Targeting ribonucleotide reductase M2 subunit by small interfering RNA exerts anti-oncogenic effects in gastric adenocarcinoma

WEI KANG[1-4], JOANNA H.M. TONG[1-3], ANTHONY W.H. CHAN[1], JUNHONG ZHAO[2], SHIYAN WANG[2], YUJUAN DONG[2], FRANKIE M.C. SIN[1-3], SAIFUNG YEUNG[1-3], ALFRED S.L. CHENG[5], JUN YU[2] and KAFAI TO[1-4]

[1]Department of Anatomical and Cellular Pathology, State Key Laboratory in Oncology in South China, Prince of Wales Hospital; [2]Institute of Digestive Disease, Partner State Key Laboratory of Digestive Disease; [3]Li Ka Shing Institute of Health Science, Sir Y.K. Pao Cancer Center, The Chinese University of Hong Kong, Hong Kong SAR; [4]Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, Guangdong; [5]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China

Received February 6, 2014; Accepted March 21, 2014

DOI: 10.3892/or.2014.3148

Abstract. Ribonucleotide reductase M2 subunit (RRM2) is one of the two subunits of human ribonucleotide reductase which plays a critical role in tumor progression. The aim of the present study was to analyze its expression, clinical significance and biological functions in gastric adenocarcinoma. We observed the upregulation of RRM2 mRNA and protein in all nine gastric cancer cell lines examined. In paired primary gastric cancers, both mRNA and protein levels of RRM2 were significantly upregulated in tumors compared with the corresponding non-tumorous gastric tissues. RRM2 protein expression correlated with higher tumor grade, advanced T stage and poor disease-specific survival. RRM2 knockdown in gastric cancer cell lines AGS, MKN1 and MKN28 significantly suppressed cell proliferation, inhibited monolayer colony formation, reduced cell invasion and induced apoptosis. Downregulation of RRM2 suppressed xenograft formation *in vivo*. Collectively, these findings suggest that RRM2 plays a crucial role in gastric tumorigenesis and may serve as a potential prognostic marker and therapeutic target in gastric cancer.

Introduction

Gastric cancer remains one of the most aggressive types of cancer in the world. The highest incidence is in eastern Asia, in China, Japan and Korea and South America and Eastern Europe (1-2). When presented, most cases are in the advanced stage. Despite the advancements in various treatment modalities, prognosis remains poor (3). Enhancing our understanding of the carcinogenetic process (4) and exploring new treatment options are important to improve the outlook of gastric cancer patients. Gastric carcinogenesis is a multistep process involving genetic and epigenetic alterations of protein-coding proto-oncogenes (5) and tumor-suppressor genes (6-7). In addition, recent studies have demonstrated the involvement of microRNAs (8) and emerging evidence underscores the importance of signaling pathways such as NF-κB, Wnt/β-catenin signaling and Notch signaling involved in the gastric cancer development (9).

Human ribonucleotide reductase (RR) is an enzyme that catalyzes the conversion of ribonucleotide 5'-diphosphates into 2'-deoxyribonucleotides which is essential for DNA synthesis and cell proliferation (10,11). RR consists of two subunits, RRM1 and RRM2. These two subunits are encoded by two distinctive genes on different chromosomes, and their mRNAs are differentially expressed throughout the cell cycle. The cellular RRM1 protein level is stabilized throughout the entire cell cycle, whereas RRM2 is only expressed during the late G1/early S phase when DNA replication occurs (12). Therefore the catalytic activity of RR is tightly controlled during the cell cycle by the level of RRM2.

Given the critical role of RR in cell growth, it has been considered as a potential therapeutic target for cancer therapy. RRM2 contributes to malignant cellular phenotype in a range of human cancers and its overexpression plays a critical role in tumor invasion (13,14). A previous study showed that sequence-specific small interfering RNA (siRNA)- mediated inhibition of RRM2 effectively blocked the cell proliferation and induced G1/S phase cell cycle arrest in melanoma cell lines (15). However, the biological functions of RRM2 in gastric cancer remain unclear. The present study investigated the tumorigenic role of RRM2 in gastric cancer.

Correspondence to: Professor Kafai To, Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, P.R. China
Email: kfto@cuhk.edu.hk

Key words: gastric cancer, ribonucleotide reductase M2 subunit, siRNA

Materials and methods

Cell lines and clinical gastric adenocarcinoma samples. The ten gastric cancer cell lines (MKN28, KATO-III, MKN45, SNU16, SNU1, MKN7, MKN1, NCI-N87, AGS and MGC-803) (16), were cultured in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin and 10 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37˚C.

Archival tissue blocks from 270 patients with gastric adenocarcinoma were retrieved from the Department of Anatomical and Cellular Pathology, Prince of Wales Hospital (PWH), Hong Kong, and arranged in tissue array blocks. Hematoxylin and eosin-stained sections were used to define tumor areas, and 3 representative 1-mm cores were obtained from each case and inserted to a recipient paraffin block using a tissue arrayer (Beecher Instruments, Silver Spring, MD, USA). Frozen tissues from another ten pairs of primary gastric cancer and non-tumorous gastric mucosa were collected from patients who underwent gastrectomy prior to any therapeutic intervention. In addition, 27 paired gastric cancer and non-tumorous gastric frozen tissue cDNAs were kindly provided by the Institute of Digestive Disease (IDD) of Prince Wales Hospital for RRM2 qRT-PCR analysis.

RNA extraction and qRT-PCR. Total RNA was extracted using RNeasy Mini Kit according to the manufacturer's instructions (Qiagen) and the concentration was measured by NanoDrop 1000 (Thermo Fisher Scientific). High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems) were applied to achieve cDNAs. For qRT-PCR, SYBR-Green PCR Master Mix (Applied Biosystems) was applied for RRM2 expression (sense, GAAGGCAGAGGCTTCCTTTT and antisense, TACTATGCCATCGCTTGCTG). Commercially available normal gastric mRNA was obtained from Ambion (AM 7996). The relative expression level was normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and calculated using the $2^{-\Delta\Delta Ct}$ method. Quantitative PCR was performed in triplicates.

Immunohistochemical staining. Immunohistochemical staining of RRM2 was performed in 4 μm sections. After de-waxing in xylene, rehydrating and rinsing in distilled water, sections were incubated at 3% H_2O_2 solution for 25 min to block endogenous peroxidase activities. Antigen retrieval was carried out by using a pressure cooker with 10 nM citrate buffer (pH 6.0) for a total of 30 min (high power 10 min followed by low power 20 min). The primary antibody RRM2 (Santa Cruz, 10846; 1:50 dilution in primary dilution buffer) was incubated at 4˚C overnight and chromogen development was performed using the standard avidin-biotin method (Dako). The cytoplasmic expression of RRM2 was assessed by combining the proportional score and the intensity score as previously described (16). The proportion score was calculated according to the proportion of tumor cells with positive cytoplasmic staining (0, none; 1, ≤10%; 2, ≤25%; 3, >25%; 4, >50%). The intensity score was assigned for the average intensity of positive tumor cells (0, none; 1, weak; 2, intermediate; 3, strong). The cytoplasmic score of RRM2 was the product of the proportion and intensity scores, ranging from 0 to 12. The cytoplasmic expression was categorized into negative (score 0), 1+ (score 1-3), 2+ (score 4-6) and 3+ (score 7-12).

Western blot analysis. Western blot analysis was performed as previously described (17). RRM2 protein was detected with a polyclonal anti-RRM2 antibody (Santa Cruz, 10846, 1:500). Other antibodies were from Cell Signaling, cleaved-caspase 3 (Asp175) (no. 9541, 1:1,000), cleaved PARP (Asp214) (no. 9541, 1:1000), p-P44/42(T202/Y204) (no. 9154, 1:1,000), p-P38MAPK(T180/Y182) (no. 4511, 1:1,000), phospho-AKT(S473) (no. 9271, 1:1,000), phospho-Stat3 (T705) (no. 9145, 1:2,000), anti-Mouse IgG-HRP (Dako, 00049039, 1:30,000) and anti-Rabbit IgG-HRP (Dako, 00028856, 1:40,000).

In vitro functional assays. Transfection was performed using Lipofectamine 2000 transfection reagent (Invitrogen) and the anti-RRM2 siRNA (verified siRNA, SI02653441; Qiagen) at the concentration of 25 nM. Mock transfection (Lipofectamine 2000 only) and scramble siRNA were included as controls. Cell proliferation was assessed using CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT; Promega, Madison, WI, USA) according to the manufacturer's instructions. Monolayer colony formation assay was performed as described in our previous report (17). Ten days after transfection of siRRM2 or scramble siRNA, the colonies were fixed and stained with 2% crystal violet. Colonies with >50 cells were counted. The experiments were performed in triplicate wells and repeated 3 times. Cell invasion assay was performed in a 24-well invasion chamber (BD Biocoat Matrigel Invasion Chamber, BD Biosciences). siRRM2 or scramble siRNA transfectants were seeded at $5x10^4$ cells/well and allowed to invade for 24 h through BD Biocoat Matrigel Invasion Chambers. Cells that invaded through the Matrigel membrane were counted from 3 microscopic fields (original magnification, x400).

Flow cytometry analysis. For cell cycle analysis, the cells were harvested 24 h after transfection, stained with propidium iodide (PI), and sorted by FACS Calibur Flow Cytometer (Becton, Dickinson, San Diego, CA, USA). The cell cycle profiles were determined using the ModFitLT software (Becton-Dickinson). For early apoptosis analysis, Annexin V-FITC Apoptosis Detection Kit (BioVision, Milpitas, CA, USA) and 7-Amino-actinomycin (7-AAD, KeyGEN BioTECH) were used according to the manufacturer's instructions.

In vivo animal study. MGC-803 cells transfected with si-scramble or siRRM2 were injected subcutaneously into the dorsal flank of five 4-week-old male Balb/c nude mice (si-scramble on the left and siRRM2 on the right). When the tumors were palpable after 8 days, the synthetic siRNA complex (25 nM) with siPORT amine transfection reagent (Ambion) in 50 μl PBS was delivered intratumorally at 3-day intervals. The mice were sacrificed after 3 weeks and the xenografts were collected.

Statistical analysis. In the functional assays such as MTT proliferation, monolayer colony formation, cell invasion and *in vivo* tumor growth, Student's t-test was used to compare the phenotype difference of siRRM2 knockdown cells and

si-scramble transfected cells. RRM2 mRNA expression in gastric cancer and paired non-cancerous tissues were compared by the Mann-Whitney U test. Correlations between RRM2 expression with other clinicopathological parameters were evaluated by non-parametric Spearman's rho rank test. The Kaplan-Meier method was used to estimate the survival rates according to the score of RRM2 cytoplasmic staining. For those statistically significant variables found in the univariate survival analysis (P<0.05), the multivariate survival was achieved by the Cox regression analysis. All statistical analysis was performed by SPSS software (version 16.0; SPSS Inc). A two-tailed P-value <0.05 was considered to indicate statistically significant differences.

Results

Upregulation of RRM2 in gastric cell lines and primary gastric tumors. The RRM2 mRNA expression levels were higher in all nine gastric cancer cell lines examined compared to the normal gastric tissue as shown in Fig. 1A. The upregulation of RRM2 in the gastric cancer cells was further confirmed by western blot analysis. High RRM2 protein expression was observed in all gastric cancer cell lines compared with the 2 normal gastric mucosal samples from patients who underwent weight reduction gastric surgery (Fig. 1B). In the panel of gastric cancer cell lines, RRM2 was strongly expressed in Kato-III, SNU16, SNU1 and AGS cell lines. When comparing tumor with the corresponding non-tumorous mucosa, upregulation of RRM2 protein was observed in 8/10 cases by western blot analysis (Fig. 1C). We further evaluated the RRM2 mRNA expression in a cohort of 27 pairs of gastric cancer and corresponding non-tumorous mucosa. Expression of RRM2 in tumor cells was significantly higher than in non-tumorous mucosal tissues (P=0.035, Fig. 1D). Immunohistochemical analysis of another 5 pairs of gastric cancer samples showed positive staining of RRM2 in all tumor tissues but not in the non-tumorous gastric glandular epithelium (data not shown).

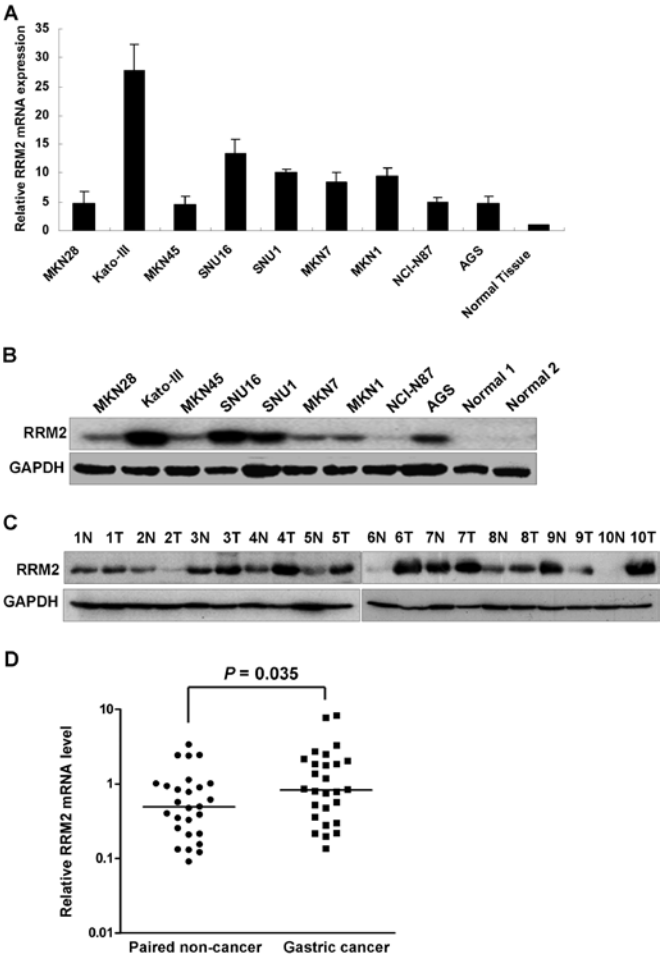

Figure 1. RRM2 upregulation in gastric cancer cell lines and primary gastric tumors. (A) RRM2 mRNA expression in 9 gastric cancer cell lines compared with normal gastric mRNA commercially available from Ambion (AM 7996). The experiment was performed in triplicates. (B) RRM2 protein expression in gastric cancer cell lines and 2 normal gastric mucosal tissues from patients with weight reduction gastric surgery. (C) Western blotting of RRM2 in 10 pairs of gastric tumors (T) and the corresponding non-tumorous mucosa (N). (D) Dot plot of qRT-PCR results from RRM2 mRNA expression level in 27 pairs of gastric adenocarcinoma and the corresponding non-tumorous mucosa (P=0.035). RRM2, ribonucleotide reductase M2 subunit.

RRM2 overexpression correlates with poor prognosis in gastric cancer patients. We studied the expression of RRM2 by immunohistochemistry in a cohort of 270 primary gastric adenocarcinoma samples. The cytoplasmic expression of RRM2 was assessed by assigning a proportional score and an intensity score as described in Materials and methods. No RRM2 expression (score 0) was observed in 14 cases (5.2%), weak cytoplasmic expression (score 1+) was observed in 98 cases (36.3%), and 116 cases (42.9%) and 42 cases (15.6%) demonstrated moderate (score 2+) and strong (score 3+) cytoplasmic staining, respectively. A total of 112 cases (41.5%) were considered negative/low expression of RRM2 (score 0 and 1+), whereas 158 cases (58.5%) with scores 2+ and 3+ were considered strongly positive for RRM2. Expression of RRM2 was primarily localized in the cytoplasm of the tumor cells (Fig. 2A). The association of RRM2 expression with clinicopathological parameters is shown in Table I. Strong RRM2 expression correlated with higher tumor grade (P=0.010) and advanced T stage (P=0.025). Univariate analysis indicated that expression of RRM2 in gastric adenocarcinoma associated with poorer disease-specific survival (P=0.012, Fig. 2B). Old age (age, >60 years, P=0.043), diffuse type histology (P<0.001), higher tumor grade (P=0.035) and advanced stage (P<0.001) were also correlated with poor disease-specific survival by Univariate analysis (Table II). Using multivariate Cox proportional hazards regression analysis, only age (P<0.001) and stage (P<0.001) were independently associated with disease-specific survival (Table II).

RRM2 knockdown reduces proliferation and invasiveness in gastric cancer cells. Upregulation of RRM2 in gastric cancer suggested a potential tumorigenic role of RRM2 in cancer development. To elucidate the functional role of RRM2 in gastric tumorigenesis, we first investigated the effect of RRM2 knockdown by siRNA *in vitro*. To avoid cell-context dependent effects, the experiments were performed in multiple gastric cancer cell lines. The 5-day MTT assay indicated that knocking down RRM2 significantly reduced cell proliferation (AGS, 62.2%, P<0.001; MKN1, 84.4%, P=0.011; MKN28, 89.1%, P=0.016; Fig. 3A) compared with the mock and the

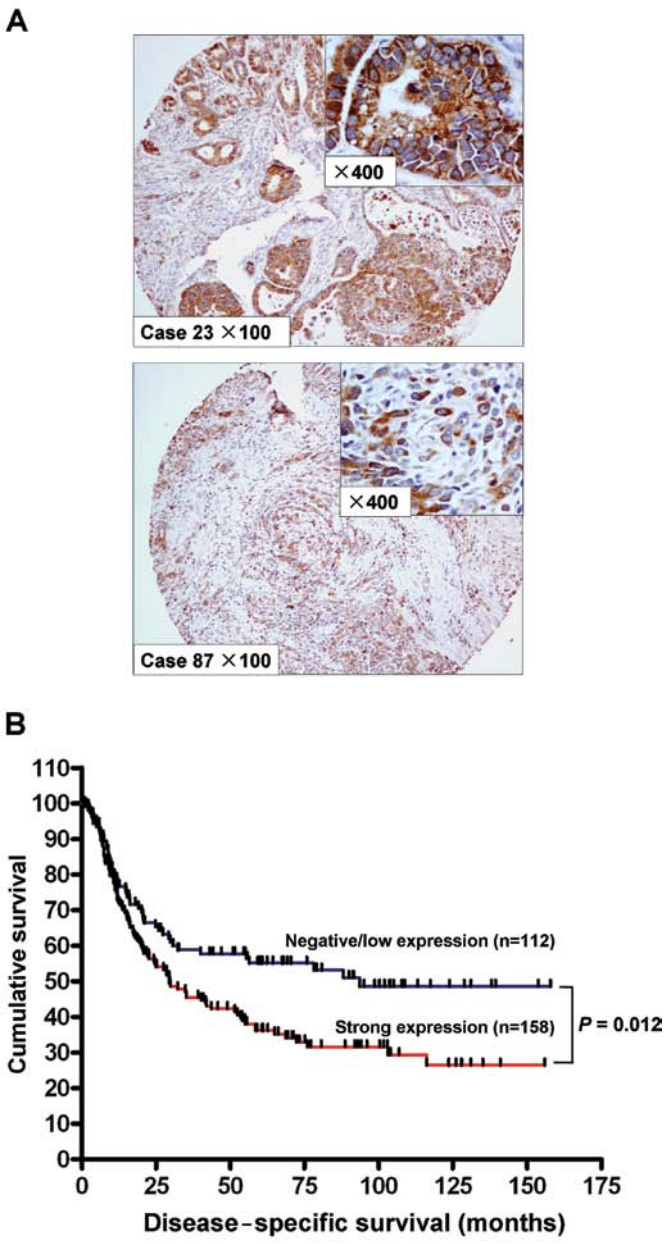

Figure 2. RRM2 upregulation correlates with poor prognosis. (A) Representative images of RRM2 immunohistochemical staining in primary gastric cancers. Case 23, intestinal type and case 87, diffuse type (original magnification ×100, insertion ×400). (B) Kaplan-Meier survival curve according to RRM2 staining status (negative and low expression groups, 112 cases; strong expression group, 158 cases; P=0.012) in gastric adenocarcinoma. RRM2, ribonucleotide reductase M2 subunit.

Table I. Correlation between RRM2 expression and other clinicopathological parameters.

	RRM2 expression (n=270)		
Characteristics	Negative and low	Strong	P-value
Gender			
Male	75	106	0.983
Female	37	52	
Age, years			
≤60	48	53	0.127
>60	64	105	
Type			
Intestinal	63	84	0.710
Diffuse	49	73	
Grade			
1	8	1	**0.010**
2	43	57	
3	61	99	
Stage			
I	29	29	0.241
II	16	16	
III	30	55	
IV	37	57	
Stage (T)			
1	20	16	**0.025**
2	31	42	
3	52	95	
4	9	4	
Stage (N)			
0	32	27	0.070
1	31	39	
2	30	50	
3	19	41	
Stage (M)			
0	93	134	0.613
1	19	23	
H. pylori			
Absence	45	75	0.254
Presence	62	75	

RRM2, ribonucleotide reductase M2 subunit. Significant P-value in bold format.

scramble siRNA control groups. In anchorage-dependent monolayer colony formation assays, we observed a significant reduction of colony number in cell lines transfected with anti-RRM2 siRNA compared with the scramble siRNA control group (reduced to 32.4, 50.2 and 25.1% in AGS, MKN1 and MKN28, respectively, P<0.001; Fig. 3B). Matrigel invasion assays were carried out to monitor the invasion potential of gastric cancer cells transfected with siRRM2. By using BD Biocoat chamber, we found that the number of invaded cells was significantly lower in siRRM2 transfectants than in the control groups (reduced to 57.3% in AGS, P=0.004; 64.9% in MKN1, P=0.009; and 36.2% in MKN28, P=0.006; Fig. 3C).

To further investigate the effects of anti-RRM2 siRNA on tumorigenicity of gastric cancer, *in vivo* tumor formation assay was carried out. A gastric cancer cell line MGC-803, which is able to form xenograft tumor in nude mice, was transfected with siRRM2. Three weeks later, mice were sacrificed and the transplanted tumors were removed and weighted. A significant decrease in xenograft weight was observed upon RRM2 knockdown (P<0.01; Fig. 3D1). Successful knockdown of RRM2 was confirmed by western blot analysis in xenograft tumors (Fig. 3D2). The findings support that RRM2 plays an important role in promoting malignant growth of gastric cancer cells *in vivo*.

Table II. Univariate and multivariate Cox regression analysis of clinicopathological factors in patients with gastric adenocarcinoma.

Characteristics	Univariate analysis RR (95% CI)	P-value	Multivariate analysis
Gender			
Male	0.832 (0.593-1.167)	0.286	
Female	1.000		
Age, years			
≤60	0.700 (0.496-0.988)	**0.043**	**<0.001**
>60	1.000		
Type			
Intestinal	0.543 (0.390-0.755)	**<0.001**	0.958
Diffuse	1.000		
Grade			
1	0.221 (0.054-0.897)	**0.035**	0.740
2	0.720 (0.508-1.021)	0.065	
3	1.000		
Stage			
I	0.098 (0.054-0.178)	**<0.001**	**<0.001**
II	0.127 (0.061-0.264)	**<0.001**	
III	0.385 (0.265-0.560)	**<0.001**	
IV	1.000		
H. pylori			
Absence	1.244 (0.884-1.750)	0.211	
Presence	1.000		
RRM2			
Negative/low	0.637 (0.448-0.905)	**0.012**	0.402
Strong	1.000		

RRM2, ribonucleotide reductase M2 subunit. Significant P-value in bold format.

RRM2 knockdown changes the cell cycle distribution in gastric cancer cells. To investigate the mechanisms underlying the growth suppressive role of anti-RRM2 siRNA, the effects of anti-RRM2 siRNA on cell cycle distribution were analyzed. Twenty-four hours after transfection, accumulation of S-phase cells was observed in the anti-RRM2-transfected group. As shown in Fig. 4A, the anti-RRM2-transfected cells showed increased percentages of S-phase cells (AGS, from 35.9 to 54.6%; MKN1, from 30.4 to 38.1%; MKN28, from 32.4 to 42.1%) and decreased percentages of G1 phase cells (AGS, from 61.6 to 33.4%; MKN1, from 54.2 to 48.4%; MKN28, from 44.0 to 34.4%) compared with the si-scramble control group cells.

Furthermore, the RRM2 knockdown was shown to induce both early and late apoptosis in AGS cells. The sub-G1 AGS cell population increased to 14.5% in siRRM2 transfectants compared with si-scramble transfectants (4.7%; Fig. 4B1). This was further validated by Annexin V-FITC apoptosis analysis, as shown in Fig. 4B2. There was an increase in early apoptotic cell population in AGS cells after anti-RRM2 siRNA transfection (from 6.3 to 13.5%, P<0.01). Late apoptosis, represented by a significant increase of cleaved-caspase 3 and cleaved-PARP (Fig. 4C), was also observed in AGS after anti-RRM2 siRNA transfection compared with the control groups.

In the meantime, the expression levels of several phosphorylated proteins which were involved in cell cycle arrest, cell growth or cell apoptosis were determined. As shown in Fig. 4C, p-P44/42(T202/Y204) and p-P38MAPK(T180/Y182) were found to be phosphorylated to high level upon anti-RRM2 siRNA transfection compared with the si-scramble control, whereas p-AKT (S473) and p-Stat3 (T705) phosphorylation status showed no change between these two groups.

Discussion

In the present study, we found both mRNA and protein levels of RRM2 were highly expressed in gastric cancer samples compared with normal gastric mucosa. The expression of RRM2 in primary gastric cancer correlated with advanced T stage and poor prognosis. These results suggested that RRM2 might play a role in gastric tumorigenesis. In keeping with this finding, a previous analysis of RRM2 genomic sequence and promoter region, including consensus TATA box, three CCAAT boxes, and several GC rich sites, provided the hypothesis of its upregulation in favor of cancer transformation and drug resistance (18). According to a previous study (19), RRM2 overexpression that was significantly associated with Epstein-Barr virus, expression of survivin and DNA

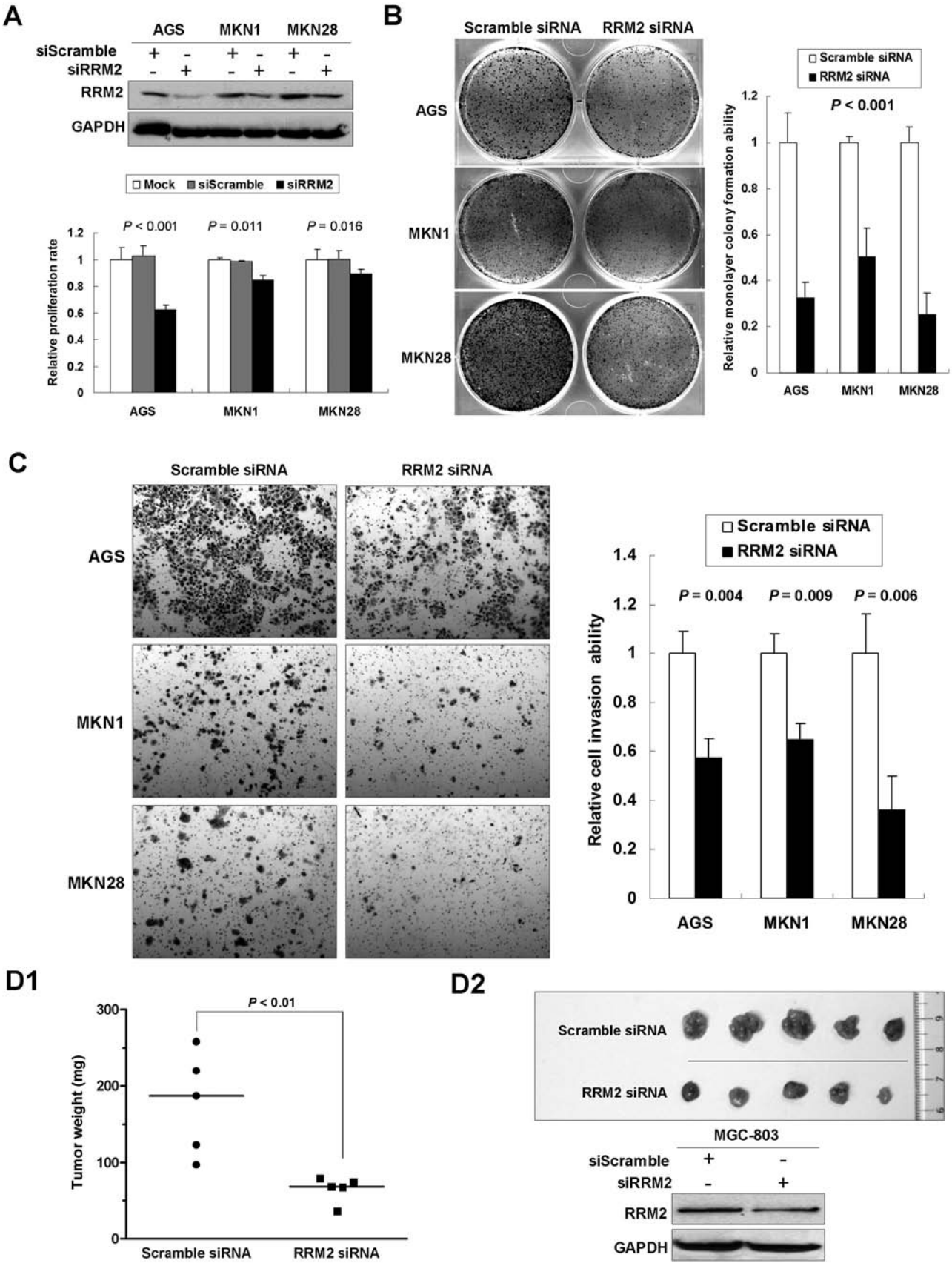

Figure 3. RRM2 knockdown by siRNA in gastric cancer cell lines. (A) Transfection with RRM2 siRNA reduced RRM2 protein expression in AGS, MKN1 and MKN28 cells. Five-day MTT assays revealed RRM2 siRNA suppressed gastric cancer cell proliferation. (B) siRRM2 reduced monolayer colony formation to 32.4, 50.2 and 25.1% in AGS, MKN1 and MKN28 cells, respectively. The experiments were performed in triplicates. (C) Representative images of invaded cells are shown. The number of invaded cells was reduced in siRRM2 transfectants compared to scramble controls in AGS, MKN1 and MKN28 cells. The number of cells was counted in three random microscopic fields and the error bars represented standard deviations. (D1) MGC-803-siRRM2 formed smaller xenograft tumors than MGC-803-si-scramble group 3 weeks after inoculation. (D2) Upper, xenograft images; lower, western blotting validation of RRM2 expression in the xenografts. RRM2, ribonucleotide reductase M2 subunit.

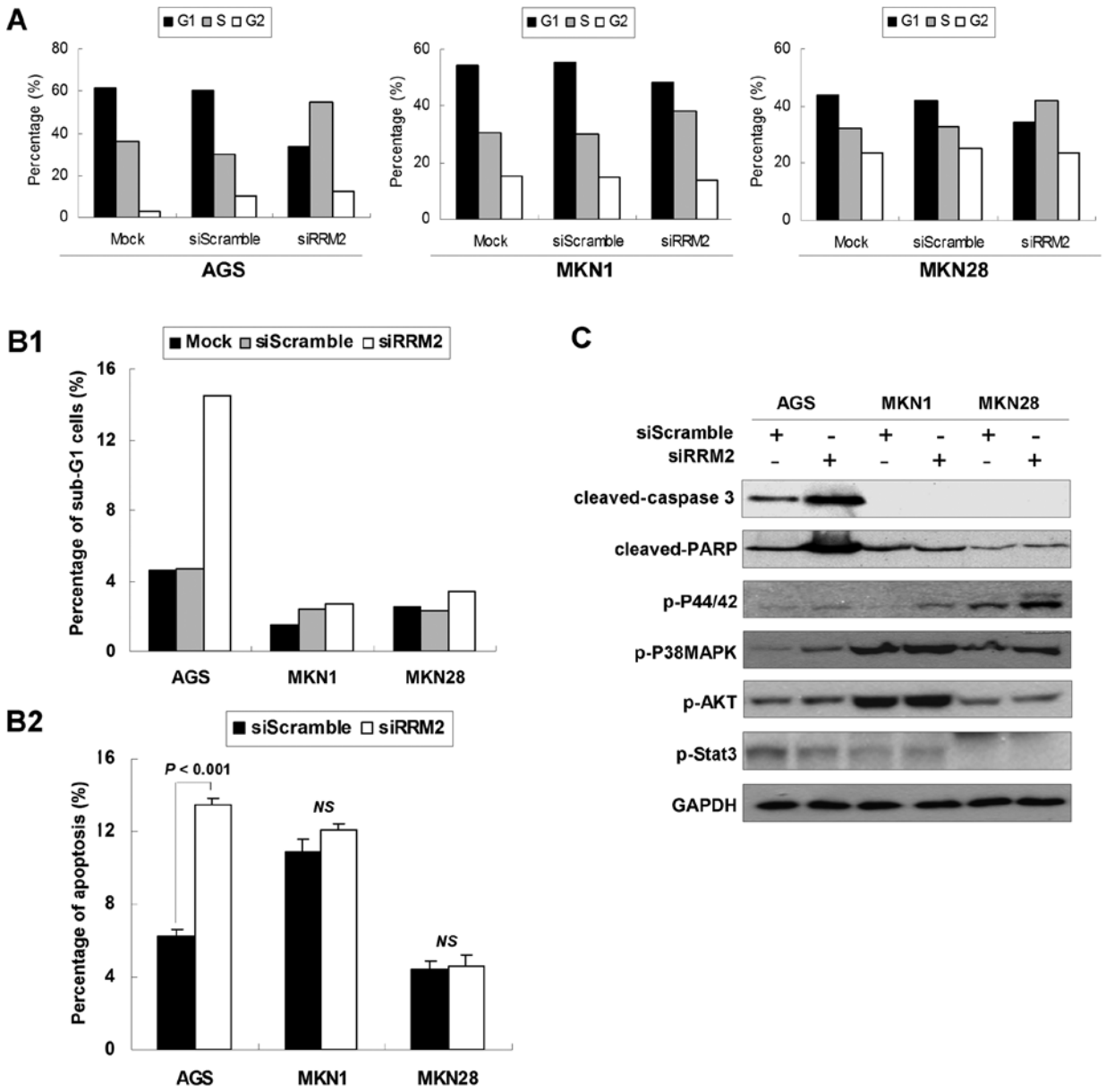

Figure 4. RRM2 knockdown inhibits cell cycle progression. (A) S phase accumulation and G1 reduction of cells were observed 24 h post-transfection. Representative figures from two independent experiments are shown. (B1) Sub-G1 population of the transfectants in AGS, MKN1 and MKN28. AGS showed increased sub-G1 cell population after siRRM2 transfection (4.7-14.5%). The experiments were repeated in two independent times. (B2) Early apoptosis analysis by Annexin V staining showed increased cell population with early apoptosis after siRRM2 transfection in AGS (6.3-13.5%, P<0.01). (C) Western blot analysis of cleaved-caspase 3, cleaved-PARP, p-P44/42 (T202/Y204), p-P38MAPK (T180/Y182), P-AKT (S473) and p-Stat3 (T705). RRM2, ribonucleotide reductase M2 subunit.

methyltransferase 1, predicted poor prognosis in patients with gastric cancer. Collectively, these results provided evidence that RRM2 served as a potential biomarker and therapeutic target for gastric cancer. In addition, RRM2 has been proposed to be a biomarker for a several types of human cancer. These previous studies suggested that RRM2 may serve as a potential diagnostic biomarker in breast cancer (20), bladder cancer (21) and ovarian cancer (10). RRM2 may also be a potential prognostic marker in non-small cell lung cancer receiving chemotherapeutic agents (22) and a potential therapeutic target for hepatocellular carcinoma (HCC) (23), bladder cancer (21) and pancreatic carcinoma (24,25).

The functional studies demonstrated for the first time that RRM2 knockdown by siRNA suppressed *in vitro* and *in vivo* growth of gastric cancer cells, and reduced cancer cell invasiveness. These results were concordant with a previous report (26) that anti-RRM2 siRNA exhibited anti-proliferative activity in various types of cancer cells. In addition to the apoptotic aspect, we found that anti-RRM2 siRNA induced both early and late apoptosis in AGS cells but not in MKN1 and MKN28 cells. Hence, the apoptotic response to siRRM2 appeared to be cell context-dependent. It is uncertain whether the different TP53 status might contribute to the different apoptotic responses as MKN1 and MKN28 cells harbor the TP53 mutation, whereas AGS cells carry the wild type TP53 (17). It has been reported that antisense RRM2 downregulated RRM2 expression and sensitized a prostate cancer cell line PC3 to UV radiation (27). In pancreatic

adenocarcinoma, synergism between RRM2 siRNA and gemcitabine resulted in increased tumor apoptosis (28). RRM2 is a downstream target of the ATM-p53 pathway that mediates radiation-induced DNA repair (29) and silencing of RRM2 was found to enhance DNA damage as measured by histone γ-H2AX. Under DNA damage and Chk1 activation mediated by ATM and ATR, upregulation of RRM2 expression level coupled with its nuclear recruitment suggests an active role of RRM2 in the cellular process in response to DNA damage (30).

RRM2 knockdown by siRNA-reduced gastric cancer cell invasion ability was also observed in this study. RRM2 overexpression in pancreatic adenocarcinoma increases cellular invasiveness and MMP-9 expression in an NF-κB-dependent manner, whereas RNA interference-mediated silencing of RRM2 expression attenuates cellular invasiveness (13). In KB cells, RRM2 plays a positive role in angiogenesis and growth through regulation of the expression of antiangiogenic thrombospondin-1 (TSP-1) and proangiogenic vascular endothelial growth factor (VEGF) (14). These results suggested that RRM2 has a significant role in driving tumor cell invasion and metastasis.

In conclusion, we demonstrated the upregulation of RRM2 in gastric adenocarcinoma and its overexpression was correlated with poor prognosis in patients with gastric cancer, suggesting that RRM2 plays a crucial role in gastric tumorigenesis and could be used as a potential prognostic biomarker in gastric cancer. Functional studies demonstrated that downregulation of RRM2 expression by RRM2-specific siRNA quenched its oncogenic properties by inhibiting cell growth *in vitro* and *in vivo*, decreasing cell invasiveness. These findings suggested that RRM2 might be involved in gastric tumorigenesis and might potentially serve as a prognostic marker and a therapeutic target in gastric cancer.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (81201591).

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