Original Article

Bioinformatic analysis of differentially expressed genes in lung cancer bone metastasis and their implications for disease progression in lung cancer patients

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Background: Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer-related death worldwide. Moreover, it is highly susceptible to distant metastasis, which is the main cause of pain in advanced lung cancer, and frequently occurs in the bone. This study aimed to identify the differentially expressed genes (DEGs) related to metastatic bone disease in lung cancer using bioinformatics methods and to analyze the risk factors influencing the incidence of secondary bone metastasis in lung cancer.

Methods: Gene expression profiles from the GSE175601 and GSE10799 datasets in the Gene Expression Omnibus (GEO) database were analyzed to screen for the DEGs associated with lung cancer bone metastasis. The STRING database was used to construct a protein-protein interaction (PPI) network, and the MCODE plugin was used to identify the key genes. The expression of these important genes in lung tumor tissues and their correlation with prognosis were validated in The Cancer Genome Atlas (TCGA) database. An examination of clinical data from patients diagnosed with stage IV lung adenocarcinoma treated at the Anhui No. 2 Provincial People's Hospital was conducted. Immunohistochemistry was used to examine the expression of key genes in lung cancer tumor tissues. A binary logistic regression analysis was conducted to examine the interactions in the expression of critical genes associated with bone metastasis in lung carcinoma patients.

Results: In total, 59 DEGs were identified in the GSE175601 and GSE10799 datasets through Venn diagram construction. The PPI network analysis revealed two significant modules and eight candidate genes (*LAPTM5*, *LCP2*, *CD53*, *ARHGAP25*, *C1QA*, *DES*, *MYH11*, and *VIM*). According to TCGA database analysis, in carcinogenic tissues of the lung, the expression of these eight critical genes is downregulated. Further, only the lung cancer patients who had high expressions of *ARHGAP25* had an improved progress-free interval (PFI) ($P<0.05$), disease-specific survival (DSS), and overall survival (OS). Of the 49 with stage IV lung adenocarcinoma patients included in the study, 27 (55.10%) developed bone metastasis. The immunohistochemical (IHC) results indicated that the expression score of ARHGAP25 was significantly lower in the group with bone metastasis (3.93±2.95) than the group without bone metastasis (6.64±3.62) ($P=0.006$). The proportion of patients with low ARHGAP25 expression was significantly higher in the group with bone metastasis (70.37%, 19/27) than the group without bone metastasis (31.82%, 7/22) ($P=0.007$). The binary logistic regression analysis identified serum alkaline phosphatase (ALP) and ARHGAP25 expression levels as independent risk factors for the occurrence of secondary bone metastatic disease in lung carcinoma patients.

Conclusions: The key gene *ARHGAP25* identified through bioinformatics for lung cancer bone metastasis was significantly downregulated. Its low expression constitutes an independent risk factor for secondary bone metastatic disease in patients with lung carcinoma.

Keywords: Lung cancer; bone metastasis; ARHGAP25; risk factors

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Introduction

Lung cancer is notorious for its insidious onset, rapid progression, and poor prognosis (1). At 16% in the United States of America (USA) and less than 10% in the United Kingdom (UK), the 5-year survival rate of advanced lung carcinoma patients is very low, making it one of the deadliest malignancies worldwide (2,3). Lung cancer frequently metastasizes to distant sites, such as the brain, bones, and lymph nodes. The impact of different metastatic sites of lung cancer on patient survival varies. Generally, local metastases (such as pleura, pericardium, bronchi) are associated with slightly longer survival time compared to distant metastases (such as liver, bone, brain). This is because patients with local metastases usually have better physical conditions and can receive more aggressive treatments such as surgical resection, radiation therapy, which can prolong survival. However, patients with distant metastases often have short survival period due to the difficulty of surgically removing the metastatic site and the challenges in treatment. Additionally, simultaneous metastases to multiple sites further complicate treatment. Key factors affecting the prognosis of lung cancer patients mainly include the following aspects: histological type of tumor; disease course and tumor staging; metastatic site; patient's physical condition; treatment methods. Bones are a common site of distant metastasis in lung cancer, with bone metastases frequently occurring in weight-bearing bones such as the axial skeleton. This is a major cause of pain in advanced lung cancer. According to a study by Hong *et al.* (4), the median time from diagnosis to the onset of bone metastasis in malignant solid tumors is 18.9 months. The average duration of survival for lung cancer patients with secondary bone metastases is 6–10 months, extending up to one year following treatment for only 40–50% of patients (5). Therefore, finding early predictive and diagnostic markers for secondary bone metastatic disease in lung carcinoma is crucial.

Currently, a common diagnostic method used to detect secondary bone metastases in clinical settings is a thorough imaging examination. Unfortunately, the rate of correct diagnosis of lung carcinoma in its early stages is quite low, and the procedures are expensive. By the time imaging shows tumor metastasis, the patient is usually in the late stages of lung cancer, and the prognosis is poor (6). Significant research has been conducted to explore

Highlight box

Key findings

- This study identified 59 differentially expressed genes associated with lung cancer bone metastasis. The protein-protein interaction network analysis pinpointed eight key genes, including ARHGAP25, whose low expression was significantly correlated with secondary bone metastasis. Patients with high ARHGAP25 expression exhibited improved progression-free intervals, disease-specific survival, and overall survival. Immunohistochemical analysis further confirmed that ARHGAP25 expression was significantly lower in patients with bone metastasis compared to those without.

What is known and what is new?

- Previous studies have established the prevalence of bone metastasis in lung cancer and its impact on patient prognosis.
- This study expands on existing knowledge by highlighting ARHGAP25 as a critical gene whose downregulation serves as an independent risk factor for secondary bone metastasis. The ARHGAP25 expression as significant risk factors provides new insights into the molecular mechanisms of bone metastasis in lung carcinoma.

What is the implication, and what should change now?

- These findings underscore the potential of ARHGAP25 as a biomarker for predicting bone metastasis in lung cancer patients. Clinically, measuring ARHGAP25 levels could aid in early detection and management of bone metastasis. Future research should focus on developing targeted therapies to modulate ARHGAP25 expression, potentially improving outcomes for patients with lung cancer bone metastasis.

predictive markers for secondary bone metastatic disease in lung carcinoma and develop predictive models. Previous studies have confirmed that increased concentration of calcium in the blood, pathological stage III, tumor size stage 4 (T4), lymph node stage 3 (N3), non-small cell lung cancer, increased carcinoembryonic antigen (CEA) levels, bone sialoprotein expression, and elevated concentrations of orthophosphoric monoester phosphohydrolase are potential risk factors for secondary bone metastatic disease in lung carcinoma (7-9). However, these indicators and methods have their limitations.

Based on developments in molecular bioinformatics in recent years and understandings of the involvement of various types of molecules, host cells, and the extracellular microenvironment in the interaction of cancer cells during the process of secondary bone metastatic disease in lung carcinoma, the current study employed analytical tools of bioinformatics. We comprehensively examined gene expression microarrays related to bone metastasis in lung cancer from the Gene Expression Omnibus (GEO) database to screen for the differentially expressed genes (DEGs) associated with bone metastasis. Further, the clinical data of patients suffering from stage IV lung adenocarcinoma treated were retrospectively analyzed. By conducting immunohistochemical (IHC) examinations of the expression of the key genes in lung cancer tumor tissues and analyzing the correlation between the expression patterns of the key genes and the development of secondary bone metastatic disease in patients with lung carcinoma, the potential of these genes as predictive biomarkers for secondary bone metastatic disease in lung carcinoma was evaluated. We present this article in accordance with the REMARK reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1081/rc).

Methods

Data source

Gene expression microarray datasets involving lung carcinoma with bone metastatic disease and normal control groups (GSE175601 and GSE10799) were downloaded from the GEO database [https://www.ncbi.nlm.nih.gov/geo/; manufacturer: National Center for Biotechnology Information (NCBI), USA; owner: National Library of Medicine (NLM) and National Institutes of Health (NIH)]. To be included in the study, the original datasets had to meet the following inclusion criteria: (I) contain whole-genome messenger RNA expression microarray data; (II) include both bone metastatic disease lung cancer data and normal control data; (III) have undergone standardization processing; and (IV) include more than three samples each. Additionally, based on the annotation information in the GEO database, all the identifier codes (IDs) were labeled with the corresponding gene symbols, and duplicate gene names were removed using the averaging method.

Identification of DEGs

The limma package (https://www.bioconductor.org/; manufacturer: Bioconductor Project, USA; owner: Bioconductor Community) was used to screen for DEGs across all datasets. A P value <0.05 and a log |fold change (FC)| >1.5 were set as the criteria for selection. DEGs with a log FC >1.5 were considered upregulated, while those with a log FC <–1.5 were considered downregulated.

Identification of hub genes and establishment of the protein-protein interaction (PPI) network

The PPI network of DEGs was assessed using the STRING database (https://cn.string-db.org/; manufacturer and owner: STRING Consortium). Cytoscape V3.9.1 (https://cytoscape.org/; manufacturer and owner: Cytoscape Consortium, USA) was used to visualize the positions and interrelationships of the key DEGs in the PPI network. The MCODE plugin in Cytoscape was used to identify central clusters to facilitate the recognition of potential functional modules and provide new insights into activating signaling pathways.

Data analysis employing TCGA

The transcriptional activity of the vital genes in lung carcinogenic tissues was investigated in The Cancer Genome Atlas (TCGA) database [https://www.cancer.gov/ccg/research/genome-sequencing/tcga; manufacturer: National Cancer Institute (NCI), USA; owner: NCI and NIH], and an analysis of their correlation with the prognosis of lung cancer patients was also conducted. TCGA data details are as follows: cancer tissue samples: 1,149; adjacent normal tissues samples: 108; total clinical data samples: 1,026 (with clinical information but no corresponding RNA sequencing data); RNA sequencing data samples containing clinical information: 1,149; and RNA sequencing data samples from the same patient: 24. In

relation to the data filtering, any samples that were normal and those lacking clinical information were removed.

Clinical data of patients with lung carcinoma included in the investigation

The clinical information of patients diagnosed with lung carcinoma who received treatment at the Anhui No. 2 Provincial People's Hospital from January 2021 to September 2023 were retrospectively analyzed. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Anhui No. 2 Provincial People's Hospital (No. 2024-036), and informed consent was taken from all the patients or family members. To be eligible for inclusion in this study, the patients had to meet the following inclusion criteria: (I) have a diagnosis of stage IV lung adenocarcinoma confirmed by cytology, histopathology, and imaging; (II) have an Eastern Cooperative Oncology Group performance status (ECOG-PS) score of 0–2; (III) have not undergone radiotherapy, chemotherapy, or other anti-tumor treatments before enrollment; (IV) have provided informed consent or have had a family member provide informed consent; and (V) have a comprehensive medical history available. Patients were excluded from the study if they met any of the following exclusion criteria: (I) incomplete medical records or examination results; (II) patients with major organ failure or accompanied by severe complications; (III) patients with active autoimmune diseases; (IV) patients who are pregnant or lactating women. Based on the Expert Consensus on the Diagnosis and Treatment of Bone Metastasis in Lung Cancer (2019 edition) (10), the detection of secondary bone metastatic disease in lung carcinoma must satisfy at least one of these conditions: (I) a clinical diagnosis of lung carcinoma, with a biopsy of the bone lesion consistent with metastasis of lung cancer; and/or (II) definitive pathological identification of lung cancer, accompanied by typical radiographic manifestations of bone metastasis.

Immunohistochemistry

Lung cancer tissue samples were collected and fixed with formalin, and then routinely embedded in paraffin. The paraffin-embedded specimens were subsequently sectioned, baked, deparaffinized, cleared with xylene, and hydrated. The slides were then heated in 0.01-M sodium citrate buffer under high pressure for 15 minutes for antigen retrieval. They were then cooled for 5 minutes, after which they were washed three times with phosphate buffered saline (PBS). The slides were then kept in a humidity chamber where they underwent a 10-minute incubation in 3% hydrogen peroxide to suppress the activity of endogenous peroxidase. Next, 10% goat's serum (diluted 20 times with PBS; BN40019, Beijing Biotechnology Co., Ltd., Beijing, China) was used to incubate the tissue sections at 37 ℃ for 10 minutes. The sections were then incubated overnight in primary antibody against ARHGAP25 (dilution 1:200; rabbit IgG1; lot Ab192020; Abcam, Cambridge, UK) at 4 ℃. Finally, the sections were then treated with horseradish peroxidase-conjugated secondary antibody (diluted 1:1,000; PR30011; Proteintech Group, Inc., Chicago, USA) at room temperature for 30 minutes. Finally, the sections underwent a 15-minute incubation at ambient temperature in a 3,3'-diaminobenzidine (DAB) staining solution to achieve the desired level of staining, counterstained with hematoxylin for 3 minutes, mounted with resin, and coverslipped. Observations were made under an Olympus inverted microscope (IX73; Olympus Corporation; Japan) at ×200 magnification. ARHGAP25 is primarily expressed in the cytosol of cells, appearing as brownish-yellow granules (11).

The cells were scored from highest to lowest based on the proportion of positive staining cells as follows: 4 (>75%), 3 (51–75%), 2 (26–50%), and 1 (≤25%). Staining intensity was scored from highest to lowest as follows: 3 (strong), 2 (medium), and 1 (null). The ultimate score for each specimen was determined by the product of these two individual scores. To determine the threshold for the high or low expression of the key genes, the average of these scores was calculated.

Statistical analysis

To analyze the DEGs, the P values and corrected P values were obtained using a *t*-test, and the false discovery rate (FDR) was used to adjust the P values. Version 26.0 of SPSS statistical software (IBM Corporation, USA) was used to analyze the complete dataset. The Chi-square test was used for group comparisons, and the categorical data are presented as the case (percentage). To determine whether the quantitative data followed a normal distribution, the Kolmogorov-Smirnov test was employed. Data conforming to a normal distribution are presented as the mean ± standard deviation, and the independent samples *t*-test was used for comparisons. Skewed data are presented as the median, and interquartile range [M (P25, P75)], and

Figure 1 Selection of the DEGs correlated with secondary bone metastatic disease in lung carcinoma (Venn diagram of DEGs from the GSE175601 and GSE10799 datasets). DEGs, differentially expressed genes.

the Mann-Whitney *U* test was used to compare groups. Univariate and multivariate analyses were carried out using the binary logistic regression tool. A P value of less than 0.05 was considered statistically significant.

Results

Selection of DEGs associated with secondary bone metastatic disease in lung carcinoma

Gene expression microarray datasets containing groups of lung carcinoma patients with secondary bone metastatic disease and normal control groups (GSE175601 and GSE10799) were retrieved from the GEO database. Following the established selection criteria, DEGs were identified and visualized using volcano plots, principal component analysis (PCA) plots, sample normalization box plots, and heat maps (Figure S1). By intersecting the DEGs from both datasets, a Venn diagram was created, resulting in the identification of 59 DEGs (*Figure 1*). *Table 1* provides a list of these particular DEGs.

Construction of PPI network of DEGs

A PPI network was built by employing the STRING database to illustrate the interactions between the proteins encoded by the DEGs (*Figure 2A*). Most of the proteins encoded by the DEGs were highly interconnected with other proteins. Additionally, based on a module analysis using MCODE, two of the most significant modules were identified from the PPI network, which included eight DEGs (*Figure 2B*). The DEGs in these modules included *LAPTM5*, *LCP2*, *CD53*, *ARHGAP25*, *C1QA*, *DES*, *MYH11*, and *VIM*.

Expression of key genes in TCGA database lung cancer tissues and their association with prognosis

We further analyzed the expression of the aforementioned eight key genes in lung cancer tumor tissues using TCGA database. We found that the transcriptional activity of these eight key genes was consistently downregulated in lung cancer tumor tissues, with statistically significant differences (P<0.05) (*Figure 3A*). Additionally, a prognostic correlation analysis showed that lung adenocarcinoma patients with increased expression of *ARHGAP25* had significantly better overall survival (OS), disease-specific survival (DSS), and progress-free interval (PFI) than those with ARHGAP25 low expression (all P<0.05) (*Figure 3B-3D*). At the same time, subgroup analysis showed that patients with high expression of ARHGAP25 in T1, T4, N0, metastasis stage 0 (M0), and stage III also had better prognosis (*Figure 3E*). Therefore, *ARHGAP25* was identified as the key gene in our study.

Clinical data from the two cohorts of lung adenocarcinoma patients

This study included 49 with stage IV lung adenocarcinoma patients with lung cancer, of whom 27 (55.10%) experienced bone metastasis. Based on the occurrence of bone metastasis, the patients were categorized into the following two cohorts: the group with bone metastasis; and the group without bone metastasis. The clinical data of the patients are presented in *Table 2*. The two groups did not differ significantly in terms of gender, age, ECOG-PS, serum neuron-specific enolase levels, CEA levels, and squamous cell carcinoma antigen levels (all P>0.05). However, a statistically significant difference was found between the two groups in terms of serum alkaline phosphatase (ALP) concentration (P<0.05), such that the serum ALP levels of the secondary bone metastatic disease group were considerably higher than those of the non-bone metastatic group.

Expression of ARHGAP25 in lung cancer tissues

As *Figure 4A* shows, the IHC scoring results indicated

Table 1 Common DEGs correlated with secondary bone metastatic disease in lung carcinoma from the GSE175601 and GSE10799 datasets

MMP1, PPP1R14A, ADARB1, PPP1R12B, KBTBD11, LAPTM5, STXBP6, IL1B, KLF11, TLR1, DES, DENND5A, CA2, PROM2, IL7R, ARHGEF15, EMILIN2, LAP3, EDNRB, KIF20A, NEDD9, SERTAD1, DNMT3A, ALDH18A1, HMGB3, ST6GALNAC5, NEXN, SAMD4A, CD53, LCP2, KCNK3, TMEM204, HMGB3P1, DST, SLC25A25, CCSER2, ADAM12, BTG2, AKAP12, EML1, KLF6, ANXA6, PID1, GRK5, ID3, EMR1, ARHGAP25, HMBOX1, MS4A7, SYNE1, C1QA, VIM, CAPN3, TMEM173, S100A12, MYH11, PIK3R5, TBX3, SLC31A2

DEGs, differentially expressed genes.

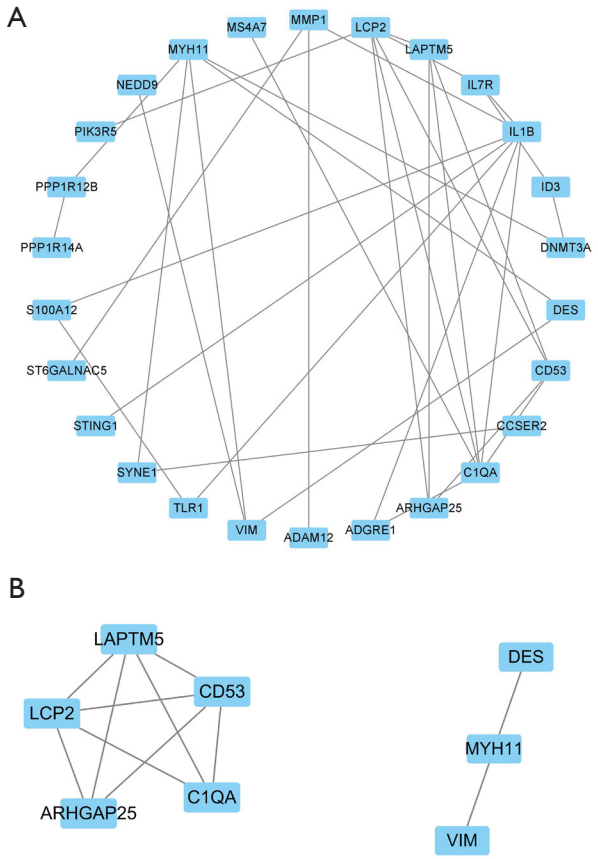

Figure 2 PPI network and key modules of DEGs. (A) PPI network of DEGs constructed using STRING. (B) Two important modules derived from the PPI network using the MCODE module in Cytoscape. PPI, protein-protein interaction; DEGs, differentially expressed genes.

that the ARHGAP25 expression score was significantly reduced in the group with secondary bone metastatic disease, which had a score of 3.93±2.95, in comparison to the group without secondary bone metastatic disease, which had a score of 6.64±3.62 (P=0.006). The IHC staining of ARHGAP25 in lung cancer tissues and adjacent tissues is shown in *Figure 4B*. The average score of all patients' lung cancer tissue immunohistochemistry was 5.14, which was used as the threshold value. Scores below 5.14 were classified as low ARHGAP25 expression, while scores above 5.14 were classified as high ARHGAP25 expression. Further, the percentage of patients with low ARHGAP25 expression was significantly more increased (70.37%; 19/27) in the group with secondary bone metastatic disease than the group without secondary bone metastatic disease (31.82%; 7/22) (P=0.007) (*Table 3*). This provides further evidence that ARHGAP25 functions as a "tumor inhibitor" in lung cancer.

Univariate and multivariate binary logistic regression analyses

A binary logistic regression analysis of the clinical data was performed to identify the univariate risk factors correlated with secondary bone metastatic disease in patients with lung carcinoma. The results indicated that serum ALP levels [hazard ratio (HR) =0.974; 95% confidence interval (CI): 0.954–0.994; P=0.01] and the low expression of ARHGAP25 (HR =5.089; 95% CI: 1.503–17.230; P=0.009) were associated with the incidence of secondary bone metastatic disease in patients with lung carcinoma. Further, the multivariate regression analysis showed that serum ALP levels (HR =0.976; 95% CI: 0.955–0.997; P=0.03) and the low expression of ARHGAP25 (HR =4.622; 95% CI: 1.158–18.456; P=0.03) were independent risk factors affecting the incidence of bone metastatic disease in patients with lung carcinoma (*Table 4*).

Discussion

Bone metastasis is one of the common sites of lung cancer metastasis. Data indicates that the average time for advanced lung cancer patients to develop bone metastasis is 9 months, and about 2/3 of patients have already developed bone metastasis at the time of lung cancer diagnosis (12,13). The primary clinical symptom of lung cancer bone metastasis is local pain, often persistent dull pain. The location of pain is related to the site of bone metastasis.

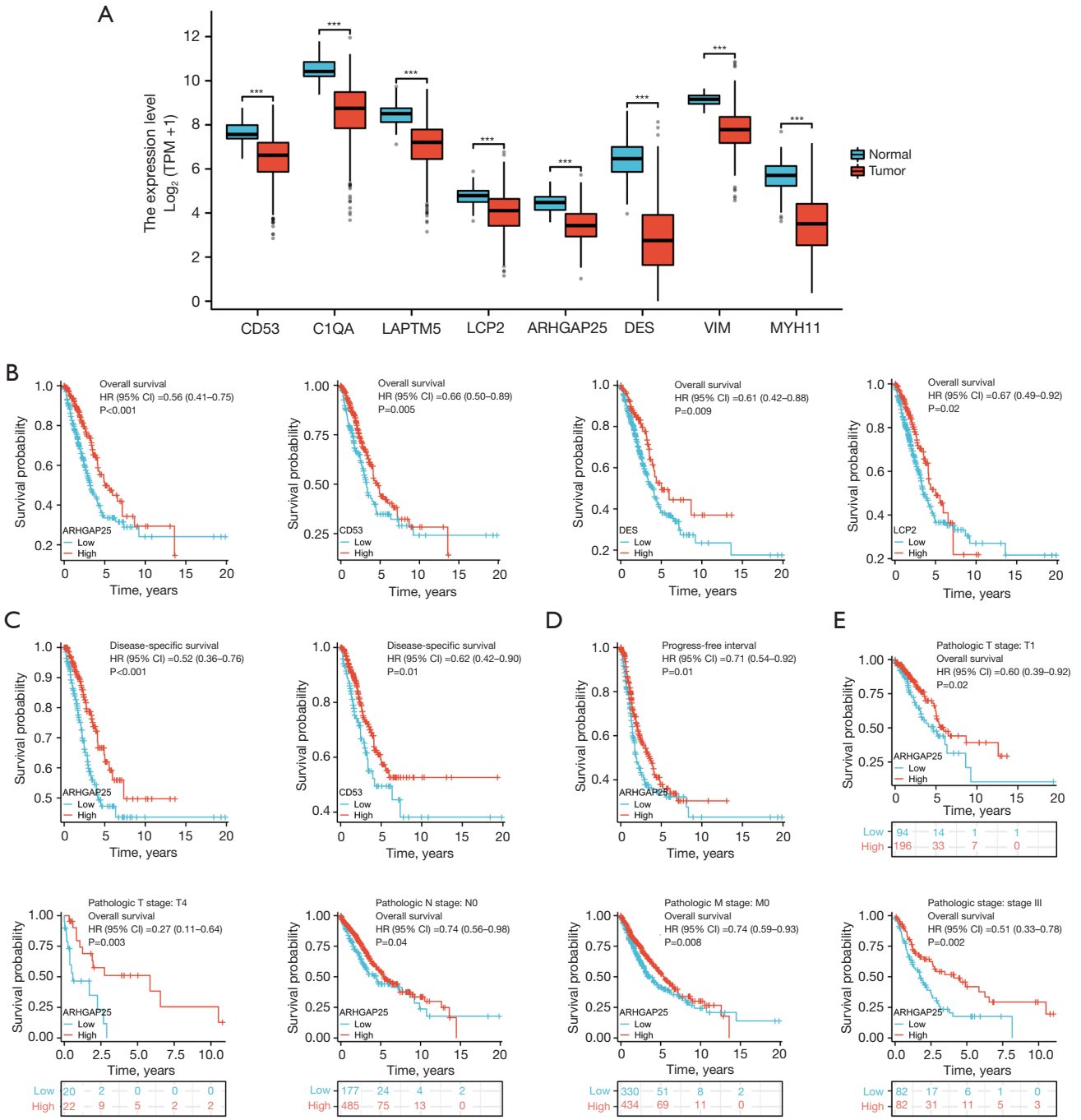

Figure 3 Expression of the key genes in TCGA database lung cancer tissues and their association with prognosis. (A) The eight key genes in the lung cancer tumor tissues from TCGA database showed downregulated expression. (B) Association between the OS of the lung carcinoma patients and the expression of the critical candidate genes (*ARHGAP25*, *CD53*, *DES*, and *LCP2*). (C) Association between the DSS of the lung carcinoma patients and the transcriptional activity of the critical candidate genes (*ARHGAP25* and *CD53*). (D) Association between the PFI of the lung cancer patients and the transcriptional activity of the pivotal gene (*ARHGAP25*). (E) Subgroup examination of the correlation between the OS of lung cancer patients and *ARHGAP25* expression. ***, P<0.001. TPM, transcripts per million; HR, hazard ratio; CI, confidence interval; TCGA, The Cancer Genome Atlas; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

Table 2 Baseline data of stage IV lung adenocarcinoma patients with and without bone metastasis

Variables	Bone metastasis group (n=27)	Non-bone metastasis group (n=22)	t/χ^2/Z	P value
Sex, n (%)			3.432	0.06
Male	17 (62.96)	8 (36.36)		
Female	10 (37.04)	14 (63.64)		
Age (years), mean ± SD	64.81±11.90	65.18±12.44	0.1061	0.92
ECOG-PS score, n (%)			2.112	0.35
0	1 (3.70)	2 (9.09)		
1	24 (88.89)	16 (72.73)		
2	2 (7.41)	4 (18.18)		
ALP ($U \cdot L^{-1}$), mean ± SD	152.59±93.83	82.73±18.43	3.433	0.001
NSE ($\mu g \cdot L^{-1}$), median (IQR)	14.90 (10.20, 24.73)	14.65 (10.89, 17.47)	–0.503	0.62
CEA (ng/mL), median (IQR)	40.34 (10.80, 68.59)	18.26 (4.70, 58.62)	–1.689	0.09
SCCA (ng/mL), median (IQR)	0.66 (0.35, 0.98)	0.58 (0.30, 1.04)	–0.151	0.88

SD, standard deviation; ECOG-PS, Eastern Cooperative Oncology Group performance status; ALP, alkaline phosphatase; NSE, neuron-specific enolase; IQR, interquartile range; CEA, carcinoembryonic antigen; SCCA, squamous cell carcinoma antigen.

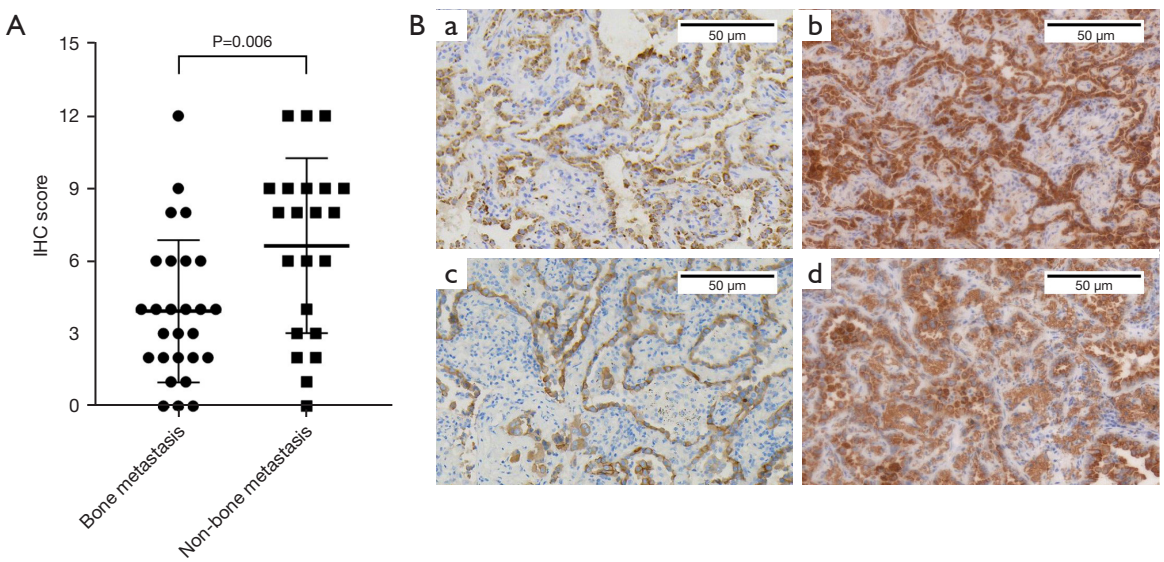

Figure 4 IHC detection of ARHGAP25 expression in tissues. (A) IHC scoring results for patients with and without bone metastasis. (B) IHC images of ARHGAP25 expression in lung cancer tissues. a: reduced expression in tissues from the bone metastasis group (×200; n=1). b: high expression in tissues from the bone metastasis group (×200; n=1). c: reduced expression in tissues from the non-bone metastasis group (×200; n=1). d: high expression in tissues from the non-bone metastasis group (×200; n=1). IHC, immunohistochemical.

Table 3 Expression of ARHGAP25 in patients with lung carcinoma

ARHGAP25 expression	Bone metastasis group (n=27), n (%)	Non-bone metastasis group (n=22), n (%)	χ^2	P value
High	8 (29.63)	15 (68.18)	7.234	0.007
Low	19 (70.37)	7 (31.82)		

Table 4 Univariate and multivariate binary logistic regression analyses

Variables	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Sex						
Male	–	–	–	–	–	–
Female	2.975	0.925–9.568	0.07	–	–	–
Age	1.003	0.956–1.051	0.91	–	–	–
ECOG-PS score						
0	–	–	–	–	–	–
1	0.333	0.028–3.990	0.39	–	–	–
2	1.000	0.053–18.915	>0.99	–	–	–
ALP	0.974	0.954–0.994	0.01	0.976	0.955–0.997	0.03
NSE	0.964	0.903–1.029	0.27	–	–	–
CEA	0.996	0.88–1.004	0.29	–	–	–
SCCA	1.183	0.603–2.323	0.63	–	–	–
ARHGAP25 expression						
High	–	–	–	–	–	–
Low	5.089	1.503–17.230	0.009	4.622	1.158–18.456	0.03

HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; ALP, alkaline phosphatase; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; SCCA, squamous cell carcinoma antigen.

When spinal metastasis further infiltrates the spinal cord, patients may experience numbness, movement disorders, and even paralysis. At present, the treatment of lung cancer bone metastasis is a comprehensive process involving the combined application of multiple treatment methods, including palliative surgical treatment, radiation therapy, chemotherapy, targeted therapy, and immunotherapy (14,15). However, these combination therapies have poor prognostic improvement for patients with lung cancer bone metastases. Over the last decade, following continuous progress in molecular biology assays, DNA microarray techniques, and next-generation sequencing, investigations of the DEGs related to secondary bone metastatic diseases in lung carcinoma at the transcriptome level and analyses of these key genes have become important methods for studying the risk factors and molecular mechanisms of lung cancer bone metastasis. Moreover, there is increasing evidence that the aberrant expression of certain genes has a vital role in the initiation and advancement of secondary bone metastatic disease in lung carcinoma cancer (16,17). Screening the expression profiles of the genes associated with secondary bone metastatic disease in lung carcinoma and identifying the key genes associated with its occurrence and development are of great importance in the assessment, prevention, and management of secondary bone metastatic diseases in lung carcinoma.

This study identified 59 DEGs through an analysis of the GSE175601 and GSE10799 datasets and the application of bioinformatics techniques. From the construction of the PPI network, eight key genes were identified (*LAPTM5*, *LCP2*, *CD53*, *ARHGAP25*, *C1QA*, *DES*, *MYH11*, and *VIM*). Further analysis of these genes in TCGA database showed that *ARHGAP25* was a key gene significantly associated with prognosis. Rho GTPases, which are members of the Ras superfamily, play critical roles in the advancement of the cellular proliferation cycle, cytoskeletal reorganization, and propagation of tumor cells, and are integral to tumor development and progression (18-20). As a Rho GTPase activating protein, ARHGAP25 activates GTPase activity to maintain Rho GTPases in an inactive state, plays a pivotal role in regulating the cytoskeleton, cell polarity control, and cell migration, and significantly affects the oncogenic processes. Based on previous research, ARHGAP25 may play the following roles in tumor occurrence and

development: (I) regulating cytoskeleton rearrangement and cell migration: ARHGAP25 regulates Rho family GTPases, affecting cytoskeletal dynamics, and consequently, the migration and invasion abilities of cancer cells (18,21). (II) Participate in tumor signaling pathways: ARHGAP25 may be involved in tumor-related pathways such as AKT/mTOR (22), which are critical for tumor cell proliferation, survival, migration, and invasion. (III) Impact on tumor microenvironment: immune regulation: ARHGAP25 may affect immune cell activity, influencing tumor immune escape and surveillance (23,24). (IV) Angiogenesis: ARHGAP25 may participate in the process of tumor angiogenesis by affecting angiogenic factors expression or the activity of angiogenic signaling pathways (25). These studies have good reference significance for further mechanism exploration in the future.

The study included patients with lung carcinoma, of whom 27 (55.10%) had bone metastasis. This relatively high proportion of patients with bone metastasis underscores the importance of identifying and employing interventions to treat high-risk metastatic patients in clinical settings. The immunohistochemistry results indicated that the IHC scoring of ARHGAP25 was greatly reduced in patients with bone metastasis compared to those without bone metastasis. A greater number of lung carcinoma patients in the bone metastasis cohort exhibited low ARHGAP25 expression. This is consistent with the bioinformatics analysis, which showed that ARHGAP25 expression was downregulated in lung cancer bone metastasis. The binary logistic regression analysis identified ARHGAP25 expression as an independent risk factor that can increase the likelihood of bone metastasis. The downregulation of ARHGAP25 might lead to an abnormal increase in Rho GTPase activity and reduced stability of the cytoskeleton. It also increases the ability of tumor cells to migrate and change shape, thereby aggravating the metastasis of tumor cells to distant sites such as bones. Xu *et al.* (26) found that the transcriptional activity of ARHGAP25 in lung cancer tissues is significantly reduced, and the overexpression of ARHGAP25 can decrease the activity of the β-catenin/Wnt signaling pathway. This in turn inhibits the expansion, migration, and infiltration of lung cancer cells. Shi *et al.* (25) found that the expression of ARHGAP25 is negatively correlated with RhoA and vasculogenic mimicry, and thus could have predictive value for non-small cell lung cancer tumor metastasis, prognosis, and targeted therapy. Tao *et al.* (27) showed that ARHGAP25 was downregulated in the colon biopsies of colorectal cancer patients, and negatively regulates the metastatic potential of colorectal cancer cells through the Wnt/β-catenin pathway.

In summary, this research revealed the probable biological function of ARHGAP25 in lung carcinoma with secondary metastatic bone disease and provided a possible biomarker for assessing the incidence of secondary bone metastatic diseases in individuals with lung carcinoma clinically. Moreover, the research results of this study are also very helpful for developing treatment strategies for lung cancer bone metastasis: (I) identifying treatment targets: treatment strategies targeting ARHGAP25, such as gene therapy, molecular targeted therapy, or immunotherapy, may help reverse its low expression state, thereby inhibiting the bone metastasis ability of lung cancer cells. (II) Guiding personalized treatment: due to the variations in ARHGAP25 expression levels among patients, personalized treatment plans can be developed by detecting ARHGAP25 expression. For patients with low expression of ARHGAP25, treatment that upregulate its expression level, such as gene therapy or specific drug interventions, can be prioritized to enhance the therapeutic effect. (III) Combination therapy: considering the complexity of bone metastasis in lung cancer, a single treatment method may not achieve ideal results. The results of this study suggest that combining treatment targeting ARHGAP25 with existing therapies such as chemotherapy, radiotherapy and targeted therapy may result in better outcomes. (IV) Assessing prognosis and monitoring treatment efficacy: the expression level of ARHGAP25 can serve as an indicator for evaluating patient prognosis and monitoring treatment efficacy. Regular monitoring of ARHGAP25 expression allows for timely understanding of changes in the patient's condition and treatment response, enabling adjustments to treatment plans and improving effectiveness. (V) Promoting new drug development: this study provides important clues and a basis for new drug development. In depth research on the molecular mechanism of ARHGAP25 can help discover new drug targets and promote the development of related drugs. In the future, new targeted drugs or immunotherapies for ARHGAP25 can provide more treatment options for lung cancer patients with bone metastases.

This study had several limitations. As the study was conducted at a single center with a limited number of participants, the statistical power and generalizability of the results might be limited. Additionally, insufficient long-term monitoring in this study did not allow for the assessment of the relationship between the expression

levels of ARHGAP25 and the progression of the disease in patients with lung carcinoma. In the future, studies will be conducted at multiple centers with larger sample sizes to address these limitations. Additionally, *in vitro* experiments will be conducted to further explore the specific molecular mechanisms of ARHGAP25 and gather more reliable data to validate ARHGAP25 as a potential molecular marker for predicting secondary bone metastatic disease in lung carcinoma.

Conclusions

In summary, multiple regulatory factors appear to be involved in the occurrence and recurrence of secondary bone metastatic disease in lung carcinoma. The identified key genes and related signaling pathways may extend understandings of the underlying molecular mechanisms. However, this study also had certain limitations, including a lack of relevant *in vitro* and *in vivo* experiments, and large sample and multicenter clinical data validation. Further validation and exploration are necessary in the future.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Anhui No. 2 Provincial People's Hospital (No. 2024-036), and informed consent was taken from all the patients or family members.

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