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Expression and clinical significance of AURKB gene in lung adenocarcinoma

Analysis based on the data-mining of bioinformatic database

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Abstract

This study aimed to investigate the expression and clinical significance of aurora B kinase (AURKB) gene in lung adenocarcinoma (LUAD) by collecting relevant data in Oncomine database.

Firstly, mRNA expression level of AURKB in LUAD was systematically analyzed using the ONCOMINE and the cancer genome atlas databases. Then, the association between AURKB expression and clinical parameters was investigated by UALCAN. The Kaplan–Meier Plotter was used to assess the prognostic significance of AURKB.

Pooled analysis showed that AURKB was frequently up-regulated expression in LUAD. In addition, immunohistochemistry showed that AURKB was highly expressed in lung adenocarcinoma tissues, while it was weakly expressed in normal tissues. Subsequently, AURKB expression was identified to be negatively associated with Overall survival ($P < 1e{-}16$), post-progression survival ($P = .017$), first progression ($P = 9.8e{-}09$).

This study confirms that increased expression of AURKB in LUAD is associated with poor prognosis, suggesting that AURKB might be used as a promising prognostic biomarker and novel therapeutic target for LUAD.

Abbreviations: AURKB = aurora B kinase, FP = first progression, LUAD = lung adenocarcinoma, OS = overall survival, PPS = post-progression survival, TCGA = the cancer genome atlas.

Keywords: aurora B kinase gene, lung adenocarcinoma, oncomine database

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All data in this study can be obtained by proper request from the authors.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Highlights

- AURKB is frequently up-regulated expression in LUAD.
- AURKB expression was identified to be negatively associated with OS ($P < 1e{-}16$).
- AURKB expression was identified to be negatively associated with PPS ($P = .017$).
- AURKB expression was identified to be negatively associated with FP ($P = 9.8e{-}09$)

1. Introduction

Lung cancer is the most common malignant tumor, accounting for 25% of cancer-related deaths and 14% of new cancers worldwide.[1] Lung adenocarcinoma (LUAD) is the most common type of pulmonary cancer. Precision medicine based on genetic alterations is considered a new strategy of lung cancer treatment that requires highly specific biomarkers for precision diagnosis and treatment. To date, a few biomarkers have been reported to provide valuable information in guiding LUAD treatment. However, due to the lack of early detection methods, the 5-year survival rate of LUAD patients is still poor, and the

5-year survival rate is less than 15%,[2] therefore, it is essential to identify specific and sensitive molecular biomarkers for improving the diagnosis and prognosis of patients with LUAD.

Aurora kinases, consisting of Aurora A, Aurora B kinase AURKB, and Aurora C (AURKC),[3] are serine/threonine kinases essential for the onset and progression of mitosis. Amplification or overexpression of Aurora kinases is frequently found in human cancers with clear evidence of oncogenic potential, implicating Aurora kinases as rational antitumor targets.[4] AURKB is the catalytic subunit of the chromosomal passenger complex, an essential regulator of chromosome segregation.[5] Overexpression of AURKB has been reported in a variety of cancers and predicts poor overall surviva.[6]l. Research indicated that AURKB was a key gene in LUAD,[7] and AURKB overexpression has been frequently identified in lung cancer patients.[8] Moreover, Yu et al[9] has demonstrated the potency of AURKB silencing in inhibiting lung cancer cell growth. Most importantly, enhanced AURKB expression correlates with therapeutic resistance in lung cancer.[10] Furthermore, selective inhibition or knockdown of AURKB with RNA interfere leads to suppression of tumor growth, cell proliferation, induction of apoptosis and increase of the sensitivity of tumor cells to chemotherapy in lung cancer, suggesting that selective targeting of AURKB may serve as a potential therapeutic target for lung cancer.[7]

In the present study, we set out to elucidate the prognostic value of AURKB in LUAD based on intergrated analysis. Firstly, we systematically evaluated AURKB expression using ONCOMINE database and the cancer genome atlas (TCGA) data. Subsequently, the association between AURKB expression and clinical parameters were investigated.

2. Material and methods

2.1. Oncomine database

ONCOMINE (http://www.oncomine.org),[11] an online microarray database for mining cancer gene information, was used to compare the mRNA levels of AURKB between tumor and corresponding normal tissues in different types of cancer. Five datasets, including Selamat lung,[12] Landi lung,[13] Stearman lung,[14] Hou lung[15] and Su lung,[16] were used to identify the expression pattern of AURKB in LUAD. Statistical differences were determined by Student *t*-test. The main thresholds were as follows: fold change≥ 1.5; *P* value ≤ 1e-4; gene rank ≥ top 10%.

2.2. UALCAN database

UALCAN (http://ualcan.path.uab.edu) is a comprehensive and interactive web resource for analyzing cancer OMICS data (TCGA and MET500).[17] UALCAN data portal can aid in the identification of candidate biomarkers for diagnostic, prognostic and therapeutic implications. The correlation between mRNA levels of AURKB and clinicopathological features (such as tumor stages, race, age, gender) was analyzed to determine the prognostic value of AURKB in patients with LUAD. The UALCAN provided the statistical significance of all results (*P*-values), and *P*-values $<.05$ were considered statistically significant.

2.3. Human protein atlas project database

The Human Protein Atlas project (https://www.proteinatlas.org/) was launched in 2003 with the aim of creating a map of protein expression patterns in normal cells,[18] tissues and cancer. At present, 26371 unique proteins corresponding to over 50% of all human protein-encoding genes have been analysed.

2.4. Survival analysis

The Kaplan–Meier Plotter database (http://kmplot.com/analysis/) is capable to assess the effect of 54k genes on survival in 21 cancer types. The largest datasets include breast (n = 6234), ovarian (n = 2190), lung (n = 3452), and gastric (n = 1440) cancer.[19] Here, the prognostic value of AURKB in patients with LUAD was verified using Kaplan–Meier survival curve. Kaplan–Meier plots for overall survival (OS), first progression (FP) and post-progression survival (PPS) were drawed. The hazard ratio with a 95% confidence interval and log rank *P*-value were calculated to evaluate the survival difference between high and low expression groups. $P < .05$ was considered statistically significant.

2.5. Patients and samples

The study was performed on a total of 100 NSCLC patients and 100 healthy donors. Specimens were collected during biopsies and surgery from Taizhou People's Hospital. Each patient participated in the study after informed consent was provided. All specimens were histologically and blindly classified by two professional pathologists. Studies were conducted in accordance with the Declaration of Helsinki and all relevant ethical regulations for work with human participants, under an approved protocol of the Institutional Review Board of Taizhou People's Hospital.

2.6. Real-time polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted using the NucleoSpin RNA Plus kit (TaKaRa Biotechnology [Dalian] Co., Ltd., Dalian, China) in accordance with the manufacturer's protocol. RNA was reverse-transcribed to complementary DNA (cDNA) using the PrimeScript RT Reagent Kit (TaKaRa Biotechnology [Dalian] Co., Ltd.). RT-PCR analysis was performed using SYBR Green Master Mixture reagent (Takara Bio, Inc., Kusatsu, Shiga, Japan) and an ABI 7500-Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA). The primers used for RT-PCR, Aurora-B:F-5'-AGAAGGAGAACTCCTACCCCT-3',R-5'-CGCGTTAAGATGTCGGGTG-3'. GAPDH:F- 5'-GTGGACATCCGCAAAGAC-3', R-5'-GAAAGGGTGTAACGCAACT-3.

2.7. Statistical analysis

Data are presented as the mean ± standard deviation. All statistical analyses were performed using Prism 7.0 software (GraphPad Software, Inc., La Jolla, CA). The gene expression data was normally distributed.[20] Student *t*-test and one-way analysis of variance were used to analyze two groups and more than two groups, respectively. $P < .05$ was considered statistically significant.

3. Results

3.1. Transcriptional expression levels of AURKB in LUAD

To address the mRNA expression of AURKB in multiple human cancers, the expression of AURKB in 20 different cancer types

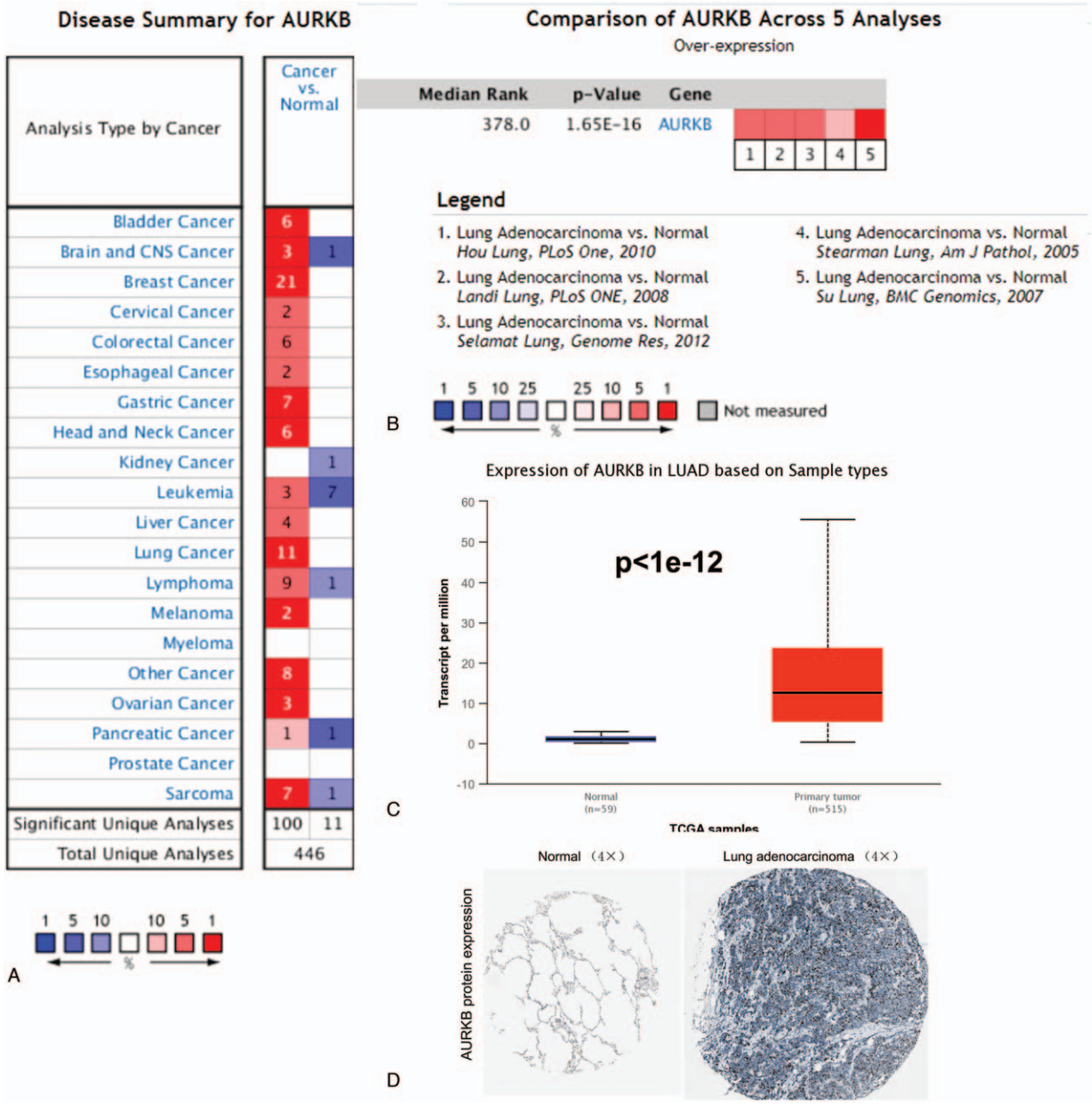

Figure 1. mRNA expression of AURKB in LUAD. (A)The expression of AURKB in 20 different cancers was evaluated using ONCOMINE database. Red represents up-regulated expression, and blue color for down-regulated expression. Cell color is determined by the best gene rank percentile for the analyses within the cell. (B) The pooled comparison of AURKB expression across seven analysis in LUAD. The rank for a gene is the median rank for that gene across each of the analyses. The P-value for a gene is its P-value for the median-ranked analysis. (C) Box plot showing mRNA levels of AURKB in LUAD and normal control from TCGA database. (D) The human project atlas project shows representative immunohistochemical images of AURKB in lung adenocarcinoma tissues compared with noncancerous tissues.

were assessed with ONCOMINE database. As shown in Fig. 1A, mRNA expressions of AURKB were significantly up-regulated in most human cancer patients except for kidney cancer and leukemia. Subsequently, we systematically analyzed mRNA expression levels of AURKB in LUAD. As shown in Table 1, our finding revealed that mRNA levels of AURKB were significantly higher in LUAD patients with a fold change of 1.5~3.5. By the comparison of AURKB expression across five datasets, pooled analysis also demonstrated that AURKB was over-expressed in LUAD (Fig. 1B; $P = 1.15e{-}16$). Similar results also were confirmed from TCGA data (Fig. 1C, $P < 1e{-}12$). In addition, the results of immunohistochemistry from Human Protein Atlas Project database showed that AURKB was highly or moderately expressed in lung adenocarcinoma tissues, while it was weakly or negatively expressed in normal tissue (Fig. 1D).

In order to verify the highly mRNA expression of AURKB in LUAD patients, we collected the lung tissues of 100 LUAD patients and 100 non-cancer patients. The RT-PCR results showed that the AURKB mRNA expression in LUAD patients was significantly higher than that of healthy donors (Fig. 1E). Moreover, there was no statistically significant difference in the expression level of AURKB in patients of different genders, ages

Table 1

significant changes of AURKB expression in LUAD using the ONCOMINE database.

NO	Datastes	Cases (Normal/LUAD)	Fold change	*t*-test	*P*-value
1	Hou lung	65/45	2.883	9.752	7.00e-14
2	Landi lung	49/58	1.526	8.102	5.35e-12
3	Selamat lung	58/58	2.449	11.037	1.65e-16
4	Stearman lung	19/20	2.097	5.136	1.17e-5
5	Su lung	30/27	3.442	6.413	2.07e-8

AURKB = aurora B kinase, LUAD = lung adenocarcinoma.

Table 2

The relationship between clinical features and AURKB expression.

Clinical characteristics		Cases	mRNA expression	F/t	*P*
Gender	Male	60	199.75 ± 38.91	0.095	.435
	Female	40	193.50 ± 39.35		
Age	<65	47	199.05 ± 37.10	1.327	.666
	≥65	53	195.65 ± 40.91		
LNM	YES	58	213.96 ± 38.60	3.307	**.000**
	NO	42	174.17 ± 26.83		
TNM	I/II	51	194.29 ± 38.54	2.299	.106
	III	21	187.64 ± 39.62		
	VI	28	209.83 ± 37.58		

AURKB = aurora B kinase, LNM = lymph node metastasis, TNM = tumor lymph node metastasis.

and tumor lymph node metastasis by *t* test ($P > .05$), but patients with LNM have the higher AURKB expression ($P < .05$, Table 2). Collectively, our finding suggests that AURKB might play an important role in the development of LUAD.

3.2. Relationship between AURKB expression and clinical parameters in LUAD

Subsequently, we explored the relationship between AURKB expression and clinicopathological features, such as tumor stages, lymph node metastasis, smoking, gender, race and age in patients with LUAD. As shown in Fig. 2, the results revealed that AURKB expression was found to be positive associated with tumor stage and lymph node metastasis (Fig. 2A and B). However, there was no relationship between AURKB expression and other clinical features such as age (Fig. 2C) and race (Fig. 2D). In addition, the expression of AURKB was observed to significantly elevate in male patients and TP53 mutation patients (Fig. 2E and G), and the mRNA levels of AURKB in smoking patients were apparently higher than that in no smoker (Fig. 2F). Taken together, our

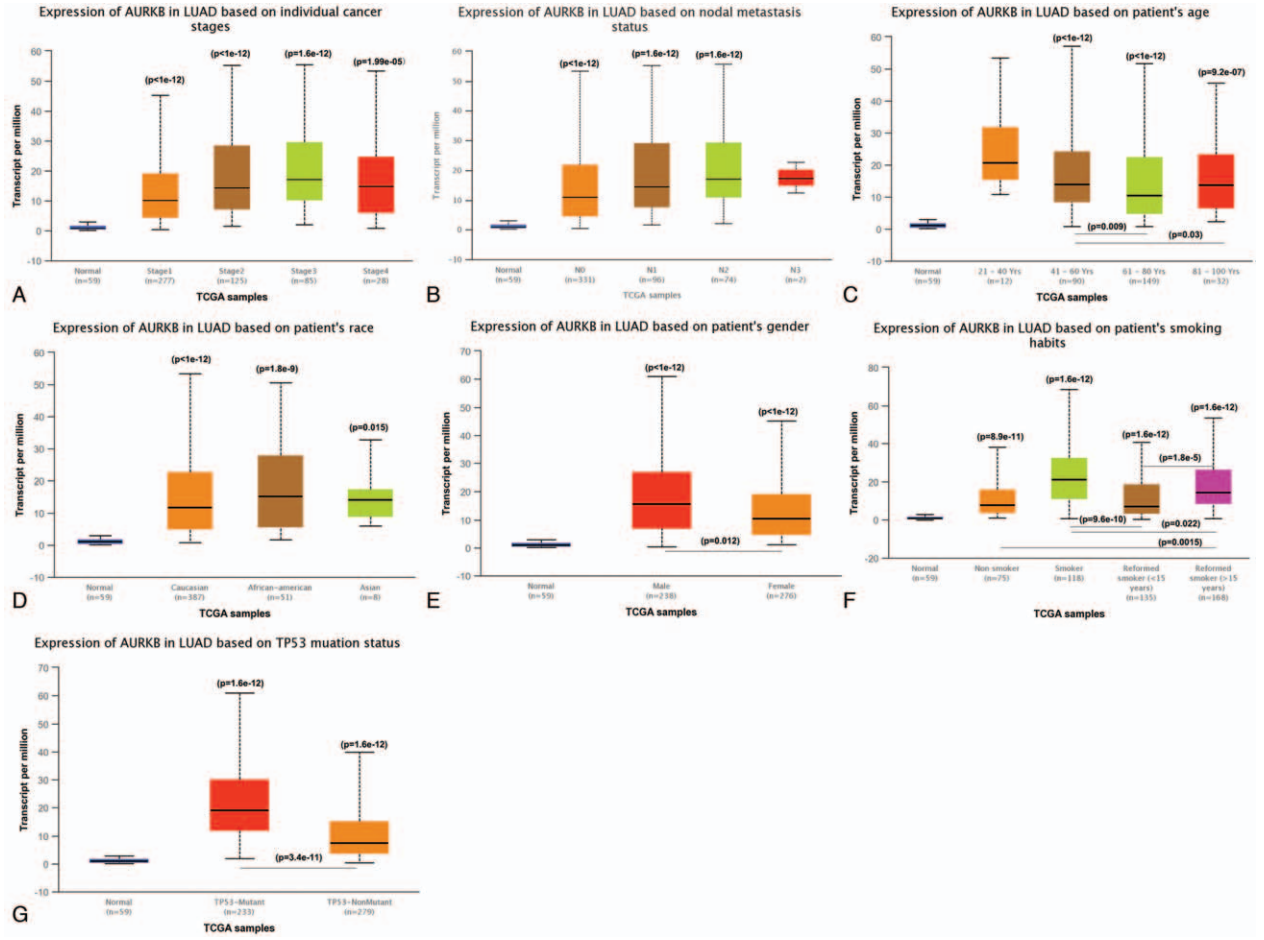

Figure 2. Box plots showing expression of AURKB mRNA in LUAD based on clinical parameters. The UALCAN database was used to assess the relationship between AURKB expression and clinical parameters such as (A)cancer stages, (B) lymph node metastasis status, (C) age, (D) race, (E) gender, (F) smoking habits and (G) TP53 mutation.

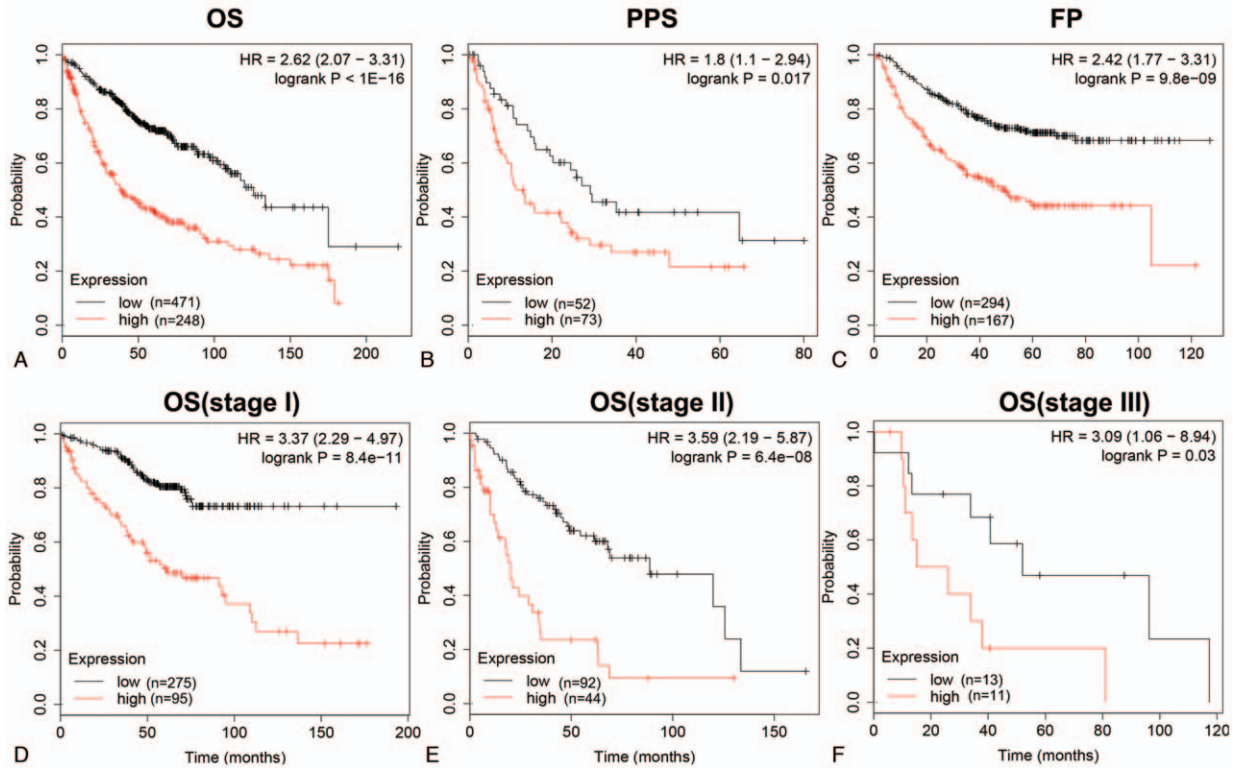

Figure 3. Survive curves evaluating the prognostic value of AURKB mRNA expression in LUAD. Patients with LUAD were split into high and low groups based on the best cutoff expression value of AURKB, (A) OS, (B) PPS and (C) FP curves were generated using the Kaplan–Meier Plotter. In addition, the relationship between mRNA levels of AURKB and survival was further evaluated in (D) stage I, (E) stage II and (F) stage III, respectively. *P*-values were calculated by log-rank test. HRs with 95% CIs are displayed. FP = first progression.

results indicated that increased AURKB might be associated with poor prognosis in LUAD.

3.3. Prognostic value of AURKB in LUAD

To further evaluate the prognostic value of AURKB, the relationship between mRNA expression of AURKB and clinical outcome was assessed using Kaplan–Meier plotter.

As shown in Fig. 3A–C, Kaplan–Meier plot revealed that over-expressed AURKB was significantly associated with worse OS (HR = 2.66; 95% CI [2.07-3.31]; $P < 1e{-}14$), PPS (HR = 1.8; 95% CI [1.1–2.94]; $P = .017$) and FP (HR = 2.42; 95% CI [1.77-3.31]; $P = 9.8e{-}09$). The tumor lymph node metastasis staging is still one of the powerful survival predictive factors. therefore, we evaluated the relationship between mRNA levels of AURKB and survival in the same staging state. The results also indicated that over-expressed AURKB was significantly associated with worse OS in the same staging state (Fig. 3D–F). Collectively, our finding suggested that over-expressed AURKB were significantly associated with poor prognosis in LUAD.

4. Discussion

At present, a limited number of studies have investigated the prognostic significance of AURKB in LUAD. To further elucidate the prognostic value of AURKB for LUAD, in the present study, we systematically analyzed the expression of AURKB in LUAC using the ONCOMINE and TCGA databases, our result confirmed that AURKB was over-expressed in patients with LUAD (Fig. 1; Table 1). Subsequently, we evaluated whether over-expression of AURKB was associated with clinicopathological features and survival outcomes for LUAD patients. In agreement with a previous study,[21] our current finding revealed that high-expression of AURKB was positively correlated with tumor stage and lymph node metastasis in LUAD (Fig. 2). Meanwhile, the survival analysis further confirmed that over-expressed AURKB was significantly associated with worse OS, FP and PPS (Fig. 3).

The AURKB expression in the lung tissues of 100 LUAD patients was significantly increased (Fig. 4), which was consistent with the database analysis. However, the result of the analysis of the relationship between clinical features and AURKB expression was not consistent with the database analysis. In database, AURKB expression was found to be positive associated with tumor stage and lymph node metastasis, while the results of clinical tissues showed that the expression of AURKB expression was only positive correlated with lymph node metastasis. In future, the more sample should be collected for further analysis and confirmation.

Research showed that p53 was a substrate of AURKB and that AURKB directly interacted with p53. AURKB phosphorylated and instigated degradation of p53 to block p53 function, thereby preventing cell apoptosis.[22] Yu et al[23] observed that AURKB could inhibit p53-related DNA damage response and apoptotic pathway in NSCLC. Furthermore, AURKB could phosphorylate MYC and CREPT/RPRD1B respectively to prevent MYC

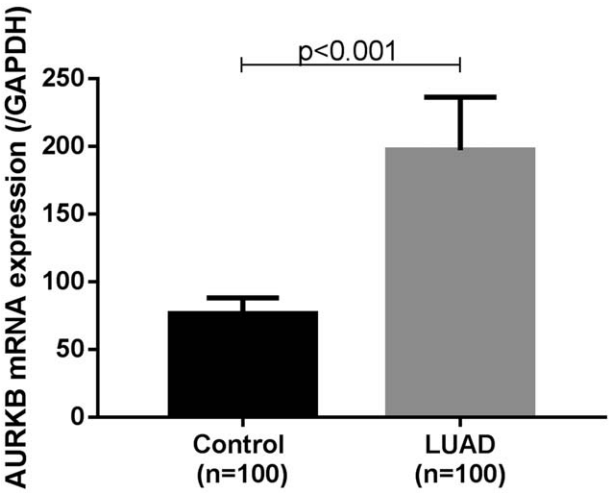

Figure 4. mRNA expression of AURKB in LUAD patients and health donors. The AURKB mRNA expression was detected by RT-PCR in the lung tissues of 100 LUAD patients and 100 non-cancer patients.

degradation and promote the transcription of Cyclin B1. Myc and cyclin B1 are the key regulators of leukemia and gastric cancer, respectively. Whether AURKB can promote the process of lung cancer through MYC or CREPT/RPRD1B remains to be further studied.

In conclusion, our study confirmed that increased expression of AURKB in LUAD is associated with poor prognosis, which suggested that AURKB might be a promising prognostic biomarker and novel therapeutic target for LUAD.

Author contributions

XYG conducted most of the experiments and wrote the manuscript; AGJ, YHS and RC conducted the experiments and analyzed the data, HYL designed the study and revised the manuscript.

All authors have read and approved the manuscript.

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