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# Expression of AUF1 in lung adenocarcinoma and its diagnostic value across three types of pathological specimens

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**Article**

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# Expression of AUF1 in lung adenocarcinoma and its diagnostic value across three types of pathological specimens

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**Abstract**

**Background** Patients with lung adenocarcinoma face challenges such as drug resistance, low detection rates of positive markers, and difficulties in diagnosing and treating those who cannot tolerate surgical biopsies due to pleural effusion. Therefore, identifying new molecular biomarkers with potential therapeutic targets is urgently needed.

**Methods** This study used differentially expressed genes between LUAD tumor tissues and adjacent normal tissues from the Gepia2 and TCGA databases to identify AU-rich element RNA-binding factor 1 (AUF1) as a novel biomarker for LUAD. The expression levels of AUF1, TFF-1, and NapsinA were analyzed in 120 surgically resected lung adenocarcinoma tissues and their paired adjacent normal tissues, 50 needle biopsy samples of lung adenocarcinoma and their matched normal tissues, and paraffin-embedded specimens of pleural effusions from 50 lung adenocarcinoma patients and 20 non-tumor controls.

**Results** Immunohistochemical analysis revealed that AUF1 was significantly upregulated in LUAD tissues compared to normal control tissues in the 120 surgical specimens, and its expression correlated with tumor size, T stage, and pleural invasion. Furthermore, AUF1 expression was assessed in cell blocks derived from needle biopsies of 50 LUAD patients, pleural effusions from 50 LUAD patients, and 20 non-tumor individuals. Results showed high AUF1 expression in both needle biopsy samples and malignant pleural effusions from LUAD patients. Moreover, AUF1 expression was consistent with that of TTF-1 and NapsinA in LUAD tissues and

malignant pleural effusions.
**Conclusion** This study highlights the potential diagnostic value of AUF1 in surgical resection specimens, needle biopsy samples, and pleural effusions of LUAD patients. Our findings have significant implications for the pathological diagnosis of lung needle biopsies and malignant pleural effusions.
Keywords: LUAD, needle biopsy, MPE, AUF1

## 1 Introduction

According to global epidemiological statistics, lung cancer is the most common and deadliest malignant tumor in China, with lung adenocarcinoma (LUAD) accounting for approximately two-thirds of all lung cancer cases[1]. Lung adenocarcinoma is characterized by high malignancy, poor prognosis, and a high recurrence rate[2]. To uncover the underlying mechanisms involved in the development and progression of lung adenocarcinoma, extensive basic and clinical research has been conducted, leading to significant advances in its treatment. Nevertheless, the incidence and mortality rates of lung adenocarcinoma remain high worldwide[3]. Therefore, there is an urgent need to identify novel biomarkers for lung adenocarcinoma to guide personalized therapy and optimize treatment strategies.

Most lung cancer cases are diagnosed at advanced or metastatic stages. Patients at these stages typically undergo minimally invasive diagnostic procedures, which in turn limit the availability of tissue samples. Maximizing the amount of usable tissue obtained via bronchoscopy or percutaneous fine-needle aspiration (FNA) is therefore crucial. Current guidelines emphasize the importance of assessing PD-L1 expression, EGFR mutations, ALK and ROS1 chromosomal rearrangements, as well as histological classification[4].

Non-small cell lung cancer (NSCLC)[5]and small cell lung cancer (SCLC) account for 85% and 15% of all lung cancer cases, respectively. Malignant pleural effusion (MPE) is a common clinical complication of lung adenocarcinoma. At initial diagnosis, 10% to 15% of patients with advanced NSCLC present with MPE, and this proportion increases further during later stages of treatment[6]. The patient was diagnosed with malignant pleural effusion (MPE). In NSCLC patients, TNM staging tends to be advanced, with a median overall survival of only 5.5 months[7]. Thus, accurate differentiation between MPE and benign pleural effusions is critical for early clinical intervention and effective management. Recently, the American Society of Clinical Oncology has approved pleural fluid cell blocks as cytological specimens suitable for EGFR and ALK testing[8]. Compared to solid tissue samples, pleural fluid cell blocks are easier to collect, safer, more cost-effective, and better accepted by patients. It is well known that TTF-1 is highly expressed in lung tissue, while Napsin A demonstrates high specificity and sensitivity for lung adenocarcinoma[9]. Studies have shown that combining Napsin A and TTF-1 improves diagnostic accuracy; co-detection of these two markers effectively distinguishes lung adenocarcinoma from other types of lung cancer and aids in differentiating malignant from benign pleural effusions[10-11]. However, no consensus has yet been reached on biomarkers for distinguishing MPE from benign pleural effusions, making it worthwhile to explore new potential markers.

In this study, we identified AUF1 from Gepia and TCGA databases. AUF1, also known as AU-rich RNA-binding factor (AUF1)/heterogeneous nuclear ribonucleoprotein D (hnRNPD), is a protein that binds to AU-rich elements (AREs) and is located on chromosome 4q21[12]. It regulates

mRNA stability by binding to ARE sites in the 3'untranslated regions (UTRs) of numerous genes[13-14]. Recent studies have shown that AUF1 promotes proliferation and invasiveness in breast cancer cells[15] and is closely associated with tumor size and malignancy in liver cancer, potentially leading to worse prognosis[16]. Moreover, mice genetically modified to express AUF1 are more likely to develop undifferentiated sarcomas[17]. The association between AUF1 and thyroid cancer was first reported in 2009. The study concluded that AUF1 may serve as an additional biomarker for thyroid cancer to distinguish the malignancy degree of tumors[18].

AUF1 is involved in the occurrence and development of various cancers, but its biological functions and mechanisms in lung adenocarcinoma and malignant pleural effusion remain unclear. This study aims to investigate the expression of AUF1 in lung adenocarcinoma and its diagnostic value for malignant pleural effusion.

## 2 Materials and methods

### 2.1 Patient information and clinical samples

The experiments utilizing human tissue specimens complied with all the relevant national regulations and institutional policies, and were in accordance the tenets of the Helsinki Declaration. The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Chengdu Medical College,Nuclear Industry 416 Hospital.All clinical specimens were collected at the hospital, and informed consent was obtained from all subjects. Primary tumor specimens and paired normal lung tissues were obtained from 120 LUAD patients during surgical resection;Biopsy tissues from 50 patients with lung adenocarcinoma. The inclusion criteria were as follows: (a) defenite diagnosis of LUAD, (b) absence of other malignant tumors, (c) TNM stages I-IV as per American Joint Committee on Cancer (AJCC) classification, and (d) availability of complete clinical and pathological data including age,sex,TNM stage, and clinical stage.In addition,pleural effusion was collected from 50 LUAD patients to 20 non-neoplastic patients. The inclusion criteria for LUAD patients were(a)pathologically confirmed LUAD and (b) absence of other malignant tumors. The inclusion criteria for the non-neoplastic subjects were (a) absence of malignant tumors and (b) lack of cancer cells in the pleural effusion[19-20].

### 2.2 ImmunohistoChemistry (IHC)

The tissue specimens were fixed in 4% phosphate-buffered paraformaldehyde and embedded in paraffin.Fresh pleural effusion was left to stand for 20-30 minutes,and the precipitate was collected and centrifuged at 2500 rpm for 5 minutes.The supernatant was discarded,and the precipitate was washed successively with 75% and 95% ethanol,then fixed in 10% formalin for 2-4 hours.The solid cell precipitate was dehydrated and processed according to the routine histological specimen procedures.The paraffin blocks were cut into 2-micron-thick sections and subjected to routine immunohistochemical detection.The samples were detected using anti-AUF1 antibody(abcam,#ab259895),anti-TTF1 antibody (MXB®Biotechnologies,Fuzhou,China, #MAB-0677),and anti-Napsin A antibody (MXB®Biotechnologies,Fuzhou,China,#MAB-0704).Rabbit-derived pre-immune serum was used as a negative control for the primary antibody.The staining was performed using the DAB substrate,and the sections were stained with hematoxylin for contrast.The sections were observed using an Olympus BX53 microscope.The staining intensity scoring criteria are as follows:0 (negative)=no staining;1 (weakly positive)=pale yellow;2 (positive)=brownish yellow.3 (strongly

positive)=brown.Patients with scores of 0 and 1 were classified into the low-expression group,while those with scores of 2 and 3 were classified into the high-expression group[19-20].

**2.3 Hematoxylin and eosin staining (H&E staining)**

Tissue specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin stain(H&E) to facilitate the histological examination of tissue morphology and to identify any pathological changes[19-20].

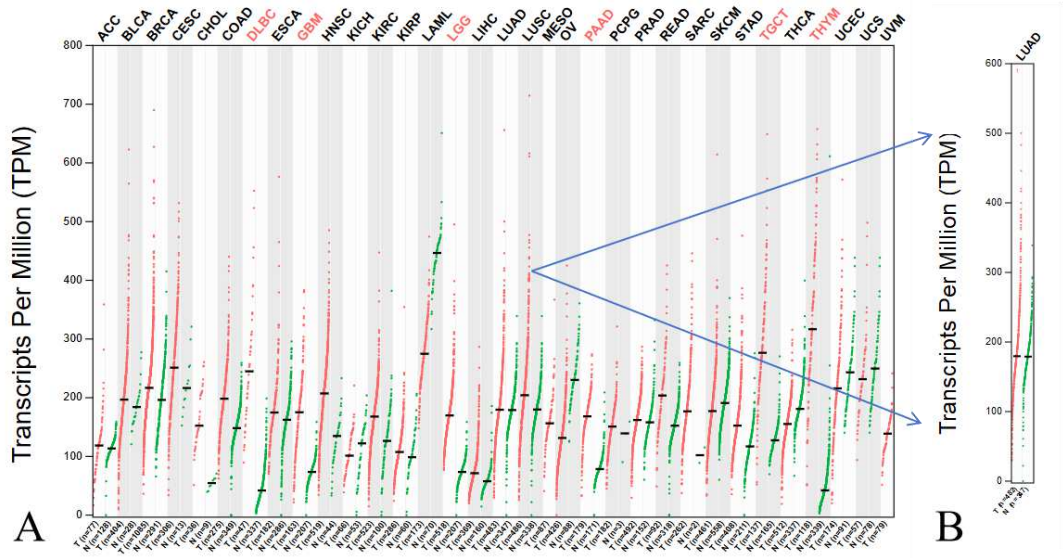
**2.4 Statistical analysis**

SPSS software (version 27.0) was used for statistical analysis. Chi-square test was used to analyze the correlation between clinicopathological characteristics and AUF1 expression. P value<0.05 was statistically significant.Multivariate logistic regression analysis:Variables with P<0.1 in univariate analysis (tumor size,T stage,pleural invasion, and differentiation) were entered into a multivariate logistic regression model using forward stepwise selection (Forward:Likelihood Ratio).The entry criterion was set at P<0.05 and the removal criterion at P>0.10.Collinearity among independent variables was assessed by variance inflation factor (VIF),with VIF>10 indicating severe multicollinearity. All statistical tests were two-sided, and P<0.05was considered statistically significant.

## 3 Result

### 3.1 Discover from the database that AUF1 is a potential diagnostic and prognostic biomarker for lung adenocarcinoma

In order to identify biomarkers for differentiating malignant from benign pleural effusion in lung adenocarcinoma patients, we first screened for AUF1 in the Gepia database (Figure 1) and TCGA database(Figure 2). Compared with normal tissues, AUF1 was significantly upregulated in lung adenocarcinoma (LUAD) tissues and was associated with prognosis, suggesting that AUF1 may be a potential diagnostic marker for lung adenocarcinoma.



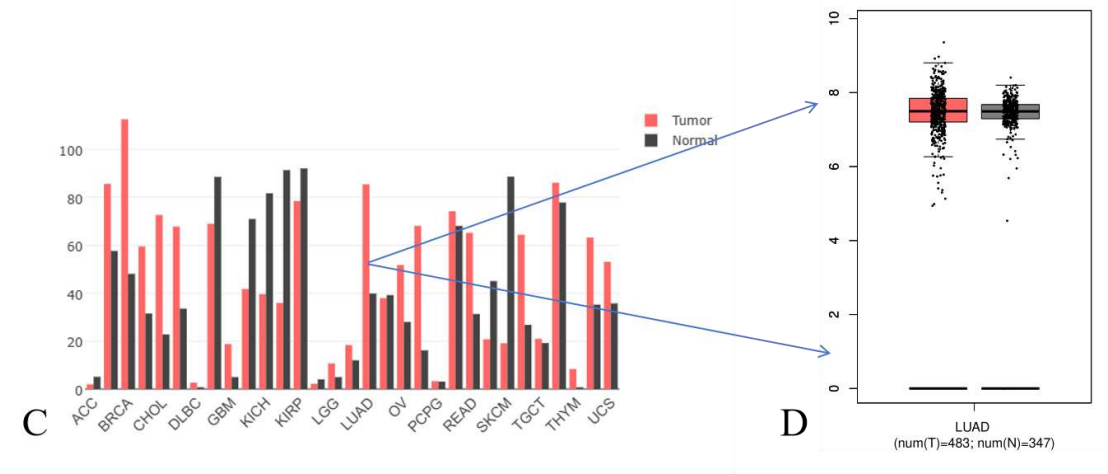
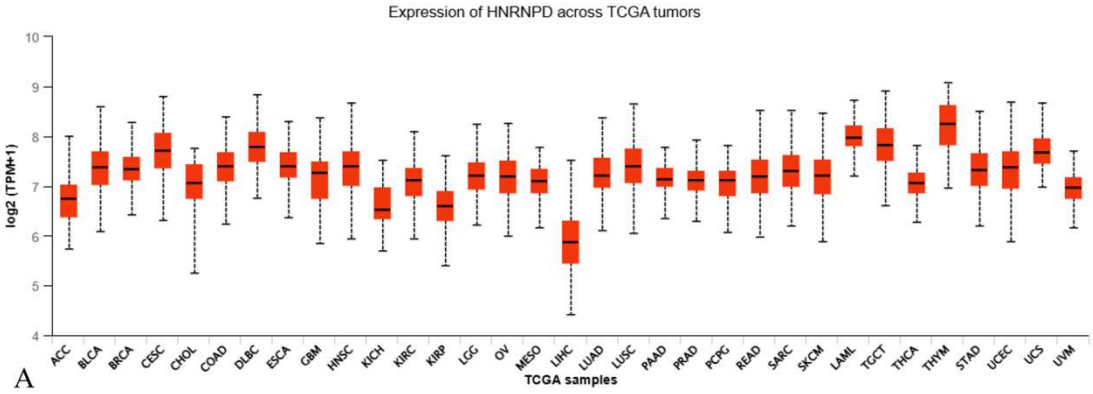
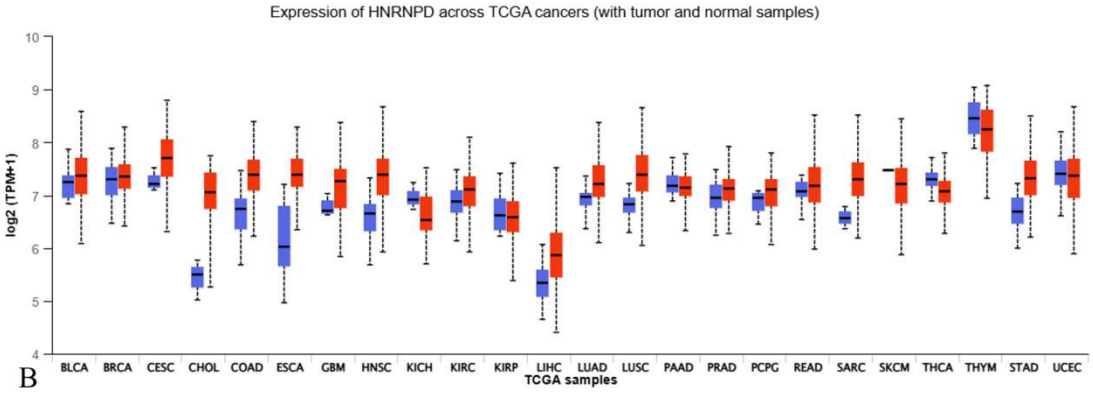



Figure 1:Gepia database:AUF1 was significantly upregulated in LUAD tissues (A-E) and was associated with prognosis (F).



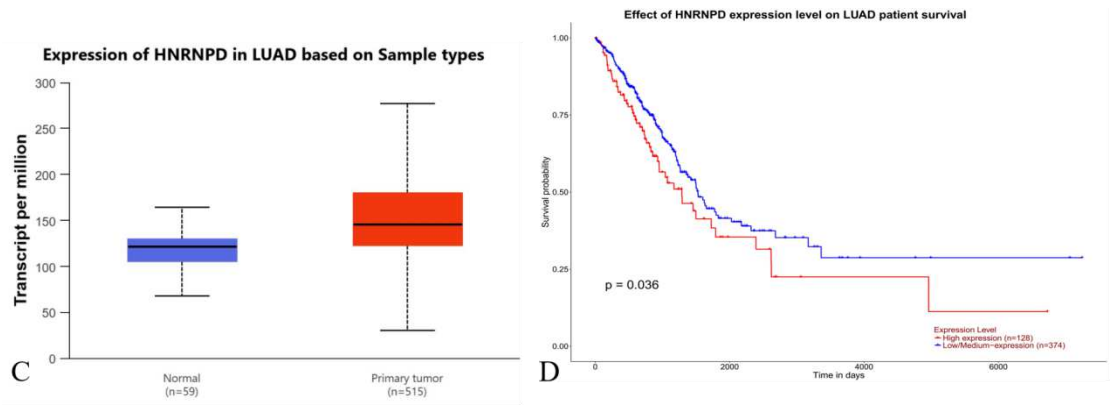



Figure 2: TCGA database:AUF1 was significantly upregulated in LUAD tissues (A-C) and was associated with prognosis (D).

**3.2 Histological verification indicates that AUF1 is a potential biomarker for lung cancer.**

To verify the actual expression of AUF1 in lung cancer, this study employed immunohistochemical methods to quantitatively analyze the expression levels of AUF1 protein in 120 lung cancer tissue samples and their adjacent normal tissue samples (Figures 3-1, 3-2; Table 1), 50 lung biopsy samples (Figures 4-1, 4-2; Table 2), 50 malignant pleural effusion samples from lung adenocarcinoma patients (Figures 5-1,5-2;Table 3). Among them, no cells were detected in the benign pleural effusion.The results showed that the expression level of AUF1 in lung cancer tissues was significantly increased, and it was consistent with the expression trends of TTF-1 and NapsinA.

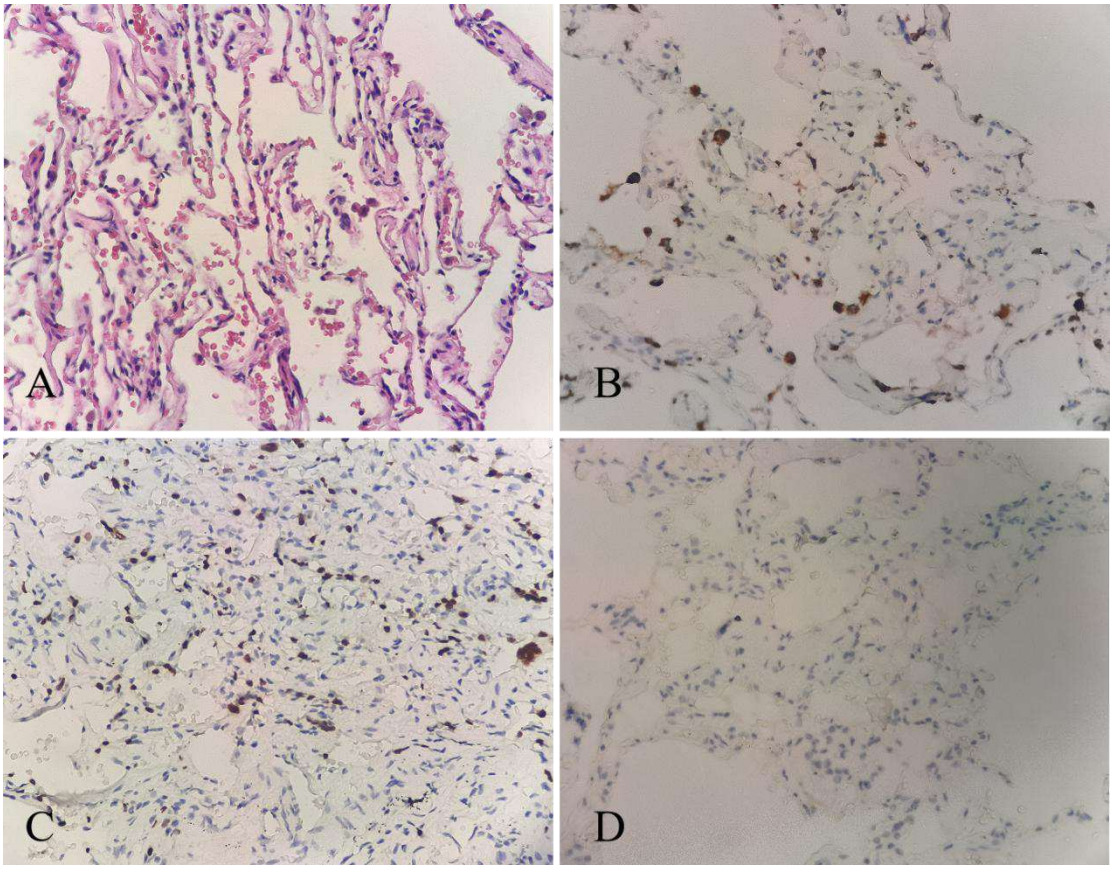


Figure 3-1: In the surgically removed normal lung tissue: A represents the HE staining of the lung tissue, B represents the NapsinA immunohistochemical staining, C represents the TTF-1 immunohistochemical staining, and D represents the AUF1 immunohistochemical staining.

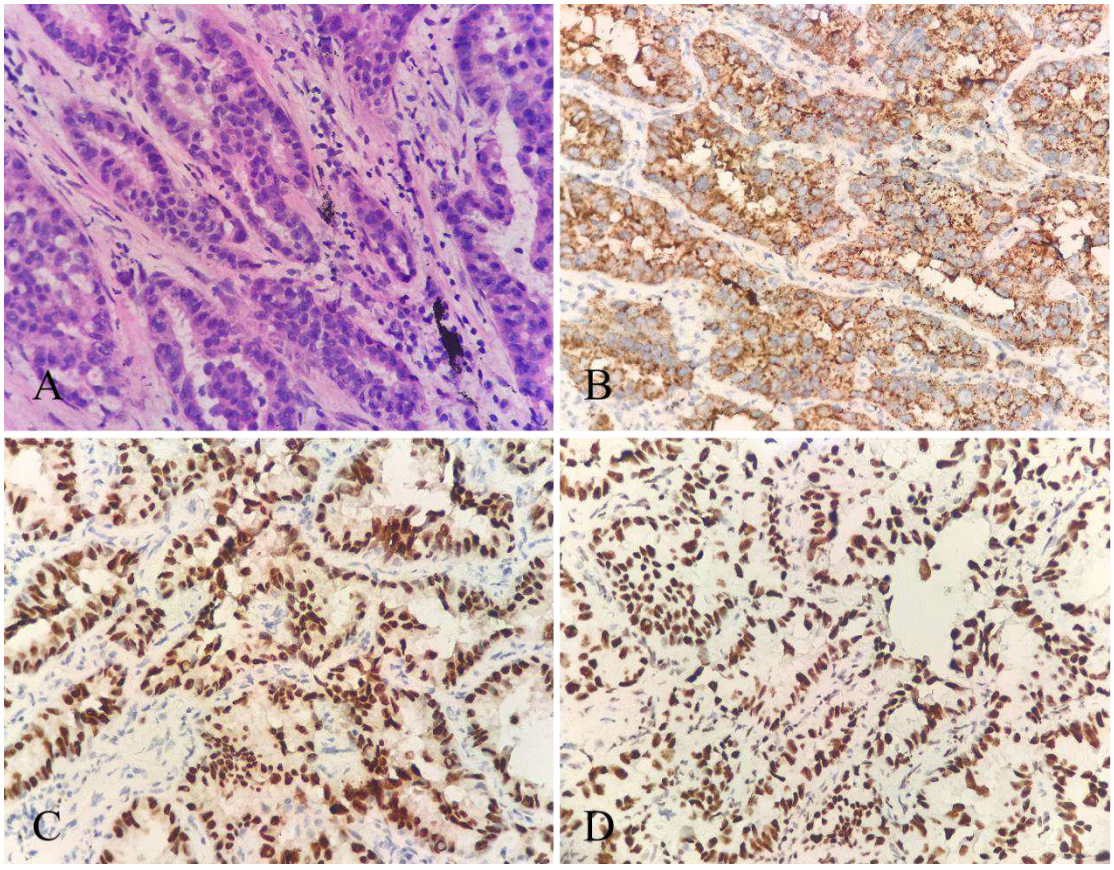



Figure 3-2: In the surgically resected lung adenocarcinoma tissue: A represents the HE staining of the lung adenocarcinoma tissue, B represents the NapsinA immunohistochemical staining, C represents the TTF-1 immunohistochemical staining, and D represents the AUF1 immunohistochemical staining.

Table 1 Comparison of AUF1 immunohistochemical results in surgical tissues from two groups of lung adenocarcinoma

| Group | n | high expression | Low expression | Positive rate | $x^2$ | $P$ |
|---|---|---|---|---|---|---|
| Observation Group | 120 | 108 | 12 | 90.00% | 210 | <0.001 |
| Control Group | 120 | 6 | 114 | 5.00% | | |

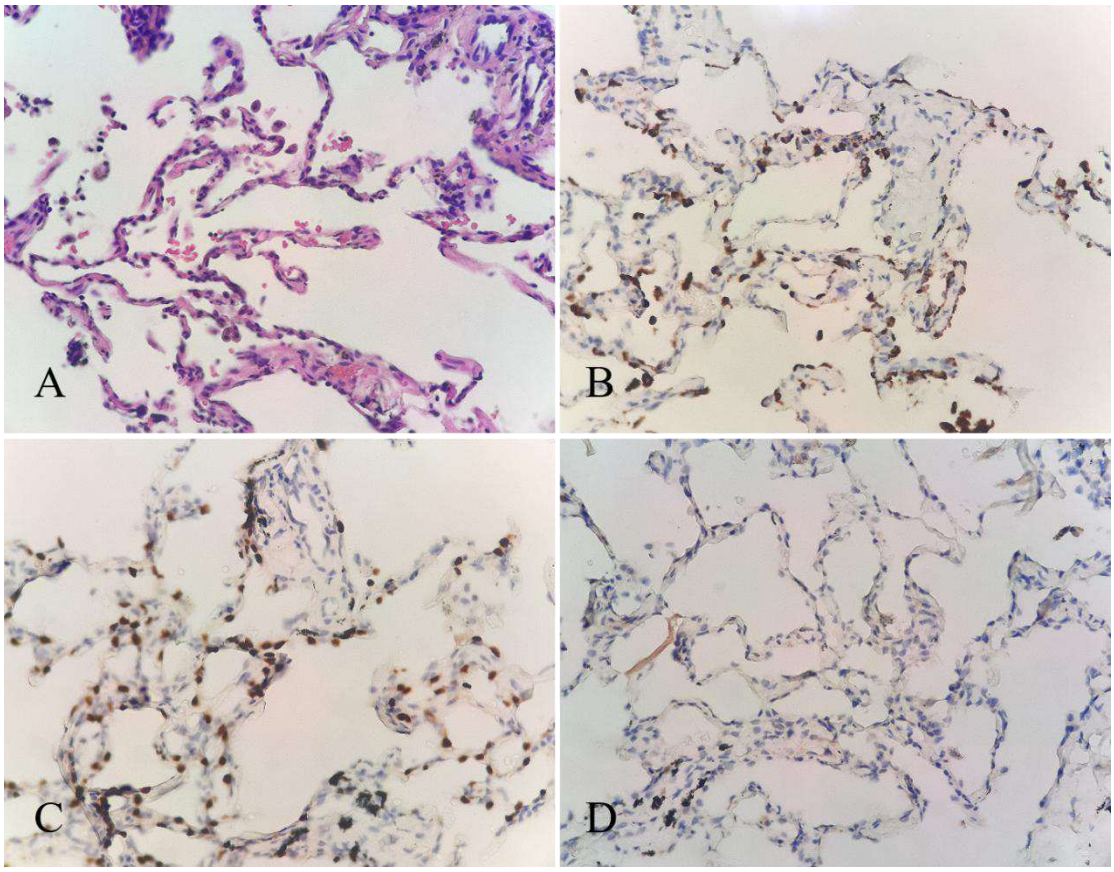



Figure 4-1: In the normal lung tissue obtained by puncture: A represents the HE staining of the lung tissue, B represents the NapsinA immunohistochemical staining, C represents the TTF-1 immunohistochemical staining, and D represents the AUF1 immunohistochemical staining.

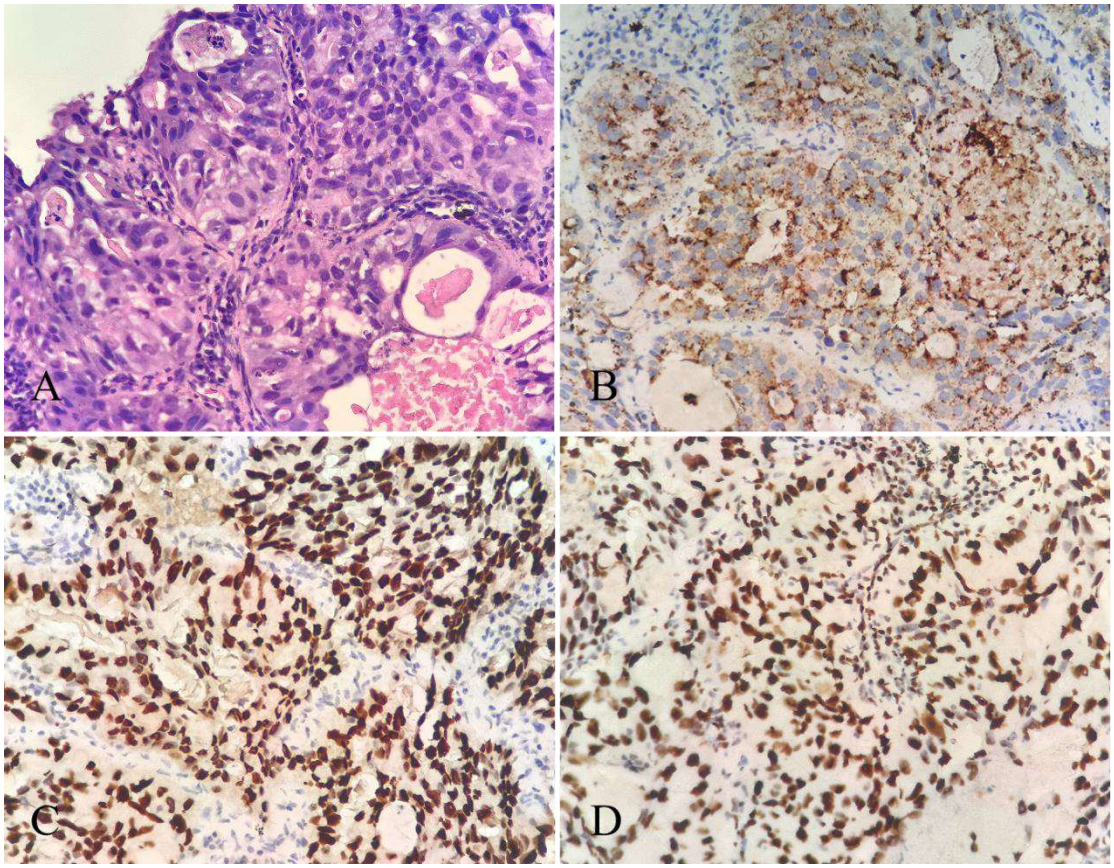



Figure 4-2: In the lung adenocarcinoma tissue obtained through biopsy: A represents the HE staining of lung adenocarcinoma, B represents the NapsinA immunohistochemical staining, C represents the TTF-1 immunohistochemical staining, and D represents the AUF1 immunohistochemical staining.

Table 2 Comparison of AUF1 immunohistochemical results in two groups of lung adenocarcinoma biopsy tissues

| Group | n | high expression | Low expression | Positive rate | $x^2$ | *P* |
|---|---|---|---|---|---|---|
| Observation Group | 50 | 41 | 9 | 82.00% | 60.03 | <0.001 |
| Control Group | 50 | 2 | 48 | 4.00% | | |

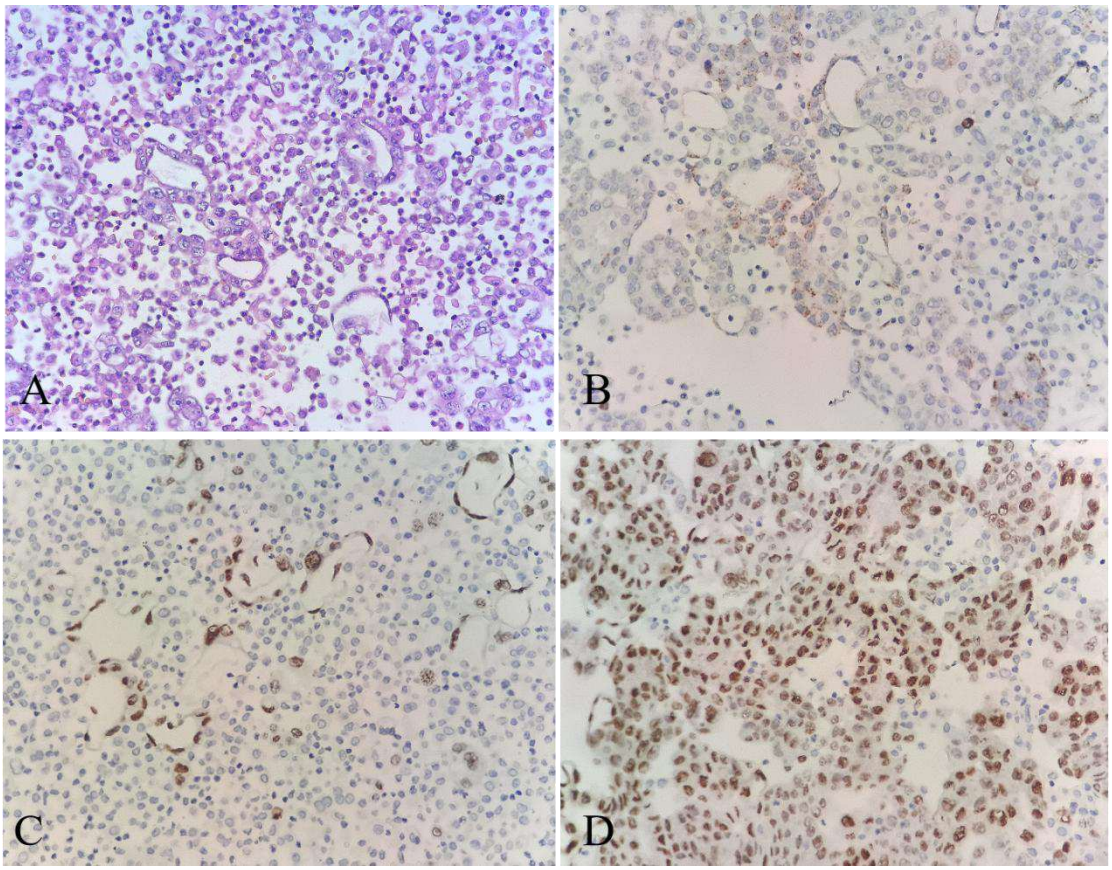



Figure 5-1: In the cell block prepared from malignant pleural effusion of lung adenocarcinoma: A represents the HE staining of lung adenocarcinoma, B represents the NapsinA immunohistochemical staining, C represents the TTF-1 immunohistochemical staining, and D represents the AUF1 immunohistochemical staining.

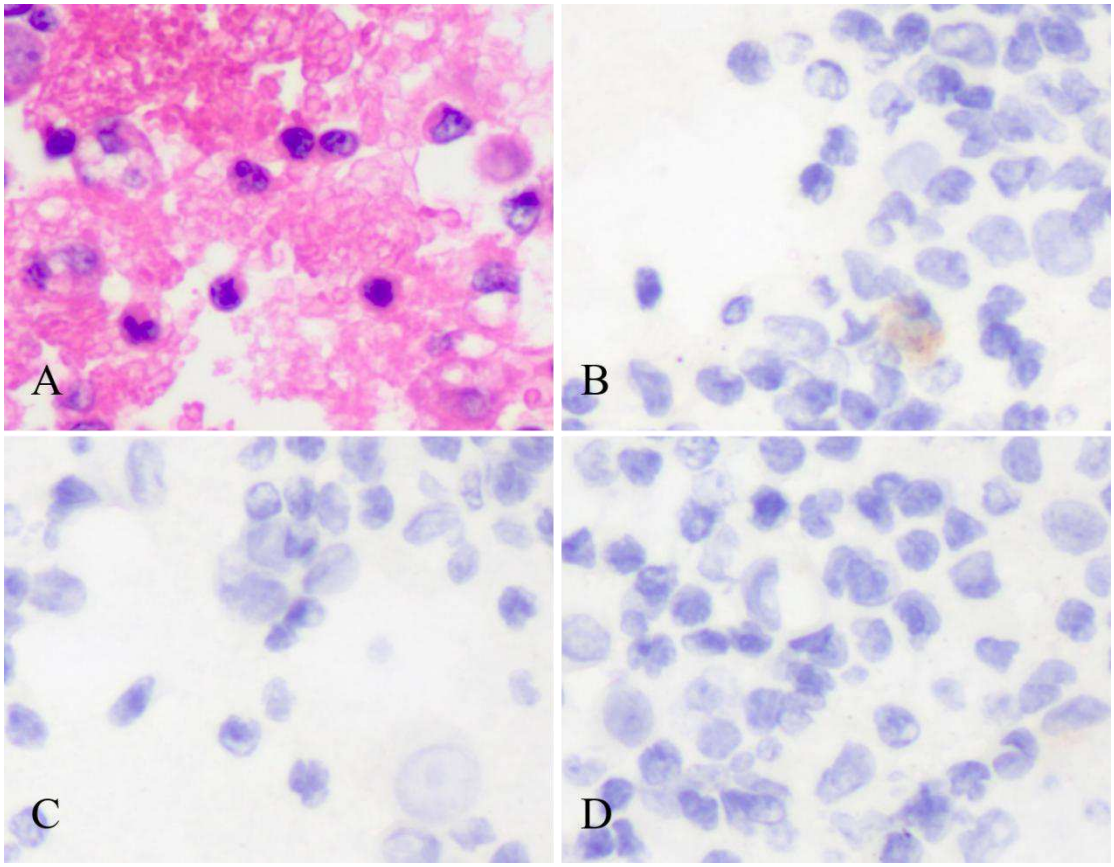



Figure 5-2: Cell blocks prepared from pleural effusion of non-tumor lung patients: A is the HE staining of lung tissue, B is the NapsinA immunohistochemical staining, C is the TTF-1 immunohistochemical staining, and D is the AUF1 immunohistochemical staining.

Table 3 Comparison of AUF1 immunohistochemical results in the pleural fluid cell blocks of the two groups

| Group | n | high expression | Low expression | Positive rate | $x^2$ | *P* |
|---|---|---|---|---|---|---|
| Observation Group | 50 | 47 | 3 | 94% | 54.23 | <0.001 |
| Control Group | 20 | 1 | 19 | 2% | | |

**3.3 The Relationship between AUF1 High Expression and Clinical Pathological Characteristics of Lung Adenocarcinoma Patients**

To verify the relationship between AUF1 and the clinical pathological characteristics of lung adenocarcinoma patients, this study found that AUF1 showed significant differences in tumor size, T classification, and pleural invasion ($p<0.05$). The specific data are shown in Table 4.

Table 4 Univariate analysis and influencing factors of AUF1 expression in lung adenocarcinoma [n (%)]

| clinical features | n | AUF1 expression | | $x^2$ | *P* |
|---|---|---|---|---|---|
| | | Low expression | High expression | | |
| Gender | | | | | |
| male | 39 | 5 | 34 | 0.51 | 0.475 |
| female | 81 | 7 | 74 | | |
| age（years） | | | | | |
| >50 | 102 | 11 | 91 | 0.466 | 0.495 |

| | | | | | |
|---|---|---|---|---|---|
| ≤50 | 18 | 1 | 17 | | |
| tumor size (cm) | | | | | |
| >1 | 61 | 10 | 51 | 5.632 | 0.018 |
| ≤1 | 59 | 2 | 57 | | |
| clinical stages | | | | | |
| I、II | 119 | 12 | 107 | - | 1* |
| III、IV | 1 | 0 | 1 | | |
| T stage | | | | | |
| T1+T2 | 62 | 10 | 52 | 5.354 | 0.021 |
| T3+T4 | 58 | 2 | 56 | | |
| N stage | | | | | |
| N0 | 93 | 10 | 83 | 0.26 | 0.61 |
| N1 | 27 | 2 | 25 | | |
| M stage | | | | | |
| M0 | 100 | 10 | 90 | 0 | 1 |
| M1 | 20 | 2 | 18 | | |
| differentiated degree | | | | | |
| Degree of differentiation | 72 | 10 | 62 | 3.024 | 0.082 |
| Poor differentiation | 48 | 2 | 46 | | |
| Pleural invasion | | | | | |
| Yes | 63 | 3 | 60 | 4.044 | 0.044 |
| No | 57 | 9 | 48 | | |

Note:The Fisher's exact test P-value for the clinical stage (I/II vs. III/IV) was 1.000. This was mainly due to the extremely small sample size of the IV stage (only 1 case), resulting in extremely low statistical power. Therefore, the reference value of this result is limited.

**3.4 Multivariate analysis of factors associated with AUF1 expression**

Multivariate logistic regression analysis was performed to identify independent factors associated with AUF1 high expression. Among the variables entered (tumor size,T stage,pleural invasion,and differentiation),T stage (T3+T4 vs.T1+T2) was identified as the sole independent risk factor (OR=9.467,95%CI:1.964-45.634,P=0.005), while tumor size,pleural invasion, and differentiation were excluded from the final model (P=0.196,P=0.446,and P=0.384,respectively) (Table 5). Collinearity diagnostics revealed no significant multicollinearity among the included variables (all VIF<10;Supplementary Table S1).To validate the robustness of our findings, we performed sensitivity analyses using alternative multivariate models with different covariate adjustment strategies;T stage remained consistently and independently associated with AUF1 high expression across all models (Supplementary Table S2).

Table 5 Multivariate logistic regression analysis (Forward LR) of factors associated with AUF1 high expression

| Variable | B | SE | Wald $\chi^2$ | *P* | OR | 95% CI |
|---|---|---|---|---|---|---|
| T stage (T3+T4 vs. T1+T2) | 2.248 | 0.802 | 7.848 | 0.005 | 9.467 | 1.964-45.634 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Constant | -2.708 | 0.571 | 22.472 | <0.001 | 0.067 | - |

### 3.5 Diagnostic Accuracy of AUF1, TTF-1 and NapsinA for Lung Adenocarcinoma

To quantitatively analyze the diagnostic accuracy of AUF1, TTF-1, and Napsin A for lung adenocarcinoma, we used ROC curves to compare the diagnostic accuracy of these three indicators. The relevant results are shown in Figures (Figures 6, 7, and 8) and Tables (Tables 6, 7, and 8). The area under the curve for AUF1 in predicting lung adenocarcinoma was greater than that of the other two indicators in all comparisons. Therefore, we believe that AUF1 has a more significant diagnostic value than TTF-1 and Napsin A.

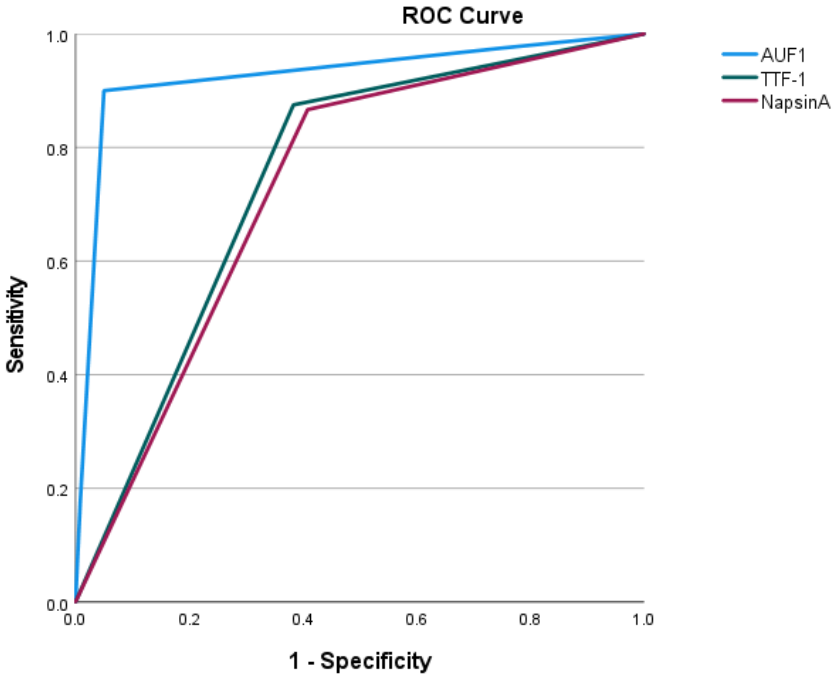



Figure 6. ROC curve analysis results of AUF1, TTF-1, and Napsin A expression in surgical specimens

Table 6. ROC curve analysis results of AUF1, TTF-1, and Napsin A expression in surgical specimens

| Marker | Area | SEa | p-value | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower | Upper |
| AUF1 | 0.926 | 0.019 | < 0.001 | 0.888 | 0.964 |
| TTF-1 | 0.746 | 0.033 | < 0.001 | 0.682 | 0.81 |
| NapsinA | 0.729 | 0.033 | < 0.001 | 0.664 | 0.794 |

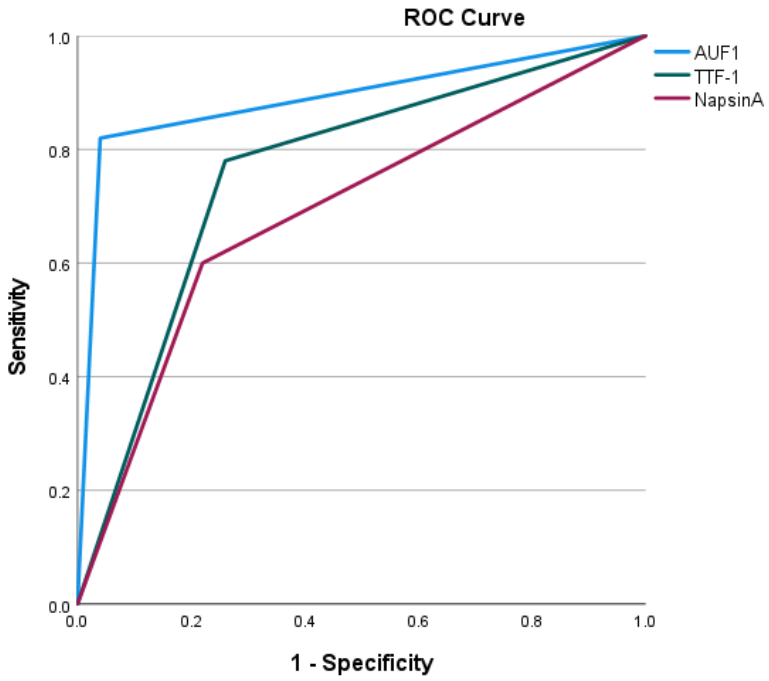



Figure 7. ROC curve analysis results of AUF1, TTF-1, and Napsin A expression in biopsy specimens

Table 7. ROC curve analysis results of AUF1, TTF-1, and Napsin A expression in biopsy specimens

| | | | | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| Marker | Area | SEa | p-value | Lower | Upper |
| AUF1 | 0.89 | 0.036 | < 0.001 | 0.819 | 0.961 |
| TTF-1 | 0.76 | 0.05 | < 0.001 | 0.663 | 0.857 |
| NapsinA | 0.69 | 0.054 | < 0.001 | 0.585 | 0.795 |

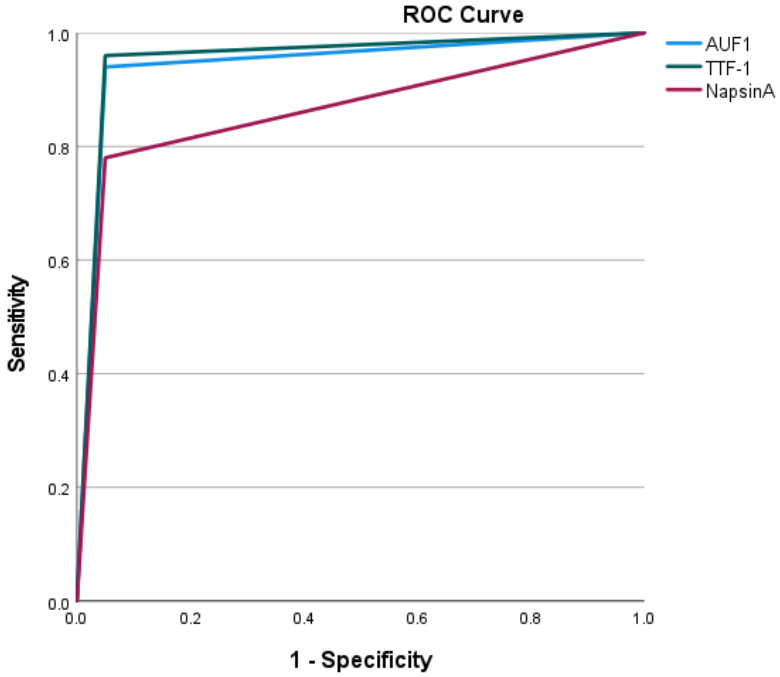



Figure 8. ROC curve analysis results of AUF1, TTF-1, and Napsin A expression in cell block specimens prepared from pleural effusion

Table 8. ROC curve analysis results of AUF1, TTF-1, and Napsin A expression in cell block specimens prepared from pleural effusion

| | | | | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| Marker | Area | SEa | p-value | Lower | Upper |
| AUF1 | 0.945 | 0.034 | < 0.001 | 0.878 | 1 |
| TTF-1 | 0.955 | 0.033 | < 0.001 | 0.891 | 1 |
| NapsinA | 0.865 | 0.046 | < 0.001 | 0.774 | 0.956 |

**4 Discussion**

The incidence and mortality rates of lung cancer are both relatively high. According to the estimation of the American Cancer Society, by the end of 2023, approximately 127,070 people may die from lung cancer, accounting for 20% of all cancer deaths[21-22]. The main histological subtype of lung cancer is lung adenocarcinoma[5]. Currently, the treatment options for patients with LUAD still mainly consist of surgical removal of the lesion, supplemented by chemotherapy and radiotherapy. However, most patients are already in the advanced stage of the disease at the time of diagnosis and have lost the opportunity for surgery. Moreover, the effects of traditional radiotherapy and chemotherapy are limited, resulting in a poor prognosis. Although targeted therapy drugs can effectively inhibit tumor proliferation and growth within a certain period after use, they often develop resistance after continuous use. Therefore, it is urgent to find new potential therapeutic targets for lung adenocarcinoma patients and to identify biomarkers for prognosis.

Serous cavity effusion cell blocks are an important component of cell pathology diagnosis and can serve as a supplement and development of cytological pathological diagnosis. The cell block

specimens, combined with immunohistochemical staining and molecular detection, help in determining the tissue origin and pathological type, and increase the positive detection rate of tumor cells. Studies have shown that the positive rate of etiological diagnosis of pleural effusion by thoracic endoscopy-guided pleural and lung biopsy is over 90%[23]. In addition, Xu Jian et al. found that compared with tissue specimens, the pleural effusion cell blocks are also a reliable pathological tissue that can be used for clinical diagnosis. When diagnosing LUAD, patients are mostly in the middle or late stage of the disease, and the body is in a cachectic state. They often cannot tolerate direct tissue biopsy at the primary lesion site. Although targeted therapy drugs can effectively inhibit tumor proliferation and growth during the initial period of use, they often develop drug resistance after continuous administration.

In recent years, various biomarkers have been discovered in pleural exfoliated cellology, such as serum lactate dehydrogenase (LDH), carcinoembryonic antigen (CEA), carbohydrate antigen 153 (CA153),and total serum cholesterol (tc) levels of adenosine deaminase (ADA)[24-26].Additionally, novel biomarkers such as reactive oxygen species regulator 1 (ROMO1), hyaluronic acid (HA), chitinase 3-like protein 1 (YKL-40), thymidine kinase 1 (TK1), pro-cathepsin D and programmed death ligand 1 (PD-L1) can enhance the diagnostic ability of pleural exfoliated cellology[8,27-31]. It is well known that TFF-1 is highly expressed in lung tissue, and Napsin A shows high specificity and sensitivity in lung adenocarcinoma[9]. Studies have shown that combining Napsin A and TTF-1 can improve diagnostic accuracy, and the combined detection of these two biomarkers can effectively differentiate lung adenocarcinoma from other types of lung cancer, and help distinguish malignant from benign pleural effusions[10-11].Therefore, it is urgent to find new potential therapeutic targets and prognostic biomarkers for patients with lung adenocarcinoma.

This study first conducted an analysis of the biological information database, revealing that AUF1 is significantly overexpressed in lung adenocarcinoma.The Kaplan-Meier survival analysis further confirmed that patients with higher expression levels of HNRNPD had significantly poorer overall survival rates, with an increased risk of death by 40% (HR=1.4,log-rank P=0.039,P(HR)=0.04). In summary, these data indicate that HNRNPD is a biomarker related to adverse clinical prognosis across the entire cancer spectrum.Notably,the opposite expression pattern in glioma and hematological malignancies suggests context-dependent dual functions of HNRNPD,which may switch between oncogenic and tumor-suppressive roles according to cell lineage and microenvironment.Further functional experiments are required to dissect distinct downstream RNA networks regulated by HNRNPD across different cancer types, and prospective clinical cohorts are warranted to validate its prognostic utility for clinical translation.The currently established biomarkers for lung adenocarcinoma include TTF-1 and NapsinA. We found that the expression of AUF1 is consistent with the expressions of lung adenocarcinoma biomarkers TTF-1 and NapsinA,suggesting that the detection of AUF1 expression has reference value for the diagnosis of lung adenocarcinoma. As a potential biomarker and therapeutic target for lung cancer, AUF1 is worthy of further research.This study first conducted an analysis of the biological information database, revealing that AUF1 is significantly overexpressed in lung adenocarcinoma.

This study found that AUF1 showed significant differences in tumor size, T classification, and pleural invasion ($p<0.05$). The above results indicate that low expression of AUF1 is more common in early-stage lung adenocarcinoma (small tumors,T1+T2 stage,no pleural invasion),and it may serve as a potential molecular marker for early diagnosis.However, due to the small number

of cases in the AUF1 low-expression group (only 12 cases),the efficacy of multivariate analysis is limited, and the conclusion needs to be verified by a large-sample prospective study.

The currently established biomarkers for lung adenocarcinoma include TTF-1 and NapsinA. We found that the expression of AUF1 is consistent with the expressions of lung adenocarcinoma biomarkers TTF-1 and NapsinA, suggesting that the detection of AUF1 expression has reference value for the diagnosis of lung adenocarcinoma. As a potential biomarker and therapeutic target for lung cancer, AUF1 is worthy of further research.

Currently, research on the role of AUF1 in tumor pathogenesis has mainly focused on tumor tissue specimens. Pleural effusion is a common clinical manifestation of advanced lung adenocarcinoma and requires differentiation from benign pleural effusion,which is crucial for determining the optimal treatment strategy. In this study, we found that AUF1 is an effective biomarker for diagnosing lung adenocarcinoma in pleural effusion cytology, with higher sensitivity for malignant pleural effusion than for non-tumor pleural effusion. Furthermore, the expression level of AUF1 in lung adenocarcinoma tissues was higher than that in corresponding adjacent normal tissues and consistent with that in malignant pleural effusion, further supporting the value of AUF1 as a reliable biomarker for lung adenocarcinoma biopsy. Regarding the quantitative comparison of the diagnostic value of AUF1, TTF-1, and Napsin A, all three markers significantly distinguished the lung adenocarcinoma group from the non-lung adenocarcinoma group. Meanwhile, we evaluated their diagnostic performance using ROC curves in surgical specimens, biopsy specimens, and pleural effusion cell blocks, and all three markers demonstrated significant diagnostic value across all specimen types (all $p<0.001$). In surgical specimens (AUC=0.926) and biopsy specimens (AUC=0.890), AUF1 exhibited the highest AUC, outperforming TTF-1 and Napsin A. In pleural effusion cell blocks,TTF-1 (AUC=0.955) and AUF1 (AUC = 0.945) showed comparable AUCs, both superior to Napsin A (AUC=0.865); however, this finding may be influenced by the relatively small sample size of pleural effusion specimens, which constitutes a limitation of the present study.

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**Author contributions**

Shigao Chen and Lu Ye: concept and design.Yulan Liu and Shuaijun Zhang: immunohistochemistry.Jinrong Chen and Zhiwei Tan: collect specimens and corresponding clinical information.Jie Hu and Sisi Li and Shuanghua Cheng: clinical data analysis.Yulan Liu: draft the manuscript.All authors have read and agreed to the published version of the manuscript.

**Data availability** The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations**

Ethics approval and consent to participate This study protocol related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Ethics Committee of the Second Affiliated Hospital of Chengdu Medical College, China National Nuclear Corporation 416

Hospital.Informed Consent for Publication: Informed consent was obtained from all individual participants included in the study for the publication of any potentially identifiable data or images presented in this manuscript. The consent forms are held by the authors and are available for review by the Editor-in-Chief if requested.

**Competing interests** The authors declare that they have no competing interests.

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**Supplementary Information**

Supplementary Table S1.Collinearity diagnostics of variables included in the multivariate logistic regression analysis.

Supplementary Table S2.Sensitivity analysis comparing different multivariate logistic regression models for factors associated with AUF1 high expression.

## Supplementary Files

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- SupplementaryTableS1.xlsx
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