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Rho GTPase Activating Protein 24 (ARHGAP24) Silencing Promotes Lung Cancer Cell Migration and Invasion by Activating β-Catenin Signaling

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Background: Rho GTPase activating protein (RhoGAPs) is an important negative regulator of the Rho signaling pathway that is involved in tumorigenesis in liver, colon, and renal cancer. However, the mechanism by which Rho GTPase activating protein 24 (ARHGAP24) regulates cell invasion and migration of lung cancer has not been fully explained.

Material/Methods: In this study, ARHGAP24 expression in lung cancer tissues and cell lines was measured by immunohistochemical and Western blot analysis. Transwell or wound healing analysis was performed to detect the cell migration and invasion of ARHGAP24 modulated A549 and NCI-H1975 cells with β-catenin inhibitor XAV-939 (10 μM) treatment, and the expression of MMP9, VEGF, and β-catenin protein was measured by Western blotting.

Results: Our results showed that ARHGAP24 expression was downregulated in lung cancer tissues and cell lines. pLVX-Puro-ARHGAP24 transfection in A549 cells significantly inhibited cell invasion and migration, along with increased E-cadherin and decreased MMP9, VEGF, Vimentin, and β-catenin protein expression. pLKO.1-ARHGAP24-shRNA transfection in NCI-H1975 cells significantly promoted cell invasion and migration, accompanied with decreased E-cadherin and increased MMP9, VEGF, and β-catenin protein expression. Moreover, NCI-H1975 cells with XAV-939 treatment showed decreased cell invasion and migration when compared with pLKO.1-ARHGAP24-shRNA transfection. ARHGAP24 silencing promoted the transcriptional activity of β-catenin in NCI-H1975 cells.

Conclusions: Our findings indicate that ARHGAP24 silencing promotes lung cancer cell migration and invasion through activating β-catenin signaling.

MeSH Keywords: beta Catenin • Cell Migration Assays • Lung Neoplasms • Neoplasm Invasiveness

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Background

Lung cancer is a pulmonary malignancy with the highest mortality rate among all cancers. Increasing morbidity and mortality were reported worldwide in the last 50 years [1,2]. Lung cancer, which mainly comprises small cell lung cancer and non-small cell lung cancer (NSCLC), has a 5-year survival rate of 15% [2]. Currently, radiotherapy and chemotherapy are generally used as therapeutic strategies against advanced lung cancer [3]. However, the effect of chemotherapy in the treatment of NSCLS is limited due to tolerance to the chemotherapeutics [1,3]. Tolerance and recurrence of tumor cells caused by the rudimentary cancer stem cells after chemotherapy have become one of the most intractable problems in the clinical treatment of lung cancer [3,4]. Therefore, exploring potential therapeutics with high efficiency against lung cancer is crucially needed.

Tumor metastasis is a highly integrated and multistep process, in which a series of molecules participate, including Rho GTPase, matrix metalloproteinases (MMPs), and vascular endothelial growth factor (VEGF) [5–7]. Rho GTPase belongs to the Ras superfamily and plays an important role in tumor development by regulating cell proliferation, cytoskeleton actin, and cell adhesion [8]. Rho GTPase activity is increased in many tumor tissues, such as gastric, breast, pancreatic, and testicular cancer [9,10]. The activation state of Rho GTPase is tightly regulated by Rho guanine nucleotide exchange factors (RhoGEFs), which promote the conversion of Rho to the active GTP-bound state, and Rho GTPase activating protein (RhoGAPs), an important negative regulator of the Rho signaling pathway combined with the functional domains of Rho family and converted Rho to the inactive GDP-bound state by promoting hydrolysis of the GTP enzymes bound to Rho [11]. ROCK, also known as Rho-associated kinase, is a downstream effector of Rho, and Rho signaling through ROCK promotes motility [12]. Rho GTPase activating protein 24 (ARHGAP24) is involved in the proliferation, cell cycle, apoptosis, migration, and invasion of renal cell carcinoma cells [13,14], the pseudopod formation induced by activated ARF6 in breast carcinoma cells [15], and tumor growth of glioblastomas through increasing Rac1 activity [16]. Although ARHGAP24 is downregulated in lung adenocarcinoma [17] and is associated with paclitaxel-resistant phenotype of lung cancer cells [18], its role in lung cancer progression, especially migration and invasion, remains largely unknown.

Increasing evidence has demonstrated involvement of the β-catenin pathway in migration and invasion of cancer cells, including oral squamous carcinoma [19], pancreatic cancer [20], and lung cancer [21]. It has been shown that ARHGAP7 can directly regulate β-catenin signaling to induce apoptosis and inhibit the invasion of colon cancer cells [22]. Furthermore, our bioinformatics analysis also suggested the correlation of ARHGAP24 and the β-catenin signaling pathway in lung cancer. Although the oncogenic role of the β-catenin pathway is well defined, it remains unclear how this pathway is aberrantly activated, and the correlation between β-catenin and ARHGAP24 in lung cancer is still unknown. In the present study, we observed the effect of altered ARHGAP24 expression on lung cancer cell migration and invasion and analyzed the relevant molecular mechanisms involving the β-catenin pathway and its downstream target genes.

Material and Methods

Clinical samples

The study was approved by the Ethics Committee of Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. Tumor tissues and corresponding non-tumorous normal lung tissues were harvested from 30 patients with lung cancer treated at Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, including 12 women and 18 men, ages 27 to 68 years (median, 49 years), who were in stage I (n=5), II (n=14), IIIa (n=9), or IIIb (n=2). All tissues were stored at –80°C until being analyzed. Informed written consent was obtained from all patients according to the guidelines of Ethics Committee. The protein expression of ARHGAP24 in tumor tissues and corresponding non-tumorous normal lung tissues was measured by immunohistochemical (IHC) studies, as previously described [23].

Cell culture

Human NSCLC cell lines, including A549 (non-metastatic adenocarcinoma), NCI-H1975 (non-metastatic adenocarcinoma), NCI-H292 (metastatic carcinoma), NCI-H1299 (non-metastatic large cell carcinoma), and NCI-H460 (non-metastatic large cell carcinoma), were obtained from ATCC (Manassas, VA, USA). Human bronchial epithelial (16-HBE) cells were purchased from Central South University (Changsha, China). All of the cells were grown in RMPI-1640 (HyClone, Waltham, MA, USA) containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (FBS), and incubated at 37°C in a humidified chamber with 5% CO_2.

Cell transfection

To elevate the expression of ARHGAP24, the coding sequence was synthesized using the following primers and cloned into pLVX-Puro plasmids (Clontech, USA). ARHGAP24 (NM_001025616.2): forward 5'-GCGAATTCATGGAGGAGAACAATGACT-3' (*Eco*R I); reverse 5'-CGGGATCCCTGAATCCATATTGTGTTT-3' (*Bam*H I). Underscores denote the cutting sites of *Eco*R I and *Bam*H I. RNA interference sequence targeting ARHGAP24 was synthesized and cloned into

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linearized pLKO.1 plasmids (Addgene, USA). The interference sites and corresponding primer sequences were: ARHGAP24 (NM_001025616.2, position 727-749: GATCGGATGACAG CAAATC): forward 5'-CCGGGATCGGATGACAGCAAATCCTCG AGGATTTGCTGTCATCCGATCTTTTTG-3'; reverse 5'-AATTC AAAAAGATCGGATGACAGCAAATCCTCGAGGATTTG CTGTCATCCGATC-3'. Recombinant plasmids together with the packaging plasmids psPAX2 and pMD2G were co-transfected into 293T cells using Lipofectamine 2000 (Invitrogen, USA). At 48 h after transfection, recombined lentivirus vectors were collected and used for infecting cells. Cells with pLKO.1-scramble shRNA or blank pLVX-Puro transfection were used as negative control.

Transwell assay

Transwell inserts (Corning, 3422) were used to detect cell migration and invasion. A549 cells were seeded into 6-well plates, transfected with pLVX-Puro-ARHGAP24 for 6 to 8 h, and serum-starved in complete RPMI-1640 medium for 24 h. NCI-H1975 cells were seeded into 6-well plates, transfected with pLKO.1-ARHGAP24-shRNA for 6 to 8 h, and serum-starved in complete RPMI-1640 medium for 24 h with or without 10 μM XAV-939. Then, the cells were digested and resuspended in serum-free RPMI-1640 medium. For invasion assay, 80 μL of matrigel (Corning, 356234) was added to the upper chamber in advance. We added 200 μL of cell suspension containing 6×10^4 cells to each insert, and different groups were divided according the treatment. We added 700 μL of complete RPMI-1640 medium to the lower chamber. After culturing for 48 h at 37°C, the inserts were doused with 4% paraformaldehyde and 0.1% crystal violet solution successively. Cells were counted in 5 different fields under a microscope (CX41RF; Olympus Corporation, Tokyo, Japan) with magnification of 200.

Wound healing assay

A549 and NCI-H1975 cells were seeded in 35-mm tissue culture dishes at a density of 8×10^5 and further seeded until they reached 100% confluence. Then, the confluence cultures were scratched using a pipette tip. After scratching, the well was gently washed twice with medium to remove the detached cells. Scratched cultures were photographed under a microscope at 0 and 48 h. Migration of cells was established by measuring the width of the scratched area at each time point in the scratched area at a magnification of ×200.

Real-time PCR analysis

Total RNA from lung cancer cell lines was extracted using the RNeasy Plus Mini Kit (Qiagen, Germany) and reversely transcribed using the TaqMan reverse transcription kit (Applied Biosystems, USA). Real-time PCR was performed using the SYBR Green qRT-PCR kit (Promega, USA) on an ABI7500 system following the manufacturer's instructions. The primers used were: ARHGAP24-F, 5'-AACTCCTGTCGCTCTTCTACC-3' and ARHGAP24-R, 5'-GCTGTTGCCCACAAATGTCTC-3'; GAPDH-F, 5'-CACCCACTCCTCCACCTTTG-3' and GAPDH-R, 5'-CCACCACCCTGTTGCTGTAG-3'. Quantification of relative expression was normalized using GAPDH expression values and calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blotting

Samples were treated with RIPA Lysis Buffer (Solarbio, Beijing, China) to extract the total protein. The proteins were quantified and stored at –20°C before use. We prepared 10% sodium dodecyl sulfate polyacrylamide gel to isolate the proteins. After transfer to nitrocellulose membrane, the bands were blocked with 5% non-fat milk. Then, the corresponding primary antibodies and secondary antibodies were diluted to appropriate concentrations and added to the protein bands, respectively. Finally, the protein bands were scanned with Tanon 5200 (Tanon, Shanghai, China). Integrated density value was used to calculate the relative protein quantity. The antibodies and reagents used were: ARHGAP24 (Abcam, ab203874, 1: 500); MMP9 (Abcam, ab76003, 1: 1000); VEGF (Abcam, ab69479, 1: 1000); Vimentin (Cell Signaling Technology, #5471, 1: 1000); E-cadherin (Cell Signaling Technology, #14472, 1: 1000); β-catenin (Abcam, ab32572, 1: 5000); GAPDH (Cell Signaling Technology, #5174, 1: 2000); HRP-labeled Goat Anti-Rabbit IgG (Beyotime, A0208, 1: 1000); HRP-labeled Donkey Anti-Goat IgG (Beyotime, A0181, 1: 1000); HRP-labeled Goat Anti-Mouse IgG (Beyotime, A0216, 1: 1000).

Plasmid construction and dual luciferase assay

A549 and NCI-H1975 cells (5×10^5 cell/well) were seeded in 6-well plates, cultured in an incubator with 5% CO_2 at 37°C for 24 h, and then co-transfected with 1.5 μg pGL3-Enhancer plasmid containing β-catenin promoter, 5 μL pLVX-Puro-ARHGAP24, pLKO.1-ARHGAP24-shRNA, or 20 ng pRL-TK as a negative control at 37°C for 6 h using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol. At 48 h after transfection, 100 μL luciferase assay reagent and 10 μL Stop&Glo reagent were added to cells. Luciferase activity (Firefly and Renilla) was measured with the Dual Luciferase Reporter assay system (Promega) according to the manufacturer's protocol.

Statistical analyses

All the results are presented as mean ±SD, and all experiments and measurements were performed at least in triplicate. All statistical analyses were carried out with GraphPad Prism software using one-way analysis of variance followed by Tukey's post hoc test. P values of less than 0.05 were considered to show a significant difference between 2 groups.

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Results

ARHGAP24 expression in lung cancer tissues and cell lines

To investigate the role of ARHGAP24 in lung cancer tumorigenesis, the expression of ARHGAP24 in lung cancer tissues and cell lines A549, NCI-H1299, NCI-H292, NCI-H1975, and NCI-H460 was measured by immunohistochemistry and Western blot analysis. As shown in Figure 1A and 1B, ARHGAP24 expression was downregulated in NSCLC tissues compared with the corresponding normal lung tissues, with the lowest expression detected in NSCLC tissues at stage III. ARHGAP24 expression was also downregulated in lung cancer cell lines compared with human bronchial epithelial (16-HBE) cells (Figure 1C). Moreover, ARHGAP24 was highly expressed in NCI-H1975 cells and expressed at low levels in A549 cells compared with other lung cancer cell lines.

ARHGAP24 overexpression inhibits A549 cell migration and invasion

In view of the ARHGAP24 expression in the 5 lung cancer cell lines, A549 cells were transfected with pLVX-Puro-ARHGAP24 to overexpress ARHGAP24, and NCI-H1975 cells were transfected with pLKO.1-ARHGAP24-shRNA to silence ARHGAP24. As shown in Figure 2A–2C, pLVX-Puro-ARHGAP24 transfection in A549 cells significantly increased the ARHGAP24 mRNA expression by 53.9-fold and the protein expression by 1.21-fold, compared with blank pLVX-Puro vector transfection.

To demonstrate the function of ARHGAP24 in lung cancer cell motility, cell migration and invasion of A549 cells after pLVX-Puro-ARHGAP24 transfection was also measured. We found that pLVX-Puro-ARHGAP24 transfection in A549 cells significantly inhibited cell migration and cell invasion by 57.2% and 46.1%, respectively, compared with the blank pLVX-Puro vector transfection (Figure 2D–2G). Moreover, the *in vitro* would healing assay also demonstrated that pLVX-Puro-ARHGAP24 transfection showed decreased migration ability compared with the blank pLVX-Puro vector transfection (Figure 3A).

ARHGAP24 overexpression inhibits MMP9, VEGF, and β-catenin expression in A549 cells

Changes in migration- and invasion-related proteins were also measured in A549 cells after pLVX-Puro-ARHGAP24 transfection. As shown in Figure 3B and 3C, pLVX-Puro-ARHGAP24 transfection in A549 cells significantly inhibited the levels of MMP9, VEGF, Vimentin, and β-catenin, but increased E-cadherin protein expression compared with the blank pLVX-Puro vector transfection. These results suggest that ARHGAP24 plays an anti-migratory and anti-invasive role in lung cancer cells.

ARHGAP24 silencing promotes NCI-H1975 cell migration and invasion

To confirm our hypothesis, the cell migration and invasion of NCI-H1975 cells after pLKO.1-ARHGAP24-shRNA transfection was also measured. We found that pLKO.1-ARHGAP24-shRNA transfection in NCI-H1975 cells significantly decreased the ARHGAP24 mRNA expression by 75.7% and protein expression by 56.2% compared with pLKO.1-scramble shRNA transfection (Figure 4A–4C). pLKO.1-ARHGAP24-shRNA transfection in NCI-H1975 cells significantly promoted the cell migration and the cell invasion by 29.1% and 34.8%, respectively, compared with pLKO.1-scramble shRNA transfection (Figure 4D–4G). The *in vitro* would healing assay also demonstrated that pLKO.1-ARHGAP24-shRNA transfection showed increased migration ability compared with the pLKO.1-scramble shRNA transfection (Figure 5A). Moreover, pLKO.1-ARHGAP24-shRNA transfection in NCI-H1975 cells significantly decreased E-cadherin and promoted the MMP9, VEGF, Vimentin, and β-catenin protein expression compared with the pLKO.1-scramble shRNA transfection (Figure 5B, 5C). These results confirm that ARHGAP24 can mediate the migration and invasion of lung cancer cells through regulating E-cadherin, Vimentin, MMP9, VEGF, and β-catenin expression.

Treatment with β-catenin inhibitor XAV-939 inhibits the migration and invasion of NCI-H1975 cells

β-catenin signaling has been previously found to be involved in regulation of the cancer cell migration and invasion, as well as MMP9, VEGF, Vimentin, and E-cadherin expression [24–27]. Therefore, the β-catenin inhibitor XAV-939 was introduced to investigate the role of β-catenin in ARHGAP24-mediated the migration and invasion of lung cancer cells. We found that 10 μM XAV-939 treatment in NCI-H1975 cells with pLKO.1-scramble shRNA transfection significantly inhibited the migration and invasion by 56.7% and 73.0%, respectively, compared with NCI-H1975 cells with only pLKO.1-scramble shRNA transfection (Figure 4D–4G). Importantly, 10 μM XAV-939 treatment in NCI-H1975 cells with pLKO.1-ARHGAP24-shRNA transfection significantly inhibited the migration and invasion by 45.0% and 48.0%, respectively, compared with that in NCI-H1975 cells with only pLKO.1-ARHGAP24-shRNA transfection (Figure 4D–4G). A similar effect was also found in the *in vitro* would healing assay (Figure 5A). Moreover, the expression of MMP9, VEGF, Vimentin, and β-catenin was also decreased by 10 μM XAV-939 treatment in NCI-H1975 cells with pLKO.1-scramble shRNA or pLKO.1-ARHGAP24-shRNA transfection (Figure 5B, 5C). These findings indicate that ARHGAP24 silencing can promote lung cancer cell migration and invasion through activating β-catenin signaling.

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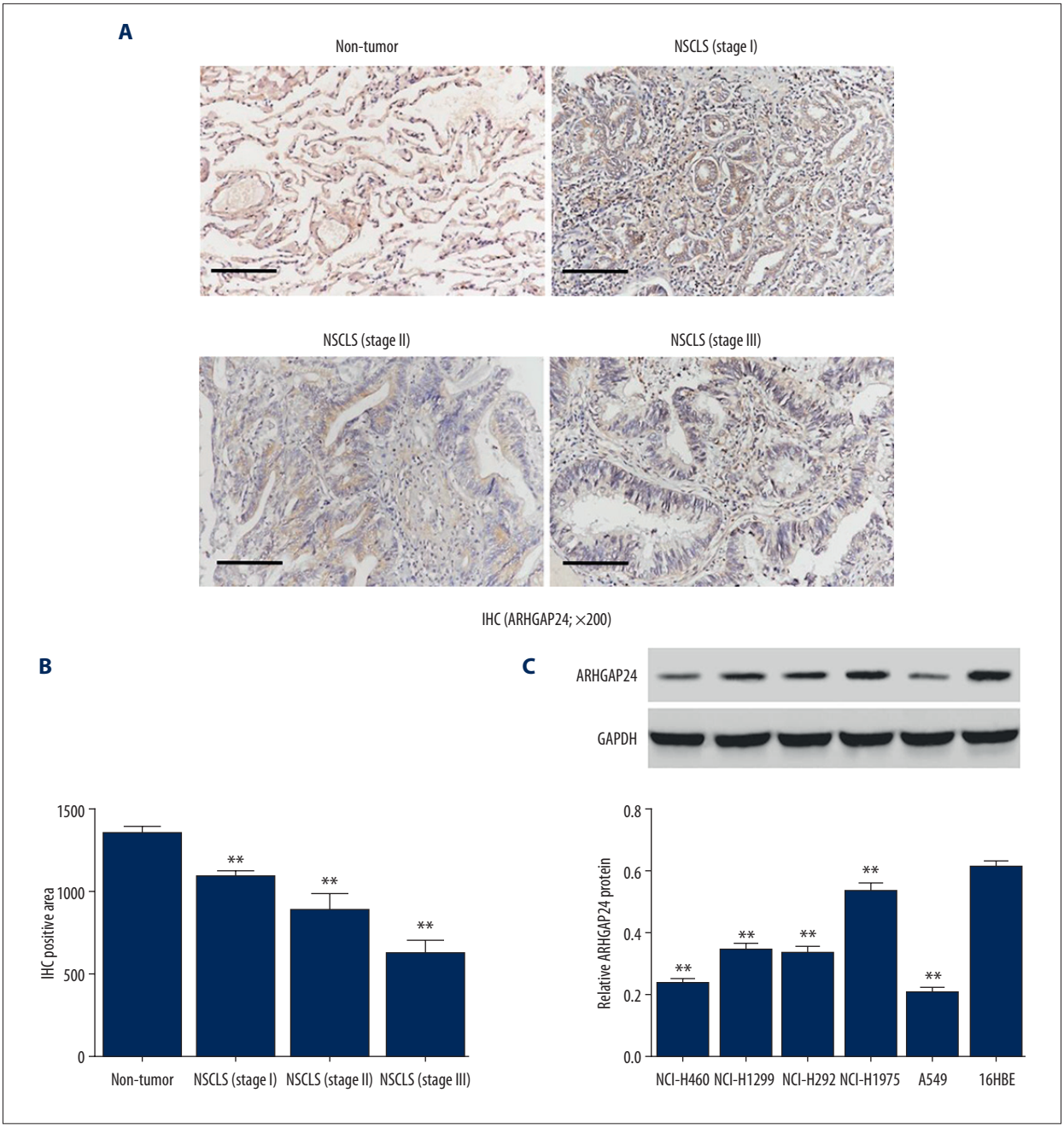

Figure 1. ARHGAP24 expression in lung cancer tissues and cell lines. The protein expression of ARHGAP24 in lung cancer tissues from patients with NSCLC and in cell lines was measured by immunohistochemistry (**A, B**) and Western blot analysis (**C**). Scale bar: 100 μm.

ARHGAP24 regulates the transcriptional activity of β-catenin

To examine the direct interaction between β-catenin and ARHGAP24, the β-catenin transcriptional activity in ARHGAP24 modulated A549 and NCI-H1975 cells was measured by dual luciferase assay, demonstrating that ARHGAP24 overexpression decreased the β-catenin transcriptional activity in A549 cells, and ARHGAP24 silencing increased the β-catenin transcriptional activity in NCI-H1975 cells (Figure 6A, 6B).

Discussion

The occurrence of lung cancer may be associated with accumulative gene damage. Studies have shown that canceration

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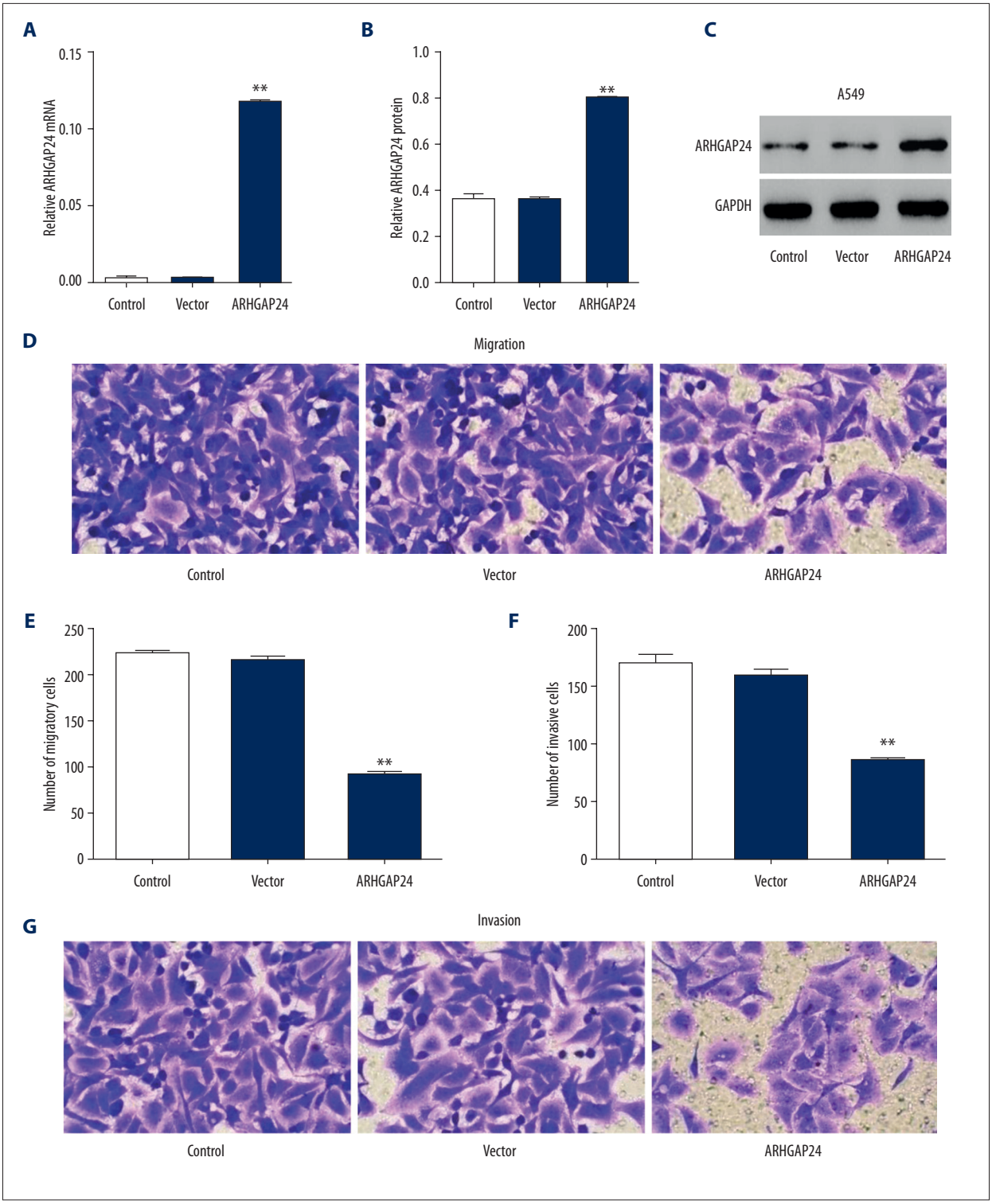

Figure 2. ARHGAP24 overexpression inhibits A549 cell migration and invasion. ARHGAP24 expression in A549 cells with blank pLVX-Puro or pLVX-Puro-ARHGAP24 transfection (**A–C**) was measured by real-time PCR and Western blotting, respectively. The cell migration (**D, E**) and invasion (**F, G**) were measured by Transwell analysis. ** P<0.01 compared with vector.

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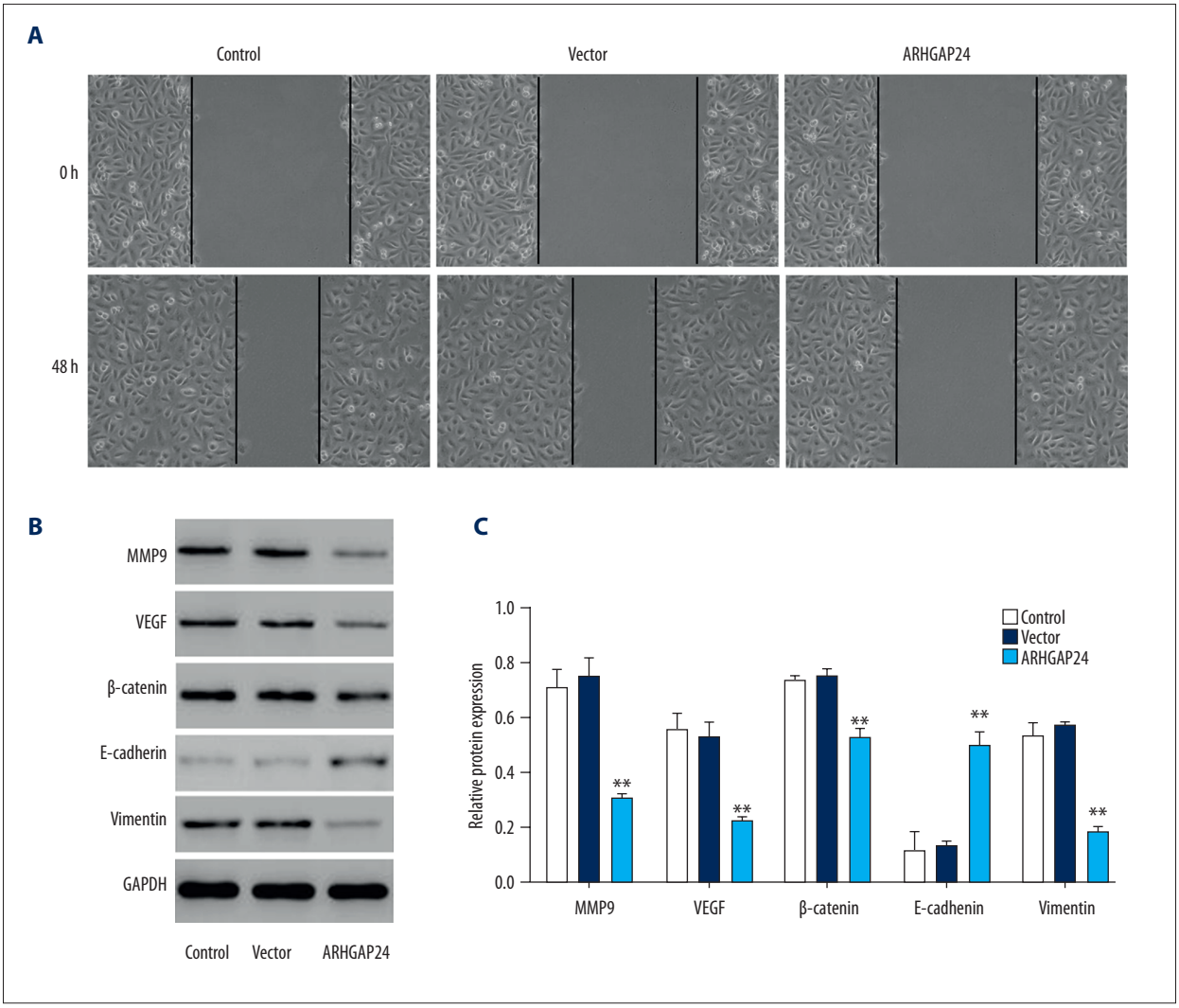

Figure 3. ARHGAP24 overexpression inhibits MMP9, VEGF, Vimentin, E-cadherin, and β-catenin expression in A549 cells. After A549 cells were subjected to blank pLVX-Puro or pLVX-Puro-ARHGAP24 transfection, the migration was assessed in *in vitro* would healing assay (**A**), and protein expression of MMP9, VEGF, Vimentin, E-cadherin, and β-catenin of A549 cells was measured by Western blot analysis (**B, C**). ** P<0.01 compared with vector.

of normal lung tissue or the deterioration of lung cancer tissue is a multistep and multigene process, in which oncogenes are activated and tumor-suppressor genes are inactivated [28,29]. Gene mutation is involved in the occurrence, development, and metastasis of cancer. Therefore, gene therapy may play an important role in cancer treatment. Recently, the relationship between tumorigenesis and RhoGAPs family has been elucidated by evidence that RhoGAPs play critical roles in gene expression, cell proliferation, apoptosis, cell cycle procession, angiogenesis, migration, invasion, and adhesion. In this study, ARHGAP24 showed anti-migratory and anti-invasive effects on lung cancer cells, which was associated with the β-catenin signaling pathway.

The role of Rho GTPase activating protein varies greatly in tumorigenesis. ARHGAP15, ARHGAP10, and ARHGAP6 overexpression suppress the tumorigenesis of glioma, ovarian cancer, and cervical carcinoma through interacting with Rac1, Cdc42, and Rac3, respectively [30–32]. However, ARHGAP18 downregulation inhibits cell migration of triple-negative breast cancer by activating RhoA [33], and ARHGAP11 inhibition in colon cancer attenuates cell mobility through enhancing Rac1 activity [34]. In the present study, ARHGAP24 overexpression in A549 cells markedly suppressed migration and invasion, while ARHGAP24 silencing in NCI-H1975 cells significantly promoted migration and invasion. In line with our findings, previous studies have reported that ARHGAP24 overexpression attenuates invasion and migration of renal cell carcinoma cells, while ARHGAP24 knockdown showed negative effects [13,14].

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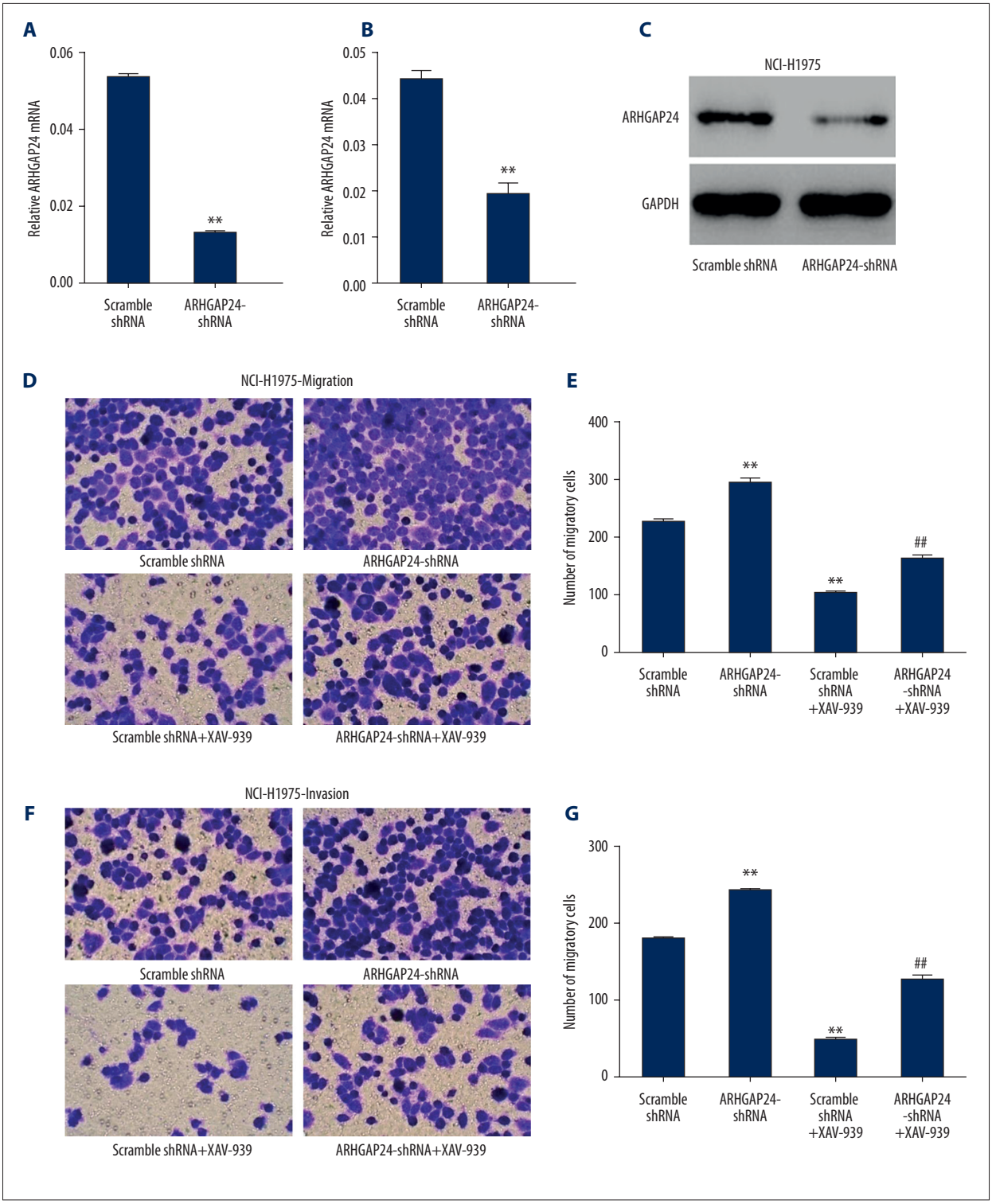

Figure 4. ARHGAP24 silencing promotes NCI-H1975 cell migration and invasion through activating β-catenin signaling. ARHGAP24 expression in NCI-H1975 cells with pLKO.1-scramble shRNA or pLKO.1-ARHGAP24-shRNA transfection (**A–C**) was measured by real-time PCR and Western blotting, respectively. The cell migration (**D, E**) and invasion (**F, G**) of NCI-H1975 cells with blank pLVX-Puro or pLVX-Puro-ARHGAP24 transfection in the absence or presence of 10 μM XAV-939 treatment were measured by Transwell analysis. ** P<0.01 compared with scramble shRNA. ## P<0.01 compared with ARHGAP24-shRNA.

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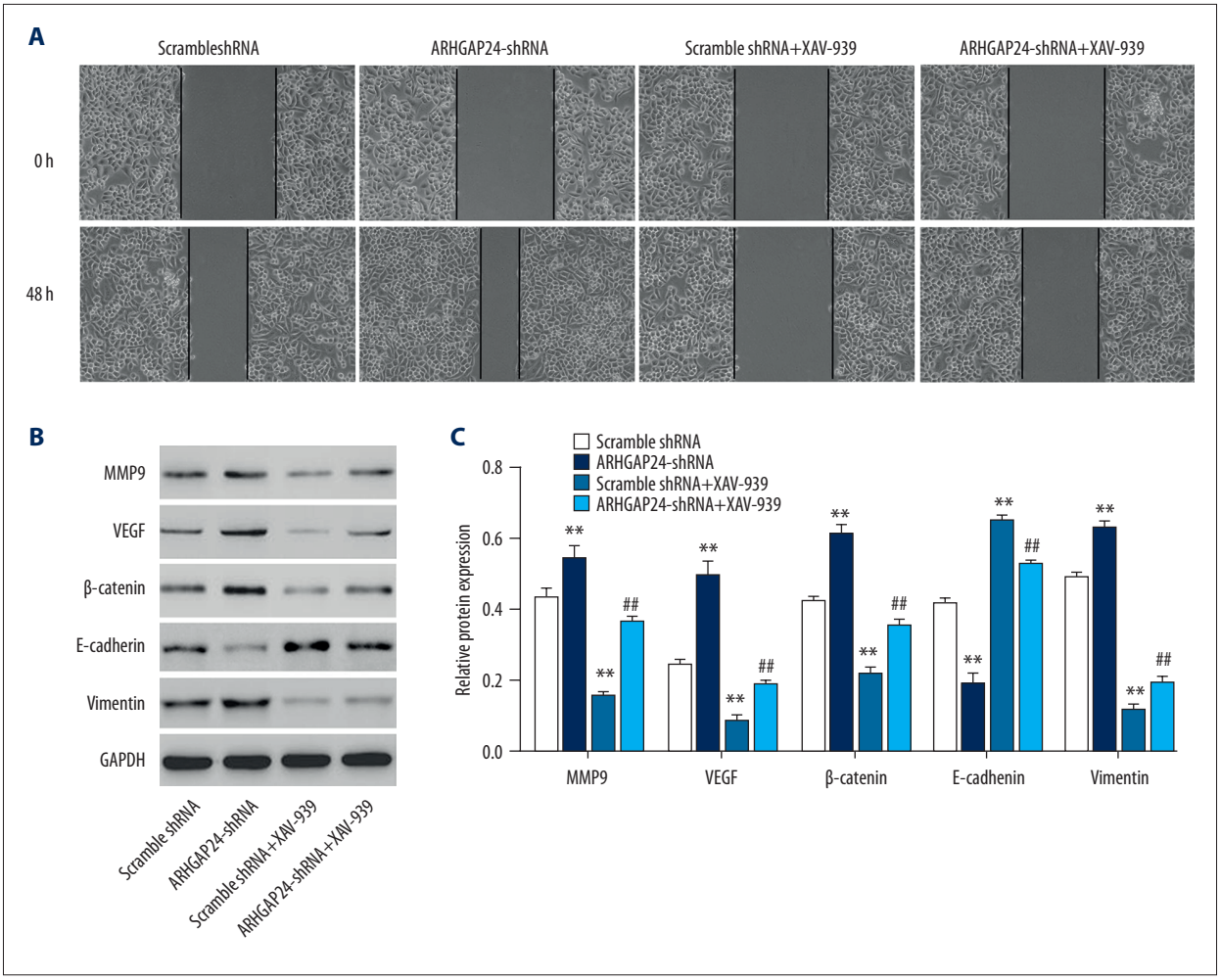

Figure 5. ARHGAP24 silencing promotes MMP9, VEGF, Vimentin, E-cadherin, and β-catenin expression in NCI-H1975 cells. The migration was assessed in *in vitro* would healing assay (**A**), and the protein expression of MMP9, VEGF, Vimentin, E-cadherin, and β-catenin in NCI-H1975 cells with blank pLVX-Puro or pLVX-Puro-ARHGAP24 transfection in the absence or presence of 10 μM XAV-939 treatment was measured by Western blot analysis (**B, C**). ** P<0.01 compared with scramble shRNA. ## P<0.01 compared with ARHGAP24-shRNA.

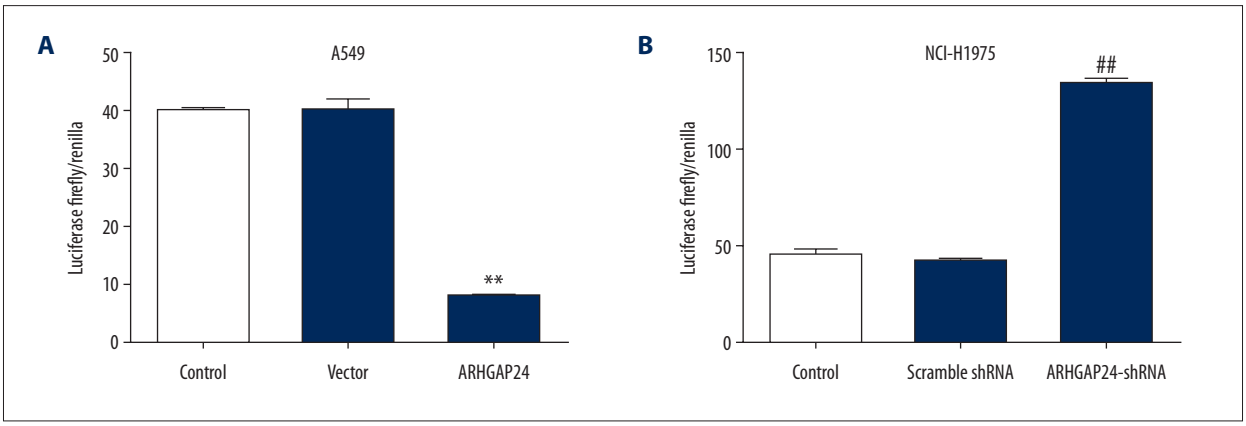

Figure 6. ARHGAP24 regulates the transcriptional activity of β-catenin. A549 (**A**) and NCI-H1975 (**B**) cells were transfected with pLVX-Puro-ARHGAP24 or pLKO.1-ARHGAP24-shRNA, and the transcription activity of β-catenin was measured by dual luciferase assay. ** P<0.01 compared with vector. ## P<0.01 compared with scramble shRNA.

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β-catenin is the core of the Wnt/β-catenin signaling pathway. β-catenin lacks cytoplasm and nucleus, and only a few stable β-catenin exists in cell adhesion connection. In the absence of external Wnt signal stimulation, β-catenin as an important regulatory protein combines with E-cadherin receptor and cytoskeletal binding protein α-catenin in cell plasma [35]. When the Wnt signal is stimulated, β-catenin accumulates in the cytoplasm, enters the nucleus, and triggers the transcriptional expression of the target gene [36]. A previous study has shown that the abnormal expression of β-catenin was related to the occurrence and development of the lung cancer, as well as invasion and metastasis [37], which is consistent with our results that inactivation of β-catenin signaling by treatment of XAV-939 significantly inhibited ARHGAP24 silencing-induced cell migration and invasion. Importantly, ARHGAP24 silencing increased the β-catenin transcriptional activity, and ARHGAP24 overexpression decreased the β-catenin transcriptional activity. These results suggest that β-catenin signaling is involved in ARHGAP24-mediated lung cancer cell migration and invasion. In agreement with the correlation of Rho GTPase activating protein and β-catenin in tumorigenesis, other research also demonstrated that ARHGAP7 may act as a tumor-suppressor gene in colon cancer through suppressing β-catenin and c-Myc expression [22].

Wnt/β-catenin signaling pathway agonist obviously abolished cell mobility, along with the decreased expression of MMP9, VEGF, Vimentin, and E-cadherin [24–27]. XAV-939 is a potential β-catenin signaling inhibitor selectively inhibiting Wnt/β-catenin-mediated transcription through tankyrase1/2 inhibition. In the present study, XAV-939 treatment also inhibited the expression of β-catenin signaling pathway-related downstream target genes, including MMP9, VEGF, Vimentin, and E-cadherin. MMP9 and VEGF have critical roles in angiogenesis, invasion and metastasis of cancer cells. Decreased migration and invasion of A549 cells was associated with the inhibition of MMP9 and VEGF [38]. Similar to our findings, XAV-939 also inhibited the cell invasion and metastasis of colon and endometrial cancer through inhibiting the expression of β-catenin-related downstream target genes, including c-Myc, CyclinD1, N-cadherin, and Vimentin expression [39,40].

Conclusions

Our results show for the first time the important role of ARHGAP24 in lung cancer cell migration and invasion. ARHGAP24 silencing promotes lung cancer cell migration and invasion through activating β-catenin signaling. Our findings may contribute the theoretical understanding of the pathogenesis of lung cancer and aid development of more effective treatment.

Conflict of interest

None.

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