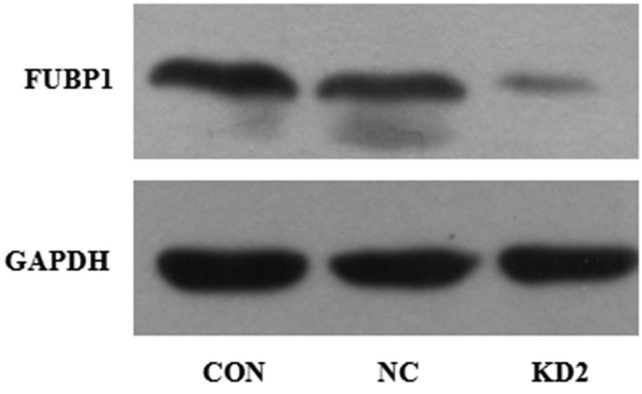

Figure 2. The protein expression of *FUBP1* of different groups by Western blot gel.

FUBP1 *Silencing Inhibits the Proliferation of Gastric Cancer Cells*

According to the OD values of each group, measured over 5 days, the values for OD450/fold of KD2 group, the NC group, and CON group were calculated, that is, the fold of OD value of each group from day 1 to day 5, compared with OD450 on day 1, representing daily proliferation times and cell proliferation times. The cell proliferation time curve was drawn in accordance with the results above, which showed (Table 2) that compared with the NO group and the CON group, the cell proliferation time of the KD2 group was significantly reduced for 5 consecutive days, indicating that the lentiviral vector transfecting the gastric cancer cell line SGC7901 could inhibit the cell proliferation.

FUBP1 *Silencing Affects the Cell Cycle Distribution of Gastric Cancer Cells*

The results of the PI-FACS cell cycle (Table 3) showed that the cell percentages in the G0/G1 phase of the CON group and the NC group were 38.16 ± 2.30 and 39.01 ± 0.20, respectively; the cell percentages in the S phase were 43.43 ± 2.18 and 43.85 ± 0.01, respectively; and the cell percentages in the G2/M phase were 18.41 ± 0.11 and 17.14 ± 0.19, respectively. Compared with those of the NC group and the CON group, the cell percentages in the S phase of the KD2 group were obviously increased (60.74 ± 1.68, $P < .01$), and the cell percentages in the G0/G1 and G2/M phases were decreased (30.48 ± 2.01, $P < .01$; 8.78 ± 0.51, $P < .01$). Silencing of the *FUBP1* gene affected the distribution of the cell cycle, and also affected the cell proliferation and division, resulting in decreased cell percentages in the G2/M phase and S-phase arrest, which was consistent with the detection results of CCK-8. It

Table 2. The Fold of OD Value of Each Group from the Day 1 to Day 5 ($\chi \pm S$)

Group	Day 1	Day 2	Day 3	Day 4	Day 5
CON	1 ± 0.029	2.394 ± 0.038	4.029 ± 0.190	5.082 ± 0.134	5.596 ± 0.181
NC	1 ± 0.026	2.118 ± 0.046	3.576 ± 0.087	4.513 ± 0.040	5.349 ± 0.091
KD	1 ± 0.035*	1.934 ± 0.057*	3.060 ± 0.036*	3.883 ± 0.099*	4.755 ± 0.087*

*$P < .05$.

Table 3. The Cell Percentages in Each Phase of the PI-FACS Cell Cycle ($\chi \pm S$)

Group	G0/G1	S	G2/M
CON	38.16 ± 2.30	43.43 ± 2.18	18.41 ± 0.11
NC	39.01 ± 0.20	43.85 ± 0.01	17.14 ± 0.19
KD	30.48 ± 2.01*	60.74 ± 1.68*	8.78 ± 0.51*

*P <.05.

was further determined that RNA interference could inhibit cell proliferation through the expression of the *FUBP1* gene of the gastric cancer cell line.

FUBP1 *Silencing Has No Significant Effect on Apoptosis of Gastric Cancer Cells*

The results of Annexin V-APC single-stain-detecting cell apoptosis (Table 4) showed that the cell apoptosis rates of the CON group and the NC group were 2.54 ± 0.138 and 2.26 ± 0.266, respectively. Compared with that of the NC group and the CON group, the cell apoptosis rate of the KD2 group was increased (3.53 ± 0.410, $P < .05$), but it was lower than 5%, which indicated that the apoptosis of gastric cancer cells was not obvious, and was still determined to be a negative result.

FUBP1 *Silencing Has No Significant Effect on Migration of Gastric Cancer Cells*

To further study on the effect of *FUBP1* silencing on migration of the gastric cancer cell line SGC7901, we designed a wound scratch assay and a transwell assay. The result of the wound scratch assay (Table 5, Figure 3) showed that compared with the CON group and the NC group, there was no significant difference in the migration rate after wound scratch for 8 hours (28%, $P > .05$) and the migration rate after wound scratch for 24 hours (54%, $P > .05$) in the KD2 group, showing no statistically significant difference.

Table 4. The Cell Apoptosis Rate of Each Group by Annexin V-APC Single-Stain ($\chi \pm S$)

Group	The Apoptosis Rate(%)
CON	2.54 ± 0.138
NC	2.26 ± 0.266
KD	3.53 ± 0.410*

*P < .05.

Table 5. The Migration Rate of Each Group After Wound Scratch for 8 Hours and 24 Hours by Transwell Assay ($\chi \pm S$)

Group	8h	24h
CON	0.21 ± 0.03	0.44 ± 0.07
NC	0.26 ± 0.05	0.48 ± 0.04
KD	0.28 ± 0.04*	0.54 ± 0.05*

*P < .05.

The result of the transwell assay (Table 6, Figure 4) showed that the migratory cell numbers of the CON group and the NC group were 160 ± 0.59 and 157 ± 1.64. Compared with the NC group and the CON group, there was no significant difference on the migratory cells per field in the KD2 group (156 ± 6.12, $P > .05$).

DISCUSSION

Gastric cancer is a kind of gastrointestinal cancer that poses a serious threat to human health. Some epidemiological studies have shown that the prevalence rate of gastric cancer is affected to a large extent by the host's susceptibility, environmental triggering factors, and other aspects, and that *Helicobacter pylori* infection plays the most important role in the etiology of gastric cancer.[9] The development and progression of gastric cancer is a complicated process involving multiple factors, multiple steps, and interactions of multiple genes. Multiple changes in the genetic and epigenetic mechanisms, such as gene mutation, gene amplification, gene deletion, and chromosomal translocation result in the activation of tumor-related oncogenes, inactivation of tumor suppressor genes, and abnormal regulation of tumor-related signal transduction pathways. These gene-level variations are the key to promote the development and progression of gastric cancer.[10-13] At present, the treatment methods of gastric cancer include the combination of endoscopic minimally invasive therapy, surgery, chemotherapy, and radiation therapy. Due to the increasing limitations of these conventional methods in the process of gastric cancer treatment, researchers begin to study the pathogenesis of gastric cancer at the gene level to find a new treatment strategy for gastric cancer.

RNA interference is also known as post-transcriptional gene silencing (PTGS),[14,15] the mechanism of which could be summarized as follows: the invasion of an exogenous gene induces the cells in vivo to produce long double-stranded RNA (dsRNA), which could be divided into small interfering RNA of about 21-23 nucleotide fragments long (siRNA), by endonuclease Dicer.[16] Then, siRNA binds with some nuclease complex to form RNA-induced silencing complex (RISC); activated RISC could bind with targeted mRNA specifically to inhibit the expression of

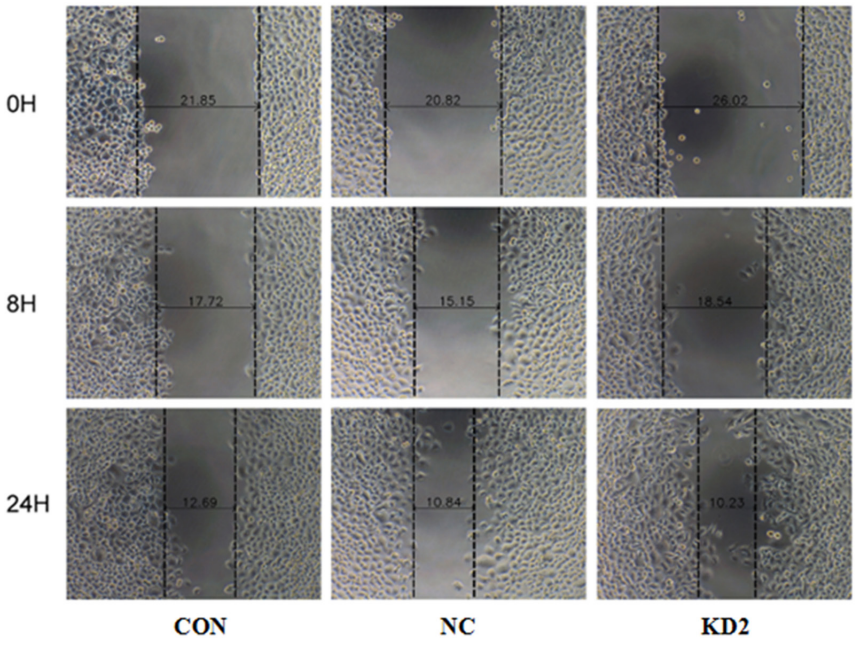

Figure 3. The photographs of the gastric cancer cell SGC7901 after wound scratch for 0 hours, 8 hours, and 24 hours in each group.

the targeted gene.[17,18] The key processes of triggering RNA interference are to synthetize siRNA and to introduce siRNA to specified cells and tissues successfully. In this study, the utilization of lentiviral vector in transfecting gastric cancer cells could not only effectively promote the expression of specific silencing of targeted genes, but could also integrate shRNA into the host chromosome, achieving the stable transfection of siRNA.

FUBP1 is a member of the ancient single-stranded DNA-binding protein family, which is located in human chromosome 1p31.1 and contains 658 amino acids. The molecular weight of *FUBP1* is about 67KD. *FUBP1*, composed of an amino-terminal domain, a central domain, and a carboxyl-terminal domain, is very conservative in structure, and the middle part is connected by variable regions.[19,20] The central domain of *FUBP1* is key to the regulatory function, and *FUBP1* could be involved in the transcriptional regulation of multiple genes through binding to the FUSE sequence at the upstream of the transcription start site. The most extensively studied gene is the oncogene *c-myc*, the activation of which is closely related to the development and progression of multiple cancers. In recent years, some studies have shown that the FUBP1 protein is overexpressed in multiple malignant tumors including medulloblastoma,[21] breast cancer,[22] renal cell carcinoma,[23] hepatocellular carcinoma,[24] and colorectal cancer,[25] and is positively correlated with the expression of the *c-myc* gene, which jointly promote the development and progression of cancer. Liu and Hu determined that the expressions of *FUBP1* and *c-myc* were up-regulated to various degrees in 12 kinds of nasopharyngeal carcinoma cell lines, showing positive correlation in the expression of both through Spearman's correlation analysis. Subsequently, qRT-PCR revealed that *FUBP1* and *c-myc* were overexpressed in the nasopharyngeal carcinoma tissues of 29 cases, compared to that of adjacent tissues, and a certain positive regulatory relationship between *FUBP1* and *c-myc* expression was determined, through analysis.[26] Based on the experiments above, Liu determined that the inhibitory effect of siRNA interference on *FUBP1* expression could inhibit cell proliferation, colony formation, and the growth of the xenograft tumor through cells experimentally in vitro and in the naked-mouse xenograft tumor experiment.[26] All of the above studies show that there

Table 6. The Migration Rate of Each Group for 18 Hours by Transwell Assay ($\chi \pm S$)

Group	Migratory Cells per Field
CON	160 ± 0.59
NC	157 ± 1.64
KD	$156 \pm 6.12^{*}$

*$P > .05$.

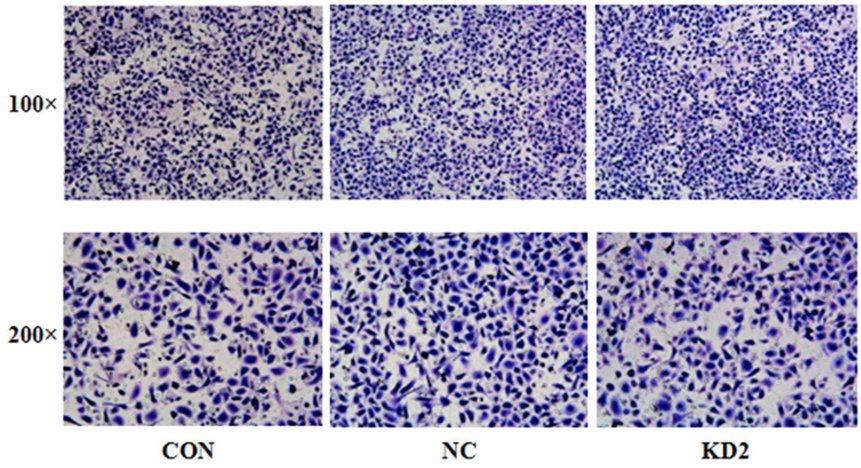

Figure 4. The photographs of Transwell assay (magnification, 100× and magnification, 200×).

is an intrinsic positive regulation relationship between FUBP1 and *c-myc* expression.

In previous studies, we have used FQ-PCR and immunohistochemical staining to determine that the expression of mRNA and protein of FUBP1 in gastric cancer tissues is obviously higher than that in adjacent tissues, chronic superficial gastritis, and chronic atrophic gastritis.[27] In order to further explore the effect of the *FUBP1* gene on the biological function of the gastric cancer cell and its role in the pathogenesis of gastric cancer, we designed this research and successfully constructed the shRNA lentiviral vectors of the targeted gene *FUBP1*. After transfecting gastric cancer cell line SGC7901, the expression of *FUBP1* was effectively inhibited. And the silencing of the *FUBP1* gene expression by RNA interference could inhibit the proliferation and division of the gastric cancer cell line SGC7901 and affect the cell cycle distribution, by decreased cell proliferation and S-phase arrest. However, *FUBP1* gene silencing had no significant effect on the apoptosis and migration of gastric cancer cells. The *FUBP1* gene may not be involved in the apoptosis and migration of gastric cancer cells. In conclusion, specifically and effectively silencing the *FUBP1* gene through RNA interference may provide a potential therapeutic strategy for gastric cancer. It is also suggested that the *FUBP1* gene may be a potential target for the genetic study and therapy of gastric cancer.

Ethics Committee Approval: This study was approved by the Ethics Review Committee of PLA 960th Hospital, Number 2013ZD06.12/2013.

Informed Consent: An informed consent was obtained from all participants with a detailed description of the potential benefits from the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.W., F.Z.; Design – Y.W., F.Z.; Supervision – Y.W., F.Z.; Resource – Y.W.; Materials – Y.Z.; Data Collection and/or Processing – F.Z., Y.Z.; Analysis and/or Interpretation – F.Z., Y.Z.; Literature Search – F.Z., Y.Z.; Writing – Y.Z.; Critical Reviews – Y.W., F.Z.

Conflict of Interest: The authors have declared that no conflicts of interest exist.

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