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Original Article

The functional role of RNF113A in cervical carcinogenesis

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Abstract: RNF113A is thought to function as an E3 ligase, engaged in the regulation of the turnover and activity of many target proteins. However, the fuctional role of RNF113A in cervical cancer remains unclear. In this study, by performing an immunohistochemistry (IHC) assay, we found that the RNF113A protein was significantly up-regulated in cervical cancer cells, and a high RNF113A expression was associated with malignant phenotypes. To determine the role of RNF113A in cervical cancer aggressiveness, we performed a gain and loss of functional experiments in cervical cancer cells with cell transfection, wound healing, transwell migration, and flow cytometry analysis. The results showed that RNF113A promotes the proliferation and survival ability of cervical cancer cells, enhances migration and invasion, and inhibits the apoptosis of cervical cancer cells. By silencing RNF113A in CSCC cell lines, we observed an up-regulation of the P53 protein level, indicating that P53 may function as a target of the RNF113A E3 ligase, and RNF113A may inhibit tumor cell apoptosis by degrading the TP53 protein.

Keywords: Cervical cancer, RNF113A, P53, apoptosis

Introduction

Cervical cancer is one of the most deadly forms of gynecological malignancy in females globally, with an estimated 570,000 cases and 311,000 deaths worldwide in 2018, the vast majority of which are in Sub-Saharan Africa and South-Eastern Asia, including China [1]. The Xinjiang Region has one of the highest regional morbidity and mortality rates of cervical cancr in China [2]. Therefore, it is important to examine the molecular mechanisms of cervical cancer.

RNF113A encodes a protein containing a C3H1-type zinc finger domain and a C3HC4 ring-type zinc finger domain. The RING-type zinc-finger proteins constitute one of the largest protein families, with over 600 members [3]. Many of these proteins have been shown to function as E3 ubiquitin ligases, and the presence of a RING domain is often sufficient for such an annotation. E3 ubiquitin ligases are engaged in the regulation of the turnover and activity of many target proteins. Together with the ubiquitin activating enzyme E1 and the ubiquitin-conjugating enzyme E2, the E3 ubiquitin ligases catalyze the ubiquitination of various biologically significant protein substrates for targeted degradation through the 26S proteasome, as well as for the non-proteolytic regulation of their functions or subcellular localizations [4]. E3 ubiquitin ligases, therefore, play an essential role in the regulation of various biological processes. Increasing evidence strongly suggests that the abnormal regulation of some E3 ligases is involved in cancer development [5-7]. RNF113A is also thought to function as an E3 ligase, and consistent with this assumption, RNF113A was found to physically interact with one of the human E2 proteins, UBE2U [8, 9]. However, the underlying mechanisms involved in the effect of RNF113A on cervical cancer remain unclear.

In the present study, we examined the expression patterns of RNF113A and its isoforms in cervical cancer tissues and cell lines. Furthermore, we determined the biological functional roles of RNF113A in the cervical cancer cell lines SiHa and C33a. By performing an immunohistochemical assay, we found that the

Table 1. Rnf113A expression in cervical carcinoma according to the patients' histopathologic characteristics

Characteristics	N	Negative	Weak	Moderate	Strong	χ^2	P
Normal cervical epithelia	24	16	5	1	0		
CSCC	85	26	13	26	20	18.354	<0.001
Differentiation							
Well	39	17	8	8	6		
Moderate/Poor	46	9	5	18	14	8.214	0.048
L/N metastasis							
Negative	53	24	6	16	7		
Positive	32	2	7	10	13	17.774	<0.001
FIGO Stage							
≤ I B	46	16	8	12	10		
> II B	39	10	5	14	10	1.666	0.645
invasion depth >1/2 muscular layer							
no	35	14	4	10	7		
Yes	50	12	9	16	13	2.699	0.44

RNF113A protein was significantly up-regulated in the CSCC tumor samples compared to the matched normal tissues. Fuctional experiments showed that the expression of RNF113A inhibited cell apoptosis in CSCC cell lines.

Materials and methods

Ethics statement

All the patients and controls provided a written informed consent and we received study approval from the ethics committee of the First Affiliated Hospital of Xinjiang Medical University.

Samples and clinical characteristics

A total of 85 patients with previously untreated, clinically diagnosed gynecological cancer, were staged in accordance with the International Federation of Gynecology and Obstetrics (FIGO) criteria and pathologically confirmed, and who underwent surgery between June 2015 and December 2018 at the Department of Gynecology in the First Affiliated Hospital in Xinjiang Medical University, were retrospectively enrolled in the present study. The patients ranged in age from 36 to 71 years (median, 53.6 years; **Table 1**). Regarding the other histopathological characteristics, 36 cases were poorly differentiated, 10 cases were moderately differentiated, and 39 cases were well differentiated. In addition, 32 cases also had lymph node metastasis.

Immunohistochemistry

IHC staining was performed with anti-RNF113A rat monoclonal antibodies (Abcam, Cambridge, MA, USA). Sections (3-mm-thick) were cut from paraffin-embedded tissue blocks. The samples were dewaxed in xylene and rehydrated in alcohol and distilled water. Antigen retrieval was then performed by heating samples for 15 min at 95°C in a citrate buffer (pH 6.0). The samples were cooled to room temperature and incubated in 3% hydrogen peroxide to quench the peroxidase activity. After incubating at 4°C overnight in a primary antibody and washing with a tris buffer, a biotin-labeled secondary antibody was added for 15 min followed by streptavidin peroxidase for 15 minutes. After eluting with PBS, diaminobenzidine and haematoxylin counterstaining were performed.

The hematoxylin-eosin (HE)-stained sections prepared using the cancerous tissues were microscopically evaluated by two independent pathologists. The amounts of protein were quantified according to their intensity (0, 1+, 2+, or 3+) and percentage (0%-100%) of staining.

Cell lines, cell culture and infection

Two human cervical cancer cell lines SiHa, C33a, and human immortalized cervical squamous epithelial (H8) cells line were purchased from Shanghai Cell Collection (Shanghai,

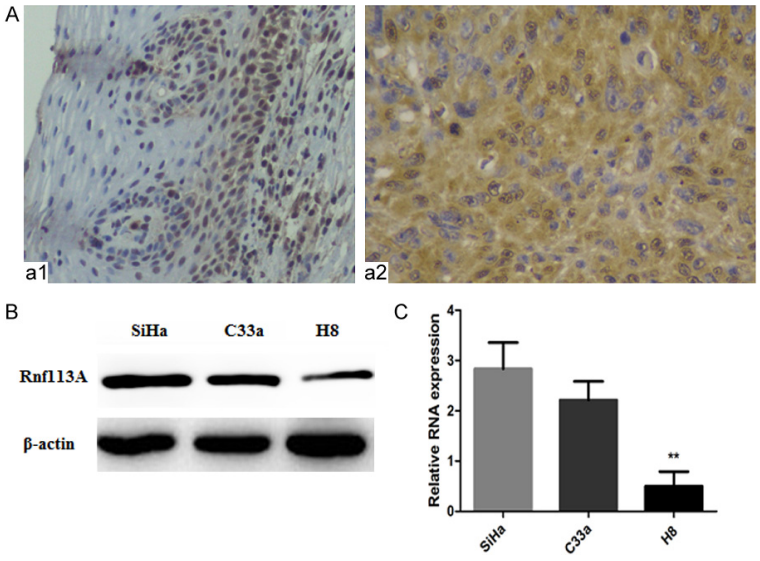


Figure 1. Representative IHC images of the RNF113A in human cervical cancer tissues and the normal controls (A). Original magnification × 100, (a1) depicts how RNF113A was expressed only in basal cells of the normal cervical epithelia; (a2) shows cervical carcinoma with a strong expression of RNF113A. (B) Western blot was applied to determine the RNF113A expression in 3 cells (C33a, and SiHa, and H8); (C) Qt-PCR was applied to detect the RNF113A mRNA in C33a, SiHa and H8 cells, Data are expressed as means ± SEM (Figs.) **p < 0.01, data are expressed as the mean ± SEM ** P<0.01.

Table 2. The influence of cell proliferation and colony formation by high expression RNF113A (x ± s, n=24)

cell	group	OD	The rate of colony formation (%)
SiHa	Control	1.256±0.064	44.333±5.132
	NC	1.263±0.008	46.333±5.132
	RNF113A	1.329±0.008◇,★	69.667±4.509◇,★
C33A	Control	1.257±0.024	42.667±3.786
	NC	1.272±0.013	45.667±2.517
	RNF113A	1.368±0.173◇,★	74.667±6.028◇,★

After being transfected with pCDNA3.1-RNF113A for 24 h in SiHa and C33A cells (◇compared with control group; ★compared with blank control group, P<0.01).

China). The cells were cultured in a DMEM medium (BI), in a 37°C incubator filled with 5% CO_2 and routinely passaged at 90% intensity. The culture medium was supplemented with 10% fetal bovine serum and 100 units/mL of penicillin and streptomycin (BI).

An Endo-Free Plasmid Mini Kit I (Omega, USA) was used to obtain the pcDNA3.1-RNF113A plasmid and vector plasmid (pcDNA3.1-RNF113A (5-TCCGCTCGAGATGATGGACTTGGAGCTGCC-3, antisense 5-ATGGGGTACCGAGTTTTTCTTAACATCTGGC-3). The SiHa cells and C33A cells were seeded into six-well plates, grown to 60% confluence and then transfected with a transfection reagent (μl) and plasmid DNA (μg) at a ratio of 1:1 at 37°C for 24 h using the X-tremeGENE HP DNA transfection reagent (Roche, USA). The cells were allocated to three groups: the normal group (non-transfection), the vector-transfection group, and the RNF113A transfection group (marked as RNF113A trans). siRNA duplexes against human RNF113A and the control (scrambled) siRNA were synthesized by Shanghai GenePharma (RNF113A siRNA-1, 5'-GCGUCUUCAAUCCAGCGA AAGAAUU-3'; RNF113A siRNA-2, 5'-CCCGAUGAGGAUGCAAUUCCCAUUA-3'; Scramble siRNA, UUCUCCGAACGUGUC ACGUTT). The SiHa cells and C33A cells were grown to a 30-50% confluence in 12-well plates. Transfections were carried out using Lipofectamine 2000. 50 pmol siRNA was added to each plate and cultured for 24 h. All transfections were performed in triplicate. The protein level of RNF113A was analyzed by western blotting. A wound healing assay and a transwell invasion experiment were performed to detect the viability of all the cells.

RNA isolation and quantitative RT-PCR analysis

Total RNA was isolated from cervical cancer cells using Trizol according to the manufacturer's protocol. Two micrograms of total RNA was subjected to a reverse transcription (RT) system (Promega, USA) for first-strand cDNA synthesis in a final volume of 20 μl. Then 2 μl of cDNA was used to perform a quantitative PCR reaction with a GoTaq qPCR Master Mix (Promega, USA) on Roche LightCycle96. β-Actin was used as the normalization control and all qRT-PCR reactions were done in triplicate. The following primer sequences were used: RNF113A forward, 5'-GCGATGCACAAGCCATCTTTG-3'; RNF113A forward, 5'-GCATTGCCCATAGACGTA TCCTT-3'; β-Actin forward, 5'-ATGATGATATCGCCGCGCTC-3'; β-Actin forward, 5'-TCGATG

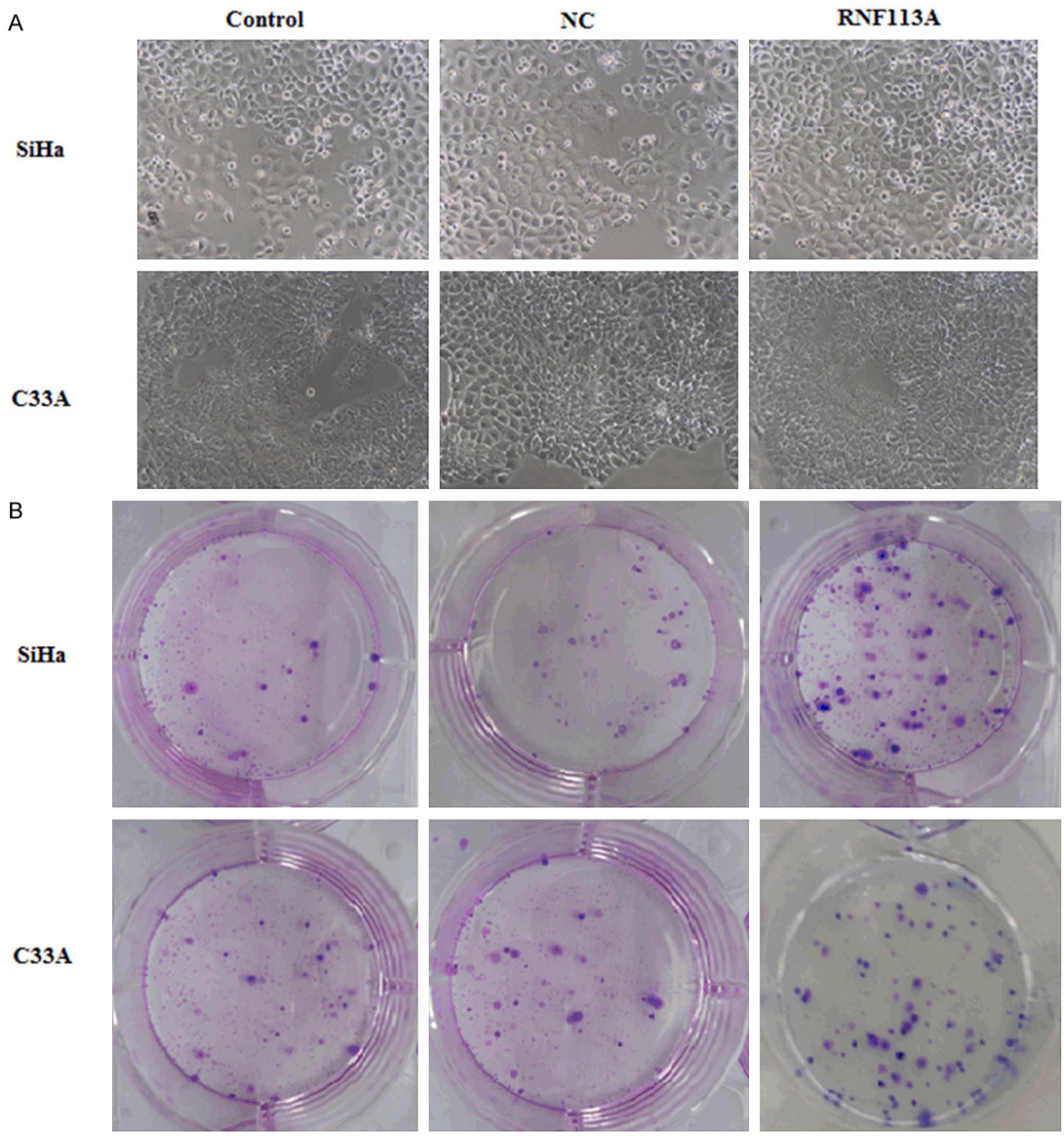


Figure 2. The clone formation and proliferation after the transfection of pCDNA3.1-RNF113A for 24 h in SiHa and C33A cells, which significantly enhanced cell clone formation and proliferation. The proliferation of the cells was determined using a microscope × 100. All experiments were performed at least three times.

GGGTACTT CAGGG-3'. $2^{-\Delta\Delta Ct}$ was used to represent expression changes.

Protein isolation and western blotting

Protease inhibitors (Boster, Wuhan, China) were also added to the cell lysates, which were maintained on ice for 20 minutes. The lysates were then centrifuged at 12,000 rpm for 10 min at 4°C. The samples (50 μg) were boiled for 5 min in a sample buffer and then separated on 12% gels by SDS-PAGE. The gels were transferred onto nitrocellulose membranes and blocked for 1 h in 5% skim milk at room temperature with shaking. Primary antibodies for rabbit anti-RNF113A and p53 (Abcam, USA) or β-Actin (Sangon, Shanghai, China) was added overnight to the blots at 4°C. Blots were washed in PBS-Tween three times, after which the secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G; Thermo, IL, USA) was added at room temperature for 2 hours. Chemiluminescent substrate (Thermo, IL, USA) was added to visualize the

Table 3. The influence on cell migration by the up-regulated or inhibited expression of RNF113A

Group	c33a cells	SiHa cells
control	79.90±6.24	188.64±16.19
Negative control	80.20±5.49	190.30±4.24
Overexpress RNF113A	104.20±4.47$^{\Delta,\nabla}$	235.00±15.87$^{\Delta,\nabla}$
Control	232.42±54.25	217.33±45.99
Negative control	270.26±49.52	201.67±50.61
RNF113A A01	77.79±29.49$^{\Delta,\nabla}$	81.81±17.81$^{\Delta,\nabla}$
RNF113A A03	100.07±36.52$^{\Delta,\nabla}$	96.87±14.21$^{\Delta,\nabla}$

The influence on cell migration which is tested by wound healing experiments by overexpressing and inhibiting RNF113A ($^{\Delta}$compared with control group, P<0.01; $^{\nabla}$compared with negative control group, P<0.01).

bands. Quantity One software was used to quantify the intensity of each band and was normalized to the intensity of the internal control β-Actin. The results were expressed as fold changes normalized to control values.

Cell proliferation assay

The cells were plated onto 96-well plates at 1000 cells per well and incubated for 1-5 days. During the incubation, 10 μl MTT Reagent was added to the wells which were measured every day and cultured for another 4 hours. Then the supernatant was discarded and 200 μl of DMSO was added to each well. Finally the absorbance of the solution was measured at 570 nm in a multifunctional microplate reader. The growth curve was constructed with time (day) as the abscissa and absorbance as the ordinate.

Wound healing assay

The cells were seeded to a 12-well plate. When the cells were grown to 80% confluence a wound was produced across the monolayer of cells using the tip of a 200 μL pipette. The adherent monolayer was washed twice to remove the non-adherent cells. After that, the cells were incubated for another 24 h. The open wound surface area and the number of cells in the area were photographed by an inverted phasecontrast microscope (Olympus, Japan) and quantified with ImagePro Plus software. All experiments were repeated three times.

Transwell migration experiment

After 48 hrs of transfection, cells were trypsinized and 2 × 10^5 cells were placed into Matrigel (with 1 mg/ml HA) coated transwell inserts with an 8-μm pore size. The cells were allowed to migrate through the Matrigel for 24 hrs. The cells in the upper chamber were removed, but the cells which had invaded the Matrigel were fixed and then stained using the Hema-3 stain kit (Millipore, Bedford, MA), and counted under a lighted microscope as described previously. All experiments were repeated three times.

Analyses of the cell cycle and the apoptotic changes by flow cytometry

SiHa cells and C33A cells were seeded in six-well culture plates at a density of 5 × 10^4 cells/well in RPMI 1640 plus 10% calf serum and 1% penicillin/streptomycin. High-fucose-content (HFC) polysaccharide (50, 100, 200, or 250 μg/mL) was added for 1 h followed by treatment with 300 μM H_2O_2 for varying time points (0-24 h). The cell cycle distributions were examined by measuring PI-fluorescence with a BD FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA) through an FL-2 filter (585 nm). We recorded 1 × 10^4 events per sample. The data were analyzed using Cell Quest.

Annexin V staining was performed to evaluate the apoptosis. Control and treated SiHa cells and C33A cells were added at 5 × 10^5 cells/mL in a binding buffer (10 mM HEPES [(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] [pH 7.4], 140 mM NaCl, 2.5 mM $CaCl_2$). FITC-annexin V (10 μl) in 190 μl of cell suspension was incubated for 10 min at room temperature. Cell mixtures were centrifuged and resuspended in 190 μl binding buffer, and 10 μl of PI (1 mg/mL) solution was added. The cells were acquired on a FACS Calibur flow cytometer at 1 × 10^4 events per sample. Necrotic cells were defined as positive for both PI and annexin V and were excluded from further analysis.

Statistical analysis

Statistical analyses were determined using SPSS Version 19. The *P* values were two-sided, and the significance level was $P<0.05$. Values were expressed as the means ± SEM. Statistical analyses were conducted using a two-tailed Student's *t*-test upon verification of the assumptions. A Mann-Whitney test was used to test continuous variables for the differences in the

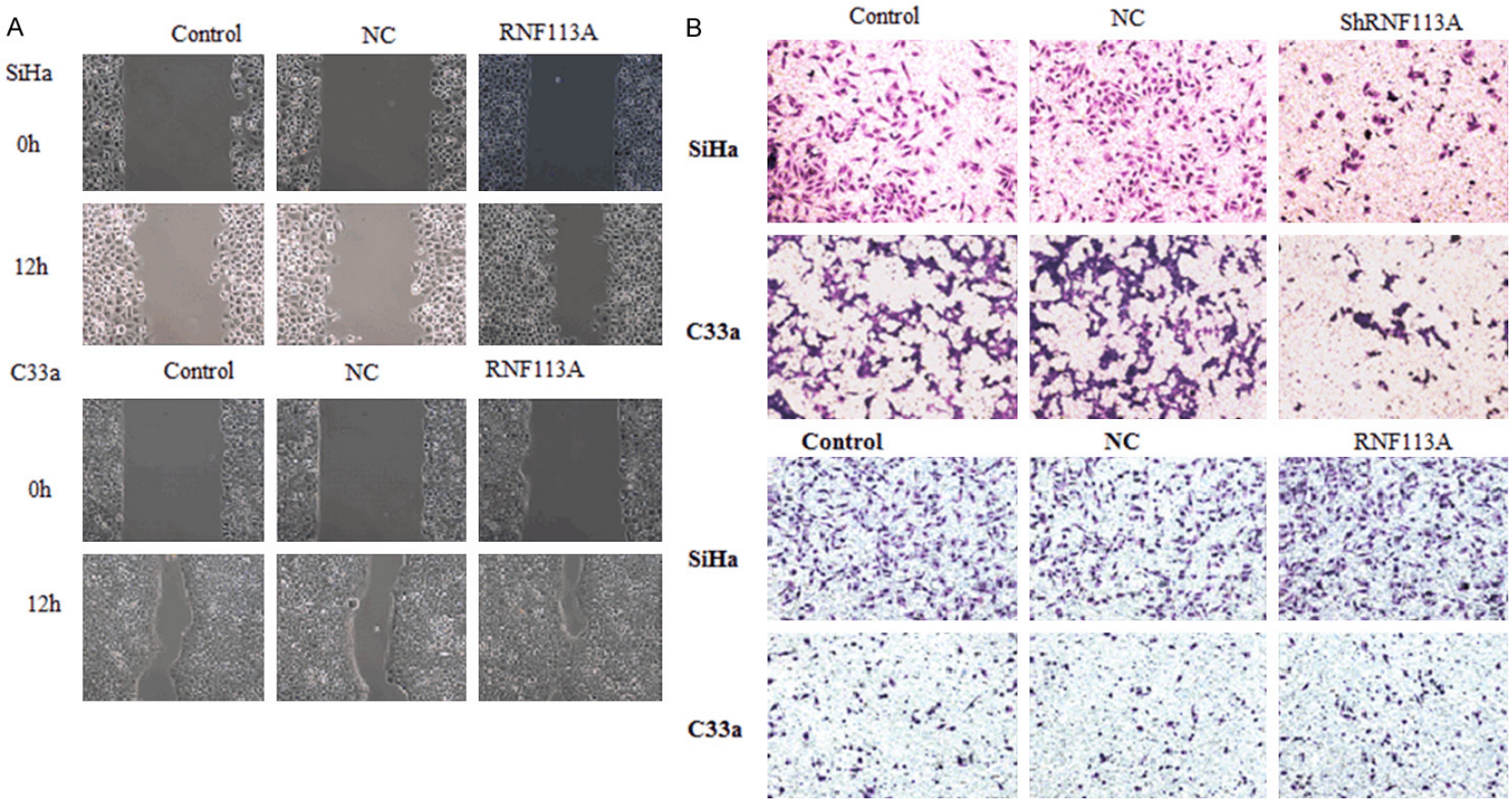


Figure 3. The influence of cell migration (A) and cell invasion (B) of SiHa and C33a by the overexpression of RNF113A and the knockdown of RNF113A. The bar graph summarizes the number of invasions per field, and the knockdown of RNF113A decreased cell migration and invasion All experiments were performed at least three times.

Table 4. The influence on cell invasion by the up-regulated or inhibited expression of RNF113A

Group	c33a cells	SiHa cells
control	42.90±3.51	117.90±11.61
Negative control	41.40±5.21	119.30±16.15
Overexpressed RNF113A	72.50±6.52$^{\Delta,\triangledown}$	162.36±8.64$^{\Delta,\triangledown}$
Control	121.67±26.47	159.00±32.00
Negative control	116.77±23.05	140.43±18.37
RNF113A A01	65.81±18.12$^{\Delta,\triangledown}$	57.85±20.92$^{\Delta,\triangledown}$
RNF113A A03	74.27±25.42$^{\Delta,\triangledown}$	88.77±7.54$^{\Delta,\triangledown,\blacktriangledown}$

The influence on cells invasion which was tested by a Transwell migration experiment by the overexpression and inhibition of RNF113A ($^{\Delta}$compared with control group, P<0.01; $^{\triangledown}$compared with negative control group, P<0.01; $^{\blacktriangledown}$compared with RNF113A D03).

Table 5. The cell apoptosis rate of the C33A and Siha cells after they were transfected with an RNF113A vector and RNF113A siRNA ($\bar{x} \pm s$, n=3)

	C33A cell apoptosis rate (%)	Siha cell apoptosis rate (%)
control	3.20±0.30	4.43±0.35
Negative control	3.43±0.40	4.40±0.44
RNF113A	3.63±0.60	4.13±0.45
Control	1.97±0.32	2.40±0.66
Negative control	1.36±0.38	2.73±0.51
RNF113A D01	12.17±1.76$^{\Delta,\triangledown}$	12.73±1.62$^{\Delta,\triangledown}$
RNF113A D03	11.13±1.46$^{\Delta,\triangledown}$	14.07±0.76$^{\Delta,\triangledown}$

($^{\Delta}$compared with control group, P<0.05; $^{\triangledown}$compared with negative control group, P<0.05).

IHC scores between the tumor and normal tissues. In addition, we performed Spearman's tests for correlations.

Results

RNF113A is upregulated in cervical cancer with poor differentiation and lymph node metastasis

To investigate whether RNF113A is dysregulated in human cervical cancer, we performed immunohistochemistry on 85 CSCC samples; the antibodies were tested on formalin-fixed, paraffin embedded, normal cervical tissues, and CSCC. Representative staining patterns for the RNF113A are shown in **Figure 1A**. RNF113A was expressed in most of the cervical cancer cells, but only in the basal cells of the normal cervical epithelia, and all positive staining was localized on the cell cytoplasms or nuclei. The expression findings of RNF113A are summarized in **Table 1**. As shown in **Table 1**, the percentage of RNF113A positive cells was 69.9% in the cervical cancer tissues. The up-regulation of RNF113A expression was significantly and positively related to lymph node metastasis (P<0.001), and the degree of cell differentiation (P= 0.048). No significant correlations between RNF113A expression and the other clinical parameters, including clinical stage and invasion depth, were observed.

To determine whether RNF113A expression exhibited similar patterns in the cervical cancer cell line, we determined the expression of RNF113A in SiHa and C33a, and immortalized the cervical (H8) cells. As expected, the expression level of RNF113A was significantly higher in the SiHa and C33a cells than in the immortalized H8 cells (**Figure 1B**). These results further supported the up-regulation of RNF113A expression in the cervical cancer cells.

The high expression of RNF113A active proliferation and the colony formation of cervical cancer cells

The above results demonstrate that overexpressed RNF113A is correlated with malignant phenotypes in cervical cancer. Thus, we wanted to explore whether an increased or decreased expression of RNF113A can change the growth of cervical cancer cells. A full-length human RNF113A plasmid was successfully transfected into SiHa and C33A cells. The Up-regulation of RNF113A promoted the colony formation of cells and increased their proliferation ability (**Table 2** and **Figure 2**). **Figure 2A**. Next we transfected pCDNA3.1-RNF113A for 24 h in the SiHa and C33A cells (**Figure 2B**). The clone formation took place after we transfected pCDNA3.1-RNF113A for 24 h in the SiHa and C33A cells.

RNF113A improves the migration and invasion of the cervical cancer cell line

A wound healing assay showed that a high expression of RNF113A led to the rapid migration rate in both cervical cancer cell lines, and decreasing the expression of RNF113A by two

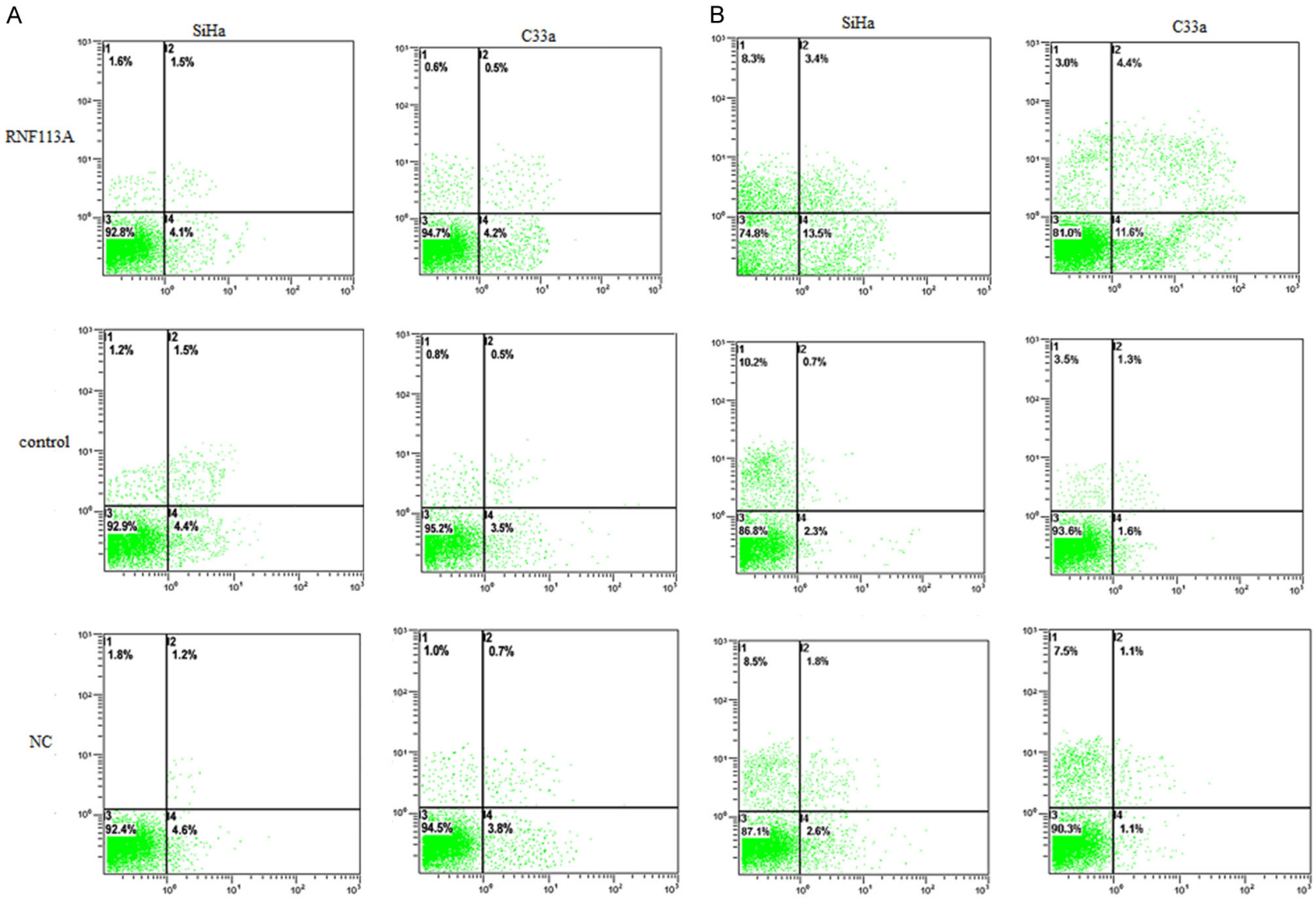


Figure 4. There is little difference in cell apoptosis after the overexpression of RNF113A by the transfected RNF113A vector; however, there was an increase of cell apoptosis both in the SiHa and C33A cells after the interference of RNF113A. All experiments were performed at least three times.

Table 6. The cell cycle of C33A after being transfected RNF113A siRNA ($\bar{x}$ ± s, n=12)

	G0/G1 (%)	S (%)	G2/M (%)
control	37.30±1.80	33.93±2.63	28.77±1.34
Negative control	36.03±1.93	34.60±4.40	29.37±2.51
RNF113A A01	59.27±0.50$^{\Delta,\blacktriangle}$	21.97±1.42$^{\Delta,\blacktriangle}$	18.77±1.05$^{\Delta,\blacktriangle}$
RNF113A A03	57.73±1.10$^{\Delta,\blacktriangle}$	23.53±2.17$^{\Delta,\blacktriangle}$	18.73±2.04$^{\Delta,\blacktriangle}$

($^{\Delta}$compared with control group, P<0.05; $^{\blacktriangle}$compared with negative control group, P<0.05).

Table 7. The cell cycle of Siha after being transfected with RNF113A siRNA ($\bar{x}$ ± s, n=12)

	G0/G1 (%)	S (%)	G2/M (%)
control	38.57±0.51	29.50±0.30	31.93±0.67
Negative control	39.17±0.59	29.40±0.79	31.43±0.21
RNF113A A01	71.73±1.37$^{\Delta,\blacktriangle}$	16.50±2.86$^{\Delta,\blacktriangle}$	12.00±4.42$^{\Delta,\blacktriangle}$
RNF113A A03	69.73±0.15$^{\Delta,\blacktriangle}$	16.07±1.12$^{\Delta,\blacktriangle}$	14.20±1.15$^{\Delta}$

($^{\Delta}$compared with the control group, P<0.05; $^{\blacktriangle}$compared with the negative control group, P<0.05).

siRNAs led to a lower migration rate in both cervical cancer cell lines, but there were fewer migrated cells in the RNF113A knockdown cells than there were in the control cells (**Table 3** and **Figure 3A**). A transwell migration assay also showed that the migrated cells were much more highly expressed in the RNF113A cells than they were in the control cells, and the inhibition of RNF113A could significantly reduce the number of invaded cells in both cervical cancer cells (**Table 4** and **Figure 3B**). These results suggest that RNF113A promotes the migration and invasion of cervical cancer cells, and migration and invasion lead to metastasis.

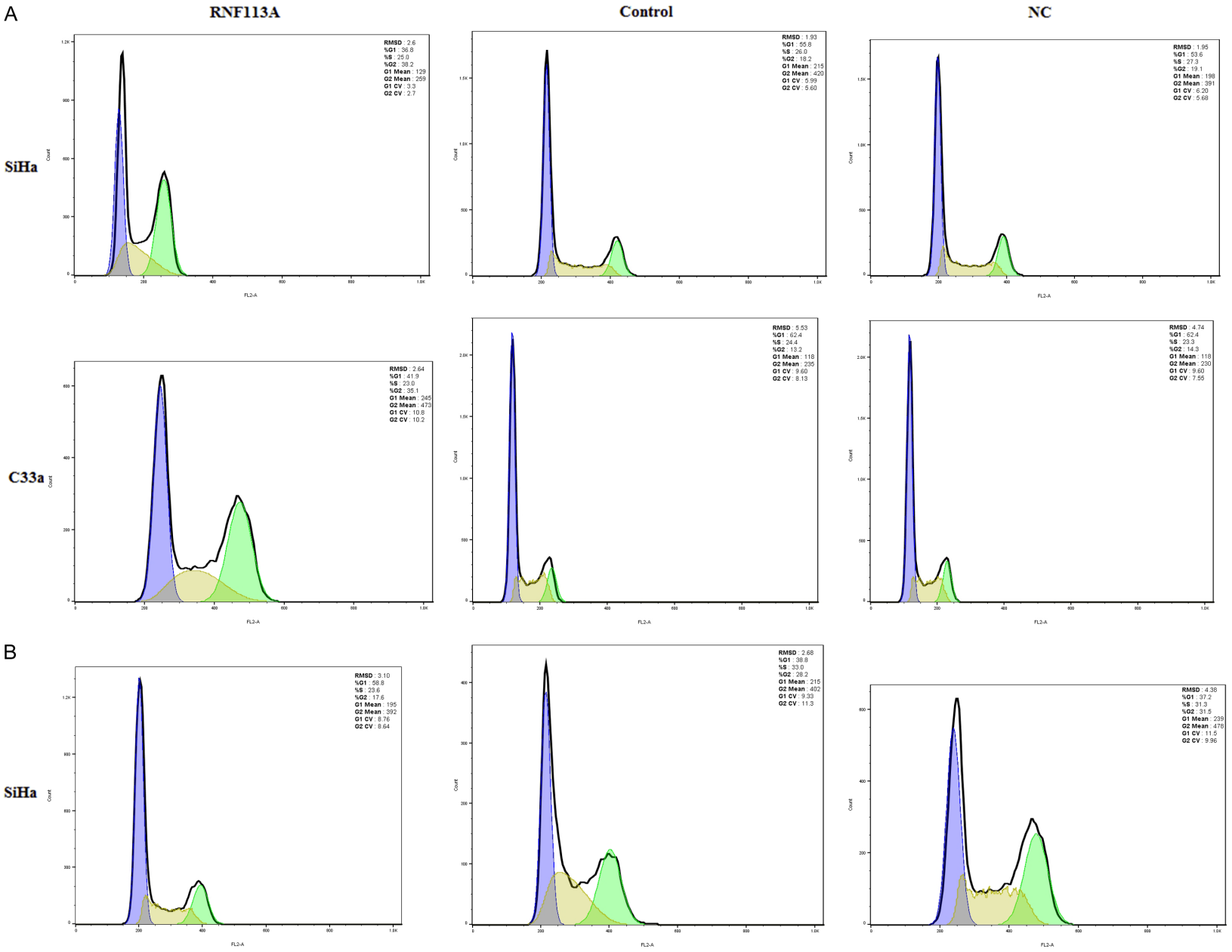
RNF113A inhibited cell apoptosis in the cervical cancer cell line

To explore the effect of RNF113A on cell apoptosis, SiHa and C33A cells were transfected with RNF113A siRNA for 48 h, and the cell apoptosis was determined by flow cytometry. There was an obvious increase of cell apoptosis in both the SiHa and C33A cells after the interference of RNF113A (**Table 5** and **Figure 4**), and the difference between the control group and the negative control group was statistically significant. There was little difference in cell apoptosis after the overexpression of RNF113A though the transfected RNF113A vector. There was an obvious increase in the number of G0/G1 phase cells in both the C33A and Siha cells, meanwhile, the number of S phases and G2/M phases decreased (**Tables 6**, **7** and **Figure 5**), and these observations demonstrate that interference in the expression of RNF113A inhibited the cell cycle and accelerated cell apoptosis. p53 acts as a tumor suppressor in many tumor types to induce apoptosis. We silenced RNF113A in cervical cancer cell lines and observed the upregulation of TP53, which indicated that RNF113A may also play a role as a modulator of P53 and that RNF113A, may inhibit tumor cell apoptosis by degrading the p53 protein (**Figure 6**).

Discussion

In recent years, a growing number of studies have suggested that there is a close relationship between ubiquitin ligase and the occurrence, development, and progression of cancer [10, 11]. The ubiquitin-proteasome pathway (UPP) degradation of many proteins that play important roles in the epithelial-mesenchymal transition, cell apoptosis and metastasis [12, 13]. Ring finger protein 113A, as a member of the UPP protein family, has been shown to function as an E3 ubiquitin ligase, and the presence of a RING domain is often sufficient for such an annotation. However, the biological role of RNF113A in cervical cancer remains unknown. In the present study, we discovered that RNF113A was upregulated in the primary site of cervical cancers with poor differention and lymph node metastasis, which indicates a high expression of RNF113A was associated with poor patient outcomes.

Tumor growth, invasion and metastasis are the main features of the malignant phenotypes of cancer. To demonstrate the potential roles of RNF113A for malignant phenotypes in cervical cancer, we implemented gain and loss of function experiments in cervical cancer cells. According to RNF113A expression in the cervical cancer cell lines, the C33a and SiHa cell lines were selected for knockdown and overexpression of RNF113A, and the resulting cell migra-


A
RNF113A
Control
NC
SiHa
RMSD : 2.6
%G1 : 36.8
%S : 25.0
%G2 : 38.2
G1 Mean : 129
G2 Mean : 259
G1 CV : 3.3
G2 CV : 2.7
RMSD : 1.93
%G1 : 55.8
%S : 26.0
%G2 : 18.2
G1 Mean : 215
G2 Mean : 420
G1 CV : 5.99
G2 CV : 5.60
RMSD : 1.95
%G1 : 53.6
%S : 27.3
%G2 : 19.1
G1 Mean : 198
G2 Mean : 391
G1 CV : 6.20
G2 CV : 5.68
C33a
RMSD : 2.64
%G1 : 41.9
%S : 23.0
%G2 : 35.1
G1 Mean : 245
G2 Mean : 479
G1 CV : 10.8
G2 CV : 10.2
RMSD : 5.53
%G1 : 62.4
%S : 24.4
%G2 : 13.2
G1 Mean : 118
G2 Mean : 235
G1 CV : 9.60
G2 CV : 8.13
RMSD : 4.74
%G1 : 62.4
%S : 23.3
%G2 : 14.3
G1 Mean : 118
G2 Mean : 230
G1 CV : 9.60
G2 CV : 7.55
B
SiHa
RMSD : 3.10
%G1 : 58.8
%S : 23.6
%G2 : 17.6
G1 Mean : 195
G2 Mean : 392
G1 CV : 8.76
G2 CV : 8.64
RMSD : 2.68
%G1 : 38.8
%S : 33.0
%G2 : 28.2
G1 Mean : 215
G2 Mean : 402
G1 CV : 9.33
G2 CV : 11.3
RMSD : 4.38
%G1 : 37.2
%S : 31.3
%G2 : 31.5
G1 Mean : 239
G2 Mean : 478
G1 CV : 11.5
G2 CV : 9.96

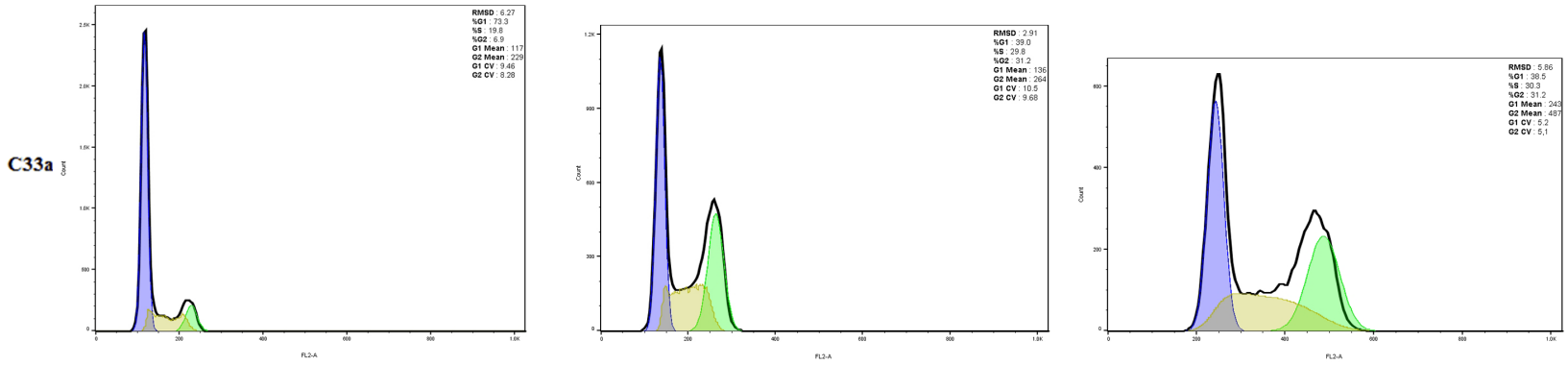


Figure 5. The influence of the cell cycle after the overexpression and knockdown of RNF113A in the SiHa and C33a cell lines. Interference in the expression of RNF113A inhibited the cell cycle. All experiments were performed at least three times. A shown the overexpression of RNF113A, and B represent the knockdown of RNF113A.

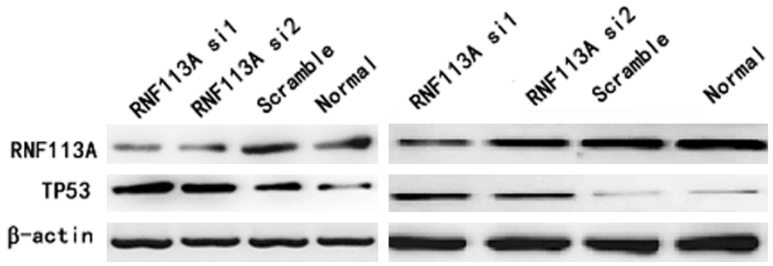


Figure 6. Up-regulation of p53 protein level after silencing RNF113A in cervical cancer cell lines, which normalized to b-tubulin. There is a statistically significant difference between the group transfected with RNF113A inhibitor and normal control.

tion, invasion, and cell cycle with apoptosis were further confirmed by transwell migration, wound healing, and by flow cytometry, respectively. The overexpression of RNF113A promotes the proliferation and survival ability of cervical cancer cells, and it also increases the migration and invasion ability in both the C33a and SiHa cell lines, but the knockdown of RNF113A inhibits migration and invasion. Interference with the expression of RNF113A inhibits the cell cycle and accelerates cell apoptosis. These results are similar to those observed with different RING finger proteins in different cancer types [14, 15]. It has been shown that ZFP36L2 promotes cancer cell proliferation, migration, and invasion in pancreatic ductal adenocarcinoma [16]. In breast cancer cells, silencing a tripartite motif-containing 59 could inhibit breast cancer cells' proliferation, migration, and invasion [17].

The tumor suppressor protein p53 is encoded by the TP53 gene the function of which is to induce cell cycle arrest and apoptosis with several target genes, including p21, BAX and Fas [18]. The half-life of p53 is very short, and is mainly regulated by ubiquitination and proteasomal degradation. Several E3 ubiquitin ligases have been shown to directly poly-ubiquitinate the p53 protein and induce its proteasomal degradation, including MDM2, RNF31 and COP1 [19, 20]. E3 ubiquitin ligases that indirectly modify p53 are highly expressed in cancers and thought to be involved in carcinogenesis by suppressing p53 function [4]. In the present study, we observed the up-regulation of the p53 protein level after silencing RNF113A in cervical cancer cell lines, which indicates that RNF113A may also play a role as a modulator of P53. p53 acts as a tumor suppressor in many tumor types to induce apoptosis. Our study strongly suggests that the degradation of p53 via abnormal E3 ligase signaling may play an important role in the attenuation of the apoptosis of cancer cells and then contribute to cancer cell development.

Overall, RNF113A as a poor prognostic biomarker for cervical cancer, promotes the proliferation, migration, and invasion and inhibits the tumor cell apoptosis of cervical cancer cells. However, the exact mechanism by which RNF113A inhibits tumor cell apoptosis by degrading the p53 protein needs further study.

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Disclosure of conflict of interest

None.

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