Cellular & Molecular Biology
Cell. Mol. Biol. 2014; 60 (1): 26-32
Published online March 25, 2014 (http://www.cellmolbiol.com)
Received on November 11, 2013, Accepted on March 16, 2014.
doi : 10.14715/cmb/2013.60.1.5

siRNA targeting prohibitins inhibits proliferation and promotes apoptosis of gastric carcinoma cell line SGC7901 *in vitro* and *in vivo*

Y. Zhang[1,2], Y. Chen[1,2], C. Qu[1,2], M. Zhou[1,2], Q. Ni[1,2] and L. Xu[1,2]✉

[1] Department of Digestive Endoscopy, Xinhua Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200092, China
[2] Key Laboratory of Pediatric Digestion and Nutrition, Shanghai 200092, China

Corresponding author: Leiming Xu, Department of Digestive Endoscopy, Xinhua Hospital, School of Medicine, Shanghai Jiaotong University, D1665 Kongjiang Road, Shanghai 200092, China. Tel: +86-21-65790000, Fax: +86-21-65791783, Email: lming_xu@163.com

Abstract

In the present study we investigate the effects of small interfering RNA (siRNA)-mediated silencing of prohibitins (Phbs) gene expressions on gastric carcinoma cell proliferation and apoptosis *in vitro* and *in vivo*. Firstly, Phbs proteins in five human gastric carcinoma cell lines were evaluated by Western blotting. Then three siRNA sequences targeting Phbs were designed and transfected into the cancer cell line with the highest Phbs protein expressions. The cell proliferation and apoptosis were analyzed by MTT and flow cytometry methods. *In vivo*, the effects of siRNAs on the proliferation and apoptosis were assayed in xenografted tumors of nude mice. Phbs proteins were expressed highest in gastric carcinoma cell line SGC7901. The cell proliferation significantly decreased upon Phbs silencing. The cell percentage in S phase was significantly lower in Phbs silenced groups, and the apoptotic cell ratio was significantly higher. siRNA targeting Phbs significantly inhibited the growth of the xenografted tumors. Further, Bcl-2 and Bax was respectively downregulated and upregulated after the Phbs silencing in the xenografted tumors. Caspase-3 and caspase-9 activities were significantly enhanced in the Phbs-silenced tumors. In summary, siRNA-mediated silencings of Phbs inhibit proliferation and promotes apoptosis of gastric carcinoma cells SGC7901 *in vitro* and *in vivo*, suggesting key roles of Phbs in the development of SGC7901 cell line.

Key words: Gastric carcinoma, RNA interference, Apoptosis, Proliferation, Nude mice.

Introduction

Currently, gastric carcinoma is the most common gastrointestinal cancer, and it has the first mortality in malignancies in China (1-3). While the etiology of gastric carcinoma is not yet fully clarified, several risk factors, including biological (i.e. inflammatory gastrointestinal disease), genetic (i.e. chromosomal defects), and behavioral (i.e. irregular diet), have been identified in some degree. Now, the most effective treatment strategy available is surgical resection followed by chemo/radiotherapy; however, this type of intervention is only successful if applied at early disease stages. Delayed diagnosis or treatment limits the options of clinical management to palliative therapy.

With the development and application of molecular biology techniques, people have recognized that the genesis and development of gastric carcinoma is a complicated process involving multiple factors including a variety of molecular mechanisms and signal transduction pathways. Even so, the mechanisms for the genesis and development of gastric carcinoma are not fully understood. Therefore, it is important to search for specific molecular markers for early diagnosis and prognosis of gastric carcinoma.

Prohibitins (Phbs), anti-proliferative proteins have been confirmed to be potential targets for tumor protein suppression. In 1989, McClung (4) firstly cloned Phbs from rat liver cells. Phbs mainly contains two subtypes Phb1 and Phb2. Prohibitin 1 (Phb1), a highly conserved protein and its homologue prohibitin 2 (Phb2) mainly localizes to the inner mitochondrial membrane.

Human Phb1 and Phb2 widely distribute in B cell surface and inner mitochondrial membrane of almost all the cells (5,6). Phbs significantly express in tubular epithelial cells and stromal cells of rat and human. An *in vitro* experiment showed that Phb1 transferred from nucleus to cytoplasm under stimulation of apoptotic signal in breast cancer cells. Zhou et al. (7) found that a small amount expressions of Phbs in fibroblast nuclei. Moreover, Phbs proteins exist in blood circulation. The biological functions of Phbs include maintaining the integrity of mitochondrial structure and function, participating early signal transduction of B cells, inhibiting transforming growth factor-β1-induced fibroblast proliferation and activation, negative control of gene transcription, and etc.

Recent studies have shown that the Phbs are promising candidate markers of tumorigenesis. A proteomic profiling analysis of lung squamous cell carcinoma found that Phbs strongly correlated with the development of tumor (8). The chromosomal location of the human Phb1 gene adjacent to the BRCA1 cancer suppressor gene has led to researchers hypothesizing its involvement in breast cancer (9), supported by a subsequent study of Jupe et al. (10) that a polymorphism in the 3′-untranslated region of Phb1 was shown to be highly correlated with premenopausal breast cancer. In addition, downregulation of the Phb1/2 complex has been observed in androgen-induced prostate cancer tissue (11), early gastric cancer and hepatocellular carcinoma (12). The studies on the correlation between Phbs and gastric carcinoma are few. Liu et al. found that suppression of miR-27a inhibits gastric cancer cell growth. And prohibitin is identified as a potential miR-27a target, combining bioinformatics and microarray analysis (13).

It has been mentioned that the anti-proliferative function once thought of Phbs were later attributed to 3' UTR of the Phbs genes. Further, the anti-apoptotic function of Phbs was also reported that while total Phbs expressions were not changed, the protein was enriched in lipid rafts after ultraviolet B (UVB) irradiation. Reduced expressions of Phbs using siRNA knockdown resulted in an increase in cellular apoptosis after UVB irradiation (14). Based on these results, in order to clarify the roles of Phbs in the development of gastric carcinoma, in the present study we employed RNA interference (RNAi) in human gastric cancer cell lines and assessed the effects on proliferation and apoptosis by a series of *in vitro* and *in vivo* experiments.

Materials and methods

Cell lines and culture conditions

Five human gastric cancer cell lines including SGC7901, AGS, BGC-27, MGC-803, and BGC-823 and a normal gastric mucosa cell line GES-1 were provided by the Cell Resource Center of the Shanghai Life Sciences Institute at the Chinese Academy of Sciences (Shanghai, China). All the lines were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and grown at 37°C in an atomosphere of 5% CO_2. When cells reached 90% confluence (about every three days), they were passaged by trypsinization. Passages 3-5 were used for experimental analyses.

Experimental animals

Specific pathogen free (SPF) BALB-c nude mice 3-4 weeks weighing 18-20 g were obtained from the Experimental Animal Center of Central South University (Changsha, China). The SPF animal room was maintained at 22 ± 2°C and 30%-60% relative humidity. The mice were given free access to food and water. All the experiments were conducted in accordance with the national guidelines for the care and use of laboratory animals. This study was approved by the Ethnic Committee of Affiliated Changzheng Hospital of the Second Military Medical University (Shanghai, China).

Real-time PCR assay

Total RNA was extracted from about 50 mg of the SGC7901 cells by the TRIzol reagent (Invitrogen) with DNase I treatment, and used as template (2 μg) for reverse transcription with AMV reverse transcriptase. The resultant cDNA (2 μL) was amplified in a 50 μL reaction system consisting of 25 μL of real-time PCR SYBR Green I master mix, 2 μL of forward and reverse primers (10 μmol/L each), and 21 μL of ddH_2O. Primers were synthesized by Invitrogen Co., Ltd (Shanghai, China) and listed in Table 1. The thermal cycling conditions included 40 cycles of denaturation at 95°C for 4.5 min and annealing at 56°C for 1 min. The data were analyzed by the Gene Expression module of the IQ5 optical system software (Bio-Rad, Hercules, CA, USA). Triplicate reactions were performed and the relative fold-change of gene expression was determined by normalizing to β-actin and calculating the $2^{-\Delta\Delta CT}$(15) (7500 system SDS software, version 1.2.3; Applied Biosystems, Foster City, USA).

Western blotting analysis

The SGC7901 cells (at 90% confluency) adhered to the culture flask were washed twice with phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.4) and lysed by covering with cell lysis buffer containing phenylmethanesulfonyl fluoride (PMSF) and incubating on ice. A cell curat was used to collect the lysates into centrifuge tubes, after which the lysates of the respective cell lines were vortexed for 30 min. Total protein was collected by centrifugation (13,000 × *g*, 10 min, C) and measured using a BCA protein assay kit (Pierce, Rockford, IL, USA).

The respective total protein samples (about 50 μg) were separated by 12% SDS–PAGE and transferred onto a polyvinylidene fluoride membrane. After blocking with 5% skim milk for 4 h and washing with Tris-buffered saline (TBS; 3 × 5 min), the membrane was incubated overnight at room temperature with the primary antibody (rabbit anti-human Phbs polyclonal antibody, 1:200 (Abcam, Cambridge, UK; internal control: GAPDH antibody, 1:1000 (Snata cruz, CA, USA)), followed by a 4 h incubation at room temperature with the appropriate secondary antibody (for Phbs: horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG, 1:2000 (Zhongshan Golden Bridge Biotechnology Co., Beijing, China); for GAPDH: Goat anti-mouse IgG, 1:2000 (Zhongshan Golden Bridge Biotechnology Co., Beijing, China). Immunoreactive bands were detected by enhanced chemiluminescence (ECL) reagent (Pierce), visualized by autoradiography, and quantified by the Quantity One analysis system (Bio-Rad, Hercules, CA, USA).

Cell transfection

Three small interfering (si)RNA sequences targeting Phbs were designed according to the Phbs genes sequence from GenBank and synthesized by Invitrogen Co., Ltd (Shanghai, China). The sequences, designated as siRNA1-P, siRNA2-P, and siRNA3-P, are listed in Table 2. An siRNA of random sequence was also synthesized for use as a negative control (NC).

The gastric cancer cell line showing the highest protein expressions of Phbs by Western blotting was selected for use in all subsequent experiments. The cells were washed twice with serum-free DMEM medium, digested with 0.25% trypsin, and made into a single cell suspension for seeding into a 6-well plate (1×10^4/well). After overnight growth at 37°C in 5% CO_2, the medium was replaced with fresh serum-free DMEM. Meanwhile, 5 μL of Lipofectamine 2000 (Invitrogen Co. Ltd., Shanghai, China) was diluted with 250 μL of serum-free DMEM and incubated for 5 min at room temperature before 5 μL of each siRNA (dissolved in DEPC-treated water, 20 μM) was respectively mixed into the solution by gentle stirring and incubated at room temperature for 20 min. The respective Lipofectamine 2000-siRNA complexes were then added to the cells. After transfection for 72 h, the cells were collected and the mRNA and protein were determined by qRT-PCR and immunofluorescence assays.

Cell proliferation assay

Cell proliferation was assessed by MTT assay. Briefly, the 3-5 passage SGC7901 cells treated with siRNA-

P or negative control were seeded in a 96-well plate (2×10^3-3×10^3/well) (200 μl/well) in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Shanghai, China) supplemented with 10% FBS. After a culture for 24, 48, or 72 hours, 20 μL of MTT solution (5 mg/mL) was added to each well and co-incubated for another 4 hours. Then the medium was removed followed by the adding of 150 μl of DMSO. The plate was shaken for 10 min to completely dissolve the crystallized precipitate. The cell viability/cytotoxicity was assessed by measuring the optical density (OD) at 450 nm in a microplate reader (EXL-800 Thermo Fisher Scientific, Waltham, MA, USA). Data from triplicate samples were averaged and plotted on a growth curve.

Cell cycle and apoptosis assays

Cell cycle assay: The SGC7901 cells (1×10^5-5×10^5) treated with siRNA-P or negative control were cultured in DMEM supplemented with 10% FBS for 3 days. After that, the cells were digested by using 0.25% trypsin and harvest. Then they were centrifuged at 80 rpm for 5 min at 4°C. The pellet was re-suspended in 0.01 M PBS. After that, the cells were fixed in 70% cold ethanol at 4ºC for 24 hours followed by an co-incubation with RNAase and then labeld by BrdU. Cell cycle was analyzed by a flow cytometry (FCM) (Olympus, Tokyo, Japan) (at 488 nm excitation wavelength and 530 nm emission wavelength).

Cell apoptosis assay (FCM): siRNA-transfected SGC7901 cells were washed with PBS twice, the cells (1×10^5-5×10^5) were then resuspended in 500 μL of binding buffer and sequentially mixed with 5 μL of Annexin V-FITC (KeyGEN Biotech, Nanjing, China) followed by 5 μL of PI. The cells were incubated at room temperature in the dark for 5-10 min. Apoptotic cells were detected by a FCM (at 488 nm excitation wavelength and 530 nm emission wavelength) and observed under a fluorescence microscopy.

Cell apoptosis assay (TUNEL): The SGC7901 cells were seeded in 24-well plates (4×10^4/mL). After culture for 72 hours, the cells were fixed using 3.7% neutral formalin for 10 minutes at room temperature. Then rehydrated with a gradient of ethanol (100%, 95%, and 70%, 5 minutes each). They were then washed with PBS for 10 minutes, and 50 μL of cytonin (R&D Systems, USA) was added to each well an another incubation for 30 minutes. They were further washed with DNase-free water twice and 2 minutes each time, and then incubated with fresh 3% H_2O_2 at room temperature for 5 minutes. 0.01 M PBS was then used to wash the cells for 1 minute, followed by TdT labeling at room temperature for 5 minutes. Each well was added with 50 μL labeling mix and incubated at 37ºC for 1 hour. The reaction was stopped with TdT stopping buffer at room temperature for 5 minutes, followed by an incubation with 50 μL of steptavidin-HRP detection solution at room temperature for 20 minutes, and then washed with PBS twice. The cell nuclei were examined under a microscope for green fluorescence labeling as indication of apoptotic cells.

Establishment of xenogrft model of human gastric cancer in nude mice

BALB-c nude mice with half males and half females were subcutaneously inoculated with 0.2 mL of prepared SGC7901 cell suspension (2×10^7/mL). Subsequently, the tumor formation was successively observed. Normally, a rice-like scleroma would be present 7 days post the inoculation, which indicated a successful establishment of transplanted tumor animal model. Then the animals were randomly divided into five groups including" a blank group, a negative control group, and three siRNA-transfected groups. Briefly, the mice in the blank group were subcutaneously injected with 50 μL of normal saline. The negativel control group was injected with 50 μL of a complex solution containing 5 μL of PGCsi3.0 plasmid in combination with 15μL of Lipofectamine 2000 and 30μL of normal saline. The siRNA-transfected group was injected with 50 μL of a complex solution containing 5 μL of siRNA-prohibitin plasmid in combination with 15 μL of Lipofectamine 2000 and 30 μL of normal saline. The general conditions of the animals, including mention, diet, activity, and etc. were observed and recorded. Tumor volume was also observed and recorded every one week to plot a growth curve. The tumor suppression rate was caculated in accordance with the following formula. Tumor suppression rate=(Tumor volume treated-Tumor volume control)/ Tumor volume control*100%.

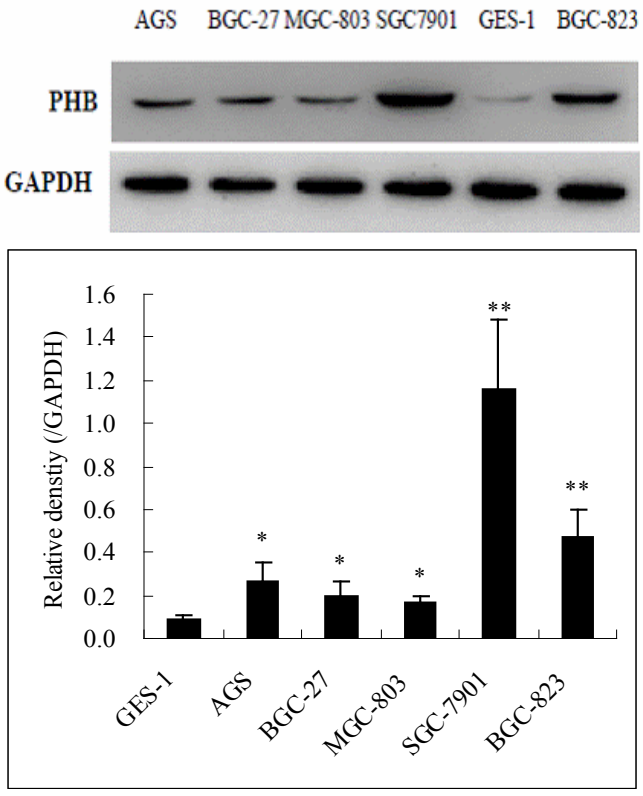

Figure 1. Western blotting analysis of Phbs protein expressions in five gastric carcinoma cell lines tested. Protein expressions of Phbs are variable in different carcinoma cell lines. There are lowest Phbs expressions in a normal gastric mucosa cell line GES-1, and the highest expressions in SGC7901 cell line. Data are presented as mean (n=3) ± SEM. $^*P<0.05$, $^{**}P<0.01$ vs. GES-1.

Results

Phbs proteins are expressed in all five gastric carcinoma cell lines tested

Phbs proteins were detected in all the five gastric carcinoma cell lines and a normal gastric mucosa cell line

GES-1, the highest level was detected in the SGC7901 and the lowest expression was found in GES-1 (Fig. 1). Thus, SGC7901 cells were selected for the subsequent RNAi analyses of the functional roles of Phbs.

Transfection of SGC7901 cells with the siRNAs targeting Phbs sequences (siRNA-P1, siRNA-P2, and siRNA-P3) led to significantly decreased Phbs mRNA and protein expressions. The siRNA-P1 had the most robust knock-down efficiency (Fig. 2).

siRNA targeting Phbs inhibits the proliferation of gastric carcinoma cell

The MTT result revealed that there was no significant difference in growth rate between negative control-treated and the control (Fig. 3). siRNA targeting Phbs (siRNA1-P, siRNA2-P, and siRNA3-P) significantly inhibited the growth of the cancer cells at 48 and 72 h (P<0.05). And siRNA1-P achieved the most efficiency in the growth inhibition (P<0.01). The results above suggested that Phbs could promote the cell growth, while the Phbs inhibition by siRNA inhibited the cell proliferation.

siRNA-silenced Phbs SGC-7091 cells are arrested at the G_1/S phase and the Phbs silencing enhances cell apoptosis

The Phbs-silenced cells showed a cell cycle distribution that was distinctive from the control cells (Fig. 4A). Specifically, the Phbs-silenced cells had a much lower proportion of cells in the S phase (siRNA1-P: 13.6±3.2%, siRNA2-P: 16.0±3.3%, and siRNA3-P: 18.5±2.1% vs. NC: 32.6±6.8%, P<0.05), which suggested that Phbs play roles in G_1/S phase transition and that Phbs exert cell cycle regulatory functions in the S phase.

In the absence of a chemotherapeutic agent 5-FU, we detected the apoptosis of gastric carcinoma cell by TUNEL. The result showed that the negative control had no significant effect on the apoptosis of the cancer cells (Fig. 4B). Actually, Phbs silencing by siRNA-P significantly promoted the cell apoptosis. Compared to the normal control, siRNA targeting Phbs (siRNA1-P, siRNA2-P, and siRNA3-P) remarkably increased apoptotic cell number (P<0.01) (Fig. 4B). Among the three siRNA, the apoptotic cell number was highest in siR-

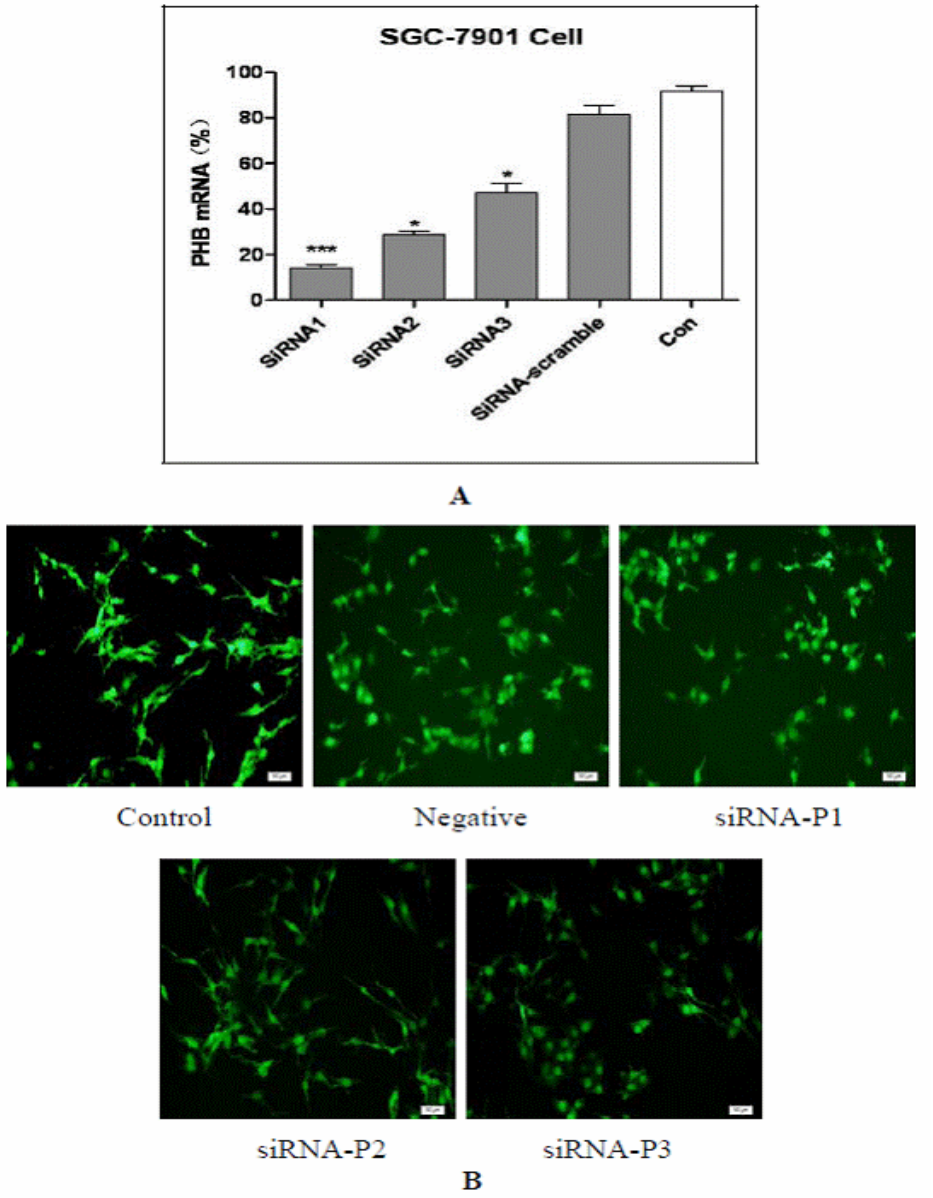

Figure 2. Assays of Phbs mRNA and protein after treatment of siRNA targeting Phbs. A, qRT-PCR assay of Phbs mRNAs after the silences in the SGC-7901 cells. B, Immunofluorescence assay of Phbs proteins after silences in the SGC-7901 cells. Phbs expressions are significant reduced both at mRNA and protein levels after the siRNAs treatments, and siRNA-P1 got the most effective inhibition. Data are presented as mean (n=3) ± SEM. $^{*}P$<0.05, $^{**}P$<0.01 vs. Control.

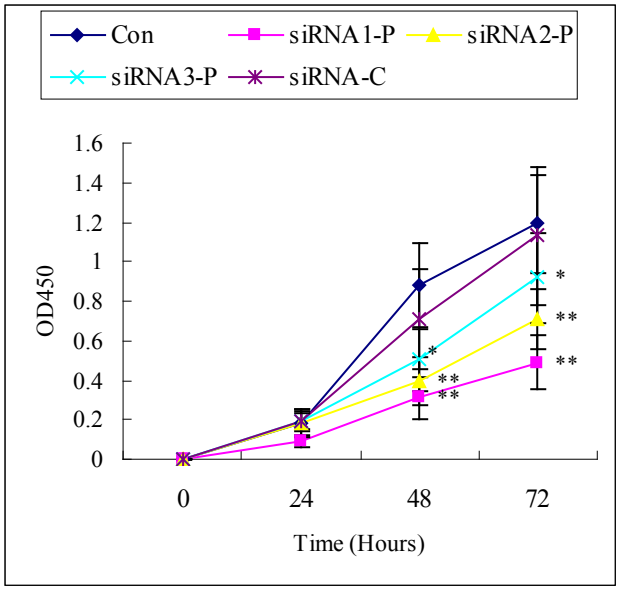

Figure 3. The effect of siRNA targeting Phbs on the growth of gastric carcinoma cell SGC7901. The cancer cell growth was not significantly inhibited by the treatment of siRNA-C. However, all the Phbs siRNAs significantly inhibited the cancer cell growth from the 24th hour. And siRNA3-P got the highest inhibition effect. Data are presented as mean (n=3) ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ vs. Control.

NA1-P-treated (Fig. 4B), which suggested siRNA1-P was most efficient in promoting cell apoptosis.

In order to investigate the effects of Phbs silencing on chemotherapeutic susceptibility, we employed gastric carcinoma cells (siRNA-treated or not) pretreated with 5-FU (50 mg/L). Then the cell apoptosis was assayed. The result revealed that compared with the control, siRNAs-P significantly increased the cell apoptosis, indicating increased susceptibility of the gastric carcinoma cells to 5-FU (Fig. 4C), suggesting key roles of Phbs in the resistance of chemotherapy.

Phbs silencings by siRNAs inhibit tumor growth in nude mice

The tumor volumes in groups were gradually increased along with the prolongation of the time. Interestingly, the growth rate was significantly lower in the siRNA-P-treated group than that in the control group 7, 14, and 21 day after the inoculation ($P<0.01$) (Fig. 5). And siRNA1-P achieved the highest inhibition efficiency.

siRNA-mediated Phbs silencing upregulates Bax, Caspase-3, -9 expressions and downregulates Bcl-2 expression in the transplanted tumor tissues from the nude mice

Western blotting results showed that siRNA-mediated Phbs significantly upregulated Bax, Caspase-3, -9 expressions and downregulated Bcl-2 level (Fig. 6). And siRNA1-P displayed a most efficient regulation on the apoptosis-related molecules. It suggested that Phbs silencing significantly increased cancer cell apoptosis,

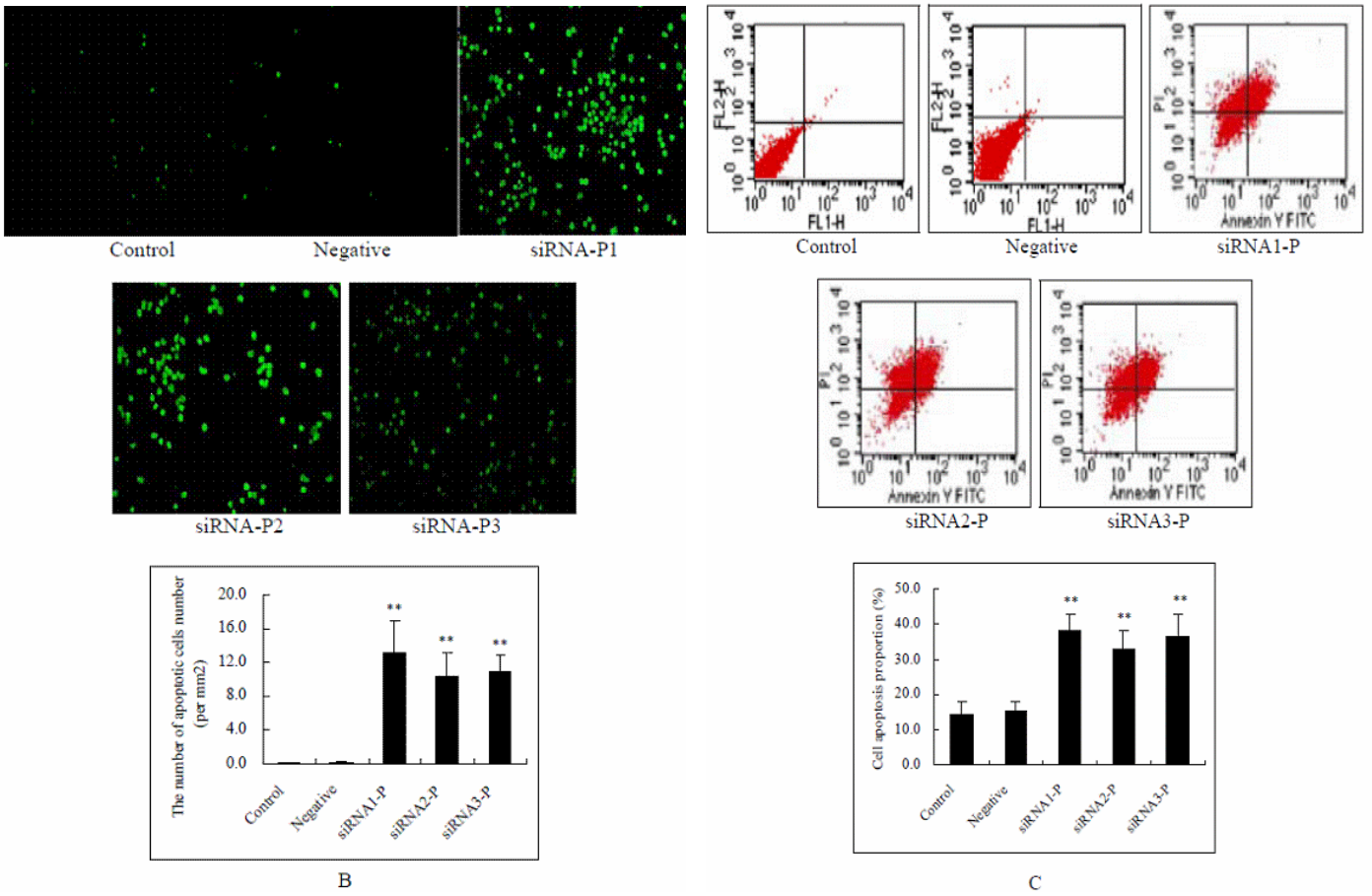

Figure 4. Apoptosis of gastric carcinoma cell SGC-7091 silenced by siRNAs targeting Phbs. A, Cell cycle assay of siRNA-silenced Phbs SGC-7091 cells. The SGC-7091 cells are arrested at the G_1/S phase; B, Immunofluorescence analysis of the apoptotic cell number of siRNA-silenced Phbs SGC-7091 cells. The apoptotic cell number is significantly increased after the silence of Phbs in the SGC-7091 cells; C, FCM assay of cell apoptosis in Phbs-silenced SGC-7091 cells pretreated with 5-FU. The cell apoptosis proportion is elevated in the siRNA-silenced Phbs SGC-7091 cells. Data are presented as mean (n=3) ± SEM. $^{**}P<0.01$ vs. Control.

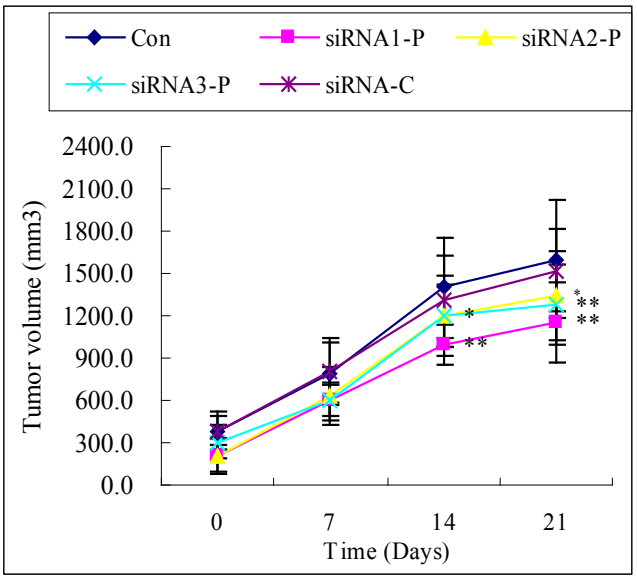

Figure 5. The tumor volumes of nude mice in groups at specific time spots. The tumor volumes of nude mice after the siRNAs treatments were significantly smaller than that in the control group. The tumor volume was smallest in the siRNA3-P group. Data are presented as mean (n=6) ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ vs. Control.

which might be associated with the growth suppression of the transplanted tumor.

Discussion

RNAi has been widely applied in gene diagnosis, gene therapy, drug screening, and etc. This technique has many sepecial advantages including high specificity, efficiency, high speed, and stability (16). In the present study we designed three sepecial siRNAs targeting Phbs. The qRT-PCR and immunofluorescence assays revealed that siRNA tareting Phbs efficiently knockdown Phbs at both mRNA and protein levels in gastric carcinoma cell line SGC7901 which has highest Phbs protein expressions among the cell lines tested.

It has confirmed that Phbs play important roles in cell growth, cell differentiation, apoptosis, and etc. Phbs interact with cell cycle regulatory proteins and their related signaling pathways to display its anti-proliferative activity (17). When pRb is inactivated, Phbs downregulate E2F activation, which in turn suppresses cell apoptosis (18). Upregulated expression of the Phbs complex was present in clinical tumor specimens, including those from bladder (19), prostate (20), and lung (21).

To the best of our knowledge, it is first time for us to investigate the roles of Phbs in proliferation and apoptosis of gastric carcinoma cell line SGC7901 *in vitro* and *in vivo*. Although it was originally identified by its ability to inhibit G1/S progression in human fibroblasts, its role as tumor suppressor is debated. In the present study we found Phbs overexpressions in the gastric carcinoma tested, compared to a normal gastric mucosa cell line GES-1. Then we silenced the Phbs by using siRNA-mediated silencing in gastric carcinoma cell lines described herein. Actually, siRNA-mediated silencing of Phbs significantly suppressed cell growth. Our finding is consistent to the study of Sievers et al (22). They found that prohibitins are required for cancer cell proliferation and adhesion. Down-regulation of prohibitin expression drastically reduced the rate of cell division. Further, loss of prohibitins did lead to a reduced growth capability independent of anchorage (22). However, our results are contradictory to some other finding (13). We speculated that these apparently inconsistent results may merely reflect differences in the cell types used or the Phbs phosphorylation state. In addition, we also found that siRNA-mediated Phbs silencing promoted cell apoptosis in the absence or presence of 5-FU and arrested cell cycle at the G_1/S phase transition. The results above suggested that on one hand siRNA-mediated Phbs silencing alone promoted cancer cell apoptosis, on the other hand the Phbs silencing significantly increased chemotherapeutic susceptibility of tumor cells that was helpful to improve chemotherapeutical efficiency and lower the chemotherapeutics dose to reduce the side effects.

It has been found that the anti-proliferative function of Phbs was later attributed to 3' UTR of the Phbs genes. Further, the anti-apoptotic function of Phbs was also reported that while total Phbs expressions were not changed, the proteins were enriched in lipid rafts after UVB irradiation. Reduced expressions of Phbs using siRNA knockdown resulted in an increase in cellular apoptosis after UVB irradiation (14). These findings were also consistent with our study. We also found that Phbs knockdown mediated by siRNA significantly promoted the SGC7901 cell apoptosis.

Subsequently, we investigated the roles of Phbs in nude mice inoculated with siRNA-P-treated gastric carcinoma cell. The *in vivo* experiment results were consistent with the finding *in vitro*. siRNA-silenced Phbs significantly inhibited the tumor growth. Cell proliferation plays a key role in the genesis and development of cancer. Accordingly, a promising therapeutic approach for inhibiting cancer cell growth is targeted repression of gene expression and/or protein activity that otherwise promotes proliferation. Further, we measured the expressions of apoptosis-related molecules such as Bax, Bcl-2, Caspase-3, and Caspase-9. As expected, siRNA-mediated Phbs silencings significantly upregulated Bax, Caspase-3, and Caspase-9 and downregulated Bcl-2, which closely associated with the increased cell apoptosis.

In summary, our findings suggest that Phbs play important roles in the proliferation and apoptosis of gastric carcinoma cell line SGC7901 *in vitro* and *in vivo,* Upregulated Bax, Caspase-3, and Caspase-9 and downregulated Bcl-2 were invovled in this event.

Acknowledgements

This work was supported by grants from the Foundation of Shanghai Key Laboratory of Pediatric Digestion and Nutrition (No. 11DZ2260500) and Science & Technology Key Program of Shanghai Committee (No. 10441901702).

References

1. Yang, L., Incidence and mortality of gastric cancer in China. *World J Gastroenterol.* 2006, **12**: 17-20. doi: 10.3978/j.issn.2224-4778

2. Zhu, X. and Li, J., Gastric carcinoma in China: Current status and future perspectives (Review). *Oncol Lett.* 2010, **1**: 407-412. doi: 10.3892/ol_00000071

3. Zhao, P., Dai, M., Chen, W. and Li, N., Cancer trends in China. *Jpn J Clin Oncol.* 2010, **40**: 281-285. doi: 10.1093/jjco/hyp187.
4. McClung, J.K., Danner, D.B., Stewart, D.A., Smith, J.R., Schneider, E.L., Lumpkin, C.K., Dell'Orco, R.T. and Nuell, M.J., Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver. Biochem. *Biophys. Res. Commun.* 1989, **164**: 1316-1322. doi: 10.1016/0006-291X(89)91813-5
5. Merkwirth, C. and Langer, T., Prohibitin function within mitochondria: essential roles for cell proliferation and cristae morphogenesis. *Biochim. Biophys. Acta.* 2009, **1793**: 27-32. doi: 10.1016/j.bbamcr.2008.05.013
6. Artal-Sanz, M. and Tavernarakis, N., Prohibitin and mitochondrial biology. *Trends. Endocrinol. Metab.* 2009, **20**: 394-401. doi: 10.1016/j.tem 2009.04.004.
7. Zhou, T.B., Zeng, Z.Y., Qin, Y.H. and Zhao, Y.J., Less Expression of prohibitin is associated with increased paired box 2 (PAX2) in renal interstitial fibrosis rats. *Int. J. Mol. Sci.* 2012, **13**: 9808-9825. doi: 10.3390/ijms13089808
8. Nan, Y., Yang, S., Tian, Y., Zhang, W., Zhou, B., Bu, L. and Huo, S., Analysis of the expression protein profiles of lung squamous carcinoma cell using shot-gun proteomics strategy. *Med. Oncol.* 2009, **26**: 215-221. doi: 10.1007/s12032-008 -9109-4
9. Najm, M.Z., Akhtar, M.S., Ahmad, I., Sadaf, Mallick, M.N., Kausar, M.A., Chattopadhyay, S., Ahad, A., Zaidi, S., Husain, S.A. and Siddiqui, W.A., Mutational analysis of prohibitin--a highly conserved gene in Indian female breast cancer cases. *Asian Pac J Cancer Prev.* 2012, **13**: 5113-5117. doi: 10.7314/APJCP.2012.13.10.5113.
10. Jupe, E.R., Badgett, A.A., Neas, B.R., Craft, M.A., Mitchell, D.S., Resta, R., Mulvihill, J.J., Aston, C.E. and Thompson, L.F., Single nucleotide polymorphism in prohibitin 3' untranslated region and breast-cancer susceptibility. *Lancet.* 2001, **357**: 1588-1589. doi: doi: 10.1016/S0140-6736(00)04747-4
11. Gamble, S.C., Odontiadis, M. and Waxman, J., Androgens target prohibitin to regulate proliferation of prostate cancer cells. *Oncogene.* 2004, **23**: 2996-3004. doi: 10.1038/sj.onc.1207444
12. Jang, J.S., Cho, H.Y., Lee Y.J., Ha, W.S. and Kim, H.W., The differential proteome profile of stomach cancer: identification of the biomarker candidates. *Oncol. Res.* 2004, **14**: 491 -499. doi: 10.3727/0965040042380441
13. Liu, T., Tang, H., Lang, Y., Liu, M. and Li, X., MicroRNA-27a functions as an oncogene in gastric adenocarcinoma by targeting prohibitin. *Cancer. Lett.* 2009, **273**: 233-242. doi: 10.1016/j.canlet.2008.08.003
14. Wu, Q. and Wu, S., Lipid rafts association and anti-apoptotic function of prohibitin in ultraviolet B light-irradiated HaCaT keratinocytes. *Exp. Dermatol.* 2012, **21**: 640-642. doi: doi: 10.1111/j.1600-0625.2012.01547.x
15. Livak, K.J. and Schmittgen, T.D., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001, **25**: 402 -408. doi: 10.1006/meth.2001.1262
16. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E. and Mello, C.C., Potent and specific genetic interference bydouble-stranded RNA in Caenorhabditis elegans. *Nature.* 1998, **391**: 806-811. doi: 10.1038/35888
17. Mishra S, Murphy LC, Nyomba BL, Murphy LJ. Prohibitin: a potential target for new therapeutics. *Trends Mol Med.* 2005, **11**: 192-197. doi: 10.1016/j.molmed.2005.02.004
18. Fellenberg, J., Dechant, M.J., Ewerbeck, V. and Mau, H., Identification of drug-regulated genes in osteosarcoma cells. *Int. J. Cancer.* 2003, **105**: 636-643. doi: 10.1002/ijc.11135
19. Wu, T.F., Wu, H., Wang, Y.W., Chang, T.Y., Chan, S.H., Lin, Y.P., Liu, H.S. and Chow, N.H., Prohibitin in the pathogenesis of transitional cell bladder cancer. *Anticancer. Res.* 2007, **27**: 895-900. doi: 10.1006/jabr.1996.0193
20. Ummanni, R., Junker, H., Zimmermann, U., Venz, S., Teller, S., Giebel, J., Scharf, C., Woenckhaus, C., Dombrowski, F. and Walther, R., Prohibitin identified by proteomic analysis of prostate biopsies distinguishes hyperplasia and cancer. *Cancer. Lett.* 2008, **266**: 171-185. doi: 10.1016/j.canlet.2008.02.047
21. Guo, F., Hiroshima, K., Wu, D., Satoh, M., Abulazi, M., Yoshino, I., Tomonaga, T., Nomura, F. and Nakatani, Y., Prohibitin in squamous cell carcinoma of the lung: its expression and possible clinical significance. *Hum. Pathol.* 2012, **43**: 1282-1288. doi: 10.1016/j.humpath.2011.10.006
22. Sievers, C., Billig, G., Gottschalk, K. and Rudel, T., Prohibitins are required for cancer cell proliferation and adhesion. *PLoS One.* 2010, **5**: e12735. doi: 10.1371/journal.pone.0012735