ORIGINAL RESEARCH

Identification and Validation of *STAT6* as a Prognostic and Predictive Biomarker in Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) is one of the most common hematological diseases in adults. The overall survival rate remains unsatisfactory. It is urgent to identify potential prognostic biomarkers and develop new molecular therapeutic strategies for AML. Signal transducer and activator of transcription (STAT) is a family of genes that encode intracellular transcription factors. STATs are associated with leukemogenesis, cellular transformation, and cell cycle in AML.

Methods: We used sequencing data and clinical data from The Cancer Genome Atlas (TCGA) and ONCOMINE to identify expression difference, gene variability and correlation as well as prognostic effects of STAT genes in AML patients. Then, we verified the expression difference of *STAT6* between healthy control and AML patients and its prognostic impact in Gene Expression Omnibus (GEO) database and our own recruited cohort.

Results: The mRNA level of *STAT6* was increased in AML patients among TCGA, GEO and ONCOMINE public datasets and was found to be an independent risk factor of overall survival in all AML patients and patients who only received chemotherapy by multivariate analysis. In our study, *STAT6* mRNA level was markedly up-regulated in AML patients (n=105) compared to healthy donor (n=39) (P=0.0435) as a validated cohort. Patients that only received chemotherapy in high STAT6 group showed significantly lower overall survival (OS) (P=0.0055).

Conclusion: *STAT6* expression was increased in AML patients. STAT6 was found to be an adverse prognosis factor in AML patients, especially those who only received chemotherapy treatments.

Keywords: acute myeloid leukemia, STAT, prognosis, TCGA, GEO

Introduction

Acute myeloid leukemia (AML) is characterized by dysfunction and abnormal clone of malignant hematopoietic stem cells.[1] It is one of the most common hematological diseases in adults.[2] Although many AML patients were able to benefit from stereotypical induction chemotherapy and allogeneic or autologous hematopoietic stem cell transplantation (HSCT), the overall survival rate of AML patients remains unsatisfactory. Moreover, patients present different chemotherapy response and prognosis to those therapies and the potential mechanisms are still under investigation. Recently, the development of molecule-targeting drugs has prolonged survival and improved clinical outcome of AML patients.[3] Advances in epigenetic mutation studies of DNA methyltransferase 3 alpha (DNMT3A), fms related receptor tyrosine kinase 3 (FLT3),

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11165

tet methylcytosine dioxygenase 2 (TET2), argininosuccinate lyase 1 (ASL1) have profoundly changed our understanding of the mechanisms of AML development. However, with the complexity in the pathogenesis of AML, the leukemogenesis is still not clearly understood. It is urgent to identify potential prognostic biomarkers and develop new molecular therapeutic strategies for AML.

Signal transducer and activator of transcription (STAT) is a family of genes that encode intracellular transcription factors in regulating cell proliferation, differentiation, and cell cycle progression in cancer. The STAT family includes seven members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6.[4] STATs play an essential role in chromatin structure and crosstalk with various oncogenic pathways in inflammation and cancer.[5] Several studies demonstrated that higher STAT protein expression improved carcinogenesis and influenced prognosis of patients. Prognostic and predictive roles of STAT gene family were also observed in glioblastoma, gastric cancer as well as lung cancer.[6–9] There were also researchers demonstrating that STATs participated in the pathogenesis of AML, and inhibition of STAT induced pathways was found to enhance leukemogenesis. Enhanced activations of STATs were also shown to be related with disease treatments.[10,11]

Improvements in sequencing methods provided us with a more comprehensive understanding of the biology of AML. The gene expression patterns in bioinformatic datasets help provide information on expression differences, related clinical characteristics and functions and clinical outcomes related to these genes. In our study, we aimed to explore the mRNA expressions of STATs on the clinical characteristics and prognostic significance of AML patients. We examined the relationship between expression profiles of STATs and clinical outcomes in AML patients not only from bioinformatic datasets such as The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) but also from AML patients we recruited.

Methods
Public Database
The gene expression database of ONCOMINE (https://www.oncomine.org/), GEPIA (http://gepia.cancer-pku.cn/), GEO (https://www.ncbi.nlm.nih.gov/geo/), Xena (https://xenabrowser.net/) and TCGA (https://www.cancer.gov/tcga) are

publicly accessible. To have an overview (Figure 1) of the mRNA expressions of STATs in leukemia in the ONCOMINE database, we defined the selection thresholds as fold-change = 1.5, p-value = 0.001. For a further detailed analysis of STAT in AML in different ONCOMINE databases, the screening threshold was identified as P <0.05 (Supplemental Table 1). For STAT family genes, the analysis of the comparison between cancer and normal sets was performed. STATs expression in AML patients and normal was analyzed using GEPIA, consisting of the AML patients' data from TCGA and the normal data from GTEXs. The TCGA Acute Myeloid Leukemia database was screened for adult AML patients with complete corresponding clinical features, STAT 1–6 expression data and excluded the overall survival time of zero from the Xena database. A total of 132 patients who met the criteria were included in the study; among them, 72 received chemotherapy treatment only, and 60 patients received auto/allo-HSCT. The major clinical features used in the diagnosis of AML patients are presented in Table 2. In addition, the gene expression datasets GSE13159 and GSE9476 of AML were also included from the Gene Expression Omnibus (GEO) database.

Patients and Ethics
A cohort of 105 AML patients and 39 healthy donors were enrolled between March 2016 to June 2019 in this study. The FAB of AML patients was classified according to the 2016 World Health Organization (WHO) criteria. The corresponding clinical features of AML patients are presented in Supplemental Table 4. The overall survival was defined as the time from diagnosis to death or the follow-up was terminated on November 15th, 2019. This study was conducted by the Declaration of Helsinki. It was approved by the ethics committee of Xiangya Hospital, Central South University.

RT-qPCR
Bone marrow mononuclear cells (BMMNCs) were separated using Ficoll-Hypaque (GE Healthcare, United States). Total RNA was extracted from BMMNCs with Trizol reagent (Life Technologies, United States). Reverse transcription to cDNA was performed using PrimeScript Kit (TaKaRa, Japan). Real-time PCR using Cham Q Universal SYBR Green Master Mix (Vazyme, China) was completed on the ViiATM7 RT-PCR system (Applied Biosystems, USA). The primers used for STAT6 expression were: forward: 5′-GTTCCGCCACTTGCCAATG-3′, reverse: 5′- TGGATCTCCCCTACTCGGTG-3′.

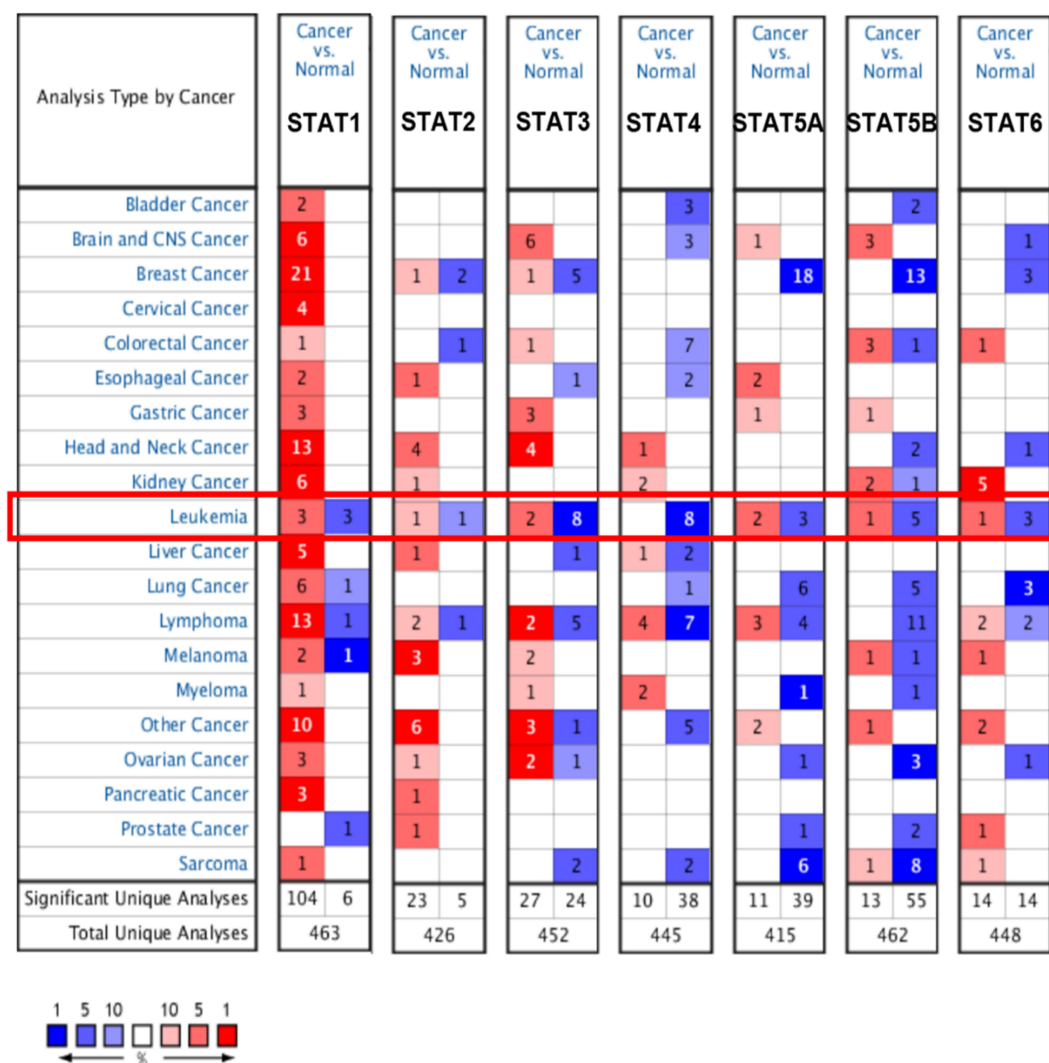| Analysis Type by Cancer	STAT1		STAT2		STAT3		STAT4		STAT5A		STAT5B		STAT6	
	red	blue	red	blue	red	blue	red	blue	red	blue	red	blue	red	blue
Bladder Cancer	2							3				2		
Brain and CNS Cancer	6				6			3	1		3			1
Breast Cancer	21		1	2	1	5				18		13		3
Cervical Cancer	4													
Colorectal Cancer	1			1	1		7				3	1	1	
Esophageal Cancer	2		1			1		2	2					
Gastric Cancer	3				3				1		1			
Head and Neck Cancer	13		4		4		1					2		1
Kidney Cancer	6		1				2				2	1	5	
Leukemia	3	3	1	1	2	8		8	2	3	1	5	1	3
Liver Cancer	5		1			1	1	2						
Lung Cancer	6	1						1		6		5		3
Lymphoma	13	1	2	1	2	5	4	7	3	4		11	2	2
Melanoma	2	1	3		2						1	1	1	
Myeloma	1				1		2			1		1		
Other Cancer	10		6		3	1		5	2		1		2	
Ovarian Cancer	3		1		2	1				1		3		1
Pancreatic Cancer	3		1		1									
Prostate Cancer		1	1		1					1		2	1	
Sarcoma	1					2		2		6	1	8	1	
Significant Unique Analyses	104	6	23	5	27	24	10	38	11	39	13	55	14	14
Total Unique Analyses	463		426		452		445		415		462		448	

1 5 10   10 5 1
%

Figure 1 Transcription levels of STAT family members in different types of cancers (ONCOMINE). The graph shows the number of datasets which had mRNA over-expression (red) or down-regulated expression (blue) of the STATs gene in ONCOMINE. The threshold was designed with following parameters: p-value of 1E-3 and fold change of 1.5.

Relative STAT6 expression mRNA levels were calculated by $2^{-\Delta\Delta CT}$ and were normalized to internal control (β-Actin).

Functional Analysis

The analyses of genetic alterations frequency, co-expression, and interaction network of STAT genes were performed with an online tool cBioPortal (https://www.cbioportal.org/). Analysis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of STATs and neighboring genes showed enrichment using the online tool Metascape (https://metascape.org/).

Statistical Analyses

All statistical analyses were completed using SPSS 22.0 and GraphPad Prism 8.0. Pearson Chi-square analysis or Fisher exact test was used for the comparison of categorical variables, whereas Mann–Whitney's *U*-test was used for the comparison of continuous variables. The prognostic effect of STAT6 expression was analyzed through Kaplan-Meier analysis using the Log rank test. Univariate and multivariate proportional hazard regression analysis was performed using Cox regression. The P-value <0.05 (two-tailed) in all statistical analyses was defined as statistically significant.

Results
Expression Levels of STATs in AML Patients

Seven STAT family members have been identified in human cancers. We compared the mRNA levels of

STATs in cancers with those in normal tissue samples by using ONCOMINE databases (Figure 1 and Supplemental Table 1). ONCOMINE analysis showed that, for *STAT1, STAT2* and *STAT5A*, they increase in some datasets and decrease in other datasets. For *STAT4, STAT5B* and *STAT6*, more datasets showed a decreased STAT in leukemia patients. For detailed analysis in ONCOMINE datasets, we further searched the expression of STATs in AML patients. In the Andersson Leukemia dataset, *STAT2, STAT5A* and *STAT5B* were increased by 1.339, 1.295 and 1.428 folds, respectively, in AML patients compared to healthy people (P = 0.018, 0.01 and 0.001, respectively). In the Valk Leukemia dataset, the increase was seen in *STAT2, STAT3* and *STAT6* and the P values compared with normal bone marrow were 0.015, 0.027 and 7.09E-4. Moreover, *STAT2* and *STAT6* were also significantly higher

in the Haferlach Leukemia dataset. *STAT2* increased by 1.171 folds while *STAT6* increased by 1.265 folds, and the P-values compared with control were 8.31E-4 and 1.85E-7 accordingly (Supplemental Table 1). To make a more detailed comparison in AML, we further analyzed the AML patients from the TCGA database. We found mRNA expression levels of *STAT2, STAT4* and *STAT6* were significantly higher in AML than in normal tissues by using GEPIA (Figure 2).

Genetic Alterations and Correlations of STATs Genes in AML

The percentages of genetic alterations in STAT family members for leukemia varied from 3 to 9% for individual genes based on the TCGA Provisional dataset (*STAT1*, 5%; *STAT2*, 5%; *STAT3*, 9%; *STAT4*, 6%; *STAT5A*, 4%; *STAT5B*,
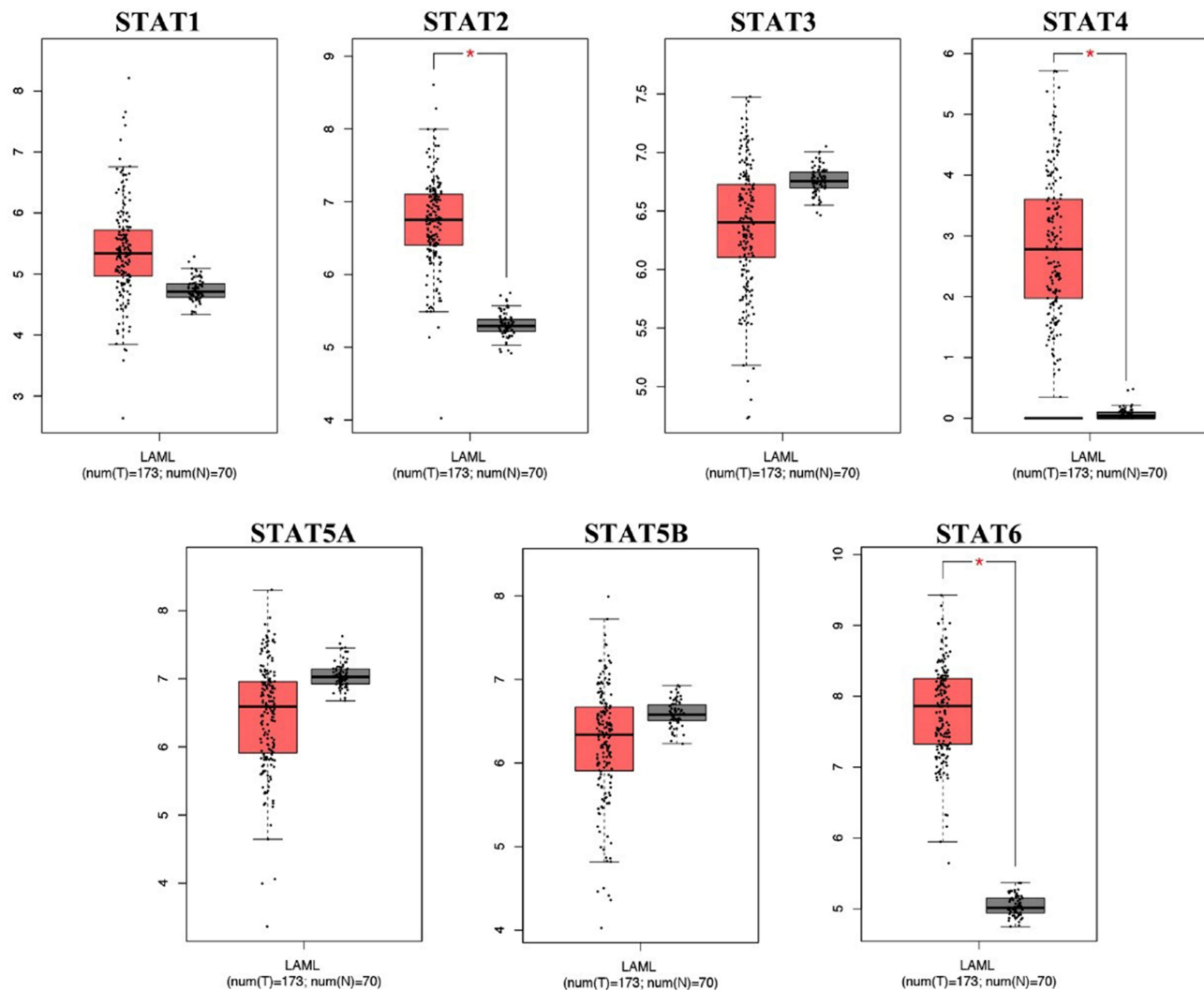

Figure 2 Expression of STAT genes in AML patients compared with healthy control.

7%; *STAT6*, 3%; Figure 3A). Pearson correlation analysis was conducted using expression data (RNA Seq V2 RSEM) of STAT family members collected from the cBioPortal online tool for Leukemia (TCGA, Provisional). The results indicated a significant positive correlation among *STAT5B* and *STAT5A*, *STAT5B* and *STAT3*, except these, only mild to moderate correlation between others (Figure 3B). Kaplan–Meier plot and Log rank test indicated no significant difference in overall survival (OS) between the cases with alterations in one of the query genes and those without (P = 0.220, Figure 3C). Next, we constructed the network for STATs

and the 50 most frequently altered neighboring genes by using cBioPortal (Figure 3D and Supplemental material).

Functional Enrichment Analysis of STATs in AML Patients

The functions of STAT family members and their co-activated genes were predicted by analyzing GO and KEGG in Metascape. The top 20 GO enrichment items were classified into 2 functional groups: biological process group (16 items) and molecular function group (4 items) (Figure 4A and B and Supplemental Table 2). STATs and
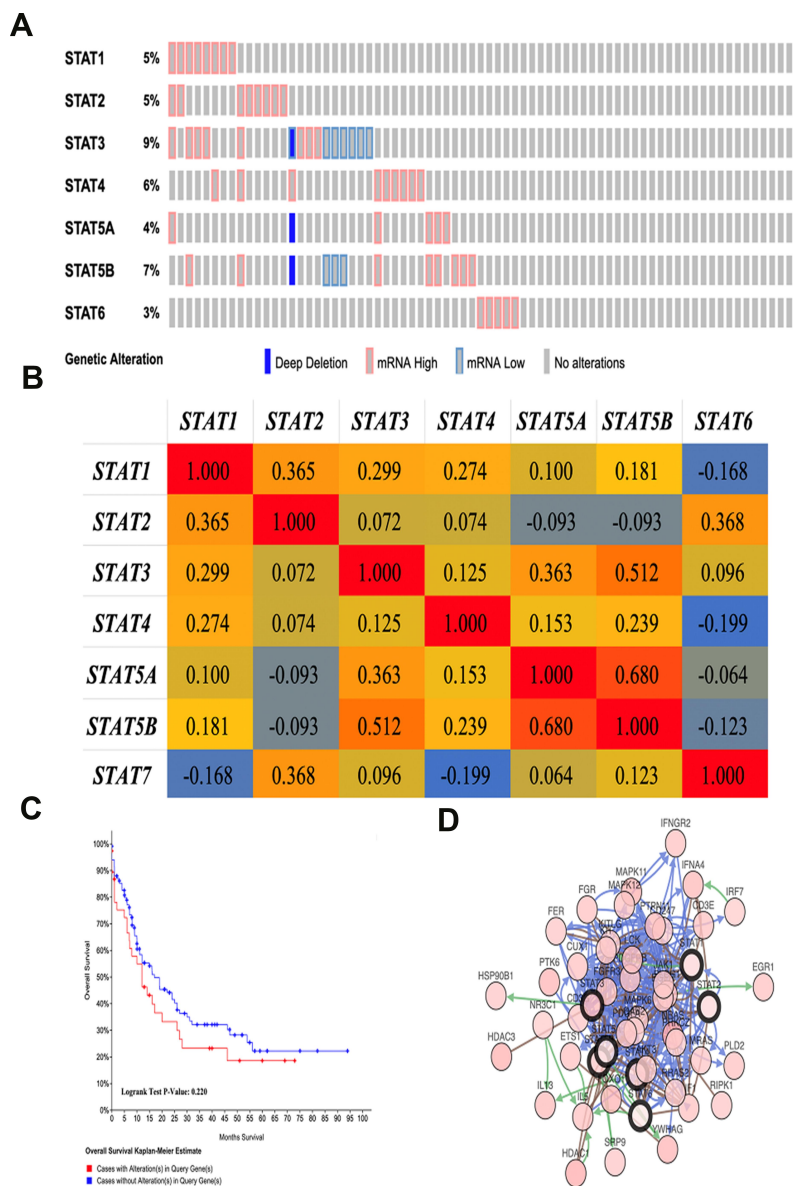

Figure 3 Alteration frequency and correlation of STAT family members and neighbor gene network in AML. (**A**) OncoPrint visual summary of alteration on a query of STAT family members; (**B**) co-expression analysis of STAT family members at the gene level; (**C**) Kaplan–Meier plots comparing OS in cases with/without STAT family member gene alterations. (**D**) Gene–gene interaction network among STAT family members and 50 most frequently altered neighboring genes.

their neighboring genes were enriched in cytokine-mediated signaling pathway and cytokine production, peptidyl-tyrosine phosphorylation, cell differentiation, such as myeloid cell leukocyte and myeloid leukocyte differentiation, positive regulation of cell migration, transmembrane receptor protein tyrosine kinase signaling pathway, positive regulation of kinase activity and so on. The functions for these genes were mainly enriched in GTPase activity, protein kinase activity, protein phosphatase binding and MAP kinase activity.

The top 12 KEGG pathways for the STAT family members and their neighboring genes are shown in Figure 4C and D and Supplemental Table 3. Among these pathways, pathways in cancer and Ras cell signaling, Cytokine-cytokine receptor interaction, Natural killer cell mediated cytotoxicity and Endocytosis were found to be related to multiple tumor development and were involved in leukemia tumorigenesis and pathogenesis.

To better understand the relationship between STAT family members and leukemia, a Metascape protein–protein interaction enrichment analysis was performed. The protein–protein interaction network and MCODE components identified in the gene lists are shown in Figure 4E and F.

Prognostic Value of STAT6 in AML Patients

To investigate the prognostic value of STATs expression in AML patients, we further studied the role of STATs genes
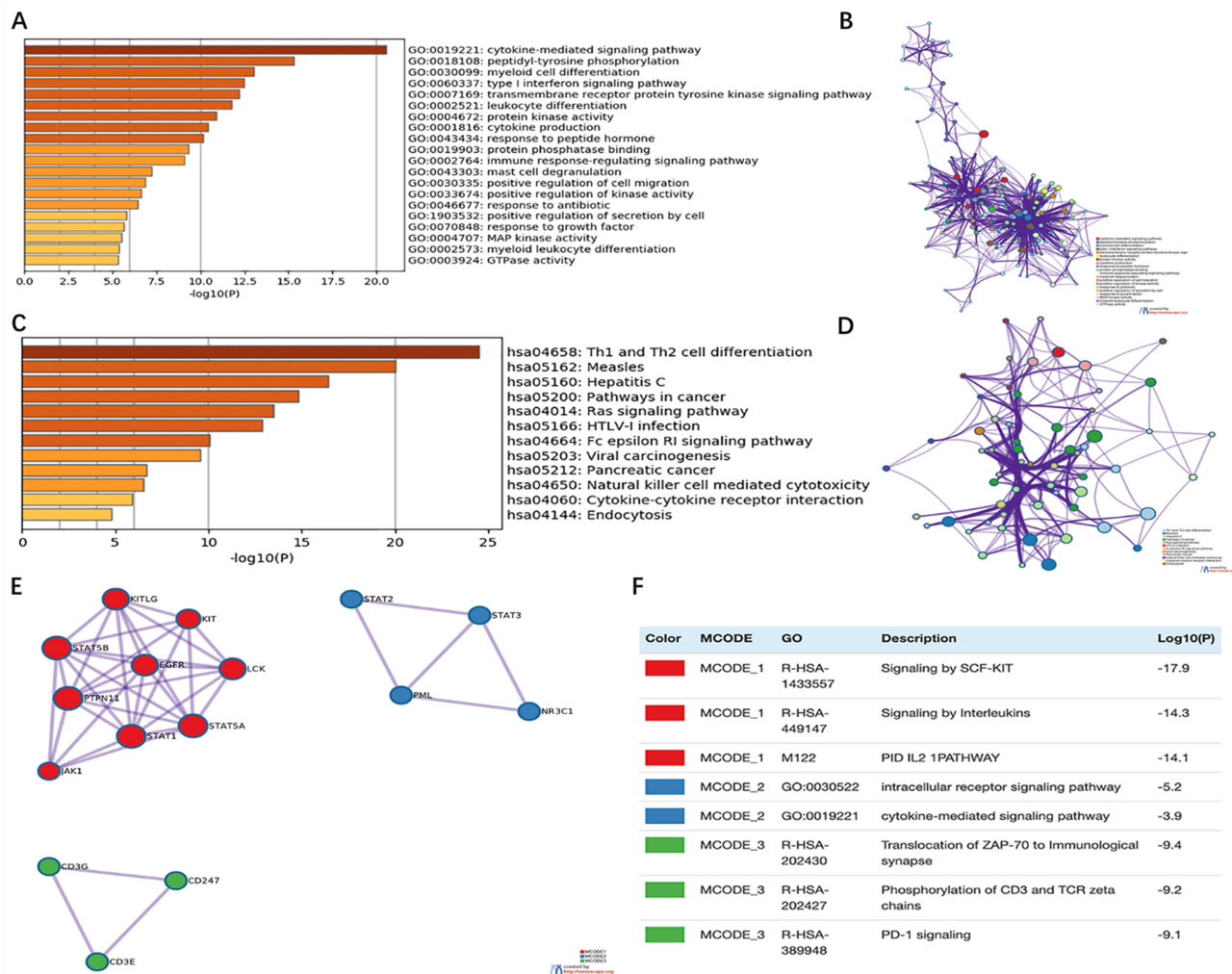

Figure 4 Enrichment analysis of STATs family members and neighboring genes in AML. (**A**) Heatmap of Gene Ontology (GO) enriched terms colored by p-values. (**B**) Network of GO enriched terms colored by p-value, where terms containing more genes tend to have a more significant p-value. (**C**) Heatmap of Kyoto Encyclopedia of Genes and Genomes (KEGG) enriched terms colored by p-values. (**D**) Network of KEGG enriched terms colored by p-value, where terms containing more genes tend to have a more significant p-value. (**E**) Protein–protein interaction (PPI) network and three most significant MCODE components form the PPI network. (**F**) Independent functional enrichment analysis of three MCODE components.
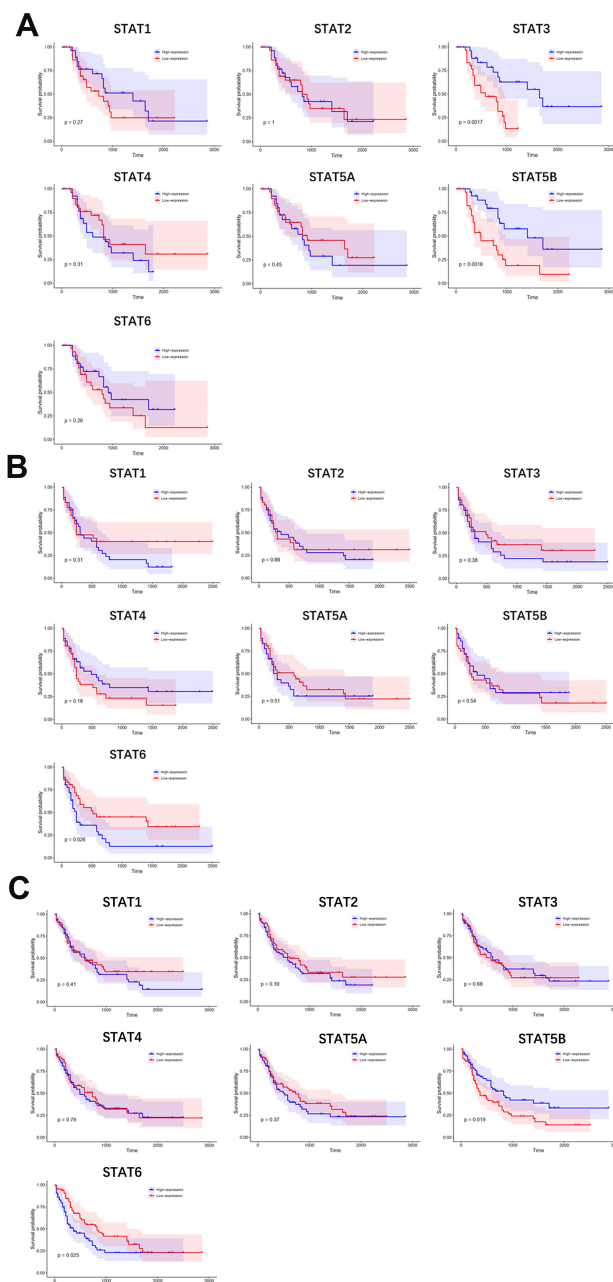
Figure 5 Prognostic value of mRNA level of STAT family members in leukemia patients with high (blue) and low (red) expression rates. (**A**) Overall Survival of AML patients. (**B**) Overall Survival of only chemotherapy-only group. (**C**) Overall Survival of HSCT group.

in the survival of AML patients from the TCGA database (divided AML patients into two groups according to the median level of STATs). Based on the Kaplan-Meier analysis, the STATs showed no correlation with the overall survival rate among total AML patients and patients with auto/allo-HSCT. However, in patients who have only received chemotherapy, higher expression of *STAT6* showed a poor prognosis (Figure 5A-C). Furthermore,

we evaluated the prognostic effect of STATs expression in AML patients by using Cox regression analysis; in both unicox and multicox analysis, *STAT6* was found as an independent predictor for overall survival in all AML patients and AML patients only received chemotherapy (Table 1).

Clinical Features of STAT6 in AML Patients

To explore the clinical features in AML patients, the comparison of clinical/laboratory characteristics of AML patients between two groups (STAT6^{high} and STAT6^{low}) was summarized in Table 2. There were no significant differences between STAT6^{high} and STAT6^{low} groups in sex, PM blasts and BM blasts (P > 0.05). However, STAT6^{high} cases had significantly higher white blood cells (WBC) and older age (P = 0.002 and 0.018). Additionally, significant differences in distributions of French-American-British (FAB) classifications and cytogenetics were observed. *STAT6* high expression was markedly negatively correlated with FAB-M3 (P = 0.005) and t (15;17) cytogenesis (P=0.009). No significant differences were found between *STAT6* expression among the gene mutations.

Verification of STAT6 in Expression and Prognosis Value in Validated AML Patients

We analyzed data from GEO datasets, GSE13159 and GSE9476, to verify the expression of *STAT6* in AML patients (Figure 6A and B). In both datasets, *STAT6* showed an increased expression in AML compared to healthy control. We next determined the expression and prognostic impact of *STAT6* in recruited AML patients. Patients' characteristics are shown in Supplemental Table 4. Follow-up data were available in 105 patients. Firstly, we observed that *STAT6* was overexpressed in AML patients compared to healthy donors (Figure 6C). By Kaplan-Meier analysis, only patients who received chemotherapy treatment and have a lower expression of *STAT6* showed higher over-all survival (Figure 6E). Cox regression analysis also identified that *STAT6* expression could be an independent factor of OS in AML patients who received chemotherapy treatment (Supplemental Table 5). Although not significant, there was also a higher survival tendency in the overall survival of STAT6^{low} group in all AML patients (Figure 6D).

Table 1 Unicox and Multicox Regression Analysis for Variablesfor Total Survival, Survival After Chemotherapy and HSCT in AML Among TCGA Cohort

	Total AML Patients				Auto/Allo HSCT				Chemotherapy Only			
	Unicox		Multicox		Unicox		Multicox		Unicox		Multicox	
	HR(95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
STAT1	1.24 (0.916–1.68)	0.164	1.616 (0.912–2.863)	0.1	0.88 (0.5–1.547)	0.656	1.167 (0.273–5.001)	0.835	1.454 (0.951–2.222)	0.084	1.085 (0.413–2.848)	0.869
STAT2	1.066 (0.666–1.706)	0.79	0.501 (0.215–1.166)	0.109	0.482 (0.191–1.212)	0.121	0.269 (0.028–2.627)	0.259	1.387 (0.754–2.549)	0.293	2.09 (0.524–8.332)	0.296
STAT3	0.813 (0.416–1.589)	0.545	0.609 (0.226–1.645)	0.328	0.431 (0.171–1.085)	0.074	2.461 (0.179–33.871)	0.501	1.307 (0.426–4.012)	0.64	0.547 (0.107–2.783)	0.467
STAT4	1.067 (0.832–1.368)	0.61	1.098 (0.794–1.518)	0.572	1.11 (0.775–1.591)	0.569	1.616 (0.942–2.775)	0.082	0.997 (0.716–1.388)	0.986	1.346 (0.688–2.631)	0.385
STAT5A	0.952 (0.636–1.426)	0.812	1.043 (0.531–2.048)	0.903	0.644 (0.336–1.235)	0.185	1.086 (0.363–3.244)	0.883	1.282 (0.72–2.281)	0.399	1.602 (0.462–5.554)	0.458
STAT5B	0.584 (0.348–0.979)	0.041	0.6 (0.228–1.577)	0.3	0.329 (0.151–0.719)	0.005	0.071 (0.007–0.707)	0.024	0.904 (0.425–1.921)	0.792	0.18 (0.036–0.911)	0.038
STAT6	1.452 (0.918–2.295)	0.111	2.414 (1.186–4.914)	0.015	0.478 (0.215–1.061)	0.07	0.336 (0.035–3.23)	0.345	2.396 (1.311–4.38)	0.005	4.563 (1.474–14.126)	0.008
AGE	1.032 (1.016–1.049)	0	1.013 (0.992–1.035)	0.211	0.999 (0.974–1.025)	0.936	0.936 (0.893–0.981)	0.005	1.047 (1.023–1.072)	0	1.027 (0.991–1.064)	0.145
BM_BLAST_	0.998 (0.987–1.009)	0.739	1 (0.985–1.015)	0.996	0.996 (0.979–1.014)	0.682	1.023 (0.987–1.061)	0.214	0.999 (0.985–1.013)	0.897	1.002 (0.981–1.025)	0.826
WBC	1.005 (1–1.01)	0.034	1 (0.993–1.007)	0.915	1.005 (0.998–1.012)	0.162	1.009 (0.996–1.023)	0.172	1.007 (1–1.014)	0.049	0.989 (0.975–1.004)	0.161
PB_BLAST_	1.002 (0.995–1.01)	0.529	1.005 (0.993–1.017)	0.397	0.993 (0.98–1.005)	0.251	0.97 (0.946–0.994)	0.016	1.011 (1.001–1.02)	0.027	1.017 (0.994–1.039)	0.146
RISK_CYTO Poor	1.657 (0.984–2.79)	0.058	2.722 (1.263–5.865)	0.011	0.954 (0.428–2.131)	0.909	1.592 (0.372–6.807)	0.531	6.011 (2.865–12.609)	0	5.856 (1.962–17.477)	0.002
TRANSPLANT	0.604 (0.383–0.954)	0.03	0.382 (0.194–0.755)	0.006								
TP53	3.268 (1.62–6.592)	0.001	4.674 (1.699–12.862)	0.003	4.78 (1.055–21.667)	0.042	1.43 (0.087–23.615)	0.803	2.823 (1.255–6.348)	0.012	2.928 (0.831–10.315)	0.094
FLT3	1.552 (0.949–2.538)	0.08	2.388 (1.195–4.772)	0.014	1.083 (0.485–2.417)	0.846	0.311 (0.075–1.297)	0.109	1.769 (0.937–3.339)	0.078	3.655 (1.091–12.242)	0.036
TET2	1.479 (0.736–2.97)	0.272	1.645 (0.708–3.821)	0.247	1.289 (0.388–4.287)	0.678	1.222 (0.274–5.458)	0.793	1.399 (0.591–3.31)	0.445	0.789 (0.249–2.507)	0.688
RUNX1	2.025 (1.057–3.879)	0.033	5.116 (2.002–13.073)	0.001	2.666 (0.988–7.189)	0.053	13.172 (1.887–91.967)	0.009	2.001 (0.839–4.771)	0.118	4.476 (1.112–18.016)	0.035
IDH2	1.042 (0.517–2.098)	0.908	0.675 (0.272–1.68)	0.399	0.749 (0.177–3.175)	0.695	1.552 (0.136–17.761)	0.724	1.129 (0.502–2.542)	0.769	0.978 (0.209–4.578)	0.978
IDH1	0.915 (0.439–1.907)	0.812	0.938 (0.295–2.979)	0.914	0.581 (0.201–1.684)	0.317	0.333 (0.056–1.985)	0.227	2.199 (0.779–6.204)	0.136	25.065 (2.334–269.15)	0.008
NRAS	1.269 (0.463–3.482)	0.643	1.087 (0.312–3.792)	0.896	0.804 (0.109–5.933)	0.831	0.089 (0.002–3.242)	0.187	1.484 (0.458–4.803)	0.51	1.241 (0.288–5.356)	0.772
NPMI	1.246 (0.759–2.044)	0.385	1.238 (0.571–2.683)	0.588	1.119 (0.524–2.389)	0.771	2.444 (0.493–12.105)	0.274	1.301 (0.669–2.529)	0.438	0.369 (0.094–1.457)	0.155
KRAS	1.565 (0.63–3.889)	0.335	2.833 (0.82–9.792)	0.1	0.544 (0.074–4.021)	0.1	1.785 (0.046–68.849)	0.756	2.303 (0.818–6.483)	0.114	5.48 (1.04–28.883)	0.045
DNMT3A	2.534 (1.532–4.191)	0	2.08 (1.004–4.308)	0.049	1.896 (0.854–4.211)	0.116	1.969 (0.489–7.937)	0.341	3.213 (1.596–6.466)	0.001	5.512 (1.51–20.118)	0.01
CEBPA	1.461 (0.699–3.055)	0.313	4.326 (1.636–11.437)	0.003	1.387 (0.525–3.669)	0.509	5.141 (0.508–52.017)	0.166	7.275 (2.114–25.037)	0.002	3.183 (0.697–14.541)	0.135

Table 2 Clinic-Pathologic Characteristics in AML Patients of Different STAT6 Expression Among TCGA Cohort

Parameters	STAT6 Expression		P-value
	Low Group (n=66)	High Group (n=66)	
Median age (years)	51 (22–81)	60 (21–88)	0.018
Sex (Female/male)	29/37	34/32	0.384
Mdeian WBC	8.3 (0.4–202.7)	22.9 (1.7–223.8)	0.002
Mdeian BM Blast	72(30–100)	72(30–99)	0.479
Mdeian PB Blast	32(0–97)	41 (0–91)	0.377
FAB			
M0	6	6	1
M1	14	19	0.315
M2	18	13	0.305
M3	12	2	0.005
M4	10	17	0.131
M5	3	9	0.069
M6	2	0	0.154
M7	1	0	0.315
Cytogenetics			
Normal	23	33	0.078
t(15;17)	11	2	0.009
t(8;21)	4	2	0.511
inv(16)	4	6	0.676
+8	4	2	0.69
del(5)	1	0	1
−7/del(7)	2	2	1
11q23	1	4	0.362
Other	7	4	0.345
t(9'22)	1	2	1
Complex	7	8	0.784
NO data	0	2	
Mutations			
TP53	5	4	1
FLT3	16	22	0.249
TET2	5	7	0.545
RUNX1	8	5	0.381
IDH1	8	6	0.572
IDH2	5	10	0.170
NPM1	14	21	0.168
DNMT3A	13	20	0.159
NRAS	4	3	0.698
KRAS	2	4	0.676
CEBPA	6	6	1

Discussion

AML is a malignant hematological disease and is associated with many different cytogenetic abnormalities with genetic alterations detected at diagnosis. It is the leading cause of leukemia-associated deaths worldwide each year. Although significant progress was made in the last two decades, etiologies, pathogenesis and biomarker of the disease are not yet clarified and are still worth researching. Meanwhile, for patients who received standard therapy, effects and prognosis vary. The STAT gene family contains seven different members, *STAT1* to *STAT6*, and is a group of proteins located mainly in nucleus and modulates target genes for cell proliferation, differentiation and apoptosis. The function of STAT genes has been validated in several cancer types.[4,12,13] STAT-related transcription factors were found constitutively activated in peripheral blood cells from AML patients.[14]

Furthermore, the JAK/STAT pathway is heavily involved in AML, and blocking phosphorylation of JAK-STAT could inhibit proliferation of AML cells in vitro and in vivo.[15–17] Among different STAT proteins, it was confirmed that STAT1 is activated in AML while inhibiting its activation decreases leukemia cell proliferation.[18,19] STAT3 and STAT5 are hyperactivated in the progression and nosogenesis of myeloid leukemia. STAT3 and STAT5 usually collaborate with upstream oncogenic drivers such as FLT3-ITD, BCL-ABL and JAK2.[20] Inhibition of STAT3 was found to have potent anti-leukemia activity, and blocking the expression of STAT5 could inhibit proliferation and enhance apoptosis of AML cells.[21,22] STAT6 induced by interleukin 4 (IL-4) also has an anti-leukemia effect in primitive AML cells.[23]

Although basic to translational research have found a potent role for STATs in the disease pathogenesis and disease inhibition, the role of STATs as biomarkers for disease diagnosis and prognosis is still worth investigating. Our research identified an expression difference in healthy control and AML patients and prognosis differences in patients with high or low expression of STATs in TCGA and ONCOMINE database. Herein, we selected STAT gene expression to analyze and investigate whether the change of expression of different STATs was related to clinical parameters as well as prognosis.

STAT6 is a member of the STAT gene family and was found to play different roles according to cancer type.[12,24–26] In AML, STAT6 was increased in AML blasts and participated in the pathogenesis of AML.[23,27] Through analysis of expression differences and prognosis values, we demonstrated that expression of STAT6 was increased in AML patients. Although it failed to achieve a significant role in predicting OS, STAT6 high expression was related to poor prognosis in patients who only received chemotherapy. Thereafter, we verified these results with the GEO databases and our cohort. Overexpression of *STAT6* was found in both GEO database GSE13159 and GSE9476. We also recruited
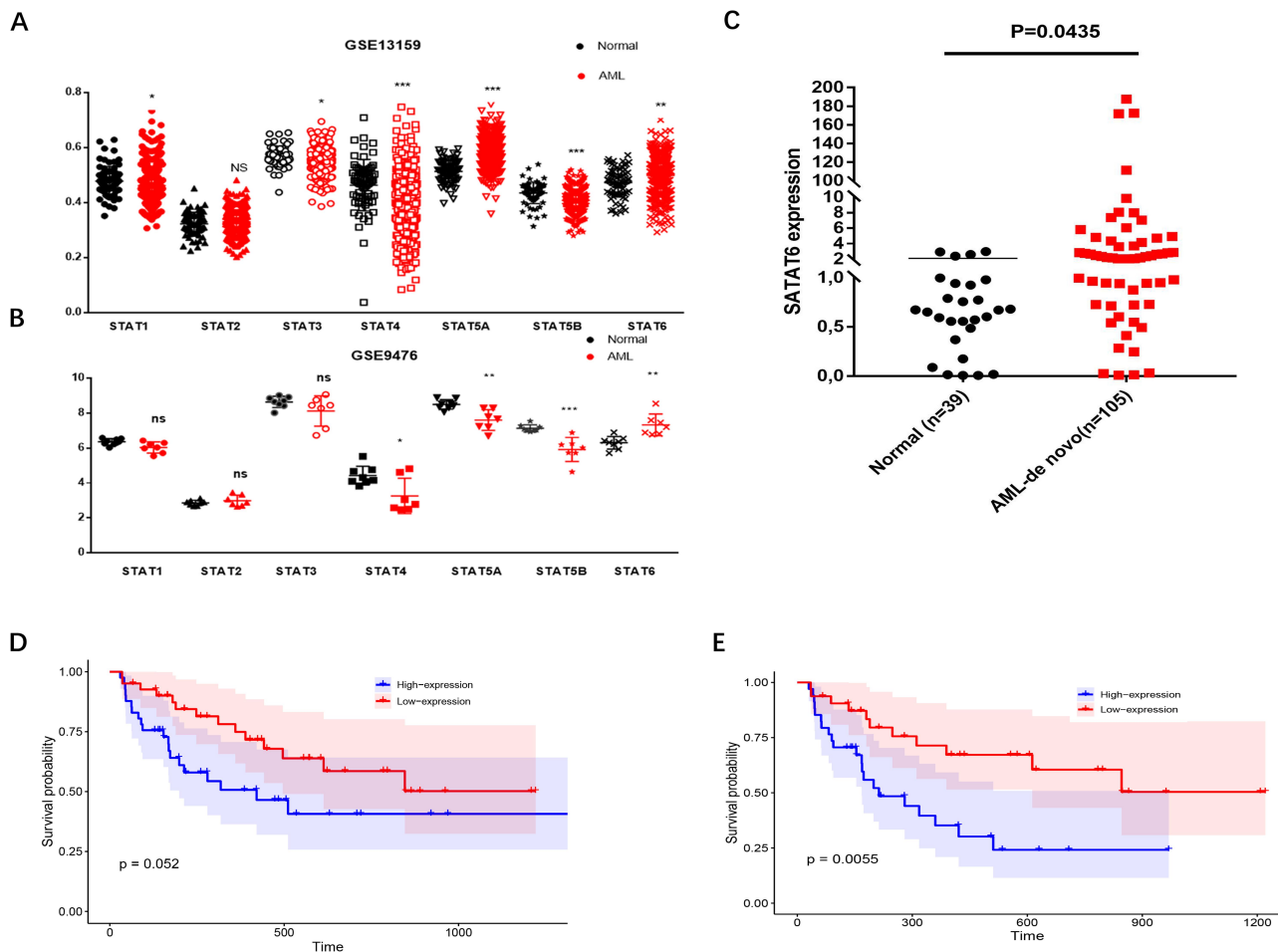
Figure 6 Expression and prognostic value of STATs in GEO datasets and recruited AML patients. (**A, B**) Expression of STATs in GEO datasets; (**C**) expression of STAT6 in recruited AML patients; (**D, E**) prognostic value of overall survival of STAT6 in total recruited AML patients (**D**) or chemotherapy-only (**E**) AML patients. *: P<0.05; **: P<0.01; ***: P<0.001.

105 AML patients, who had undergone chemotherapy or HSCT. *STAT6* increased in these patients while a poor prognosis with higher *STAT6* expression in patients only received chemotherapies was also verified. In accordance with our finding, STAT6 level and activation were confirmed to have a prognostic value in patients who underwent chemotherapy in several cancer types, such as primary CNS lymphoma, B cell lymphoma and colon cancer,[28–30] and may relate to the patients' immune function.[31]

It is well known that STAT signaling can be activated by diverse cytokines that are involved in many biological processes, such as immunity, cell division, cell death, and cancer development. From our GO and KEGG analyses, we demonstrated that STATs are enriched in cell differentiation, cell migration and pathways in cancer, which are all risk factors in AML progression. In the AML research field, STAT6 is mainly triggered by IL-4, SHP-1 and JAK. In these signaling pathways, STAT6 usually get triggered

and phosphorylated.[24,32,33] Our research was the first to find and validate the expression differences of STATs in AML patients and the prognostic value of the *STAT6* gene in AML patients only-received chemotherapy treatment. These results may lead to further investigations of the gene function of STAT6. Whether STAT6 could be a prognostic factor or some important target, more evidence should be proved.

Abbreviations

AML, Acute myeloid leukemia; STAT, signal transducer and activator of transcription; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GEPIA, Gene Expression Profiling Interactive Analysis; TCGA, The Cancer Genome Atlas; HSCT, hematopoietic stem cell transplantation; OS, Overall Survival; GeneMANIA, Gene Multiple Association Network Integration Algorithm; PPI, Protein–protein interaction.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Xiangya Hospital, Central South University in China, and all subjects gave written informed consent.

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Author Contributions

JyC designed the study, WL performed most of the experiments, FyZ, JzY, YL, WL, CC, KxZ and XlZ collected the clinical sample and data, performed rest necessary experiments. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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