Upregulation of EMID1 accelerates to a favorable prognosis and immune infiltration in lung adenocarcinoma.

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Research

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Abstract

Background

Lung cancer is a kind of refractory cancer. Lung adenocarcinoma (LUAD) is the main subtype of lung cancer. Although there are many ways to treat lung cancer, the survival rate of patients has not improved. Therefore, more new molecules need to be found for the diagnosis and treatment of LUAD.

Methods

The data from The Cancer Genome Atlas (TCGA) database were used to analyze the value of EMID1 in diagnosis and prognosis of LUAD. The relationship between clinic pathological features and EMID1 was analyzed with the Wilcoxon signed-rank test and logistic regression by R (v.3.5.1). Gene Set Enrichment Analysis (GSEA) was performed to investigate the potential mechanism of EMID1 expression on the prognosis of LUAD. The correlation between tumor infiltrating immune cells and genes was assessed by CIBERSORT. In addition, GEPIA and Gene Expression Omnibus (GEO) database were used to verify the results.

Results

The decreased expression of EMID1 was significantly related to the late stage and metastasis of lung cancer. Kaplan Meier survival analysis showed that patients with low expression of EMID1 had worse prognosis than those with high expression of EMID1. Notch signaling pathway may be an important biological pathway for EMID1 to play a role in LUAD. In addition, CIBERSORT also found that the infiltration level of B cells was positively correlated with the expression of EMID1, which played an important role in the immune environment of LUAD. All results were validated in GEO and GEPIA database.

Conclusion

The analysis of EMID1 was helpful to understand the immune microenvironment of LUAD and improve the survival status of patients with LUAD. All the results suggested that EMID1 might be a new immune related prognostic marker of LUAD.

Introduction

Lung cancer, as one of the refractory cancer types, is the main cause of cancer death. (1)Lung cancer has the lowest five-year survival rate among some major cancers, such as colon cancer, breast cancer and prostate cancer.(2) According to histology, non-small cell lung cancer (NSCLC) is one of the main subtypes of lung cancer, accounting for 85% of all lung cancer cases. NSCLC can be divided into three types: squamous cell carcinoma, adenocarcinoma and large cell carcinoma. Lung adenocarcinoma (LUAD) is the most common type of lung cancer, accounting for about 40% of all lung cancer. LUAD develops from small airway epithelial type II alveolar cells that secrete mucus and other substances.(3–

5) At present, the treatment methods of lung cancer mainly include surgery, radiotherapy, chemotherapy, targeted cancer therapy and immunotherapy. However, the survival rate of patients has not been improved, and remains at 15% within five years of treatment.(6) Therefore, it is urgent to find more new molecules for the treatment and diagnosis of LUAD, so as to improve the survival status of patients with LUAD.

The environment of tumor growth is called tumor microenvironment, which consists of blood vessels, lymphatic vessels, extracellular matrix, immune cells, stromal cells, secretory proteins, RNA and small organelles.(7) Tumor microenvironment plays an important role in tumor occurrence, development, metastasis, recurrence and drug resistance. Immune cells are an important part of tumor microenvironment. Previous studies have proved that immune cells play an indispensable role in tumor development. For instance, regulatory T cells (Tregs) can produce IL-10, transforming growth factor - β (TGF - β) and cell-mediated cell contact (CTLA4) to play an immunosuppressive function and inhibit the recognition and clearance of tumor cells by the immune system.(8) High expression of Tregs in tumor microenvironment has also been shown to be associated with poor prognosis in some cancers, for example breast cancer, ovarian carcinoma and pancreatic ductal adenocarcinoma. (9–11) On the contrary, it has been proved that Tregs may be related to a good prognosis in Hodgkin's lymphoma by directly inhibiting the growth of tumor cells. (12–14) Most of the B cells exist at the edge of tumor invasion. Some studies have also found that the B cell infiltration in tumor microenvironment is related to the good prognosis of some cancers.(15, 16) However, the role of immune cells in tumor microenvironment in LUAD is not clear.

Emilin (elastin microfibril interphase juxtaposition protein) is a short collagenous A-column region at the C-terminal of a self-globular C1q protein binding domain composed of elastin microfibril proteins, which enlarges the potential helical coil structure and enlarges the cysteine-rich domain at the N-terminal (EMI domain).(17) Sandy Larson et al. showed that EMID1 is associated with Pca bone metastasis, because EMID1 was highly expressed in osteoblasts.(18) However, no studies have shown that EMID1 is involved in the development of cancer at present.

Thus, we acquired the data from The Cancer Genome Atlas (TCGA) and evaluated the prognostic value of EMID1 expression in human LUAD by R-3.5.1. GSEA afforded additional insight into the biological pathways engaged in the LUAD-pathogenesis-linked EMID1 regulatory network. Moreover, CIBERSORT as a recent metagene approach and Tumor Immune Estimation Resource (TIMER) were deployed to assess the density of distinct Tumor- infiltrating immune cells (TIICs) in varied microenvironments, and study the EMID1 and TIICs correlation. The conclusions we establish could help us further comprehend and present a potentially positive EMID1 role, as also a tumor-immunity-interactions correlation, and mechanism involved.

Materials And Methods

Data acquisition

This study identified and downloaded an open data set containing gene expression profiles and prognosis information of tumor and normal tissues from TCGA (https://portal.gdc.cancer.gov/), including 535 tumor groups and 59 normal tissues. Then, 522 clinical data were used for clinical correlation analysis and 494 data for survival analysis. In order to study the effect of emid1 expression in tumor microenvironment, 535 tumor tissues were used for CIBERSORT analysis.

Gene set enrichment analysis (GSEA)

We analyzed GO item and KEGG pathway with GSEA to explore the possible biological function of emid1 in LUAD. In the enrichment results, a false discovery rate (FDR < 0.25) and the nominal P value (P < 0.05) were considered statistically significant.

Assessment of tumor-infiltrating immune cells in LUAD

CIBERSORT (http://cibersort.stanford.edu/) is a deconvolution algorithm based on gene expression, which uses a set of reference gene expression values and is considered as the minimum description of any cell type. Based on these values, support vector regression (SVR) is used to infer the proportion of cell types from a large number of mixed cell type samples. Therefore, CIBERSORT can be used to accurately estimate the proportion of tumor infiltrating lymphocytes. In this study, we aimed to determine the proportion of 22 kinds of immune cells in LUAD by CIBERSORT to evaluate their correlation with survival rate and molecular subsets. In order to evaluate the effect of EMID1 expression, we upload the gene expression data of 535 samples obtained from TCGA to CIBERSORT portal. The algorithm uses 1000 default signature matrices, estimates the p value of deconvolution through Monte Carlo sampling, and establishes the confidence of the results. According to P < 0.05, the immune cells that may be affected by EMID1 were selected. In addition, correlation thermography was used to detect the correlation of 22 immune cells.

Verification analysis

GSE8894 data set was obtained from Gene Expression Omnibus (GEO) database and contains clinical information of 61 samples. 61 clinical data were used for survival analysis. GEPIA is an online database that uses standard processing flow to analyze 8587 normal and 9736 tumor samples in GTEX and TCGA. (19) We used the survival module of GEPIA to analyze the relationship between the prognosis of patients with LUAD and the expression of EMID1. The differential expression of EMID1 between tumor and normal tissues was observed by boxplot, and the differential expression of EMID1 in different pathological stages was compared.

Statistical analysis

R version 3.5.1 analyzes all the statistics. The Wilcoxon signed-rank test, along with a logistic regression, helped evaluate the correlation of clinic-pathological features with EMID1. The correlation between tumor infiltrating immune cells and genes was assessed by CIBERSORT. A P value < 0.05 in all tests is regarded statistically significant.

Results

Association with EMID1 expression and clinic-pathological variables, and survival outcomes

We obtained clinical and gene expression data of 522 samples from TCGA and 61 samples from GEO database. Specific patient characteristics of LUAD were shown in Table 1. We evaluated EMID1 expression data from TCGA. As shown in Fig. 1A-1E, the decreased expression of EMID1 was significantly correlated with clinical stage (P = 0.017) and tumor status (P = 0.008). According to logistic regression analysis, the median expression of the dependent variable of EMID1 expression classification was 2.5, indicating poor prognosis. See Table 2 for details. In patients with LUAD, the decreased expression of EMID1 was significantly correlated with clinical stage (stage III vs. stage I, p = 0.012; stage IV vs. stage I, p = 0.009), tumor status (T3 vs. T1, p = 0.041), lymph node (N2 vs. N0, p = 0.007) and distant metastasis (M1 vs. M0, p = 0.030). Therefore, compared with the high expression group, the patients with low EMID1 expression had a higher risk of developing lung cancer. Meanwhile, a Kaplan-Meier survival analysis also suggested poorer prognosis in low EMID1 expression LUAD, with p = 0.024. (Fig. 1F)

GSEA helps to identify an EMID1 linked signaling pathway

In this study, GSEA was performed between low and high expression data of EMID1 to determine the signal pathway significantly related to EMID1 in LUAD. See Table 3 for specific results. Figure 2 showed ten KEGG pathways associated with the high expression phenotype of EMID1: melanogenesis, basal cell carcinoma, vasoconstriction, glycosaminoglycan biosynthesis of heparin sulfate, Notch signaling pathway, neuroactive ligand receptor interaction, hedgehog signaling pathway, ganglioside biosynthesis series, GnRH signaling pathway and dilated heart Myopathy.

Relationship between EMID1 expression and tumor-infiltrating immune cells

In order to study whether the expression of EMID1 affects the immune microenvironment of lung adenocarcinoma, the gene expression profile of the samples was analyzed by using the CIBERSORT computing resources, so as to infer the density of 22 immune cells in LUAD. First, according to the expression of EMID1, 535 tumor samples were divided into two parts: 267 cases of low expression and 268 cases of high expression. Then, the relative proportion of 22 immune cells in these tumor samples was estimated by CIBERSORT. The results were shown in Fig. 3A. B cells naïve (p = 0.001), B cells memory (p = 0.012), Plasma cells (p = 0.034), T cells CD4 memory resting (p = 0.051), T cells regulatory(Tregs) (p < 0.001), Mast cells resting (p = 0.002) were significantly increased in high expression group. By contrast, T cells CD4 memory activated (p < 0.001), Macrophages M1 (p = 0.035) were significantly increased in low expression group. The diverse TIIC subgroups moreover presented a weak to moderate correlation (Fig. 3B).

Verification analysis of EMID1

As shown in Fig. 4A, Kaplan Meier survival analysis showed that the prognosis of patients with high expression of EMID1 was better than that of patients with low expression of EMID1 ($P < 0.001$). At the same time, we found that the low expression of EMID1 was significantly related to the low OS ($P < 0.001$) and the late stage of pathology through the GEPIA database. (Fig. 4B and 4C) The expression of EMID1 in tumor tissue was significantly lower than that in normal group. (Fig. 4D)

Discussion

Lung cancer is the main cause of cancer death, with adenocarcinoma as the main subtype. In order to improve the prognosis of LUAD patients, it is necessary to identify new biomarkers of LUAD.(18) Our study is the first to show that the expression of emid1 is related to cancer and may be a prognostic biomarker for LUAD.

Our study confirmed that the low expression of EMID1 in LUAD is related to the poor survival time and prognosis, as well as the progress of clinical pathology such as late stage and metastasis of lung cancer. The study deployed GSEA to further explore EMID1 functionality in LUAD, and specified the following as differentially enriched in its high expression phenotype: melanogenesis, basal cell carcinoma, vasoconstriction, glycosaminoglycan biosynthesis of heparin sulfate, Notch signaling pathway, neuroactive ligand receptor interaction, hedgehog signaling pathway, ganglioside biosynthesis series, GnRH signaling pathway and dilated heart Myopathy. We also evaluated the relationship between the expression of EMID1 and the level of immune infiltration in LUAD by CIBERSORT. The expression of EMID1 has an effect on a variety of immune cells. All the results suggested that EMID1 might be an independent prognostic marker of LUAD.

Notch pathway is involved in cell proliferation, differentiation and survival. Notch signaling pathway is one of the common signaling pathways in cancer. Notch activated mutations and amplification of Notch pathway play a key role in the progression of cancer. (20) Notch signaling pathway is a highly conserved ligand receptor signaling pathway, which contains four Notch receptors and five ligands. The four receptors are notch 1, notch 2, notch 3 and notch 4, which have similar structures.(21–23) Anja Baumgart et al. found that the lack of Notch 1 led to the reduction of early tumor formation, suggesting that notch 1 plays a role in promoting cancer. However, the expression of notch 2 receptor in NSCLC is weak, suggesting that notch 2 may play an anti-cancer role in NSCLC.(24) Compared with notch 1 and 2, notch 3 receptor has received less attention, but its role can not be ignored. Min Zhou et al. showed that the activation of Notch 3 can promote the development of lung cancer, suggesting that Notch 3 may be a carcinogen of lung cancer.(25) Therefore, we speculated that the increased expression of emid1 might play an anti-cancer role by inhibiting the activity of Notch 1 and notch 2, or by stimulating the activity of notch 2. In a word, through the study of biological function, we can further understand the function of EMID1.

Tumor infiltrating lymphocyte, as a primary prognostic biomarker in tumor progression, can also serve to independently predict sentinel lymph node status and survival in cancer.(26, 27) A significant aspect of our study entailed EMID1 expression with reference to immune infiltration levels in LAUD, and concluded a positive correlation with B cells, thereby indicating EMID1 regulated tumor immunology. Research evidence increasingly submits tumor-infiltrating B cells correlate with positive clinical outcomes in several cancers, producing antibodies whilst also acting as APCs or antigen presenting cells that intrinsically regulate cellular immunity in a tumor microenvironment.(28–30) Moreover, B cells also have the opposite effect on tumor immunity and progression. For example, B cells regulate adaptive immunity by releasing circulating cytokines or chemokines, thereby recruiting immunosuppressive myeloid cells, which eventually lead to chronic inflammation or neonatal cancer.(31) Kuo-Hao. et al. also correlated B cell infiltration with anti-PD-L1 therapy to potentially advance prospective treatment options for lung cancer patients.(32) However, the mechanism of EMID1 regulating tumor-infiltrating B cells are not clear, and more research is needed.

Our study still has several shortcomings. First of all, the clinical data types of our samples were less, which inevitably leads to the loss of some useful information. Secondly, our study did not analyze the signal mechanism at the cytological level. Finally, this study did not carry out protein level analysis because there was not enough clinical sample data. In a word, our conclusions still require validation via an expanded clinical sampling in future research.

In conclusions, our study assessed the relationship between EMID1 and clinicopathologic variables and survival outcomes and explored the mechanism of EMID1 in lung adenocarcinoma. Notch signaling pathway may be the main regulation pathway of EMID1 in LUAD. In addition, the change of EMID1 expression was related to the proportion of B cells in LUAD, EMID1 may play an important role in the immune environment of LUAD. Therefore, EMID1 may be a promising prognostic marker for lung adenocarcinoma.

Declarations

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Contributions

CR-L, RF-W, FW-Q and Z-Z conceived the study and participated in the study design, performance, coordination and manuscript writing. WJ-D, F-F, LL-W carried out the assays and analysis. FW-Q, Z-Z revised the manuscript. All authors reviewed and approved

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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None

Availability of data and material

All data are included in the article.

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Tables

Tables 1-3 are not provided with this version.

Figures

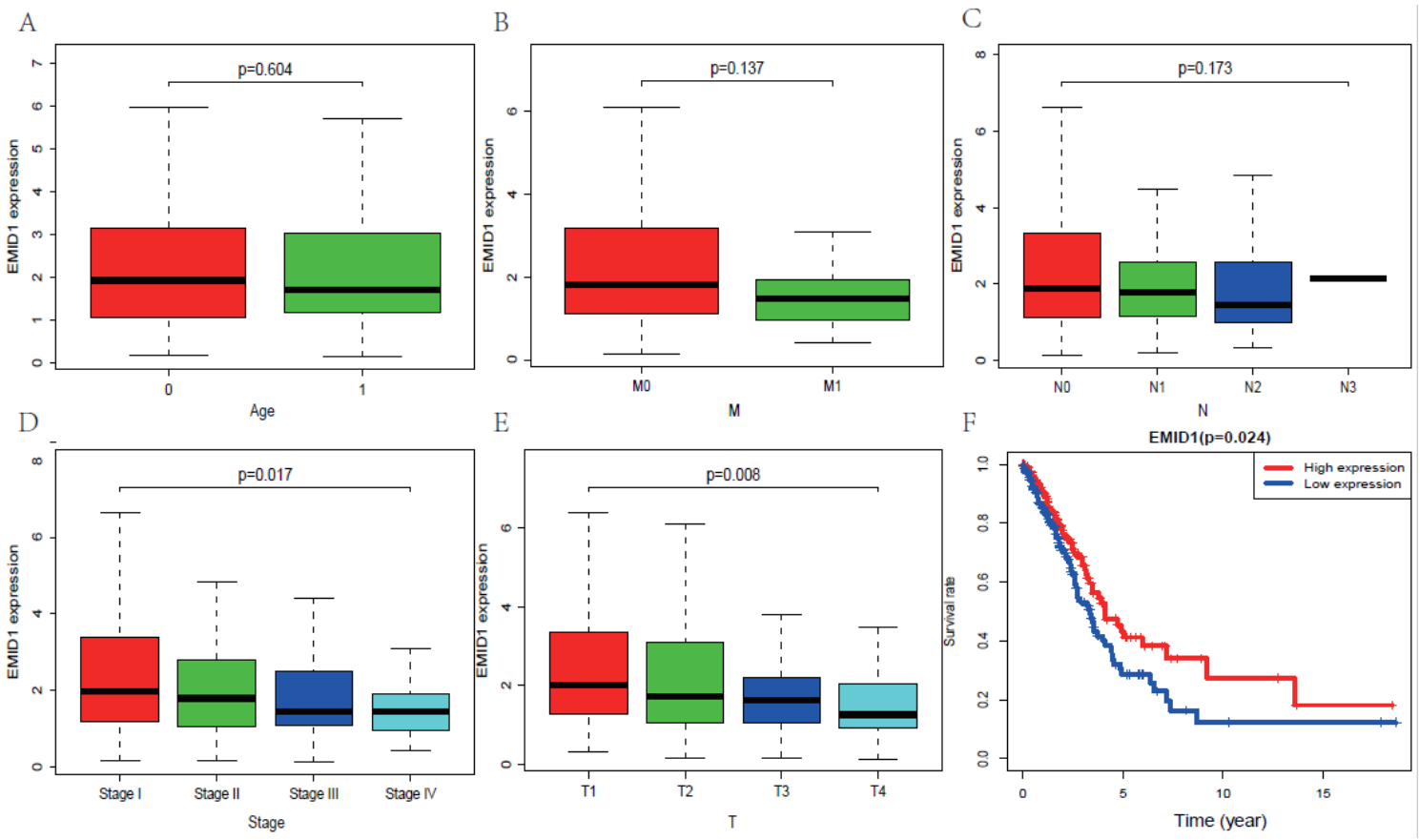

Figure 1

(A-E). Association with EMID1 expression and clinicopathologic characteristics, including A: Age, B: M, C: N, D: stage, E: T, (T: tumor status, N: lymph node, M: distant metastasis). (F). The relationship between the expression of EMID1 and the prognosis of patients

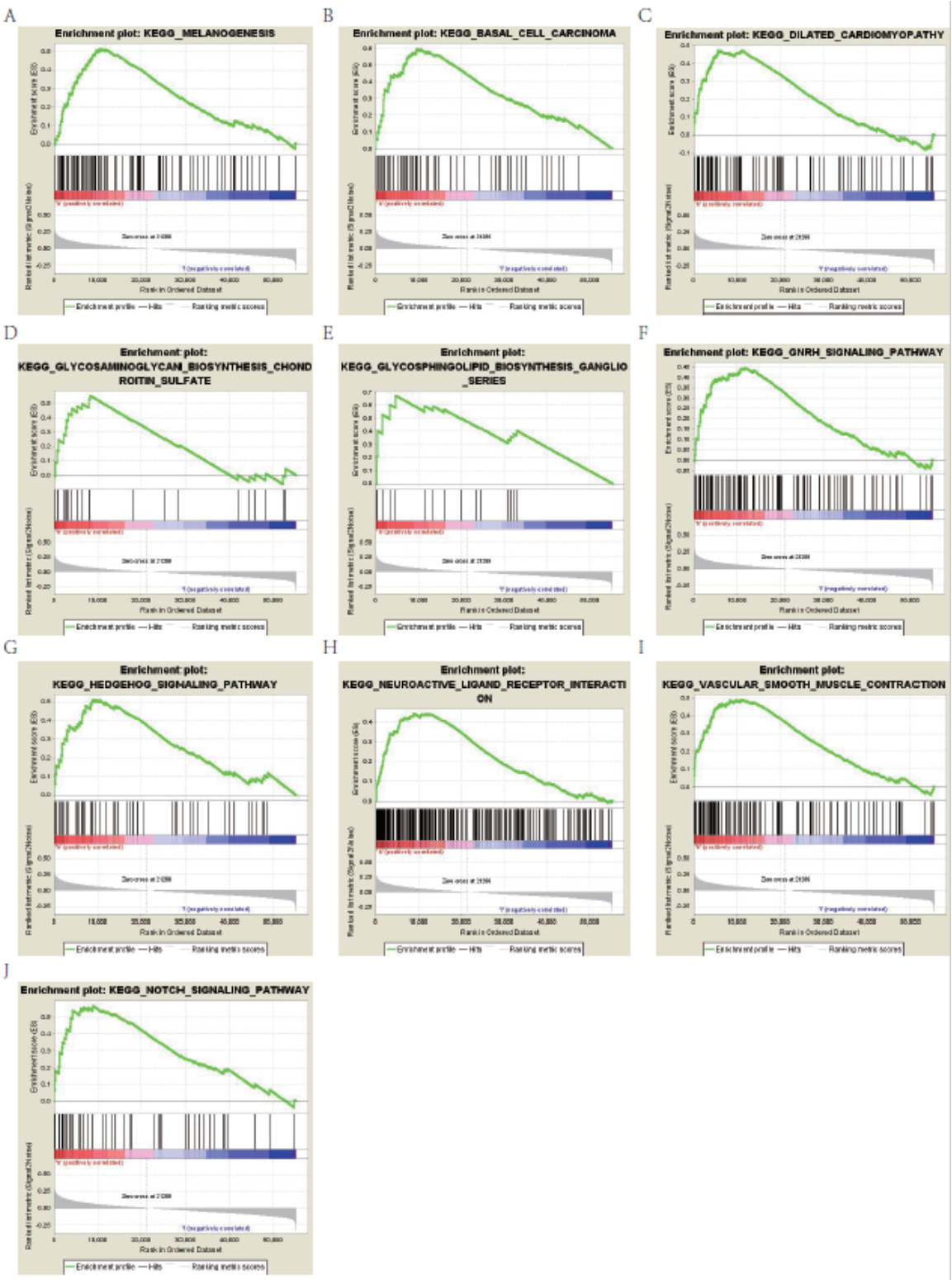

Figure 2

Enrichment plots from (GSEA). The results showing melanogenesis (A), basal cell carcinoma(B), dilated cardiomyopathy(C), glycosaminoglycan biosynthesis heparin sulfate(D), glycosphingolipid biosynthesis ganglio series(E), gnrh signaling pathway(F), hedgehog signaling pathway(G), neuroactive ligand receptor interaction(H), vascular smooth muscle contraction(I), notch signaling pathway(J) are differentially

enriched in high EMID1 expression. ES, enrichment score; NES, normalized ES; NOM p-val, normalized p-value.

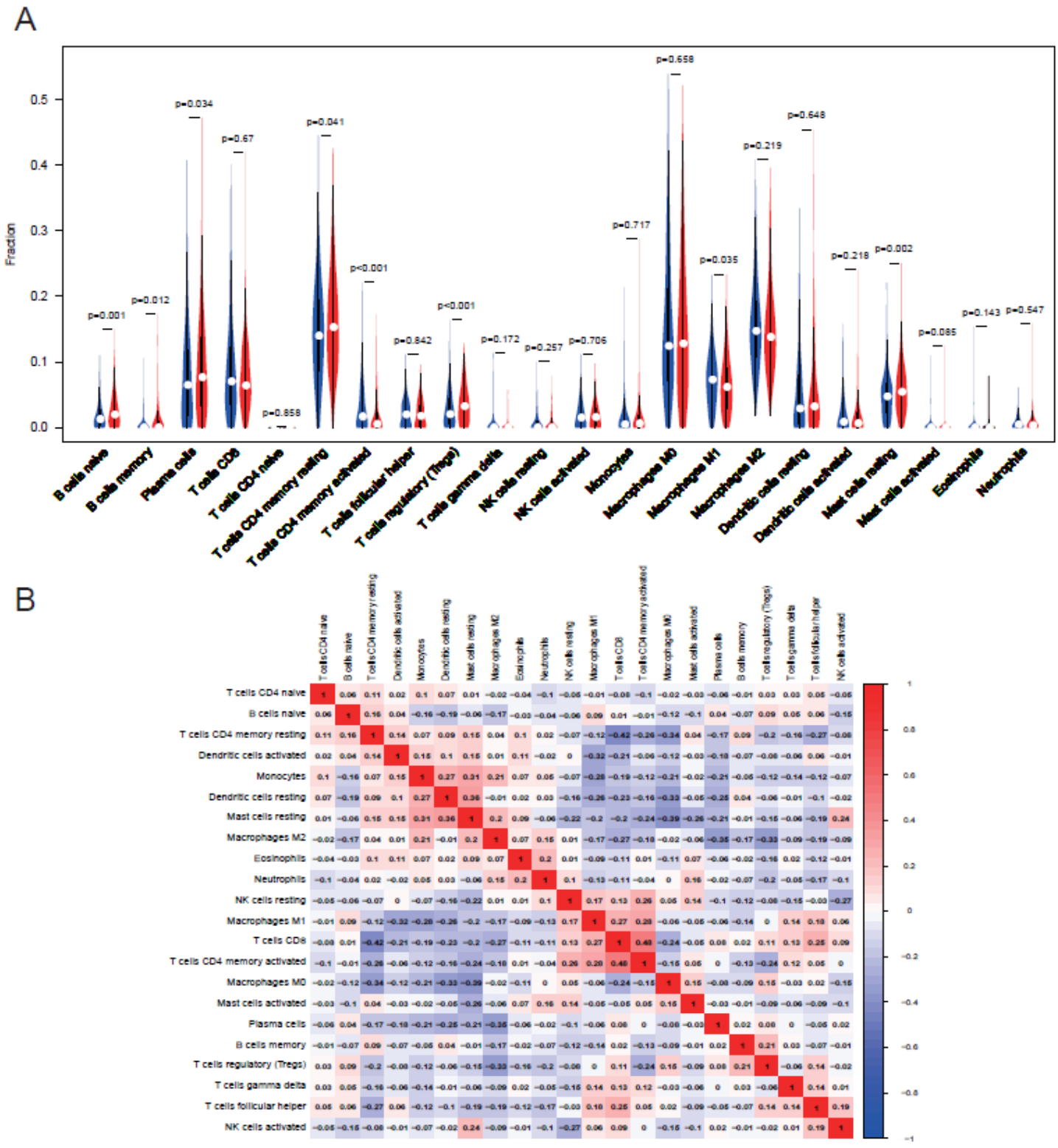

Figure 3

(A). The relative proportion of 22 kinds of immune cell infiltration of lung adenocarcinoma in high EMID1 expression group and low EMID1 expression group were estimated by CIBERSORT. (B). The correlation of different proportion of infiltrating immune cell subsets in lung adenocarcinoma.

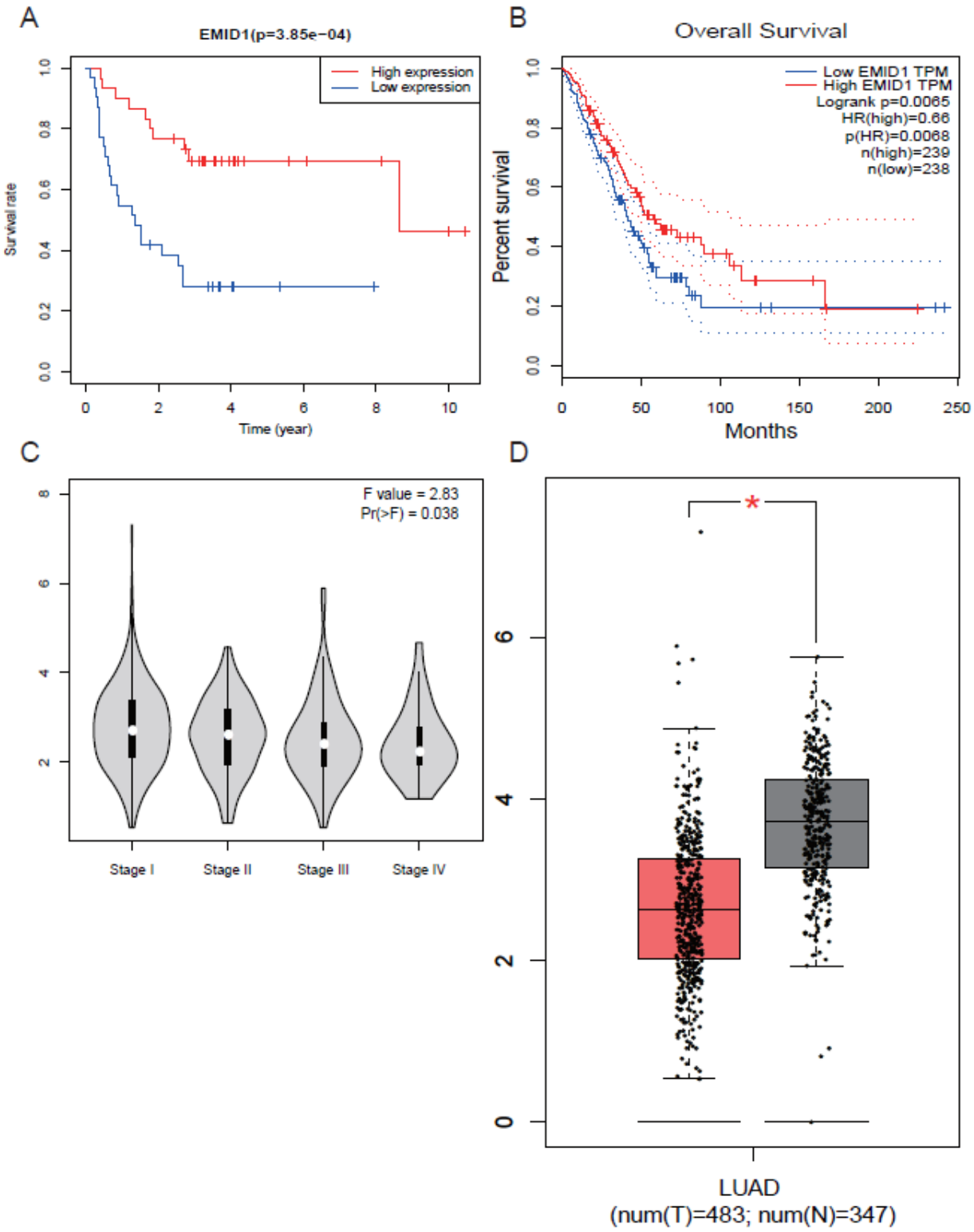

Figure 4

(A) The relationship between the expression of EMID1 and the prognosis of LUAD patients in GEO database. (B) The expression level and overall survival rate of EMID1 in GEPIA database. (C) The expression of EMID1 was different in different pathological stages. (D) The expression level of EMID1 in normal and cancer tissues.