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The expression of POSTN and immune cell infiltration are prognostic factors of lung adenocarcinoma

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Abstract

The objective of this study is to identify useful prognostic factors of lung adenocarcinoma (LUAD) by evaluating the changes of periostin (POSTN) expression activity and immune cell infiltration using public data repositories. The gene and protein expressions of POSTN in LUAD were collected and analyzed from Ualcan and Human Protein Atlas online database. The cell infiltration data of immune cells in LUAD patients were retrieved and processed using the TIMER tool. Cox regression analysis was employed to screen and characterize prognosis factors, Kaplan–Meier Plotter was used to analyze the survival curve of LUAD patients, and receiver operating characteristic curve was used to calculate the relationship between temporal POSTN expressions and the prognosis of LUAD. The expression activity of POSTN in LUAD was higher than that in normal tissues. With the exception of B cell which showed opposite correlationship, the infiltration of most immune cells, namely $CD8^+$ T cells, macrophages, neutrophils, and dendritic cells, was positively correlated with the POSTN expression activity. Together, our investigation suggested that the POSTN expression activity and B-cell infiltration were the prognostic factors of LUAD. In addition, the 1-year negative predictive value of the POSTN expression activity for overall survival, disease-free survival, and progression-free interval was 0.902, 0.926, and 0.838, respectively. Along with decreased B-cell infiltration, the elevated expression of POSTN is an important factor of LUAD prognosis.

Abbreviations: CAFs = cancer-associated fibroblasts, ECM = Extracellular matrix, LUAD = lung adenocarcinoma, OS = overall survival, POSTN = Periostin, RFS = recurrence-free survival, ROC = receiver operating characteristic curve, TCGA = The Cancer Genome Atlas.

Keywords: bioinformatics, expression, lung adenocarcinoma, periostin, prognosis

1. Introduction

Lung adenocarcinoma (LUAD) is a subtype of non-small cell lung cancer. The vast majority of LUAD are derived from small bronchial mucosa epithelia, while only few are from the mucous glands of the larger bronchus. Compared with lung squamous cell carcinoma, which is a prevalent subtype of non-small cell lung cancer, LUAD is more likely to occur in women and non-smokers. In addition, LUAD is characterized with rapid early metastasis, high degree malignancy, and high mortality. Reports have been shown that many confirmed LUAD patients lost their surgical opportunity when they were lately identified at advanced stages with a poor prognosis,[1,2] strengthening the urgent need of useful biomarkers for diagnosis and prognosis in LUAD.

The extracellular matrix (ECM) is a dynamic structural network that can regulate the activity of extracellular signaling molecules, as well as cell function by directly activating signaling pathways through binding to receptors on the cell surface, which is an essential part of the tissue microenvironment. Matricellular proteins can regulate the microenvironment,[3] the behavior of surrounding cells, and the homeostasis of tissues. Increasing evidence shows that matricellular proteins play a vital role in the occurrence and development of tumors.

Periostin (POSTN) is an ECM secreted protein, the expression pattern of which is periodontal and periosteum-specific in mice. POSTN can bind to many ECM proteins through its different domains. POSTN usually presents at low levels in most adult tissues but is highly expressed in pathological sites such as in tumors and inflamed organs.[4] Oncological studies have shown that POSTN is expressed in a variety of tissues and is involved in the incidence of gastric cancer,[5] breast cancer,[6] and prostate cancer.[7] However, whether and how POSTN is associated with LUAD are still ambiguous.

In this paper, we obtained the POSTN expression-related data from The Cancer Genome Atlas (TCGA) database and analyzed the expression of POSTN and immune cell infiltration in the pathogenesis and prognosis of LUAD to present

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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their correlations, providing potential prognostic markers of LUAD.

2. Materials and Methods

2.1. Analysis of POSTN expression

RNA-seq data related to Htseq-FPKM (fragments per kilobase per million) were downloaded from TCGA database. Fragments per kilobase per million was converted to transcripts per million. All data were filtered and transformed into log2 before analysis. The samples included 535 tumor tissues, 288 normal tissues, and 57 paired samples from bronchus and lung tissue, and were pathologically diagnosed as LUAD. R language (base package and ggplot2 package) was used to analyze gene expressions. Ualcan web (http://ualcan.path.uab.edu/index.html) and Human Protein Atlas online database (https://www.proteinatlas.org/) were used to analyze POSTN protein expression in LUAD and normal tissues. The above-mentioned public databases are in the framework of proper ethical approval. We declared non ethical issues or conflicts of interest when the public data were included in this study. Besides, 57 cases of LUAD tissues and 21 cases of normal tissues were collected between May 2019 and November 2020. All specimens were fresh and were pathologically diagnosed. In accordance with the Declaration of Helsinki, this study was approved by the ethics committee of XiangZhou Hospital.

Total RNA were isolated from tissues with TRIzol (Thermo Fisher Scientific, Inc., USA, MA) before reverse transcription to synthesize cDNA with test kit (Thermo Fisher Scientific, Inc.). Reverse transcription-quantitative polymerase chain reaction was used to analyze POSTN gene expression in LUAD tissues and normal tissues using the following primers: 5′-GAAGGTGAAGGTCGGAGTC-3′ (forward), 5′-GAAGATGGTGATGGGATTTC-3′ (reverse) for the internal reference gene *GAPDH*; and 5′-GCCATCACAT CGGACATA-3′ (forward), 5′-CTC CCA TAA TAG ACT CAG AAC A-3′ (reverse) for *POSTN*. The thermocycling conditions were as follows: 95°C denaturation for 5 minutes, 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 45 seconds with 35 cycles. POSTN expression levels were normalized to GAPDH and were determined using the $2^{-\Delta\Delta Ct}$ method.

2.2. Univariate/multivariate Cox regression

Clinical data related to LUAD were downloaded from TCGA database. To define prognostic factors in LUAD, the TNM stage, pathological stage, age, gender, smoking history, and POSTN expression data were included to construct Cox regression model using the survival package of R (http://www.r-project.org/).

2.3. Analysis of prognostic markers

The prognostic scan database (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html) and Kaplan–Meier Plotter database (https://kmplot.com/analysis/) were used to analyze the relationship between POSTN expression and a variety of prognostic markers, including overall survival (OS) and recurrence-free survival (RFS). R packages (time ROC and ggplot2) were used to predict prognostic indicators such as OS, disease-free survival, and progression-free interval at 1, 3, and 5 years.

2.4. Analysis of immune cell infiltration

The TIMER (http://timer.cistrome.org/) tool (https://cistrome.shinyapps.io/timer/) was used to analyze the correlation between POSTN expressions and immune cell infiltration through Gene module. Survival model was executed to construct Cox regression models of POSTN expressions and different types of immune cell infiltration to present the influence of immune cell infiltration on the prognosis of LUAD. The correlation between POSTN expression and cancer-associated fibroblasts (CAFs) was analyzed through Outcome module.

2.5. Statistical analysis

Statistical calculations were performed using SPSS (SPSS Inc., Chicago, IL) software version 16.0. $P < .05$ was considered to indicate a statistically significant difference. The measured data were expressed as mean ± standard deviation. A Student *t* test was compared using the 2-sided data log-rank method.

3. Results

3.1. The expression activities of POSTN among tissues

We first compared gene transcriptional expression activities of POSTN in LUAD and normal tissues (Fig. 1A and B), demonstrating that gene expression levels of POSTN in cancer tissues were significantly higher than that in normal tissues in both paired samples and nonpaired samples. In consistence, the Ualcan tool was used to analyze the differential expression of proteins in cancer tissues and normal tissues, showing that POSTN protein expression levels in cancer tissues were higher than that in normal tissues (Fig. 1C). Immunohistochemical images in Human Protein Atlas database were used to confirm the differential protein expressions of POSTN among tissues (Fig. 1D and E).

At the same time, we examined gene expression levels in cancer and normal tissues to recognize the relationship between POSTN gene expressions and clinicopathological features. The expression levels in cancer tissues and normal tissues were 0.45 ± 0.27 and 0.18 ± 0.10, respectively, with significant differences ($P < .001$). However, we observed no significant differences between gene expression and age, gender, smoking, clinical stage, differentiated degree, and other parameters..

3.1. Prognostic factors analysis of LUAD

To identify prognostic factors of LUAD, we simulated univariate/multivariate Cox regression model using clinical data and POSTN expression data. Univariate regression analysis suggested that the TNM stage, pathological stage, and POSTN gene expressions were related to OS in LUAD (Fig. 2A), while multivariate regression analysis supported that the POSTN gene expression was the one closely related to OS (Fig. 2B).

3.2. Survival curve analysis

We looked into the gene expression levels of POSTN related to the 2 prognostic indicators, OS and RFS using the Kaplan–Meier Plotter database. The results showed that in comparison with that in the high POSTN expression group, the values of both OS and RFS were constantly higher in the low POSTN expression group of LUAD patients (Fig. 3A and B). In consistence, we employed PrognoScan database to reevaluate OS and RFS in the prognosis of LUAD, showing that the values of RFS of patients with low POSTN expression was constantly higher and vice versa (Fig. 3C). Totally, these results suggested that the expression levels of POSTN might be a useful prognostic indicator to LUAD patients.

Next, we used ROC curve to evaluate the demonstration of POSTN expression activities in LUAD diagnosis and prognosis prediction. First, we analyzed the diagnostic value of POSTN expression in LUAD (Fig. 4A), showing that the parameter values of area under the curve, sensitivity, specificity, positive predictive value, and negative predictive value were 0.792, 0.763, 0.718, 0.230, and 0.965, respectively. Meanwhile, time-dependent ROC was used to analyze the value of POSTN expressions in predicting 1-, 3-, and 5-year prognostic indicators (Fig. 4B–D and Table 1), indicating that POSTN expressions represent a

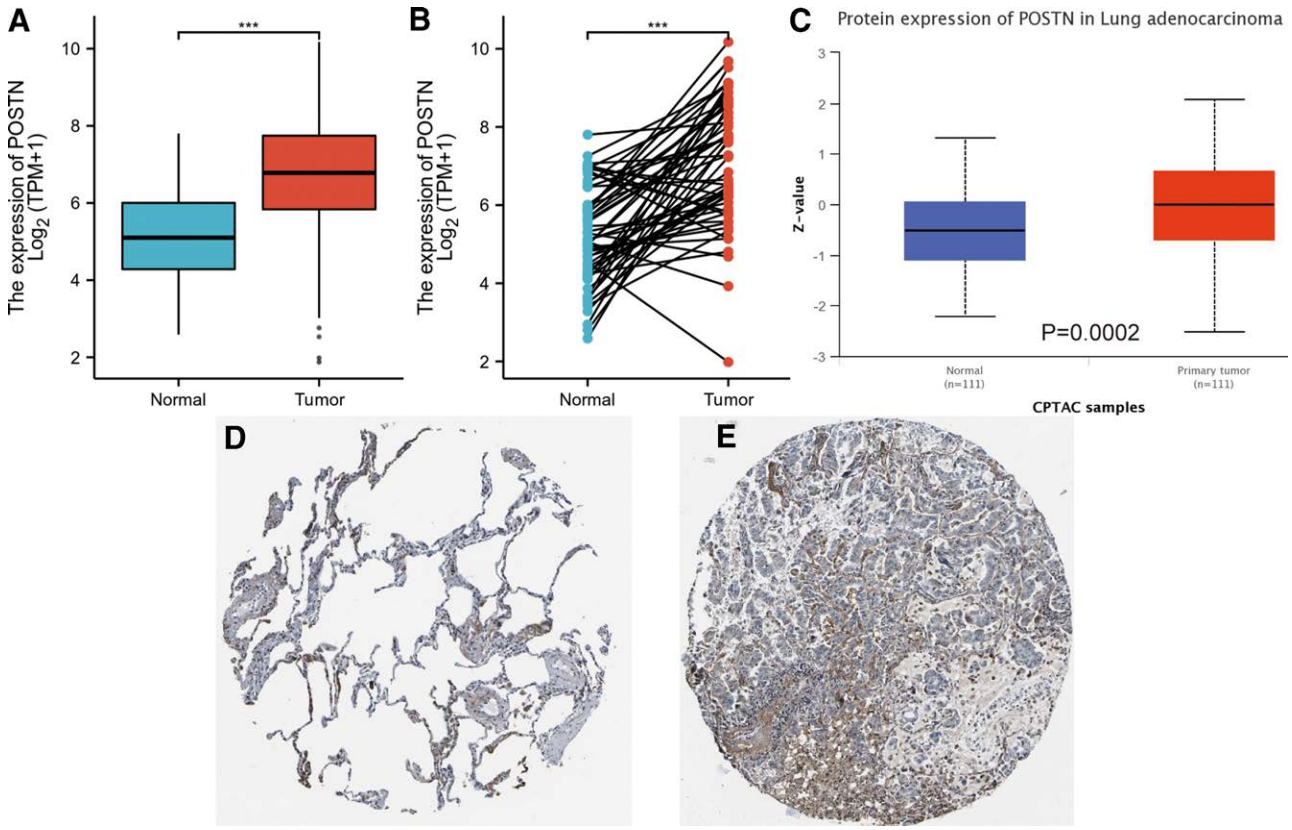

Figure 1. Expression analysis of POSTN in LUAD. Analysis of gene expression patterns in nonpaired samples (A) and paired samples (B). (C) Analysis of POSTN protein expression. (D) Immunohistochemical analysis of POSTN protein accumulations. ***P < .001. LUAD = lung adenocarcinoma, POSTN = periostin.

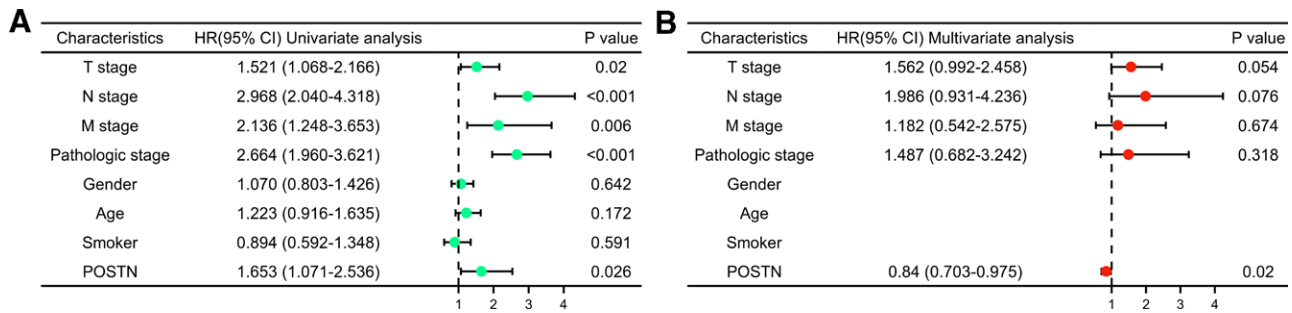

Figure 2. Univariate/multivariate Cox regression analysis of prognostic factors. (A) Single factor analysis and (B) multi-factor analysis.

certain value in diagnosing and predicting 1-year prognostic indicators, especially in terms of NPV.

3.3. Immune cell infiltration analysis

The TIMER is an online analysis tool to analyze gene expression, gene correlation, immune cell infiltration, etc. We first constructed a Cox regression model using age, gender, race, pathological stage, and POSTN expression to recognize prognostic factors (Table 2). Again, the POSTN expression was found to be a prognostic factor for LUAD patients. Then the relationship between the POSTN expression and prognosis was analyzed (Fig. 5), and it was consistently found that the survival time of patients with low POSTN expression was longer than that of patients with high expression.

In addition to POSTN, the infiltration of immune cells play an important role in development, progression, and prognosis of tumors. We then analyzed the correlation between the POSTN gene expression and immune cell infiltration (Fig. 6A) and found that regardless of $CD4^+$ T cells, other 5 immune cell infiltration were correlated with the POSTN expression. To be specific, the B-cell infiltration was negatively correlated with the POSTN expression, although cell infiltrations of macrophages, neutrophils, $CD8^+$ T cells, and dendritic cells were positively correlated with the POSTN expression. Cox regression model was further constructed by combining the data of different types of immune cells and gene expressions (Table 3). It was found that B cells, CD4 T cells, and the POSTN gene expression were the affecting factors of prognosis in LUAD patients. We then divided patients into high expression group and low expression group according to gene expression levels of POSTN. Similarly, we divided patients into high-level group and low-level group according to the levels of the immune cell infiltration, and drew the survival curve (Fig. 6B). It was found that the high-level B-cell infiltration in the low POSTN expression group had a longer survival time, and the high-level B-cell infiltration in the high POSTN expression group also had a higher survival time. In addition, we analyzed the correlation between CAFs infiltration

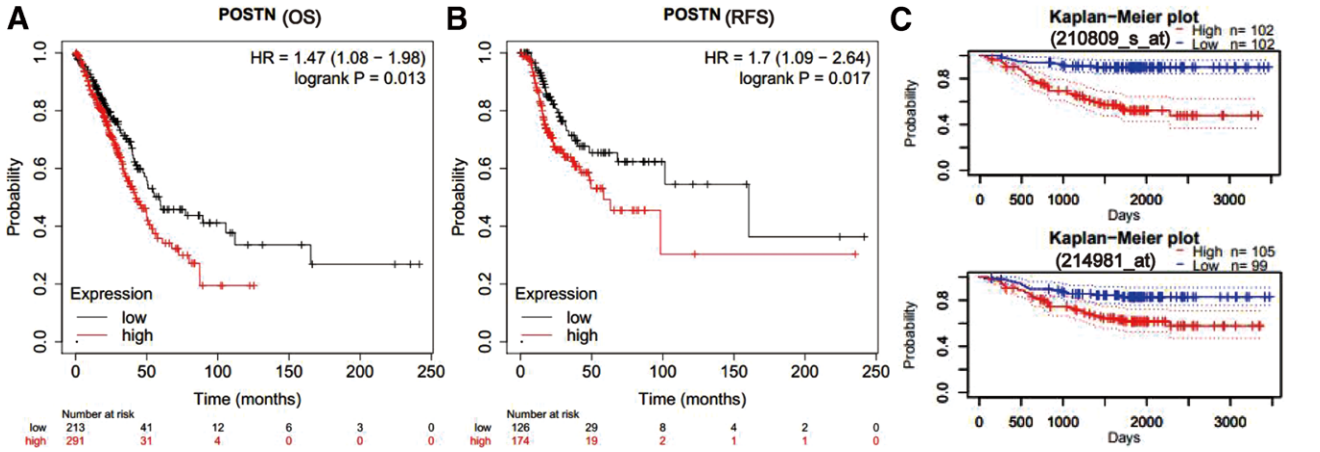

Figure 3. K-M survival curve of LUAD patients. (A) Analysis of OS by K-M plotter database. (B) Analysis of RFS indicators by K-M plotter database. (C) Analysis of RFS indicators by PrognoScan database. K-M plotter = Kaplan–Meier plotter, LUAD = lung adenocarcinoma, OS = overall survival, RFS = recurrence-free survival.

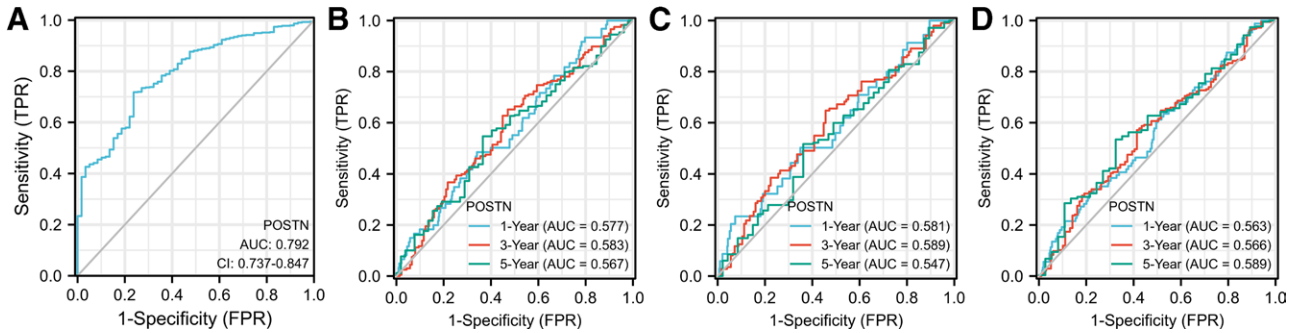

Figure 4. ROC curve analysis of the value of POSTN in diagnosis and prognosis prediction. (A) Diagnostic value; (B) predictive value in OS; (C) predictive value in DFS; (D) predictive value in PFI. DFS = disease-free survival, OS = overall survival, POSTN = periostin, ROC = receiver operating characteristic.

Table 1

ROC analysis of the relationship between POSTN expression and prognosis of LUAD at different times.

	OS				DFS				PFI			
Time(yr)	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
1	0.485	0.659	0.164	0.902	0.234	0.926	0.207	0.936	0.328	0.788	0.26	0.838
3	0.651	0.529	0.453	0.717	0.647	0.544	0.378	0.783	0.571	0.586	0.6	0.557
5	0.546	0.635	0.683	0.493	0.516	0.638	0.528	0.628	0.534	0.676	0.743	0.452

DFS = disease-free survival, NPV = negative predictive value, OS = overall survival, PFI = progression-free interval, POSTN = periostin, PPV = positive predictive value, ROC = receiver operating characteristic curve.

Table 2

Cox model analysis of prognostic factors.

	HR	95%CI_l	95%CI_u	*P*
POSTN	1.156	1.027	1.301	.016*
Age	1.008	0.99	1.026	.374
Gender male	1.035	0.744	1.44	.838
Race, Black	9,739,809.673	0	Inf	.993
Race, White	11,480,783.81	0	Inf	.993
Stage 2	2.221	1.494	3.303	0***
Stage 3	2.604	1.696	4	0***
Stage 4	2.569	1.335	4.944	.005**
Purity	2.49	1.226	5.059	.012*

CI = confidence interval, HR = hazard ratio, POSTN = periostin.

*$P < 0.05$, ***$P < 0.001$. 95%CI_low, 95%CI_up.

and POSTN expressions (Fig. 7) and found that in LUAD, CAFs infiltration was positively correlated with the POSTN expression (R = 0.813, 0.819, 0.713, 0.187, P < .001).

4. Discussion

ECM is an important component in the tissue microenvironment, which regulates the tissue microenvironmental homeostasis. Accelerating evidence showed that ECM proteins played an important role in the process of tumor development.[8,9] Recent reports have shown that POSTN, a new identified ECM protein, not only regulates homeostasis in the tissue microenvironment, but is also closely related to the occurrence of various malignant tumors.[10,11] Integrating with bioinformatic assays, this study uncovered and discussed the value of POSTN expressions in occurrence and prognosis of LUAD, providing a certain theoretical basis for future investigation and clinic applications.

We first comprehensively analyzed the POSTN gene and protein expression patterns in LUAD tissues in assistance of multiple softwares and online database to show that POSTN were highly expressed in tumor tissues. Moreover, through univariate/multivariate Cox regression analysis and Kaplan–Meier survival analysis, it was found that the high POSTN expression could be used as an affecting factor of prognosis in LUAD. Our finding is in line with previous studies. Thongchot et al[12] reported that POSTN promotes proliferation, migration, invasion of colorectal cancer cells, and reduces cell autophagy, suggesting that the increasing POSTN expression level in colorectal cancer is supposed to be an independent poor prognostic indicator of colorectal cancer. Meanwhile, the high POSTN expression in ovarian cancer has been associated with poor OS.[13] It seems that the linear relationship of the POSTN expression in processing of certain tumors is somehow related to the source of tumor specimens (most of which originate from the tubal epithelium).

The above results indicated that the POSTN expression might play an important role in LUAD prognosis, even though the molecular mechanism was unclear. In LUAD, we showed that a low POSTN expression predicted a better prognosis, the idea of which is supported by our immunoinfiltration analysis that high level of infiltration of B cells also predicted a better prognosis. Given that POSTN has been reported to mediate cell-to-cell communication,[13,14] we speculated that POSTN

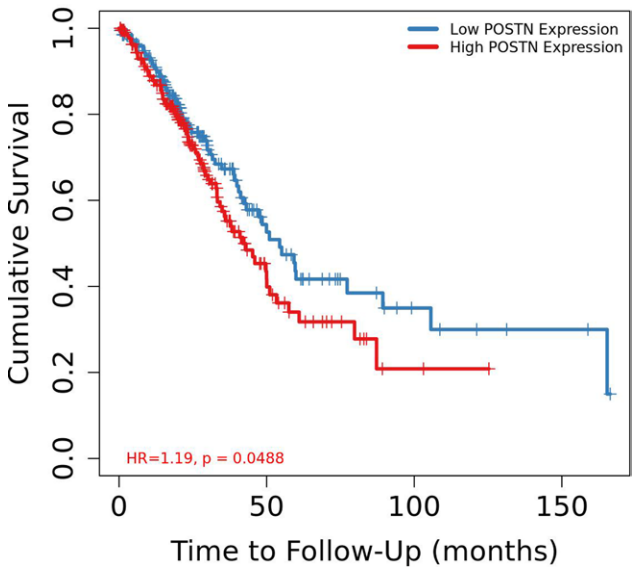

Figure 5. Prognostic analysis of LUAD by the TIMER tool. LUAD = lung adenocarcinoma.

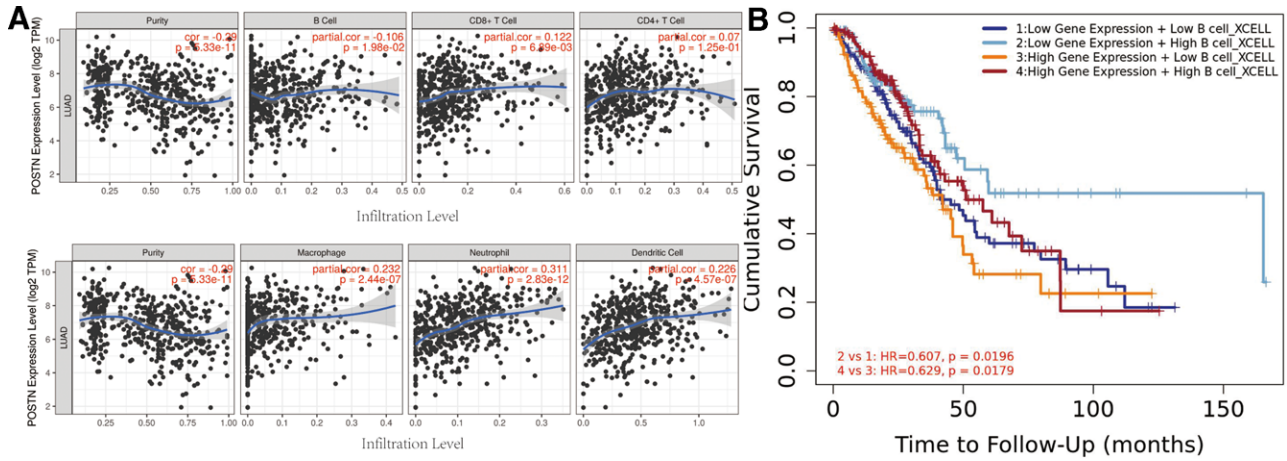

Figure 6. Relationship of immune cell infiltration, gene expression, and clinical prognosis. (A) TIMER analyzes the correlation between the gene expression and the cell infiltration level. (B) The survival curves of different infiltration levels and expression levels.

Table 3

Relationship between immune cell infiltration and prognosis.

	HR	95%CI_l	95%CI_u	*P*
B_cell	0.007	0.001	0.091	0***
CD8_Tcell	1.5	0.252	8.939	.656
CD4_Tcell	17.059	1.183	245.985	.037*
Macrophage	0.655	0.052	8.206	.743
Neutrophil	0.258	0.005	12.264	.491
Dendritic	0.722	0.195	2.676	.627
POSTN	1.165	1.032	1.314	.013*

CI = confidence interval, HR = hazard ratio, POSTN = periostin.

*P < 0.05, ***P < 0.001. 95%CI_low, 95%CI_up.

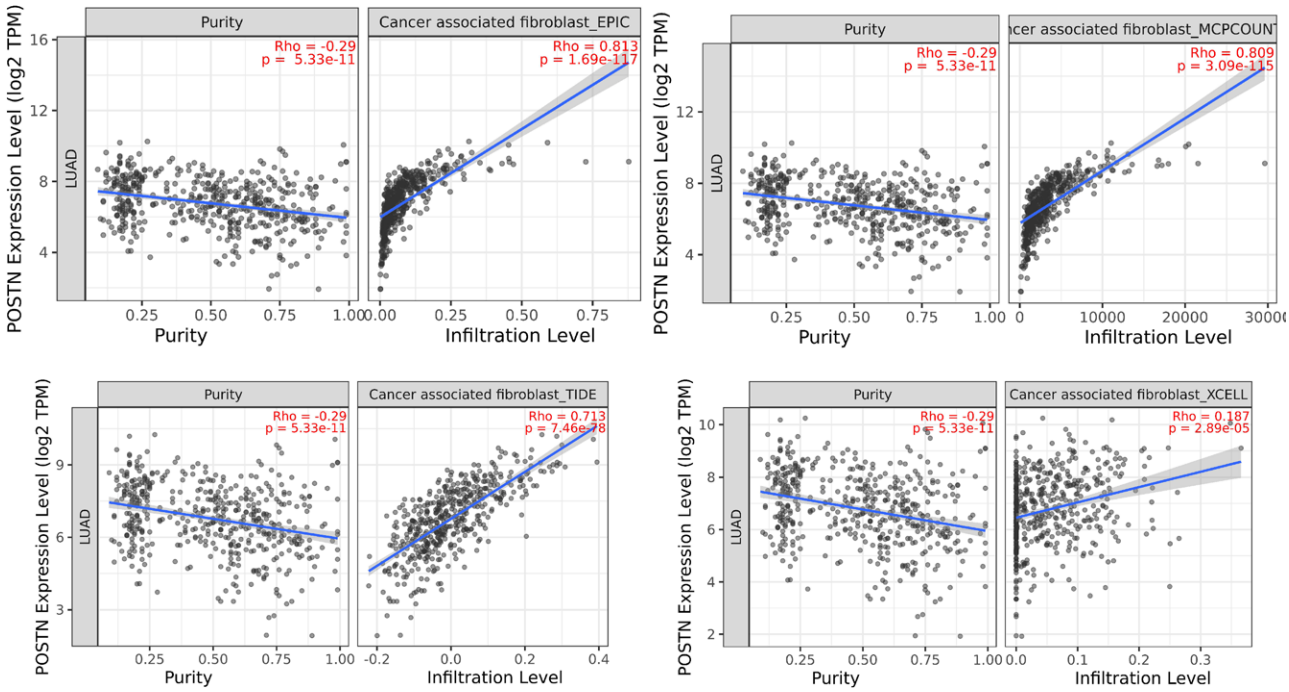

Figure 7. Correlation between CAFs infiltration and the POSTN expression. CAF = cancer-associated fibroblast, POSTN = periostin.

acts as a poor prognostic factor of LUAD due to its potential involvement in the infiltration of B cells. In most cases, including especially CAFs, POSTN was synthesized and secreted in mesenchymal cells. As expected, this study also found that the infiltration of CAFs was positively correlated with the POSTN expression in LUAD. Yue et al[15] found that POSTN was highly enriched in serous ovarian cancer cells that are produced by CAFs and its overexpression is related to the decrease of OS. POSTN from CAFs can be used as a ligand of integrin to induce mesenchymal transformation and promote the migration and invasion of ovarian cancer cells through activating PI3K/AKT pathway. CAFs could regulate the biological characteristics between tumor cells and other stromal cells through cell-cell contact, releasing multiple regulatory factors, to synthesize and reshape the ECM, and thus affect the occurrence and development of cancers.[16]

POSTN was an important component of tumor microenvironment to activate Wnt pathway, maintain tumor cell activity, and promote cell metastasis. The Wnt pathway can recognize and screen tumor stem cells in a variety of tumor cells, and maintain the activity of tumor cells and further promote the occurrence and development of tumors.[17,18] Also, it could improve the mobility, invasiveness, and antiapoptotic ability of tumor cells by activating AKT/PKB signaling pathway.[19]

In summary, POSTN was highly expressed in LUAD, and its increasing expression and decreasing B-cell infiltration jointly affect the prognosis of LUAD patients. POSTN had a certain value in predicting prognostic indicators within 1 year. Altogether, our data provided a certain theoretical basis for POSTN as a prognostic marker. To verify the biological function of POSTN in LUAD prognosis will be a meaningful and challenging task in the next period.

Author contributions

Data curation: Fang Gao.
Formal analysis: Hua Gan, Jin Liu.
Investigation: Hua Gan, Jin Liu.
Resources: Hua Gan.
Software: Hua Gan.
Writing – original draft: Fang Gao.
Writing – review & editing: Hua Gan, Jin Liu.

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