Junctional Adhesion Molecule-Like Protein (JAML) Is Correlated with Prognosis and Immune Infiltrates in Lung Adenocarcinoma

Likui Fang
Wenfeng Yu
Guocan Yu
Fangming Zhong
Bo Ye

Corresponding Author: Bo Ye, e-mail: B1618142@zju.edu.cn
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Background: Junctional adhesion molecule-like protein (JAML) is a member of the junctional adhesion molecule family and mediates migration of immune cells, but its function in cancers remains unclear. This study aimed to evaluate the role of JAML in the prognosis and immune infiltrates of lung adenocarcinoma (LUAD).

Material/Methods: JAML expressions in LUAD tissues and normal tissues were compared using The Cancer Genome Atlas (TCGA) database and datasets from the Gene Expression Omnibus (GEO) database. The influence of JAML expression on prognosis was analyzed by Kaplan-Meier curve and Cox regression model. Interactive and functional analyses of JAML were performed by LinkedOmics and GeneMANIA databases. TIMER2.0, TISIDB, and GEPIA2 databases were used to investigate the correlation between JAML expression and immune infiltrates.

Results: JAML expression was decreased in LUAD ($P<0.001$), and lower JAML expression was associated with worse outcomes of LUAD patients. High JAML expression was the protective factor for overall survival (OS) (HR 0.706, 95% CI 0.500-0.997, $P=0.048$). Interactive and functional analyses suggested that co-expressed genes with JAML have an obvious link to immune-related pathways. In addition, JAML expression was positively associated with infiltrating levels of CD8+ T cells, CD4+ T cells, B cells, dendritic cells, macrophages, and neutrophils, and had significant correlations with diverse immune marker sets in LUAD.

Conclusions: JAML expression was significantly correlated with prognosis and immune infiltrates. These preliminary findings suggested JAML could be considered as a potential prognostic biomarker and therapeutic target for LUAD.

Keywords: Adenocarcinoma of Lung • JAML Protein, Human • Prognosis

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Background

Lung cancer is one of the most common malignant tumors and is the leading cause of cancer-related death worldwide [1,2]. Lung adenocarcinoma (LUAD) is the most frequent histological subtype [3]. More than half of cases have advanced or metastatic disease at the time of initial diagnosis and require systematic therapy [4]. Although chemotherapy remains an important component of systemic therapy, targeted therapy such as tyrosine kinase inhibitors (TKIs) has shown a better activity in oncogene-driven disease [5,6]. The recent development of immunotherapy has fundamentally shifted the treatment paradigm of patients without sensitive molecular mutations [7]. Immune checkpoint inhibitors (ICIs) have become the front-line treatment in patients with high PD-L1 expression [8]. However, immunotherapy remains ineffective for most patients, and secondary drug resistance is also a challenging problem [9].

Junctional adhesion molecule-like protein (JAML), also named AMICA1, is a junctional adhesion molecule (JAM) that belong to the immunoglobulin superfamily [10]. JAML can mediate the transendothelial migration (TEM) of immune cells and might be involved in modulating cellular responses during tissue immunity [11,12], suggesting that JAML influences the tumor immune microenvironment. In head and neck squamous cell carcinoma, JAML is one of the genes that play a potential role in tumor evolution mechanisms related to the tumor immune microenvironment [13]. However, the function of JAML in LUAD is poorly investigated. This study explored JAML expression in LUAD and its impact on prognosis based on bioinformatics analysis. The association between JAML expression

Table 1. Baseline characteristics of the LUAD patients.

| Characteristics | N   | (%) |
|-----------------|-----|-----|
| Age            |
| >65            | 261 | 50.6|
| ≤65            | 255 | 49.4|
| Gender         |
| Male           | 249 | 46.6|
| Female         | 286 | 53.5|
| Smoking history|
| Yes            | 446 | 85.6|
| No             | 75  | 14.4|
| T stage        |
| T1             | 175 | 32.9|
| T2             | 289 | 54.3|
| T3             | 49  | 9.2 |
| T4             | 19  | 3.6 |
| N stage        |
| N0             | 348 | 67.1|
| N1             | 95  | 18.3|
| N2             | 74  | 14.3|
| N3             | 2   | 0.4 |
| M stage        |
| M0             | 361 | 93.5|
| M1             | 25  | 6.5 |
| Pathologic stage|
| Stage I        | 294 | 55.8|
| Stage II       | 128 | 23.3|
| Stage III      | 84  | 15.9|
| Stage IV       | 26  | 4.9 |
| JAML expression|     |     |
| High           | 268 | 50.1|
| Low            | 267 | 49.9|

Figure 1. (A) JAML expression was lower in LUAD tissues than in normal tissues in TCGA database. (B) The ROC curve of JAML expression in LUAD. TPR – true positive rate; FPR – false positive rate. ***P<0.001. The figure was created using R statistical software (version 3.6.3).
and the tumor immune microenvironment was investigated using the TIMER2.0, TISIDB, and GEPIA2 databases. The results revealed the significant impact of JAML expression on prognosis and tumor immune microenvironment of LUAD.

**Material and Methods**

**Data Acquisition**

The data of mRNA expression and clinicopathologic characteristics of LUAD patients were obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). The database is publicly open-access and local ethics committee approval is not necessary. A total of 535 LUAD samples and 59 normal tissue samples from the TCGA database were included in the study. The differential expression of JAML between normal tissues and LUAD was verified by the gene expression profiling datasets GSE10799, GSE27262, GSE40791, and GSE118370, which were obtained from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). GSE10799 included 16 LUAD samples and 3 normal lung tissue samples. GSE27262 included tumor and adjacent normal tissue pairs from 25 stage I LUAD patients. GSE40791 included 100 non-neoplastic lung samples, and 69, 12, and 13 stage I, II, and III LUAD frozen tissues, respectively. GSE118370 included 6 pairs of invasive LUAD tissues and normal lung tissues. The expressions of JAML protein were compared between LUAD and normal tissues, and were further compared in the Human Protein Atlas (https://www.proteinatlas.org/).

**Figure 2.** Validation of lower JAML expression in LUAD than that in normal tissues in (A) GSE10799, (B) GSE27262, (C) GSE40791, and (D) GSE118370 datasets. **P** < 0.01; ***P** < 0.001. The figure was created using R statistical software (version 3.6.3).
which provides protein immunohistochemistry in normal human tissues and tumor tissues.

LinkedOmics Database Analysis

The LinkedOmics database is a platform (http://www.linkedomics.org) to access, analyze, and compare cancer multi-omics data within and across tumor types [14]. The differentially expressed genes related to JAML were screened through the LinkFinder module in the database, and the correlation was tested using the Pearson correlation coefficient. The gene set enrichment analyses (GSEA) of Gene Ontology biological process (GO_BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were performed in the LinkInterpreter module.

GeneMANIA Database Analysis

GeneMANIA is a website (http://genemania.org) for generating hypotheses about gene function, analyzing gene lists, and prioritizing genes for functional assays [15]. Given a query gene list, GeneMANIA finds functionally similar genes using a wealth of genomics and proteomics data. We obtained the protein–protein interaction (PPI) network information of JAML using the online tool GeneMANIA.

TIMER2.0 Database Analysis

The Tumor Immune Estimation Resource (TIMER) is a public website (http://timer.cistrome.org/) providing comprehensive
analysis and visualization functions of tumor-infiltrating immune cells [16]. The correlations of JAML expression with the abundance of 6 types of infiltrating immune cells (CD8+ T cells, CD4+ T cells, B cells, dendritic cells, macrophages, and neutrophils) in LUAD were evaluated in the TIMER2.0 database.

TISIDB Analysis

The Tumor-Immune System Interaction Database (TISIDB) is a user-friendly web portal (http://cis.hku.hk/TISIDB/index.php) which provides comprehensive investigation of tumor-immune interactions [17]. The association between JAML expression and immune features was analyzed in TISIDB to verify the results from TIMER2.0 database analysis.

GEPIA2 Database Analysis

The Gene Expression Profiling Interactive Analysis (GEPIA) is an online database which provides key interactive and customizable functions, including differential expression analysis, correlation analysis, survival analysis, similar gene detection, and dimensionality reduction analysis [18]. The associations of JAML expression with multiple markers for immune cells were investigated in the GEPIA2 database.

Statistical Analysis

The t test was used to analyze the measurement data if the observed variables were normally distributed. Otherwise, the

Figure 4. (A) JAML expression was lower in T2 and T3 stage than in T1 stage, but the difference was not observed in T4 stage. (B) Low JAML expression was associated with positive N stage. (C) There was no significant difference between JAML expression and M stage. (D) Low JAML expression was associated with advanced pathologic stage. ns, P ≥ 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001. The figure was created using R statistical software (version 3.6.3).
Figure 5. Kaplan-Meier survival curves for (A) overall survival (OS) and (B) progression-free survival (PFS) of the LUAD patients with high and low JAML expression level. The figure was created using R statistical software (version 3.6.3).

Table 2. Cox regression analysis of JAML and clinicopathologic characteristics with survival outcomes in LUAD.

| Characteristics                        | Univariate analysis | Multivariate analysis |
|----------------------------------------|---------------------|-----------------------|
|                                        | HR                  | 95% CI                | P         | HR                  | 95% CI                | P         |
| Overall survival                       |                     |                       |           |                     |                       |           |
| Age (>65 vs ≤65)                       | 1.223               | 0.916-1.635           | 0.172     | 1.642               | 1.050-2.568           | 0.030     |
| Gender (Male vs Female)                | 1.070               | 0.803-1.426           | 0.642     | 1.373               | 0.875-2.124           | 0.160     |
| Smoking history (Yes vs No)            | 0.894               | 0.592-1.348           | 0.591     | 1.029               | 0.652-1.610           | 0.873     |
| T stage (T2 & T3 & T4 vs T1)           | 1.728               | 1.229-2.431           | 0.002     | 1.373               | 0.875-2.124           | 0.160     |
| N stage (N1 & N2 & N3 vs N0)           | 2.601               | 1.944-3.480           | <0.001    | 1.435               | 0.769-2.677           | 0.257     |
| M stage (M1 vs M0)                     | 2.136               | 1.248-3.653           | 0.006     | 1.373               | 0.875-2.124           | 0.160     |
| Pathologic stage (Stage II & Stage III & Stage IV vs Stage I) | 2.933               | 2.173-3.958           | <0.001    | 1.435               | 0.769-2.677           | 0.257     |
| JAML (High vs Low)                     | 0.618               | 0.460-0.831           | 0.001     | 0.706               | 0.500-0.997           | 0.048     |
| Progress free survival                 |                     |                       |           |                     |                       |           |
| Age (>65 vs ≤65)                       | 1.023               | 0.784-1.335           | 0.867     | 1.509               | 1.090-2.090           | 0.013     |
| Gender (Male vs Female)                | 1.172               | 0.901-1.526           | 0.236     | 1.851               | 1.562-2.288           | 0.445     |
| Smoking history (Yes vs No)            | 0.968               | 0.658-1.426           | 0.870     | 1.509               | 1.090-2.090           | 0.013     |
| T stage (T2 & T3 & T4 vs T1)           | 1.882               | 1.379-2.570           | <0.001    | 1.509               | 1.090-2.090           | 0.013     |
| N stage (N1 & N2 & N3 vs N0)           | 1.512               | 1.152-1.984           | 0.003     | 0.851               | 0.562-1.288           | 0.445     |
| M stage (M1 vs M0)                     | 1.513               | 0.855-2.676           | 0.155     | 1.509               | 1.090-2.090           | 0.013     |
| Pathologic stage (Stage II & Stage III & Stage IV vs Stage I) | 1.960               | 1.502-2.557           | <0.001    | 1.851               | 1.221-2.806           | 0.004     |
| JAML (High vs Low)                     | 0.758               | 0.581-0.989           | 0.041     | 0.846               | 0.644-1.111           | 0.229     |
Mann-Whitney U test was used. The receiver operating characteristic (ROC) curve was used to explore the diagnostic value of JAML expression for LUAD. The patients were divided into 2 groups according to the median expression of JAML. Kaplan-Meier method and the log-rank test were performed to analyze the impact of JAML expression on overall survival (OS) and progression-free survival (PFS). Cox regression analysis was performed to determine the degree of contribution of JAML expression on survival outcomes. All the above analyses were conducted using R statistical software (version 3.6.3) and SPSS software (version 24.0). Statistical significance was set at P value <0.05 (All P values presented were 2-sided).

Results

Decreased JAML Expression in LUAD

The baseline characteristics of the patients with LUAD are presented in Table 1. The JAML expression was significantly lower in LUAD samples in comparison to normal tissue samples in TCGA database (P<0.001, Figure 1A). The ROC curve showed the potential diagnostic value of JAML expression for LUAD (AUC 0.956, 95% CI 0.937-0.975) (Figure 1B). The analyses of datasets (GSE10799, GSE27262, GSE40791, and GSE118370) from the GEO database verified the decreased expression of JAML in LUAD compared to normal tissues (Figure 2A-2D). Correspondingly, the expression of JAML protein was down-regulated in LUAD tissue in comparison to that in normal tissue in the Human Protein Atlas (Supplementary Figure 1).

Associations Between JAML Expression and Clinicopathologic Characteristics

The associations between JAML expression and clinicopathologic characteristics were analyzed to explore the role of JAML in LUAD progress. The results showed no significant association between JAML expression and age (Figure 3A), with lower expression of JAML in males and smokers (P=0.003 and 0.019, respectively, Figure 3B, 3C). T1 stage had higher expression of JAML than T2 and T3 stage (P=0.001 and 0.042, respectively), but the differential expression was not observed in T4 stage (Figure 4A). In addition, JAML expression decreased in positive N stage (N1, N2, and N3) and advanced pathologic stage (Stage III and IV) compared to N0 and early pathologic stage (Stage I and II) (P=0.021 and 0.006, respectively, Figure 4B, 4D), but JAML expression was not significantly associated with M stage (Figure 4C).

Low JAML Expression Showing Poor Survival Outcomes

Survival analyses by Kaplan-Meier method and the log-rank test showed that the patients with low JAML expression were correlated with inferior OS (P=0.001) and PFS (P=0.041) (Figure 5A, 5B). Univariate Cox regression analysis demonstrated that JAML expression was the significant factor for OS (HR 0.618, 95% CI 0.460-0.831) and PFS (HR 0.758, 95% CI 0.581-0.989) (Table 2). Multivariate Cox regression analysis proved that high JAML expression was the independent protective factor for OS (HR 0.706, 95% CI 0.500-0.997, P=0.048) (Figure 6).

Interaction Analysis of JAML in LUAD

The LinkFinder module in the LinkedOmics database was used to explore the co-expression pattern of JAML in LUAD. The number of observations in the search dataset was 515. A total of 10 820 genes (red dots) were positively correlated with JAML and 9168 genes (green dots) were negatively correlated (Supplementary Figure 2). The top 50 genes that were positively associated with JAML are presented in Figure 7A and the top 50 genes negatively associated with JAML are presented in Figure 7B. These genes are listed in Supplementary Tables 1 and 2, respectively.

In the LinkInterpreter module, GO term analysis showed that co-expressed genes of JAML were mainly involved in...
Fang L. et al: JAML expression in lung adenocarcinoma
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Figure 7. The co-expression genes with JAML in LUAD from the LinkedOmics database. (A, B) Heat maps of top 50 genes positively and negatively correlated with JAML in LUAD, respectively. (C, D) GO annotations and KEGG pathways of JAML in LUAD. The figure was created using the LinkedOmics database (http://www.linkedomics.org).
interleukin-4 production, cellular defense response, interferon-gamma production, interleukin-1 production, interleukin-2 production, leukocyte proliferation, T cell activation, adaptive immune response, leukocyte cell–cell adhesion, and immune response-regulation signaling pathway (Figure 7C). KEGG pathway analysis indicated enrichment in autoimmune thyroid disease, *Staphylococcus aureus* infection, asthma, hematopoietic cell lineage, viral myocarditis, allograft rejection, intestinal immune network for IgA production, cell adhesion molecules, and Th1 and Th2 cell differentiation (Figure 7D).

The results from GeneMANIA database analysis revealed that the functions of JAML and their associated molecules were primarily related to leukocyte and neutrophil migration, cell adhesion molecule binding, neutrophil chemotaxis, and leukocyte cell–cell adhesion (Figure 8).

### Figure 8. Protein–protein interaction network of JAML. The figure was created using the GeneMANIA database (http://genemania.org).

### Correlations of JAML Expression with Immune Infiltrates

The correlation of JAML expression with immune cell infiltration was explored in the TIMER2.0 database. The result showed that JAML expression was significantly and positively associated with the infiltrating levels of CD8+ T cells, CD4+ T cells, B cells, dendritic cells, macrophages, and neutrophils (Figure 9). In TISIDB, JAML expression was also correlated with abundance of activated CD8+ T cells, activated CD4+ T cells, activated B cells, activated dendritic cells, macrophages, and neutrophils (Figure 10).

To further investigate the relationship between JAML and diverse immune infiltrating cells, the correlations between JAML and immune marker sets of immune cells in LUAD were analyzed in the GEPIA2 database. We found that the expression levels of most marker sets marking different T cells, B cell, regulatory T cell (Treg), tumor-associated macrophage (TAM), monocytes, neutrophils, and dendritic cell (DC) had significant
Figure 9. Correlation of JAML expression with immune infiltration in LUAD from the TIMER2.0 database. The figure was created using the TIMER2.0 database (http://timer.cistrome.org/).
correlations with JAML expression in LUAD (Table 3). Marker genes of T cell exhaustion also had a positive correlation with JAML expression, especially TIM-3.

**Discussion**

Tumorigenesis involves a succession of complex genetic alterations, and avoiding immune destruction is one of the critical processes [19]. T cell infiltration is widely accepted as a key component of adaptive cancer immune and tumor-infiltrating lymphocytes within the tumor microenvironment and are considered prognostic markers in various cancers [20,21]. JAMs are intercellular adhesion molecules that importantly participate in the transmigration of monocytes, neutrophils, and some T cells through homophilic and heterophilic interactions [22]. Increasing evidence suggests that JAM family members could have potential clinical relevance in cancer development and function as tumor suppressors [23,24]. JAML has been reported as a new JAM family member, and its role in LUAD has not yet been fully elucidated.

This study explored the effect of JAML expression on the progression and prognosis of LUAD on the basis of various public databases. By analyzing the data from TCGA and GEO databases, we found that LUAD samples had lower JAML expression compared to normal tissue samples. Decreased JAML expression was observed in advanced T stage, N stage, and pathologic stage, while the differential expression was not significant in T4 stage and M stage. In addition, the survival analysis revealed that high expression of JAML in LUAD had favorable OS and PFS in comparison to low expression. Cox regression analysis further proved the protective role of high JAML expression in OS. These results suggested that JAML is closely correlated with LUAD tumorigenesis and progression and might act as a tumor suppressor. Two recent bioinformatics studies have also demonstrated that JAML is one of the favorable genes in LUAD, and low JAML expression could increase lung cancer risk [25,26]. However, its role in the tumor immune microenvironment was unknown.

Previous studies indicated that JAML plays an important role in γδ T cell activation and tissue homeostasis [27,28]. JAML

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**Figure 10.** Correlation of JAML expression with immune infiltration in LUAD from the TISIDB. The figure was created using TISIDB (http://cis.hku.hk/TISIDB/index.php).
### Table 3. Correlation analysis between JAML and markers of immune cells in GEPIA.

| Cell type | Gene marker | Normal | Tumor |
|-----------|-------------|--------|-------|
|           |             | R      | P     | R     | P     |
| B cell    | CD19        | 0.051  | 0.7   | 0.26  | 5.1e-09 |
|           | CD79A       | 0.034  | 0.8   | 0.17  | 0.00019 |
| CD8+ T cell | CD8A       | 0.0034 | 0.98  | 0.26  | 4.4e-09 |
|           | CD8B        | 0.046  | 0.73  | 0.14  | 0.0022  |
| Tfh       | BCL6        | -7e-04 | 1     | 0.11  | 0.019  |
|           | IL21        | -0.049 | 0.71  | 0.24  | 9.5e-08 |
| Th1       | TBX21       | -0.096 | 0.47  | 0.095 | 0.036  |
|           | STAT4       | -0.023 | 0.86  | 0.27  | 8.2e-10 |
|           | STAT1       | -0.02  | 0.88  | 0.14  | 0.0016 |
|           | IFNG        | -0.011 | 0.94  | 0.19  | 4.2e-05 |
|           | TNF         | 0.15   | 0.26  | 0.37  | 0      |
| Th2       | GATA3       | -0.077 | 0.56  | -0.0012 | 0.98  |
|           | IL13        | -0.1   | 0.44  | 0.19  | 1.7e-02 |
|           | STAT6       | 0.11   | 0.41  | 0.31  | 3.8e-12 |
|           | STAT5A      | 0.5    | 5.5e-05 | 0.62 | 0      |
| Th17      | STAT3       | -0.0029 | 0.98 | 0.078 | 0.085 |
|           | IL17A       | 0.079  | 0.55  | 0.12  | 0.0085 |
| Treg      | FOXP3       | 0.11   | 0.42  | 0.47  | 0      |
|           | STAT5B      | -0.077 | 0.56  | 0.36  | 2.2e-16 |
|           | CCR8        | 0.14   | 0.3   | 0.48  | 0      |
|           | TGFB1       | 0.51   | 3.8e-05 | 0.35 | 3.1e-15 |
| M1        | NOS2        | -0.24  | 0.065 | -0.022 | 0.63  |
|           | IRF5        | 0.79   | 1.13  | 0.42  | 0      |
|           | PTGS2       | -0.086 | 0.52  | -0.14 | 0.0029 |
| M2        | CD163       | 0.29   | 0.027 | 0.39  | 0      |
|           | VSIG4       | 0.68   | 4.3e-09 | 0.64 | 0      |
|           | MS4A4A      | 0.7    | 5.1e-10 | 0.6  | 0      |
| TAM       | CCL2        | -0.039 | 0.77  | 0.2   | 7.4e-06 |
|           | CD68        | 0.66   | 1.2e-08 | 0.61 | 0      |
|           | IL10        | -0.044 | 0.74  | 0.4   | 0      |
| Monocyte  | CD86        | 0.45   | 0.00037 | 0.64 | 0      |
|           | CD115       | 0.49   | 7.6e-05 | 0.64 | 0      |
Table 3 continued. Correlation analysis between JAML and markers of immune cells in GEPIA.

| Cell type               | Gene marker | Normal R | Normal P | Tumor R | Tumor P |
|-------------------------|-------------|----------|----------|---------|---------|
| Neutrophil              | CD66b       | 0.057    | 0.67     | 0.27    | 1.5e-09 |
|                         | CCR7        | 0.097    | 0.47     | 0.46    | 0       |
|                         | CD11b       | 0.6      | 5e-07    | 0.72    | 0       |
| Natural killer cell     | XCL1        | -0.18    | 0.17     | -0.031  | 0.49    |
|                         | CD7         | 0.031    | 0.81     | 0.1     | 0.028   |
|                         | KIR3DL1     | 0.045    | 0.73     | 0.0069  | 0.13    |
| Dendritic cell          | CD1C        | -0.0066  | 0.96     | 0.53    | 0       |
|                         | CD141       | -0.11    | 0.4      | 0.18    | 4.9e-05 |
|                         | CD11c       | 0.8      | 2.6e-14  | 0.65    | 0       |
| T cell exhaustion       | PDCD1       | 0.052    | 0.7      | 0.25    | 3.2e-08 |
|                         | CTLA4       | 0.055    | 0.68     | 0.37    | 0       |
|                         | LAG3        | -0.097   | 0.47     | 0.11    | 0.013   |
|                         | TIM-3       | 0.36     | 0.0047   | 0.62    | 0       |

Tfh – follicular helper T cell; Th – T helper cell; Treg – regulatory T cell; TAM – tumor-associated macrophage.

was also identified as a crucial component of DC migration and can promote response to DC-based cancer immunotherapy [29]. Similarly, our study demonstrated the significant association between JAML and the tumor immune microenvironment. LinkedOmics database analysis pointed out that most co-expressed genes with JAML had obvious links to immune-related pathways, which agrees with the result of GeneMANIA database analysis. Moreover, JAML expression had a significantly consistent correlation with the infiltration levels of CD8+ T cells, CD4+ T cells, B cells, dendritic cells, macrophages, and neutrophils. The correlation between JAML expression and the marker genes of immune cells also suggests the role of JAML in recruitment and regulation of immune infiltrating cells. First, JAML expression was associated with most markers of T helper cells, which suggested that JAML could potentially regulate T cell functions in LUAD. Second, significant correlations were also found between JAML expression and markers of monocytes. Intriguingly, JAML expression was positively correlated with the expression of Treg and T cell exhaustion markers, which contributed to the formation of an immunosuppressive microenvironment. A recent report demonstrated that high frequency of circulating Tregs was associated with a favorable response to ICIs therapy [30]. In addition, M2 macrophage markers such as VSG4 and MS4A4A showed strong correlations with JAML expression, while M1 macrophage markers showed weak correlations. These results revealed the potential role of JAML in polarization of TAM. Taken together, our findings suggest that JAML is specifically correlated with immune infiltrating cells and plays a vital role in immune regulation in the LUAD microenvironment, but the interaction between JAML and the immune microenvironment is complicated, and does not just lead to a completely positive or negative effect.

There were several limitations in our study. First, all the data analyzed in our study were obtained from online databases, and data heterogeneity was difficult to avoid. Cox regression analysis showed M stage was not significantly associated with PFS. This discrepancy might be caused by data heterogeneity and limited samples. Second, the role of JAML in regulating immune microenvironment seemed to be complicated, and the details of mechanisms by which JAML regulates T cell functions remain unknown. Finally, our results need to be validated by further studies.

**Conclusions**

Our preliminary findings showed that JAML was downregulated in LUAD, and low JAML expression was associated with tumor progression and poor prognosis. High JAML expression was an independent protective factor for OS and could be considered as a prognostic biomarker. Because of the significant association between JAML expression and the infiltration of diverse immune cells, JAML might play important roles in regulation of the immune microenvironment in LUAD.
Declaration of Figures’ Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Supplementary Materials

**Supplementary Figure 1.** The level of JAML protein in LUAD tissue was lower than that in normal tissue in the Human Protein Atlas (Antibody HPA047919, 10X). The figure was download in Human Protein Atlas (https://www.proteinatlas.org).

**Supplementary Figure 2.** All genes significantly associated with JAML as distinguished by Pearson test in LUAD from the LinkedOmics database. The figure was created using the LinkedOmics database (http://www.linkedomics.org).
## Supplementary Table 1. The top 50 genes that were positively associated with JAML.

| Gene     | Statistic | P value     | FDR          |
|----------|-----------|-------------|--------------|
| SPN      | 0.87776   | 4.59E-166   | 4.59E-162    |
| BTK      | 0.868331  | 2.42E-158   | 1.61E-154    |
| DOK2     | 0.864833  | 1.25E-155   | 6.25E-152    |
| FGD2A    | 0.84968   | 2.38E-148   | 9.13E-145    |
| CD37     | 0.851339  | 7.85E-146   | 2.61E-142    |
| P2RY13   | 0.850216  | 4.64E-145   | 1.32E-141    |
| CD300LF  | 0.84648   | 5.41E-144   | 1.35E-140    |
| CL1orf162| 0.847151  | 5.49E-143   | 1.22E-139    |
| FGR      | 0.844632  | 2.57E-141   | 5.14E-138    |
| SASH3    | 0.844313  | 4.16E-141   | 7.56E-138    |
| SELPLG   | 0.841924  | 1.49E-139   | 2.48E-136    |
| CD3      | 0.837684  | 7.36E-137   | 1.13E-133    |
| CD4      | 0.837089  | 1.73E-136   | 2.47E-133    |
| NCKAP1L  | 0.836384  | 4.75E-136   | 6.33E-133    |
| CD52     | 0.833422  | 3.14E-134   | 3.93E-131    |
| DPEP2    | 0.833195  | 4.32E-134   | 4.79E-131    |
| MYO1F    | 0.831197  | 6.93E-133   | 7.29E-130    |
| CLEC10A  | 0.830327  | 2.30E-132   | 2.30E-129    |
| TNFAIP8L2| 0.82949   | 7.23E-132   | 6.88E-129    |
| LPXN     | 0.829777  | 9.66E-132   | 8.78E-129    |
| SNX20    | 0.828918  | 1.58E-131   | 1.37E-128    |
| LY6     | 0.828598  | 2.43E-131   | 2.03E-128    |
| AIF1     | 0.818807  | 9.59E-126   | 7.67E-123    |
| GAB3     | 0.818328  | 1.76E-125   | 1.36E-122    |

| Gene     | Statistic | P value     | FDR          |
|----------|-----------|-------------|--------------|
| EVI2B    | 0.818197  | 2.08E-125   | 1.54E-122    |
| SPI1     | 0.817413  | 5.64E-125   | 4.02E-122    |
| LST1     | 0.816492  | 1.80E-124   | 1.24E-121    |
| GMFG     | 0.814586  | 1.95E-123   | 1.30E-120    |
| IGSF6    | 0.814375  | 2.54E-123   | 1.64E-120    |
| ARHGAP30 | 0.807086  | 2.08E-121   | 1.30E-118    |
| WAS      | 0.809297  | 1.26E-120   | 7.64E-118    |
| NLRC4    | 0.809137  | 1.53E-120   | 8.99E-118    |
| CD53     | 0.806027  | 6.24E-119   | 3.56E-116    |
| DOCK2    | 0.804383  | 4.31E-118   | 2.39E-115    |
| HLA-DPB1 | 0.800373  | 4.46E-116   | 2.35E-113    |
| PIK3R5   | 0.799322  | 1.48E-115   | 7.59E-113    |
| GGTA1    | 0.798294  | 4.74E-115   | 2.37E-112    |
| C17orf87 | 0.798012  | 6.52E-115   | 3.18E-112    |
| PARVG    | 0.793511  | 9.84E-113   | 4.57E-110    |
| OSCAR    | 0.790969  | 1.58E-111   | 7.18E-109    |
| IRF8     | 0.790198  | 7.66E-117   | 4.14E-114    |
| PRAM1    | 0.790017  | 4.43E-111   | 1.97E-108    |
| ARHGEF6  | 0.788939  | 1.41E-110   | 6.15E-108    |
| ITGAL    | 0.788848  | 1.56E-110   | 6.63E-108    |
| LAIR1    | 0.788761  | 1.71E-110   | 7.13E-108    |
| CSF2RB   | 0.788263  | 2.92E-110   | 1.19E-107    |
| KIAA0748 | 0.786265  | 2.44E-109   | 9.76E-107    |
| SPN      | 0.87776   | 4.59E-166   | 4.59E-162    |

**FDR** – false discovery rate. FDR is calculated by Benjamini-Hochberg method.
### Supplementary Table 2.
The top 50 genes that were negatively associated with JAML.

| Gene     | Statistic | P value   | FDR       |
|----------|-----------|-----------|-----------|
| NOL10    | -0.51092951 | 1.37E-35  | 3.83E-34  |
| PAICS    | -0.492426981 | 8.24E-33  | 2.10E-31  |
| EPT1     | -0.479806564 | 5.21E-31  | 1.23E-29  |
| CCT3     | -0.450196909 | 4.56E-27  | 9.22E-26  |
| GTF3C2   | -0.470759665 | 5.51E-26  | 1.23E-25  |
| CGC3     | -0.393560742 | 3.81E-30  | 8.72E-29  |
| DDX1     | -0.421238332 | 1.44E-25  | 2.37E-24  |
| LCLAT1   | -0.451984988 | 2.70E-27  | 5.51E-26  |
| RIOK1    | -0.413496507 | 3.48E-24  | 6.36E-23  |
| MRPL19   | -0.410446848 | 2.75E-22  | 4.18E-21  |
| KDM1A    | -0.408779249 | 2.55E-21  | 3.66E-20  |
| HDP5     | -0.401157197 | 2.74E-21  | 3.43E-20  |
| HSPD1    | -0.402243284 | 1.89E-21  | 2.74E-20  |
| PCKR1    | -0.407570770 | 1.62E-21  | 2.37E-20  |
| MRPP5    | -0.400465620 | 1.93E-21  | 2.78E-20  |
| C16orf59 | -0.395736583 | 1.13E-21  | 1.66E-20  |
| SUV39H2  | -0.395639639 | 1.32E-21  | 1.98E-20  |
| OX1       | -0.407134370 | 1.54E-21  | 2.24E-20  |
| C17orf94 | -0.395736583 | 1.06E-21  | 1.51E-20  |
| EEF1E1   | -0.407134370 | 1.24E-21  | 1.66E-20  |
| PDK1      | -0.400465620 | 1.32E-21  | 1.98E-20  |
| MTPAP     | -0.395639639 | 1.32E-21  | 1.98E-20  |
| CFS1A     | -0.395736583 | 1.13E-21  | 1.66E-20  |
| XPOS     | -0.401034671 | 2.55E-21  | 3.66E-20  |
| MS1H2     | -0.400699429 | 2.77E-21  | 3.96E-20  |

FDR – false discovery rate. FDR is calculated by Benjamini-Hochberg method.

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