RAD52 motif-containing protein 1 promotes non-small cell lung cancer cell proliferation and survival via cell cycle regulation

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Abstract. DNA repair proteins such as RAD52 have been implicated in tumor progression and response to chemotherapy. RAD52 motif-containing protein 1 (RDM1) has been implicated in the response to chemotherapeutic agent cisplatin; however, its function in lung cancer progression remains unclear. This study aimed to investigate the role of RDM1 in the progression of non-small cell lung cancer (NSCLC). We found elevated RDM1 mRNA and protein expression in NSCLC tissues and cell lines compared to levels in normal lung cells. RDM1 protein expression in lung cancer tissues was found to correlate with tumor size, histological differentiation, lymph node metastasis and tumor-node-metastasis (TNM) stage. Knockdown of the *RDM1* gene with siRNA significantly reduced the cellular proliferation rate and increased apoptosis in the human NSCLC cell line, NCI-H1299. Compared to wild-type NCI-H1299 cells, *RDM1* knockdown enhanced the activity of caspase-3 and caspase-7, and decreased the proportion of cells in the S-phase of the cell cycle. Taken together, these data imply that RDM1 promotes the survival and proliferation of NSCLC cells. Due to its similarity to RAD52, we hypothesized that RDM1 potentially repairs DNA double-strand breaks arising through DNA replication, thereby preventing G_2/M cell cycle arrest. Accordingly, specific targeting of RDM1 may be a novel therapeutic strategy in the treatment of NSCLC.

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Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NSCLC, non-small cell lung cancer; qPCR, quantitative polymerase chain reaction; RDM1, RAD52 motif-containing protein 1; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastasis

Key words: non-small cell lung cancer, RAD52 motif-containing protein 1, RDM1, DNA repair, cell cycle, apoptosis

Introduction

Lung cancer is a leading cause of tumor-related morbidity and mortality worldwide (1,2). Despite recent breakthroughs in the diagnosis and treatment of this disease, the prognosis of patients remains poor, especially for those with metastatic disease (2). Standard treatment for lung cancer includes traditional chemotherapeutic agents such as cisplatin and docetaxel; however, their efficacy is somewhat hindered by the development of drug resistance (3). In the case of non-small cell lung cancer (NSCLC), newer targeted-molecular therapies have shown great promise (4,5), yet the majority of patients with advanced disease still develop drug resistance within one year (6). Therefore, there is a pressing need to develop new therapeutic options in the fight against lung cancer, and to better understand the mechanisms leading to drug resistance.

Drug resistance has been linked to changes in the DNA repair capacity within tumor cells (7). Chemotherapeutic drugs such as cisplatin induce DNA damage in tumor cells, and, in turn, activate the DNA damage response. Activation of the DNA damage response by chemotherapeutic drugs can have the desired effect of tumor cell apoptosis, or the cells may repair the lesion, leading to chemoresistance.

Components of the DNA damage response pathway have not only been implicated in chemoresistance, but they themselves may play oncogenic roles (8,9). Indeed, the capacity to repair damaged DNA through homologous recombination has previously been implicated in patient susceptibility to lung cancer (10,11). For example, NSCLC patients expressing high levels of RAD51, a protein involved in homologous recombination and DNA repair, had significantly poorer 5-year survival rates compared to those with low RAD51 expression (12). Similarly, the DNA repair protein RAD52 was implicated as an oncogene in lung squamous cell carcinoma, with enhanced tumor cell death observed in mouse cells ablated of RAD52 (9,13). Therefore, it is worth exploring components of the DNA damage response pathway, such as RAD1/2, as antitumor drug targets in lung cancer.

In the present study, we examined the role of RAD52 motif-containing protein 1 (RDM1) in NSCLC. The *RDM1* gene was identified through a database search looking for

proteins similar to RAD52 (14). Like RAD52, RDM1 is involved in DNA double-strand break repair and homologous recombination (14,15). Previous research has shown that RDM1 binds to cisplatin-damaged DNA *in vitro*, suggesting its possible role in the development of chemoresistance (14). As further evidence, RDM1 ablation in a chicken B cell line DT40 resulted in a more than 3-fold increase in cisplatin sensitivity (14). However, RDM1-knockout cells were not hypersensitive to DNA damage caused by ionizing radiation, ultraviolet irradiation, or alkylation (14). Therefore, the precise role of RDM1 in cisplatin resistance remains unclear.

Subsequent research has shown that RDM1 expression is modulated by heat shock (16). More recent studies revealed that the DNA-binding capability of RDM1 is enhanced at low pH, indicating that it may function in acidic conditions such as the tumor microenvironment (17). Furthermore, RDM1 has been shown to be upregulated at the mRNA level in lung squamous cell carcinoma (18). Nonetheless, despite the growing evidence, the precise function of RDM1 in NSCLC remains largely unknown. Therefore, this study aimed to clarify the role of RDM1 in NSCLC.

Materials and methods

Patients and tissue specimens. Paraffin-embedded tumors and adjacent non-cancerous tissue specimens were collected from a series of 103 patients with NSCLC who had only undergone surgical resection, but had not received chemotherapy between January 1, 2015 and 31 December 31, 2016 at the Inner Mongolia Autonomous Region People's Hospital, Hohhot, Inner Mongolia, China. The study group consisted of 77 men (75%) and 26 women (25%), and the mean age was 60.7 years (range, 32-81 years). The specimens were classified by their tumor-node-metastasis (TNM) stage (19). Detailed patient information including gender, age, tumor size, histological differentiation, and lymph node metastasis is documented in Table I. The study was approved by the Medical Ethics Committee of Inner Mongolia Autonomous Region People's Hospital, and all participants provided written, informed consent.

Immunohistochemistry. Paraffin-embedded 4-μm tissue sections were stained with the anti-RDM1 antibody. In brief, deparaffinized tissue sections were processed for antigen retrieval using target retrieval solution (pH 9.0; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) at 120˚C for 4 min. Non-specific immunoreactions were blocked at room temperature for 30 min using the Dako Protein-Block kit (Agilent Technologies, Inc.). Sections were incubated at 4˚C overnight with the Invitrogen RDM1 polyclonal primary antibody (1:100; cat. no. PA5-50103; Thermo Fisher Scientific, Inc., Waltham, MA, US)A, followed by incubation with 3,3'-diaminobenzidine tetrahydrochloride (Dako; Agilent Technologies, Inc.) for 5 min. Nuclear counter staining was conducted with hematoxylin. Images were captured using a light microscope (Olympus BX50; Olympus Corp., Tokyo, Japan) and processed using Photoshop software (version 7.0; Adobe, San Jose, CA, USA).

Cell culture. Human lung cancer cell lines, including NCI-H1299, A549, H1688 and H1975, were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). Human normal lung epithelial BEAS-2B cells were used as control. Cells were cultured in Gibco™ RPMI-1640 medium (Thermo Fisher Scientific, Inc.), containing 10% HyClone™ fetal bovine serum (FBS; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and 100 U/ml penicillin/streptomycin. All cells were incubated at 37˚C in a humidified atmosphere containing 5% CO_2.

Knockdown of RDM1 by lentivirus. The target sequence for RDM1-specific shRNA (shRDM1: 5'-GGCCCATCCTGG TTTCTAT-3') and the negative control shRNA (shCtrl: 5'-TTC TCCGAACGTGTCACGT-3') were obtained from GeneChem Biotech (Shanghai, China). The NCI-H1299 cell line was transfected with shRDM1 or shCtrl using transfection reagents (c1507; Applygen Technologies, Inc., Beijing, China) according to the manufacturer's instructions.

Western blot analysis. Lung tissue samples and human lung cancer cell lines were homogenized in RIPA buffer (50 mmol/l Tris-HCl, 150 mmol/l NaCl, 0.5% sodium deoxycholate, 1% NP-40, 0.1% SDS, 2 mmol/l Na3VO4, 500 mmol/l NaF, 58 μg/ml aprotinin, 10 μg/ml leupeptin, and 2 mmol/l phenylmethylsulfonyl fluoride) using the Multi Beads Shocker system (Yasui-Kikai, Osaka, Japan) at 4˚C. Protein samples (15 μg) were separated by SDS-PAGE using a gradient gel (5-20%; ATTO, Tokyo, Japan), followed by electroblotting onto polyvinylidene difluoride membranes (ATTO) and incubation in a blocking buffer of 5% non-fat milk for 1 h at room temperature.

Membranes were incubated with the following primary antibodies: rabbit anti-RDM1 (1:1,000; cat. no. ab201293; Abcam) and mouse anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:5,000; Chemicon, Temecula, CA, USA) at 4˚C overnight. Membranes were visualized by incubation with appropriate horseradish peroxidase-linked secondary antibodies, followed by enhanced chemiluminescence (ECL) detection using ECL Plus western blotting detection reagents (Amersham Biosciences, Little Chalfont, UK). The immunoreactive bands of targeted proteins were quantified by VH analyzer software (VH-H1A5; Keyence Corp., Osaka, Japan), normalized by GAPDH levels, and determined relative to the sham values. All experiments were repeated three times.

Quantitative polymerase chain reaction (qPCR). Total RNA was extracted from lung tissue samples and human lung cancer cell lines using the RNeasy Mini Kit (Qiagen Sciences, Inc., Gaithersburg, MD, USA), purified with the RNase-Free DNase Set (Qiagen), and used as a template for cDNA synthesis by the PrimeScript RT Reagent Kit (Takara, Shiga, Japan). PCR reactions were performed in a Thermal Cycler Dice Real Time System Tp800 (Takara) using SYBR Premix ex *Taq* (Takara) according to the manufacturer's instructions as follows: 30 sec at 95˚C, then 40 cycles of 5 sec at 95˚C and 30 sec at 60˚C; then for the dissociation stage, 15 sec at 95˚C, 30 sec at 60˚C, and 15 sec at 95˚C. The sequences of primers were: RDM1 forward primer, 5'-GCCCATCCTGGTTTCTATGCC-3' and reverse primer, 5'-AGACGAACCTTGACTGGAGAT-3'; GAPDH forward primer, 5'-TGACTTCAACAGCGACAC CCA-3' and reverse primer, 5'-CACCCTGTTGCTGTAGCC

Table I. Association between RDM1 expression and the clinicopathological characteristics of the NSCLC cases.

Clinicopathological parameters	Total	RDM1 expression		P-value
		Negative (n=45)	Positive (n=58)	
Age (mean ± SD, years)	103	61.8±10.2	59.6±11.7	0.368
Gender, n (%)				
Male	77	32 (41.5)	45 (58.5)	0.447
Female	26	13 (50)	13 (50)	
Tumor size (cm), n (%)				
≤3	38	27 (71.1)	11 (28.9)	0.007[a]
>3	65	18 (27.7)	47 (72.3)	
Histological differentiation, n (%)				
Well	7	5 (71.4)	2 (28.6)	0.013[a]
Moderate	35	19 (54.3)	16 (45.7)	
Poor	61	21 (34.4)	40 (65.6)	
Histological classification, n (%)				
Squamous carcinoma	61 (59)	24 (39.3)	37 (61.7)	0.653
Adenocarcinoma	12 (12)	7 (58.3)	5 (41.7)	
Adenosquamous	10 (10)	4 (40)	6 (60)	
Others	16 (3)	9 (56.2)	7 (43.8)	
Lymph node metastasis, n (%)				
Absent	42	24 (57.1)	18 (42.9)	0.034[a]
Present	61	21 (34.4)	40 (65.6)	
TNM stage, n (%)				
I+II	59	37 (62.7)	22 (37.3)	0.004[a]
III+IV	44	8 (18.2)	36 (81.8)	
1-year survival rate, n (%)	48 (46.6)	31 (68.9)	16 (27.6)	0.045[a]

[a]P<0.05, statistical significance. RDM1, RAD52 motif-containing protein 1; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastasis.

AAA-3'. Relative gene expression data were determined by the standard curve method, and fold-changes were normalized by GAPDH, and relative to control values. Each sample was tested in triplicate.

Detection of cell proliferation. The NCI-H1299 cell line was seeded into 96-well plates at a density of 2000 cells per well, and infected with the control or RDM1-siRNA lentivirus. Cell numbers were then counted via Celigo S Image Cytometer (Nexcelom Bioscience, Ltd., Manchester, UK) every day for 5 days to determine the growth rate. All samples were assayed in triplicate.

Detection of caspase-3/-7 activity. After lentivirus infection, caspase-3/-7 activity was detected using the Caspase-Glo® 3/7 Assay (G8091; Promega, Madison, WI, USA) according to the manufacturer's instructions. In brief, the NCI-H1299 cell line was seeded into 96-well plates at a density of 2,000 cells per well, and harvested when the confluence reached 80%. Caspase-Glo® 3/7 Reagent (100 μl) was added to each well of a white-walled 96-well plate containing 100 μl of blank, negative control cells, or treated cells in culture medium, and incubated in the dark at room temperature for 30 min to 3 h. The reaction product was detected at 405 nm using a plate-reading luminometer (M2009PR, Tecan Infinite, Switzerland). All samples were assayed in triplicate.

Determination of apoptosis by flow cytometry. The Invitrogen™ eBioscience™ Annexin V Apoptosis Detection Kit APC (88-8007; Thermo Fisher Scientific, Inc.) was used for labeling of apoptotic cells according to the manufacturer's protocol. The cells from each group were collected after lentivirus infection, washed twice with ice-cold phosphate-buffered saline (PBS) and resuspended in 200 μl binding buffer containing 10 μl Annexin V-APC. Fluorescence intensity was measured by flow cytometry (BD Biosciences). All samples were assayed in triplicate.

Cell cycle analysis by flow cytometry. After lentivirus infection, the NCI-H1299 cell line was seeded into 6-well plates at a density of $2x10^5$ cells per well, and harvested when the confluence reached 80%. The collected cells were fixed in 75% cold ethanol (10009269; Sinopharm Chemical Reagent, Co., Ltd., Shanghai, China) for 1 h at 4˚C. The cells were then washed with cold PBS and stained with 500 μl propidium iodide (PI) buffer (P4170 Sigma; Merck KGaA, Darmstadt, Germany) for 1 h at 37˚C in the dark. The cell cycle stages were analyzed by flow cytometry. All samples were assayed in triplicate.

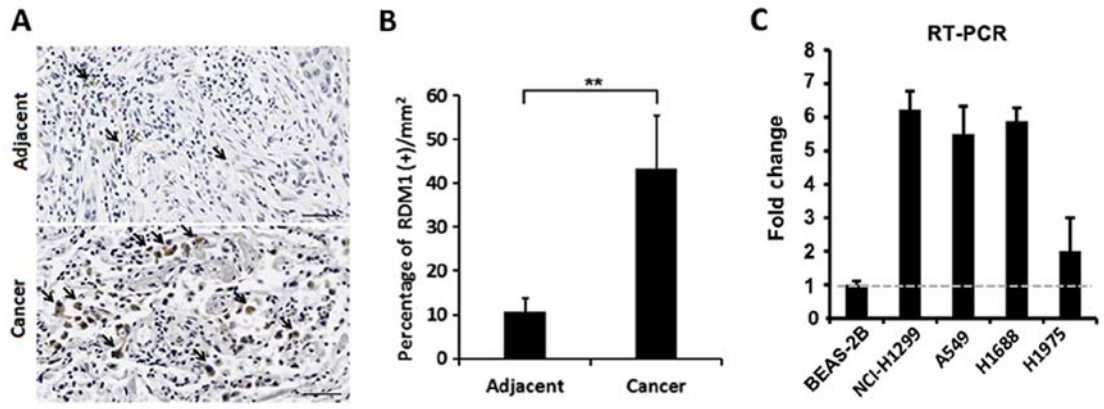

Figure 1. Expression of RDM1 in lung cancer tissue and cell lines. (A) Representative images of immunohistochemical staining for RDM1 in lung cancer and adjacent tissues. Arrows indicate RDM1-positive cytoplasm. Scale bar, 100 μm. (B) Quantification of the percentage of RDM1-positive cells per area (mm^2) in lung cancer and adjacent tissues (**P<0.01; Student's t-test). (C) Quantitative PCR analysis of relative *RDM1* gene expression in the lung cancer cell lines NCI-H1299, A549, H1688, H1975 and human normal lung epithelial BEAS-2B cells. GADPH expression was used to normalize the data. RDM1, RAD52 motif-containing protein 1.

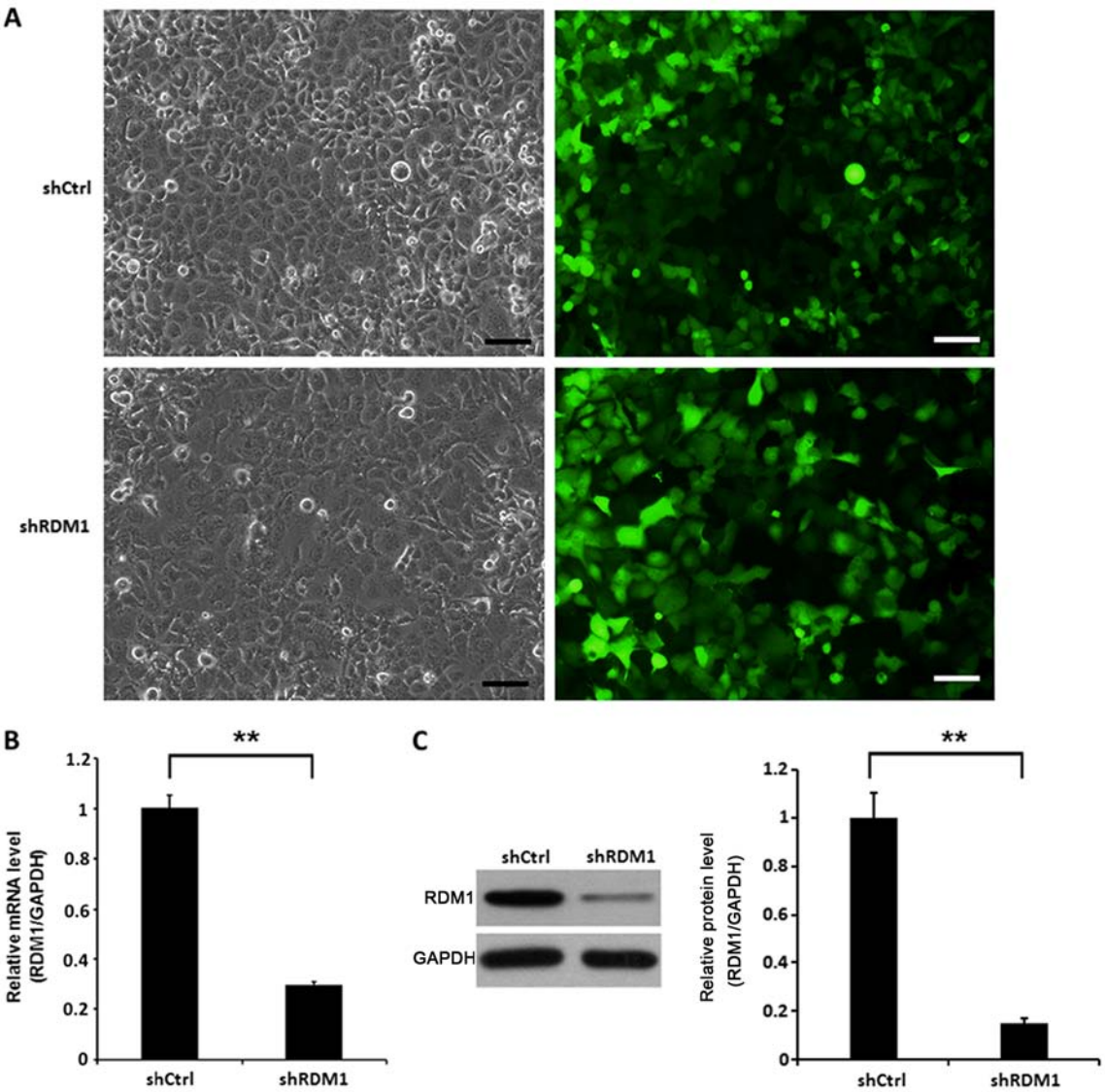

Figure 2. Knockdown of *RDM1* by lentiviral siRNA. (A) NCI-H1299 cells were infected with control (shCtrl) or RDM1-siRNA lentivirus (shRDM1) and examined by light microscopy and fluorescence microscopy at day 3 post-infection. Note: most cells expressed GFP. Scale bar, 50 μm. (B) Quantitative PCR analysis showing a significant decrease in *RDM1* mRNA level after *RDM1* knockdown in the NCI-H1299 cell line (**P<0.01; Student's t-test). (C) Western blot analysis showing a significant decrease in the RDM1 protein level after *RDM1* knockdown in the NCI-H1299 cell line (**P<0.01; Student's t-test). GADPH was used as a control. RDM1, RAD52 motif-containing protein 1.

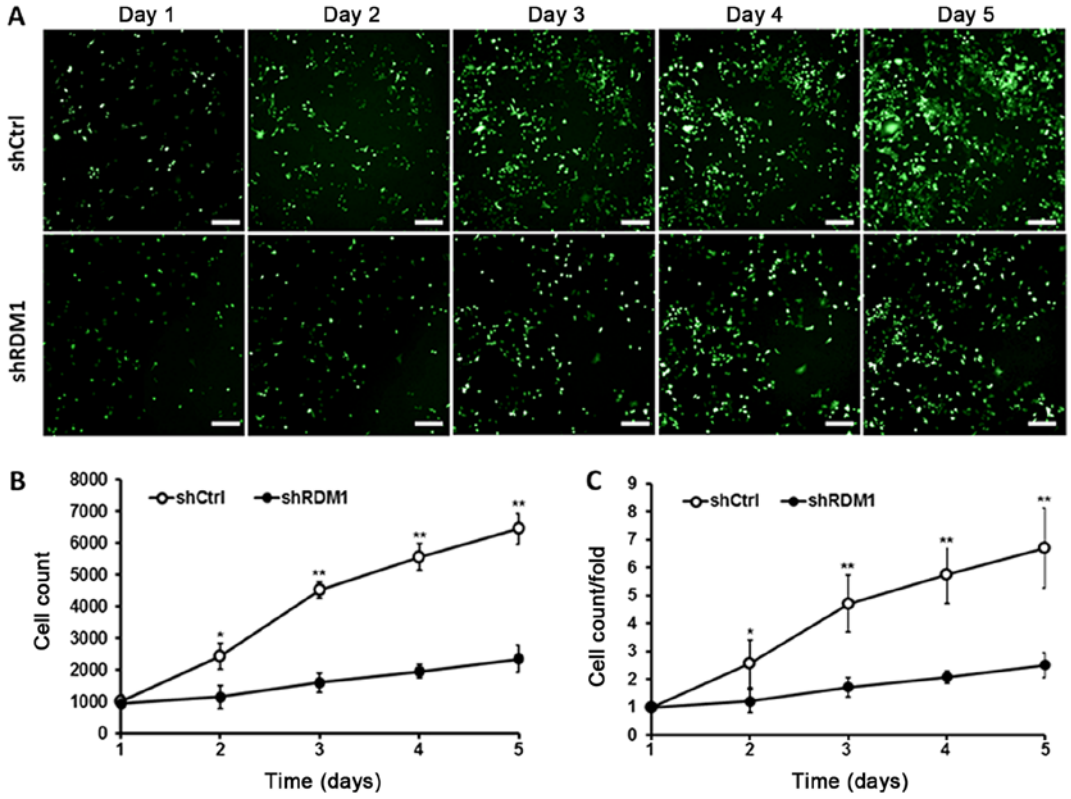

Figure 3. Effect of *RDM1* knockdown on NCI-H1299 cell growth. (A) NCI-H1299 cells were infected with control (shCtrl) or RDM1-siRNA lentivirus (shRDM1) and high content cell imaging was applied every day as indicated to acquire raw images of cell growth. Scale bar, 100 μm. (B and C) Cells were seeded into 96-well plates and infected with control or RDM1-siRNA lentivirus and cell growth was assayed every day for 5 days (**P<0.01; Student's t-test). RDM1, RAD52 motif-containing protein 1.

Statistical analysis. All experiments were repeated three times. SPSS software v. 17.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the results using either Student's t-test or one-way analysis of variance. A P-value <0.05 was considered statistically significant.

Results

RDM1 is highly expressed in lung cancer tissues and cell lines. Using immunohistochemistry, we found that RDM1 protein expression was significantly elevated in the cell nuclei of lung cancer tissues compared to that in adjacent non-cancerous tissue (Fig. 1A and B). We also found that expression of RDM1 mRNA was elevated in lung cancer cell lines (NCI-H1299, A549, H1688 and H1975) when compared with that noted in the human normal lung epithelial BEAS-2B cells (Fig. 1C). Therefore, the expression of RDM1 appears to be upregulated in lung cancer.

RDM1 expression correlates with tumor size, histological differentiation, lymph node metastasis and TNM staging in lung cancer. We then assessed the correlation between RDM1 expression and the clinicopathological parameters of lung cancer patients. Among the 103 tissue samples obtained from NSCLC patients, 58 showed positive RDM1 protein expression and 45 were negative. As shown in Table I, RDM1 expression in NSCLC tissues was significantly correlated with tumor size, histological differentiation, the degree of lymph node metastasis, TNM stage and 1-year survival rate (all P<0.05); however, it was not directly associated with age, gender or histological classification.

RDM1 knockdown significantly reduces the cellular proliferation rate and increases apoptosis in a lung cancer cell line. To investigate the role of RDM1 *in vitro*, we knocked down *RDM1* in a human NSCLC cell line (NCI-H1299) using lentiviral vector-expressed siRNA. NCI-H1299 cells infected with RDM1-siRNA showed significantly reduced RDM1 mRNA and protein levels at day 3 post-infection compared to the controls (P<0.01; Fig. 2).

We then monitored the effect of *RDM1* knockdown on lung cancer cell growth and apoptosis. We found that NCI-H1299 cells transfected with RDM1-siRNA showed a significant reduction in the cell growth rate over 5 days compared to the controls (P<0.01; Fig. 3). In addition, the activity of caspase-3/-7 in NCI-H1299 cells infected with RDM1-siRNA was enhanced compared to that noted in the controls (P<0.01; Fig. 4A). Flow cytometric analysis confirmed there was a significant increase in the apoptosis rate in the NCI-H1299 cells in which *RDM1* had been knocked down (P<0.01; Fig. 4B). Therefore, RDM1 promoted cell proliferation and inhibited apoptosis in a lung cancer cell line.

RDM1 regulates the cell cycle. Next, we examined the effect of *RDM1* knockdown on the cell cycle using flow cytometry. *RDM1* knockdown resulted in a significant increase in the

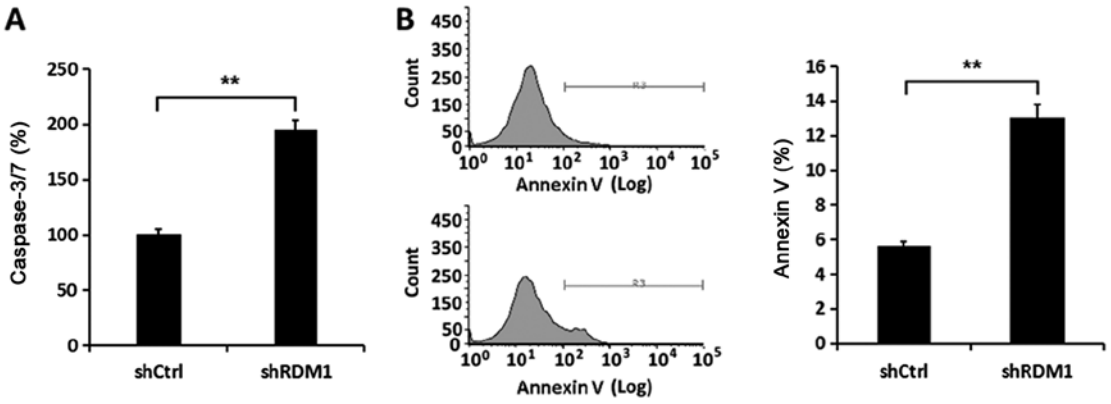

Figure 4. Effect of *RDM1* knockdown on NCI-H1299 cell apoptosis. (A) Activity of caspase-3/-7 in NCI-H1299 cells infected with control (shCtrl) or RDM1-siRNA lentivirus (shRDM1) (**P<0.01). (B) Apoptosis rate in NCI-H1299 cells infected with the control (shCtrl) or RDM1-siRNA lentivirus (shRDM1) (**P<0.01; Student's t-test). RDM1, RAD52 motif-containing protein 1.

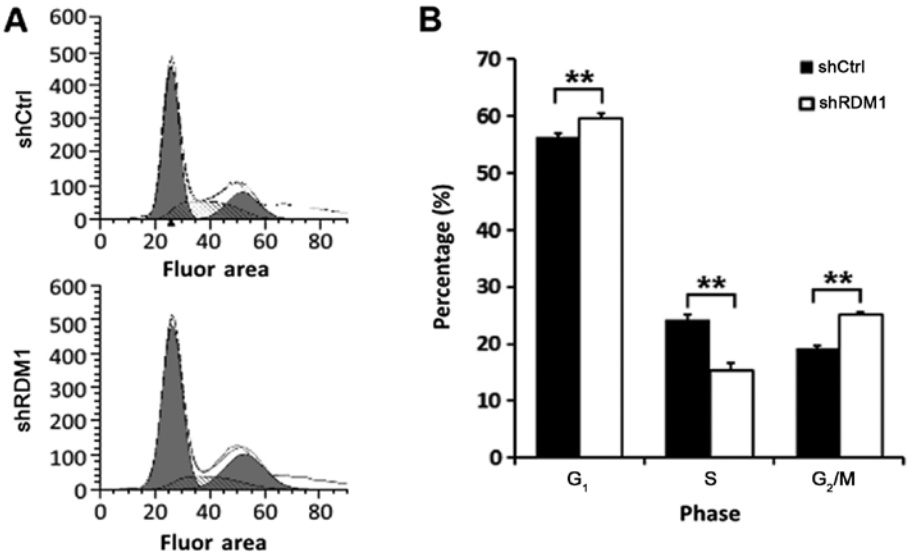

Figure 5. Effect of *RDM1* knockdown on the NCI-H1299 cell cycle. (A) The cell cycle of NCI-H1299 cells was analyzed by flow cytometry after infection with control (shCtrl) or RDM1-siRNA lentivirus (shRDM1). (B) Compared with shCtrl, RDM1-siRNA cultures showed a significant decrease in the proportion of cells in the S phase (**P<0.01; Student's t-test); however, there was a significant increase in the proportion of cells in the G_1 and G_2/M phases (**P<0.01; Student's t-test). RDM1, RAD52 motif-containing protein 1.

proportion of lung cancer cells in the G_1 and G_2/M phases and a decreased proportion in the S phase (P<0.01; Fig. 5). These results suggest that RDM1 may be involved in DNA repair in lung cancer cells, thereby promoting progression through the DNA damage checkpoint (G_2/M) of the cell cycle and avoiding apoptosis.

Discussion

The present study aimed to clarify the role of RAD52 motif-containing protein 1 (RDM1) in non-small cell lung cancer (NSCLC). We found elevated expression of RDM1 at both the mRNA and protein levels in NSCLC tissues and cell lines. To the best of our knowledge, this study first reported that RDM1 protein expression in NSCLC tissues correlates with tumor size, histological differentiation, lymph node metastasis, TNM stage and 1-year survival rate. Meanwhile, *RDM1* knockdown in a human NSCLC cell line significantly reduced the cellular proliferation rate and increased apoptosis. In addition, *RDM1* knockdown enhanced the activity of caspase-3/-7. Finally, we provide evidence that RDM1 may function as a DNA repair protein that promotes cell cycle progression of lung cancer cells. Taken together, our results indicate that RDM1 could be a molecular marker of NSCLC and may represent a novel drug target.

DNA repair proteins, such as RAD52 and RDM1, have previously been implicated in the development of resistance to cancer therapy, including resistance to platinum-based chemotherapy (14,15,20) and radiotherapy (21). In addition to drug resistance, proteins involved in DNA repair pathways have been implicated in tumor progression (22). For example, RAD52 has been linked to both the progression and risk of small cell lung cancer (SCLC) and NSCLC (8,9,23). In addition, RDM1 was previously shown to undergo stress-induced nucleolar accumulation, indicating it may function in the heat-shock response that is implicated in tumorigenesis (16).

As further evidence of the role of RDM1 in lung cancer progression, here we showed that RDM1 expression was correlated with tumor size and metastasis in NSCLC patients. In addition, we found that increased RDM1 expression promoted cell growth and reduced apoptosis in an NSCLC cell line. This indicates that RDM1 plays a similar role to RAD52 in promoting the progression of lung cancer, through increasing cell survival and proliferation. However, the mechanism by which RDM1 promotes cell proliferation requires further investigation.

A previous study found an abundance of *RDM1* transcripts in the testis, which implies that RDM1 may play a possible role in spermatogenesis (14). During spermatogenesis, immature sperm cells undergo rounds of mitotic and meiotic division and the control of genetic stability is paramount. We hypothesize that RDM1 may have a similar function in controlling genomic stability in NSCLC cells, which allows the cells to proliferate. Indeed, we found that knockdown of RDM1 resulted in a decreased proportion of NSCLC cells in the S phase, with an increased proportion in the G_1 and G_2/M phases. This indicates that the cells were arrested at the G_2/M DNA damage checkpoint, which serves to protect genomic integrity and prevent cells with damaged DNA from entering the S phase. In turn, cells with unrepairable DNA lesions undergo permanent arrest or apoptosis, whereas if the damage is repaired, for example by RDM1, checkpoint-arrested cells may resume cell cycle progression (24). Therefore, RDM1 appears to promote cell cycle progression, likely through repairing DNA double-strand breaks.

Our results are similar to those previously observed for the related DNA repair protein, RAD52, in lung cancer (8,9). RAD52 is predominantly recruited for DNA repair during the S phase of the cell cycle and plays a crucial role in the regulation of homologous recombination-related genomic instability in humans (25). Indeed, RAD52 has been shown to promote mitotic DNA synthesis following replication stress, such as that occurring during tumor proliferation (26). In particular, RAD52 appears to repair collapsed DNA replication forks in cancer cells (27). In addition, the recruitment of Rad52 to stalled DNA replication forks in yeast has been shown to be controlled by various checkpoint and cell cycle signals (28). Therefore, the recruitment of RDM1 in proliferating lung cancer cells may be under similar control by cell cycle regulators or signals.

Similar findings have been made for RAD51. Inhibition of RAD51 transcription in a chicken DT40 cell line resulted in a high G_2/M phase arrest, with high levels of chromosome-type breaks (29). Conversely, overexpression of RAD51 protein in tumor cells was associated with high DNA repair capacity and elevated recombination rates (30). Therefore, together, these results imply that lung cancer cells may specifically overexpress and recruit DNA repair proteins such as RDM1, RAD51 and RAD52 in order to generate genetically stable clones suitable for sustained proliferation.

While inhibiting RDM1 seems a promising therapeutic option for NSCLC, we must remember that DNA repair proteins are multifunctional. Therefore, any RDM1 inhibitor that is developed may have unwanted side effects or toxicities. Furthermore, multiple isoforms of RDM1 have been identified, each with a different subcellular location, and potentially possessing different functions (16). Thus, unraveling the specific functions of each of these isoforms will be key for reducing any off-target effects. Indeed, specific inhibitors of proteins involved in homologous recombination for the treatment of cancer remain in their infancy (22). Nonetheless, better characterization of DNA repair proteins, such as that performed here for RDM1, will aid in the discovery of not only more effective DNA repair inhibitors, but also more efficient anticancer combination therapies that overcome the issues of drug resistance.

In summary, our data revealed that RDM1 plays a pivotal role in the survival and proliferation of NSCLC cells by regulating the cell cycle, likely through its DNA repair capabilities. Further studies are warranted to clarify the role of RDM1 in various subtypes of NSCLC, as well as to determine its prognostic and therapeutic value.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

GX, DS and JS conceived and designed the study. GX, JD, EW, FZ and RH performed the experiments. GX, DS and JS wrote the paper. GX, JD, EW, FZ, RH, DS and JS reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Inner Mongolia Autonomous Region People's Hospital, and all participants provided written, informed consent.

Consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

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