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Knockdown of 14-kDa phosphohistidine phosphatase expression suppresses lung cancer cell growth *in vivo* possibly through inhibition of NF-κB signaling pathway

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In previous study, we reported that 14-kDa phosphohistidine phosphatase (PHP14) was associated with lung cancer cell migration and invasion. We also found that the expression of PHP14 was markedly increased in a part of human lung cancer tissues. In this study, we investigated the impact of PHP14 knockdown on lung cancer cell tumorigenesis *in vitro* and *in vivo*, as well as the regulatory pathway. Depletion of endogenous PHP14 expression in lung cancer cells reduced colony formation activity of lung cancer cells *in vitro* and inhibited the xenograft tumor growth *in vivo*. Further experiments revealed that the NF-κB signal pathway inhibitor PDTC inhibited the upregulated expression of MMP9 induced by PHP14 overexpression in lung cancer cells. Furthermore, knockdown of PHP14 in lung cancer cells correlated with decreased expression of a subset of NF-κB-regulated genes, such as BCL-2, COX-2, MCP-1, MMP9 and VEGF-C, which play an important role in tumor progression. Together these data suggest that knockdown of PHP14 in lung cancer cells inhibits lung cancer tumor growth *in vivo*, possibly via regulating the NF-κB pathway.

Key words: 14-kDa phosphohistidine phosphatase, NF-κB signaling pathway, lung cancer, RNA interference

Lung cancer is the most common malignancy and the leading cause of cancer-related death worldwide [1]. Lung cancer development is a complex process involving the accumulation of genetic and epigenetic intreraction, resulting in uncontrolled proliferation, cellular transformation and the acquisition of aggressive behaviour [2]. Although much effort has been invested in identifying the genes involved in lung cancer, the mechanisms underlying this disease are not fully understood.

The 14-kDa phosphohistidine phosphatase (PHP14), also known as PHPT1, is the first histidine phosphatase protein identified in vertebrates and is similar to the janus proteins of *Drosophila* [3,4]. Several studies have demonstrated various physiological functions for PHP14, such as its ability to dephosphorylate ATP-citrate lyase [5] and the β-subunit of G proteins *in vitro* [6]. A recent report also revealed that PHP14 could negatively regulate CD4 T cells through inhibition of the K channel KCa3.1 [7] and regulate the epithelial Ca^{2+} channel TRPV5 by reversible histidine phosphorylation [8]. Some studies have revealed that histidine kinases and histidine phosphorylated proteins, such as Nm23, P-selectin, and annexin I, were associated with signal transduction in cancer [9,10].

In our previous studies, we demonstrated that knockdown of PHP14 expression in lung cancer cells significantly inhibited lung cancer cell migration, invasion and metastasis *in vitro* and *in vivo*, and that the expression of PHP14 was higher in cancer patients with lymph node metastasis compared with patients without lymph node metastasis [11]. Further study found that the expression level of PHP14 was significantly higher in lung tumor samples (53.42%) than normal tissues adjacent to tumor lesions (23.33%) (P=0.003) [12]. However, the roles of PHP14 in primary lung cancer growth and its mechanisms of action are still unknown. In this study, we examined the effect of PHP14 expression on lung cancer cell growth *in vitro* and *in vivo*, as well as the possible mechanism.

Materials and methods

Cell lines. Non-small cell lung cancer cell line A549 and small cell lung cancer cell line NCI-H446 were originally obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco LifeBTechnologies, Gaithersburg, MD, USA), supplemented with 10% FCS (HyClone, Logan, UT) at 37°C in 5% CO_2. U0126 was purchased from Cell Signaling Technology, pyrrolidinedithiocarbamate (PDTC) was purchased from Sigma-Aldrich. Mouse anti-human PHP14 polyclonal antibody and pcDNA3.1/PHP14 was generated as previous described [11].

RNA interference and stably transfected clone selection. As previously described [11], short interfering RNA (siRNA)-coding oligos against human PHP14 targeting sequence CGGACATCTACGACAAAGT was cloned into pGPU6/GFP/Neo siRNA expression vector. A siRNA oligo that did not match any known human coding cDNA was used as negative control. The insert-containing vector or control vector was stably transfected into A549 and H446 cells using the FuGENE 6 transfection reagent (Roche, Indianapolis, IN, USA). After 8 hours, cells were treated with 400μg /mL G418-sulfate (G418, Merck). G418-sulfate-resistant clones were selected, and those clones were then expanded for further studies.

In vitro colony formation assay. To detect the anchorage-independent growth, six-well plates were precoated with 0.6% agarose in RPMI-1640 with 10% FBS, and cells (A549 or H446) treated with siRNA were seeded at 1×10^3 cells per well in 0.3% agarose/ DMEM with 10% FBS, respectively. The plates were incubated for 2 weeks. Colonies with a diameter greater than 100 μm were counted under an inverted microscope.

Zymography assay. Gelatin zymography was used to determine the expression and activity of MMP2 and MMP9. Briefly, cells were seeded onto 100 mm plates using serum-free medium. After 24 h, conditioned medium was collected, and protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). Culture supernatants were subjected to electrophoresis on gelatin substrate gels (10 % SDS-polyacrylamide gels containing 1 mg/mL of gelatin). The gels were treated with 2.5% Triton X-100 for 30 min and incubated at 37°C for 24 h in a buffer containing 100 mM Tris–HCl (pH 7.4), 0.15 M NaCl, and 15 mM $CaCl_2$. The gels were stained with Coomassie Blue R-250 and then de-stained with water until clear zones appeared.

RT-PCR. Total RNA was isolated from cell lines and tissues using Trizol reagent (Invitrogen, Carlsbad, CA), and 2 μg was reverse transcribed into single-strand cDNA in 20 μL of reaction buffer using Molony murine leukemia virus reverse transcriptase (Promega, Madison, WI) and oligo (dT)15 (Promega) as a primer. Specific primers of PHP14 were designed according to National Center for Biotechnology Information reference sequence, and β-actin was chosen as an internal control. The primers were used as follows: PHP14: sense: 5'-GCCACAGAGCCACCCCCA-3', antisense: 5'-ATCCAGACAAATCCTTCCAGCA-3'; β-actin: sense: 5'-GAGCTACGAGCTGCCTGACG-3', antisense: 5'-CCTAGAAGCATTTGCGGTGG-3'. The RT-PCR primers of MMP9 are the same as the real time PCR primers. The PCR amplification was carried out in a reaction buffer containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, all four dNTPs (each at 167 μM), 2.5 U of Taq DNA polymerase, and 0.1 μM primers. The reactions were performed in the ABI GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with the following program: denaturing for 30 sec at 94°C, annealing for 30 sec at 60°C, and elongating for 30sec at 72°C for a total of 25 cycles (for β-actin) or 27 cycles (for PHP14 and MMP9); the final extension took place at 72°C for 7 min. Equal volumes of each PCR sample were subjected to electrophoresis in a 1% agarose gel, which was then stained with ethidium bromide and photographed with Chemilmager 440 (Alpha Innotech Corp., San Leandro, CA).

Real time PCR. Primers of BCL-2, BCL-XL, COX-2, IL-8, IkBα, MCP-1, MMP2, MMP9, PHP14, TIMP1, TIMP2, uPA, VEGF-C and GAPDH used for quantitative PCR were designed using Primer 5 software (Table 1). The reactions were performed on the ABI 7500 Real-Time PCR System (Applied Biosystems) with SYBR® Green (Applied Biosystems). GAPDH was chosen as internal control. Each experiment was performed in 20 μl of reaction volume containing 10 μl SYBR®

Table 1. Primers used for real-time PCR.

Genes		Primer sequences (5'-3')	Product sizes
BCL-2	Sense	GAGGATTGTGGCCTTCTTTG	151bp
	Anti-sense	CCCAGCCTCCGTTATCCT	
BCL-XL	Sense	ACATCCCAGCTCCACATCAC	174bp
	Anti-sense	CGATCCGACTCACCAATACC	
COX-2	Sense	TGCATTCTTTGCCCAGCACT	144bp
	Anti-sense	AAGGCGCAGTTTACGCTGTCT	
IL-8	Sense	AACTTCTCCACAACCCTC	247bp
	Anti-sense	TGGCAGCCTTCCTGATTT	
IkBα	Sense	GTCAAGGAGCTGCAGGAGAT	109bp
	Anti-sense	ATGGCCAAGTGCAGGAAC	
MCP-1	Sense	TCTGTGCCTGCTGCTCATAG	167bp
	Anti-sense	GCTTCTTTGGGACACTTGCT	
MMP2	Sense	TGGCAAGTACGGCTTCTGTC	179bp
	Anti-sense	TTCTTGTCGCGGTCGTAGTC	
MMP9	Sense	TGCGCTACCACCTCGAACTT	200bp
	Anti-sense	GATGCCATTGACGTCGTCCT	
PHP14	Sense	TCAGTAGCCGTCGTTAGCC	174bp
	Anti-sense	TGCGACTGTGAGTGTCTGGG	
TIMP1	Sense	CCGGGGCTTCACCAAGACCTA	111bp
	Anti-sense	AGGTCCGTCCACAAGCAATGAGT	
TIMP2	Sense	AAGAGCCTGAACCACAGGTA	175bp
	Anti-sense	GAGCCGTCACTTCTCTTGAT	
uPA	Sense	GCCTTGCTGAAGATCCGTTC	91bp
	Anti-sense	GGATCGTTATACATCGAGGGGCA	
VEGF-C	Sense	CTACAGATGTGGGGGTTGCT	240bp
	Anti-sense	GCTGCCTGACACTGTGGTAA	
GAPDH	Sense	GAGTCAACGGATTTGGTCGT	185bp
	Anti-sense	GACAAGCTTCCCGTTCTCAG	

Green PCR Master Mix (Applied Biosystems), 1 μl of cDNA or H_2O as negative control, 2 μl of forward and reverse primers (Table 1, 10mM each), and 7 μl of distilled H_2O. Thermal cycle conditions for all the genes were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 60°C for 1 min. A uniform amplification of the products was rechecked by analyzing the melting curves of the amplified products. All reactions were carried out in triplicate to access the reproducibility. The relative quantitation of mRNA expression was calculated with the comparative Ct (the threshold cycle) method using the follow formula:

$$Ratio = 2^{-\Delta\Delta Ct} = 2^{-[\Delta Ct(sample) - \Delta Ct(calibrator)]},$$

where $\Delta Ct = Ct$ of target genesof $- Ct$ endogenous control gene (GAPDH) [13].

Western blot analysis. The cells were treated with lysis buffer [50 mM Tris-HCl pH8, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 1% NP40 and a protease inhibitor cocktail (dissolve 1 tablet in 50 ml lysis buffer)]. Equal quantities of protein were separated by 12% SDS-PAGE and transferred onto nitrocellulose membrane (Amersham Biosciences) using a Bio-Rad wet transfer unit. After blocking with 5% (w/v) non-fat dried milk in TBST solution [25 mM-Tris, pH7.5, 150 mM NaCl, 0.05 %(v/v) Tween-20] for 1hr at room temperature, the membranes were incubated with mouse anti-human PHP14 polyclonal antibody (1:200 dilution) and rabbit anti-human α-tubulin antibody (1:1000, Santa Cruz Biotechnology) for 1hr at 37°C or overnight at 4°C, followed by alkaline phosphatase-conjugated horse anti-mouse or goat anti-rabbit IgG (1:1000 dilution, Santa Cruz Biotechnology) for 1 h at 37°C. Target proteins were detected by 5-bromo-4-chloro-3-indoyl phosphate (BCIP) and nitro blue tetrazolium (NBT). α-tubulin was chosen as an internal control for equal protein loading.

Immunohistochemistry. The immunohistochemical staining of PHP14 was performed as previous [12]. Briefly, the slide was dried at 60°C for 4 h, deparaffinized in xylene, and then rehydrated through a graded ethanol series. The endogenous peroxidase activity was blocked for 20 min with 3% H_2O_2. Antigen retrieval was carried out in an antigen retrieval buffer (10 mmol/L citrated buffer (pH 6.0)) at 95°C in a microwave cooker for 10 minutes.. After incubating the slide with bovine serum for 30 min at room temperature, the PHP14 antibody [1:50 dilution in PBST (PBS, 0.05% Tween-20, pH 7.4)] was applied for one night at 4°C and then washed before incubated with the secondary antibody, HRP-conjugated, affinity purified donkey anti-mouse, for 1 h at 37°C. Detection was performed with 3, 30-diaminobenzidine tetrahydrochloride peroxidase substrate and counterstaining with hematoxylin, followed by dehydration, clearing, and mounting.

Xenograft assay. Four to six weeks old female athymic nude mice were injected with tumor cells (5×10^6) subcutaneously into the left and right flank regions (5 mice per group). All animals were purchased from the Institute of Animal Science of the Vital River Co., Ltd. The animals had not received any drugs or previous procedures. Injected mice were examined every 3 or 5 days for tumor appearance, and tumor volumes were estimated from the length (a) and width (b) of the tumors, as measured by calipers, using the formula $V= ab^2/2$ [14]. Mice injected with cells derived from A549 cells carrying control-siRNA developed tumors approximately 9 days after inoculation, compared to 19 days of the group with A549 cells carrying PHP14-siRNA. Five mice per group were sacrificed 26 days after injection, and subcutaneous tumors were surgically excised, weighed, and photographed, and a portion of tumor was frozen at -80°C. All experiments and procedures involving mice were approved by an animal care and use committee at the Beijing Friendship Hospital, Capital Medical University. Our care and use of animals followed the guidelines of the Experimental Animal Management Ordinance.

Statistical analysis. We used SPSS software version 18.0 (SPSS INC., Chicago, IL, USA) to conduct all statistical comparisons. The data were presented as the mean ± SD. Mann–Whitney test was used to compare the differences between the control and experimental groups. All statistical tests were two sided. *P* values of less than 0.05 were considered to be statistically significant.

Results

Inhibition of PHP14 expression in lung cancer cells suppresses anchorage-independent growth *in vitro*. To determine the function of PHP14 in cellular transformation, we first constructed an expression vector harboring PHP14 siRNA and stably transfected the PHP14 siRNA vector into A549 and H446 cells. As shown in Figure 1A and 1B, the mRNA and protein levels of PHP14 were significantly reduced in A549-siRNA and H446-siRNA cells compared with control cells harboring scramble siRNA ($p < 0.05$).

Anchorage-independent growth assay was performed to measure the effect of PHP14 knockdown on colony formation potential of lung cancer cells *in vitro*. Compared to cells transfected with control vector, cells with PHP14 knockdown showed significantly lower colony formation ability and produced smaller colonies ($p < 0.05$) (Figure 1C).

Knockdown of PHP14 expression in lung cancer cells inhibits xenograft tumor growth *in vivo*. To further explore the biological function of PHP14 *in vivo*, we generated tumor xenografts by subcutaneously inoculating tumor cells stably expressing PHP14 siRNA or control vector at the left and right flank regions of athymic nude mice. As shown in Figure 2A, comparison of the average tumor latency and volume revealed that tumors derived from A549 cells carrying PHP14 siRNA emerged later and proliferated to a smaller size compared to the control group. Moreover, the tumor incidence was also lower in the xenograft model (10/10 control, 7/10 PHP14 knockdown). The final weights of PHP14 knockdown tumors were significantly lower than controls (Figure 2B). However, the overall weight of mice did not show any differences among groups. Lungs of mice bearing xenograft tumors were also ex-

amined but revealed no metastases in either control or PHP14 knockdown groups (data not shown). Semi-quantitative RT-PCR and immunohistochemistry of PHP14 in xenograft tumors confirmed that suppressed PHP14 expression had been maintained throughout the experimental time course (Figure 2C and D).

Depletion of endogenous PHP14 suppresses MMP expression *in vitro*. Matrix metalloproteinase (MMP)-2 and MMP9 are extracellular proteinases that contain fibronectin type II inserts within the catalytic domain and have implicated roles in tumorigenesis and metastasis [15]. We first examined the effect of PHP14 knockdown on MMP2 and MMP9 ex-

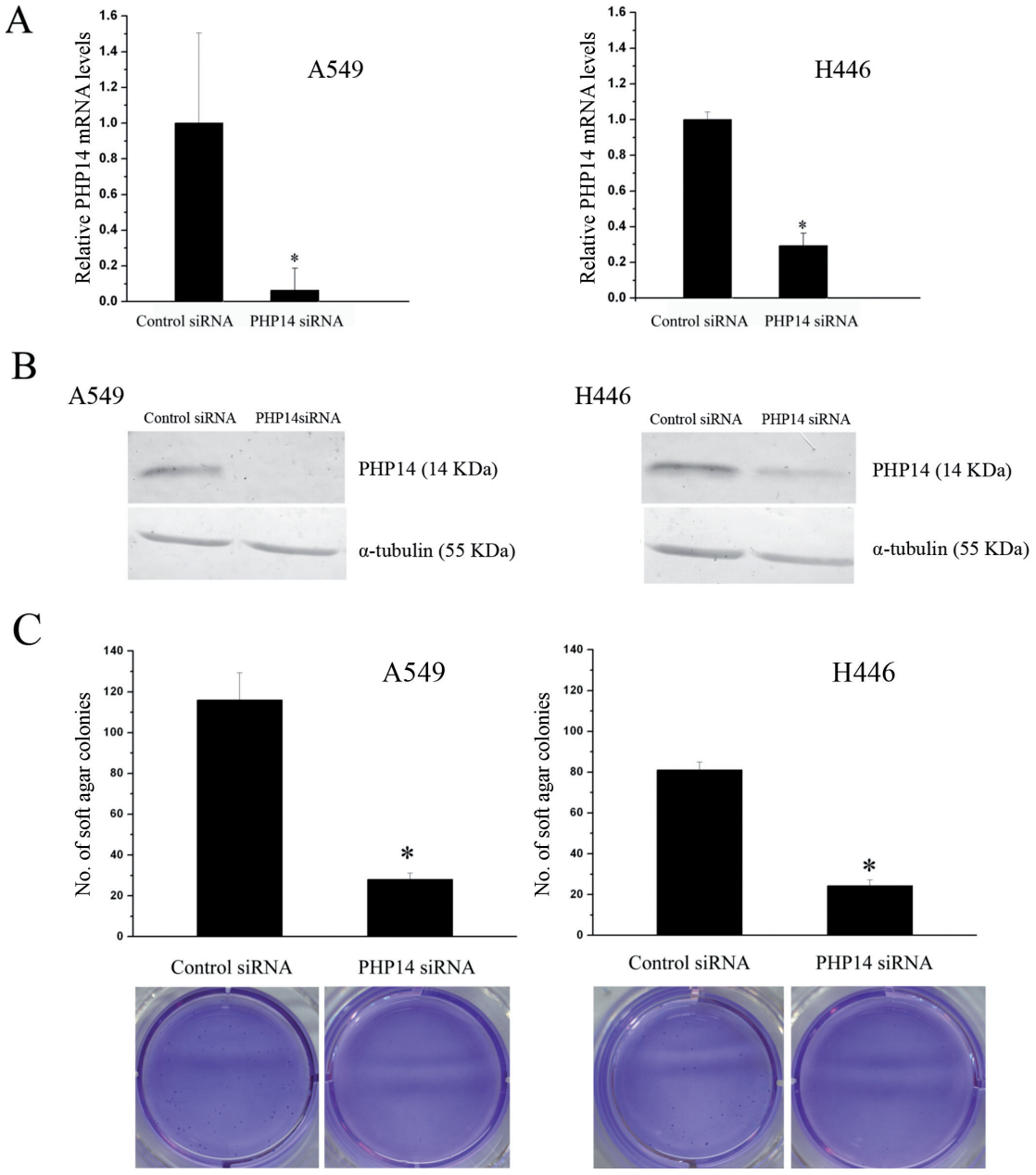

Figure 1. Knockdown of PHP14 expression by siRNA inhibited lung cancer cell colony formation. (A) Expression of PHP14 in A549 and H446 cells stably expressing control-siRNA or PHP14-siRNA as determined by quantitative PCR. GAPDH mRNA was used as an internal control. (B) Expression of PHP14 in A549 and H446 cells stably expressing control-siRNA or PHP14-siRNA as determined by western blot analysis. α-tubulin was used as an internal control. (C) Colony formation of A549 and H446 cells carrying control-siRNA or PHP14-siRNA as assessed by soft agar assay. Bottom, representative photos of soft agar colonies formed by cells carrying control-siRNA or PHP14-siRNA. Each data represents the mean ± SEM value of triplicate wells. *$p < 0.05$, Mann–Whitney test.

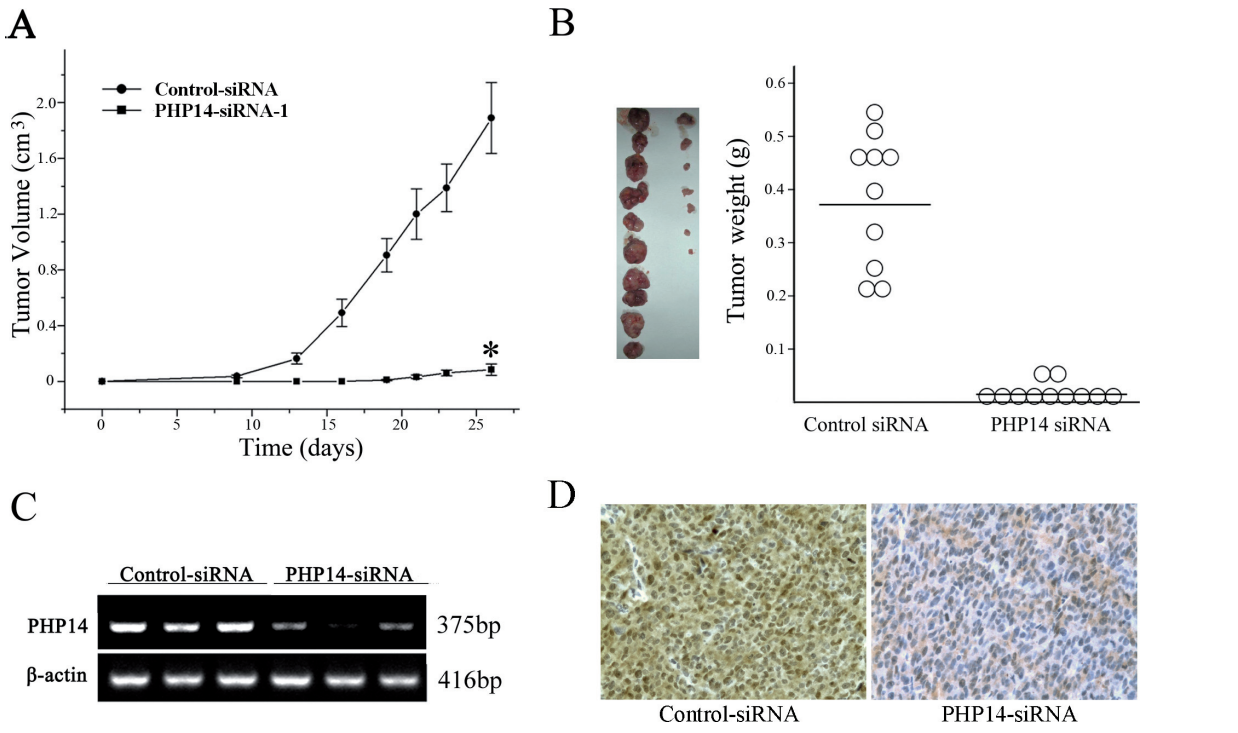

Figure 2. Knockdown of PHP14 expression in A549 cells inhibited xenograft tumor growth *in vivo*. (A) Volumes of tumors derived from control-siRNA (closed circle) or PHP14-siRNA (closed square) cells measured over time. Each data point represents the mean ± SEM of ten xenograft tumors. (B) Xenograft tumors derived from control-siRNA or PHP14-siRNA at 26 days. Left, photographs of excised xenograft tumors. Right, weights of xenograft tumors. Tumors from two independent xenograft experiments were weighed immediately after removal. Mean xenograft tumor weight is indicated. (C) Expression of PHP14 mRNA was examined by semi-quantitative PCR of tumor cells from xenograft tumors of individual mice carrying either A549 control-siRNA tumors or A549 PHP14-siRNA tumors. β-actin was used as an internal control. (D) The expression of PHP14 in the primary tumors was examined by immunohistochemistry. *$p < 0.05$, log-rank statistical analysis.

pression and activity. MMP2 and MMP9 were significantly downregulated after PHP14 knockdown in A549 and H446 cells, while their inhibitors (TIMP1 and TIMP2) were upregulated. These results were confirmed by real time PCR (Figure 3A) and gelatin zymography assays (Figure 3B).

Table 2. PHP14 knockdown decreased expression of a subset of NF-κB regulated genes.

Cell line	A549		H446	
siRNA	Control	PHP14	Control	PHP14
BCL-2	1±0.282	0.231±0.178*	1±0.073	0.186±0.075*
BCL-XL	1±0.053	0.559±0.03	1±0.069	0.277±0.112*
COX-2	1±0.174	0.341±0.335*	1±0.074	0.174±0.308*
IkBα	1±0.052	0.924±0.037	1±0.051	0.841±0.072
IL-8	1±0.052	1.437±0.029	1±0.065	0.145±0.143*
MCP1	1±0.091	0.256±0.301*	1±0.081	0.234±0.272*
MMP2	1±0.087	1.022±0.213	1±0.054	0.074±0.072*
MMP9	1±0.081	0.428±0.377*	1±0.023	0.093±1.059*
uPA	1±0.045	1.119±0.027	1±0.065	0.63±0.264
VEGF-C	1±0.136	0.456±0.048*	1±0.038	0.308±0.123*

*: $p<0.05$, Mann–Whitney test.

Knockdown of PHP14 expression may inhibit lung cancer xenograft tumor growth through the NF-κB signal pathway. MMP2 and MMP9 are gelatinases secreted by different cell types [15,16]. The expression of MMPs is regulated by the MEK1 pathway and NF-κB pathway [17-19]. To investigate the pathway by which PHP14 regulates the expression of MMPs in lung cancer cells, PHP14-overexpressing A549 and H446 cells were treated with U0126 or PDTC, which selectively inhibit the MEK1 or NF-κB pathway, respectively. As shown in Figure 4, PHP14-overexpressing A549 and H446 cells showed increased MMP9 expression. The induction of MMP9 expression by PHP14 overexpression was not changed in the presence of MEK1 inhibitor U0126 (10 μM for 2 h). However, the expression of MMP9 became undetectable in the presence of NF-κB inhibitor PDTC (50 μM for 2 h) regardless of PHP14 status, suggesting that PHP14 may affect lung cancer xenograft tumor growth and metastasis via NF-κB pathway.

To confirm whether the NF-κB pathway was regulated by PHP14, we examined the expression of 10 NF-κB regulated genes in A549 siRNA and H446 siRNA cells using real time PCR. Besides the expression of IL-8 and uPA genes, which were slightly increased in A549 siRNA cells, the expression of all other examined genes were decreased in siRNA cells (Table 2).

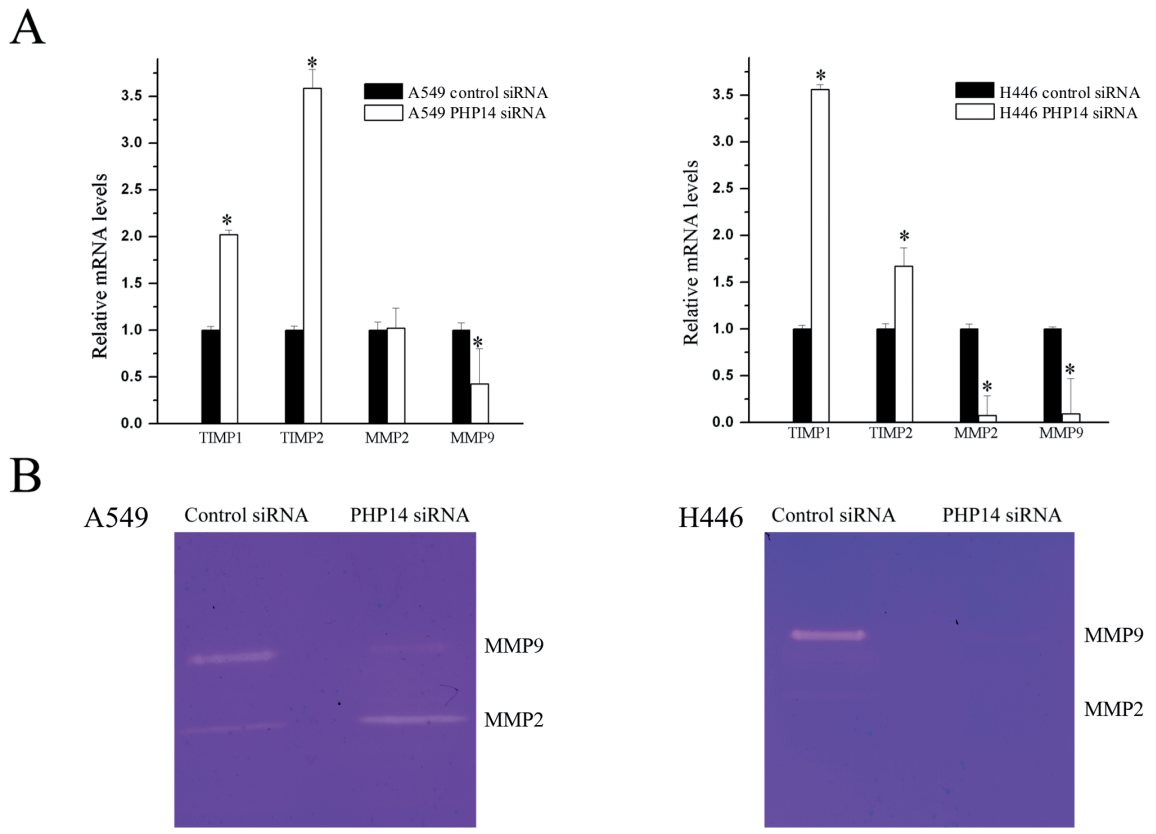

Figure 3. Depletion of endogenous PHP14 suppresses MMP expression *in vitro*. (A) Quantitative PCR analyses of TIMP1, TIMP2, MMP2, MMP9 in A549 and H446 cells carrying control-siRNA or PHP14-siRNA. (B) Levels of MMP-2 and MMP-9 in conditioned medium derived from siRNA-transfected A549 and H446 cells were determined by gelatin zymography. *$p < 0.05$, Mann–Whitney test.

Five of ten genes were decreased more than 2-fold in A549 siRNA cells, while eight of ten genes were decreased more than 2-fold in H446 siRNA cells compared to control siRNA cells. These data confirm that NF-κB regulates these genes in a cell type-dependent manner [20]. Among the decreased genes, BCL-2, COX-2, MCP-1, MMP9 and VEGF-C were all downregulated in A549 and H446 siRNA cells.

Discussion

MMPs are the predominant extracellular proteolytic enzymes in mediating the interactions of extracellular matrix and tumor cells [21, 22]. One of the earliest events in angiogenesis is basement membrane degradation, which requires MMP function [23]. MMP2 and MMP9 have been detected in malignant tissues and associated with tumor aggressiveness, angiogenesis and metastasis potential [24]. Our previous and present studies demonstrate that PHP14 can significantly affect lung cancer cell tumorigenesis and metastasis *in vivo*. To examine the mechanism underlying the effects of PHP14 knockdown, we evaluated the expression and activity of MMP2 and MMP9 in siRNA cells. This also provided a hint of the mechanism through which PHP14 affects lung cancer tumorigenesis and metastasis.

Multiple signaling transduction pathways are involved in the regulation of MMP production in human cancer cells. The expression of MMP9 is mostly regulated by the MEK1 and NF-κB pathways [24]. In our study, the upregulated expression

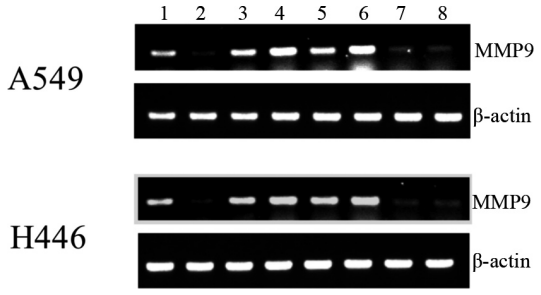

Figure 4. PHP14 may affect lung cancer xenograft tumor growth and metastasis via NF-κB pathway. PHP14-overexpressing A549 and H446 cells were treated with U0126 or PDTC, which selectively inhibit the MEK1 or NF-κB pathway, respectively, and MMP9 expression was detected. Lane 1, control-siRNA cells; lane 2, PHP14-siRNA cells; lane 3, pcDNA3.1 transfected cells; lane 4, pcDNA3.1/PHP14 transfected cells; lane 5, pcDNA3.1 transfected cells treated with U0126; lane 6, pcDNA3.1/PHP14 transfected cells treated with U0126; lane 7, pcDNA3.1 transfected cells treated with PDTC; and lane 8, pcDNA3.1/PHP14 transfected cells treated with PDTC.

of MMP9 in PHP14 overexpressing A549 and H446 cells was suppressed by the broad spectrum NF-κB inhibitor PDTC but not the MEK 1 inhibitor U0126, suggesting that PHP14 may regulate MMP9 expression via the NF-κB pathway or that PHP14 may be a downstream gene of MEK1 to regulate the NF-κB pathway. The NF-κB pathway regulates the expression of a wide range of genes that are implicated in many normal and protective biological processes, but is also usurped during tumorigenesis. NF-κB pathway regulates critical processes related to cancer progression, including apoptosis, angiogenesis, invasion, metastasis, growth, and proliferation [25-26]. The NF-κB pathway is regulated by many endogenous molecules and extracellular signals [27]. In our present study, knockdown of PHP14 inhibited the NF-κB pathway and downregulated the expression of many NF-κB regulated genes, such as BCL-2, COX-2, MCP-1, MMP9 and VEGF-C, both in A549 and H446 siRNA cells. Our data suggest that inhibition of PHP14 expression in lung cancer cells also inhibits the NF-κB pathway, which inhibits tumorigenesis and metastasis *in vivo*.

PHP14 is a protein histidine phosphatase. Histidine kinases and histidine phosphorylated proteins, such as Nm23, P-selectin, annexin I, have been found to play important roles in tumor growth and metastasis [10]. In our previous study, we found that knockdown of PHP14 expression in lung cancer cells inhibited cell migration and invasion both *in vitro* and *in vivo* [11], and the expression of PHP14 was significantly upregulated in lung cancer cells and tissues [12]. A recent report suggested that PHP14 might play an important role in hepatocellular carcinoma cell proliferation [28], but the mechanism was not fully elucidated. Here we show that knockdown of PHP14 expression also inhibits the lung cancer cell colony formation *in vitro* and xenograft tumor growth *in vivo*. Furthermore, we elucidate that PHP14 functions in lung cancer xenograft tumor growth and metastasis possibly via regulating the NF-κB pathway. Although our data show that the NF-κB pathway is regulated by PHP14, the functional relevance of PHP14 and NF-κB is still unknown, and future studies will be focused on how PHP14 regulates NF-κB.

In conclusion, we demonstrate for the first time that knockdown of PHP14 in lung cancer cells inhibits lung cancer xenograft tumor growth and metastasis possibly via regulating the NF-κB pathway. The results of our study provide insight into a novel mechanism through which PHP14 plays a critical role in tumor progression.

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