

RESEARCH

Open Access



SOX9 promotes the invasion and migration of lung adenocarcinoma cells by activating the RAP1 signaling pathway

Jun-fa Yang¹, Qing Liao¹ and Chen-lin Lu^{1*}

Abstract

Objective SOX9 has been shown to be related to the metastasis of various cancers. Recently, it has been reported that SOX9 plays a regulatory role in lung adenocarcinoma (LUAD) cell metastasis, but the specific mechanism remains to be explored. Therefore, the objective of this study was to observe the effect and mechanism of SOX9 on the invasion and migration of LUAD cells.

Methods RT-qPCR was applied to observe the expression of SOX9 and RAP1 in tumor tissues and corresponding normal lung tissues collected from LUAD patients. Co-immunoprecipitation and Pearson correlation to analyze the expression correlation of SOX9 with RAP1. To observe the role of SOX9, the invasion and migration levels of LUAD A549 cells in each group were observed by Transwell invasion assay and Scratch migration assay after knocking down or overexpressing SOX9. Besides, the expression levels of RAP1 pathway-related proteins (RAP1, RAP1GAP and RasGRP33) were observed by RT-qPCR or western blot. Subsequently, RAP1 was overexpressed and SOX9 was knocked down in A549 cells, and then the cell invasion/migration level and RAP1 pathway activity were assessed.

Results The expression levels of SOX9 and RAP1 in tumor tissues and A549 cells of LUAD patients were significantly increased and positively correlated. Overexpression of SOX9 or RAP1 alone in A549 cells enhanced the invasion and migration ability of cells, as well as up-regulated the expression levels of RAP1, RAP1GAP and RasGRP33. However, knocking down SOX9 decreased cell invasion and migration levels and weakened the activity of RAP1 pathway. Notably, overexpressing RAP1 while knocking down SOX9 significantly activated RAP1 pathway and promoted cell invasion and migration.

Conclusion Overexpression of SOX9 in LUAD can significantly activate the RAP1 signaling pathway and promote cell invasion and migration.

Keywords Lung adenocarcinoma (LUAD) cell, SOX9, RAP1 signaling pathway, Invasion, Migration

*Correspondence:

Chen-lin Lu
luchenlin@yeah.net

¹Department of Respiratory and Critical Care Medicine, Taizhou School of Clinical Medicine, The Affiliated Taizhou People's Hospital of Nanjing Medical University, Nanjing Medical University, Taizhou 225300, Jiangsu, China



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Introduction

Lung cancer is a well-known fatal malignancy and the main cause of cancer-related death [1]. Currently, lung cancer has become the second most prevalent cancer in both men and women, accounting for 25% of all cancer-related deaths [2]. More terribly, the incidence of lung cancer still remains high. In 2020, 220,600 new cases of lung cancer and 179,600 deaths were reported worldwide [3]. Moreover, the survival rate of lung cancer patients is not high because they are diagnosed at an advanced stage. During 2007–2013, the 5-year survival rate of lung cancer patients was only 18% [4]. There are various types of lung cancer, such as non-small cell lung cancer (NSCLC) that accounts for about 85% of all lung cancers. Lung adenocarcinoma (LUAD) is the most common histological subtype of NSCLC [5]. At present, LUAD treatments mainly include surgical resection, chemotherapy, radiation therapy and immunotherapy. Despite advances in these treatments, the overall survival rate (OS) nor the mortality rate of LUAD has been improved, mainly owing to the lack of effective therapeutic targets [6]. Furthermore, it cannot be ignored that surgical resection is the only cure for LUAD, and that LUAD cannot be treated if distant metastasis has taken place [7]. Therefore, it is urgent to reveal the molecular mechanism of LUAD metastasis and identify effective biomarkers and therapeutic targets.

SOX9, belonging to the SOX group, has three domains [8], including a DNA binding domain with a high mobility group box [9] and a transactivation domain that plays a crucial role in embryonic development [10]. Studies have shown that SOX9 contributes significantly to cell lineage limitation and terminal differentiation through specific time and space expression patterns [8]. According to a recent study, the imbalance of SOX9 expression is a driving factor for the occurrence and development of cancer and a boosting factor for the malignant growth of cells under different conditions [11]. But whether SOX9 functions specifically as an oncogene [12] or a tumor suppressor [13] is a matter of some debate. Some studies have pointed out that the expression level of SOX9 is correlated with tumor invasion, metastasis and patient survival [14, 15]. Interestingly, Huang et al. discovered that SOX9 overexpression was not only significantly associated with tumor (T, $P=0.03$), node (N, $P=0.000$), and metastasis (M, $P=0.032$) categories in clinical samples of NSCLC, but also significantly promoted the migration, invasion and epithelial-mesenchymal transition (EMT) of NSCLC cells [16]. Besides, Pei et al. discovered that the long non-coding RNA rhabdomyosarcoma 2-associated transcript (lncRNA-RMST) could promote the ubiquitination of SOX9 protein, thereby inhibiting the growth and metastasis of LUAD [17]. Current investigations have revealed the importance of SOX9 in the development

of LUAD. For example, Capaccione et al. proposed that SOX9 may be used as a hub to mediate crosstalk across key carcinogenic pathways in LUAD [18]. However, the mechanism by which SOX9 regulates the progress of LUAD has not been fully uncovered. In addition, RAP1 pathway is considered as a crucial signaling pathway for regulating tumor metastasis [19]. The relevance of this pathway in LUAD has, however, received relatively little research, and only some studies have mentioned that the target gene of exosomes in pleural effusion of patients with LUAD may be related to RAP1 [20]. The relationship between SOX9 and RAP1 is not clear, either. Therefore, this study made an attempt to explore the regulatory correlation between SOX9 and the RAP1 signaling pathway as well as their effects on LUAD metastasis, thereby providing new insights for revealing the mechanism of LUAD metastasis.

Materials and methods

Clinical sample collection

A total of 40 samples including tumor tissue samples (Tumor group 20) and matched normal lung tissue (Normal group 20) samples were collected from LUAD patients who received histopathology diagnosis in The Affiliated Taizhou People's Hospital of Nanjing Medical University, Taizhou School of Clinical Medicine, Nanjing Medical University from December 2021 to January 2023 and then stored at -80°C . The inclusion criteria were as follows: patients (1) diagnosed as LUAD pathologically; (2) aged from 18 to 75 years old; (3) who never received any treatment before participating in this study; (4) who volunteered to participate in this study; (5) informed of the contents of this study and providing written consent. The exclusion criteria: patients (1) participating in other clinical studies at the same time; (2) with any other cancers in the past 5 years or diagnosed during screening; (3) with active brain metastases [21]. This study was conducted according to the Declaration of Helsinki and clinical practice guidelines, and the agreement was given by the local ethics committees of the participating centers.

Cell culture and treatment

Both human LUADA549 cells and human normal lung epithelium BEAS-2B cells were purchased from National Collection of Authenticated Cell Cultures. BEAS-2B and A549 cells were separately cultured in a Dulbecco's Modified Eagle Medium (DMEM) and a Roswell Park Memorial Institute (RPMI) 1640 Medium at 5% CO_2 under 37°C , both of which contained 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Subsequently, pCMVp-NEO-BAN vector with SOX9 and RAP1 overexpression and shRNA-SOX9 expression was transfected into A549 cells (SOX9, RAP1 and sh-SOX9) by FuGENE[®] 6 transfection reagent (E2691, promega, USA). Besides,

A549 cells were transfected with shRNA-SOX9 and RAP1 overexpression vectors. After 72 h of cell culture under conditions of 37 °C and 5% CO₂, the next experiment was performed. In this study, shRNA-SOX9 vector and its negative control vector (sh-NC), and SOX9 overexpression vector and its negative control vector were all synthesized by Guangzhou Tsingke Biotech Co., Ltd.

RT-qPCR

The separation of total RNA from lung tissue samples was carried out under the instructions of the RNeasy Pure Tissue Kit (DP431, TIANGEN). As for the RNA extraction from A549 cells, the transfected cells were placed on ice at first, then the cell culture solution was removed, the cells were washed three times using pre-cooled PBS, and finally the total RNA of cells was isolated and purified by RNA Easy Fast Kit (DP451, TIANGEN). The concentration of RNA was determined by NanoDrop spectrophotometers (840-317400, ThermoFisher, USA). Upon diluting the RNA concentration to 500 ng/μL, 2 μg of RNA was reversely transcribed into complementary deoxyribonucleic acid (cDNA) according to the instructions of FastKing cDNA First Chain Synthesis Kit (KR116, TIANGEN). Finally, the expression levels of SOX9, RAP1 and GAPDH (an internal control) in tissue or cell samples were detected by RealUniversal Color PreMix (FP201, TIANGEN). Based on the acquired data, the relative expression of the gene was calculated using 2^{-ΔΔCt} method. The primer sequences shown in Table 1 were synthesized by Sangon Biotech (Shanghai) Co., Ltd.

Transwell invasion assay

Transwell invasion assay was adopted to observe the invasion of cells in each group. Firstly, the matrix gel and the culture medium were fully mixed and coated in the upper chamber. Then, A549 cells were seeded in a 6-well plate at a density of 5×10⁶ cells/well. After complete adherence to the wall, the cells were subjected to a 72-h transfection. Next, the cells were resuspended with trypsin, and then introduced in the upper chamber at a density of 5×10⁵ cells/well. And the culture medium containing 10% FBS was added into the lower chamber. Following 24 h of cell invasion, the lower chamber was fixed with 4% paraformaldehyde and then stained with 0.05% crystal violet. Images of cell invasion were captured by a

microscope, a representative area was selected for photographing, and the IMAGE-J software was employed for quantification. Finally, cell invasion in each group was observed.

Scratch migration assay

The migration of A549 cells in different groups was measured by scratch migration assay in vitro. After transfection for 72 h, A549 cells were collected and seeded into 6-well plates at a density of 5×10⁶ cells/well. An overnight adherence was allowed for the cell confluence of 80%. Monolayer cells were scratched vertically with a 10μL pipette tip, and the dropped cells were gently washed with PBS. Next, the wound healing image was captured by a Nikon inverted microscope at the specified time point. The gap width was calculated using GraphPad Prism software, the healing area was measured, and the cell movement was calculated according to the healing percentage.

Western blot

After transfection, A549 cells were collected and the culture medium was removed. Next, the cells were washed with pre-cooled PBS for three times. Following that, the cells were supplemented with 1 ml of RIPA buffer (Beyotime) containing protease inhibitors, and then transferred into a centrifuge tube. Subsequently, the cells were ultrasonically broken for 3 min under the conditions of a 3-s stop for every 2-s work. Subsequent to a 10-min cell centrifugation at 4 °C and 12,000 g, the concentration of total protein in cells of each group was determined by a BCA protein assay kit (Beyotime). Next, 20 μg of total protein was mixed with 5× SDS loading buffer and then boiled for 10 min. Later, the protein was separated by 10% SDS-PAGE, and the target protein was transferred to polyvinylidene fluoride (PVDF) membranes by electrotransfer. Following a 2-h incubation with 5% skim milk, the target protein was incubated with primary antibodies at 4 °C overnight. The used primary antibodies included RAP1 (1:1000, ab272863, Abcam), RAP1GAP (1:1000, ab32373, Abcam), RasGRP3 (1:1000, ab124823, Abcam). Then, the membranes were incubated with goat anti-rabbit or mouse secondary antibody for 1 h. The enhanced chemiluminescence (SuperSignal ECL, ThermoFisher) was applied to develop proteins in the membranes. The expression level of each protein was observed through Gel Doc XR+Gel Documentation System (Bio-red, USA).

Co-immunoprecipitation (CO-IP)

RIPA buffer (Beyotime Biotechnology) containing a protease inhibitor (Roche Applied Science, USA) was added to the transfected A549 cells, which were later placed on a low-speed rotating shaker at 4 °C for a 30-min lysis.

Table 1 Primer sequences

Gene name	Sequences (5' to 3')
SOX9	F: AGGAAGCTCGCGACCACTAC R: GGTGGTCTTCTTGCTGCAC
RAP1	F: ACTTACAGGACCTGAGGGAACAG R: CCTGCTCTTTGCCAACTACTCG
GAPDH	F: GTCTCTCTGACTTCAACAGCG R: ACCACCCTGTTGCTGTAGCCAA

The lysate was then centrifuged at 12,000 rpm at 4 °C for 10 min. After the centrifugation, the supernatant was collected and part of the lysate was removed as an input sample. The remaining supernatant were shaken with specific primary antibody overnight on a rotating shaker at 4 °C. Protein A/G plus-agarose (sc-2003, Santa-Cruz Biotechnology, USA) was then added to the mixture and incubated in a rotating shaker at 4 °C for 6 h. Finally, immunoprecipitates and whole cell lysates were collected for western blot analysis.

Statistics and analysis

SPSS16.0 software was employed for data analysis, and GraphPad Prism 9.2.0 software for drawing. Variance analysis was adopted for comparisons between groups, t test for two independent samples, and one-way ANOVA for more than two independent samples. Additionally, Pearson analysis was performed to clarify the correlation between SOX9 and RAP1 expression in clinical samples. $P < 0.05$ indicated a significant difference.

Results

Up-regulation of SOX9 expression in lung adenocarcinoma

The change of gene expression is one of the factors that affect the development of diseases. Therefore, the expression changes of SOX9 in LUAD were first observed in this study. Based on the RT-qPCR results, it was evident that the SOX9 expression level was significantly increased in the Tumor group compared with that in the Normal group in the collected clinical samples (Fig. 1A, $P < 0.05$). Notably, the SOX9 expression level was also up-regulated in human LUAD A549 cells relative to that in human normal lung epithelium BEAS-2B cells (Fig. 1B, $P < 0.05$). Therefore, changes in the SOX9 expression level may play a key role in the development of LUAD.

Knockdown of SOX9 inhibits A549 cell invasion and migration

To explore the role of SOX9 in LUAD, changes in cell invasion and migration were observed after knocking down or overexpressing SOX9 in A549 cells. The results of RT-qPCR showed that knocking down SOX9 could significantly reduce the expression level of SOX9 in A549 cells in contrast to the sh-NC group. Moreover, compared with the Vector group, the overexpression vector of SOX9 could significantly raise the expression level of SOX9 in cells (Fig. 2A, $P < 0.05$). Subsequently, the invasion and migration ability of cells were observed by Transwell and Scratch assays. The assay results showed that in comparison with the sh-NC group, the invasion and migration ability of A549 cells in the sh-SOX9 group were weakened (Fig. 2B-C, $P < 0.05$). However, compared with the Vector group, the invasion and migration ability of A549 cells in the SOX9 group were enhanced (Fig. 2B-C, $P < 0.05$). In short, suppression of SOX9 may be a potential strategy to suppress LUAD metastasis.

Up-regulation of SOX9 in lung adenocarcinoma activates the RAP1 signaling pathway

To reveal the mechanism by which SOX9 controls LUAD metastasis, the expression level of RAP1 was observed in clinical samples. The observation results demonstrated a much higher expression level of RAP1 in the Tumor group compared with the Normal group (Fig. 3A, $P < 0.05$). Then, according to Pearson correlation analysis, the expression of RAP1 was positively correlated that of SOX9 in LUAD tissues (Fig. 3B, $P = 0.004$). The CO-IP results further indicated the interaction between RAP1 and SOX9 (Fig. 3C). Relative to BEAS-2B cells, the expression level of RAP1 was up-regulated in A549 cells (Fig. 3D-E, $P < 0.05$). Moreover, in comparison with the NC group, sh-SOX9 could inhibit the mRNA and protein levels of RAP1 while decreasing the protein levels of RAP1GAP and RasGRP3. As opposed to the Vector

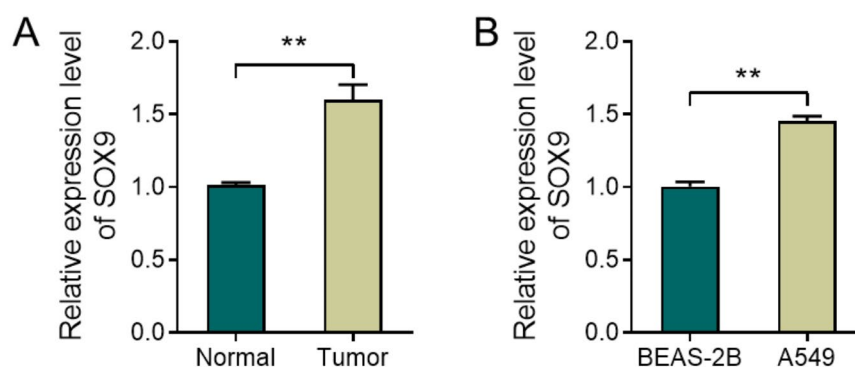


Fig. 1 Up-regulation of SOX9 expression in lung adenocarcinoma. A-B, RT-qPCR to detect the expression level of SOX9 in clinical samples (A) and cells (B). ** $P < 0.01$

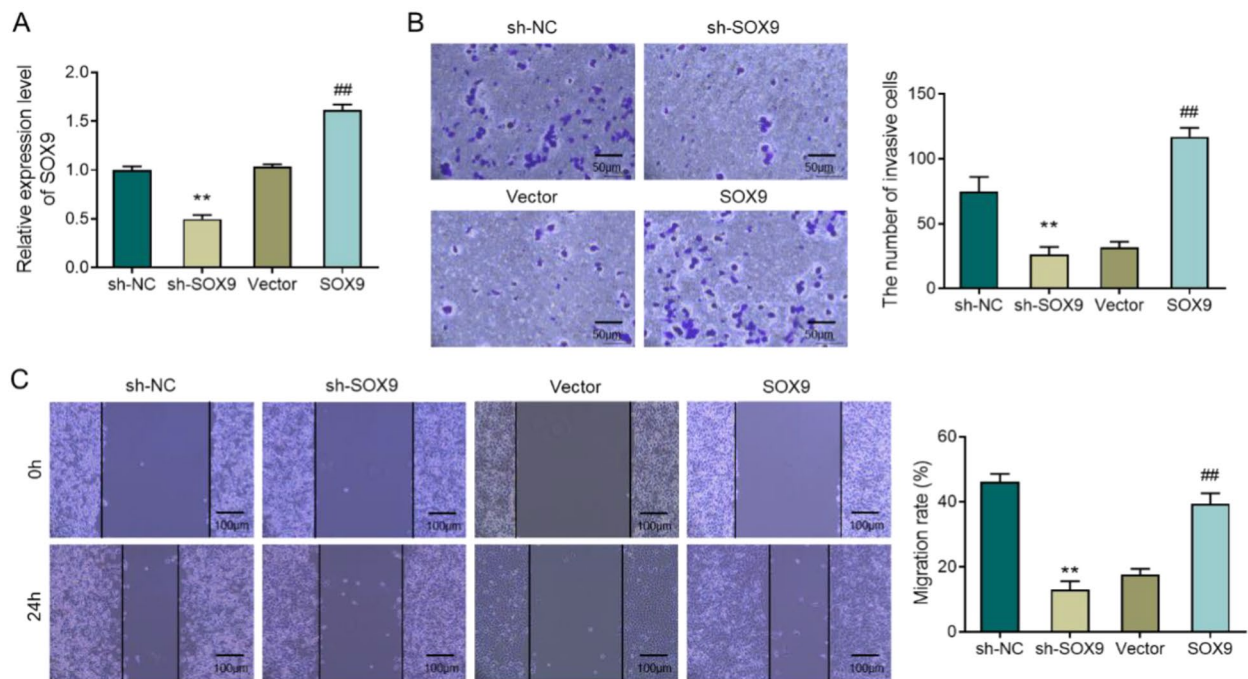


Fig. 2 Knocking down SOX9 inhibits invasion and migration of A549 cells. **A**, RT-qPCR to detect the expression level of SOX9 in cells of each group; **B**, Transwell to observe the invasion level of cells in each group; **C**, Scratch migration assay to check the migration ability of cells in each group. ** $P < 0.01$, vs. sh-NC; ## $P < 0.01$, vs. Vector

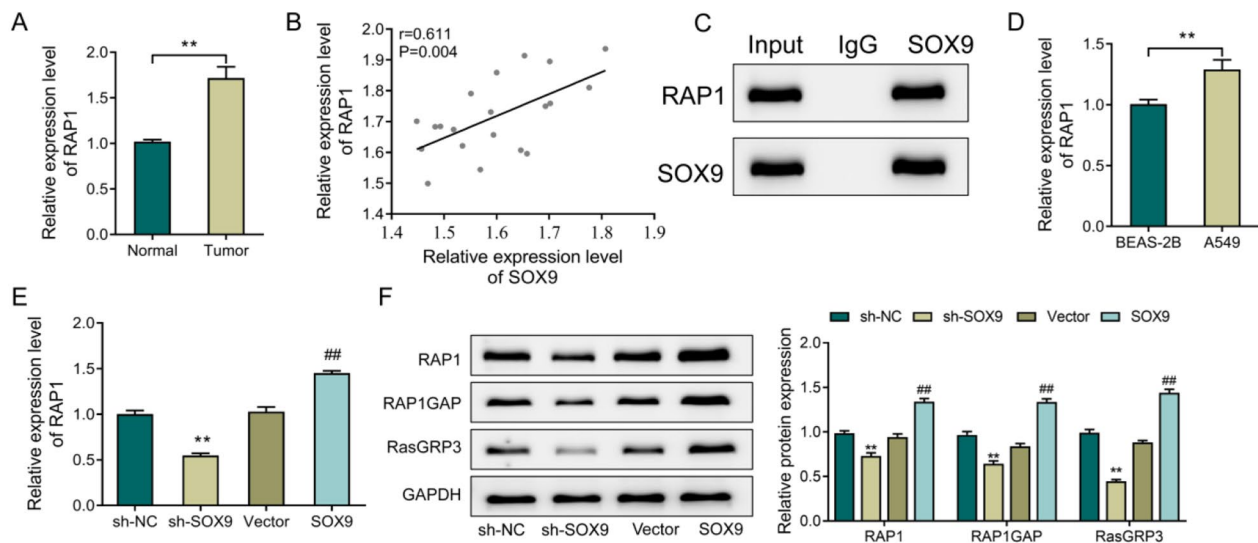


Fig. 3 Up-regulating SOX9 in lung adenocarcinoma activates the RAP1 signaling pathway. **A**, RT-qPCR to check the expression of RAP1 in clinical samples; ** $P < 0.01$; **B**, Pearson analysis for the correlation between SOX9 and RAP1 expression in clinical samples; **C**, CO-IP is used to analyze the interaction between RAP1 and SOX9. **D-E**, RT-qPCR to measure the expression of RAP1 in BEAS-2B cells, A549 cells (**D**) ** $P < 0.01$; and different expression levels of SOX9 cells (**E**); ** $P < 0.01$, vs. sh-NC; ## $P < 0.01$, vs. Vector; **F**, Western blot to detect the expression levels of RAP1, RAP1GAP and RasGRP3 in each group of cells. ** $P < 0.01$, vs. sh-NC; ## $P < 0.01$, vs. sh-SOX9.

group, overexpression of SOX9 could up-regulate the mRNA and protein levels of RAP1 as well as the protein levels of RAP1GAP and RasGRP3 (Fig. 3E, $P < 0.05$). All in all, up-regulating SOX9 in LUAD could activate the RAP1 signaling pathway.

SOX9 regulates the invasion and migration of A549 cells by activating the RAP1 signaling pathway

To further verify the role and correlation of SOX9 and RAP1 in LUAD, SOX9 was knocked down and RAP1 was overexpressed in A549 cells. The results of RT-qPCR revealed that the mRNA expression levels of SOX9 and RAP1 in the sh-SOX9+RAP1 group were higher than those in the sh-SOX9 group. Compared with the RAP1 group, the sh-SOX9+RAP1 group showed a significant drop in levels of SOX9 and RAP1 (Fig. 4A-B, $P < 0.05$). In terms of the western blot results, a notable rise was observed in the expression levels of RAP1, RAP1GAP and RasGRP3 in the sh-SOX9+RAP1 group compared

with the sh-SOX9 group; the sh-SOX9+RAP1 group exhibited lower protein expression levels of RAP1, RAP1GAP and RasGRP3 than the RAP1 group (Fig. 4C-D, $P < 0.05$). Subsequently, the invasion and migration ability of cells were observed by Transwell and Scratch assays. Specifically, compared with the sh-SOX9 group, the sh-SOX9+RAP1 group displayed increased invasion and migration ability of cells; the invasion and migration ability of A549 cells in the sh-SOX9+RAP1 group decreased in comparison with the RAP1 group (Fig. 4E-F, $P < 0.05$). The above findings proved that SOX9 could enhance the invasion and migration ability of A549 cells by activating the RAP1 signaling pathway.

Discussion

The mining of LUAD biomarkers has been the subject of numerous research reports as of late. For instance, FAM72 has been discovered to serve as a biomarker of poor prognosis in patients with LUAD [7], ERO1L has

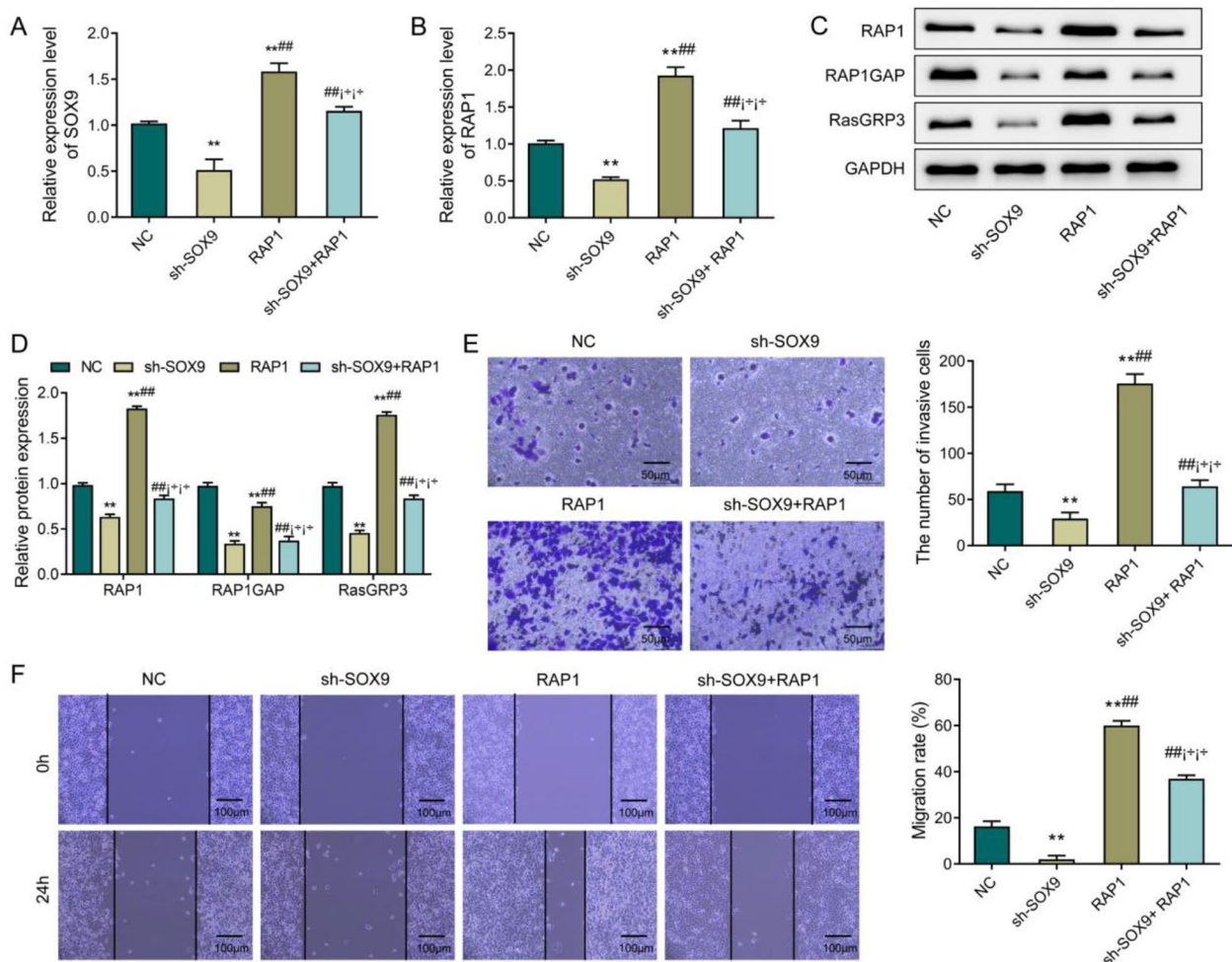


Fig. 4 SOX9 regulates the invasion and migration of A549 cells by activating the RAP1 signaling pathway. **A-B**, RT-qPCR to test the expression levels of SOX9 and RAP1 in cells of each group; **C-D**, Western blot to detect the expression levels of RAP1, RAP1GAP and RasGRP3 in cells; **E**, Transwell to observe cell invasion levels; **F**, Scratch migration assay to observe the migration ability of cells. ** $P < 0.01$, vs. sh-NC; ## $P < 0.01$, vs. sh-SOX9; i+ $P < 0.01$, vs. RAP1.

been reported to be related to the drug resistance of immunotherapy in LUAD patients [22], and lncRNA DGCR5 is considered to be involved in the progress of LUAD [23]. These biomarkers, which are constantly being discovered, provide important clues for the formulation of therapeutic regimen for LUAD. However, the known biomarkers are not enough to improve the poor survival of LUAD patients, hence additional research into biomarkers associated with LUAD development and metastasis is required. In this study, the expression level of SOX9 was significantly increased in LUAD tissues and cells. Moreover, research by Jana et al. showed considerably up-regulated expression level of SOX9 in breast cancer [24]. Carrasco-Garcia et al. discovered that metastatic colorectal cancer cells had higher expression of SOX9 than static colorectal cancer cells [25]. Therefore, we speculated that the up-regulation of SOX9 expression may function as a protooncogene in LUAD. In this paper, after altering the expression level of SOX9 in A549 cells, we observed that high expression of SOX9 significantly increased the migration and invasion levels of cells. Furthermore, Wang et al. disclosed that SOX9 acted as the transcription factor of FOXA1 to significantly promote the tumorigenicity of lung cancer cells [26]. Overexpression of SOX9 can significantly raise the migration and invasion level of H1299 cells, as revealed by previous studies [18, 27, 28]. The expression level of SOX9 was proved again to be closely related to the migration and invasion level of lung cancer cells (A549 cells) in this article.

RAP1, a member of the Ras-like small GTP binding protein family, can bind to guanosine triphosphate (GTP) or guanosine diphosphate (GDP) [29]. Studies have demonstrated that RAP1 activity is positively regulated by guanine nucleotide exchange factors (GEFs) and negatively regulated by GTPase-activating proteins (GAPs) [30]. Compared with non-cancer cells, the activated RAP1 is highly expressed in cancer cells. For example, in breast epithelial cells, increased sugar uptake induces the malignant phenotype of non-cancerous breast cells via activating integrins through RAP1 up-regulation [31]. Studies have also revealed that the expression of RAP1 in lung cancer is significantly up-regulated, while down-regulation of RAP1 expression can reduce the migration and invasion of NSCLC cell lines [32]. Notably, various drugs or Chinese herbal medicine preparations have been proved to be able to inhibit cancer via directly or indirectly mediating the RAP1 signaling pathway. For instance, Han et al. claimed that He-Chan Pian inhibited the metastasis of NSCLC through the regulation of GREM1/RAP1 signaling pathway mediated by miR-205-5p [33]. Additionally, it's reported that propofol can regulate the activity of RAP1 pathway, thereby increasing the chemosensitivity of NSCLC cells [34]. Overall, RAP1

plays an important role in the development of LUAD. In this research, the expression level of RAP1 was markedly increased in LUAD tissues and cell lines and positively correlated with the expression of SOX9. On the other hand, overexpression of RAP1 significantly enhanced the invasion and migration ability of LUAD cells. It is logical to speculate that SOX9 may promote cancer through activating RAP1. Besides, after knocking down or overexpressing SOX9 in cells, the expression levels of RAP1 signaling pathway related proteins and RasGRP3 changed with the change of SOX9 expression. Interestingly, the expression level of RAP1GAP also increased with the increase of RAP1 expression. Previous reports on gastric cancer and colorectal cancer reckoned RAP1GAP as a tumor suppressor gene [35, 36]. However, other investigations have demonstrated that RAP1GAP can promote the invasion and migration of squamous cell carcinoma cells and myeloid leukemia cell lines [37, 38]. Hence, RAP1GAP appears to have more complex roles in addition to acting as a tumor suppressor gene. SOX9 was knocked down and RAP1 was overexpressed in cells to investigate their roles in LUAD in this study. The investigation results indicated that the expression level of RAP1GAP also changed in tandem with the RAP1 expression. Moreover, the effect of knocking down SOX9 on the invasion and migration of LUAD cells was reversed by the overexpression of RAP1. Therefore, SOX9 could affect the invasion and migration of LUAD by activating the RAP1 signaling pathway activity. In hepatocellular carcinoma, CD73 has been reported to activate AKT signaling via a Rap1/P110 β cascade. Specifically, CD73 activates SOX9 transcription through c-Myc and prevents SOX9 degradation by inhibiting glycogen synthase kinase 3 β , promoting SOX9 expression and enhancing its protein stability. These actions are essential for maintaining the traits of CSCs [39]. Therefore, we speculate that SOX9 may be a transcription factor for proteins associated with the RAP1 signaling pathway, or it may play other roles in this signaling pathway.

However, neither the specific mechanism by which SOX9 activates RAP1 nor the reason why RAP1 and RAP1GAP increased at the same time in LUAD cell lines was clarified in this study. It's possible put down to the fact that the expression of RAP1GAP decreases accordingly as RAP1 decreases to prevent the decrease of RAP1 in cells. Moreover, excess RAP1GAP mRNA will be also quickly degraded; otherwise, it may lead to other diseases.

In addition, we only performed in vitro experiments and verified our results in a single cell line; we did not further explore the prognosis and expression of SOX9 and RAP1 at different stages of primary tumors and in non-metastatic and metastatic LUAD samples. Further

research is needed to make up for these shortcomings in study design.

Conclusion

To sum up, the up-regulated expression of SOX9 is closely related to the increase of cell invasion and migration ability in patients with LUAD. Furthermore, SOX9 can play a biological role by activating the RAP1 signaling pathway. Therefore, SOX9 may serve as a new biological target for the treatment of LUAD.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12890-023-02740-w>.

Supplementary Material 1

Acknowledgements

Not applicable.

Authors' contributions

J.Y. conceptualized and designed the study, drafted the initial manuscript. J.Y. and Q.L. collected the data and carried out the initial analyses. C.L. critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding

This study did not receive any funding in any form.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

This study was conducted according to the Declaration of Helsinki and clinical practice guidelines. The study was approved by the Ethics Committee of The Affiliated Taizhou People's Hospital of Nanjing Medical University. The informed consent was obtained from the patients.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 29 June 2023 / Accepted: 29 October 2023

Published online: 02 November 2023

References

- Sung H, Ferlay J, Siegel RL, et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and Mortality Worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–49.
- Kocher F, Hilbe W, Seeber A, et al. Longitudinal analysis of 2293 NSCLC patients: a comprehensive study from the TYROL registry. *Lung Cancer*. 2015;87(2):193–200.
- Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7–33.
- Siegel RL, Miller KD, Fuchs HE, et al. Cancer Stat 2021. *CA Cancer J Clin*. 2021;71(1):7–33.
- Yao G, Chen K, Qin Y, et al. Long non-coding RNA JHDM1D-AS1 interacts with DHX15 protein to Enhance Non-small-cell Lung Cancer Growth and Metastasis. *Mol Ther Nucleic Acids*. 2019;18:831–40.
- Chen D, Wang R, Yu C, et al. FOX-A1 contributes to acquisition of chemoresistance in human lung adenocarcinoma via transactivation of SOX5. *EBioMedicine*. 2019;44:150–61.
- Yu Y, Wang Z, Zheng Q, et al. FAM72 serves as a biomarker of poor prognosis in human lung adenocarcinoma. *Aging*. 2021;13(6):8155–76.
- Jo A, Denduluri S, Zhang B, et al. The versatile functions of Sox9 in development, stem cells, and human Diseases. *Genes Dis*. 2014;1(2):149–61.
- Belo J, Krishnamurthy M, Oakie A, et al. The role of SOX9 transcription factor in pancreatic and duodenal development. *Stem Cells Dev*. 2013;22(22):2935–43.
- Geraldo MT, Valente GT, Nakajima RT, et al. Dimerization and Transactivation Domains as candidates for functional modulation and diversity of Sox9. *PLoS ONE*. 2016;11(5):e0156199.
- Li X, Yang L, Chen LL. The Biogenesis, functions, and challenges of Circular RNAs. *Mol Cell*. 2018;71(3):428–42.
- Wang L, He S, Yuan J, et al. Oncogenic role of SOX9 expression in human malignant glioma. *Med Oncol*. 2012;29(5):3484–90.
- Wang HY, Lian P, Zheng PS. SOX9, a potential Tumor suppressor in Cervical cancer, transactivates p21WAF1/CIP1 and suppresses cervical Tumor growth. *Oncotarget*. 2015;6(24):20711–22.
- Mesquita P, Freire AF, Lopes N, et al. Expression and clinical relevance of SOX9 in gastric Cancer. *Dis Markers*. 2019;2019:8267021.
- Jeselsohn R, Cornwell M, Pun M, et al. Embryonic transcription factor SOX9 drives Breast cancer endocrine resistance. *Proc Natl Acad Sci U S A*. 2017;114(22):E4482–E91.
- Huang JQ, Wei FK, Xu XL, et al. SOX9 drives the epithelial-mesenchymal transition in non-small-cell Lung cancer through the Wnt/beta-catenin pathway. *J Transl Med*. 2019;17(1):143.
- Pei Y, Zhou B, Liu X. The long non-coding RNA rhabdomyosarcoma 2-associated transcript exerts anti-tumor effects on lung adenocarcinoma via ubiquitination of SOX9. *Ann Transl Med*. 2022;10(1):10.
- Capaccione KM, Hong X, Morgan KM, et al. Sox9 mediates Notch1-induced mesenchymal features in lung adenocarcinoma. *Oncotarget*. 2014;5(11):3636–50.
- Bailey CL, Kelly P, Casey PJ. Activation of Rap1 promotes Prostate cancer Metastasis. *Cancer Res*. 2009;69(12):4962–8.
- Zhang X, Bao L, Yu G, et al. Exosomal miRNA-profiling of pleural effusion in lung adenocarcinoma and Tuberculosis. *Front Surg*. 2022;9:1050242.
- Yang JC, Wu YL, Schuler M, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol*. 2015;16(2):141–51.
- Liu L, Wang C, Li S, et al. ERO1L is a novel and potential biomarker in lung adenocarcinoma and shapes the Immune-suppressive Tumor Microenvironment. *Front Immunol*. 2021;12:677169.
- Dong HX, Wang R, Jin XY, et al. LncRNA DGCR5 promotes lung adenocarcinoma (LUAD) progression via inhibiting hsa-mir-22-3p. *J Cell Physiol*. 2018;233(5):4126–36.
- Jana S, Madhu Krishna B, Singhal J, et al. SOX9: the master regulator of cell fate in Breast cancer. *Biochem Pharmacol*. 2020;174:113789.
- Santos JC, Carrasco-Garcia E, Garcia-Puga M, et al. SOX9 Elevation acts with canonical WNT signaling to Drive Gastric Cancer Progression. *Cancer Res*. 2016;76(22):6735–46.
- Wang X, Yin Y, Du R. SOX9 dependent FOXA1 expression promotes tumorigenesis in lung carcinoma. *Biochem Biophys Res Commun*. 2019;516(1):236–44.
- Wang X, Ju Y, Zhou MI, et al. Upregulation of SOX9 promotes cell proliferation, migration and invasion in lung adenocarcinoma. *Oncol Lett*. 2015;10(2):990–4.
- Wang X, Liu Y, Liu X, et al. MiR-124 inhibits cell proliferation, migration and invasion by directly targeting SOX9 in lung adenocarcinoma. *Oncol Rep*. 2016;35(5):3115–21.
- Zhang YL, Wang RC, Cheng K, et al. Roles of Rap1 signaling in Tumor cell migration and invasion. *Cancer Biol Med*. 2017;14(1):90–9.
- Looi CK, Hii LW, Ngai SC et al. The role of ras-Associated protein 1 (Rap1) in Cancer: bad actor or good Player? *Biomedicines*. 2020;8(9).
- Onodera Y, Nam JM, Bissell MJ. Increased sugar uptake promotes oncogenesis via EPAC/RAP1 and O-GlcNAc pathways. *J Clin Invest*. 2014;124(1):367–84.

32. Lu J, Zhou L, Wu B, et al. MiR-501-3p functions as a Tumor suppressor in non-small cell Lung cancer by downregulating RAP1A. *Exp Cell Res.* 2020;387(1):111752.
33. Kan J, Fu B, Zhou R, et al. He-Chan Pian inhibits the Metastasis of non-small cell Lung cancer via the mir-205-5p-mediated regulation of the GREM1/Rap1 signaling pathway. *Phytomedicine.* 2022;94:153821.
34. Ling Q, Wu S, Liao X, et al. Anesthetic propofol enhances cisplatin-sensitivity of non-small cell Lung cancer cells through N6-methyladenosine-dependently regulating the miR-486-5p/RAP1-NF-kappaB axis. *BMC Cancer.* 2022;22(1):765.
35. Yang Y, Zhang J, Yan Y, et al. Low expression of Rap1GAP is associated with epithelial-mesenchymal transition (EMT) and poor prognosis in gastric cancer. *Oncotarget.* 2017;8(5):8057–68.
36. Gao WL, Ye GC, Liu LW, et al. The downregulation of Rap1 GTPase-activating protein is associated with a poor prognosis in Colorectal cancer and may impact on Tumor progression. *Oncol Lett.* 2018;15(5):7661–8.
37. Mitra RS, Goto M, Lee JS, et al. Rap1GAP promotes invasion via induction of matrix metalloproteinase 9 secretion, which is associated with poor survival in low N-stage squamous cell carcinoma. *Cancer Res.* 2008;68(10):3959–69.
38. Qiu T, Qi X, Cen J, et al. Rap1GAP alters Leukemia cell differentiation, apoptosis and invasion in vitro. *Oncol Rep.* 2012;28(2):622–8.
39. Ma XL, Hu B, Tang WG, et al. CD73 sustained cancer-stem-cell traits by promoting SOX9 expression and stability in hepatocellular carcinoma. *J Hematol Oncol.* 2020;13(1):11.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.