Research Article

CD86 Is Associated with Immune Infiltration and Immunotherapy Signatures in AML and Promotes Its Progression

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Background. Cluster of differentiation 86 (CD86), also known as B7-2, is a molecule expressed on antigen-presenting cells that provides the costimulatory signals required for T cell activation and survival. CD86 binds to two ligands on the surface of T cells: the antigen CD28 and cytotoxic T lymphocyte-associated protein 4 (CTLA-4). By binding to CD28, CD86—together with CD80—promotes the participation of T cells in the antigen presentation process. However, the interrelationships among CD86, immunotherapy, and immune infiltration in acute myeloid leukemia (AML) are unclear. Methods. The immunological effects of CD86 in various cancers (including on chemokines, immunostimulators, MHC, and receptors) were evaluated through a pan-cancer analysis using TCGA and GEO databases. The relationship between CD86 expression and mononucleotide variation, gene copy number variation, methylation, immune checkpoint blockers (ICBs), and T-cell inflammation score in AML was subsequently examined. ESTIMATE and limma packages were used to identify genes at the intersection of CD86 with StromalScore and ImmuneScore. Subsequently, GO/KEGG and PPI network analyses were performed. The immune risk score (IRS) model was constructed, and the validation set was used for verification. The predictive value was compared with the TIDE score. Results. CD86 was overexpressed in many cancers, and its overexpression was associated with a poor prognosis. CD86 expression was positively correlated with the expression of CTLA4, PDCD1LG2, IDO1, HAVCR2, and other genes and negatively correlated with CD86 methylation. The expression of CD86 in AML cell lines was detected by QRT-PCR and Western blot, and the results showed that CD86 was overexpressed in AML cell lines. Immune infiltration assays showed that CD86 expression was positively correlated with CD8 T cell, Dendritic cell, macrophage, NK cell, and Th1 cell and also with immune examination site, immune regulation, immunotherapy response, and TIICs. ssGSEA showed that CD86 was enriched in immune-related pathways, and CD86 expression was correlated with mutations in the genes RB1, ERBB2, and FANCC, which are associated with responses to radiotherapy and chemotherapy. The IRS score performed better than the TIDE website score. Conclusion. CD86 appears to participate in immune invasion in AML and is an important player in the tumor microenvironment in this malignancy. At the same time, the IRS score developed by us has a good effect and may provide some support for the diagnosis of AML. Thus, CD86 may serve as a potential target for AML immunotherapy.

1. Introduction

Acute myeloid leukemia (AML) is a common hematological disease characterized by the clonal proliferation, abnormal differentiation, and cell death evasion of bone-marrow-derived hematopoietic stem and progenitor cells [1]. These cells proliferate in the peripheral blood and infiltrate the bone marrow. The tumor microenvironment in AML is characterized by immunosuppression, which promotes immune tolerance and the immune escape of malignant cells
The main components of the AML bone marrow microenvironment (BMM) include T cells, B cells, and NK cells [3]. The immune imbalance of T helper cells (Th cells) is a major contributor to the sudden progression of AML [4].

T-cell-mediated cellular immunity is primarily achieved by the specific binding of antigen peptides to the major histocompatibility complex (MHC) (first signal) and the binding of costimulatory molecules located on the surface of antigen-presenting cells (APCs) to their receptors (second signal) [5]. The absence of costimulatory molecules leads to immune unresponsiveness, which promotes tumor escape in AML. Owing to advancements in research, immunotherapies that retarget effector cells (T cells, NK cells) have been developed, and these have become the key for AML treatment [6]. Typically, tumors suppress the immune system, resulting in the impairment of T-cell function. The goal of immunotherapy is to eliminate this impairment. Studies have shown that vaccines for AML/dendritic cell fusion can amplify T-cell populations and prevent AML recurrence [7]. Therefore, effective immunotherapy approaches that target specific proteins are the key to AML treatment.

B7-2, better known as CD86, is a member of the B7 family [8]. CD28 and cytotoxic T lymphocyte antigen 4 (CTLA4) are regulated. CD86 can bind to CD28, leading to signal production and the recognition of antigenic peptides by T-cell receptors (TCRs), which leads to T-cell proliferation and IL-2 production [9]. CD86 has been reported to be overexpressed in samples from AML patients [10]. CD86 is a marker for monocytes and dendritic cells and is involved in the progression of AML [11]. Improvements in sequencing technology have promoted extensive research on molecular networks using gene sequencing data from public databases [12]. However, the correlation between CD86 and immunomodulators (chemokines, receptors, and MHC proteins), immunotherapy results, and immune checkpoint proteins in AML has not been reported. Therefore, it is very important to explore the associations among CD86-related molecules, immune infiltration, and immunotherapy.

2. Methods

2.1. Data. The Cancer Genome Atlas (TCGA) data: Pan-carcinoma (33 species) RNA sequencing (RNA-SEQ) data (FPKM values) were downloaded from the UCSC Xena data portal (https://xenabrowser.net/). They were converted to TPM format, and somatic mutation data and survival information were downloaded. Log2 transformation was performed on the RNA-SEQ data, and somatic mutation data were analyzed using MuTect. Copy number variation (CNV) data processed using GISTIC were downloaded. Further, StromalScore and ImmuneScore were calculated. Ten, ‘ggplot2’ was used to draw volcano maps and heat maps of the DEGs. A total of 308 up-regulated genes and 16 down-regulated genes were identified through this analysis.

2.2. Differential Gene Analysis. The R limma package was used to filter out immune-related genes (IRGs) (https://Bioconductor.org/packages/limma/). The differentially expressed genes (DEGs) were identified based on a cutoff value of false detection rate (FDR) < 0.05 and Log2 [fold change] > 1. The differentially expressed IRGs were then extracted from the list of all DEGs.

2.3. Analysis of Immunological Characteristics of AML. First, using the web portal TISIDB (https://cis.hku.hk/TISIDB) [13], genes related to the immune response, including those encoding immune stimulators, MHC proteins, immune receptors, and chemokines, were identified. ‘ggplot2’ in R software was used for visualization, and the R package ‘Corrlot’ was used to calculate the Spearman correlation coefficients between the expression of CD86 and that of the abovementioned genes. In order to calculate the correlation between CD86 expression and that of various oncogenes expressed in the tumor microenvironment, single-sample gene enrichment analysis (ssGSEA) was performed and the correlation between CD86 and immune cell scores was calculated. The association of CD86 with immune risk scores (IRs) and the inflammatory coefficient of T cells was calculated using the generalized T-cell inflammation score formula [14].

Then R package ‘limma’ was used to analyze the differences in the expression of chemokines, immunostimulators, MHC proteins, and immune receptors based on high vs. low CD86 expression. CIBERSORT, MCPcounter, TIMER, Quantiseq, and Xcell were used to examine the immune-infiltrating cells in AML. The correlation between CD86 and common immune checkpoint blockers (ICBs) was calculated. Further, StromalScore and ImmuneScore were calculated for AML samples using the R package ‘ESTIMATE.’ ‘Limma was used to identify the DEGs in the high vs. low CD86 expression, StromalScore, and ImmuneScore groups. Then, ‘ggplot2’ was used to draw volcano maps and heat maps of the DEGs. A total of 308 up-regulated genes and 16 down-regulated genes were identified through this analysis.

2.4. Immune Risk Score (IRS) Calculation. IRSs were calculated based on the time of patient enrollment. The 324 DEGs were randomly sampled from TCGA to establish the training and validation sets at a 1:1 ratio. The R package ‘SurvMiner’ was used to conduct univariate Cox regression analysis for the DEGs, and the optimal characteristic genes
were identified according to the Least Absolute Shrinkage and Selection Operator (LASSO) method. Multivariate Cox regression analysis was performed, and based on the median IRS, the sample was divided into groups. The Kaplan–Meier method was used to compare survival outcomes between these groups. Univariate Cox analysis was used to screen PRGs with a prognostic value. The $P$ value threshold for significance was set at 0.05, and 17 survival-related genes were selected for further analysis. LASSO-penalized Cox regression analysis and GLMNET R software package were used to establish a prognostic model to reduce overfitting. Finally, six genes and their coefficients were retained to determine the penalty parameter ($\lambda$) with the minimum criterion. The risk score was obtained using the formula $\text{IRS} = \sum_{i=1}^{n} \beta_i * x_i$, where $\beta = \text{the regression coefficient}$. AML patients were divided into two groups: high-risk and low-risk group. The 'SurvMiner' R software package was used to compare survival status between the two risk groups, and 'Survival' and 'timeROC R' software packages were used for receiver operating characteristic (ROC) curve analysis. In addition, univariate and multivariate Cox regressions were used to determine the independent prognostic value of the three genes. To verify the validity of the model, analyses were performed using data from the GEO internal test queue or ICGC external validation queue. Median risk scores were performed using data from the GEO internal test queue or ICGC external validation queue. Median risk scores were obtained using the GEO training cohort, whereas patients in the GEO test cohort were divided into low- and high-risk groups.

2.5. GO, KEGG and PPI Analysis. Based on CD86 expression, StromalScore, and ImmuneScore, the patients were divided into two groups. Using the limma package and subsequent filtering based on $|\log2\text{FC}| \geq 1$ and FDR < 0.05, DEGs were identified in the high vs. low CD86 expression, StromalScore, and ImmuneScore groups. The ‘cluster analyzer’ R package was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Normalized $P$ values < 0.05 and an FDR $q < 0.05$ were considered statistically significant.

PPI analysis was performed for the 324 DERs using STRING, Cytoscape was used for visualization, and the MCODE plug-in was used to identify critical clusters.

2.6. Cell Culture. Myelodysplastic SKM-1 cells and human myeloid leukemia OCI-AML2, SH-1, KU812, MEG01, and K562 cells were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences. SKM-1 cells were cultured in DMEM high-glucose medium (Gibco), and OCI-AML2, SH-1, HL-60, MEG01, and K562 cells were cultured in IMDM medium (Gibco). All media were supplemented with 10% fetal bovine serum (Biological Industries) and 1% cyanin-streptomycin (Biosharp). All cells were cultured at 37.5°C in a 5% CO₂ incubator.

2.7. Total RNA Extraction and RT-PCR. The TRIzol reagent (Invitrogen) was used to extract total RNA from cells after treatment. The Prime Script RT Master Mix kit (TaKaRa) was used to reverse transcribe the extracted RNA into cDNA. Subsequent RT-PCR was performed based on the manufacturer’s instructions of the amplification kit. PCR primers: CD86: R: 5’-CTGCTCATCTATACACGGTACC-3’; F: 5’-GGAAACGTCGTACAGTTCTGTG-3’. GAPDH: R: 5’-AGAAGGCTGGGGCTCATTTG-3’, F: 5’-AGGGCCATCCACAGTCTTC-3’.

2.8. Western Blot Assay. After cell digestion and centrifugation, RIPA lysis buffer (Beyotime Biotech) was added, and the cells were lysed on ice for 30 min. Then, cells were centrifuged at 12000 rpm for 30 min. The supernatant was removed, and protein levels were quantified using the BCA kit (Beyotime Biotech). The proteins were separated using SDS-PAGE and electrotransferred to PVDF membranes. The CD86 primary antibody (Proteintech) was incubated overnight at 4°C after 2 hours of rapid blocking solution (BSA; Beyotime Biotech). On the following day, the corresponding secondary antibody was added. Protein bands were detected using the ECL exposure solution.

2.9. Statistical Analysis. Data were plotted using R package (V 4.0.0). The $T$ test and $U$ test were used to compare variables between two groups. Categorical variables were evaluated using the Chi-square test. Pearson and Spearman coefficients were used for correlation analysis. The Kaplan–Meier method was used to plot survival outcomes, and the logarithmic rank sum test was used to analyze statistical differences. $P < 0.05$ was considered statistically significant.

3. Results

3.1. CD86 Is Overexpressed in Many Cancers and Is Associated with the Prognosis and Immune Response of AML. Using TCGA data on the expression profiles of 33 cancers, CD86 expression was examined. The findings showed that CD86 was highly expressed in most of the cancers, such as breast cancer, cholangiocarcinoma, colorectal cancer, esophageal cancer, glioma, renal clear cell carcinoma, renal papillary cell carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, pancreatic cancer, rectal adenocarcinoma, gastric cancer, thyroid cancer, and endometrial cancer (Supplementary Figure 1). CD86 was also overexpressed in AML (Supplementary Figure 1). Then, based on the median expression value of CD86, patients were divided into high- and low-expression groups.

Kaplan–Meier analysis was performed to examine high vs. low CD86 expression in various cancers using TCGA data, and log-rank tests were used for survival analyses. The results showed that low CD86 expression was associated with bladder urothelial carcinoma, cervical squamous cell carcinoma, and endocervical adenocarcinoma. In AML, low expression of CD86 had a statistically significant better prognosis (Supplementary Figure 2). The results of univariate Cox regression analysis were then used to create a forest map, which showed that CD86 expression was statistically significant in various cancers (Supplementary Figure 3A). Subsequently, using the TISIDB website...
established by Ru et al., four gene sets—chemokines, immunostimulators, MHC proteins, and receptors—were downloaded (Supplementary Table 1). Spearman correlation coefficients were used to analyze the association between CD86 and these four gene sets in different cancer types (Figure 1(a)). Subsequently, the correlation between key pan-carcinoma molecules (including CTLA4, PDCD1LG2, IDO1, and HAVCR2) and CD86 was calculated. These genes were found to be positively correlated with CD86 in AML (Figures 1(b)–1(d)). ssGSEA method was then used to evaluate the scores of 28 immune cell types in different cancer types, and then calculated the correlation between CD86 and them. The results showed that CD86 expression was positively correlated with 28 types of immune cells (Figure 1(f)).

3.2. Single Nucleotide Variation (SNV), Gene Copy Number Variation (CNV), and Methylation Analysis of CD86 in AML.

Site mutations are a key pathogenic factor causing abnormal proliferation in AML. To investigate whether CD86 is mutated in AML, SNV, and gene CNV data for AML were analyzed. The results showed that CD86 was not mutated in AML. The AML samples were divided into two groups according to a CD86-expression-based cutoff. The group with high CD86 expression had a higher risk, indicating that the high CD86 level was a risk factor for leukemia (Figure 2(a)). Then, the 10 genes with the highest mutation frequencies in the high- vs. low-expression groups were plotted. Accordingly, we found that DNMT3A, FLT3, NPM1, IDH2, and other genes had a relatively high mutation frequency in the low expression group (Figure 2(b)). Differences in tumor mutation load (TMB) were examined in the CD86 high- vs. low-expression groups, but the results revealed no significant differences (Figure 2(c)). The amplification and deletion of CD86 was examined. However, most samples showed no copy number changes in the CD86 gene (Figure 2(d)). The expression of the CD86 gene was compared across different groups. Meanwhile, the correlation between the expression of CD86 and the degree of methylation was calculated and plotted. CD86 expression showed a significant negative correlation with CD86 methylation (Figure 2(e)). All previous experiments were conducted using public databases. To validate whether CD86 was associated with AML, we examined CD86 expression in vitro. QRT-PCR and Western blot were used to detect CD86 expression in SKM-1 (myelodysplastic syndrome), OCI-AML2 (human myeloid leukemia cell), SH-1 (human myeloid leukemia cell), HL-60 (human myeloid leukemia cell), MEG01 (human megakaryoblastic leukemia cell), and K562 cells (Figure 2(f) and 2(g)). These results demonstrated that while CD86 was not mutated in AML and was not related to the TMB, the degree of CD86 methylation decreased with an increase in CD86 expression.

3.3. Immune Status of CD86 High- vs. Low-Expression Groups in AML. To further understand the association between CD86 expression and immunoassay sites in AML, the differences in chemokine, immunostimulator, MHC protein, and immune receptor expression were compared between the high vs. low CD86 expression groups (Supplementary Table 2). A heat map was drawn to represent the DEGs (Figure 3(a)). The distribution of 28 types of immune cells in the high vs. low CD86 expression groups was analyzed. The results showed that for 24 types of immune cells, the group with the high expression of CD86 had a higher immune score (Figure 3(b)). To further understand the correlation between CD86 expression and tumor-infiltrating immune cells (TIICs) in AML, CIBERSORT, MCPCounter, TIMER, Quantiseq, and Xcell were used. Immune infiltration analysis was performed, and correlation between CD86 expression and immune scores was calculated. Further, given that CD8+ T cell recruitment and dendritic cell, macrophage, NK cell, and Th1 activation are required during the migration of immune cells to tumors, the marker genes of these cell types were analyzed in the CD86 high- vs. low-expression groups (Supplementary Table 3). The heat map is shown in Figure 3(c). In addition, the correlation between CD86 and immune checkpoints was calculated. The results indicated that CD86 was positively correlated with these aforementioned immunoassay sites (Figure 3(d)).

3.4. CD86 Is Associated with Immune Checkpoint Blockers (ICBs) in AML. Immune checkpoint blockers (ICBs) are the key to enhancing the body’s endogenous anti-tumor immune effect. It is critical to find markers that predict the clinical efficacy of ICBs. The correlation between CD86 expression and the inflammatory scores of ubiquitous T cells were examined. Interestingly, a significant positive correlation was identified (Figure 4(a)). This suggested that high CD86 expression promotes the inflammatory response. In addition, the correlation between CD86 and the immune characteristics of different ICB response subgroups, including immune regulators, tumor-infiltrating immune cell-effector genes, immune checkpoints, and immunotherapy-related genes was examined. CD86 was also found to be positively correlated with these factors (Figure 4(b)). Subsequently, ssGSEA was used to evaluate the scores for tumor and immune-related pathways, including Immune_differentiation and Interferon_response. The results showed that high CD86 expression was present in these pathways (Figure 4(c)). Molecular subtypes also have a great impact on adjuvant chemotherapy. Subsequently, based on a literature survey [15–17], we compared the mutations in RB1, ERBB2, FANCC, and other genes that could be associated with chemoradiotherapy responses. Accordingly, different mutation frequencies were observed in the high and low CD86 groups (Figure 4(d)). Common pathways of tumor growth (EGFR_network, Immune_inhibit_Oncogenic_pathways, and Radiotherapy_predicted_pathways) were compared between the high- and low-CD86-expression groups (Figure 4(e)). The results suggested that CD86 is associated with different subtypes of ICB, and that the high expression of CD86 can result in higher mutation frequencies in chemoradiation-related genes.
Figure 1: Continued.
Figure 1: *CD86* was associated with immunoassay sites in pan-cancers. (a) Correlation between *CD86* and immunomodulators (chemokines, receptors, MHC, and immunostimulators). (b–e) Correlation between *CD86* and four immune checkpoints, PDCD1, CTLA4, CD274, and LAG3. The dots represent cancer types. The Y-axis represents the Pearson correlation, while the X-axis represents –log10P. (f) Correlation between diffuse carcinoma and 28 tumor-associated immune cells calculated with the ssGSEA algorithm. The color indicates visual cues the correlation coefficient (red is positive, blue is negative). The asterisks indicate a statistically significant *P* value calculated using Spearman correlation analysis. (*P* < 0.05).

Figure 2: Continued.
3.5. Identification of Immune-Associated Differential Genes (DERs), Protein–Protein Interaction (PPI) Network, and KEGG/GO Analysis. To determine whether CD86 is associated with the tumor microenvironment in AML, StromalScore and ImmuneScore scores were calculated for AML samples using the ESTIMATE algorithm. Ten, samples were divided based on CD86 expression cutoffs, and limma package was used to identify the DEGs in high vs. low CD86 expression, StromalScore, and ImmuneScore groups (Supplementary Table 4). Volcano and heat maps of the DEGs were plotted (Supplementary Figure 4). The DEGs common to the CD86, StromalScore, and ImmuneScore groups were determined. Accordingly, 308 common up-regulated genes and 16 common down-regulated genes were identified (Figures 5(a) and 5(b)). Then, we used WebGestaltR for the GO and KEGG functional enrichment analysis of the DERs.
Figure 3: Continued.
Figure 3: CD86 was correlated with immunoassay sites. (a) Differences in expression of immunomodulators (chemokines, receptors, MHC, and immune stimulants) in LAML between the high and low CD86 groups; (b) differences in immune cell scores between high CD86 and low CD86 groups; (c) effector gene differences in immune cells associated with 5 TICs (CD8 + T cells, NK cells, macrophages, Th1 cells, and dendritic cells) between the high and low CD86 groups; (d) correlation between CD86 and immune checkpoints. The colors and values represent Spearman correlation coefficients. (* P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; blank, P > 0.05).

Figure 4: Continued.
**Figure 4:** CD86 predicts progression of immune checkpoint blockades (ICBs) in LAML. (a)–(b) Correlations between CD86 and the pan-cancer T-cell inflamed score and the individual genes included in the T-cell inflamed signature. The T-cell inflamed score is positively correlated with the clinical response to cancer immunotherapy; (c) correlations between CD86 and molecular subtypes using seven different algorithms and AML signatures; (d) mutational profiles of neoadjuvant chemotherapy-related genes in low- and high-CD86 groups. (e) correlations between CD86 and the enrichment scores of several therapeutic signatures such as targeted therapy and radiotherapy.

**Figure 5:** Continued.
The DERs were found to be closely related to tumorigenesis and immune pathways (Figures 5(c)–5(f)). There were 3 clusters with more than five genes, namely, Mcode1, Mcode2, and Mcode4. Subsequently, WebGestaltR was used for GO and KEGG functional enrichment analysis to identify the functions of the clusters (Supplementary Figures 5 and 6). The Mcode1 module was closely related to immune pathways (Figure 6(a)), including the toll-like receptor (TLR) signaling pathway and cytokine–cytokine receptor interaction (Figures 6(b)–6(e)).

3.6. IRS Model Construction and Verification. After a series of analyses, 324 DERs were identified. Subsequently, 65 prognostic genes were obtained through random sampling based on TCGA samples (training: test = 1:1) and univariate Cox regression ($P < 0.05$, Supplementary Table 5). Then, the LASSO method was used to select the best genes, and six genes were obtained according to the minimum lambda cutoff of 0.1452 (Figure 7(a)). Multivariate Cox regression analysis was performed using these six genes, and the risk coefficients of related genes were obtained and represented by a forest map (Figure 7(b)). Then, the risk score of each sample in the TCGA training and verification datasets was calculated. The samples were divided into two groups (high vs. low expression) based on the best cutoff, and Kaplan–Meier curves were drawn. Further, ROC curve analysis was also performed. The results showed that the low-expression group had a better survival prognosis (Figures 7(c) and 7(d)). Subsequently, IRS model validation was performed using all TCGA datasets, GSE10358 datasets, and GSE37642 (GPL570) datasets. The results showed that patients in the low-expression group had a good prognosis (Figures 7(e)–7(g)). In order to further verify the accuracy of the IRS, evaluations were performed using the following GEO datasets: GSE146173, GSE106291, GSE37642 (a subset of the GPL96 platform), GSE12417 (a subset of the GPL97 platform), and GSE12417 (a subset of the GPL96 platform). Here too, the results revealed a better prognosis in the low-risk group (Supplementary Figures 7A–7E).

3.7. Association between IRS and Immunity. Meanwhile, based on the TCGA dataset, we compared the differential expression of high and low IRC expression groups and concentration of chemokine, immunostimulator, MHC, and receptor genes. These were represented by heat maps (Figure 8(a)). The difference in CD86 expression between the high- and low-expression groups was detected (Figure 8(b)). Analyses of inflammation scores for pan-cancer T cells revealed significantly higher scores in the high-expression group (Figure 8(c)). Subsequently, we plotted the correlation between IRC and 28 types of immune cells using ssGSEA method. The results suggested that the high-expression group was enriched for a variety of immune cells (Figure 8(d)). Differences in immunoassay sites and IRC groupings were also examined (Figure 8(e)). The results suggested that a high IRS is correlated with immune cells in AML.

3.8. Performance Comparison between IRS and TIDE. To verify the effect of the IRS model constructed by us, we collected data from the IMvigor210, GSE91061, GSE78220, and GSE135222 datasets after immunotherapy. We used our method to calculate the IRS, and the TIDE website was used to evaluate the TIDE score (https://tide.dfci.harvard.edu/) for immune treatment effects. The predictive value of the IRS and TIDE for the response to treatment was then compared. Survival prediction curves and Kaplan–Meier curves (median cutoff) were used for analysis. Our IRS score was found to be better than the TIDE score (Figures 9(a)–9(k)).

4. Discussion

AML is a malignancy tightly linked to the bone marrow microenvironment [18]. The BMM is mainly composed of immune cells and stromal cells, with the former playing a key role in AML progression [2]. T cells are important cells of the immune system [19]. According to findings, a high lymphocyte count in the bone marrow is directly associated with better overall survival in patients with AML.
Figure 6: PPI model and KEGG/GO analysis. (a) PPI analysis diagram of module Mcode1; (b)–(e) GO and KEGG functional enrichment analysis of Mcode1 gene.
Myo7a (N=65)  1.1  (0.79 – 1.6)
CLCN5 (N=65)  1.1  (0.69 – 1.9)
BCL2L11 (N=65)  1.0  (0.60 – 1.8)
TRPV4 (N=65)  1.1  (0.81 – 2.1)
RASL11A (N=65)  1.5  (0.88 – 2.5)
RNF144B (N=65)  1.2  (0.84 – 1.6)
PTK2 (N=65)  1.3  (0.97 – 1.8)
ZNF532 (N=65)  1.1  (0.80 – 1.5)

# Events: 40; Global p-value (Log–Rank): 0.0017028
AIC: 269.81; Concordance Index: 0.72

Hazard ratio

Figure 7: Continued.
Figure 7: IRS construction and validation. (a) LASSO coefficient profiles of 40 prognostic RNAs in GEO training cohort. The coefficient profile plot was developed against the log (lambda) sequence; (b) the forest map shows the genetic multifactorial results of the final IRS model; (c) KM and ROC analysis of IRS model on TCGA training dataset; (d) KM and ROC analysis of IRS model on TCGA validation dataset; (e) KM and ROC analysis of IRS model on all TCGA data sets; (f) KM and ROC analysis of IRS model on all datasets of GSE10358; (g) KM and ROC analysis of the IRS model on the entire dataset of GSE37642.
In AML, T cell dysfunction is caused by the immunological conflict between a dysfunctional cytokine regulatory network and overactivated T cells [20]. The complete activation of T cells requires two stimuli. During the first signal, TCRs need to bind to the antigenic peptide-bound MHC on APCs [21]. The secondary signal is provided by costimulatory molecules on APCs that interact with receptors on the surface of T cells. The most important costimulatory molecules are CD86 and CD80.

A molecule can be a central target for cancer immunotherapy depending on its specific expression in the tumor microenvironment. CD86 (B7-2), a member of the B7 family of proteins, is one of the surface proteins of APCs [22]. The B7 family has been implicated in the progression of AML. The levels of CD80 (B7-1) are elevated in AML [23]. Moreover, programmed cell death ligand (PD-L1, B7H-1) is abnormally expressed in AML patients and is directly associated with a poor prognosis [24]. T cells can be activated to exert immune effects only when CD86 is expressed on APC membranes and binds to CD28 on the surface of T cells [11]. Using data from public databases, we found that CD86 is overexpressed in many cancers, and especially in AML. We also demonstrated this in AML cell lines. In AML, a high expression of CD86 was found to be associated with a poor prognosis. Further, clinical data from GEO and TCGA datasets show that high CD86 expression is directly associated with a poor prognosis in AML.

In AML, the immunomodulatory network in the BMM is an important factor promoting cancer progression [25]. These regulatory networks include chemotactic cytokines, immunostimulatory molecules, MHC, and receptors. Interestingly, the expression of CD86 is positively correlated with the expression of these genes [26]. Using TCGA data, we observed increased infiltration of dendritic cells, NK cells, CD4+ cells, CD8+ cells, macrophages, and Th1 cells in the group with high CD86 expression. This was confirmed using external validation data. The upregulation of immune inspection sites by infiltrating immune cells is also a key factor in cancer progression. Some immune targets—including cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death protein 1 ligand 2 (PDCD1LG2), indoleamine 2,3-dioxygenase 1 (IDO1), and hepatitis A virus cellular receptor 2 (HAVCR2)—promote the progression of AML [27–29]. However, immune checkpoints act as double-edged swords in AML. Clinical studies on targeted ICBs have shown that drug resistance is a key factor leading to a poor prognosis after AML treatment using ICBs. Our study showed that CD86 expression was positively correlated with these...
Figure 9: Performance comparison of IRS and TIDE. (a) IRS survival curve and ROC curve of dataset IMvigor210; (b) TIDE survival curve and ROC curve of dataset IMvigor210; (c) ROC curves of IRS and TIDE effect on immunotherapy in dataset IMvigor210; (d) IRS survival curve and ROC curve of dataset GSE135222; (e) TIDE survival curve and ROC curve of dataset GSE135222; (f) ROC curves of IRS and TIDE effect on immunotherapy in dataset GSE91061; (g) IRS survival curve and ROC curve of dataset GSE78220; (h) TIDE survival curve and ROC curve of dataset GSE78220; (i) ROC curves of IRS and TIDE effect on immunotherapy in dataset GSE78220; (j) IRS survival curve and ROC curve of dataset GSE78220; (k) TIDE survival curve and ROC curve of dataset GSE78220; (l) ROC curves of IRS and TIDE effects on immunotherapy in dataset GSE135222.
immunoassay sites. This may be because \textit{CD86} promotes the expression of immune-infiltrating cells in BMM, thus stimulating the expression of immune checkpoints. This indicates that AML patients with low \textit{CD86} expression may not be responsive to ICBs. Meanwhile, we calculated the differentially expressed genes based on \textit{CD86} expression and the immune and matrix components in the BMM. These DEGs were mainly concentrated in the TLR signaling pathway, cytokine–cytokine receptor interaction, and other immune-related pathways.

Our findings also confirmed the involvement of \textit{CD86} in the immune response in AML. The pan-cancer T-cell inflammation score indicates the efficacy of anti-PD-1 immunotherapy for various cancers [30]. The high expression of \textit{CD86} was positively correlated with a high T-cell inflammation score [31, 32], indicating that high \textit{CD86} expression was negatively correlated with the effects of immunotherapy.

In AML, mutation sites not only affect disease classification but also affect risk stratification and chemotherapeutic resistance. For example, FLT3 mutations are detected in about one-third of AML patients, and these mutations are directly related to the poor prognosis of AML [33]. However, interestingly, the mutation rates of DNMT3A, FLT3, NPM1, and IDH2 were higher in the low \textit{CD86} expression group in our study [34, 35]. The mutation rate of \textit{RUNX1} was higher in the \textit{CD86} group, which could be because of the number of samples. Our study also showed that \textit{CD86} expression was negatively correlated with DNA methylation. This was noteworthy because methylation has been found to predict chemotherapy outcomes in AML [36]. Meanwhile, we predicted that mutations in RB1, ERBB2, and FANCC increased as \textit{CD86} expression increased, suggesting that \textit{CD86} may be related to radiotherapy and chemotherapy resistance in AML. However, further verification is still needed.

The IRS is a genetic prognostic model calculated using a formula to assess the risk of a disease. An IRS can predict the survival and prognosis of AML patients undergoing chemotherapy. Immune risk scores can be used to predict chemotherapy outcomes in AML [36]. We developed an IRS model to predict the overall prognosis of AML. Veriﬁcation with external datasets showed that our model is superior to the TIDE score. This complements the enrichment of AML risk scores.

Nevertheless, there are some limitations to our study. First, all our samples were obtained from public databases, and a large number of patient samples are still needed for follow-up veriﬁcation. Second, no \textit{in vivo} experiments or mechanistic studies were performed. This area needs to be explored further.

5. Conclusion

This study found that \textit{CD86} is involved in the progression of AML and is closely related to the BMM in AML. The expression of \textit{CD86} could be used to predict immunotherapy efficacy. Therefore, the development of \textit{CD86}-targeting drugs could lead to advancements in AML treatment.

Data Availability

The data used to support the ﬁndings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conﬂicts of interest.

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Supplementary Materials

Supplementary Figure 1. \textit{CD86} mRNA expression in various cancers. Supplementary Figure 2. OS survival and prognosis of \textit{CD86} in high- and low-expression group in various cancers. Supplementary Figure 3. \textit{CD86} and univariate analysis in various cancers. Supplementary Figure 4. Heat and volcano maps showed the high and low expression of \textit{CD86}, immune score and matrix score. A. Gene heat map and volcano map of \textit{CD86} high- and low-expression group; B. Gene heat map and volcano map of the high- and low-expression immune score group; C. Gene heat map and volcano map of high- and low-expression group with matrix score. Supplementary Figure 5. The PPI network, GO and KEGG analyzed of Mcode2. A. the PPI network diagram of Mcode2; B-C. Enrichment of the top ten of Go analysis; D-E. KEGG was used to analyze the top ten enrichment pathways. Supplementary Figure 6. The PPI network, GO and KEGG analyzed of Mcode4. A. the PPInetwork diagram of Mcode4; B-C. Enrichment of the top ten of Go analysis; D-E. KEGG was used to analyze the top ten enrichment pathways. Supplementary Figure 7. IRS different risk groups scores of survival prognosis in GEO data set were correlated with GSE146173, GSE106291, GSE37642 (the GPL96 platform), GSE12417 (the GPL97 platform), And GSE12417 (the GPL96 Platform) OS curve and ROC curve. Supplementary Table 1. Chemokines, immunostimulators, MHC proteins, and receptors in TISIDB website. Supplementary Table 2. Differences in expression of chemokines, immune stimulators, MHC proteins and immune receptors between \textit{CD86} high-expression group and low expression group. Supplement Table 3. Differences between \textit{CD86} high- and low-expression group and CD8+ T cell recruitment, macrophage, NK cell, and Th1. Supplementary Table 4.
Differential genes in StromalScore, ImmuneScore and CD86 high- and low-expression groups. Supplement Table 5. 65 genes screened out by Cox regression analysis. (Supplementary Materials)

References


