ITGB2 Expression is Negatively Correlated with the Prognosis of Acute Myeloid Leukemia

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Primary research

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Abstract

Objective: Acute myeloid leukemia (AML) is a clonal malignant hematological neoplasm with a poor prognosis and high heterogeneity. Many studies have been conducted on the diagnosis and treatment of AML, but the immune microenvironmental mechanisms underlying AML disease progression have not been fully elucidated. The aim of this study was to find the potential genes in tumor microenvironmental mechanisms underlying the initiation and progression of AML through relevant biological informatics analysis, and investigate the potential influence of the gene in tumor microenvironment (TME).

Methods: AML samples of genes were retrieved from The Cancer Genome Atlas (TCGA) databases. The number of tumor-infiltrating immune cells (TIC) as well as immune and stromal components in AML cases was calculated using the ESTIMATE and CIBERSORT algorithms. Two methods, COX regression analysis and protein-protein interaction (PPI) network, were applied to obtain related genes, and the intersection of related genes was taken to obtain differentially expressed genes (DEGs). Gene Set Enrichment Analysis (GSEA) was used for explore the biological signaling pathway. CIBERSORT analysis for the proportion of TICs was performed to reveal that TICs which are related of the target gene.

Results: Cross-tabulation analysis of univariate COX regression analysis and PPI network known the β2 integrin factor (ITGB2) as a major predictor of AML prognosis. High expression of ITGB2 was correlated with low survival of AML patients. GSEA revealed that the higher the ITGB2 gene expression, the more active the immune-related activity. CIBERSORT analysis of the TICs ratio revealed that 9 kinds of TICs were negatively correlated with the expression of ITGB2, including CD4 memory resting T cells, CD8 T cells, naive B cells, resting NK cells, Plasma cells, follicular helper T cells, resting Mast cells, Eosinophils and activated mast cells. Only monocytes were positively correlated with ITGB2 expression. These results provided further evidence that ITGB2 levels may determine the prognosis of AML patients by modulating the immune status of TME, which provides an additional suggestion for the treatment of AML.

1. Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy with a high degree of molecular heterogeneity [1]. The main treatment options for AML are chemotherapy and hematopoietic stem cell transplantation. However, the toxicity of chemotherapeutic agents may lead to serious and even fatal complications. Effective treatment options for advanced malignancies are cancer immunotherapy, such as chimeric antigen receptor (CAR) T cells, bi-specific T cell engagers (BiTEs), and immune checkpoint blockers (ICBs).

Cancer immunotherapy is a revolutionary approach to the treatment of new therapeutic malignancies. It can use immune cells to destroy patient's cancer cells [2]. A deeper understanding of immune mechanisms in the tumor microenvironment (TME) could facilitate the discovery of biomarkers that predict clinical outcomes[3, 4].The tumor microenvironment (TME) includes various immune and stromal cells as well as extracellular components. It is associated with tumor inflammation, immunosuppression,

and angiogenesis.[5]. Among them, immune cells and stromal cells are the main components of the immune microenvironment necessary to promote the growth and development of tumor cells[6–8]. A growing number of studies have shown that tumor-infiltrating immune cells (TIC) in immune microenvironment have a role in the treatment of malignant tumors [9]. Data suggested the presence of reactive γ-interferon-secreting tumor-infiltrating lymphocyte (TIL) in AML patients. They have antitumor activity, which may have great potential in cellular therapy for AML[10].

In this paper, we applied both ESTIMATE and CIBERSORT calculation methods to calculate the relationship between immune and stromal components and survival in AML samples from The Cancer Genome Atlas (TCGA) database, and identified a predictive biomarker, *Integrin subunit beta 2* (ITGB2), by PPI and COX analysis. ITGB2 gene is located on chromosome 21 (21q22.3). It is 40 kb in length, contains 16 exons, and encodes the β2 subunit (CD18) of the β2 integrin family[11]. Mutations in the ITGB2 gene can result in defective expression or abnormal function of the CD18 molecule, affecting leukocyte adhesion and preventing leukocytes from reaching the site of inflammation[12]. CD18, products of Integrin subunit beta 2 (ITGB2) genes, participate in several functional pathways of immune system. CD18 may play a role in the development of thrombotic complications in PMF patients [13]. CD11/CD18-deficient MDS is considered a completely different subtype with a poorer prognosis [14]. Liu et al. recently reported that YAP promotes tumor cell invasion by inducing ITGB2 expression in tumor cells [15]. A study found that CAFs with high ITGB2 expression could promote tumor proliferation in OSCC by activating the PI3K/AKT/mTOR signaling pathway through NADH oxidation in the mitochondrial oxidative phosphorylation system[16]. These studies suggest that ITGB2 might play an important role in TME. We applied PPI networks and COX regression analysis to compare differentially expressed genes (DEGs) in the immune and stromal components of AML samples and concluded that ITGB2 may be an important potential indicator of altered immune microenvironment status in AML.

Thus, this article systematically integrated and analyzed the DEGs in the immune microenvironment of AML, and the functional enrichment and validation were identified by using PPI networks and COX regression analysis. Then, ITGB2 were related to the prognosis of AML were screened out. Our results provide novel information of ITGB2, an important gene in the immune microenvironment, for the prognosis of AML, a hematologic tumor with poor prognosis, and provide new research ideas for the early diagnosis and treatment of AML.

2. Results

2.1 Analysis Process of This Study

To estimate the number of immune and matrix components and the proportion of TICs in AML samples, clinical data of 200 cases and 151 transcriptomic data were downloaded from the TCGA database, and then ImmuneScore and StromalScore were obtained by ESTIMATE algorithm. DEGs were obtained by differential analysis. The network core genes were found using PPI network. The prognosis-related genes obtained using univariate COX regression analysis, and both were cross-tabulated to finally find a single

gene, ITGB2. ITGB2 was used to perform a series of subsequent analyses regarding AML survival, GSEA, TICs correlation, etc.

2.2 Correlation between Immune/Stromal/ESTIMATE Score and AML survival

To determine the potential association of overall survival with immune scores and stromal scores, we classified the 200 AML cases into high and low subgroups based on the median score of ImmuneScore or StromalScore. Higher scores estimated in the ImmuneScore or StromalScore indicate more immune or stromal components in the TME. ESTIMATEScore was the sum of the ImmuneScore and StromalScore indicating the combined proportion of both components in the TME. As shown in Fig. 1, the results revealed that the proportion of immune components and ESTIMATEScore had negative correlation with the overall survival rate, with no statistically significant differences, while StromalScore was not correlated with the survival rate. These results implied that the immune components of TME may have implications for the prognosis of AML patients.

2.3 Identification of DEGs based on immune scores and stromal scores in AML

The AML patient's immune and stromal scores were divided into two groups with high and low scores, and the scores were compared between the two groups, setting the cut-off criteria as $p < 0.05$ and $|\text{fold change}| > 1$ to find the genes with differences. The higher the patient's immune cell content, the higher the gene expression level. Comparing the gene expression levels of the high and low scoring subgroups, 897 DEGs were obtained, of which, 655 genes were upregulated and 242 genes were downregulated (Figs. 2B,C,D). Similarly, 785 DEGs were obtained from StromalScore, consisting of 567 up-regulated genes and 218 down-regulated genes (Figs. 2A,C,D). The DEGs of immune cells and stromal cells were taken to intersect to obtain common DEGs, which were represented by differential Venn diagrams. A total of 502 genes were upregulated and 122 genes were downregulated. These 624 DEGs may be determinants of the tumor microenvironment in AML patients.

Results from gene ontology (GO) enrichment analysis indicated that the DEGs almost mapped to the immune-related and inflammation-related GO terms, such as neutrophil activation and positive regulation of cytokine production (Fig. 2E). The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis also displayed the enrichment of cytokine–cytokine receptor interaction, and osteoclast differentiation, phagosome, staphylococcus aureus infection, tuberculosis (Fig. 2F). Thus, the overall functions of DEGs are associated with the immune and inflammatory response, which implied that the involvement of immune factors was a predominant feature of TME in AML.

2.4 Intersection Analysis of PPI Network and Univariate COX Regression

To investigate whether there are protein interactions between these DEGs, we constructed a PPI network based on the STRING database and Cytoscape software. The interconnections between the 624 genes can be seen in Fig. 3A. Among them, the top 30 genes ranked by the number of gene-linked nodes were shown in the bar chart (Fig. 3B). Univariate COX regression analysis was performed for survival of AML patients, and the top 20 genes were obtained in order of P-value (Fig. 3C). Then, a cross-tabulation analysis was performed between the 30 genes of the leading nodes in the PPI network and the top 20 genes of the COX regression analysis. Only one factors, ITGB2, was overlapping from the above analyses (Fig. 3D).

2.5 The Correlation of ITGB2 Expression with the Survival in AML Patients

The gene product of ITGB2 is one of the βintegrins, which is reported to be expressed mainly on immune cells. It has been suggested that it can be involved in leukocyte extravasation, binding and clearance of complement fragments, phagocytosis and killing of intracellular pathogenic microbes [17, 18]. ITGB2 has only been reported in CLL in hematologic tumors. However, it has never been reported in acute leukemia. In this study, all AML samples were divided into high and low groups by median of ITGB2 expression. The survival analysis showed that AML patients with ITGB2 low expression had longer survival than that of ITGB2 high expression ($p = 0.007$) (Fig. 4A). Given the levels of ITGB2 were negatively correlated with the survival of AML patients, ITGB2 expression-associated signal pathways were investigated by GSEA enrichment analysis. ITGB2 is an immune-related factor, and the higher the gene expression, the more active some signaling pathways are, such as B cell receptor singnaling pathway, chemokine signaling pathway, Toll like receptor singnaling pathway (Fig. 4B). These results suggested that ITGB2 might be a potential indicator of the tumor microenvironment in AML patients.

2.6 Correlation between ITGB2 expression and TICs

To further confirm the correlation between ITGB2 gene and TICs, we analyzed the proportion of tumor-infiltrating immune subgroups using the CIBERSORT algorithm. We used both immune cell differential analysis and correlation tests to find 22 immune cells in AML samples (Fig. 5). In the difference analysis of immune cells, there were 12 kinds of immune cells in the two groups with high and low expression of ITGB2 have statistical difference (Fig. 6A). The results from the correlation analysis showed that a total of 10 kinds of TICs were correlated with the expression of ITGB2 (Fig. 6B). Among them, only one kind of TICs, monocytes was positively correlated with ITGB2 expression; nine kinds of TICs were negatively correlated with ITGB2 expression, including native B cell, CD8 + T cells, resting NK cells, and resting Mast cells, activated NK cells, activated mast cells, resting CD4 memory T cells, Plasma cells and follicular helper T cells. These results further proved that the levels of ITGB2 expression was closely correlated with immune cells in the TME.

3. Discussion

The treatment of AML is challenging. Although most AML patients can achieve remission quickly after chemotherapy, the remission period is relatively short. The median survival is 46 months in non-elderly patients (age < 65 years) and only 4 months in elderly AML patients[19, 20]. Bone marrow is considered a reservoir of leukemic stem cells (LSCs) that persist after chemotherapy and cause disease relapse. One of the important reasons for AML chemoresistance has long been related to the interaction of leukemic cells with the bone marrow microenvironment. In this study, we demonstrated that immune scores in the tumor microenvironment are associated with poor prognosis (Fig. 1), which may be related to ITGB2-related activation of immune and inflammatory pathways.

The pathogenesis of hematologic malignancies such as acute lymphoblastic leukemia (ALL), lymphoma, chronic myeloid leukemia (CML) has been reported to be related to the immune microenvironment[21–24], but the immune microenvironment of AML is relatively poorly understood. AML is a disease with great molecular heterogeneity, and many studies on the immune microenvironment of AML lack sufficient patient numbers or molecular data results, which makes it more difficult to study the pathogenesis of AML in the tumor microenvironment. In the presented study, we attempted to identify immune microenvironment-related genes from TCGA database to further determine the survival time of AML patients. We have shown through a series of bioinformatics analyses that ITGB2 may be an important indicator in the immune microenvironment associated with poor prognosis in AML patients (Fig. 4).

AML is a highly heterogeneous disease with rapid progression and poor prognosis that may depend on the bone marrow microenvironment[25]. Several studies have shown that immune cells and stromal cells in the bone marrow microenvironment are important components affecting AML cell proliferation, apoptosis and drug resistance[26]. In recent years, somatic mutations such as FLT3 and NPM1 have been shown to have strong prognostic significance and have been included in NCCN and ELN guidelines.[27, 28]. In this study, we identified immune genes from the TCGA database in the bone marrow microenvironment that have prognostic significance in AML.PPI network and COX regression analysis were applied to obtain the only common DEGs, ITGB2, and overall survival analysis was performed. We also used bioinformatics approaches to explore DEGs in depth, including GO analysis, signaling pathway enrichment analysis (Fig. 2).

ITGB2 was identified to be involved in immune activities. Applying the collected expression and CGH microarray for analysis, some investigators pointed out that the ITGB2 gene may be one of the reasons why acute leukemia cells antagonize anthracyclines and are not sensitive to relevant chemotherapy regimens. In AML resistance of blasts to idarubicin and mitoxantrone may reflect impaired integrins pathway[29]. The high expression of ITGB2 in triple negative breast cancer affects the prognosis of patients[30]. There were similar results in our study. We concluded from a series of bioinformatics analyses that ITGB2 may be an important indicator of the status of the immune microenvironment in AML patients. Here, we analyzed AML transcriptome data from the TCGA database and revealed that the overexpression of ITGB2 was significantly associated with poor prognosis (Fig. 4). Thereby, we further analyzed the relationship between the ITGB2 gene and the immune microenvironment. The GSEA results showed that immune-related signaling pathways, such as B cell receptor signaling pathway, chemokine

signaling pathway, Toll like receptor signaling pathway, were significantly enriched in the ITGB2 high expression group. These results implied that the ITGB2 gene may be involved in the state transition of the AML tumor microenvironment from immune-dominant to inflammatory-dominant.

4. Conclusion

In conclusion, we used the TCGA database as the basis of our study to find important immune and stromal cell-related differential genes that affect the immune status of AML patients through an integrated bioinformatics approach, and investigated the relationship between disease progression and immune microenvironment differential genes in AML patients. Considering the specific properties of the hematopoietic microenvironment of leukemia[31], ESTIMATE may not accurately predict infiltrating stromal and immune cells for the AML microenvironment, and we need to develop a more suitable and accurate algorithm. Due to the limited number of AML patients in the TCGA database, there is a need to further investigate AML disease-associated stromal and immune cell features in large multicenter clinical trials in AML patients, which may provide new prognostic biomarkers for achieving precision tumor therapy. We performed transcriptomic analysis of AML data and the results suggested that the ITGB2 gene in TME had an impact on the prognosis of AML patients. These results highlighted the significance of exploring the interactions between tumor cells and immune cells, providing a new area of research for further effective treatment options for AML.

5. Materials And Methods

5.1 Database

Transcriptome RNA-seq data of 200 AML cases and the corresponding clinical data were downloaded from TCGA database (https://portal.gdc.cancer.gov/). Transcriptomic data and clinical data were obtained from TCGA database, where transcriptomic data consisted of 3 files, cart, manifest, metadata; clinical data was 1 file: cart. The downloaded files were calculated using the ESTIMATE algorithm to obtain the immune score and the stromal score. Clinical characteristics statistics in AML patients from TCGA were showed in Table 1.

5.2 Generation of Immune/Stromal/ESTIMATE Score and acquisition of DEGs

Stromal cells and immune cells from AML patients were scored. The higher the score, the higher the stromal or immune cell content in the sample. The higher the combined score, the lower the purity of the tumor cells. Patients were classified into high and low groups based on the median values of the immune and stromal cell scores. The data analysis was done by using R 4.0.1 and the R packages “limma” and “pheatmap”. DEGs were obtained by differential analysis of high and low subgroups. In our work, genes with p-values < 0.05, fdr < 0.05 and |fold change| > 1 were defined as DEGs.

5.3 GO and KEGG enrichment analyses of DEGs

The 624 DEGs were used to perform GO and KEGG enrichment analyses with R language with the aid of R packages "enrich plot," "Cluster Profiler," "ggplot2," and "org. Hs.eg.db." Only terms with both p- and q-value of < 0.05 were considered significantly enriched.

5.4 PPI Network Construction

We built the PPI network in the STRING website, and then visualized the network using the Cytoscape version 3.8. The number of linkage nodes per gene was found by PPI. The higher the number of linkage nodes of a gene, the more critical its role in PPI and may be a PPI core gene.

5.5 COX Regression Analysis

To identify prognosis-related genes, we performed Cox regression analysis of DEGs using the R package "survival". HR > 1 indicates high risk genes, representing higher gene expression and higher patient risk. HR < 1 indicates low risk genes, representing higher gene expression and lower patient risk. $p < 0.01$ was considered statistically significant and genes were associated with prognosis. The top 20 genes associated with AML prognosis obtained by univariate COX regression analysis were shown in the forest plot.

5.6 GSEA enrichment analysis

The gene expression matrix file and cls file of group description were used as input files. C2 kegg gene set v7.1 were selected as main gene set with which GSEA performed using the software GSEA-4.0.

Declarations

6.1 Ethics approval and consent to participate

Not applicable

6.2 Consent for publication

Not applicable

6.3Availability of data and materials

Not applicable

6.4 Competing interests

The authors declare that they have no competing interests with respect to this manuscript.

6.5 Funding

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6.6 Authors' contributions

Jianan Zhou, Pei Li analysed the data. Jianan Zhou and Bobin Chen wrote the paper. Jianan Zhou, Bobin Chen and Pei Li designed the study.

6.7 Acknowledgements

Not applicable

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Tables

Table 1 Clinical characteristics statistics in AML patients from TCGA

Clinical characteristics	Total	%
Age at diagnosis (year)		
Young age (<60)	108	54
Old age (>=60)	92	46
Gender		
Male	109	54.5
Female	91	45.5

Figures

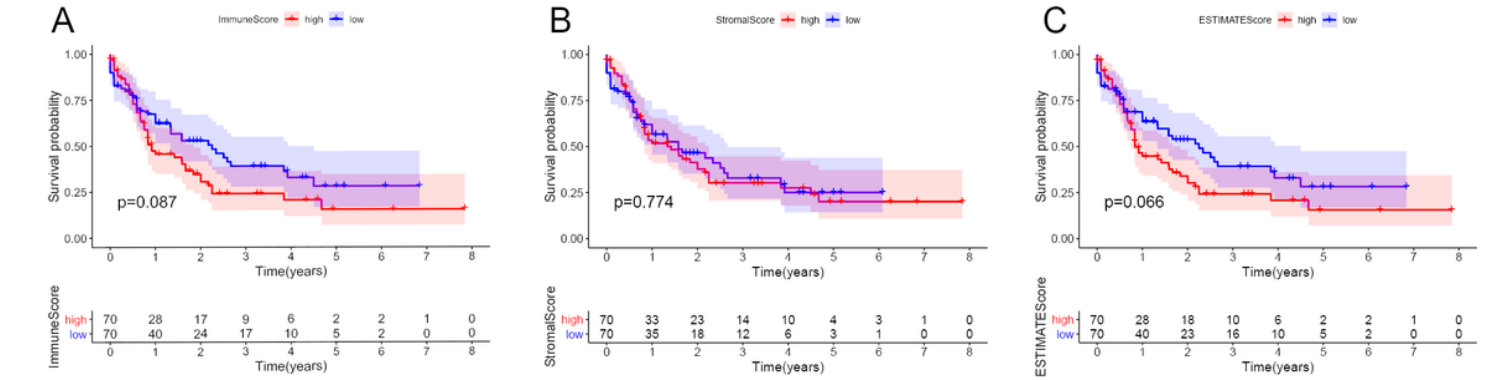

Figure 1

Correlation between scores and overall survival in patients. A. Kaplan-Meier curve of overall survival for AML patients with low vs. high ImmuneScore (p = 0.087 by log-rank test). B. Kaplan-Meier survival curve for StromalScore with p = 0.774 by log-rank test. C. Survival analysis curves for AML patients grouped by ESTIMATEScore (p = 0.066 by log-rank test).

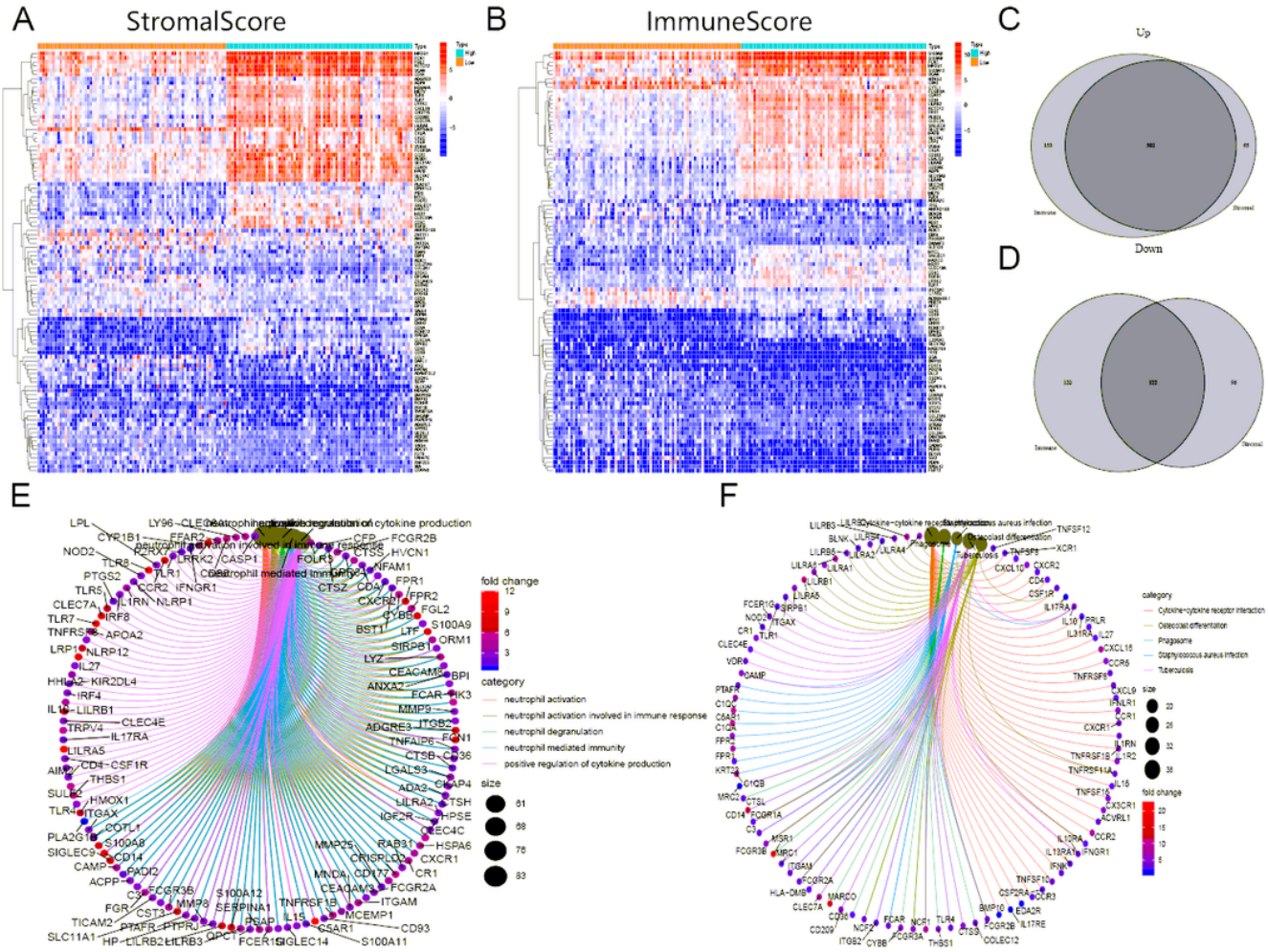

Figure 2

Heatmaps, Venn plots, and enrichment analysis of GO and KEGG for DEGs. A. Heatmap for DEGs generated by comparison of the high score group vs.the low score group in StromalScore. B. Heatmap for

DEGs in ImmuneScore, similar with (A). C,D. Venn diagram showing the common up- and down-regulated DEGs in ImmuneScore and StromalScore. E,F. GO and KEGG enrichment analysis for 624 DEGs.

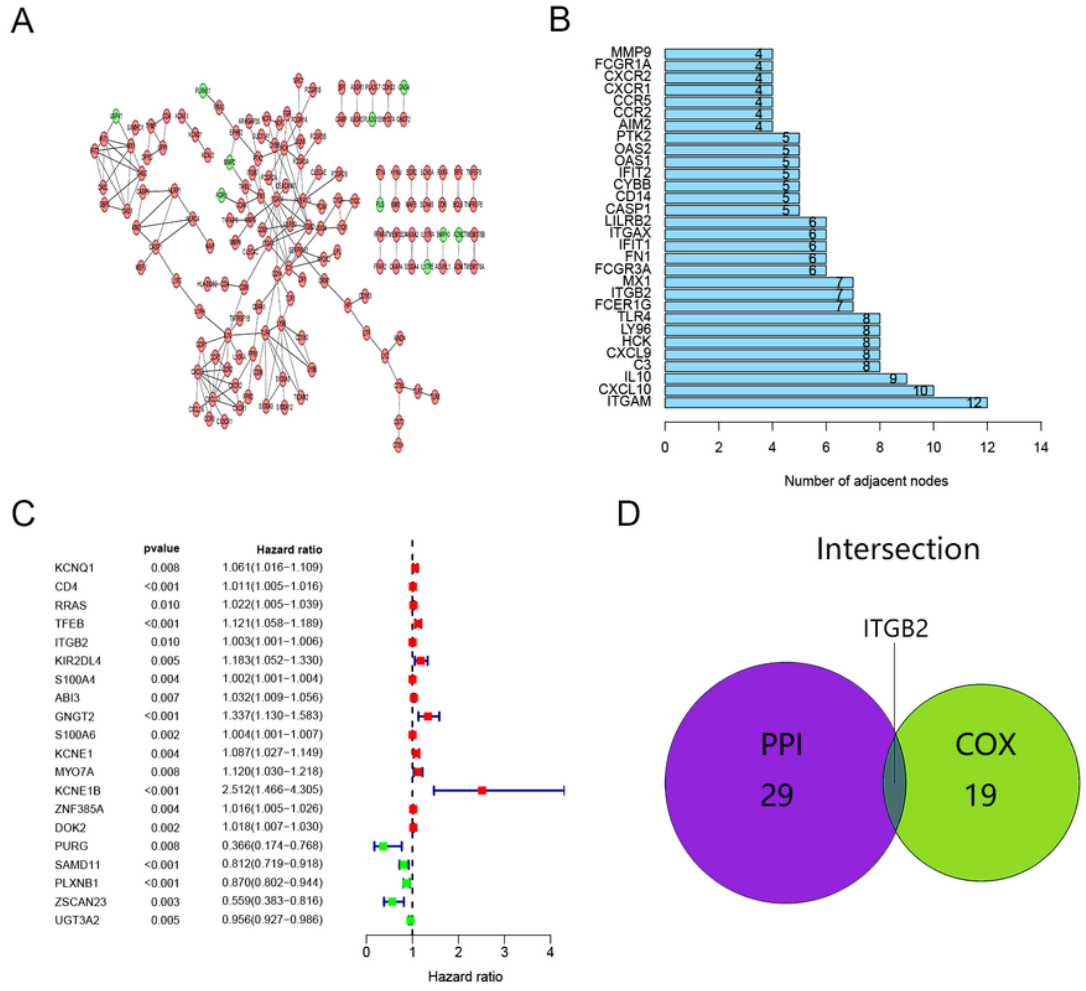

Figure 3

Enrichment plots of PPI and Cox. A. The minimum interaction requirement was set as high reliability (0.95). B. The top 30 genes ordered by the number of nodes. C. The forest map represented the results of the univariate Cox regression analysis of DEGs. D. Vennplot showing the one common factor shared by leading 30 nodes in PPI and top 20 factors in univariate COX.

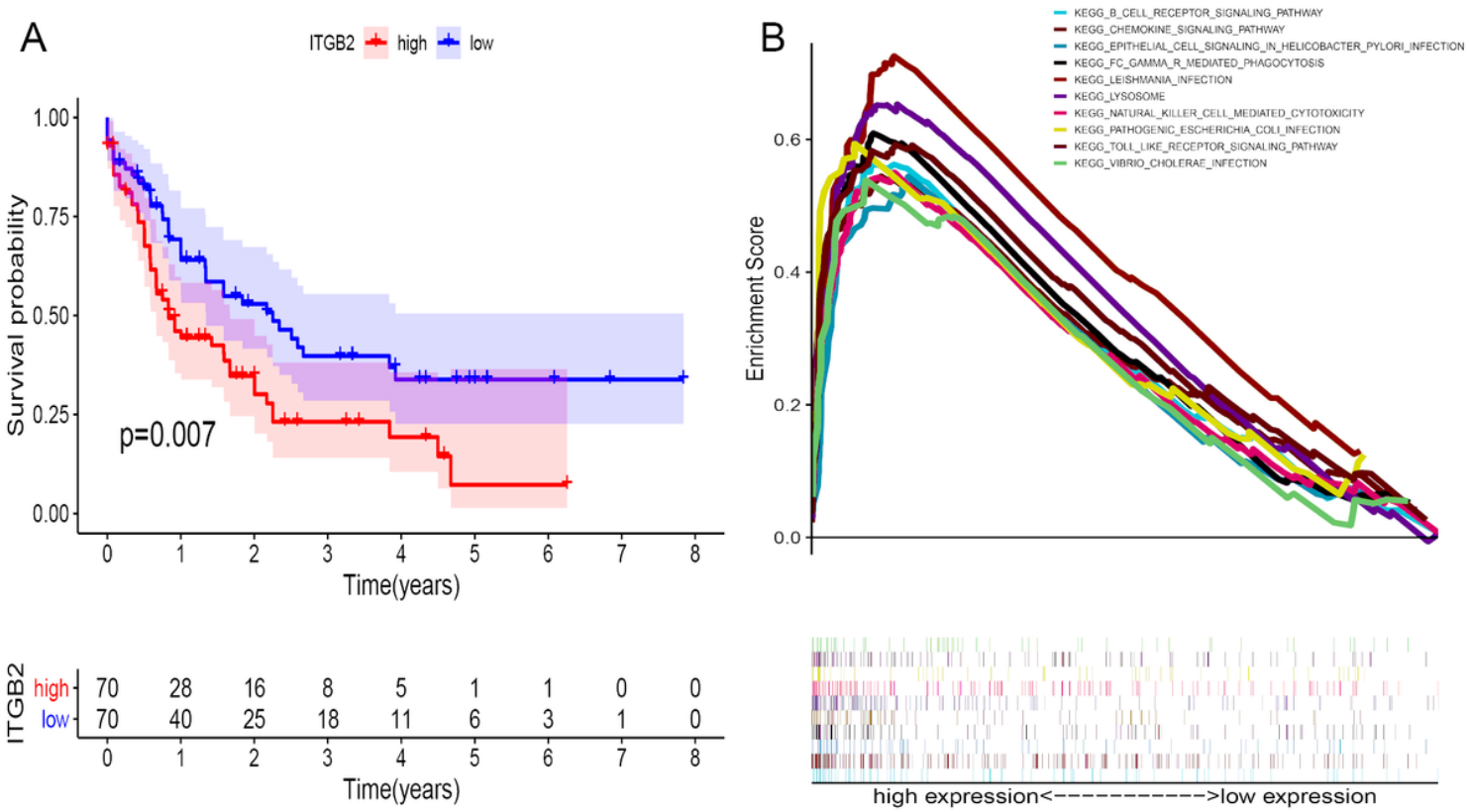

Figure 4

Correlation of ITGB2 expression with prognosis in AML. A. The relationship between ITGB2 expression and AML patient survival rate was analyzed by Kaplan–Meier survival analysis. p = 0.007 by log-rank test. B. GSEA for samples with high ITGB2 expression. Only 10 leading gene sets were displayed in the plot.

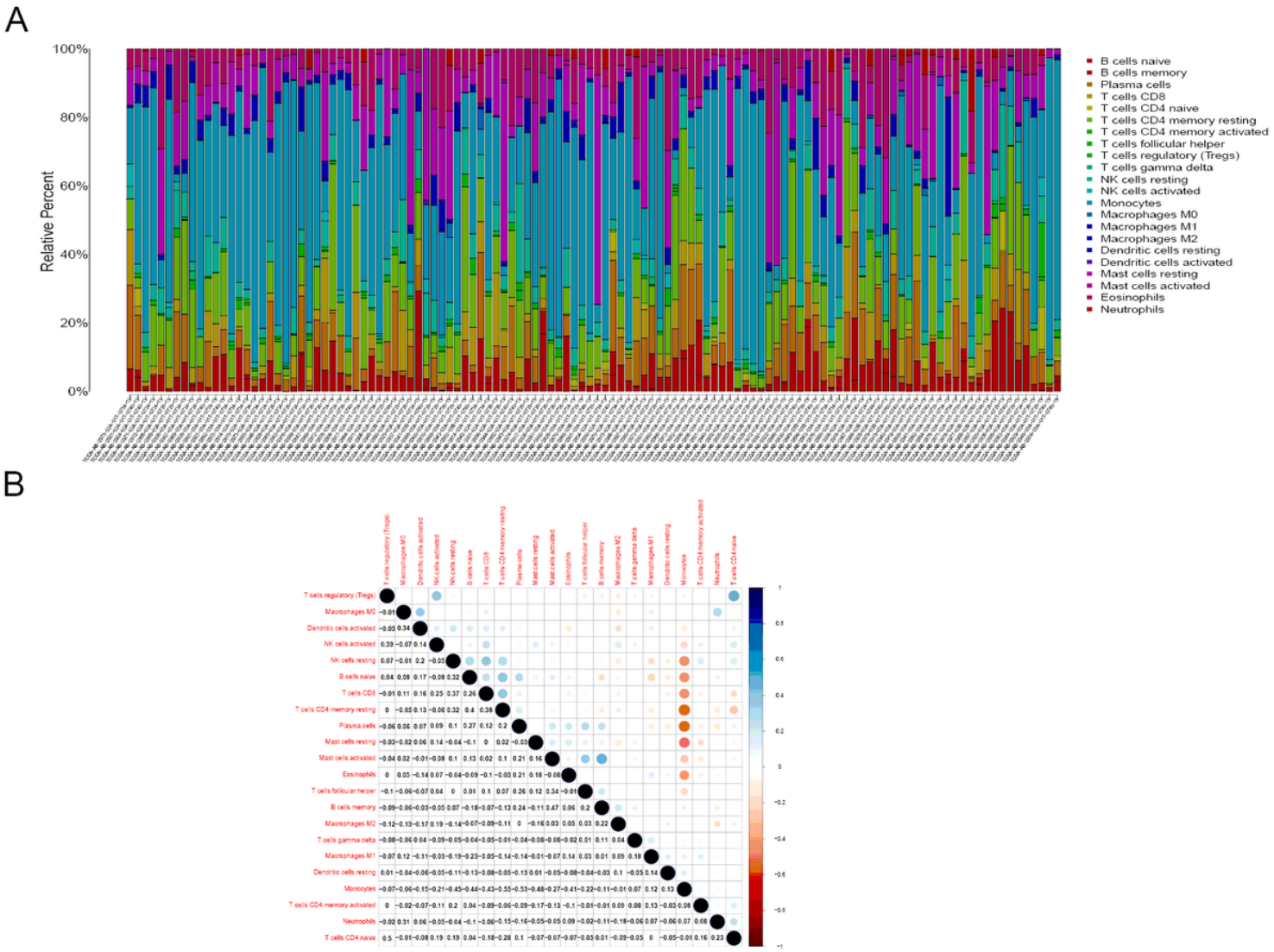

Figure 5

Correlation of ITGB2 expression with TICs in AML. A. The bar chart showed a ratio of 22 kinds of TICs in AML samples. Column names of plot were sample ID. B. Correlation matrix showing the correlation between 22 kinds of TICs. 0-1 represents the level of positive correlation, while -1-0 represents the level of negative correlation.

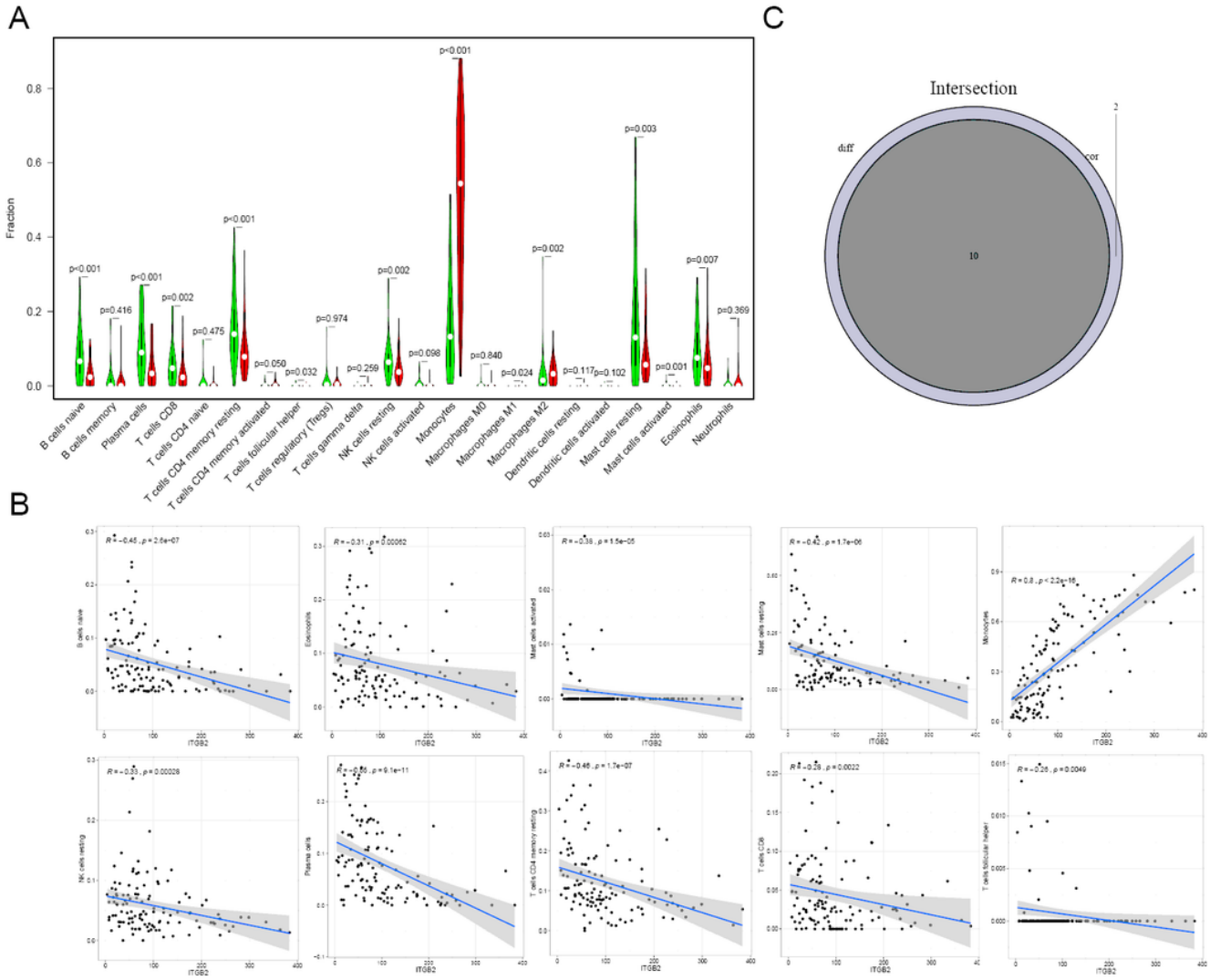

Figure 6

Correlation of TICs proportion with ITGB2 expression. A. Violin diagrams is used to show the proportion of 22 kinds of TIC between AML samples with high or low ITGB2 expression. Data were assessed by the Wilcoxon rank-sum test. B. Scatter plots showed 10 kinds of TICs that correlated with ITGB2 expression ($p < 0.05$). C. Venn diagram showed 10 kinds of TICs associated with ITGB2 expression, verified by difference and correlation tests, respectively.