Gastric cancer is the most commonly diagnosed malignant cancer and is one of the most frequent causes of cancer mortality worldwide (1). From 1985 to 1996, 50,169 patients with gastric carcinoma, diagnosed and treated with gastrectomy, were registered in the National Cancer Data Base. Analysis of these data showed that the 5-year survival rate was 78% in stage IA, but 8% to 58% with more advanced disease (2). In Shanghai, the overall 5-year survival rate for patients with gastric cancer treated in Ruijin Hospital was 37.9% (in 2,145 patients) were registered in the National Cancer Data Base. Analysis of these data showed that the 5-year survival rate was 78% in stage IA, but 8% to 58% with more advanced disease (2). In Shanghai, the overall 5-year survival rate for patients with gastric cancer treated in Ruijin Hospital was 37.9% (in 2,145 patients) in the 1990s (3). Although population screening programs in Japan have improved the early detection of gastric cancer and improved the outcome, the majority of patients with gastric cancer are still diagnosed at an advanced stage in areas outside of Japan. The survival rate for patients with gastric cancer has also been increased by the combination of surgery and chemoradiotherapy, but the outcome of this common malignancy remains unsatisfactory mainly due to the poor understanding of the mechanism of gastric cancer development and the lack of specific target gene therapy (4). Therefore, prevention and early detection of the tumor is essential to reduce gastric cancer mortality. It has been known that the pathogenesis of gastric carcinomas is multifactorial, which includes genetic predisposition and environmental factors. Genetic predisposition has been found to have a number of genetic alterations including tumor suppressor genes, oncogenes, cell adhesion molecules, growth factors, and genetic stability (5). However, little is known about the exact molecular events leading to its development and progression.

The metallopanstimulin-1 (MPS-1) gene was first identified from human mammary carcinoma MDA-468 cells and is a growth factor–inducible gene which has been reported to play an important role in mediating cellular proliferation in response to various growth factors and other environmental signals (6). MPS-1 encodes a 9.4 kDa multifunctional ribosomal S27 protein homologous to the rat S27 ribosomal protein, and MPS-1 is ubiquitously expressed in normal tissues except for the brain and the placenta. However, MPS-1 is highly expressed in actively proliferating cells and cancer cell lines (6, 7). In addition, MPS-1 is up-regulated in a wide variety of cancer and improved the outcome, the majority of patients with gastric cancer are still diagnosed at an advanced stage in areas outside of Japan.
cancer tissues such as breast cancer, prostate cancer, colon cancer, head and neck carcinoma, melanoma, and hepatocellular carcinoma, suggesting that this protein may play a role in progression toward malignancy (7–12). MPS-1 has been considered to be an attractive target for anticancer therapy because MPS-1 is underexpressed in most normal human tissues and is overexpressed in a variety of tumors. The mechanisms of MPS-1 contributing to the progression of malignant tumor are still poorly understood, therefore, understanding this mechanism is of paramount interest in the target design for medical intervention in malignant tumor transformation.

The MPS-1 protein belongs to DNA-binding proteins containing one zinc finger domain of the C4 type, and it is likely a transcriptional factor (6). MPS-1 is also regarded as potentially being involved in the DNA repair and recognition of altered mRNA (6, 13), just like the proteins of the adenovirus E1A gene family and the steroid/thyroid hormone receptor superfamily, which are involved in the recognition of DNA damage. Recently, studies have also shown that MPS-1 is a tumor marker for early detection in certain types of cancer cells and could be an immunotherapeutic target in breast cancer (7, 14–16).

In a previous study, we identified MPS-1 as a gastric cancer–associated antigen and our RT-PCR analysis showed that the MPS-1 mRNA levels were significantly higher in gastric cancer tissues than those in the adjacent normal tissues. In this study, we examined MPS-1 protein levels in gastric cancer cell lines and tissue levels using immunohistochemical staining and Western blotting. To assess MPS-1 as a potential anticancer therapeutic target, we used the small interfering RNA (siRNA) method to down-regulate its expression in gastric cancer cell line SGC7901 and analyzed these cancer cells’ spontaneous apoptosis, growth ability, and tumorigenicity in vitro and in vivo. Our results showed that specific MPS-1 siRNAs could significantly suppress MPS-1 expression. Furthermore, we also showed that stable suppression of MPS-1 expression by vector-based siRNA increased spontaneous apoptosis, inhibited the growth ability and tumorigenicity of gastric cancer cells in vitro and in vivo.

Materials and Methods

Cell lines and tissue samples. Gastric cancer cell lines N87 (ATCC: CRL-5822), SNL-1 (ATCC: CRL-5971), AGS (ATCC: CRL-1739), and KATOIII (ATCC: HTB-103) were originally obtained from the American Type Culture Collection (Rockville, MD). Another two gastric cancer cell lines, MRK45 and SGC7901, were preserved in our institute. Immortalized gastric mucosal epithelial cell line GES-1 was a gift from Prof. Feng Bi (Institute of Digestive Disease, Xi’Jing Hospital, The Fourth Military Medical University, Xian, Shaanxi Province, PRC). All cell lines were routinely cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum in a humidified cell incubator with an atmosphere of 5% CO2 at 37°C.

A total of 90 primary gastric cancer and 18 adjacent normal tissues were studied in immunohistochemistry. The paraffin-embedded tissue specimens were derived surgically from patients with pathologic proven gastric carcinoma at the Department of General Surgery, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, from 2001 to 2003. The pathologic tumor staging was determined according to the American Joint Committee on Cancer tumor-node-metastasis classification (17). In addition, we also collected fresh cancer tissues and adjacent normal tissues from 20 patients with gastric cancer in Ruijin Hospital to evaluate the MPS-1 expression by Western blotting.

Preparation of anti-MPS-1 antibody. A full-length MPS-1 cDNA was subcloned into the pGEX-5X-1 vector (Amersham Biosciences, Piscataway, NJ) and GST-MPS-1 fusion protein was expressed in Escherichia coli BL-21 and purified by glutathione Sepharose chromatography. Rabbit antiserum specific to the recombinant protein was generated by s.c. inoculation of New Zealand white rabbits with 200 µg of the purified protein, again emulsified in complete Freund’s adjuvant. The animals were given booster doses a total of six times at 2-week intervals, and the booster doses contained the same amount of the respective purified proteins. Sera from the immunized animals were harvested 7 days after the last immunization and affinity-purified by an Affi-gel 10 (Bio-Rad, Hercules, CA) column with immobilized glutathione S-transferase and GST-MPS-1 fusion proteins as described previously (18). IgG fractions of preimmune sera were purified by another affinity chromatography on a Sepharose Protein A (Rockland Immunochemicals, Inc., Gilbertsville, PA) according to the manufacturer’s protocols. The purified antibodies were stored at −80°C until use.

Immunohistochemistry. Paraffin-processed sections were cut at 5 µm and mounted on xylene-treated glass slides. Each slide was dewaxed in xylene and rehydrated in grade alcohol, followed by boiling in 10 mmol/L of citrate buffer (pH 6.0) for antigen retrieval. After inhibition of endogenous peroxidase activity for 30 minutes with methanol containing 0.3% H2O2, the sections were blocked with 2% bovine serum albumin in PBS for 30 minutes and incubated overnight with specified diluted anti-MPS-1 antibody (1:400) at 4°C. Following washes with PBS, the slides were incubated for 10 minutes with the appropriate biotinylated linked anti-rabbit IgG secondary antibody (Dako, Carpinteria, CA), washed thrice for 10 minutes with the appropriate biotinylated linked anti-rabbit IgG secondary antibody (Dako, Carpinteria, CA), washed thrice for 10 minutes and incubated for 10 minutes with horseradish peroxidase–conjugated streptavidin (Dako) for 10 minutes. After extensive washing with PBS, 3,3′-diaminobenzidine + substrate + Chromogen (Dako) was added to the sections to visualize the antibody staining for 1 minute, and sections were counterstained with hematoxylin, dehydrated, and evaluated under a light microscope. Normal rabbit-purified IgG was used as a negative control for the staining reactions of MPS-1 expression.

The stained slides were scored independently by two observers without clinico-pathologic information. When there were different opinions, agreement was reached by careful discussion of evaluators. MPS-1 expression was determined in three categories (negative, weak positive, and strong positive) by assessing the percentage and intensity of stained tumor cells as described previously (19): the percentage of positive cells was classified according to five grades (percentage scores): <10% (grade 0), 10% to 25% (grade 1), >25% to 50% (grade 2), >50% to 75% (grade 3), and >75% (grade 4). The staining intensity was classified according to four grades (intensity scores): no staining (grade 0), light brown staining (grade 1), brown staining (grade 2), and dark brown staining (grade 3). MPS-1 staining positivity was determined with the formula: overall score = percentage score + intensity score. An overall score of ≤3, >3 to ≤6, and >6 was defined as negative, weak positive, and strong positive, respectively.

Plasmid construction. The pRNAT-H1.1/neco plasmid (GenScript, Piscataway, NJ), a siRNA expression vector containing a green fluorescent protein gene under a separate promoter for tracking the transfection efficiency, was used for the cloning of small synthetic oligonucleotides that encode two complementary sequences of 19 nucleotides separated by a short spacer region of 9 nucleotides. Three sequences unique to the coding region of MPS-1 were designed, inserted between the BamHI and HindIII sites of the pRNAT-H1.1/neco vector.
plasmid. The positive clones were confirmed by sequencing. Three sequences were deposited as follows:

- pRNAT-92 (92-110 nucleotides) oligo 1: 5'-GAGGAAAAACAGAGG-AAATTCAGAGATTTCTCTGTGTCTCTC-3',
- pRNAT-133 (133-151 nucleotides) oligo 2: 5'-CCTACTTCTCTGGATGTGGTTCTC-3',
- pRNAT-259 (259-277 nucleotides) oligo 3: 5'-GGCTTACAGAGGATGTTC-3',
- pRNAT-110 (110-128 nucleotides) oligo 4: 5'-CTCTGTGTCTCTGTAAGCC-3'.

**siRNA transfection and selection of SGC7901 stable transfec-tants.** SGC7901 is an adherent, moderately differentiated, human gastric cancer cell line which we previously used to clone MPS-1 as a gastric cancer–associated antigen gene.1 One day before transfection, SGC7901 cells were plated in a six-well plates with 1 x 10^5 cells per well using culture medium without antibiotics. The cells were transfected with 3.0 μg/well of pRNAT-vector and pRNAT-siRNA plasmids, respectively, using N-[1-(2,3-dioleoyloxy)propyl]-NNN-trimethylammoniummethyl sulfate (Roche, Indianapolis, IN) according to the manufacturer’s protocol. Cells transfected with medium but lacking DNA served as controls. Fresh growth medium was replaced to the manufacturer’s protocol. Cells transfected with medium but lacking DNA served as controls. Fresh growth medium was replaced after 4 hours of transfection. Cells were passaged at a 1:10 dilution at 24 hours after transfection and cultured in medium supplemented with G418 (Promega, Madison, WI) at 1,000 μg/mL for 4 weeks. Stably transfected clones were picked and maintained in medium containing 350 μg/mL G418 for further study.

**RT-PCR.** Total RNA was extracted from 2 x 10^5 cells by Trizol reagent (Invitrogen, Carlsbad, CA). One microgram of RNA was converted into cDNA using the Reverse Transcription System (Promega) with oligo-dT. PCR was carried out with a set of primers for MPS-1 included 5'-TGC AGA GCC CCA ATT CCT ACT-3' (F), 5'-GGC TAA AGA CCG TGG TGA TTTT-3' (R), β-actin 5'-TGC TAT CCC TGT ACG CCT CT-3' (F), and 5'-AGT ACT TGC CCT CAG GAG CA-3' (R), respectively. PCR amplification was initiated by 5 minutes of incubation at 95°C, ended after a 5-minute extension at 72°C, 30 cycles for denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension 72°C for 1 minute. We did RT-PCR in triplicate to confirm the reproducibility of the experiment and internal controls for β-actin were done on all specimens simultaneously.

**Western blotting.** Cells and tissues were lysed in M-PER Mammalian Protein Extraction Reagent (Pierce, Rockford, IL.) containing a cocktail of protease inhibitors (Bio-Rad). The lysated proteins were quantified by bicinchoninic acid protein assay kit from Pierce. Subsequently, equal amounts of proteins were separated by SDS-PAGE, then transferred to polyvinylidene difluoride membranes (Bio-Rad). Nonspecific binding sites were blocked by incubating with 5% nonfat milk in TBST buffer (TBS plus 0.1% Tween 20). The blots were washed with TBST and then incubated with a specific primary antibody overnight at 4°C. The blots were again washed with TBST and then incubated with horseradish peroxidase– conjugated anti-rabbit antibody (Santa Cruz Biotechnol ogy, Santa Cruz, CA) for 2 hours at room temperature. Proteins were visualized by enhanced chemiluminescence detection system (Amer sham, Freiburg, Germany). Autoradiograms were quantified by densitometry (Quantity One software, Bio-Rad). As a loading control, β-actin-specific antibody (Sigma, St. Louis, MO) was used. Relative protein levels were calculated by referring them to the amount of β-actin protein. The mean values from three independent experiments were taken as results. Polyclonal antibody recognizing caspase-3 and monoclonal antibody recognizing glutathione S-transferase were purchased from BD Biosciences PharMingen (Clontech, Palo Alto, CA).

**Cell growth assay.** The parental and stably transfected siRNA cells (1 x 10^5) were seeded into six-well plates in triplicate. Total cell numbers were counted every 2 days with a hemocytometer and an Olympus inverted microscope. The cell viability was assessed by using trypan blue. The number of cells per well was reported as the mean ± SD at the indicated number of days after plating.

**Soft agar colony formation assay.** Cells (3 x 10^3) were trypsinized to single-cell suspension and then plated in triplicate onto six-well plates.
in complete culture medium containing 0.3% agar on top of 0.6% agar in the same medium. The plates were incubated at 37°C in 5% CO₂ for 16 days, colonies were fixed with 70% ethanol, and stained with 0.2% crystal violet. The colonies containing at least 50 cells were counted.

Flow cytometric analysis of apoptosis and cell cycle status. Cells were routinely harvested by trypsin digestion at 24, 48, and 72 hours after seeding, washed with PBS, and then fixed in cold 75% ethanol at 4°C overnight. After staining with 250 μg/mL propidium iodide, 5 μg/mL RNase A, and 5 mmol/L EDTA in PBS (pH 7.4) for 30 minutes, cell cycle analysis was done on a FACSscan (Beckman Instruments, Fullerton, CA) to determine the percentage and distribution of cells in the apoptotic sub-G₁, and the G₁, S, and G₂-M phases. The apoptotic cells were assessed by flow cytometric detection of sub-G₁ DNA content of 1 × 10⁴ cells.

Cancer xenograft model and tumorigenicity assay. Six-week-old male BALB/c nu/nu nude mice (Institute of Zoology, Chinese Academy of Sciences) were housed in a specific pathogen–free environment at the Animal Laboratory Unit, School of Medicine, Shanghai Jiao Tong University. China. SGC7901 cells, SGC7901/vector, and SGC7901/Animal Laboratory Unit, School of Medicine, Shanghai Jiao Tong Sciences) were housed in a specific pathogen–free environment at the University, China. The terminal nucleotidyl transferase–mediated nick end labeling assay was carried out according to the manufacturer’s instructions of the FragEL DNA Fragmentation Detection kit (Calbiochem, La Jolla, CA) with some modifications. In brief, after endogenous peroxidases in the tissue sections were inhibited with 3% H₂O₂, the terminal nucleotidyl transferase–mediated nick end labeling reaction mixture was incubated with the tissues for 1.5 hours at 37°C in a humidified chamber. After the conjugate was incubated with tissue sections for 30 minutes, the 3,3′-diaminobenzidine substrate (Dako) was added at room temperature and maintained for 10 minutes. The slides were then counterstained with hematoxylin, dehydrated, and mounted with a glass coverslip and examined by light microscopy.

Statistical analysis. Results were expressed as mean ± SD. Statistical analysis was done using SAS 6.12 software package (School of Medicine, Shanghai Jiao Tong University). The χ² test and Fisher exact test were used to test the significance of the difference in frequency of MPS-1 between normal and tumor samples. The correlation between MPS-1 expression in the tumors and clinicopathologic variables was calculated with the Kruskal-Wallis rank test and the Mann-Whitney U test. P < 0.05 was selected as the statistically significant value.

Results

The expression pattern of MPS-1 in gastric cancer tissues. Over-expression of MPS-1 has been reported in many human cancers. However, its expression status in gastric cancer remains unclear. In the present study, expression and subcellular localization of MPS-1 were evaluated via immunohistochemistry in 90 primary gastric cancer tissues and 18 normal mucosal specimens. Normal mucosal specimens showed either no protein expression (n = 10) or very weak protein expression in the epithelium (Fig. 1A). Conversely, the immunoreactive

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patterns of MPS-1 were predominantly positively identified in the cytoplasm of cancer tissues (Fig. 1B-D) with a high frequency of 86% ($P < 0.001$).

The clinical and pathologic characteristics of 90 patients with gastric cancer are listed in Table 1. In the analysis of MPS-1 protein levels in gastric cancer tissues and various gastric cancer patients’ clinicopathologic variables, our results clearly showed a positive association of MPS-1 staining intensities with the tumor-node-metastasis stage. The index of MPS-1 staining was much higher in patients with stage III + IV than that of stage I + II disease ($P < 0.01$). However, there were no significant correlations between the levels of MPS-1 expression and other clinicopathologic characteristics, such as histologic classification, lymphatic invasion, and neoplastic metastasis.

**Western blotting analysis of MPS-1 expression in gastric cancer tissues and cell lines.** We used Western blotting to detect the expression of MPS-1 protein in gastric cancer tissues (Fig. 2A) and in gastric cell lines as well as in an immortalized gastric mucosa cell line (Fig. 2B). In 16 of 20 patients, MPS-1 protein levels were relatively higher in the cancer tissues than adjacent normal tissues. This finding was consistent with results from the immunohistochemical staining analysis. In six different gastric cancer cell lines, N87, SNU-1, AGS, KATOIII, MKN45, and SGC7901, and one immortalized gastric mucosa cell line,GES-1, our results showed that MPS-1 protein levels were highly expressed in all investigated gastric cancer cell lines as compared with the normal gastric mucosa cell line GES-1 (Fig. 2B). Particularly, AGS, SUN-1, and SGC7901 cell lines had ~24-fold, 18-fold, and 12-fold, respectively, higher MPS-1 protein levels than that in the GES-1 cell line, a normal gastric mucosa cell line (Fig. 2C). These results suggest that MPS-1 may have a role in the carcinogenesis of gastric cancer.

**Identification of the effective siRNA target sequence.** Because there is considerable variability in the effectiveness of different siRNA sequences, we first investigated three recombinant MPS-1-specific siRNA plasmids, pRNAT-92, pRNAT-133, and pRNAT-259. These MPS-1-specific siRNA plasmids and pRNAT-H1.1/neo vectors were transfected into SGC7901 cells. After 4 weeks of selection with G418, stable transfected clones were established. Three clones were examined for MPS-1 mRNA expression by semiquantitative RT-PCR and Western blotting. As shown in Fig. 3A and B, MPS-1 levels were different in the transfected SGC7901 cells containing pRNAT-92, pRNAT-133, pRNAT-259, and the pRNAT-H1.1/neo vector. There were no significant changes of MPS-1 expression in pooled SGC7901/v vector, SGC7901/pRNAT-92, and SGC7901/pRNAT-259 cells. However, In SGC7901/pRNAT-133 cells, MPS-1 mRNA and protein levels were significantly low, reduced by 85% and 90%, respectively, compared with parental SGC7901 cells. These results suggested that pRNAT-133 plasmid could specifically knock-down MPS-1 expression in the stable transfected SGC7901 cells.

**Inhibition of cell proliferation and colony formation by MPS-1 knockdown.** To study the effect of the down-regulation of MPS-1 in gastric cancer cell proliferation and colony formation, we selected two clones derived from stable transfectants with both control vector pRNAT-H1.1/neo and pRNAT-133 plasmids for further study. As shown in Fig. 3C, MPS-1 knockdown suppressed the cancer cell growth significantly in regular medium. The number of pooled SGC7901/pRNAT-133 cells was reduced by ~70%, 73%, and 76% by days 3, 5, and 7 after plating, respectively, compared with the SGC7901 cells ($P < 0.001$). Subsequent soft agar colony formation assay was done to evaluate the tumorigenicity of MPS-1 down-regulated cells in vitro. Colony formation rates were $5.4 \pm 2.2\%$, $27.5 \pm 4.2\%$ and $32.3 \pm 5.6\%$ in SGC7901/pRNAT-133, SGC7901/vector transfectants, and parental SGC7901 cells, respectively. Compared with the controls, SGC7901/pRNAT-133 exhibited not only a lower amount, but also a smaller size of colonies (Fig. 3D). Furthermore, we also discovered that ~30% to 40% of the SGC7901/pRNAT-133 transfectants exhibit aberrant morphology with larger and flatter cells observed under phase-contrast microscope (data not shown).

**Down-regulation of MPS-1 expression induces spontaneous apoptosis.** We have shown that down-regulation of MPS-1 expression levels inhibited the gastric cell proliferation and colony formation (see above). We also suspected that the down-regulation of MPS-1 expression also induces spontaneous gastric cell apoptosis. Indeed, our data showed that ~10% to 15% of SGC7901/pRNAT-133 transfectants exhibited morphologic features typical of apoptosis, including condensed chromatin and nuclear fragmentation by Hoechst 33342 staining (Fig. 4A) and flow cytometry for DNA content (Fig. 4B). The percentage of cells with hypodiploid DNA content ($sub-G_1$) was: SGC7901/vector transfectants, 1.2 ± 0.1%, 1.4 ± 0.1%, and 1.9 ± 0.2%; SGC7901/pRNAT-133 transfectants, 6.7 ± 0.8%, 9.6 ± 1.0%, and 13.5 ± 1.1% at
Stable suppression of MPS-1 expression inhibits cell growth and tumorigenicity of gastric cancer cells SGC7901 in vitro. A, specific gene silencing of MPS-1 appeared in siRNA-treated cells by RT-PCR analysis. More obviously, the knockdown effect on MPS-1 mRNA (120 bp) expression was shown in transfectants with pRNAT-133 compared with pRNAT-92, pRNAT-259, and pRNAT-vector plasmids. B, the same effects of siRNA on MPS-1 protein expression were confirmed by Western blotting. C, role of MPS-1 in regulating SGC7901 cell proliferation was determined by cell growth assay; cells from triplicate wells were collected and counted every other day. Points, mean of three independent experiments; bars, ±SD; *, P < 0.001 compared with SGC7901/vector. D, colony formation rates of SGC7901 parental cells, SGC7901/vector, and SGC7901/pRNAT-133 transfectants were analyzed by soft agar assay and compared between SGC7901/vector and SGC7901/pRNAT-133 cells. Columns, mean of at least three independent experiments; bars, ±SD; *, P < 0.001.

24, 48, and 72 hours, respectively (P < 0.01). However, the distribution of G1, S, and G2-M phases in different transfected cells with altered MPS-1 expression did not show remarkable change by fluorescence-activated cell sorting analysis (data not shown). Moreover, the cells with sub-G1 DNA content increased significantly, suggesting that MPS-1 knockdown cells undergo promoted spontaneous apoptosis. To further analyze this phenotype in SGC7901/pRNAT-133 transfectants, we treated the cells with serum deprivation. The SGC7901/pRNAT-133 transfectants were more susceptible to the stimuli and underwent an increased spontaneous apoptosis than parental and control vector–transfected cells (Fig. 4C). Anti-caspase-3 Western blotting was also done to analyze the apoptosis in SGC7901/pRNAT-133 transfectants. Caspase-3, the executioner caspase in apoptosis, was clearly activated, as shown by the cleavage of full-length protein (Fig. 4D).

**Suppression of MPS-1 inhibits tumorigenicity in vivo.** To examine whether down-regulation of MPS-1 expression could inhibit the tumorigenicity of SGC7901 cells in nude mice, the growth of xenograft tumor models from SGC7901, SGC7901/vector, and SGC7901/pRNAT-133 transfectants were compared (Fig. 5A). Our results showed that after the same amount of three types of cells were injected to mice, tumors appeared in all of the animals from two control groups at day 7. However, only three of eight mice that received injection of the SGC7901/pRNAT-133 transfectants developed a relatively small tumor at a later time (day 19). The other five animals remained tumor-free for an additional 1 month. Immunohistochemical staining and Western blotting also showed that MPS-1 protein was lowly expressed in tumors derived from SGC7901/pRNAT-133 transfectants but was highly expressed in tumors derived from SGC7901/vector transfectants and parental SGC7901 cells (Fig. 5B and C).

**Suppression of MPS-1 increased apoptosis in vivo.** We used terminal nucleotidyl transferase–mediated nick end labeling assay to assess tumor cell apoptosis in nude mice xenografts. As shown in Fig. 5D, the SGC7901/pRNAT-133 transfectants showed a more tumor cell positive staining and a significantly higher apoptotic index than the cells that were not transfected or those transfected with the vector (P < 0.001). These results suggested that inhibition of tumor growth was partly attributable to increased spontaneous apoptosis in vivo.

**Discussion**

MPS-1 belongs to the S27E family of ribosomal proteins and is highly expressed in a wide variety of actively proliferating cells (6, 7). Convincing evidence has also shown that MPS-1 is overexpressed in many types of malignant tumors and might be involved in the progression towards malignancy (7–9, 12). However, little is known about the expression of MPS-1 in gastric carcinoma and its correlation with the clinicopathologic features of these patients. In the current study, we showed that the expression of MPS-1 protein levels was higher in gastric tumor tissues than that in the adjacent normal tissues. Immunohistochemical analysis also confirmed that tumor cells exhibited abundant MPS-1 expression in the cytoplasm, in contrast to normal gastric tissues which displayed lower MPS-1 expression. Given the oncogenic properties of the expression patterns of MPS-1 in cancer tissues, the high expression of MPS-1 protein observed in gastric cancer supports its involvement in the development of human gastric cancer in vivo. To investigate whether MPS-1 expression might be associated with the progression of gastric cancer, the MPS-1 expression levels and the clinicopathologic characteristics of 90 patients with gastric cancer were compared. We did not find the relationship between the MPS-1 expression and gender, tumor size, differentiation grade, Lauren classification, and lymphatic invasion. However, there was a significant association between MPS-1 expression...
and tumor-node-metastasis stage. The MPS-1 expression was much higher in tumors with late stage than those with early stage, which implied that MPS-1 might also play an important role in malignant transformation of gastric cancer. These findings in gastric cancer are consistent with studies in other cancers (7–9, 12). Our results also showed that there was a tendency of high MPS-1 expression with metastasis, suggesting that MPS-1 might play a potential role in gastric cancer metastasis. However, this difference is not statistically significant (0.074), possibly because of our relatively small sample size.

We have clearly shown that the MPS-1 gene is highly expressed in gastric cancer cells from patient samples and cell

Fig. 4. MPS-1 knockdown induces spontaneous apoptosis in gastric cancer cells in vitro. A, nuclear morphology of SGC7901/vector and SGC7901/pRNAT-133 transfectants were stained with Hoechst 33342 and analyzed by fluorescence microscopy (magnification, ×200). Arrow, apoptotic cells. B, DNA content, measured by propidium iodide staining and flow cytometry of SGC7901/vector and SGC7901/pRNAT-133 transfectants at the indicated time intervals after seeding in regular medium. M, positions of sub-G₀ populations. C, SGC7901 parental cells and stable transfectants were incubated in medium with 10% FCS for 24 hours and switched to serum-free medium for another 48 hours. The percentage of apoptotic cells was measured by flow cytometry. Columns, mean of three independent experiments; bars, ±SD. *, P < 0.001 compared with parental cells and control transfectants; #, P < 0.001 compared with those cultured in 10% serum. D, Western blotting analysis of active caspase-3 expression in stable SGC7901/pRNAT-133 transfectants.
It is unclear whether down-regulation of the MPS-1 gene also affects gastric cancer cell tumorigenesis. Therefore, we have constructed specific MPS-1 siRNA plasmids and established permanent transfected cell lines to investigate the potential role of MPS-1 in the tumorigenicity of gastric cancer. We showed, for the first time, that a siRNA sequence contained 133 to 151 bp of the MPS-1 coding region, which significantly down-regulated the MPS-1 expression in cancer cell lines. Cellular growth assay and tumorigenicity assay, both in vitro and in vivo, revealed that MPS-1 depletion resulted in the inhibition of tumor growth in vivo. A, progression of tumors in nude mice. A total of \(3 \times 10^5\) parental SGC7901 cells and stable transfectants were inoculated into male nude mice. Tumor diameters were measured every 3 days. Points, mean of each group of mice (\(n = 8\)); *, \(P < 0.001\) versus SGC7901/vector. B, the expression level of MPS-1 protein in excised tumors was analyzed by Western blotting. C, MPS-1 expression was determined by immunohistochemical staining in solid tumors derived from parental SGC7901 cells and stable transfectants (original magnification, \(\times 200\)). D, apoptosis of tumor cells induced by MPS-1 knockdown was determined by terminal nucleotidyl transferase–mediated nick end labeling assay done as described in “Materials and Methods” (original magnification, \(\times 200\)). Arrows, positive cells. The percentage of apoptotic cells was counted. Columns, mean; bars, ± SD (\(n = 5\)); *, \(P < 0.01\) compared with SGC7901/vector transfectants.
growth and tumorigenicity of SGC7901 cells. These findings have provided evidence that MPS-1 indeed may function as key mediators of cell growth and tumorigenicity and will be a promising target for gastric cancer treatment.

One of the mechanisms of MPS-1 in tumorigenesis is likely involved in the decrease of cell apoptosis. This is supported by our results that down-regulation of MPS-1 activated caspase-3. Tumors develop precisely because of an imbalance between cellular proliferation and death. Although increased cellular proliferation has long been regarded as the predominant cause of neoplasia, in recent years, a growing body of evidence has also supported the hypothesis that cancer cells survive because they fail to undergo apoptosis, or programmed cell death. Previous studies have shown that gastric cancer is moderately resistant to apoptosis and that the incidence of spontaneous apoptosis in cancer cells was 1% to 2% of gastric carcinomas (21, 22). In this study, judged by the appearance of a sub-G1 population in fluorescence-activated cell sorting profiles, caspase-3 activation, and the formation of fragmented nuclei, promoted spontaneous apoptosis was the second major phenotype observed in gastric cancer cells with stable suppression of MPS-1 expression. Moreover, MPS-1 knockdown–induced apoptosis in transfecants could also be magnified by serum deprivation. Meanwhile, the analysis of cell cycle in this study did not detect any obvious cell cycle arrest. Combining these results, down-regulating MPS-1 expression did not induce cell cycle arrest but instead caused an increased incidence of spontaneous apoptosis to occur, we suggest that MPS-1 has an antiapoptotic effect in gastric cancer cells. However, the MPS-1 pathways involved in apoptosis remain obscure. Albert and colleagues have reported that MPS-1 protein contains one zinc finger domain, and could specifically bind the DNA oligomer containing a consensus cyclic AMP responsive elements sequence (6, 13). Considering that cyclic AMP responsive element–binding proteins can act as either activators or repressors of transcription, MPS-1 protein might be involved in regulating the transcription of specific genes related to growth control and apoptosis. Thus, further studies are also needed to elucidate the exact apoptotic mechanism revealed in this study and to determine whether MPS-1 knockdown–induced apoptosis is a general phenomenon in the various types of cells derived from different tumors.

In summary, we have shown that strong expression of MPS-1 occurred in gastric cancer and that the expression level of MPS-1 in gastric cancer tissues correlated with the advancement of tumor stage. With a vector-based siRNA method, we showed that stable down-regulation of MPS-1 expression inhibited gastric cancer cell growth, increased the incidence of spontaneous apoptosis, and suppressed tumorigenicity in nude mice. Therefore, MPS-1 is likely to play an important role in the tumorigenicity of human gastric cancer and is a promising molecular target for gastric cancer therapy. Given our previous studies in which MPS-1 was also found to be a gastric cancer–related antigen, the vector-based MPS-1 siRNA, combined with immune intervention, might be useful as a potential therapeutic method for the treatment of gastric cancer.

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**References**

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