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High Expression of AFF1 Associate with Poor Prognosis for Patients with Acute Myeloid Leukemia

Xi Chen[1,2], Qin Yan[1,2], Xiang Qin[1,2], Qian Chen[1,2], You Yang[1,2], Jing Liu[1,2], Yan Zeng[1,2], Sili Long[1,2] and Wenjun Liu[1,2*]

[1]*Department of Pediatrics Hematology, Children Hematological Oncology and Birth Defects Laboratory, The Affiliated Hospital of Southwest Medical University, Sichuan, China*

[2]*Sichuan Clinical Research Center for Birth Defects, Sichuan, China*

Abstract

Background: AFF family genes (AFFs) are closely related to the occurrence, development and prognosis of leukemia, but its relationship with Acute Myeloid Leukemia (AML) is not clear. This study aims to explore the relationship between AFFs and the prognosis of AML through bioinformatics methods and clinical data.

Methods: The expression and prognostic value of AFFs in AML were analyzed by databases such as ONCOMINE, GEPIA, EMBL-EBI, TCGA and CCLE. We collected 32 clinical cases, including 24 cases of AML (non-M3 type) and 8 cases of benign individuals. The expression level of AFF1 was detected by real-time fluorescence quantitative PCR (RT-qPCR) and western blotting.

Results: In multiple databases and cell lines, the RNA expression levels of AFF1, AFF2, AFF3 and AFF4 in AML were higher than those in the control group. This relationship was further verified in AML patients by RT-qPCR. Among them, the high expression of AFF1 was statistically significant ($P<0.05$). At the protein level, the expression of AFF1 in AML patients and cell lines was also higher than that in controls ($P<0.05$). High expression of AFF1 was significantly associated with poor prognosis in AML ($P<0.05$).

Conclusion: AFFs expression levels was significantly higher in AML. Particularly, the high expression of AFF1 was significantly related to a poor prognosis of AML. It indicated that AFF1 might be a potential therapeutic target for AML.

Keywords: AFFs; Acute myeloid leukemia; Bioinformatics analysis; Prognosis

Abbreviations: AFFs: Aff Family Genes; AML: Acute Myeloid Leukemia; RT-qPCR: Real-Time fluorescence-based Quantitative PCR; NPM1: Nucleophosmin 1; CEBPA: CCAAT/Enhancer Binding Protein Alpha; FLT3: FMS-like Tyrosine kinase 3; ITD: Internal Tandem Duplication; OS: Overall Survival; MLL: Mixed Lineage Leukemia; SECs: Super Elongation Complexes; KMT2A: Lysine Methyltransferase 2A; MS: Mass Spectrometry; CPTAC: Clinical Proteomics Tumor Analysis Consortium; CR: Complete Remission; ECL: Enhanced Chemiluminescence; PBMCs: Peripheral Blood Mononuclear Cells

Introduction

Acute Myeloid Leukemia (AML) is a malignant disease of myeloid hematopoietic stem/progenitor cells, characterized by abnormal proliferation of primitive and naive myeloid cells in the bone marrow and peripheral blood [1]. The incidence of AML is approximately 5.06 per 100,000 and AML in children accounts for about 20% of childhood leukemia cases [2]. It's prognosis closely related to cytogenetics and molecular abnormalities, such as NPM1 (nucleophosmin 1, NPM1), CEBPA (CCAAT/enhancer binding protein alpha, CEBPA), FLT3 (Fms-Like Tyrosine Kinase 3,) ITD (Internal Tandem Duplication), P53, and chromosome aneuploidy mutations, etc [3,4]. Although recent research on FLT3 mutations as a therapeutic target has made major breakthroughs in the treatment of AML, the morbidity and mortality rates are still high, with a 5-year Overall Survival (OS) of 20% [5-7]. The 5-year OS for patients under the age of 60 is 35%-40% while the patients over 60 years is 5%-15% [8]. Therefore, finding more targets that are therapeutic is very important for AML.

The AFF Family Genes (AFFs), namely the AF4/FMR2 family, is a family of RNA binding proteins (AFF1, AFF2, AFF3 and AFF4), which are located in the nucleus and act as transcriptional activators to have a positive effect on RNA elongation [9]. The main function of AFFs gene is to regulate transcription, which plays an important role in the occurrence and development of a variety of tumors. AFF1 is involved in inhibiting the migration and invasion of Serous Epithelial Ovarian Cancer (SEOC) cells [10] and down regulating indicators related to poor prognosis of lung adenocarcinoma [11]. AFF2 is not only related to the prognosis of patients with Thymoma [12] and Neuroblastoma [13] but also involved in the occurrence and development of non-keratinizing squamous cell carcinoma of the nasal cavity [14]. AFF3 participates in the occurrence and development of Adrenocortical Cancer (ACC) [15]. AFF4 may become the key to the pathogenesis of leukemia through many Mixed Lineage Leukemia (MLL) partners [16]. Among them, AFF1 is an important part of the backbone of Super Elongation Complexes (SECs). SECs incorporating AFF1 exhibit histone methyltransferase activity (H3K79me2/3 and H3K36me2) and P-TEFb kinase activity, which are required for oncogenic transformation

***Corresponding author:** Dr. Wenjun Liu, Department of Pediatrics Hematology, Children Hematological Oncology and Birth Defects Laboratory, The Affiliated Hospital of Southwest Medical University, Sichuan, China, E-mail: wenjun_liu@swmu.edu.cn

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[17]. Studies have reported that AFF1 plays a role in leukemia mainly by rearranging with Mixed-Lineage Leukemia (MLL, Lysine-Specific Methyltransferase 2A and KMT2A). It not only induces apoptosis and hinders cell proliferation [18], but also regulates hematopoietic stem cell properties and self-renewal of human leukemia cells by encoding a hematopoietic stem cell developmental regulator [19]. And then, it can also drive leukemia by directly binding and inducing the anti-apoptotic factor BCL2 and the proto-oncogene MYC [20].Some researchers pointed out that AFF1-KMT2A rearrangement frequently occurs for children in acute lymphoblastic leukemia and means high risk [21]. However, the expression of AFFs in AML and its relationship with prognostic value remain unclear. Therefore, this article aims to explore the value of AFFs in predicting the prognosis of AML and to provide a basis for clinical intervention and treatment through recognized databases and clinical samples of AML patients (Figure 1).

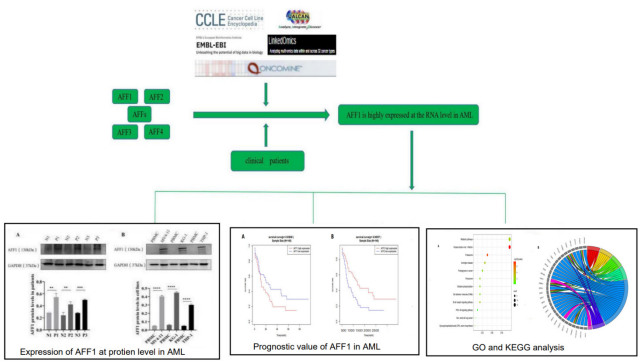

Figure 1: Flow chart of the study.

Materials and Methods

Bioinformatics methods

GEPIA database: TCGA and GTEx have generated a large amount of RNA sequencing data. GEPIA provides key interactive and customizable functions based on TCGA and GTEx, including differential expression analysis, contour mapping, correlation analysis, similar gene detection and dimensionality reduction analysis [22]. Use GEPIA to analyze the expression changes of AFFs in AML samples and normal samples.

ONCOMINE database: The oncomine database has collected 18,000 genes, pathways, and networks in cancer gene expression microarrays, which span most cancer types and subtypes. The database aims to collect, standardize, analyze and provide cancer transcriptome data to the biomedical research community [23]. Use the oncomine database to compare the expression data of AFFs in AML and normal samples.

Linked omics database: The Linked omics database is a multi-omics database based on Mass Spectrometry (MS) global proteomics data generated by the Clinical Proteomics Tumor Analysis Association (CPTAC) on selected TCGA tumor samples. Contains multi-omics data and clinical data of 32 cancers and 11 158 patients from the Cancer Genome Atlas (TCGA) project [24]. Analyze the related genes of AFFs through the Linked omics database

Cancer Cell Line Encyclopedia (CCLE) database: CCLE database includes gene expression, sequencing and Single Nucleotide Polymorphism (SNP) array copy number of 1,650 genes in 947 cell lines [25]. The CCLE data was used to prove the expression of AFFs in cancer cell lines.

The European Bioinformatics Institute (EMBL-EBI) database: EMBL-EBI database maintained by the European Institute of Bioinformatics (EBI) collects nucleotide sequences and annotations available from public sources. In September 2006, the size of the EMBL nucleotide sequence database has increased from 58.7 million to 80.5 million. The database is part of international cooperation with DDBJ (Japan) and GenBank (USA) [26]. The goal is to collect and present nucleotide sequences and annotations as comprehensively as possible, and to further verify the expression of AFFs in AML cell lines.

UALCAN database: UALCAN database uses TCGA transcriptome and clinical patient data to study gene expression levels, not only comparing tumor and normal tissue samples, but also comparing tumors at different pathological stages, different grades and different races specialty. We compared the AFFs' difference expression level of different stages in AML by using the UALCAN database [27].

TCGA database: We downloaded the transcriptome data and clinical data of AML patients through the TCGA database, and converted the probe ID to the gene name according to the annotation file. We matched the patient's ID number with the corresponding RNA expression profile and clinical data, and excluded patients whose ID numbers cannot match. In the end, we selected 140 patients with complete clinical data for survival analysis. The survival curve was generated by running the R package "survival" using Rstudio (Version 4.0.4), and the difference in survival curve was compared by log-rank test. A P value <0.05 is considered significant.

DAVID and KOBAS database: The DAVID database and the KOBAS database are based on the gene function categories in the gene list and the signal pathways involved in regulation. Classification can quickly help clarify new biological processes related to cell functions and pathways. GO analysis and KEGG analysis of AFFs-related genes were carried out through DAVID database and KOBAS database.

Patients and specimens

Clinical specimens: We collected 24 AML cases (non-M3 type) admitted to the Affiliated Hospital of Southwest Medical University as the experimental group, and 8 benign individuals as the control group. The diagnostic criteria of AML patients refer to the WHO 2016 standard and are classified according to the FAB classification. The treatment of AML patients is mainly based on the Chinese adult AML diagnosis and treatment guidelines (non-APL) (2017) [28]. Collecting clinical data such as patient age, gender, gene mutation/fusion, subtype classification, risk, Complete Remission (CR), etc.

RT-qPCR: Total RNA was extracted using RNA simple Total RNA Kit (TIANGEN, China, Beijing). cDNA was synthesized using TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR kit (TransGen Biotech, China, Beijing), and PerfectStart Green qPCR SuperMix (Transgen, China, Beijing) was used to detect AFFs mRNA levels. The primers sequences were AFF1- forward, 5′-GCTTCTCTGGGGTTTGTTCA-3′, AFF1-reverse, 5′-AAGGAACGTCATCCATGCTC-3′, AFF2-forward, 5′-TGGAGAGGGTATCTGTGCGA-3′, AFF2- reverse, 5′-AAGTCC CTTGGCTTCGGATG-3′, AFF3-forward, 5′-CAGGTTTGGGAACTC CAACG-3′, AFF3-reverse, 5′-CTCAACAGGATGATGGCACG-3′, AFF4- forward, 5′-AAGAGGCGGGTGACACTTTA-3′, AFF4-reverse, 5′-GCAAATGAGCCATCCCAGAG-3′, β-Actin-forward, 5' GGCGGCACCACCATGTACCC-3',β-Actin-reverse,5'-CCACACGGA

GTACTTGCGC-3'. The2−ΔΔCT method was used to calculate the relative mRNA expression.

Western-blot: The cells were collected and lysed with lysis buffer (Biyuntian, Beijing, China). The extracted proteins were loaded in equal amounts, separated by SDS-PAGE (Solarbio, China), and transferred to polyvinylidene fluoride membranes (0.45 μm, Millipore, Burlington, MA, USA). The membranes incubated with primary antibodies overnight at 4°C and secondary antibodies for 2 hours at room temperature. Finally, protein expression was detected by an Enhanced Chemiluminescence (ECL) system (Vilber, France).

Statistical analysis

Statistical analysis was performed using SPSS (version 17.0, IBM Corp. Armonk, NY) and GraphPad Prism (version 8.0, GraphPad Software). Student's t-test was used to test the expression difference between the experimental group and the control group. According to the median value of AFFs expression, patients were divided into high expression group and low expression group, and the difference between high expression group and low expression group was statistically compared by Fisher's exact test analysis. When the P value is less than 0.05, it is considered statistically significant.

Results

AFFs are highly expressed at RNA level In AML

AFFs are highly expressed in AML database (GEPIA and ONCOMINE database): We used the GEPIA database to compare the transcription level of AFFs in AML samples and normal samples. The results showed that AFF1, AFF2 and AFF3 were highly expressed and had a statistical significance (P<0.05), the high expression of AFF4 had no statistical significance (P>0.05) (Figure 2).

By using the ONCOMINE database, we compared the transcription level of AFFs in AML with normal samples. ONCOMINE's research showed that AFF1, AFF2, AFF3 and AFF4 were highly expressed in AML (Table 1) (P<0.05). In the Stegmaier Leukemia data set the mRNA transcription level of AFF1 was 4.216 times that of normal people (P<0.05), the mRNA transcription level of AFF2 was 2.646 times that of normal people (P<0.05), the transcription level of AFF3 was 1.628 times that of normal people (P<0.05), the transcription level of AFF4 was 1.855 times that of normal people (P<0.05).

AFFs are highly expressed in AML clinical samples: We analyzed the mRNA expression level of 24 AML patients (non-M3 type) in our hospital by real-time fluorescent quantitative PCR (RT-qPCR). The results showed that the expressions of AFF1 (P=0.006), AFF2 (P=0.021) and AFF4 (P=0.014) in AML samples were significantly higher than those in the control group and the high expression of AFF3 (P=0.084) had no statistical significance (Figure 3). Further analysis of the post-treatment remission of patients in high expression group (AFF high) and low expression group (AFF low) (Table 2), we found that there was no significant difference in the expression of AFFs between different gender, age, prognosis and complete remission (P>0.05). The relationship between the expression of AFFs and gene mutations and fusion genes has no statistical significance (P>0.05) which might be related to the small sample size.

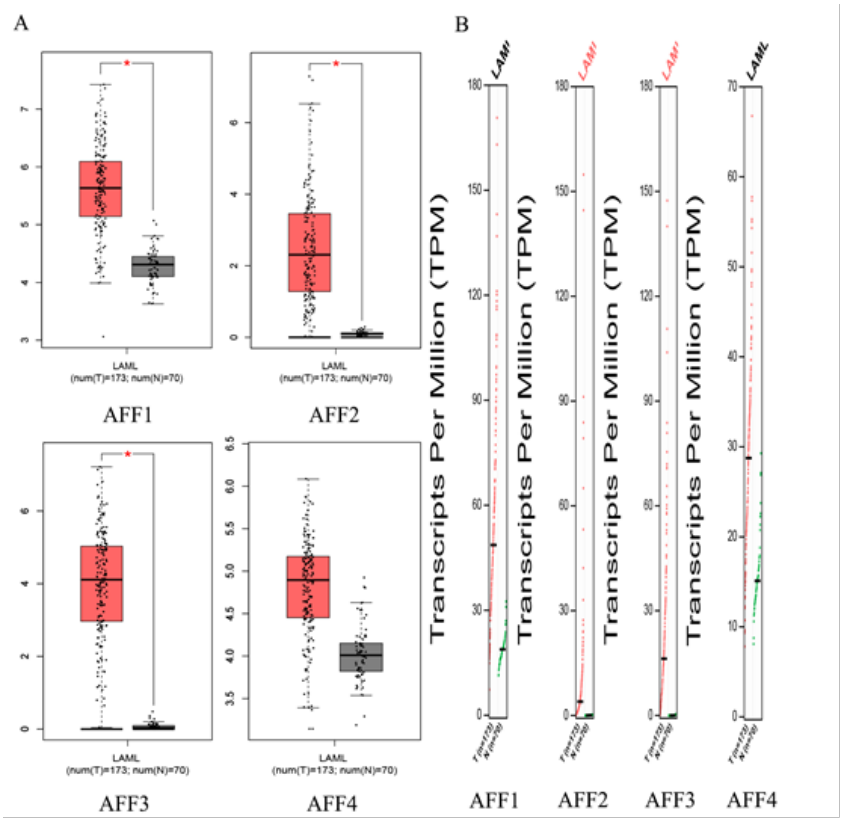

Figure 2: The Expression of AFFs in Leukemia (GEPIA); (A,B) The expression levels of AFFs in AML *vs.* normal samples. **Note:** *P<0.05.

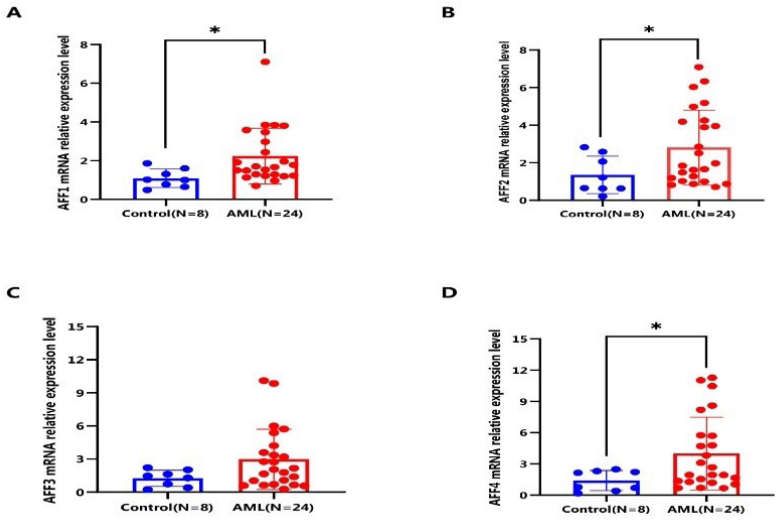

Figure 3: Expression of AFF genes in AML and normal samples. (A) The expression levels of AFF1 in AML compared with normal samples; (B) The expression levels of AFF2 in AML compared with normal samples; (C) The expression levels of AFF3 in AML compared with normal samples; (D) The expression levels of AFF3 in AML compared with normal samples. **Note:** * : P<0.05.

GENE	Types of leukemia *vs.* normal Samples	Fold change	P Value	t-test	Reference
AFF1	Acute Myeloid Leukemia *vs.* Normal	4.216	1.00E-03	4.752	Stegmaier Leukemia
AFF2	Acute Myeloid Leukemia *vs.* Normal	2.646	3.19E-04	4.5	Stegmaier Leukemia
AFF3	Acute Myeloid Leukemia *vs.* Normal	1.628	4.40E-02	1.902	Stegmaier Leukemia
AFF4	Acute Myeloid Leukemia *vs.* Normal	1.855	8.84E-05	5.233	Stegmaier Leukemia

Table 1: The significant changes of AFFs' expression in transcription level (ONCOMINE database).

			AFF1 low	AFF1 high	P	AFF2 low	AFF2 high	P	AFF3 low	AFF3 high	P	AFF4 low	AFF4 high	P
Age	<60	14	7.00E+00	7	1	9	5	0.214	8	6	0.68	8	6	0.68
	≥ 60	10	5.00E+00	5		3	7	-	4	6		4	6	-
Gender	Male	6	3.00E+00	3	1	2	4	0.64	2	4	0.64	3	3	1
	Female	18	9	9	-	10	8	-	10	8	-	9	9	-
Immunophenoyping	M0	1	0	1	-	0	1	-	0	1	-	1	0	-
	M1	6	1	5	-	3	3	-	4	2	-	3	3	-
	M2	8	4	4	-	3	5	-	5	3	-	4	4	-
	M4	3	3	0	-	2	1	-	1	2	-	1	2	-
	M5	6	4	2	-	4	2	-	2	4	-	3	3	-
Gene mutation /fusion	FLT3	5	2	3	-	1	4	-	4	1	-	1	4	-
	N-RAS	3	1	2	-	2	1	-	2	1	-	1	2	-
	c-KIT	3	1	2	-	2	1	-	2	1	-	3	0	-
	NPM1	4	2	2	-	2	2	-	2	2	-	1	3	-
	WT1	10	6	4	-	5	5	-	4	6		4	6	-
	IDH2	5	3	2	-	3	2	-	4	1	-	3	2	-
	DNMT3A	4	3	1	-	3	1	-	2	2	-	2	2	-
	CEBPA	2	1	1	-	1	1	-	1	1	-	1	1	-
	ETO	3	0	3	-	2	1	-	2	1	-	3	0	-
	RUNX1	2	2	0	-	0	2	-	2	0	-	1	1	-
	MPL	1	0	1	-	0	1	-	1	0	-	0	1	-
	ASXL1	1	0	1	-	0	1	-	1	0	-	0	1	-
	MLL	1	0	1	-	1	0	-	0	1	-	0	1	-
	BCOR	3	2	1	-	2	1	-	2	1	-	1	2	-
Risk	Good	2	0	2	-	1	1	-	0	2	-	1	1	-
	Intermediate	7	3	4	-	4	3	-	5	2	-	5	2	-
	Poor	15	9	6	-	7	8	-	7	8	-	6	9	-
Complete remission		4	3(75%)	1(25%)	0.59	3(75%)	1(25%)	0.59	3(75%)	1(25%)	0.59	3(75%)	1(25%)	0.59

Table 2: Relationship between baseline characteristics and AFFs' mRNA expression (Fisher exact test).

Expression of AFFs in different cancer cell lines: In the CCLE database, the expression level of AFFs in different cancer cells was systematically clarified (Figures 4A-4D). We found that AFF1 and AFF4 were highly expressed in acute myeloid leukemia cell lines. Subsequently, we analyzed the expression level of AFFs in acute myeloid leukemia cell lines through EMBL-EBI, and found that AFF1 and AFF4 were highly expressed in most acute myeloid leukemia cell lines (Figure 4E).

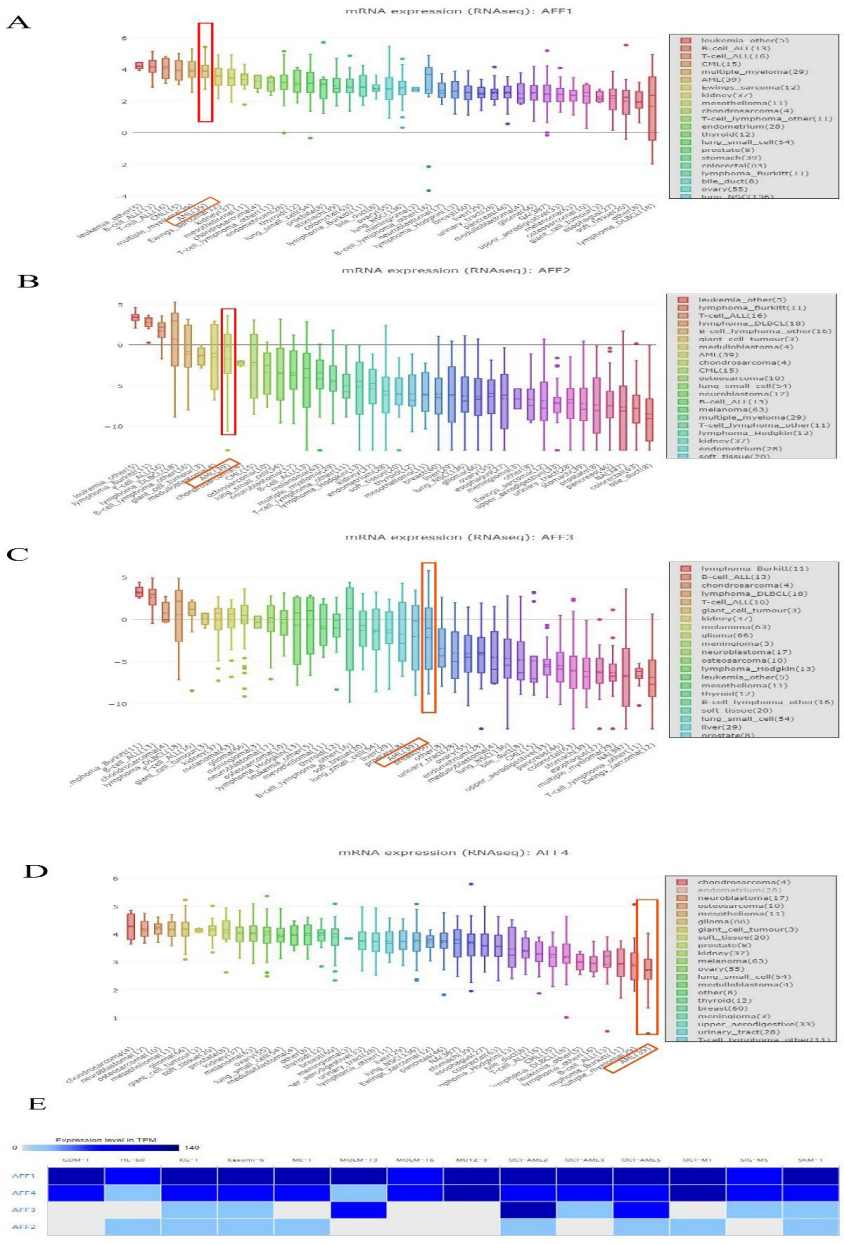

Figure 4: Expression of AFFs in Pan-cancers and AML cell lines (CCLE and EMBL-EBI). (A) The expression levels of AFF1 in Pan-cancers cell lines by CCLE; (B) The expression levels of AFF2 in Pan-cancers cell lines by CCLE; (C) The expression levels of AFF3 in Pan-cancers cell lines by CCLE; (D) The expression levels of AFF4 in Pan-cancers cell lines by CCLE; (E) The expression levels of AFFs in AML cell lines by EMBL-EBI.

Expression of AFFs in French-American-British (FAB) subtypes of AML: In UALCAN, according to the FAB classifications of acute leukemia and myelodysplastic syndromes, the expression levels of AFFs were different in different types (Figure 5). The expression of AFF1 in the M3 type was significantly lower than other types, and the differences between M3 and M0 (P=4.36E-05) M3 and M1 (P=1.73E-02) had statistical significance (Figure 5A). The expression of AFF2 in M3 type was significantly higher than that of other types, and the differences between M3 and M0 (P=1.16E-04), M3 and M1 (P=1.79E-04), M3 and M2 (P=2.04E-04), M3 and M4 (P=2.19E-04), M3 and M5 (P=1.16E-04) had statistical significance (Figure 5B). The expression of AFF3 in M3 type was significantly lower than that of other types, and the differences between M3 and M0 (P=6.01E-04), M3 and M1 (P=1.62E-02), M3 and M2 (P=8.30E-03), M3 and M4 (P=2.83E-04), M3 and M5 (P=2.53E-02) had statistical significance (Figure 5C). The expression of AFF4 in M3 type was significantly lower than that of M0 (P=2.36E-02), and there was no statistical significance between other types and M3 (Figure 5D).

Protein expression level of AFF1 in clinical patients and cell lines of AML

At the mRNA level, only AFF1 was highly expressed in all databases, cell lines and clinical patients involved in this study and it has statistical significance. Therefore, we further performed the analysis and validation at the protein level. We collected PBMCs of AML patients and benign individuals for detection. The result showed that AFF1 was significantly highly expressed in AML ($P<0.05$) (Figure 6A). At the same time, we used the AML cell lines of KG-1, MV4-11 and THP-1 as experimental groups, and Peripheral Blood Mononuclear Cells (PBMCs) from benign individuals as controls. AFF1 was also highly expressed in cell lines ($P<0.05$) (Figure 6B).

The prognostic value of AFF1 in AML

We downloaded the transcription data and clinical data of AML through the TCGA database, and analyzed the relationship between the expression level of AFF1 and the prognosis of AML patients through Kaplan-Meier. High expression of AFF1 was negatively correlated with the Overall Survival (OS) of AML ($P<0.05$) (Figure 7A). The prognostic value of AFF1 was further validated by the Bloodspot database (Figure 7B).

Correlation analysis of AFF1 related genes in AML

The related genes of the AFF1 were obtained from the Linked omics database. The red dots in the volcano map showed positively related genes, and the green dots showed negatively related genes. The heat map showed the top 50 genes that were positively correlated and negatively correlated with the AFF1 (Figure 8A). Among them, CCDC6 (Pearson-Correlation=0.5772, P=9.351E-17), MAP3K5 (Pearson-Correlation=0.4879, P=9.878E-12) and PTP4A3 (Pearson-Correlation=2081, P=6.011E-03) were positively correlated with AFF1 (Figure 8B). High expression of these genes predicted poor prognosis ($P<0.05$) (Figure 8C).

Enrichment analysis of AFFs related genes in AML

The AFFs related genes obtained by Linked omics were used for functional enrichment analysis and KEGG signal pathway enrichment analysis (Figure 9). The main function is to regulate the stability of mRNA, the regulation of cell cycle, and the transcription of DNA. Signal pathway analysis speculates that AFFs may be involved in the regulation of proteoglycan pathways, cell adhesion molecules (CAMs), and Wnt signaling pathways in cancer.

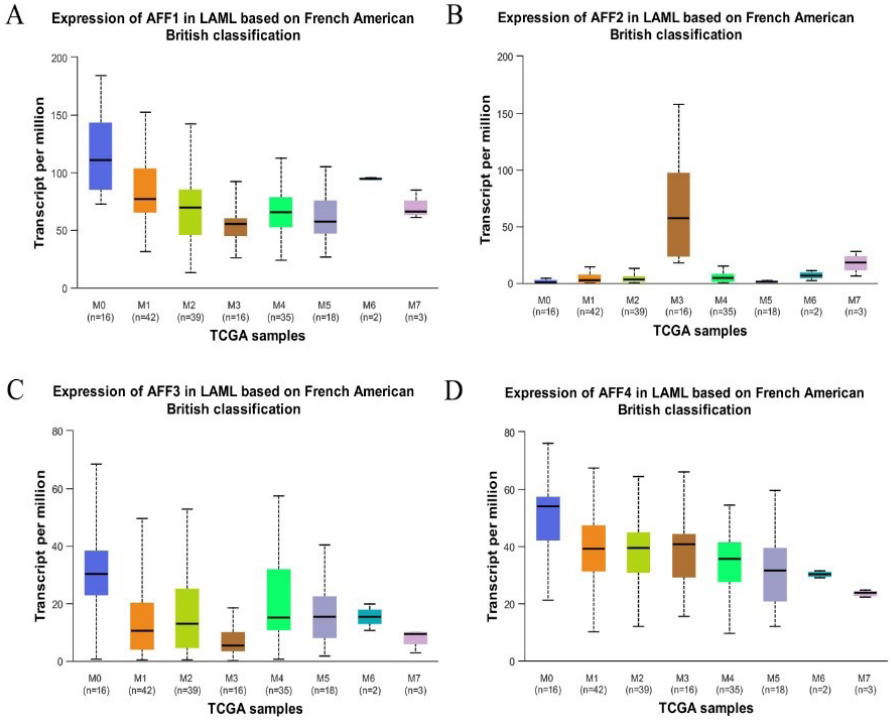

Figure 5: The Expression of AFFs in leukaemia based on FAB classification (UALCAN). (A) The expression level of AFF1 in AML based on FAB classification; (B) The expression level of AFF2 in AML based on FAB classification; (C) The expression level of AFF3 in AML based on FAB classification; (D) The expression level of AFF4 in AML based on FAB classification.

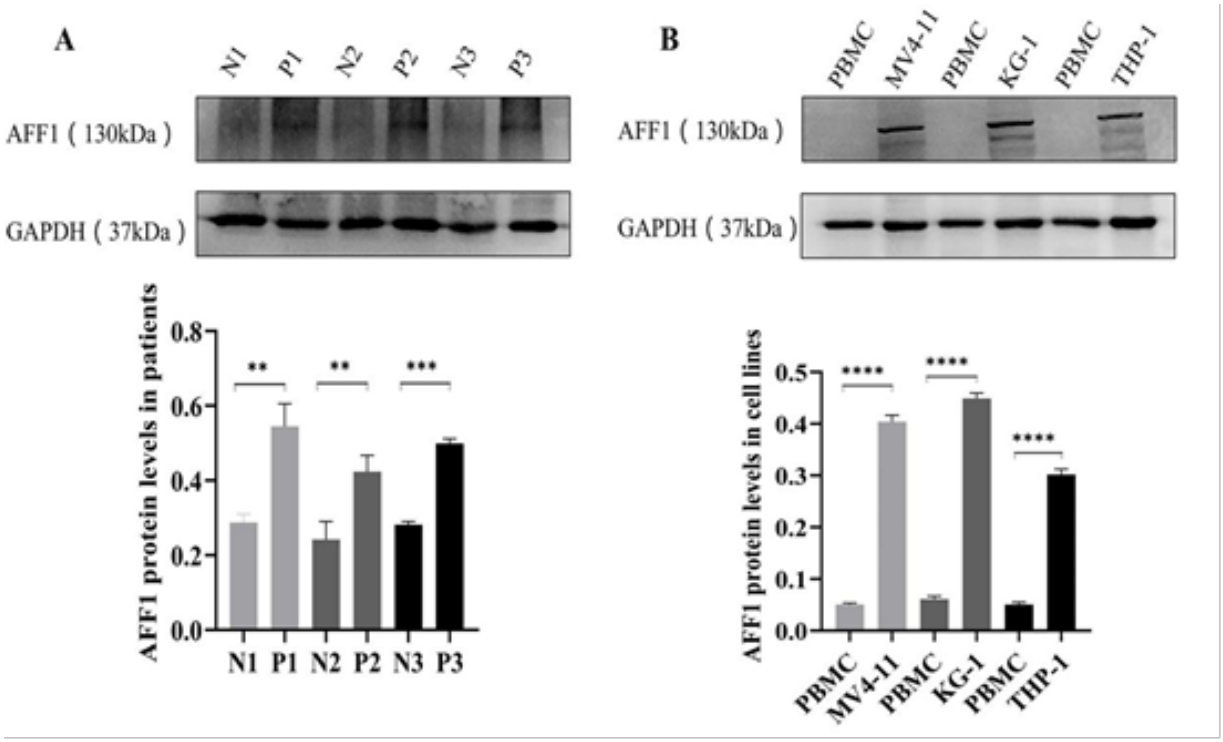

Figure 6: Protein expression level of AFF1 in clinical cases of AML and cell lines (A) Protein expression level of AFF1 in AML patients and normal samples. (B) Protein expression level of AFF1 in AML. **Note:** N1,2,3 : Normal sample 1,2,3; P1,2,3:Patient 1,2,3; **: AFF1 protein presence in first and second group; ***: AFF1 protein presence in third group; ****: AFF1 protein comparison with PBMC along with MV4-11, KG-1, THP-1 in all the groups.

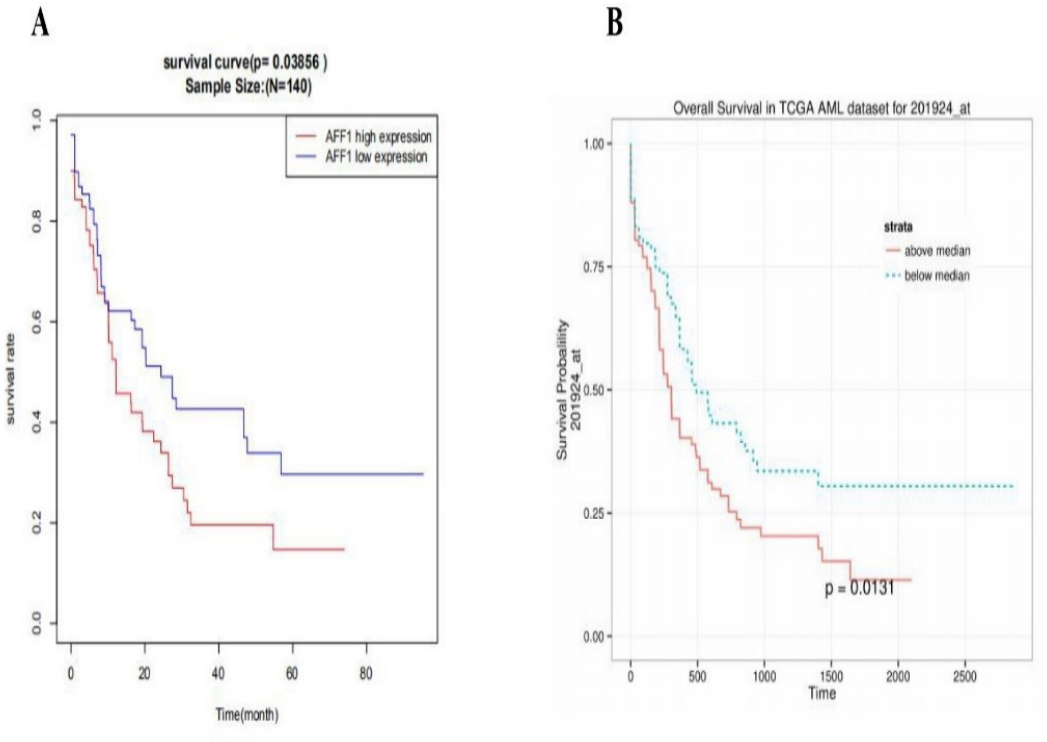

Figure 7: The prognostic values of AFF1 expression in AML patients. (A) The prognostic values of AFF1 expression analyzed by TCGA; (B) The prognostic values of AFF1 expression analysed by Bloodspot. **Note:** (—) AFF1 high expression; (—) AFF1 low expression, above median; (·····) below median

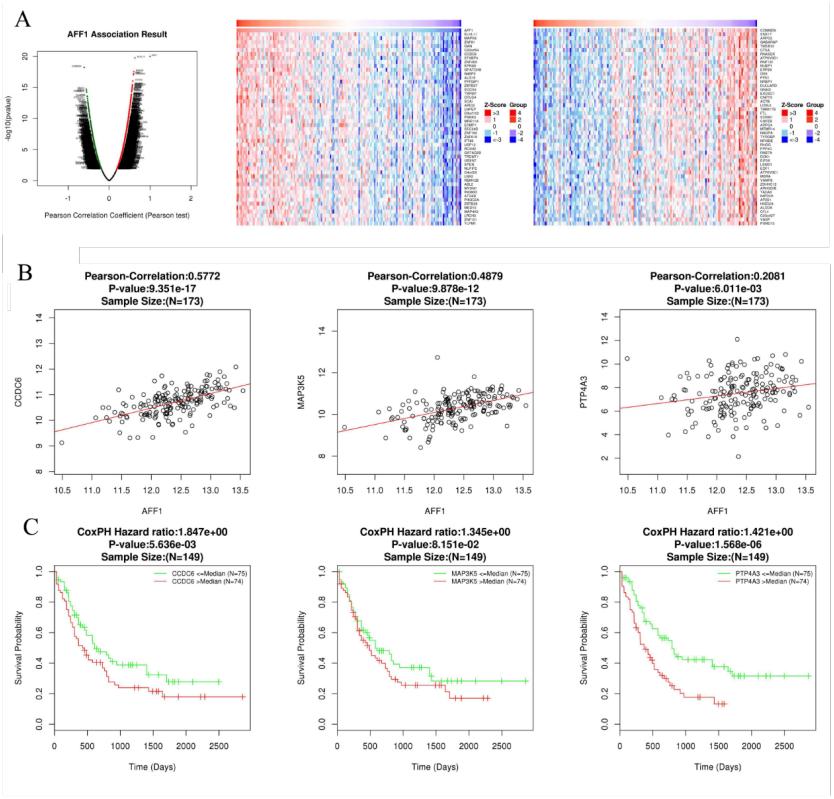

Figure 8: Differentially expressed genes correlated with AFF1 in AML (LinkedOmics). (A) Volcano plots and heat maps indicating genes positively and negatively genes correlated with AFF1 in AML; (B) The scatter plots show Pearson's correlation of AFF1 expression with expression of CCDC6, MAP3K5, PTP4A3; (C) Prognostic analysis of CCDC6, MAP3K5.

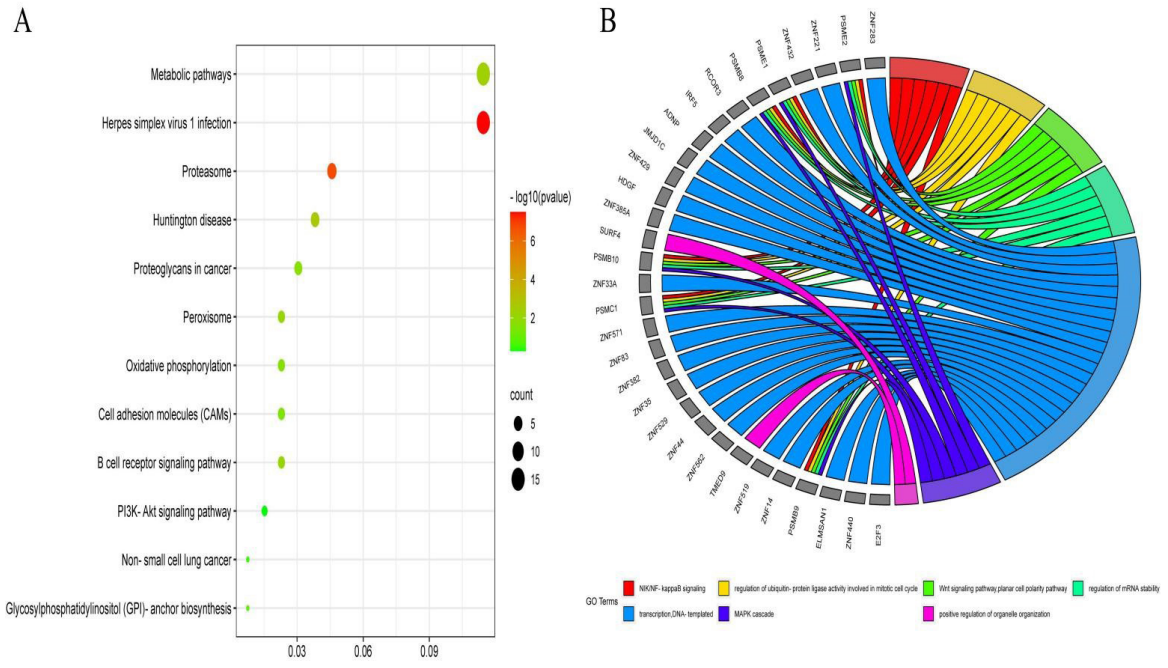

Figure 9: Functional enrichment analysis of AFFs (A) KEGG pathway analysis of AFFs (Kobas); (B) GO-BP terms of AFF (DAVID). **Note:** ( ) NIK/NF-kappaB signaling; ( ) Regulation of ubiquitin-protein ligase activity involved in mitobic cell cycle; ( ) Wnt signaling pathway planar cell polarity pathway; ( ) Regulation of mRNA stability; ( ) Transcription DNA-templated; ( ) MAPK cascade; ( ) Positive regulation of organelle organization.

Discussion

As a kind of RNA binding protein, AFFs' biological function involves various tumors, including leukemia, but there is a lack of research on the occurrence and development of AML. In order to explore the potential functions of AFFs in AML, we used online bioinformatics database and clinical patients for analysis.

The analysis of Oncomine database and GEPIA database showed that AFF1, AFF2 and AFF3 were highly expressed in AML (Figure 2 and Table 2). In this study, the high expression of AFF1, AFF2 AFF3 and AFF4 was further verified by RT-qPCR in AML clinical patients. The high expression of AFF1, AFF2, and AFF4 had statistically significant ($P<0.05$) (Figure 3). In addition, CCLE database showed that AFF1, AFF2, and AFF3 were mainly expressed in hematological tumors, while AFF4 was mainly expressed in osteosarcoma. Among them, AFF1 was significantly highly expressed in AML (Figures 4A-4D). Among cell lines, AFF1 and AFF4 were highly expressed in most AML cell lines through EMBL-EBI (Figure 4E). The expression of AFF family genes varies in different databases, cell lines, and our clinical data. Among them, AFF1 was highly expressed in all databases, cell lines and our clinical data. We further validated this conclusion at the protein level by western blot.

Studies have shown that KMT2A/AFF1 rearrangement occurs frequently in high-risk children, which is related to poor prognosis [29]. Through analysis of TCGA and Bloodspot, we found that high expression of AFF1 was negatively correlated with OS in AML (Figure 7). We also used the Linked omics database to perform correlation analysis of AFF1-related genes (Figure 8A). Among them, CCDC6, MAP3K5 and PTP4A3 were significantly positively correlated with AFF1 (Figure 8B). The high expression of CCDC6, MAP3K5 and PTP4A3 were negatively correlated with OS in AML (Figure 8C). They were respectively involved in the occurrence and development of papillary thyroid cancer [30], tumor metastasis [31] and the prognosis of papillary renal cell carcinoma [32].

Using AFFs-related genes found in the Linked omics database for functional enrichment analysis and KEGG signaling pathway analysis. The result showed that function of AFFs-related genes involved the stability of mRNA, transcription of DNA and the regulation of proteoglycan pathway in cancer (Figure 9B), so AFFs might be related to the occurrence and development of a variety of tumors. Studies reported that AFF1 and AFF4 became key regulators in the pathogenesis of leukemia through MLL [33]. AFF1 candrive leukemia [20] and AFF4 can regulate tumor progression in cancer mice [34]. This was consistent with our research. In addition, also relate AFFs-related genes to CAMs and proteoglycans in cancer (Figure 9A). CAMs were involved in the metastasis and invasion of cancer cells [35]. Proteoglycans, such as HSPG2 [36], which can promote the metastasis and invasion of solid tumors. This was consistent with the promotion of tumor cell migration and invasion by AFFs. Studies also confirmed that AFF4 was involved in the invasion and migration of melanoma cells [37], Lung Adenocarcinoma (LUAD) cells [38], and Head and Neck Squamous Cell Carcinoma (HNSCC) cells [39] while AFF1 inhibited migration and invasion of Serous Epithelial Ovarian Cancer (SEOC) cells [10]. Our research found that AFFs were involved in Wnt signal pathway and cell cycle regulation. The signal transduction of the Wnt/Fzd pathway was related to the maintenance of hematopoietic stem cells and the establishment of acute leukemia [40]. At the same time, wnt pathway also regulated the apoptosis of leukemia cells in AML [41]. These results suggested that AFFs may be involved in tumor cell proliferation and apoptosis in AML.

Conclusion

In this study, we analyzed the expression and prognostic value of AFFs, AFFs were highly expressed in AML. In particular, AFF1 has prognostic value and is a promising therapeutic target for AML. High AFF1 expression was associated with poor prognosis in AML patients, suggesting its potential role as a prognostic marker. Additionally, functional enrichment analysis suggested that AFFs might be involved in key processes related to mRNA stability, DNA transcription, and signaling pathways, such as the Wnt pathway, which are crucial in AML pathogenesis. These findings indicate that AFF1 and other AFF family genes may serve as promising therapeutic targets in the treatment of AML, offering new avenues for further research and clinical intervention.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with a set of principles of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Affiliated Hospital of Southwest Medical University. All authors for publication approve the manuscript. Since we used the remaining blood after the blood routine test, we only need to pass the ethical review of the hospital and do not require the informed consent of the participants, hence the need for informed consent was waived by the ethics committee of Affiliated Hospital of Southwest Medical University.

Consent for publication

Not applicable

Availability of data and materials

The raw data of this study are derived from：

GEPIA database: http://gepia.cancer-pku.cn/

Oncomine database: https://www.oncomine.com/

LinkedOmics database: http://www.linkedomics.org/login.php

CCLE database: https://sites.broadinstitute.org/ccle

EMBL-EBI database: http://www.ebi.ac.uk/embl

UALCAN: http://ualcan.path.uab.edu/

TCGA database: https://portal.gdc.cancer.gov/

DAVID database: https://portal.gdc.cancer.gov/

KOBAS database: http://kobas.cbi.pku.edu.cn/

Bloodspot database: https://servers.binf.ku.dk/bloodspot

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

XC, QY, XQ, YY, JL,YZ, SLL and WJL are the principle investigator. XC and QY completed specimen collection and data processing. XC and SLL designed experiments. XC, QY, XQ, YY, JL, YZ, SLL and WJL participated in the writing and revision of the article. All authors read and approved the final manuscript.

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