

# RNA interference-mediated knockdown of RhoGDI2 induces the migration and invasion of human lung cancer A549 cells via activating the PI3K/Akt pathway

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**Abstract** Rho GDP dissociation inhibitor 2 (RhoGDI2) has been identified as a tumor suppressor gene for cellular migration and invasion. However, the underlying mechanism and effector targets of RhoGDI2 in lung cancer are still not fully understood. In this study, a vector-expressed small hairpin RNA (shRNA) of RhoGDI2 was transfected into the human lung cancer cell line A549. After the successful transfection, the down-regulation of RhoGDI2 promoted the proliferation, migration, and invasion of lung cancer cells *in vitro* through the increasing expression and activities of the matrix metalloproteinase 9 (MMP-9) and PI3K/Akt pathways. Transiently transfecting the small interfering RNA (siRNA) of MMP-9 into the RhoGDI2 shRNA cells reduced the MMP-9 expression. Both transfecting the siRNA and adding the MMP-9 antibody into the RhoGDI2 shRNA cells led to a decrease in the invasion and migration of the lung cancer cells. The blockade of the PI3K/Akt pathway by LY294002 resulted in abolishment of the effects of RhoGDI2 shRNA in Akt phosphorylation and MMP-9 expression. This result suggests that the down-regulated RhoGDI2 contributed to the migration and invasion of the lung cancer cell line via activating the PI3K/Akt pathway and the ensuing increase in the expression and activity of MMP-9. In conclusion, we report that the shRNA-mediated knockdown of RhoGDI2 induces the invasion and migration of lung cancer due to cross-talk with the PI3K/Akt pathway and MMP-9. Verifying the role and molecular mechanism of the participation of RhoGDI2 in the

migration and invasion of lung cancer may provide a target for better treatment.

**Keywords** RhoGDI2 · Lung cancer · Metastasis · MMP-9 · PI3K/Akt

## Introduction

Despite advances in anti-cancer therapies, lung cancer remains the most frequently diagnosed cancer and the leading cause of death in the world [1]. Although low-dose CT can reduce the mortality of lung cancer by 20 %, it is nevertheless a disease with a dismal prognosis because of both the poor early detection and the poor therapeutic options for its highly metastatic forms [2, 3]. Therefore, identifying biomarkers that are related to the migration and invasion of lung cancer is necessary for the prediction of patients' prognoses and to help design effective target therapy.

The Rho GDP dissociation inhibitor 2 (RhoGDI2) is a member of the RhoGDI family, which contains the central regulatory molecules for the activation of Rho GTPase and then participates in the regulation of different cellular processes, including the cell cycle, migration, and apoptosis; it is also important for tumor growth and progression [4, 5]. Although it has been reported that RhoGDI2 targets the endothelin axis in lung cancer [6], the underlying mechanism and biological functions of RhoGDI2 during lung cancer progression are still unclear, a lack of understanding that has become a major challenge for investigating cancer and the development of drugs for its treatment.

It has been proven that RhoGDI2 contributes both to cell migration and invasion by participating in activating the PI3K/Akt pathway in colorectal carcinomas [7]. The signals

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of the phosphatidylinositol 3-kinases (PI3Ks) can induce the cells to grow and proliferate, and they assist with survival and motility. As the end point of the PI3K pathway, Akt contributes to the malignant characteristics in several cells through phosphorylated activation [8]. As a downstream target of the PI3K/Akt pathway, the mesenchymal transition related gene matrix metalloproteinase 9 (MMP-9) contributes to all stages of tumor progression, especially promoting the process of invasiveness and metastasis by degrading major components of the extracellular matrix and basement membrane proteins [9]. RhoGDI2 has not been known to cross-talk with the PI3K/Akt pathway during the regulation of cell migration and invasion in cancer progression based on our previous work [10].

In this study, to elucidate the role of RhoGDI2 in the migration and invasion of lung cancer cells, we transfected the plasmid expressing small hairpin RNA (shRNA) against the RhoGDI2 into A549 cells and subsequently clarified the relationships among the RhoGDI2.

## Materials and methods

### Cell lines and transfections

Human lung cancer cell line A549 was obtained from the Institute of Biochemistry and Cell Biology (SIBS, CAS, Shanghai, China) and grown in DMEM media (Gibco, Grand Island, NY, USA), maintained at 37 °C in a 5 % CO<sub>2</sub> incubator. pGCsi-H1 plasmid (GeneChem, Shanghai, China) expressed shRNA was targeted against RhoGDI2 as well as against a scrambled nonspecific sequence as a control. The A549 cells were transfected with pGCsi-H1-RhoGDI2 and pGCsi-H1-control, and the corresponding RhoGDI2 shRNA and control shRNA cells were selected by G418 (Invitrogen, Carlsbad, CA, USA). The MMP-9 small interfering RNA (MMP-9 siRNA) and noncontrolled (NC) vectors were purchased from GenePharma (Shanghai, China). The RhoGDI2 shRNA cell line was transiently transfected with MMP-9 siRNA and NC plasmids using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cells treated with the transfection reagent alone were marked as the controls (mock). After

transfection with the plasmids, the cells were incubated for 48 h before being collected for further analysis.

### Real-time PCR

The total RNA was extracted separately from the A549, control shRNA, and RhoGDI2 shRNA cells using an RNAsimple Total RNA Kit (TIANGEN, Beijing, China), treated by DNase and then were used for the synthesis of first strand cDNA using Super M-MLV Reverse transcriptase (BioTeke, Beijing, China). A real-time PCR analysis was performed using the specific primers listed in Table 1. The amplified products were quantitated with SYBR Green (Solarbio, Beijing, China) fluorescence.

### Western blot analysis

Protein was extracted from the treated cells. After the protein concentration was determined, the equivalent of 40 µg of protein was boiled in a phosphate buffer solution (PBS) with a 5× loading buffer. The bands of the protein separated by SDS-PAGE (PAGE: 8, 10, and 12 % gel) were transferred onto PVDF membranes (Millipore, Billerica, MA, USA) using an electroblot apparatus (DYCZ-40D, Beijing, China). The filters were blocked in 5 % nonfat milk and incubated separately with the primary antibodies of RhoGDI2, MMP-9, p-Akt, Akt, p85α, p110α, and phosphatase and tensin homolog (PTEN) (Wanleibio, Shenyang, China) at 4 °C overnight with gentle rocking. The membranes were then washed with Tris–Tween-buffered saline (TTBS) and incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies for 45 min at 37 °C. After extensive washing with TTBS, the protein bands were visualized using an enhanced chemiluminescence (ECL) reagent.

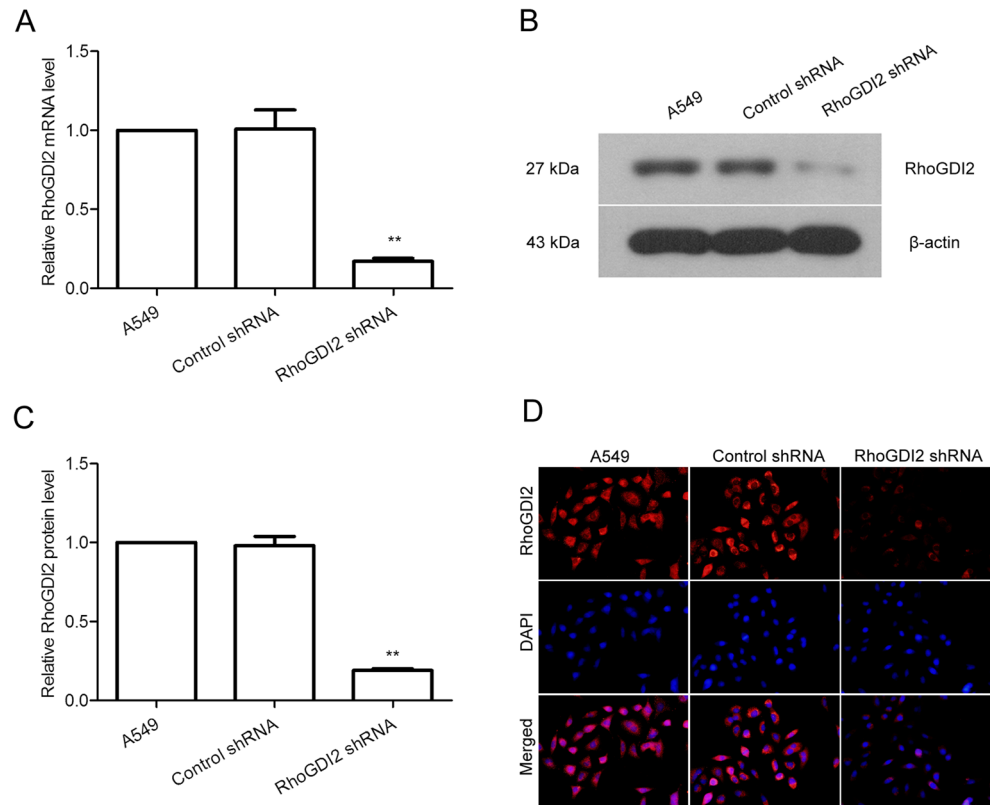
### Immunofluorescence

The glass slides with the grown cells were washed with PBS, and the cells were fixed with 4 % paraformaldehyde for 15 min before blocking with normal goat serum (Solarbio, Beijing, China). After blocking for 15 min, the slides were soaked in anti-RhoGDI2 antibody (1:50) at 4 °C overnight. After washing away the unbound primary antibodies,

**Table 1** Oligonucleotide primer sets for real-time PCR

Gene symbol	Primer sequence (5'–3')	Length (bp)	T <sub>m</sub> (°C)	Amplicon size (bp)
RhoGDI2	F: 5'-ACCGACGATGACAAGCAAGAC-3'	21	59.9	132
	R: 5'-GCTGAACACGCCTGAGAGAAT-3'	21	59.4	
MMP-9	F: 5'-CGAACTTTGACAGCGACAAGA-3'	21	58.9	214
	R: 5'-ATAGGGCGAGGACCATAGAGG-3'	21	60	
β-actin	F: 5'-CTTAGTTGCGTTACACCCTTCTTG-3'	25	62	156
	R: 5'-CTGTACCTTACCCTTCCAGTTT-3'	24	64.4	

**Fig. 1** shRNA-mediated down-regulation of RhoGDI2 at the RNA and protein levels. Human lung cancer cell line (A549) was transfected with a pGCsi-H1 plasmid expressing either a scrambled nonspecific sequence as a control (control shRNA) or shRNA targeted against RhoGDI2 (RhoGDI2 shRNA). **a** After 48 h of transfection, the total RNA extracted from the control and transfected cells was used as a template for the RT-PCR analysis using RhoGDI2 primers. **b** For the Western blot analysis, the total cell lysates were immunoblotted with an RhoGDI2 antibody.  $\beta$ -actin was used as the control. **c** The protein levels of RhoGDI2 were quantified by a gray analysis. **d** The immunofluorescence of the cellular RhoGDI2 was tested using Texas red, DAPI, and merged channels



Cy3-labeled goat anti-rabbit secondary antibody (1:100) was added to the slides in the darkroom, and the slides were incubated for 1 h. After washing off the unbound secondary antibodies, all of the slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and mounted in an aqueous mounting medium. Finally, the stained slides were observed via a laser scanning confocal microscope BX3 (Olympus, Tokyo, Japan).

#### MTT assay

Approximately  $3 \times 10^3$  A549, control shRNA, and RhoGDI2 shRNA cells were seeded separately in 96-well plates, incubated for various time intervals (12–72 h), and treated for 4 h with a 0.2 mg/ml 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution at 37 °C. After incubation, the MTT solution was removed, and for quantification, 200  $\mu$ l of dimethyl sulfoxide (DMSO) was added to each well to completely dissolve the dark blue crystals. The absorbance was measured at 490 nm by an ELISA ELX-800 (BioTek, Beijing, China).

#### Clone formation assay

Briefly, the A549, control shRNA, and RhoGDI2 shRNA cells were seeded as single cells in 35-mm dishes (300 cells per dish), and the dishes were allowed to culture for 14 days

until large colonies were visible. The colonies were fixed with paraformaldehyde and stained with Wright-Giemsa dye for 5 min. The dishes were photographed, and the number of colonies was counted under a phase-contrast microscope AE31 (MOTIC, Xiamen, China).

#### Wound-healing assay

Cells were grown to their full confluence in the plates. On the cell monolayer, a scratch was made using a sterile 200- $\mu$ l pipette tip. The rest of the cells were washed twice with a serum-free medium, photographed, and then incubated for another 12 and 24 h in a serum-free medium. The distances of the cells' migration into the denuded area were calculated later.

#### Matrigel invasion assay

The cells were detached, washed three times in PBS, and prepared;  $2 \times 10^4$  suspended cells in 200- $\mu$ l medium were seeded in the upper chamber of a Transwell (Corning, Corning, NY, USA) insert that was coated with 50- $\mu$ l diluted Matrigel (Matrigel: serum-free medium = 1:2). The lower chamber was filled with an 800- $\mu$ l complete medium with 20 % fetal bovine serum (FBS). After 24 h of incubation, the cells in the upper chamber were scraped away, and the cells present on the lower surface of the insert were stained with

0.5 % crystal violet dyes (Amresco, Solon, OH, USA) for 5 min. Photographs of the invaded cells were taken, and the number of cells was counted under an AE31 phase-contrast microscope.

#### Gelatin zymography assay

The enzymatic activity and molecular weight of MMP-9 were determined by gelatin zymography. The proteins of the A549, control shRNA, and RhoGDI2 shRNA cells were separated by SDS-PAGE, and the gels were incubated for 40 h in Tris-CaCl<sub>2</sub>+ZnCl<sub>2</sub> buffer and then stained with Coomassie brilliant blue G250.

#### Treatments of the inhibitor and antibodies

The PI3K inhibitor LY294002 was purchased from Beyotime (Shanghai, China) to treat A549 cells, control shRNA, and RhoGDI2 shRNA cells, and DMSO (Sigma, St. Louis, MO, USA) treated corresponding cells were used as the controls. Anti-MMP-9 was obtained from Wanleibio (Shenyang, China) for treating RhoGDI2 shRNA cells, and IgG added cells were treated as control.

#### Statistical analysis

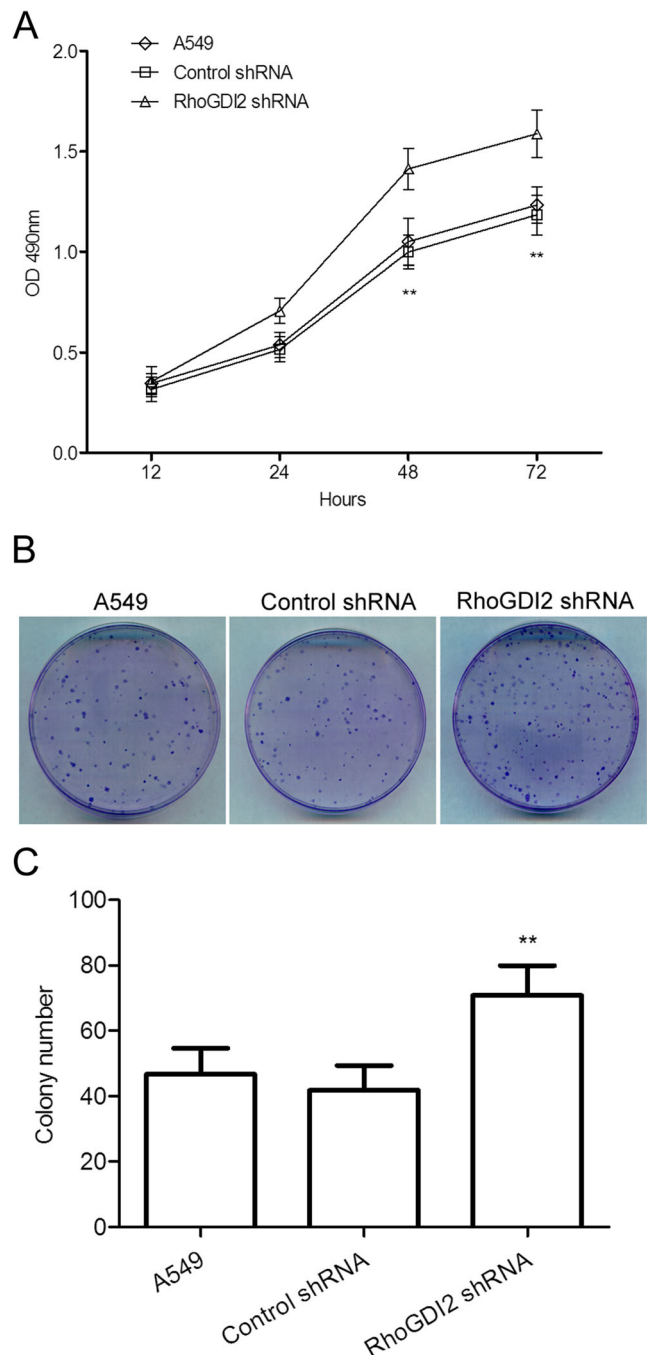
The results are expressed as the means  $\pm$  standard deviation (SD). All of the raw data were analyzed by a one-way analysis of variance (ANOVA) followed by the Bonferroni test for post hoc comparisons. A *P* value less than 0.05 was considered statistically significant.

## Results

The down-regulation of RhoGDI2 at the RNA and protein levels led to a more rapid proliferation and enhanced colony-forming potential of the lung cancer cells in vitro

The RhoGDI2 protein has drawn a great deal of attention from researchers because it has been identified as a prognostic marker in patients after cystectomies [11]. To investigate the transfection and interference of RhoGDI2, the transcription and expression levels of RhoGDI2 were tested in A549, control shRNA, and RhoGDI2 shRNA cells. We used plasmid vectors that express shRNA to inhibit the RhoGDI2 expression. A real-time PCR analysis of the total RNA and a Western blot analysis of the total protein extracted from the transfected RhoGDI2 shRNA cells showed significant reductions in the mRNA and protein levels of RhoGDI2 compared with the A549 and control shRNA cells (Fig. 1a–c). Moreover, we determined the

effect of the pGCsi-H1-RhoGDI2 plasmid on the expression of RhoGDI2 using immunofluorescence, and less fluorescence was detected in the RhoGDI2 shRNA cells (Fig. 1d), demonstrating that a stable RhoGDI2 shRNA



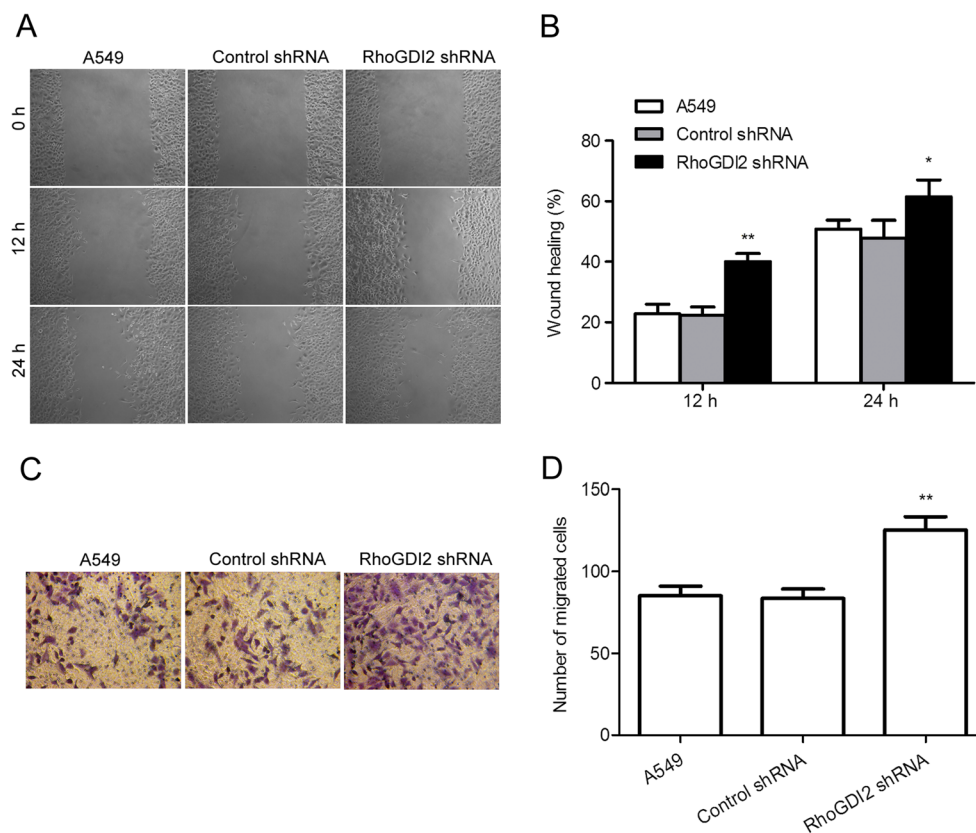
**Fig. 2** MTT and clone formation assays of the A549, control shRNA, and RhoGDI2 shRNA cells. **a** Cell proliferation of the transfected cells and control was assessed over 12–72 h by an MTT assay. **b** Cells were cultured until visible colonies were evident and then stained with Wright-Giemsa dye and presented as photographs. **c** The cells on the plate were enumerated and plotted as colony numbers. Each bar represents the mean of the triplicate analysis of the mean  $\pm$  SD. (*P* < 0.05). The data are representative of three independent studies. \*\**p* values, < 0.05

cell line was successfully constructed. An MTT assay was performed to determine the properties of the cells' proliferation, which were significantly increased in the RhoGDI2 shRNA cells compared with the A549 and control shRNA cells (Fig. 2a). The clone formation assays showed that more colonies of the RhoGDI2 shRNA cells existed and that they progressed with greater survival compared with the A549 and control shRNA cells, as observed in the photographs (Fig. 2b) as well as in the colony numbers (Fig. 2c). These results suggest that the down-regulation of RhoGDI2 endowed lung cancer cells with more rapid proliferation and increased colony formation.

The down-regulation of RhoGDI2 showed the increased invasive and migratory properties of the lung cancer cells in vitro

*RhoGDI2* has been reported as a new metastasis suppressor gene of bladder cancer [12]. To determine the functional significance of RhoGDI2 in lung cancer

metastasis, we compared the wound-healing migratory and Matrigel invasive abilities of the RhoGDI2 shRNA cells with that of the A549 and control shRNA cells. The results from the wound-healing assays over a 12–24 h period are presented as photomicrographs (Fig. 3a) and as the distance traveled rates of the cells (Fig. 3b), which showed the increased motility of the RhoGDI2 shRNA cell line. The quantification of the wound-healing assay illustrated that the migration of the RhoGDI2 shRNA cells was increased by nearly 18 and 14 % in 12 and 24 h, respectively, compared with the A549 and control shRNA cells. The Matrigel invasion assay similarly showed that more RhoGDI2 shRNA cells migrated over a 24-h period (Fig. 3c). The quantitative analysis showed that approximately 30 more RhoGDI2 shRNA cells penetrated through the Matrigel-coated Transwell insert compared with the A549 and control shRNA cells (Fig. 3d). These results suggest that RhoGDI2 suppressed the migration and invasion of the lung cancer cells.



**Fig. 3** Down-regulation of RhoGDI2 induced the migration and invasion of the lung cancer cells. **a** The A549, control shRNA, and RhoGDI2 shRNA cells were wounded and allowed to migrate into the denuded area over 0–24 h to determine the motility of the cells. The cultures were visualized by microscopy. **b** The wound-healing rates were measured by the distance traveled by the cells to the front of the denuded area. **c** A Matrigel invasion assay was performed after 48 h of transfection, and the

cells were allowed to invade through the Transwell inserts, which were coated with Matrigel. After 24 h of incubation, the invaded cells were stained with crystal violet dyes and photographed under the microscope. **d** The number of migrated cells were counted and plotted. *Bars* represent the mean of the triplicate analysis of the mean±SD. ( $P<0.05$ ). The data are representative of three independent studies. \* $p$  values,  $<0.01$ ; \*\* $<0.05$

Down-regulation of RhoGDI2 induced more expression and higher activity of MMP-9 in lung cancer cells in vitro

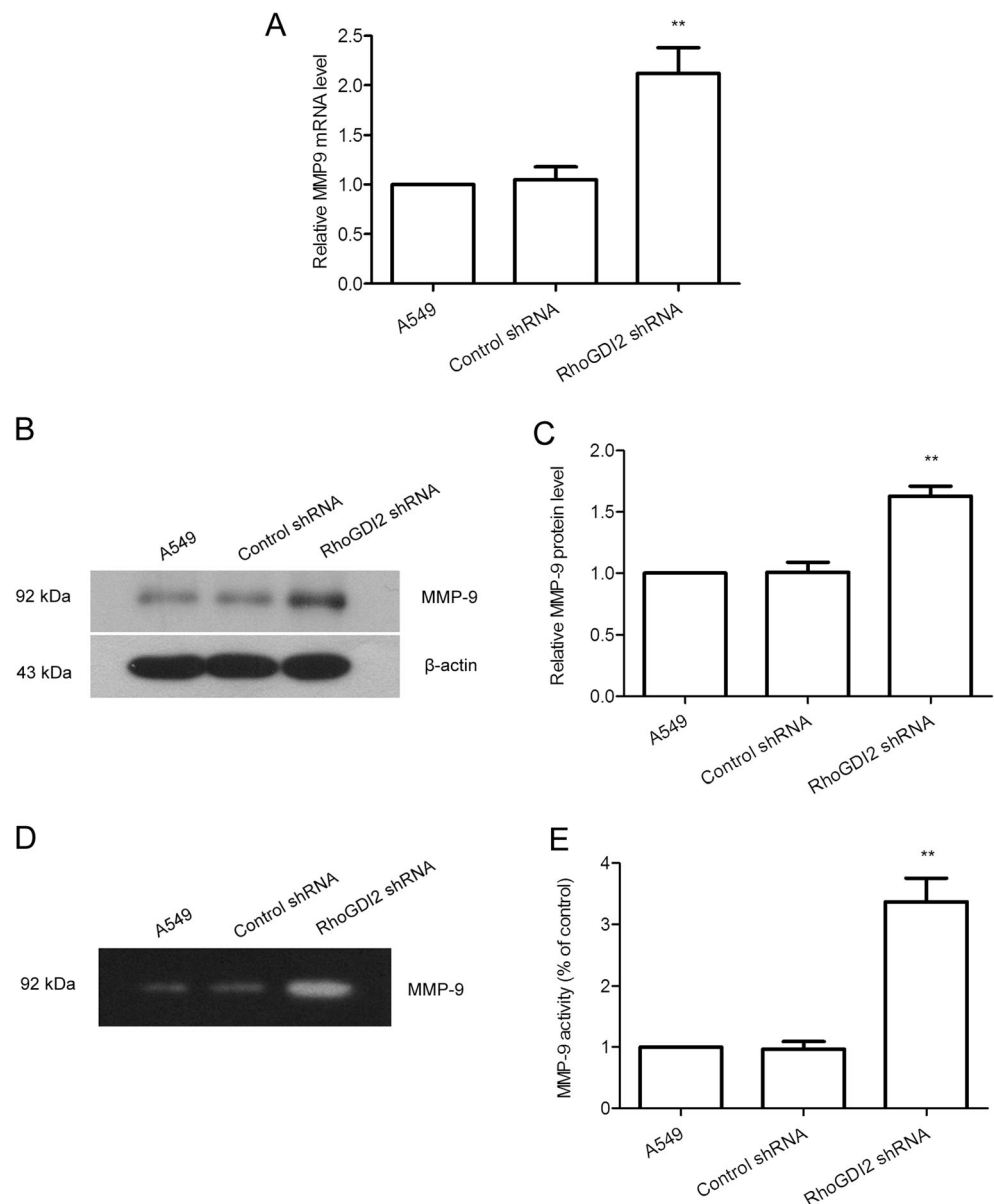
The expression levels of MMP-9 have been reported in gastric carcinoma, bladder, breast, and lung cancers [13–15]. To better understand the relationship between RhoGDI2 and MMP-9 in lung cancer, the expression levels and activities of MMP-9 were tested in the A549, control shRNA, and RhoGDI2 shRNA cell lines. The down-regulation of RhoGDI2 markedly enhanced the expression of MMP-9 at the mRNA (Fig. 4a) and protein (Fig. 4b and c) levels. A gelatin zymography assay was used to assess the matrix proteolytic activity, which showed that the down-regulation of RhoGDI2 led to a 3.5-fold increase in MMP-9 activity

(Fig. 4d and e). These results suggest that RhoGDI2 is inversely correlated to the MMP-9 in lung cancer cells.

Decreased activity of MMP-9 reduced the migration and invasion of RhoGDI2 shRNA cells

As an important factor participating in the degradation of the basement membrane collagen, the activity of MMP-9 plays an important role in invasiveness and metastasis [9]. To explain the interaction of RhoGDI2 and MMP-9 in lung cancer metastasis, we investigated the role of MMP-9 in the RhoGDI2 shRNA cell lines by transiently transfecting the RhoGDI2 shRNA cells with NC and MMP-9 siRNA plasmids, and alternately, by adding IgG and the MMP-9

**Fig. 4** siRNA-mediated down-regulation of the MMP-9 at the RNA, protein, and activation levels. **a** After 48 h of transfection, the total RNA extracted from the A549, control shRNA, and RhoGDI2 shRNA cells was used as a template for the RT-PCR analysis using MMP-9 primers. **b** For the Western blot analysis, the total cell lysates were immunoblotted with an MMP-9 antibody.  $\beta$ -actin was used as the control. **c** The protein levels of MMP-9 were quantified by a gray analysis. **d** A gelatinase zymography assay was performed to investigate the gelatinolytic activity of the cells. The MMP-9 is represented by the band at 92 kDa. **e** The activity of the MMP-9 in cells was calculated and presented as magnification. Each *bar* represents the mean of the triplicate analysis of the mean  $\pm$ SD. ( $P < 0.05$ ). The data are representative of the three independent studies. \*\* $p$  values,  $< 0.05$

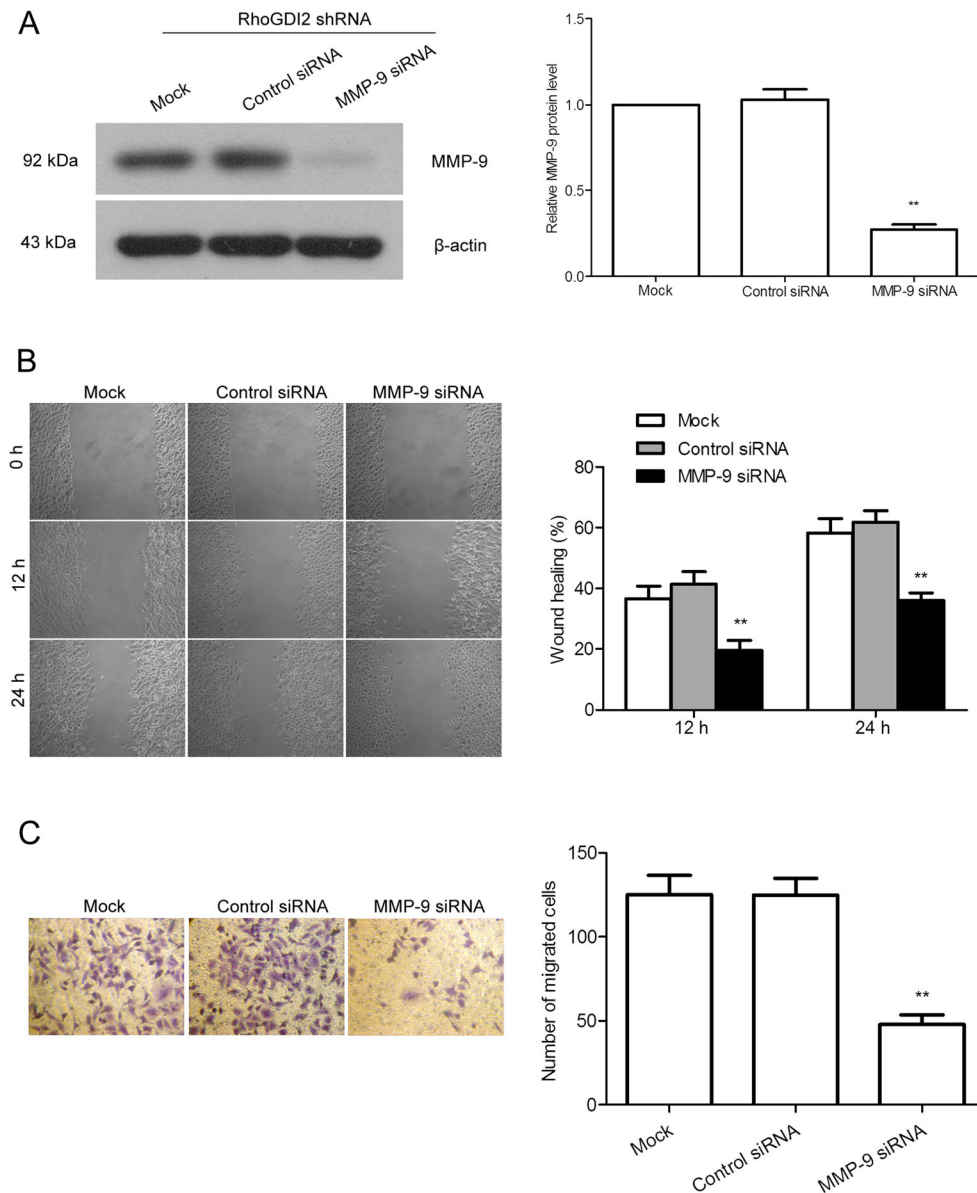


antibody. After successful transfection of the MMP-9 siRNA into the RhoGDI2 shRNA cells, a significant down-regulation of MMP-9 at the protein level was observed (Fig. 5a). In the cells with the confirmed decreases in MMP-9 activity, the migratory and invasive abilities of the cells were evaluated, and both the MMP-9 siRNA and anti-MMP-9 cells showed decreased cell migration and invasion compared with the mock cells and the cells transfected with NC (Fig. 5b–e). These results suggest that the decreased MMP-9 activity abolished the effect of

RhoGDI2 shRNA in the migration and invasion of the lung cancer cells.

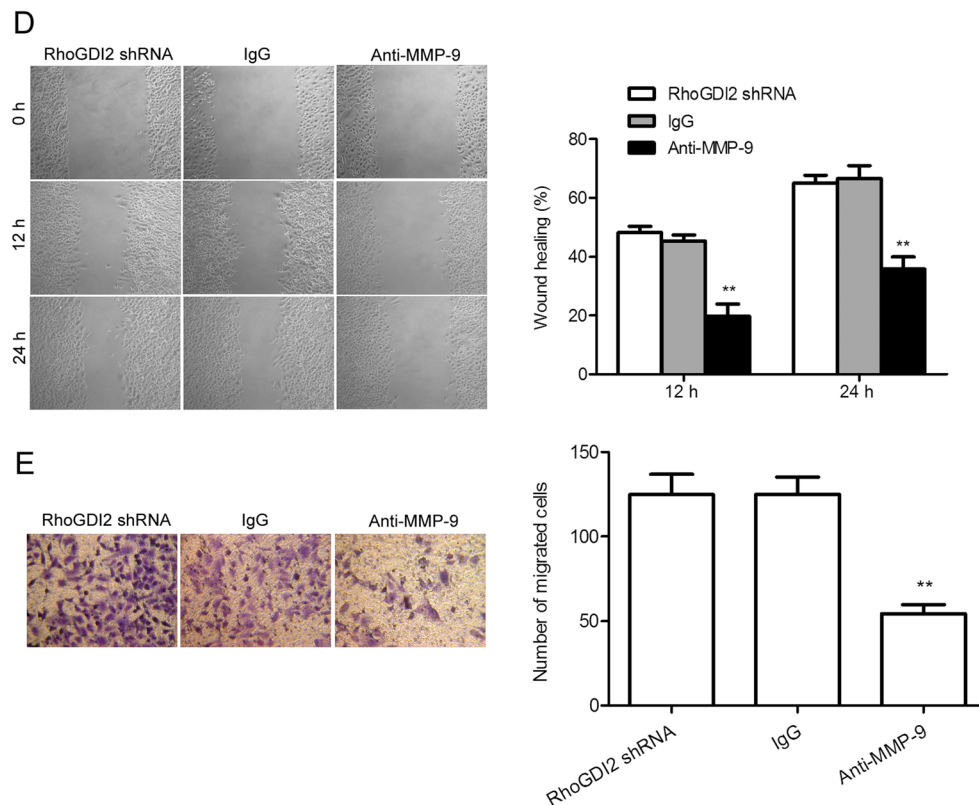
Effect of RhoGDI2 interference on the activation of the PI3K/Akt pathway and metastasis of lung cancer

It is likely that the PI3K/Akt pathway had cross-talk with the RhoGDI2 [10]. To elucidate the relationship between RhoGDI2 and the PI3K/Akt pathway in lung cancer metastasis, we measured the expression levels of the PI3K subunits



**Fig. 5** Down-regulation of MMP-9 inhibits the migration and invasion of RhoGDI2 shRNA cells. **a** siRNA mediated decrease of the MMP-9 expression in the RhoGDI2 shRNA cells compared with the control siRNA transfected and mock cells. The protein levels of the MMP-9 were quantified by gray analysis. The migration and invasion of the MMP-9 siRNA cells were confirmed by the photographs, the data on wound-healing (**b**) and the Matrigel invasion assay (**c**) after transfecting

for 12 and 24 h. The same assays were performed to investigate the migration (**d**) and invasion (**e**) of the RhoGDI2 shRNA cells after adding the antibody of MMP-9 (anti-MMP-9) for 12 and 24 h, with IgG added and RhoGDI2 shRNA cells as contrast. Bars represent the mean of the triplicate analysis of the mean±SD. ( $P < 0.05$ ). The data are representatives of three independent studies. \*\* $p$  values,  $< 0.05$



**Fig. 5** (continued)

(p85 $\alpha$  and p110 $\alpha$ ), downstream target Akt and phosphorylated Akt (p-Akt) in the cell lines. The down-regulation of RhoGDI2 by shRNA increased the Akt phosphorylation and the expressions of p85 $\alpha$  and p110 $\alpha$  (Fig. 6a). The blocking of the PI3K/Akt pathway by the LY294002 inhibitor resulted in the reduced expressions of both the p-Akt and MMP-9 (Fig. 6b) and in inhibiting the invasiveness and metastasis of the RhoGDI2 shRNA cell line (Fig. 6c). These findings demonstrate that the down-regulated RhoGDI2 increased the migration and invasion of the lung cancer cells in vitro through activating the PI3K/Akt pathway.

## Discussion

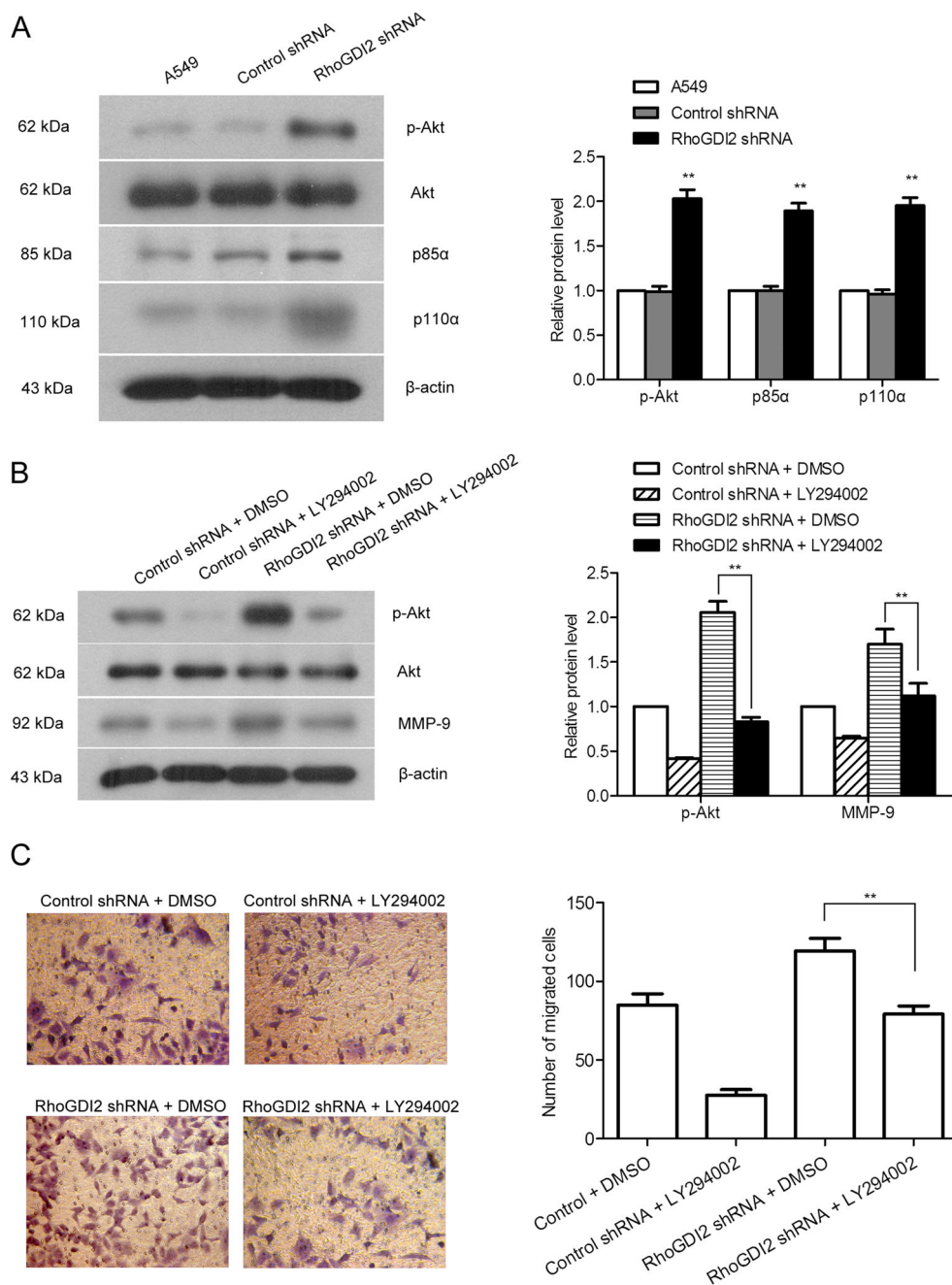
Cancer is one of the most prevalent causes of death worldwide. The aberrant expression of RhoGDI2 has been found in a variety of human cancers, and its dual roles in regulating the Rac GTPases activities that contribute to aggressive phenotypes of cancer cells have been reported [16]. However, the precise role and mechanism of RhoGDI2 in lung cancer metastasis remains unclear. In this study, we found that the shRNA mediated down-regulation of RhoGDI2 in lung cancer cells (1) increased the proliferation, migration, and invasion of cancer cells in vitro, (2) enhanced MMP-9 expression, and (3) activated the PI3K/Akt pathway by increasing the

p-Akt, p85 $\alpha$ , and p110 $\alpha$  levels. Similar RhoGDI2 characteristics have been described in bladder cancer, ovarian carcinoma, and Hodgkin's lymphoma [17–19], while an opposite function has been found in breast cancer for RhoGDI2 [20]. The conflicting roles of RhoGDI2 in different types of cancer cells have been described [21, 22], which may result from the variant mechanisms of RhoGDI2 regulation in cancer progression.

The migration and invasion of the tumor cells determine the aggressive potential of the cancer. MMP-9 is an important signal in controlling cell proliferation and maintaining normal tissue structure and epithelial integrity, and the increased expression level of MMP-9 plays an important role in tumor metastasis [23, 24]. The factors that regulate MMP-9 in the development of lung cancer have been reported [25, 26], but it is not known how MMP-9 is affected by RhoGDI2 in lung cancer. Here, we have shown that down-regulated RhoGDI2 promoted the expression and activity of MMP-9 and then increased the invasion and migration of the lung cancer cells. Furthermore, as MMP-2 is another member of the matrix metalloproteases (MMPs), its activity was increased after the RhoGDI2 knockdown (data not shown). When the expression of MMP-9 was down-regulated by the transfected siRNA or the activity of MMP-9 was decreased by adding the antibody to the RhoGDI2 shRNA cells, the cell motility was significantly inhibited. These results suggest that down-regulated RhoGDI2 promotes the invasion and migration of lung cancer



**Fig. 6** Elucidation of the relationships of the RhoGDI2, MMP-9, and PI3K/Akt pathway. **(a)** Down-regulated RhoGDI2 led to an increased expression of p85 $\alpha$  and p110 $\alpha$  as well as phosphorylation of Akt (p-Akt), which are presented as gel images on the Western blot and data on the gray analysis. Blocking the PI3K/Akt pathway by LY294002 abolished the effect of RhoGDI2 shRNA in Akt phosphorylation, MMP-9 expression (b) and invasive ability (c). Each bar represents the mean of the triplicate analysis of the mean  $\pm$  SD. ( $P < 0.05$ ). The data are representative of three independent studies. \*\* $p$  values,  $< 0.05$



cells, partly through the up-regulation of MMP-9. Perhaps RAC1, as a member of the Rho GTPase family of proteins, is a target of RhoGDI2 for stimulating the activity of MMP-9 in lung cancer. Moreover, apoptosis in cancer can be induced via reducing the MMP-9 expression [27–29]. Thus, MMP-9 offers great therapeutic potential for treating cancers, and the development of its inhibitor as a new drug is warranted.

PI3K/Akt is an intensively explored and crucial intracellular pathway in tumorigenesis, and numerous oncogenes and suppressor genes closely related to PI3K/Akt may be considered as targets for treating tumors [30, 31]. Based on our

previous research, the expression of RhoGDI2 is inversely correlated to the activity of the PI3K/Akt pathway [10]. Our results demonstrate that down-regulated RhoGDI2 enhances the proliferative and metastatic potentials of the lung cancer cells through the up-regulation of p-Akt, p85 $\alpha$ , and p110 $\alpha$ . Moreover, the down-regulated RhoGDI2 resulted in the decreased expression of the tumor suppressor phosphatase and tensin homolog deleted on chromosome ten (PTEN), which may terminate the PI3K signaling (data not shown). All of the results verify that down-regulated RhoGDI2 can induce the PI3K/Akt signaling cascade. Interestingly, it was apparent that

the LY294002 treatment blocked the metastasis of the RhoGDI2 shRNA cells, and the eliminated Akt phosphorylation as well as the MMP-9 expression by LY294002 suggest that the down-regulated RhoGDI2 contributed to the migration and invasion of the lung cancer cells in vitro via, activating the PI3K/Akt pathway and then inducing the expression of MMP-9. The same tendencies of the PI3K/Akt pathway and MMP-9 also exist in gastric carcinoma and prostate cancer [13, 32]. This evidence suggests that RhoGDI2 may be considered to be a target of depressing the migration and invasion of lung cancer.

In summary, our study shows that down-regulated RhoGDI2 is related to lung cancer progression. In this study, *RhoGDI2* functions as a tumor suppressor gene of lung cancer in vitro, as the down-regulated RhoGDI2 promotes the proliferation, migration, and invasion of lung cancer cells by activating the PI3K/Akt pathway and the ensuing increase in the expression and activity of MMP-9. Deciphering the role and molecular mechanism of the RhoGDI2 regulating system in lung cancer not only increases the understanding of cancer progression but also provides a therapeutic molecular target for treating lung cancer.

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