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RESEARCH ARTICLE

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A-kinase interacting protein 1 might serve as a novel biomarker for worse prognosis through the interaction of chemokine (C-X-C motif) ligand 1/chemokine (C-X-C motif) ligand 2 in acute myeloid leukemia

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Abstract

Background: This study aimed to explore the association of A-kinase interacting protein 1 (AKIP1) with chemokine (C-X-C motif) ligand (CXCL) 1/CXCL2, and further investigate their correlation with clinical features and prognosis in acute myeloid leukemia (AML) patients.

Methods: Totally 160 de novo AML patients were recruited, and their bone marrow samples were collected before treatment for detecting the expressions of AKIP1, CXCL1, and CXCL2 by the quantitative polymerase chain reaction. Complete remission (CR) was assessed after induction treatment, and event-free survival (EFS) and overall survival (OS) were calculated.

Results: AKIP1 expression was positively associated with CXCL1 ($P < .001$) and CXCL2 expression ($P < .001$). AKIP1 high expression was correlated with FAB classification ($P = .022$), monosomal karyotype ($P = .001$), and poor risk stratification ($P = .013$), while CXCL2 high expression was associated with monosomal karyotype ($P = .001$). As for treatment response, AKIP1 high expression exhibited a trend to be increased in non-CR patients compared with CR patients, while without statistical significance ($P = .105$). However, no correlation of CXCL1 ($P = .418$) or CXCL2 ($P = .685$) with CR achievement was observed. Most importantly, AKIP1 and CXCL1 were negatively correlated with accumulating EFS and OS (all $P < .05$), while CXCL2 only showed a trend to be negatively associated with accumulating EFS ($P = .069$) and OS ($P = .055$; but without statistical significance).

Conclusion: AKIP1 might serve as a novel biomarker for worse AML prognosis through the interaction of CXCL1/CXCL2.

KEYWORDS

acute myeloid leukemia, AKIP1, complete remission, CXCL1, CXCL2

1 | INTRODUCTION

According to the most recent global statistics report, the incidence and mortality of leukemia account for 2.4% and 3.2% of all cancers, and acute myeloid leukemia (AML), as the most common acute leukemia, is characterized by the accumulation of immature and non-functional myeloid precursor cells in the blood and bone marrow.[1,2] Current therapies, such as chemotherapy and allogeneic stem cell transplantation, bring in improvements to the survival rate in patients with AML, while the majority of patients with AML still experience unfavorable prognosis with below 50% 5-year overall survival (OS).[3] Besides, patients with AML experience heterogeneous responses and outcomes to the treatments which make the exploration of markers for prognosis necessary. Therefore, it is essential to discover novel molecular markers which would serve as novel prognostic indexes and guide AML management in the future.

A-kinase interacting protein 1 (AKIP1), a molecular regulator of protein kinase A, is reported to serve as an adaptor or structural intracellular protein and is localized to the cytoplasm, nucleus, and mitochondria.[4] Recent studies reveal that AKIP1 contributes to the tumorigenesis angiogenesis, lymph angiogenesis, and invasiveness in several solid tumors, including breast cancer, colorectal cancer, and gastric cancer, and it predicts worse survival profiles in patients with cancer, suggesting that AKIP1 functions as an oncogene and may be an effective prognostic marker in these cancers.[5-7] Whereas in hematologic malignancies, the role of AKIP1 is limitedly studied. Interestingly, AKIP1 is shown to upregulate the expressions of the chemokine (C-X-C motif) ligand 1 (CXCL1)/chemokine (C-X-C motif) ligand 2 (CXCL2) and induce the activation of CXCL1/CXCL2 downstream oncogenic signaling pathway (Wnt/β-catenin signaling), leading to the development and progression of cervical cancer and hepatocellular carcinoma.[7,8] Furthermore, previous reports illuminating that CXCL1 functions as pro-angiogenic gene in AML, and CXCL2 enhances survival of primary chronic lymphocytic leukemia cells, meanwhile, both of which possess prognostic value in leukemia.[9,10] And Wnt signaling pathway is also shown to be an important oncogenic signaling functioning by regulating progression and chemosensitivity in AML.[11] Collectively, we speculated that AKIP1 was positively associated with CXCL1/CXCL1, and they had potential to be prognostic biomarkers in patients with AML. Thus, we conducted this study to explore the association of AKIP1 with CXCL1/CXCL2 and further investigate their correlation with clinical features and prognosis in AML patients.

2 | METHODS

2.1 | Participants

A total of 160 de novo AML patients in our hospital were consecutively enrolled from May 2016 to April 2019. The inclusion criteria were as follows: (a) diagnosed as primary AML based on peripheral blood and bone marrow examinations including morphology, cytochemistry, immunophenotyping, cytogenetics, and molecular genetics; (b) age above 18 years old; (c) no history of chemotherapy, radiotherapy, or other systematic treatments; (d) not complicated with other malignant myeloid diseases; and (e) negative serology for human immunodeficiency virus (HIV). And patients were excluded if they met the following criteria: (a) M3 in French-American-Britain (FAB) classification (acute promyelocytic leukemia); (b) life expectancy was <12 months. (c) unable to be followed up regularly; and (d) pregnant or lactating woman. This study was approved by the Ethics Committee of Yantaishan Hospital, and all patients signed informed consents.

2.2 | Baseline data collection

Baseline characteristics of patients were collected after enrollment, which included (a) demographic characteristics: age and gender; (b) morphology classification (French-American-Britain [FAB] classification); (c) cytogenetic abnormalities: NK, CK, inv(16) or t(16;16), t(8;21), −7 or 7q−, +8, 11q23, t(9;11), −5 or 5q−, t(9;22), inv(3) or t(3;3) and t(6;9); (d) risk stratification; (e) karyotype (monosomal karyotype); and (f) molecular genetics variation: internal tandem duplications in the FMS-like tyrosine kinase 3 (FLT3-ITD) mutation, isolated biallelic CCAAT/enhancer-binding protein α (CEBPA) mutation, and nucleophosmin 1 (NPM1) mutation. FAB classification criterion was referred to a report of the FAB cooperative group,[12] and risk stratification was performed according to NCCN Clinical Practice Guidelines in Oncology: Acute Myeloid Leukemia (Version 1.2016).

2.3 | Samples collection and detection

Bone marrow samples of patients with AML were collected before initiation of treatment. Subsequently, the mononuclear cells were separated by density gradient centrifugation, then the expressions of AKIP1, CXCL1, and CXCL2 in mononuclear cells were detected by quantitative polymerase chain reaction (qPCR).

2.4 | Quantitative polymerase chain reaction

Total RNA was firstly extracted from bone marrow mononuclear cells using PureZOL RNA isolation reagent (Bio-Rad) and then reversely transcribed to cDNA using ReverTra Ace qPCR RT Kit (Toyobo) according to the instructions of the manufacturer. Following that, qPCR was performed using KOD SYBR qPCR Mix (Toyobo) to quantify AKIP1, CXCL1, and CXCL2 expressions. And the data were calculated using $2^{-\Delta\Delta Ct}$ method with GAPDH as an internal reference, and the first enrolled AML patient as relative control for calculating. The primers used in RT-qPCR were listed as follows:

AKIP1 primer: forward CCCAACCCTTAGTGCTTCCTTC, reverse CGACTCGCCTCTGTGATAACG; CXCL1 primer: forward TGCTGCTC CTGCTCCTGGTA, reverse AGGATTGAGGCAAGCTTTCC; CXCL2 primer: forward TCACCTCAAGAACATCCAAAGT, reverse AGACA AGCTTTCTGCCCATTC; and GAPDH primer: forward GAGTCCAC TGGCGTCTTCAC, reverse ATCTTGAGGCTGTTGTCATACTTCT. We searched the NCBI database to acquire the CDS sequences of AKIP1/

CXCL1/CXCL2 and designed the primers for AKIP1/CXCL1/CXCL2 via Primer Premier 6.0 (Premier, Canada).

2.5 | Treatment

All the patients with AML received appropriate induction treatments that were based on their physical condition, clinical status, and willingness, according to AML guideline.[13] Complete remission (CR) was assessed for patients with AML after induction treatment, and CR was defined as bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 × 10^9/L (1000/μL), platelet count >100 × 10^9/L (100 000/μL), and independence of red cell transfusions. Then, hematopoietic stem cell transplantation (HSCT) was performed in CR patients with suitable bone marrow donors.

2.6 | Follow-up

Regular follow-up was performed by telephone or clinic visit, the last follow-up date was April 30, 2019, and the median follow-up duration was 17.5 months ranging from 2.0 to 36.0 months. Event-free survival (EFS) was measured from the date of entry into the study to the date of induction treatment failure, or relapse from CR or death, and patients without any of these events were censored on the date they were last examined.[14] Overall survival (OS) was measured from the date of entry into the study to the date of death, and patients not known to have died at last follow-up were censored on the date they were last known to be alive.[14]

2.7 | Statistical analysis

Statistical analysis was performed using SPSS 24.0 software (IBM), and figures were plotted using GraphPad Prism 7.00 software (GraphPad Software). Categorical variables were summarized using frequency and percentage, while continuous variables were displayed as mean ± standard deviation (SD) or median and interquartile range (IQR). Chi-square test was used to determine the correlation between categorical variables, and Spearman's rank correlation test was used to examine the correlation between continuous variables. Kaplan-Meier curve was used to display OS and EFS, and the log-rank test was used to compare the difference of EFS and OS between groups. All tests were two-tailed, and *P* value <.05 was considered significant.

3 | RESULTS

3.1 | Baseline characteristics

There were totally 160 AML patients with the mean age of 45.7 ± 13.3 years included in the present study (Table 1). Among them, 84 (52.5%) were males and 76 (47.5%) were females. The median WBC was 17.5 (8.7-32.2) × 10^9/L. Regarding the FAB classification, there were 1 (0.6%), 58 (36.3%), 43 (26.9%), 45 (28.1%), and 13 (8.1%) patients in M1, M2, M4, M5, and M6, respectively. Other detailed baseline characteristics were listed in Table 1.

TABLE 1 Characteristics of AML patients

Items	AML patients (N = 160)
Age (y), Mean ± SD	45.7 ± 13.3
Gender, No. (%)	
Male	84 (52.5)
Female	76 (47.5)
WBC (×10^9/L), Median (IQR)	17.5 (8.7-32.2)
FAB classification, No. (%)	
M1	1 (0.6)
M2	58 (36.3)
M4	43 (26.9)
M5	45 (28.1)
M6	13 (8.1)
Cytogenetics, No. (%)	
NK	78 (48.8)
CK	21 (13.1)
inv(16) or t(16;16)	10 (6.2)
t(8;21)	7 (4.4)
−7 or 7q−	7 (4.4)
+8	6 (3.8)
11q23	5 (3.1)
t(9;11)	3 (1.9)
−5 or 5q−	1 (0.6)
t(9;22)	1 (0.6)
inv(3) or t(3;3)	1 (0.6)
t(6;9)	1 (0.6)
Others (non-defined)	19 (11.9)
MK, No. (%)	17 (10.6)
FLT3-ITD mutation, No. (%)	37 (23.1)
Isolated biallelic CEBPA mutation, No. (%)	14 (8.8)
NPM1 mutation, No. (%)	53 (33.1)
Risk stratification, No. (%)	
Favorable	39 (24.4)
Intermediate	61 (38.1)
Poor	60 (37.5)

Abbreviations: AML, acute myeloid leukemia; CEBPA, CCAAT/enhancer-binding protein α; and NPM1: nucleophosmin 1; CK, complex karyotype; FAB classification, French-American-Britain classification; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; IQR, interquartile range; MK, monosomal karyotype; NK, normal karyotype; SD, standard deviation; WBC, white blood cell.

3.2 | Correlation of AKIP1 with CXCL1 and CXCL2

AKIP1 relative expression was observed to be positively associated with CXCL1 relative expression (r = 0.409, P < .001; Figure 1A) and CXCL2

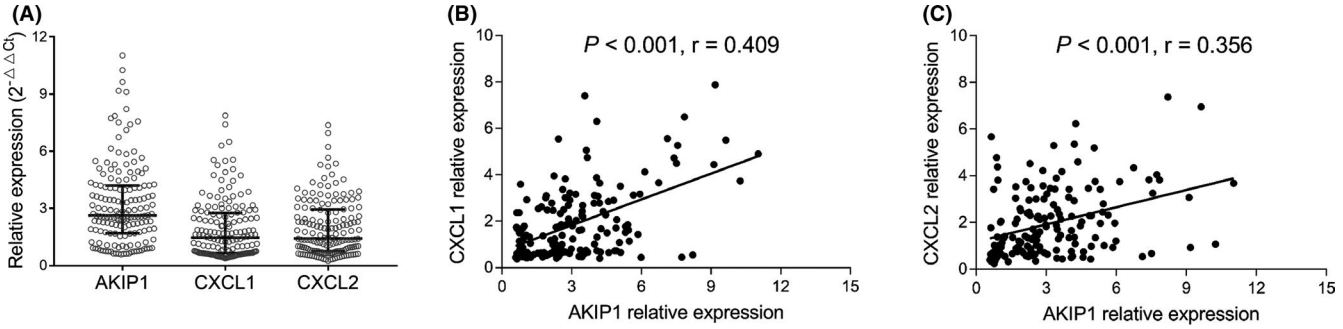

FIGURE 1 Correlation of AKIP1 with CXCL1 and CXCL2. AKIP1/CXCL1/CXCL2 expressions (A). The correlation of AKIP1 relative expression with CXCL1 relative expression (B) and CXCL2 relative expression (C) in AML patients. The correlation between continuous variables was detected by Spearman's rank correlation test. $P < .05$ was considered significant. AKIP1, A-kinase interacting protein 1; AML, acute myeloid leukemia; CXCL1, chemokine (C-X-C motif) ligand 1; and CXCL2, chemokine (C-X-C motif) ligand 2

relative expression ($r = 0.356$, $P < .001$; Figure 1B) in patients with AML. AKIP1/CXCL1/CXCL2 relative expressions were shown in Figure 1C.

3.3 | Correlation of AKIP1, CXCL1, and CXCL2 with clinical characteristics

According to the median level of AKIP1, CXCL1, or CXCL2, these indexes were divided into high and low expressions (Table 2). AKIP1 high expression was correlated with FAB classification ($P = .022$), positively associated with MK ($P = .001$), poor risk stratification ($P = .013$), while there was no correlation between AKIP1 expression with age, gender, WBC, cytogenetics, FLT3-ITD mutation, isolated biallelic CEBPA mutation, or NPM1 mutation (all $P > .05$). As for CXCL1 expression, no association was found between CXCL1 expression with age, gender, WBC, FAB classification, cytogenetics, MK, FLT3-ITD mutation, isolated biallelic CEBPA mutation, NPM1 mutation, or risk stratification (all $P > .05$). With regarding to CXCL2 expression, its high expression was associated with MK ($P = .001$), however, there was no association between CXCL2 expression with age, gender, WBC, FAB classification, cytogenetics, FLT3-ITD mutation, NPM1 mutation, isolated biallelic CEBPS mutation, or risk stratification (all $P > .05$).

3.4 | Correlation of AKIP1, CXCL1, and CXCL2 with CR and allo-HSCT realization

There was no difference of AKIP1 expression ($P = .105$; while a trend of lower AKIP1 expression in CR patients), CXCL1 expression ($P = .418$), or CXCL2 expression ($P = .685$) between AML patients with CR and AML patients with no CR (Table 3). And there was also no difference in AKIP1 expression ($P = .417$), CXCL1 expression ($P = 1.000$), or CXCL2 expression ($P = .258$) between AML patients with allo-HSCT post-CR and AML patients without allo-HSCT post-CR.

3.5 | Correlation of accumulating EFS with AKIP1, CXCL1, and CXCL2

According to the median level of AKIP1, CXCL1, or CXCL2, all AML patients were divided into those with high expression and with low expression. Accumulating EFS was reduced in AML patients with AKIP1 high expression compared with AML patients with AKIP1 low expression ($P < .001$; Figure 2A). Besides, accumulating EFS was decreased in AML patients with CXCL1 high expression compared with AML patients with CXCL1 low expression ($P = .008$) (Figure 2B). However, accumulating EFS was similar in AML patients with CXCL2 high expression and AML patients with CXCL2 low expression ($P = .069$; Figure 2C).

3.6 | Correlation of accumulating OS with AKIP1, CXCL1, and CXCL2

Accumulating OS was reduced in AML patients with AKIP1 high expression compared with those with AKP1 low expression ($P < .001$; Figure 3A). And accumulating OS was decreased in AML patients with CXCL1 high expression compared with those with CXCL1 low expression ($P = .011$; Figure 3B). While accumulating OS was similar between in AML patients with CXCL2 high expression and those with CXCL2 low expression ($P = .055$; Figure 3C).

4 | DISCUSSION

In this present study, we observed that (a) AKIP1 expression was positively associated with CXCL1 and CXCL2 expression, and AKIP1 high expression was correlated with FAB classification, MK as well as poor risk stratification, and CXCL2 high expression was associated with MK in AML patients. (b) AKIP1 and CXCL1 were negatively correlated with accumulating EFS and accumulating OS in AML patients.

Accumulating researches indicate that AKIP1 dysregulation is associated with pathological conditions, and AKIP1 participates in the development and progression of various types of solid tumors.[5-7] For example, clinical experiments in patients with gastric cancer illustrate that AKIP 1 high expression correlates with advanced TNM stage and lymph node metastasis, and mechanistic experiments exhibit that AKIP1 promotes gastric cancer cell proliferation, migration, and invasion by activating slug-induced EMT.[5] Another study displays that increased AKIP1 expression is associated with early

TABLE 2 Correlation of AKIP1, CXCL1, and CXCL2 expression with clinical characteristics of patients

	AKIP1 expression			CXCL1 expression			CXCL2 expression		
Items	**High**	**Low**	***P* value**	**High**	**Low**	***P* value**	**High**	**Low**	***P* value**
Age, No. (%)									
<45 y	40 (51.9)	37 (48.1)	0.635	41 (53.2)	36 (46.8)	0.429	44 (57.1)	33 (42.9)	0.082
≥45 y	40 (48.2)	43 (51.8)		39 (47.0)	44 (53.0)		36 (43.4)	47 (56.6)	
Gender, No. (%)									
Male	42 (50.0)	42 (50.0)	1.000	42 (50.0)	42 (50.0)	1.000	44 (52.4)	40 (47.6)	0.527
Female	38 (50.0)	38 (50.0)		38 (50.0)	38 (50.0)		36 (47.4)	40 (52.6)	
WBC, No. (%)									
$<10 \times 10^9$/L	21 (45.7)	25 (54.3)	0.485	23 (50.0)	23 (50.0)	1.000	21 (45.7)	25 (54.3)	0.485
$\geq 10 \times 10^9$/L	59 (51.8)	55 (48.2)		57 (50.0)	57 (50.0)		59 (51.8)	55 (48.2)	
FAB classification, No. (%)									
M1	0 (0.0)	1 (100.0)	0.022	1 (100.0)	0 (0.0)	0.078	0 (0.0)	1 (100.0)	0.850
M2	20 (34.5)	38 (65.5)		22 (37.9)	36 (62.1)		28 (48.3)	30 (51.7)	
M4	28 (65.1)	15 (34.9)		28 (65.1)	15 (34.9)		21 (48.8)	22 (51.2)	
M5	24 (53.3)	21 (46.7)		22 (48.9)	23 (51.1)		24 (53.3)	21 (46.7)	
M6	8 (61.5)	5 (38.5)		7 (53.8)	6 (46.2)		7 (53.8)	6 (46.2)	
Cytogenetics, No. (%)									
inv(16) or t(16;16)	6 (60.0)	4 (40.0)	0.121	3 (30.0)	7 (70.0)	0.552	6 (60.0)	4 (40.0)	0.064
t(8;21)	0 (0.0)	7 (100.0)		3 (42.9)	4 (57.1)		1 (14.3)	6 (85.7)	
+8	3 (50.0)	3 (50.0)		3 (50.0)	3 (50.0)		4 (66.7)	2 (33.3)	
t(9;11)	1 (33.3)	2 (66.7)		2 (66.7)	1 (33.3)		1 (33.3)	2 (66.7)	
NK	37 (47.4)	41 (52.6)		41 (52.6)	37 (47.4)		37 (47.4)	41 (52.6)	
CK	14 (66.7)	7 (33.3)		10 (47.6)	11 (52.4)		12 (57.1)	9 (42.9)	
−7 or 7q−	5 (71.4)	2 (28.6)		2 (28.6)	5 (71.4)		2 (28.6)	5 (71.4)	
11q23	1 (20.0)	4 (80.0)		1 (20.0)	4 (80.0)		0 (0.0)	5 (100.0)	
−5 or 5q−	1 (100.0)	0 (0.0)		1 (100.0)	0 (0.0)		1 (100.0)	0 (0.0)	
t(9;22)	0 (0.0)	1 (100.0)		1 (100.0)	0 (0.0)		1 (100.0)	0 (0.0)	
inv(3) or t(3;3)	1 (100.0)	0 (0.0)		1 (100.0)	0 (0.0)		1 (100.0)	0 (0.0)	
t(6;9)	0 (0.0)	1 (100.0)		0 (0.0)	1 (100.0)		0 (0.0)	1 (100.0)	
Others (non-defined)	11 (57.9)	8 (42.1)		12 (63.2)	7 (36.8)		14 (73.7)	5 (26.3)	
MK, No. (%)									
No	65 (45.5)	78 (54.5)	0.001	12 (70.6)	5 (29.4)	0.073	65 (45.5)	78 (54.5)	0.001
Yes	15 (88.2)	2 (11.8)		68 (47.6)	75 (52.4)		15 (88.2)	2 (11.8)	
FLT3-ITD mutation, No. (%)									
No	58 (47.2)	65 (52.8)	0.189	60 (48.8)	63 (51.2)	0.574	59 (48.0)	64 (52.0)	0.348
Yes	22 (59.5)	15 (40.5)		20 (54.1)	17 (45.9)		21 (56.8)	16 (43.2)	
Isolated biallelic CEBPA mutation, No. (%)									
No	72 (49.3)	74 (50.7)	0.576	73 (50.0)	73 (50.0)	1.000	71 (48.6)	75 (51.4)	0.263
Yes	8 (57.1)	6 (42.9)		7 (50.0)	7 (50.0)		9 (64.3)	5 (35.7)	
NPM1 mutation, No. (%)									
No	54 (50.5)	53 (49.5)	0.867	54 (50.5)	53 (49.5)	0.867	54 (50.4)	53 (49.5)	0.867
Yes	26 (49.1)	27 (50.9)		26 (49.1)	27 (50.9)		26 (49.1)	27 (50.9)	
Risk stratification, No. (%)									
Favorable	13 (33.3)	26 (66.7)	0.013	15 (38.5)	24 (61.5)	0.252	16 (41.0)	23 (59.0)	0.420

(Continues)

TABLE 2 (Continued)

Items	AKIP1 expression			CXCL1 expression			CXCL2 expression		
	High	Low	*P* value	High	Low	*P* value	High	Low	*P* value
Intermediate	29 (47.5)	32 (52.5)		33 (54.1)	28 (45.9)		33 (54.1)	28 (45.9)	
Poor	38 (63.3)	22 (36.7)		32 (53.3)	28 (46.7)		31 (51.7)	29 (48.3)	

Note: Correlation was determined by Chi-square test.

Abbreviations: AKIP1, A-kinase interacting protein 1; CEBPA, CCAAT/enhancer-binding protein α; and NPM1: nucleophosmin 1; CK, complex karyotype; CXCL1, chemokine (C-X-C motif) ligand 1; CXCL2, chemokine (C-X-C motif) ligand 2; FAB classification, French-American-Britain classification; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; MK, monosomal karyotype; NK: normal karyotype; WBC, white blood cell.

TABLE 3 Correlation of AKIP1, CXCL1, and CXCL2 expression with CR and allo-HSCT post-CR

Items	AKIP1 expression			CXCL1 expression			CXCL2 expression		
	High	Low	*P* value	High	Low	*P* value	High	Low	*P* value
CR, No. (%)									
No	19 (63.3)	11 (36.7)	0.105	17 (56.7)	13 (43.3)	0.418	14 (46.7)	16 (53.3)	0.685
Yes	61 (46.9)	69 (53.1)		63 (48.5)	67 (51.5)		66 (50.8)	64 (49.2)	
Allo-HSCT post-CR, No. (%)									
No	53 (45.7)	63 (54.3)	0.417	58 (50.0)	58 (50.0)	1.000	56 (48.3)	60 (51.7)	0.258
Yes	8 (57.1)	6 (42.9)		7 (50.0)	7 (50.0)		9 (64.3)	5 (35.7)	

Note: Correlation was determined by Chi-square test.

Abbreviations: AKIP1, A-kinase interacting protein 1; allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR, complete response; CXCL1, chemokine (C-X-C motif) ligand 1; CXCL2, chemokine (C-X-C motif) ligand 2.

recurrence and AKIP1 is a potential mediator of tumor metastasis via regulating Wnt/β-catenin signaling in hepatocellular carcinoma.[7] Furthermore, Wnt/β-catenin signaling, as an oncogenic signaling in both solid tumors and hematologic malignancies, is reported to be associated with the AML-related fusion proteins and FLT3-1TD mutation, which correlated with unfavorable survival profiles in AML patients.[15] Nevertheless, the role of AKIP1 in hematologic malignancies has been rarely investigated. In addition, recent studies illustrate that CXCL1 and CXCL2 are activated by AKIP1 during the oncogenic development, and CXCL1/CXCL2 regulate AML cell migration during the leukemogenesis.[8,9,16] Accordingly, we hypothesized that AKIP1 might be correlated with clinical outcomes via reaction with CXCL1/CXCL2 in AML patients. In the present study, we found that AKIP1 expression was positively associated with both CXCL1 and CXCL2 expressions, and AKIP1 high expression was correlated with FAB classification, MK as well as poor risk stratification, and CXCL2 high expression was associated with MK in AML patients. The possible reasons might include that (a) increased AKIP1 expression might

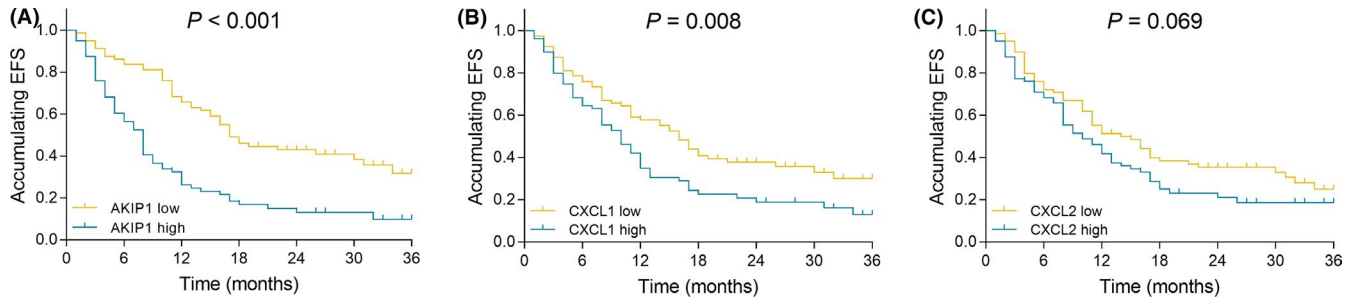

FIGURE 2 Comparison of accumulating EFS between AML patients with high expression and low expression of AKIP1/CXCL1/CXCL2. Comparison of accumulating EFS between AML patients with high expression of AKIP1 and those with low expression of AKIP1 (A). Comparison of accumulating EFS between AML patients with high expression of CXCL1 and those with low expression of CXCL1 (B). Comparison of accumulating EFS between AML patients with high expression of CXCL2 and those with low expression of CXCL2 (C). The survivals for AML patients were exhibited by Kaplan-Meier curve and the comparisons of survival between patients with AKIP1/CXCL1/CXCL2 high expression and low expression were performed by log-rank test. $P < .05$ was considered significant. AKIP1, A-kinase interacting protein 1; AML, acute myeloid leukemia; CXCL1, chemokine (C-X-C motif) ligand 1; CXCL2, chemokine (C-X-C motif) ligand 2; and EFS, event-free survival

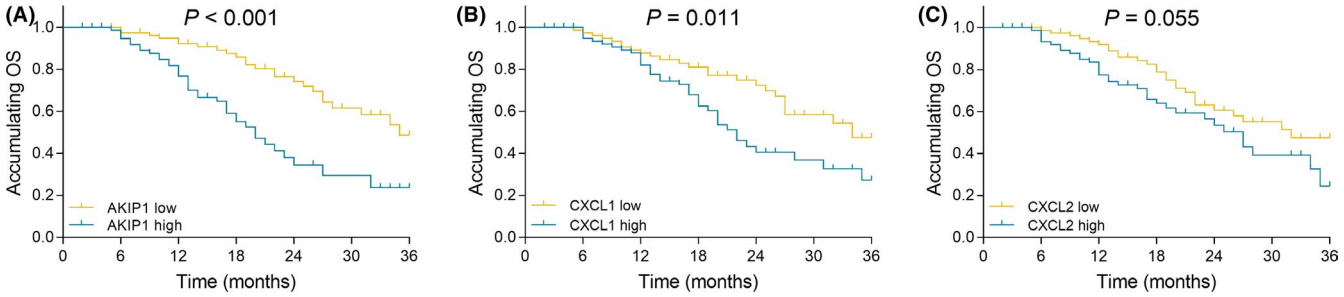

FIGURE 3 Comparison of accumulating OS between AML patients with high expression of AKIP1/CXCL1/CXCL2 and those with low expression of AKIP1/CXCL1/CXCL2. Comparison of accumulating OS between AML patients with high expression of AKIP1 and those with low expression of AKIP1 (A). Comparison of accumulating OS between AML patients with high expression of CXCL1 and those with low expression of CXCL1 (B). Comparison of accumulating OS between AML patients with high expression of CXCL2 and those with low expression of CXCL2 (C). The survivals for AML patients were exhibited by Kaplan-Meier curve and the comparisons of survival between patients with AKIP1/CXCL1/CXCL2 high expression and low expression were performed by log-rank test. $P < .05$ was considered significant. AKIP1, A-kinase interacting protein 1; AML, acute myeloid leukemia; CXCL1, chemokine (C-X-C motif) ligand 1; CXCL2, chemokine (C-X-C motif) ligand 2; and OS, overall survival

enhance the level of CXCL1/CXCL2, which promoted AML cell proliferation and migration, further contributing to the progression of AML and the chromosome abnormality. (b) Upregulated AKIP1 might activate the oncogenic CXCL1/CXCL2 downstream signaling pathway (Wnt/β-catenin signaling), leading to increased AML-related genes mutations, which further contributed to poor risk stratification. Conversely, overexpression of CXCL1/CXCL2 might also activate the oncogenic pathway, which increased the expression of AKIP1, while the underlying mechanism needed further exploration via cellular experiments.

Recent studies reveal that AKIP1 has potential to be a prognostic marker in several solid cancers.[6,17,18] For example, AKIP1 serves as a mediator of tumor metastasis, and its high expression is correlated with unfavorable prognosis in patients with non-small cell lung cancer.[18] In another study AKIP1 is an independent predictive factor for decreased accumulating OS in patients with colorectal cancer.[6] As for CXCL1/CXCL2 are found to be of prognostic value in various cancers including hematologic malignancies, and their involvements in leukemia have been illustrated by several studies.[9,19] CXCL1 downregulation is correlated with increased cell apoptosis and inhibited cell proliferation in AML, and CXCL2 overexpression is exhibited to enhance chronic lymphocytic leukemia cell survival.[9,10,19] Given the previous studies, we speculated that AKIP1/CXCL1/CXCL2 might associate with unfavorable prognosis in patients with AML. In the study, AKIP1 and CXCL1 were negatively correlated with accumulating EFS and accumulating OS in AML patients. The possible explanations might consist of that (a) based on our previous data, AKIP1 high expression was correlated with unfavorable cytogenetic change (such as: MK occurrence) and poor risk stratification, which indirectly led to poor prognosis.[20,21] (b) AKIP1 high expression might activate CXCL1/CXCL2 downstream oncogenic signaling pathway (such as: Wnt/β-catenin signaling), which further promoted the self-renewal of AML stem cell and enhanced the resistance to the chemotherapy; therefore, patient with AKIP1 high expression had poor survival profiles in a long-term period.

However, there were still several limitations in our study as follows: (a) The follow-up duration was relatively short; thus, the long-term prognostic value of AKIP1/CXCL1/CXCL2 needed to be observed in a longer follow-up. (b) Considering that the underlying molecular mechanism was not evaluated in the present study, further cellular experiments were needed in the future. (c) The sample size was relatively small; thus, more patients from multiple regions throughout China were needed for validation. (d) Although AKIP1 expression was found to be positively associated with CXCL1/CXCL2 expression, the detailed regulatory mechanism of AKIP1/ CXCL1/ CXCL2 signaling needed further cellular experiments for exploration.

In conclusion, AKIP1 might serve as a novel biomarker for worse AML prognosis through the interaction of CXCL1/CXCL2.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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