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Knockdown of RhoGDIα induces apoptosis and increases lung cancer cell chemosensitivity to paclitaxel

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This study aimed to investigate the effects of RhoGDIα knockdown on apoptosis and the chemosensitivity of lung cancer cells to paclitaxel. The signaling proteins involved were also assessed. RhoGDIα expression was assessed by RT-PCR, Western blotting and immunohistochemistry. Apoptosis was determined by flow cytometric assessment, and cell viability was measured with the MTT assay. Phosphorylation levels of signaling proteins, ERK, JNK, Akt, Bad and IκBα were tested by Western blotting and immunohistochemistry. Positivity for RhoGDIα in lung cancer tissues was significantly higher than in paracancerous tissues. Downregulation of RhoGDIα was associated with significantly increased apoptosis and repressed cell viability. This effect could be due to the consequent upregulation of p-JNK, as well as decreased levels of p-ERK, p-Bad and p-IκBα. Knockdown of RhoGDIα strengthened the effect on apoptosis and inhibition of cell viability induced by paclitaxel treatment. This chemosensitization effect could be a result of the intensification of pro-apoptotic JNK activation, and repression of anti-apoptotic p-ERK, p-Bad and p-IκBα expression stimulated by paclitaxel. In summary, our study indicated that RhoGDIα could be a promising therapeutic target, and the combination of RhoGDIα siRNA and paclitaxel might be a valuable potential therapy for lung cancer treatment.

Key words: lung cancer, RhoGDIα, apoptosis, chemosensitivity

Lung cancer is the one of the leading cause of cancer-related deaths worldwide with less than 16% of patients surviving beyond 5 years [1, 2]. Cytotoxic chemotherapy remains the key component of treatment in both the adjuvant and metastatic settings. However, many patients do not respond to chemotherapy. Therefore, new treatment modalities against this aggressive neoplasm are urgently needed.

Rho proteins, a group of small guanosine triphosphate (GTP)-bound proteins, have been shown to be involved in a variety of fundamental cellular processes, including mitosis, intracellular transport, cytoskeletal rearrangement and cell morphogenesis. The Rho GDP dissociation inhibitor (RhoGDI) is a cellular regulatory protein that acts primarily by controlling the cellular distribution and activity of Rho GTPases. As one of three GDIs indentified as being specific for the Rho proteins, RhoGDIα is ubiquitously expressed in a variety of tissues. RhoGDIα is known to function as a GDI for Rac and involved in various fundamental cellular processes [3].

RhoGDIα is often thought of as a negative regulator of the Rho GTPases, as it has the ability to bind and hold the GTPases in inactive, cytosolic forms that are unable to interact with guanine-nucleotide exchange factors (GEFs) effectively, and/or other downstream target molecules. However, there is evidence that RhoGDIα can also positively regulate RhoGTPases [4, 5]. For example, RhoGDIα positively regulates Rac1 by protecting it from apoptosis-associated inactivation [5]. Therefore, the function of RhoGDIα is yet to be fully established.

It has been shown that RhoGDIα is frequently upregulated in ovarian and breast cancers [6-8]. Overexpression of RhoGDIα protects cancer cells from the induction of apoptosis and plays a role in chemotherapy drug resistance [5, 9, 10]. Nonetheless, the expression of RhoGDIα in lung cancers and

the mechanism underlying the action of RhoGDIα as an anti-apoptotic molecule have not yet been elucidated.

In this study, we measured the expression of RhoGDIα in lung cancer tissues. The anti-apoptotic and chemoresistant effects of RhoGDIα were tested in the A549 lung cancer cell line. The effects on related cell signaling pathways were also investigated.

Patients and methods

Patient specimens and characteristics. Surgical specimens from human lung tumors were collected from 40 patients who attended our hospital. All human tissue samples were obtained and handled in accordance with an approved Institutional Review Board application (the Committee on Medical Ethics, the First Affiliated Hospital of Soochow University). All samples were collected before any chemotherapy or radiotherapy had been given. Samples of normal tissue from areas distant to the tumor or tissue taken from adjacent to the tumor were available from 75% of the patients. Each specimen was fixed in 10% formalin and embedded in paraffin, before 4-μm sections were cut and stained with hematoxylin and eosin for the assessment of tumor morphology. Two pathologists evaluated the tumors. There were 18 squamous cell carcinomas, 16 adenocarcinomas, and six small-cell lung carcinomas (SCLC). Tumor histopathological grading was performed according to the WHO grading system: two cases were well differentiated, 20 cases showed intermediate levels of differentiation and 18 cases were poorly differentiated. Tumor stage was determined according to the tumor-node-metastasis staging system (TNM, 6th edition). Nine cases were identified as stage I, 18 as stage II, 12 as stage III and one case was stage IV. The mean age of patients at the time of primary surgery was 61.8 ± 8.4 years (range, 39–80 years).

Antibodies. The polyclonal antibodies used in this study were sc-360 to detect RhoGDIα (Santa Cruz Biotechnology, Santa Cruz, CA, USA), ab8932 to detect p-ERK and ab28825 to detect p-Bad (Abcam PLC, Cambridge, UK). The monoclonal antibodies used were anti-p-IκBα (B-9) and anti-p-JNK (G-7; Santa Cruz Biotechnology).

Immunohistochemical staining. Immunohistochemical (IHC) staining was performed on 4-μm sections of formalin-fixed, paraffin-embedded tissues. Samples were heated at 56°C, deparaffinized, and rehydrated through a graded series of ethanol. Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 10 min. Microwave antigen retrieval was performed for p-IκBα; heat-mediated antigen retrieval for other antibodies, followed by incubation with the primary antibody for 1 hour at room temperature. After incubation with the corresponding secondary antibodies for 20 min, the bound complex was visualized by using the SuperPicTure polymer detection kit (No.87-8963; Invitrogen). The fetal kidney was used as a positive control. Negative controls were prepared by using phosphate-buffered saline (PBS) as a substitute for the primary antibody.

Evaluation of immunostaining. Immunostaining was independently evaluated by two pathologists who were blinded to the clinicopathological findings of the patients. Staining intensity was scored between given a score of 0 and –3 for negative, weak, moderate, or strong staining, respectively. The distribution was scored as 0–4 for staining distributions of 0%, <10%, 10–50%, 50–90%, and >90%, respectively. The sum of these scores was then used to determine four groups as follows: (I) negative = 0–1, (II) weak = 2–3, (III) moderate = 4–5, and (IV) strong = 6–7. A total score of between 4 and 7 indicated positive immunostaining [11].

Cell culture. The human lung cancer cell lines, A549 and H157, were purchased from the American Type Culture Collection (Manassas, Virginia, USA). Cells were cultured at 37°C in Dulbecco's modified Eagle's medium (Invitrogen, Camarillo, CA, USA) containing 10% fetal bovine serum (Invitrogen), 100 U/mL penicillin and 100 μg/mL streptomycin. The cell line was incubated in a humidified atmosphere of 95% air and 5% CO_2.

Transfection of small interfering RNA. Target-specific small interfering RNA (siRNA; sc-36417) designed to knock down RhoGDIα gene expression, irrelevant (Control) siRNA (sc-37007), and the transfection medium (sc-36868), were purchased from Santa Cruz Biotechnology. SiRNA transfections were performed according to the instructions of the manufacturer.

Reverse transcription-polymerase chain reaction. The quantification of gene expression was performed by reverse transcription-polymerase chain reaction (RT-PCR) using an Eppendorf PCR system (Eppendorf, Hamburg, Germany). cDNA was made from total RNA extracted from cultured A549 cells. The following primers were designed to span exon–intron junctions: RhoGDIα, 5'-CAGATTGCAGCGGAGAACGAG-3' (forward), 5'-ATGTACTTCATGCCGGACA-3' (reverse); GAPDH, 5'-CAACTACATGGTCTACATGTTCC-3' (forward), 5'-CAACCTGGTCCTCAGTGTAG-3' (reverse). The products of the RT-PCR reaction were electrophoresed on 1% agarose gels, visualized by ethidium bromide staining, and quantified using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA). Each sample was normalized against GAPDH mRNA expression.

Western blot analyses. Cells were collected in the log phase and lysed with 100 μL PIPA lysis buffer. Protein (50 μg) from the lysate was electrophoresed in 10–15% sodium dodecyl sulfate polyacrylamide gel and transferred to a polyvinylidene difluoride immobilon P membrane (Millipore, Bedford, MA, USA) according to the instructions of the manufacturer. Membranes were probed with the previously detailed antibodies and anti-β-actin (Cell Signaling, Beverly, MA, USA). The protein expression was determined using horseradish peroxidase-conjugated antibodies followed by enhanced chemiluminescence (ECL Amersham Pharmacia Biotech, Buckinghamshire, UK) detection, and further quantified using Quantity One software (Bio-Rad Laboratories).

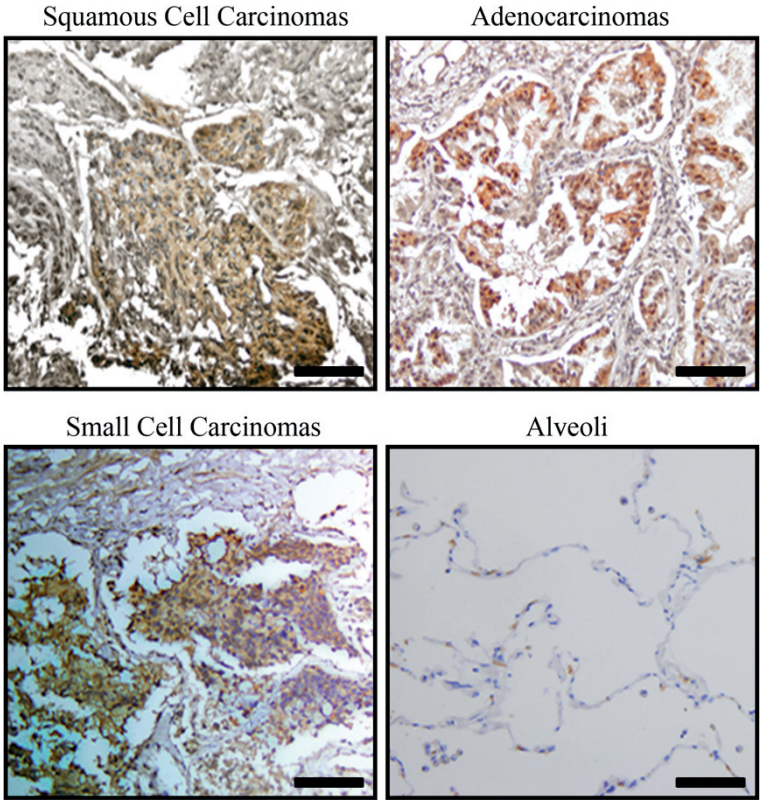

Figure 1. Immunohistochemistry for RhoGDIα. Examples of various lung cancer pathological types and areas of normal lung tissue stained with an anti-RhoGDIα antibody: squamous cell carcinoma, adenocarcinoma, small-cell carcinoma and alveoli. Immunostaining is observed in the areas of lung cancer tissue, but not obviously in the alveoli. Scale bar=50 μm.

MTT assay. Cellular viability was evaluated by the MTT (3-[4,5-dimethyltiazol-2-yl] 2,5-diphenyl-tetrazolium bromide) assay [12]. Cells were seeded in 96-well tissue culture plates at concentrations of 5×10^3/well. After treatment, MTT (Sigma, St. Louis, MO, USA) was added to each well at a final concentration of 0.5 mg/ml, followed by incubation at 37°C for 4 h. Then, the medium was removed and 200 μl of dimethyl sulphoxide (DMSO) was added to each well. The absorbance of the mixture was measured at 490 nm using a microplate ELISA reader (Bio-Rad Laboratories). The relative cell viability was calculated as follows: relative cell viability = (mean experimental absorbance/mean control absorbance) × 100%.

Apoptosis analysis. The apoptosis level was assessed by quantification of the sub-G_1 peak by flow cytometry (FCM). Briefly, adherent and detached cells were collected with trypsin, resuspended at 1×10^6/mL and fixed in ice-cold 70% ethanol for 4 hours at 4°C. Each sample was resuspended in PI/RNase solution for 30 min and analyzed using a fluorescence-activated cell sorter (FACS) (Beckman, Miami, USA). The relative level of apoptosis was calculated as follows: relative apoptosis = (mean experimental apoptosis percentage/mean control apoptosis percentage) × 100%.

Statistical analysis. Statistical analysis was carried out using the SAS Statistical program version 8.2 for Windows. The association between RhoGDIα and the clinicopathological data was explored and assessed by the χ^2 or Fisher's exact tests, depending on the cellular frequencies observed in the 2×2 tables. The correlations between the protein level of RhoGDIα and the phosphorylation levels of p-ERK, p-JNK, p-Akt, p-Bad and p-IκBα were assessed by Spearman's rank correlation coefficient. $P<0.05$ was considered statistically significant.

Results

Expression of RhoGDIα in tissue specimens of patients with lung cancer. The level of RhoGDIα protein in 40 specimens of cancer tissue and 30 paired paracancerous tissue specimens from patients with lung cancer was assessed by IHC techniques. In the lung cancer specimens, high levels of RhoGDIα were observed in a variety of pathological types of lung cancer. In contrast, no obvious RhoGDIα immunoreactivity was identified in the alveoli (Fig. 1). The level of RhoGDIα positivity was significantly higher in lung cancer tissues than in paracancerous tissues (Table 1). An analysis by stratification for sex, age, pathological subtype, tumor

Table 1. The expression of RhoGDIα in lung cancer tissue and para-tumor tissue.

	No. of patients	Positive (n)	Negative (n)	Positive (%)	χ^2	P
Lung cancer tissue	40	34	6	85	5.609	0.027
Para-tumor tissue	30	18	12	60		

Table 2. Clinicopathological features and their relationship to RhoGDIα staining.

	No. of patients (n=40)	The expression of RhoGDIα (%)	P
Gender			0.654
Male	25	88	
Female	15	80	
Age (yrs)			1
≤60	14	85.7	
>60	26	84.6	
Histology			0.422
Squamous cell carcinoma	18	88.9	
Adenocarcinoma	16	75	
Small cell carcinoma	6	100	
Tumor grading			1
Well and intermediate differentiated	22	86.4	
Poor differentiated	18	83.3	
Metastatic lymph nodes			0.195
Positive	24	91.7	
Negative	16	75	
Stage			0.671
Early (I/II)	27	81.5	
Advanced (III/IV)	13	92.3	

grade, lymphatic metastasis, and TNM stage was performed, but RhoGDIα positivity was not significantly different within these stratification factors, although there was a trend toward increased RhoGDIα positivity in cases with metastatic lymph node involvement (Table 2).

Downregulation of RhoGDIα induces apoptosis in lung cancer cells. It has already been shown that RhoGDIα plays an important role in protecting tumor cells from apoptosis induction [5, 9, 10]. As RhoGDIα has been found to be overexpressed in lung cancer tissues, we assessed whether the knockdown of RhoGDIα could trigger apoptosis in lung cancer cells. Cells were divided into three treatment groups: solvent control (Control), control siRNA and RhoGDIα siRNA. Cells were initially treated for 24, 48, or 72 hours, and were then tested for the expression of RhoGDIα mRNA in A549 cells. RT-PCR analysis revealed decreased levels of RhoGDIα mRNA in the cells transfected with RhoGDIα siRNA for 24 and 48 hours (Fig. 2A). Western blot analysis revealed similar changes in the levels of RhoGDIα protein (Fig. 2B) in both A549 and H157 cells. Therefore, 48 hours was selected as the standard time for transfection in all further experiments.

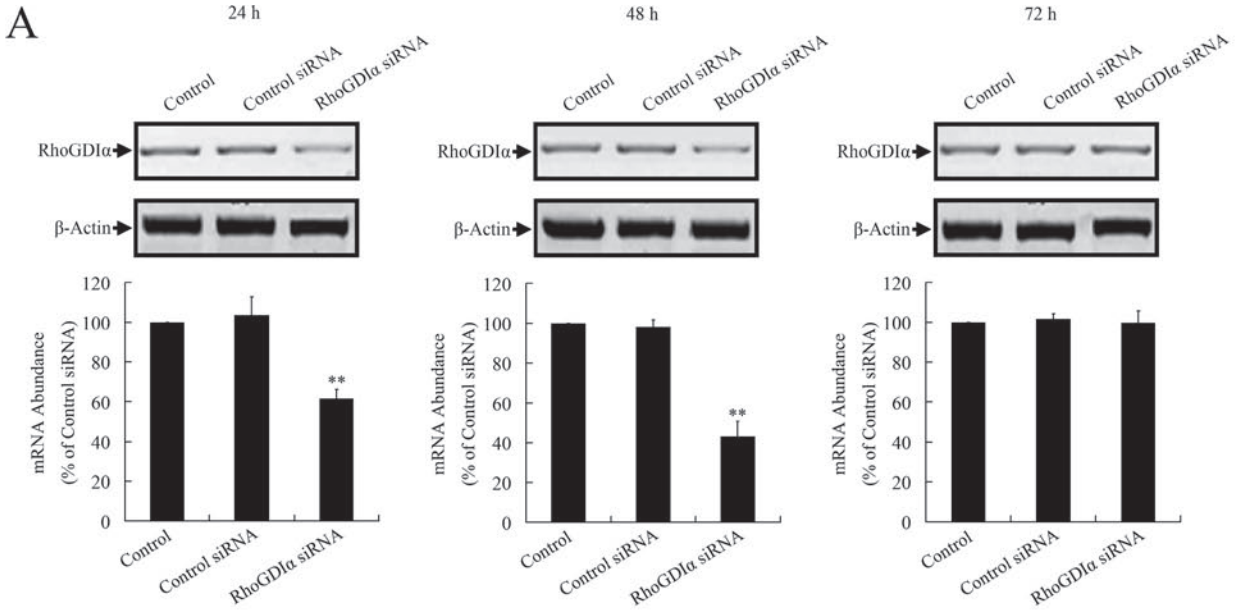

Figure 2. The role of RhoGDIα in tumor cell apoptosis and growth. (A) The expression of RhoGDIα mRNA was decreased in A549 cells transfected with RhoGDIα siRNA. Cells were transfected with RhoGDIα or Control siRNA for 24, 48, or 72 hours, and RhoGDIα mRNA was assayed by RT-PCR.

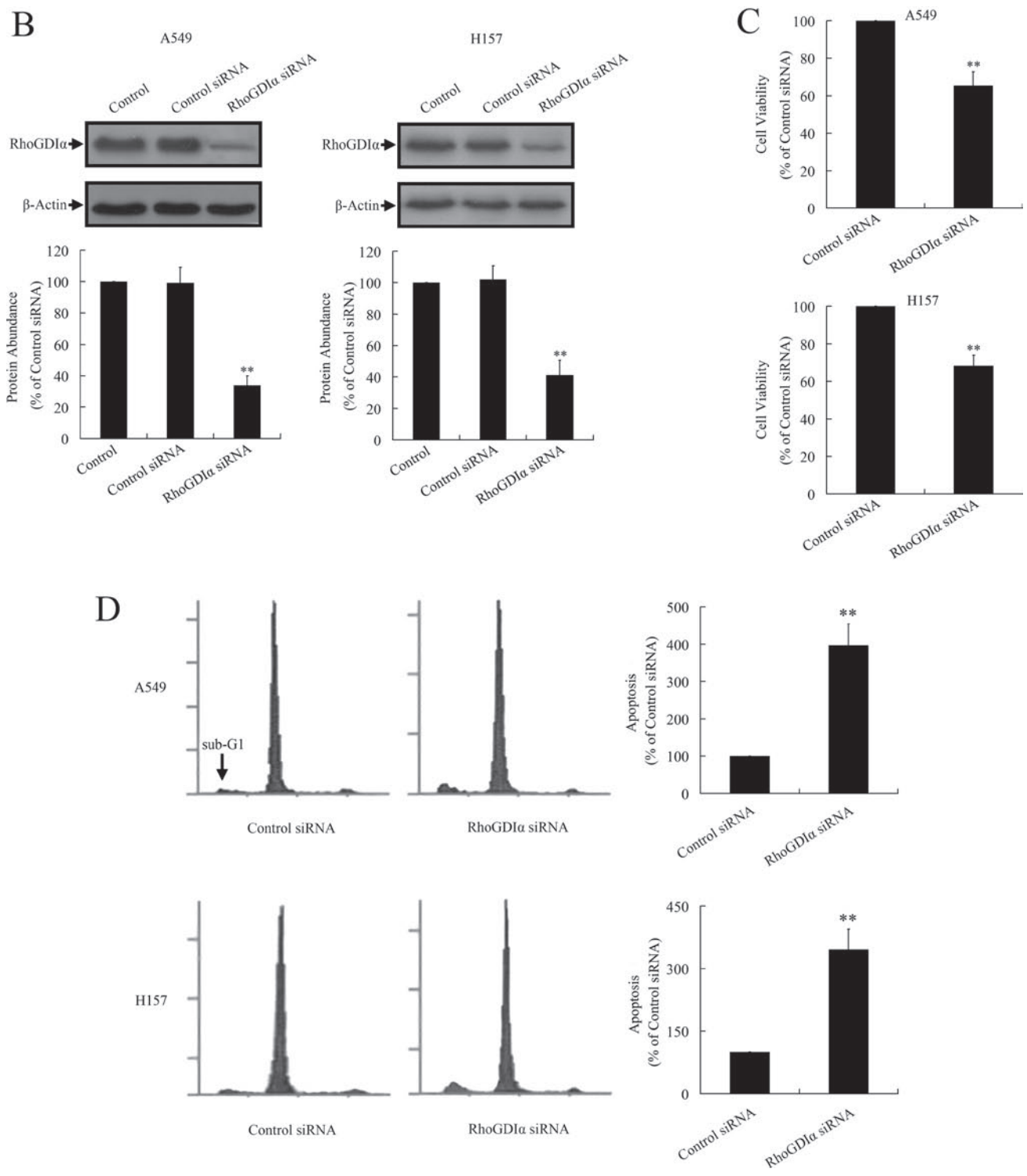

Figure 2. The role of RhoGDIα in tumor cell apoptosis and growth. (B) The expression of RhoGDIα protein was decreased in A549 and H157 cells transfected with RhoGDIα siRNA. After transfection for 48 hours, the lysates from A549 or H157 cells transfected with RhoGDIα or Control siRNA were analyzed by Western blotting. (C) Downregulation of RhoGDIα repressed cell viability in A549 and H157 cells. Viability of cells transfected with control siRNA and RhoGDIα siRNA for 48 hours were tested using MTT assay. RhoGDIα siRNA presented a significant repress on cell viability. **P < 0.01 indicate significant differences from the control siRNA group. (D) Knockdown of RhoGDIα triggered apoptosis in A549 and H157 cells; 48 hours after transfection cells were stained with PI for apoptosis analysis. RhoGDIα siRNA induced significant apoptosis in A549 and H157 cells. **P < 0.01 indicate significant differences from control siRNA group.

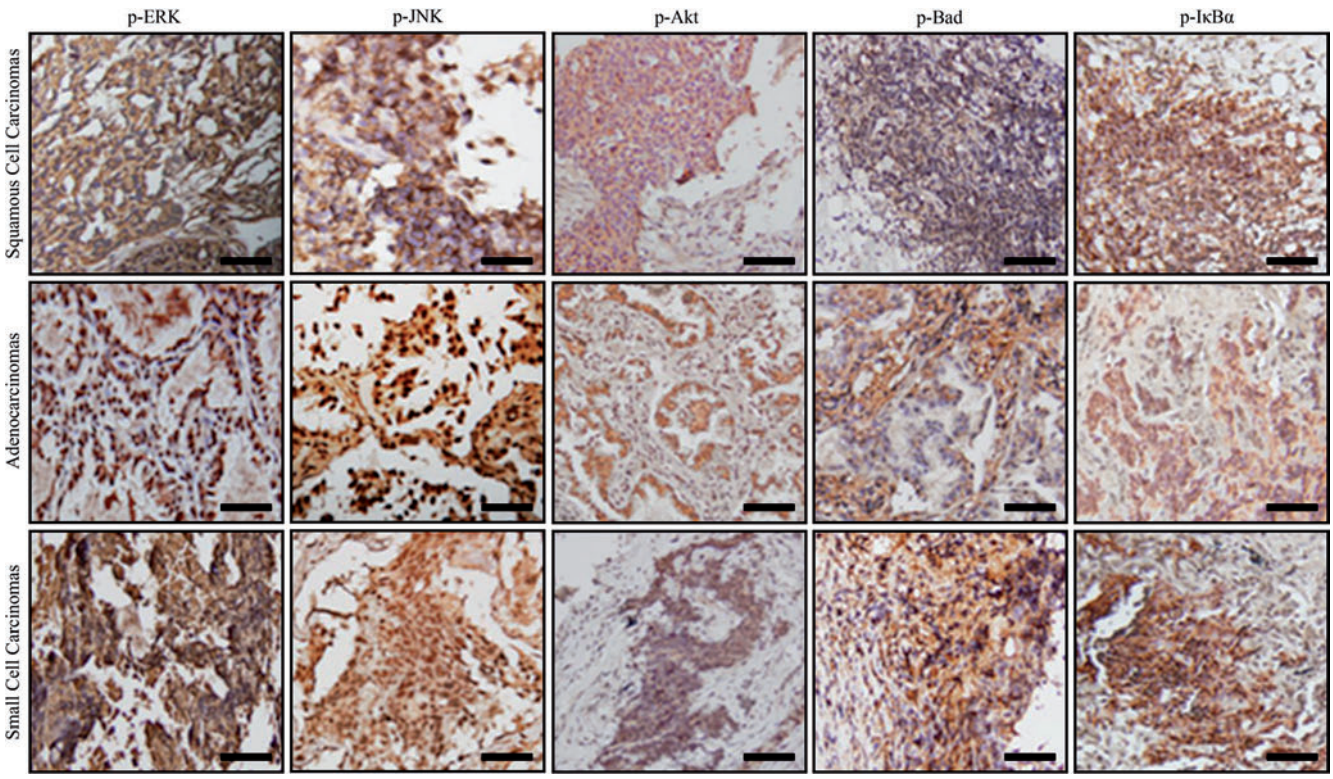

Figure 3. Phosphorylation levels of ERK, Akt, JNK, Bad and IκBα in sections of squamous cell carcinoma, adenocarcinoma and small-cell lung carcinoma. Scale bar=50 μm.

MTT assays were applied to quantify the growth inhibition effect of RhoGDIα siRNA. As shown in Fig. 2C, the knockdown of RhoGDIα significantly repressed cell viability. The role of RhoGDIα downregulation on apoptosis in A549 and H157 cells was assessed by flow cytometry. Cells with downregulated levels of RhoGDIα exhibited a significantly higher level of apoptosis than the control siRNA groups (Fig. 2 D). These results indicate that downregulation of RhoGDIα induced apoptosis and repressed cell growth in lung cancer cells.

Relationships between the expression of RhoGDIα and the phosphorylation levels of apoptosis-relating proteins in lung cancer tissues. As RhoGDIα played a role in protecting cells from the induction of apoptosis, the mechanism of its action as an anti-apoptotic molecule was explored further. Several pathways have been proved to participate in the regulation of apoptosis, such as the MAPK [13], PI3K/Akt [14], Bcl-2 [15] and NF-κB pathways [16]. These pathways are downstream of Rac-1 [17-22], which is a target of RhoGDIα [5]. Therefore, these pathways may be involved in the regulation of RhoGDIα-related apoptosis. To confirm this hypothesis, the phosphorylation levels of several proteins, including ERK, JNK, Akt, Bad and IκBα, which are kinases that belong to these signaling pathways, were assessed in lung cancer tissues (Fig. 3). The correlations between RhoGDIα expression level and the phosphorylation levels of these signaling proteins were analyzed. IHC analysis revealed that the level of RhoGDIα protein was positively correlated with the phosphorylation levels of Bad (p-Bad), and negatively correlated with p-JNK (Table 3). No correlations were identified between the expression levels of RhoGDIα and the phosphorylation levels of other signaling proteins.

The effects of RhoGDIα knockdown on the phosphorylation levels of signaling proteins involved in apoptosis. To confirm the above observation in lung cancer specimens, we

Table 3. The relationship of RhoGDIα with p-ERK, p-Akt, p-Bad, p-JNK and p-IκBα.

	p-ERK		p-Akt		p-Bad		p-JNK		p-IκBα	
	R	P	R	P	R	P	R	P	R	P
RhoGDIα	0.135	0.405	0.196	0.225	0.459	0.003**	-0.336	0.034*	0.116	0.478

R, rank correlation coefficient; * $P < 0.05$, ** $P < 0.01$.

then evaluated the effects of the RhoGDIα knockdown on the phosphorylation levels of apoptosis-relating proteins in A549 cells using Western blotting (Fig. 4). Upon the downregulation of RhoGDIα, the level of p-JNK increased, the level of p-Bad decreased, and the level of p-Akt was unchanged, which was in accordance with the observations in lung cancer tissues. Although we found no relationships between RhoGDIα expression and p-IκBα or p-ERK in the IHC analysis, the downregulation of RhoGDIα in A549 cells increased the levels of IκBα and ERK phosphorylation significantly, which suggests that these two pathways could also be involved in the regulation of apoptosis by RhoGDIα.

Downregulation of RhoGDIα sensitizes A549 cells to apoptosis induced by paclitaxel. Paclitaxel, a microtubule inhibitor, is one of the most effective chemotherapeutic agents against lung cancer. The mechanism of the anticancer action of paclitaxel involves inducing mitotic arrest of the cells due to microtubule stabilization, which eventually results in apoptosis [23, 24]. Unfortunately, the emergence of chemoresistance is a major obstacle in paclitaxel treatment. As RhoGDIα could protect NSCLC cells from the induction of apoptosis, we ascertained whether the knockdown of RhoGDIα could sensitize NSCLC cells to the treatment of paclitaxel.

Following 10 nM paclitaxel treatment for 24 hours, the level of apoptosis was quantified by FCM. Treatment with paclitaxel led to a 2.12-fold (±0.09) increase in the level of apoptosis in the control siRNA-transfected groups, compared to cells transfected with RhoGDIα siRNA, where paclitaxel results in 3.04-fold (±0.21) higher levels of apoptosis (Fig. 5A).

The MTT assays further confirmed the increase of paclitaxel cytotoxicity against cell viability when RhoGDIα was knocked down. In the control siRNA-treated groups, paclitaxel repressed cell viability by 25.67% ± 10.07%. In the RhoGDIα siRNA-treated groups, the decrease in cell viability after paclitaxel treatment was 52.12% ±10.84% (Fig. 5B).

In order to investigate the mechanism involved in the chemosensitization effect of RhoGDIα siRNA, the phosphorylation levels of the signaling proteins, including ERK, JNK, Akt, Bad and IκBα, were assessed by Western blotting. When treated with paclitaxel for 24 hours, the phosphorylation level of JNK, IκBα, Bad and ERK increased. Downregulation of RhoGDIα reduced the upregulation of p-IκBα, p-Bad and p-ERK induced by paclitaxel, but increased the paclitaxel-stimulated upregulation of p-JNK (Fig. 5C). These results indicate that the downregulation of RhoGDIα may sensitize A549 cells to paclitaxel-induced apoptosis through diminishing the phosphorylation of IκBα, Bad and ERK, and increasing the activation of JNK.

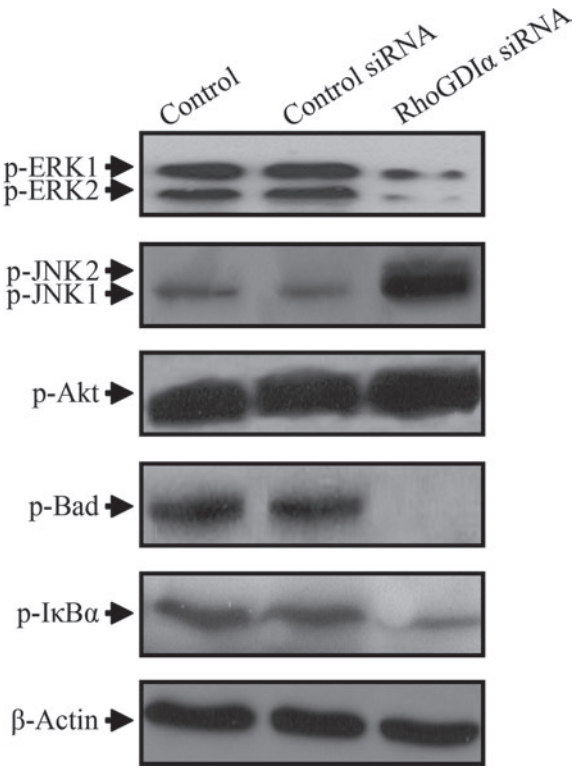

Figure 4. The changes in the phosphorylation levels of the signaling proteins involved in apoptosis. Cells were divided into three treatment groups: solvent control (Control), control siRNA and RhoGDIα siRNA. The lysates from A549 cells were assayed by Western blotting 48 h after transfection.

Discussion

RhoGDIα, a member of the GDI family, is involved in a variety of cellular processes that include cell differentiation, cytoskeletal rearrangement and apoptosis [3]. Recent studies have shown that the level of RhoGDIα expression is increased in various cancers, including ovarian [6] and breast cancer [7]. Some studies have even demonstrated that the degree of RhoGDIα overexpression correlates with cancer grade [6] and drug resistance in vitro [9]. This study has shown that the inhibition of RhoGDIα by targeted siRNA led to the induction of apoptosis and the inhibition of growth in lung cancer cells. These results show that the downregulation of RhoGDIα induced apoptosis in cancer cells and suggest that RhoGDIα plays an important role in protecting lung cancer cells from apoptosis. However, the cell signaling transduction mechanism involved in the RhoGDIα-related regulation of apoptosis remains unclear.

Upon treatment with chemotherapeutics, RhoGDIα can form a tight complex with the Rac1 GTPase in the cytoplasm, which may shield Rac1 from caspase-mediated cleavage, thus maintaining Rac1 in an intact and functional state [5]. Rac1 inhibits apoptosis, as well as promote cancer cell proliferation and metastasis through the phosphorylation of Bad [21], ERK [17] JNK [18, 19], Akt [20] and IκBα [22]. The RhoGDIα-dependent inhibition of apoptosis might be executed through the phosphorylation of these proteins. Thus, the relationships between the expression of RhoGDIα and the phosphorylation levels of Bad, ERK, JNK, Akt and IκBα were assayed in this study.

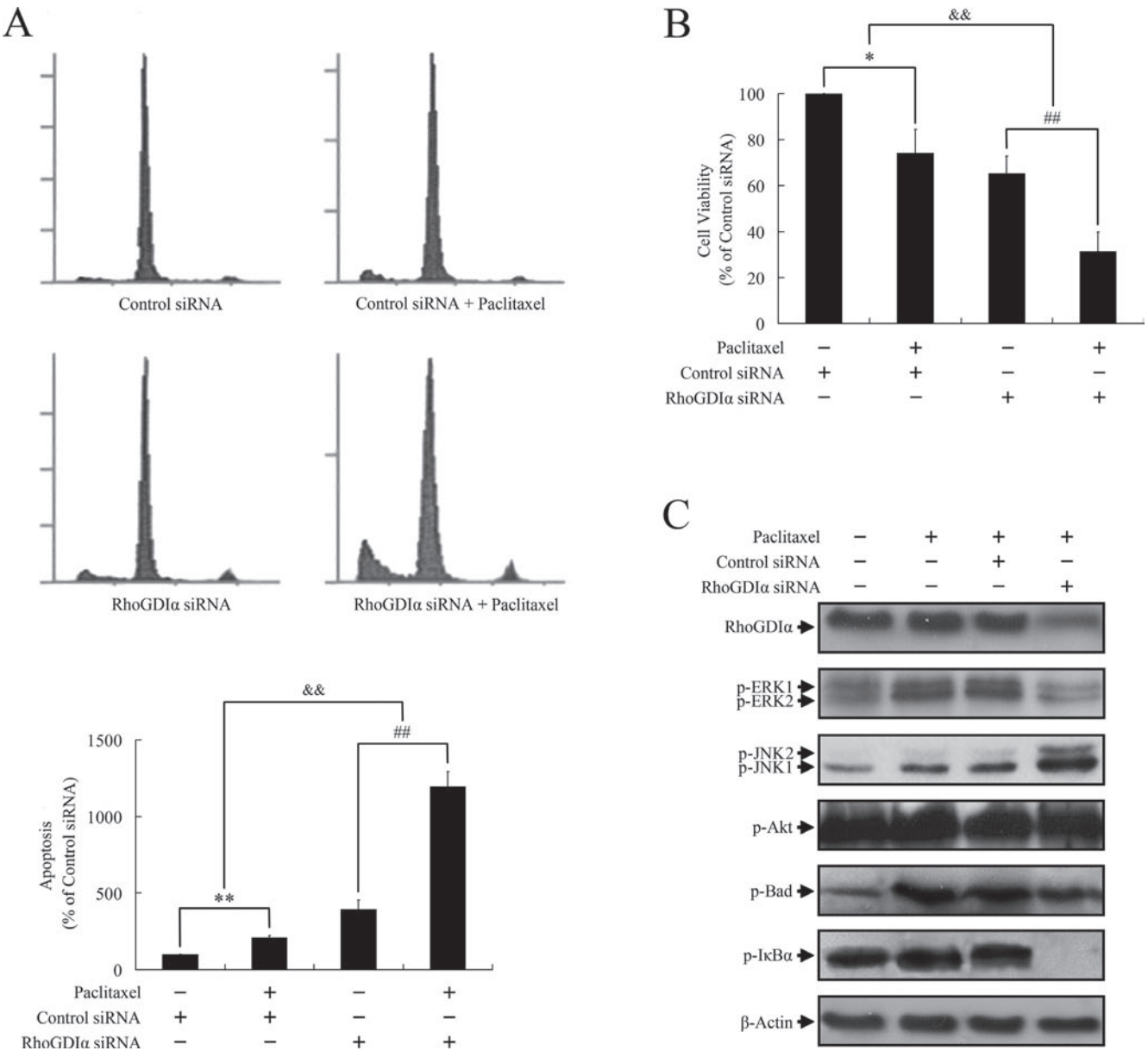

Figure 5. The role of RhoGDIα in paclitaxel-induced apoptosis and growth inhibition. (A) and (B) Downregulation of RhoGDIα increased the sensitivity of cells to paclitaxel-induced apoptosis (A) and growth inhibition (B). After transfection with control siRNA or RhoGDIα siRNA for 48 hours, cells were further treated with 10 nM paclitaxel for 24 hours followed by apoptosis assay using flow cytometry or cell viability evaluation using MTT assay. *$P < 0.05$ and **$P < 0.01$ vs. control siRNA group; $^{\#\#}P < 0.01$ vs. RhoGDIα siRNA group; $^{\&\&}P < 0.01$ between folds induction. (D) The changes in the phosphorylation levels of the signaling proteins involved in apoptosis. After transfection with control siRNA or RhoGDIα siRNA for 48 hours, cells were further treated with 10 nM paclitaxel for 24 hours. The lysates from A549 cells were assayed by Western blotting.

Figures 3 and 4 show that RhoGDIα expression levels positively correlated with those of p-Bad, p-ERK and p-IκBα. Considering the anti-apoptotic effect of p-Bad [25], p-ERK [26] and p-IκBα [22], RhoGDIα potentially inhibits apoptosis through the phosphorylation of these three proteins. Interestingly, a negative correlation was found between RhoGDIα expression levels and p-JNK. The reason for this might be due to the complex function of JNK, the activation of which can promote proliferation [27] or induce apoptosis [28, 29], depending on the stimulation and cell type. As JNK-dependent induction of apoptosis has been widely explored in lung cancer cells [29, 30], the activation of JNK in the lung cancer tissues and cells used in this study could to be pro-apoptotic. Therefore, the up-regulation of RhoGDIα might repress apoptosis through the inhibition of JNK.

It has been previously shown that RhoGDIα protects breast cancer cells against apoptosis induced by treatment with etoposide (VP-16) and doxorubicin [5]. In the present study, the downregulation of RhoGDIα using siRNA appeared to sensitize A549 cells to paclitaxel treatment, which is a drug in common use for the treatment of lung cancer. These studies suggested that the combination of RhoGDIα knockdown could be a promising strategy for chemotherapy.

Paclitaxel induces apoptosis through the activation of JNK [31], while the phosphorylation of ERK [32, 33], Bad [34] and IκBα [35] results in paclitaxel resistance. Our study revealed that treatment with paclitaxel increased the phosphorylation levels of JNK, IκBα, Bad and ERK, which was in line with previous studies. These observations suggested that the phosphorylation of JNK mediated cytotoxicity, but the phosphorylation of ERK, Bad and IκBα reduced the curative effect of paclitaxel. Moreover, knocking down RhoGDIα increased the phosphorylation of JNK, but prevented the up-regulation of p-ERK, p-Bad and p-IκBα, suggesting the downregulation of RhoGDIα could enhance JNK-dependent induction of apoptosis and decrease the ERK, Bad and IκBα-dependent protection. These results indicate that the chemosensitization effect of RhoGDIα siRNA to paclitaxel could be a result of the increase in the induction of apoptosis, in addition to reducing levels of chemoresistance.

In summary, we have shown that RhoGDIα was overexpressed in lung cancer tissue. RhoGDIα protected cells from apoptosis. This effect could be due to the phosphorylation of ERK, Bad and IκBα, as well as the dephosphorylation of JNK. The knockdown of RhoGDIα induced apoptosis and repressed cell viability, which suggests that RhoGDIα might be a new therapeutic target for lung cancer. Moreover, the knockdown of RhoGDIα sensitized lung cancer cells to the cytotoxic effects of paclitaxel. This chemosensitization effect could be due to the repression of the anti-apoptotic factors, p-ERK, p-Bad and p-IκBα, as well as the upregulation of the pro-apoptotic protein, p-JNK. Thus, the combination of RhoGDIα siRNA and paclitaxel might be an effective strategy for lung cancer treatment.

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