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Expression level and prognostic potential of beta-catenin–interacting protein in acute myeloid leukemia

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Abstract

Inhibitor of beta-catenin and TCF (*ICAT*) is a key protein in the Wnt-β-catenin signaling pathway. However, its role in acute myeloid leukemia (AML) remains unknown. In this study, we evaluated its expression level as well as its prognostic value in AML patients. A total of 72 patients with AML and 30 control subjects were enrolled in this study during the period of January 2017 and December 2019 at Zhongshan Hospital of SunYat-sen University. *ICAT* and *β-catenin* expression levels in peripheral blood were determined via enzyme-linked immunosorbent assays. *ICAT* levels in AML patients were significantly lower and *β-catenin* levels were higher than those of the control group. After the first course of standard chemotherapy, the concentration of *ICAT* in the partial remission group (93.79 ng/mL) was significantly higher than that in the initial diagnosis group (49.38 ng/mL) and the no response group (39.94 ng/mL). AML subtypes had lower *ICAT* expression levels than controls, and *ICAT* levels were significantly correlated with body mass index, bone marrow/peripheral blood blast cell proportions, and white blood cell and red blood cell counts at initial diagnosis. Furthermore, low *ICAT* expression was found to be associated with poor disease-free survival and overall survival in AML. *ICAT* is closely associated with AML progression and can be used as an indicator to monitor AML treatment efficacy.

Abbreviations: AML = acute myeloid leukemia, ATRA = all-trans retinoic acid, BM = bone marrow, BMI = body mass index, CEBPA = CCAAT enhancer binding protein alpha, CR = complete remission, DFS = disease-free survival, DNMT3A = DNA methyltransferase 3A, ELISA = enzyme-linked immunosorbent assay, EZH2 = histone methyltransferase enhancer of zeste homolog 2, FAB = French-American-British, FLT3-ITD = Fms-like tyrosine kinase 3 internal tandem duplication mutations, Hb = hemoglobin, ICAT = inhibitor of beta-catenin and TCF, IDH2 = isocitrate dehydrogenase isoform 2, LDH = lactate dehydrogenase, LEF = lymphocyte enhancement factor, NPM1 = Nucleophosmin, NR = no response, NRAS = neuroblastoma Ras, OS = overall survival, PB = peripheral blood, PLT = platelet, PR = partial remission, RBC = red blood cell, RUNX1 = Runt-related transcription factor 1, TA = pirarubicin and cytarabine, TCF = T-cell factor, TET2 = Tet methylcytosine dioxygenase 2, TP53 = tumor protein p53, WBC = white blood cell, WHO = World Health Organization, WT1 = Wilms tumor 1.

Keywords: acute myeloid leukemia, beta-catenin, interacting protein

1. Introduction

Acute myeloid leukemia (AML), with a high incidence, is a type of hematological malignancy with clinical and biological diversity.[1] It is characterized by rapid onset, severe clinical symptoms, and rapid progression,[2] it can be diagnosed at any age. However, the disease is relatively uncommon before 45 years, and the patient survival rate is <25% after a confirmed diagnosis for >5 years.[3] Due to the biological heterogeneity associated with cytogenetics, epigenetics, and transcriptional diversity,[1,4] risk stratification and targeted therapy for AML are challenging.[5] To date, karyotypic analysis remains the most critical indicator for AML classification. However, many patients lack the associated clonal chromosome aberrations and molecular markers. A variety of acquired gene changes, such as nucleophosmin (*NPM1*), Fms-like tyrosine kinase 3 (*FLT3*), DNA methyltransferase 3A (*DNMT3A*), and CCAAT enhancer binding protein alpha (*CEBPA*) mutations, lead to significant differences in prognosis with AML patients. Based on the World Health Organization classification of AML,[6] *NPM1* mutations in cytogenetically normal patients are associated with a favorable outcome, while Runt-related transcription factor 1 (*RUNX1*) mutations are associated with poor overall survival in AML patients. The combination of chemotherapy and targeted drug

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therapy is reported to prolong survival for patients with AML. However, due to the significant heterogeneity of the disease, patients with similar stages of the disease and under the same clinical treatment plan often have different clinical outcomes. In particular, some patients develop multiple drug resistance, leading to relapse.[7] The identification of reliable markers in AML patients may provide more evidence for clinical treatment and disease monitoring.

In our previous study, HL60 cells were stimulated with all-trans retinoic acid and methanesulfonate sterol (NSC67657) for comparative proteomics. We screened differentially expressed proteins before and after cell differentiation. Thus, we observed that catenin, beta-interacting protein 1 (inhibitor of beta-catenin and TCF [*ICAT*] or *CTNNBIP1*), an inhibitor of *β-catenin* is upregulated during monocyte differentiation, can enhance drug sensitivity of HL60 cells.[8] The *ICAT* molecule plays a crucial role in the *Wnt/β-catenin* signaling pathway, and in a competitive region with T-cell factor (*TCF*)/lymphocyte enhancement factor (*LEF*), binds to the tandem protein and E-cadherin and inhibits *Wnt* signal transduction as well as intracellular adhesion,[9,10] which plays a critical role in tumorigenesis. Therefore, it has been observed in several studies that *ICAT* acts as a caner suppressor.[11–13] Specifically, Hu et al[10] found that *ICAT* inhibited colorectal cancer cell proliferation and prevented their translocation. However, several conflicting results have also been reported. For example, some studies have shown that *ICAT* promotes cancer invasion and metastasis in some cancers.[14,15] Based on the above studies, *ICAT* may play a different role in different types of cancer. In addition, previous studies on the role of *ICAT* in patients with AML are limited; thus, its role in human AML is unclear. Therefore, in this study, our objective was to determine the significance of *ICAT* and *β-catenin* in patients with AML who received chemotherapy, while focusing on morphological classification. Furthermore, we investigated the correlation between *ICAT* and *β-catenin* levels and the clinical characteristics and prognosis of the disease. The levels of *ICAT* and *β-catenin* in peripheral serum samples from patients with AML were determined using enzyme-linked immunosorbent assays (ELISAs).

2. Materials and Methods

2.1. Sample collection

Seventy-two patients with AML (42 males and 30 females; ages range, 18 to 86 years; mean age, 46 years) were recruited at Zhongshan Hospital of SunYat-sen University (Guangzhou, China) from January 2017 to December 2019. Inclusion criteria were as follows: all patients were newly diagnosed according to the French-American-British classification, which classifies AML as M0–M7 based on cell type, degree of differentiation, and cytochemical staining, as well as the World Health Organization blood tumor classification standard.[16] The morphology of bone marrow (BM) cells (under a microscope, Wright-Giemsa stain, Zhuhai BaSO Biotechnology Co., Ltd, Zhuhai, China), cellular immunology (flow cytometer assay, MPO, CD13, CD33, CD34, CD19, CD64, CD65, and CD117 monoclonal antibodies, Beckman Coulter, Inc, USA), cytochemical staining (the smear staining method, peroxidase and periodic acid-Schiff staining, Zhuhai BaSO Biotechnology Co., Ltd, ZhuHai, China), cytogenetics, and leukemia fusion gene detection (real-time quantitative reaction) were performed on all samples. Patients with hepatic and renal insufficiency, malnutrition, and diabetes were excluded. Additionally, patients who could not tolerate chemotherapy drugs were also excluded. We recruited 30 patients with nonmalignant hematological diseases (20 cases of proliferative anemia, 10 cases of idiopathic thrombocytopenic purpura) without damage to the heart, liver, lungs, kidneys, or other important organs to form the control group. A total of 16 males and 14 females were involved, with an average age of 41.69 years (20–62 years). Moreover, these patients in the control group were not on any treatment when they were initially diagnosed, and their serum samples were taken before treatment (see Figure S1, Supplemental Digital Content, http://links.lww.com/MD/G997, which illustrates the flowchart for collecting samples). Informed consent was obtained from all the participants following the Declaration of Helsinki. The study was also approved by the Ethics Board of the affiliated Zhongshan Hospital of Sun Yat-Sen University.

2.2. Detection of ICAT and β-catenin

Patients' serum samples were collected before and after treatment and stored at temperatures – 70°C. The same batch of ELISA (Shanghai Jianglai Biotechnology Co., Ltd, Shanghai, China) was used to detect the levels of *ICAT* and *β-catenin* proteins in peripheral blood (PB). Before analysis, the serum samples and kit were equilibrated at room temperature (25°C) for 60 minutes (min), and standard wells and sample wells were set. After that, the assay procedure was carried out according to manufacturer's instructions. Western blotting was used to analyze ICAT protein. The total proteins of AML samples were extracted using serum protein extraction kit (Beyotime Biotechnology Co., Ltd, Beijing, China). Protein concentration was determined by bicinchoninic acid method according to the manufacturer's protocol (KeyGEN Biotech, China).The PVDF membranes were incubated with the ICAT antibodies (Abcam, England) at 4°C overnight, and membranes were probed with goat anti-rabbit IgG (H + L) (Cell Signaling Tech, USA), then developed by ECL substrate (Pierce, USA).

2.3. Criteria for evaluation of prognosis

AML patients received a standard dose of chemotherapy. Specifically, newly diagnosed AML cases were considered the initial group. For patients with AML, the remission induction protocol was: non-M3 patients (i.e., patients with AML subtypes M1, M2, M4, M5, M6, and M7) were treated with a standard dose of daunorubicin and cytarabine (Actavis Italy S.p.A, Inc., Italy; daunorubicin and cytarabine, 44 and 200 mg/m^2, respectively; mode, intravenous continuous infusion; period, days 1–7), demethoxydaunorubicin and cytarabine (IA) (Zhejiang Hisun Pharmacentical. Co., LTD, China; 8 mg/m^2; mode, intravenous continuous infusion; period, days 1–7), pirarubicin and cytarabine (Zhejiang Hisun Pharmacentical Co., Ltd, China;35 mg/m2; mode, intravenous continuous infusion; period, days 1–7), homoharringtonine and cytarabine (Shanghai Xinyi Pharmaceutical Co., Ltd, China; 4 mg/D; mode, intravenous continuous infusion, days 1–6), while patients with subtype M3 were treated with all-trans retinoic acid (Shandong Liangfu Pharmaceutical Co., Ltd, China; 10 mg each time, 2–3 times a day) or arsenic trioxide (Beijing SL Pharmaceutical Co., Ltd, China; 10 mg/D; mode, intravenous continuous infusion; period weeks 1–4) to induce differentiation. After 1 course of treatment, the prognosis of all the patients was determined according to the *Diagnostic and Therapeutic Criteria of Hematologic Diseases*[17] as complete remission (CR), partial remission (PR), and no response (NR). The patients were also followed up after 1 course of treatment and thereafter blood samples were collected for ELISA analysis. In addition, clinical data were collected including the number of BM blast cells (manual counting 200 nucleated cells), the number of white blood cells (WBCs) (laser flow cytometry, Sysmex XN-9000, Japan) in PB, platelets (PLTs) (electronic resistance method, Sysmex XN-9000, Japan), hemoglobin (Hb) concentration (sodium dodecyl lauryl sulfate colorimetric method), and lactate dehydrogenase (LDH) levels

(lactic acid method) from our hospital's computerized test system (Nanfang Huiqiao).

2.4. Detection of AML fusion transcripts and gene mutations

Real-time quantitative reverse transcription-polymerase chain reaction was performed on BM samples to detect AML fusion transcripts and gene mutation sites (including *TEL/AML1*, *AML1/ETO*, *PML/RARα, CBFβ/MYH11*, *FLT3-ITD, NPM1, CEBPA, DNMT3A, NRAS, TET2, RUNX1, IDH2, EZH2, TP53, WT1*, etc). Specifically, the polymerase chain reaction tests were performed at the clinical laboratory of Guangzhou Kingmed Center (Guangzhou, China).

2.5. Follow-up

Patient follow-up was done via phone or through consultation of medical records. The follow-up deadline for this study was May 2020, and the median follow-up time of 14 (6–30) months. Additionally, overall survival (OS) was defined as the time from the date of diagnosis to the date of death for any reason or the final follow-up and disease-free survival (DFS) as the time from CR to recurrence.

2.6. Statistical analysis

GraphPad Prism 8.0 software version (GraphPad Software, San Diego, CA) and SPSS software, version 21.0.0 (International Business Machine, Armonk, NY) were used for statistical analysis. We analyzed qualitative data by using the chi-square test, while quantitative data were expressed as the median (range). The independent samples *t* test was used for data analysis if the data followed a normal distribution. The Mann–Whitney *U* test was used if the data did not follow a normal distribution. Furthermore, Spearman correlation analysis was performed to determine the degree of correlation between different variables, and paired nonparametric analysis was conducted to compare data corresponding to the initial diagnosis with those obtained after treatment. After stratifying by each characteristic, hierarchical proportional hazards, regression analysis was used to estimate the adjusted hazard ratio and 95% confidence interval with OS and DFS. The Kaplan–Meier method was used to generate the survival curve, and the log-rank method was used for testing. The Cox risk model was used for multivariate analysis. Statistical significance was set at a P value of <.05. There were no statistically significant differences in sex or age between the 2 experimental groups ($P > .05$).

3. Results

3.1. Differential levels of ICAT and β-catenin in AML and control subjects

ELISA tests were performed to detect *ICAT* and *β-catenin* protein levels in 72 patients with initial AML and 30 patients with nonmalignant blood disease (control group). It was found that *ICAT* levels in the AML group were lower than those in the control group ($P < .001$; **Fig. 1A**), but *β-catenin* levels were higher ($P < .05$; **Fig. 1B**). After the first course of standard chemotherapy, 39 AML patients showed CR, 15 patients showed PR, while 18 patients showed NR. ICAT levels in the CR and PR groups were higher than those in the initial diagnosis and NR groups ($P < .01$; **Fig. 1C**). The similar results were obtained by western blotting (see Figure S2, Supplemental Digital Content, http://links.lww.com/MD/G998, which illustrates western blotting analysis of ICAT levels). However, for all the different groups, the levels of β-catenin before and after treatment showed no significant difference (all $P > .05$; **Fig. 1D**).

3.2. Correlation between ICAT and β-catenin levels and the clinical characteristics of patients with AML

The correlation between the levels of *ICAT* and *β-catenin* with clinical characteristics of 72 AML is illustrated in **Table 1**. Spearman correlation analysis showed positive correlation between ICAT and body mass index, but it showed negative correlation with BM, PB blast cells, WBCs, and red blood cells at initial diagnosis of AML (all $P < .05$). However, *ICAT* levels showed no correlation with age, gender, Hb, PLT, LDH, or existence of mutation sites at initial diagnosis (all $P > .05$). Furthermore, *β-catenin* levels showed significant positive correlation with the proportion of blast cells in PB as well as LDH levels but negatively correlated with red blood cells count. All other markers were nonsignificant (all $P > .05$).

3.3. French-American-British classification and expression of ICAT and β-catenin expression in different AML subtypes

The expression levels of *ICAT* and *β-catenin* were determined by ELISA. Thus, a significant decrease in the expression levels of *ICAT* was observed in all the AML subtypes compared to the control group ($P < .05$). AML subtypes M4 and M5 as well as unclassified leukemia showed higher levels of *β-catenin* expression than the control group. While no significant difference was observed between M1, M2, and M3 subtypes of AML (**Table 2**).

3.4. Association between ICAT level and molecular biomarkers

Risk stratification was performed based on cytogenetic abnormality and molecular mutations. Our hospital's computerized test system (Nanfang Huiqiao) provided the data for risk stratification.

We categorized 72 patients with AML into favorable, intermediate, and poor groups and found that the concentration of *ICAT* in the favorable group was significantly higher than that in the intermediate and poor groups (**Fig. 2**). Furthermore, the level of *ICAT* also significantly decreased along with increasing cytogenetic risk. Regarding other possible prognostic markers (see Table S1, Supplemental Digital Content, http://links.lww.com/MD/G999, which illustrates the molecular markers being detected), there were no significant differences observed between low- and high-level *ICAT* groups ($P > .05$).

3.5. Correlation between ICAT level and AML prognosis

The Kaplan–Meier method was used to construct survival curves for patients with AML, stratified by *ICAT* expression levels (high or low). The log-rank survival analysis showed that the DFS and OS of AML patients with high *ICAT* expression were significantly higher than those with low *ICAT* expression.

Patients with AML who had low levels of *ICAT* expression had poor DFS and OS (**Fig. 3**). An univariate analysis was performed to determine the factors (such as age, sex, proportion of BM blast cells, WBCs count at time of diagnosis, levels of Hb, PLT, and LDH, and mutations) that affect the survival of patients with AML. Factors associated with values of $P < .5$ were included in the multivariate analysis. Thus, *ICAT* was identified as an independent factor that affects DFS as shown in Table 3.

4. Discussion

The advancements in cell morphological, immunological, and cytogenetic techniques have greatly improved the molecular and clinical diagnosis and typing of AML.[18,19] In addition, BM

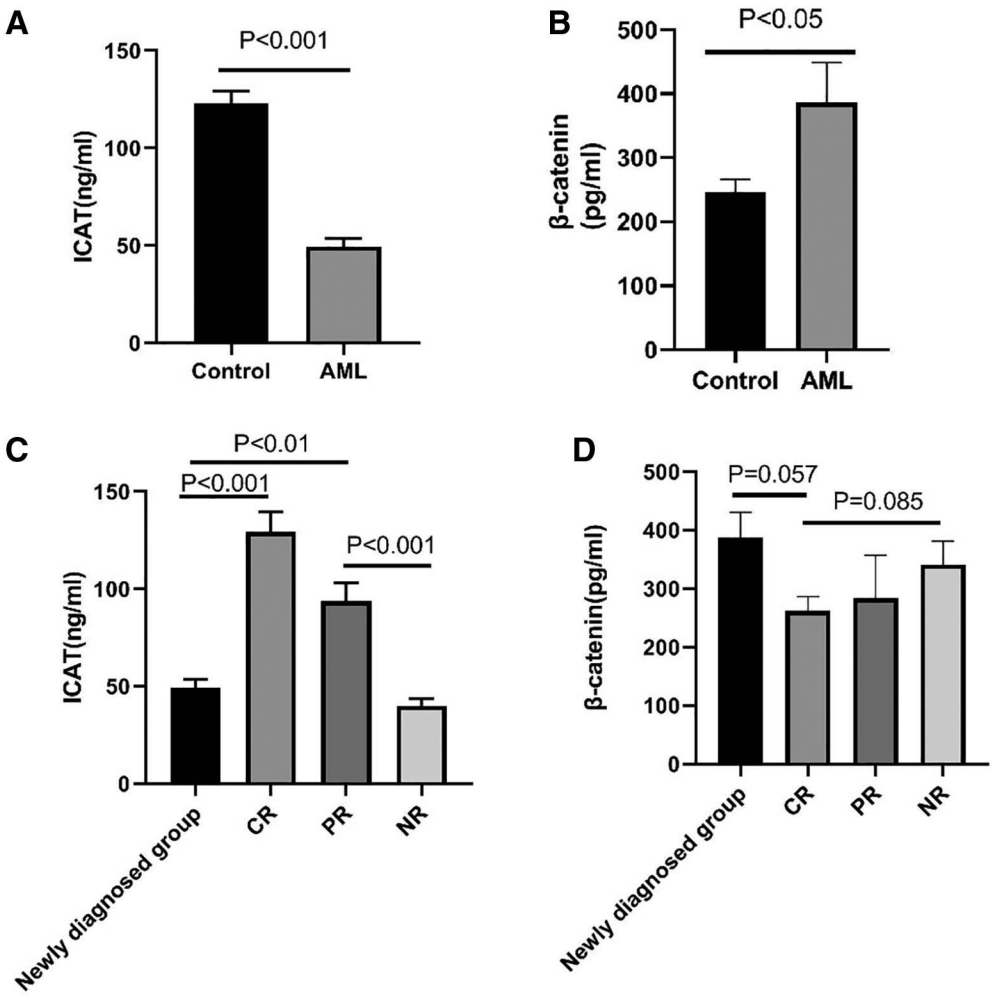

Figure 1. The concentration of ICAT and β-catenin in control and acute myeloid leukemia groups. (A) The level of ICAT in the control group (123.9 ± 34.79ng/ml, n = 30) and the AML group at initial diagnosis (49.38 ± 36.49 ng/mL, n = 72). (B) The level of β-catenin in the control group (170.0 ± 77.01 pg/mL, n = 30) and the AML group at initial diagnosis (386.9 ± 370.3 pg/mL, n = 72). (C) The level of ICAT at initial diagnosis and the AML subgroups after the first course of chemotherapy. Newly diagnosed group, 49.38 ± 36.49 ng/mL (n = 72); CR, 129.3 ± 63.83 ng/mL (n = 39); PR, 93.79 ± 36.86 ng/mL (n = 15); NR, 39.94 ± 16.26 ng/mL (n = 18). (D) The level of β-catenin at initial diagnosis and the AML subgroups after the first course of chemotherapy. Newly diagnosed group, 386.9 ± 370.3 pg/mL (n = 72); CR, 262.3 ± 151.7 pg/mL (n = 39); PR, 283.4 ± 271.9 pg/mL (n = 15); NR, 341.2 ± 170.3 pg/mL (n = 18). AML = acute myeloid leukemia, CR = complete remission, ICAT = Inhibitor of beta-catenin and TCF, NR = no response, PR = partial remission.

Table 1

Correlation of ICAT and β-catenin levels with clinical parameters in acute myeloid leukemia patients.

		ICAT		β-catenin	
Clinical parameter	**Median (range)**	***r***	***P* value**	***r***	***P* value**
Sex	M/F	–0.149	.380	–0.108	.524
Age (yr)	46 (18–86)	0.013	.939	–0.005	.978
BMI (kg/m^2)	23.1 (15.7–31.96)	0.442	.008**	–0.311	.069
Blasts in BM (%)	58 (17–93)	–0.345	.003**	–0.019	.871
Blasts in PB (%)	48 (1–94)	–0.325	.009**	0.403	.000**
WBC count (×10^9/L)	8.3 (0.99–389)	–0.244	.039	–0.037	.759
RBC count (×10^{12}/L)	2.51 (1.46–5.10)	–0.287	.015*	–0.257	.029*
Hb (g/L)	84 (45–154)	–0.015	.199	–0.194	.102
PLT (×10^{12}/L)	46 (3–239)	–0.155	.193	–0.050	.676
LDH (U/L)	352 (134–1858)	0.217	.067	0.488	.000**
Mutant	Y/N	0.001	.991	0.110	.361

Characteristics of the 72 AML patients before therapy used in this study.

AML = acute myeloid leukemia, BM = bone marrow, BMI = body mass index, Hb = hemoglobin, ICAT = inhibitor of beta-catenin and TCF, LDH = lactate dehydrogenase, M/F = Male/Female, PB = peripheral blood, PLT = platelet, RBC = red blood cell, WBC = white blood cell, Y/N = yes/no.

**$P < .01$.

*$P < .05$.

Table 2

The levels of ICAT and β-catenin in acute myeloid leukemia and control groups.

Grouptyping	N	Average (range) ICAT (ng/mL)	Average (range) β-catenin (pg/mL)
AML (n = 72)			
M1	4	65.43 (12.39–94.76)*	345.56 (210.12–678.90)
M2	23	53.36 (17.41–176.12)*	342.92 (242.79–1378.21)
M3	18	50.34 (9.39–81.47) *	339.19 (32.49–1452.1)
M4	12	45.21 (18.78–74.13)*	491.15 (162.09–856.02)*
M5	6	33.87 (15.41–78.21)*	516.87 (77.79–783.52)*
No-known type	9	59.72 (15.95–124.45)*	420.79 (103.04–856.02)*
Control (n = 30)	30	123.89 (73.94–205.41)	254.60 (76.38–321.18)

Characteristics of the 72 AML patients before therapy, 30 control individuals used in this study. AML = acute myeloid leukemia, ICAT = Inhibitor of beta-Catenin and TCF, M1 = acute myeloid leukemia without maturation, M2 = acute myeloid leukemia with maturation, M3 = acute promyelocytic leukemia, M4 = acute myelomonocytic leukaemia, M5 = acute monoblastic leukemia.

*$P < .05$ vs control group.

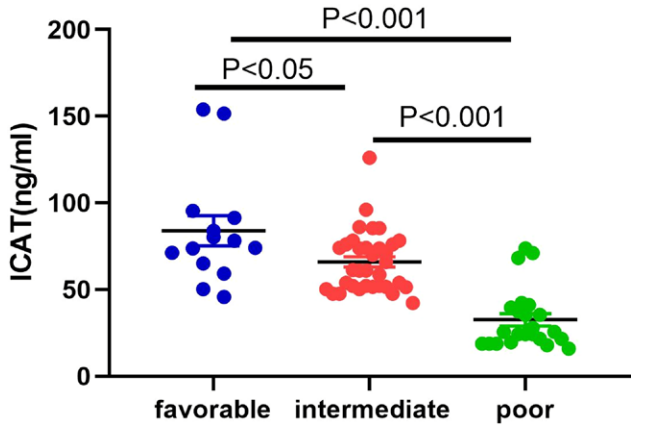

Figure 2. The concentration of ICAT before therapy in favorable, intermediate, and poor groups. ICAT = inhibitor of beta-catenin and TCF.

transplantation and chemotherapy, as well as the use of targeted drugs have increased the survival rate for patients with AML. Despite this, the CR rate among patients with AML is still insufficient; the development of resistance in cells to chemotherapy is also a major obstacle in the treatment of AML.[20] Thus, the identification of reliable biomarkers for real-time monitoring of AML progression is very important.

It is known that *ICAT* regulates the *Wnt/β-catenin* signaling pathway by inhibiting the binding of *β-catenin* to *TCF/LEF*, thus preventing the activation of genes downstream of *Wnt* signaling. According to previous studies,[21] HL60 cells exhibit low levels of ICAT protein expression, which can be significantly upregulated by NSC67657 during the monocyte differentiation of HL60 cells. Furthermore, exogenous injection of ICAT protein can significantly improve HL60 cells' sensitivity to NSC67657. NSC67657 is significantly inhibited if the gene encoding intracellular ICAT is silenced.[21] It has been suggested in a few studies[8] that the expression level of intracellular *ICAT* protein level can interfere with chemotherapy and may represent a tumor suppressor in leukemia cells. Although *ICAT* is a differential protein expressed specifically during monocyte differentiation in HL60 cells and plays an important role in monocyte differentiation,[8] its regulatory role may interfere with granulocyte induction chemotherapy.

In this study, at initial diagnosis, patients with AML had lower *ICAT* levels than individuals in the control. Our results also indicated that patients with mononuclear leukemia and granular leukemia in the AML group had low *ICAT* levels. Moreover, different AML subtypes and the control group showed statistically significant differences in *ICAT* levels. Consistent with our findings, low *ICAT* levels have also been reported in human lung cancer, glioma, and breast cancer.[13,22,23] In this study, the *ICAT* concentration was lowest in the M5 subgroup, which is consistent with previous studies that have shown a decrease in *ICAT* levels in mononuclear cells. *FLT3-ITD* is a constitutively activated variant of the *FLT3* tyrosine kinase receptor. Its expression in AML is associated with a poor prognosis.[24] Notably, our results indicated that patients with low *ICAT* expression levels were more likely to harbor *FLT3- ITD, NPM1, CEBPA*, and *DNMT3A* mutations than those with high *ICAT* expression levels. Conversely, *TP53* mutations were rare in the low ICAT expression group. These results indirectly indicated that there might be a pathogenic relationship between these mutations and *ICAT* expression, as shown by a previous study.[25] However, there was no statistically significant difference in the expression level of *ICAT* in low and high groups. A larger sample size is needed for further verification. It is well known that patients with AML in the favorable-risk group show good survival, and previous studies have also demonstrated the prognostic value of the classification of patients based on cytogenetic abnormalities.[26] Similarly, we found that *ICAT* decreased significantly with increasing cytogenetic risk. In the favorable group, the concentration of *ICAT* was significantly higher than that in the intermediate and poor groups. These findings suggested that low *ICAT* expression was associated with a poor prognosis of acute leukemia.

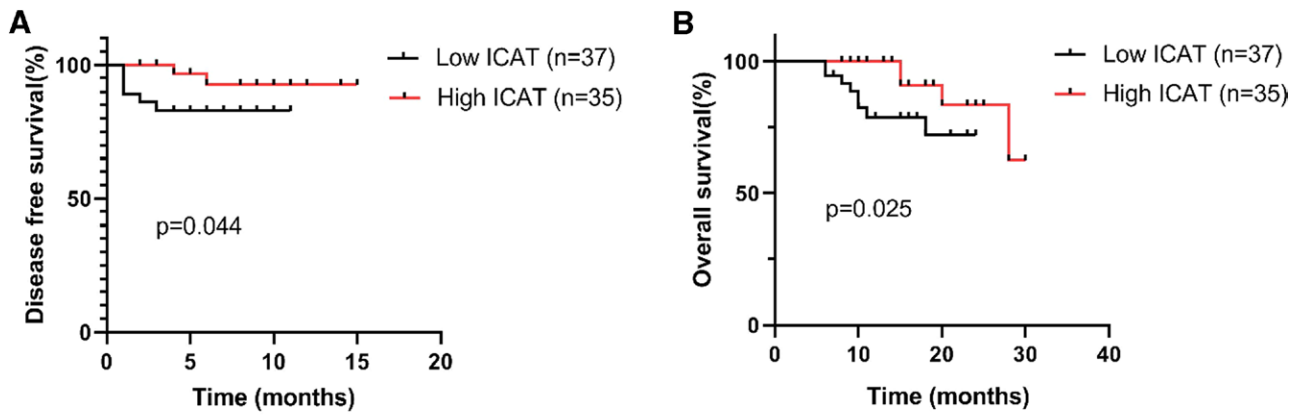

Figure 3. Probability of disease-free survival and overall survival in patients with different ICAT concentration. (A) Disease-free survival. (B) Overall survival.

Table 3

Univariate and multivariate analyses of factors influencing disease-free survival and overall survival

	Univariate analysis		Multivariate analysis	
Factor	HR (95% CI)	*P* lvalue	HR (95% CI)	*P* value
DFS				
ICAT (≥40 ng/mL) (n = 35)	0.2 (0.0–0.9)	.040	0.1 (0.0–0.8)	.034
Sex (male) (n = 42)	1.5 (0.4–5.9)	.571		
Age (≥45 yr) (n = 35)	0.5 (1.1–2.2)	.364	0.6 (0.1–2.6)	.462
Blasts (≥60%) (n = 33)	1.9 (0.5–1.8)	.393	1.0 (0.2–6.5)	.979
WBC ($\geq 30 \times 10^9$/L) (n = 24)	1.0 (0.2–4.3)	.971		
Hb (≥90 g/L) (n = 28)	0.8 (0.2–3.3)	.745		
PLT ($\geq 50 \times 10^{12}$/L) (n = 27)	0.4 (0.1–1.6)	.177	0.2 (0.0–1.3)	.087
LDH (≥540 U/L) (n = 33)	1.9 (0.4–9.3)	.438	0.7 (0.1–5.5)	.733
OS				
ICAT (≥40 ng/mL) (n = 35)	0.3 (0.1–1.1)	.080	0.3 (0.1–1.3)	.114
Sex (male) (n = 42)	1.5 (0.5–4.6)	.499	1.3 (0.4–4.3)	.694
Age (≥45 yr) (n = 35)	0.4 (0.1–1.1)	.172	0.5 (0.1–1.9)	.290
Blasts (≥60%) (n = 33)	0.3 (0.0–1.1)	.080	0.8 (0.2–4.0)	.820
WBC ($\geq 30 \times 10^9$/L) (n = 24)	1.2 (0.3–4.0)	.735		
Hb (≥90 g/L) (n = 28)	0.5 (0.2–1.5)	.196	0.6 (0.2–2.3)	.483
PLT ($\geq 50 \times 10^{12}$/L) (n = 27)	0.5 (0.2–1.6)	.232	0.4 (0.1–1.6)	.173
LDH (≥540 U/L) (n = 33)	1.4 (0.4–4.9)	.564		

Characteristics of the 72 AML patients before therapy used in this study.

$P < .05$, indicating a statistical significant difference.

AML = acute myeloid leukemia, CI = confidence interval, DFS = disease-free survival, Hb = hemoglobin, HR = hazard ratio, ICAT = inhibitor of beta-catenin and TCF, LDH = lactate dehydrogenase, OS = overall survival, PLT = platelet, RBC = red blood cell, WBC = white blood cell.

The *β-catenin* plays a key role in the *Wnt* signaling pathway; therefore, the regulation of its activity is crucial. Specifically, the level of *β-catenin* at initial diagnosis was significantly higher in the AML group than that in the control group. This is consistent with previous findings.[27] Additionally, the level of *β-catenin* in M4, M5, and unclassified leukemia was significantly higher than that in the control group. We also observed that the level of *β-catenin* in M1, M2, and M3 was higher than in the control group. Nevertheless, these differences were not statistically significant. Studies have shown that *β-catenin*, which is primarily located in the cell membrane, can detach from the intercellular connection complex and enter the cytoplasm via 2 approaches: by binding to the coupling protein and then decomposing; and by entering the nucleus to affect gene expression; notably, the amount of free *β-catenin* in the cytoplasm is lower than that in the nucleus.[28] Recently, some researchers have reported that *β-catenin* can be detected in serum.[29,30] In line with these previous studies, we reported that ELISA can be used to detect significant differences between AML subtypes based on differences in *β-catenin* levels.

In this study, after the first course of standard chemotherapy, there were 39 patients who achieved CR, 15 patients achieved PR, and 18 showed NR. We also observed that the level of *ICAT* in the CR group was significantly higher than that in the initial diagnosis group, indicating that this dynamic change could reflect the disease course. Notably, the concentration of *ICAT* gradually increased with the improvement of the disease but decreased with disease recurrence. This was confirmed via paired analysis of changes in *ICAT* level changes in the same patients with AML at initial diagnosis and after the first course of treatment. Consistent with the above observations, Chang et al also observed an upward trend in pathological stage and poorer patient survival with low expression of *ICAT*. However, *ICAT* knockdown resulted in cancer cell migration.[13] Kosari-Monfared et al[12] observed that *CTNNBIP1* expression decreased from stage 1 to 4 in gastric adenocarcinoma, suggesting *CTNNBIP1* plays a critical inhibitory role in tumor initiation procedures. Furthermore, we observed significant correlations between the level of *ICAT* and the proportion of BM and PB blast cells as well as the WBC count in patients with AML at initial diagnosis. This observation indicates that the concentration of *ICAT* could reflect the tumor burden of leukemia. The greater the tumor burden, the lower the concentration of *ICAT*. However, no correlation was found between *ICAT* levels and sex, age, and LDH level in this study.

In a previous study, female patients showed significantly lower expression of *CTNNBIP1* than their male counterparts.[12] This was not the case in this study, possibly due to the small sample size. There was also a significant correlation between *β-catenin* levels and LDH. This is because the level of LDH may be related to cell destruction and tumor load. According to the survival analysis, patients with low levels of *ICAT* had a shorter survival time. Accordingly, AML patients with low *ICAT* expression had shorter DFS and OS. The analysis of the prognosis of patients with AML using univariate analysis and multivariate analyses, including *ICAT* level, sex, age, proportion of BM blast cells, as well as WBC count and PLT indicated that the level of *ICAT* was a protective factor that affects the DFS of patients with AML. Our analysis further highlighted the existence of an association between a low *ICAT* expression level and a poor prognosis for AML. A previous study found that a low expression of *CTNNBIP1* is significantly associated with poorer survival in lung cancer. Since *CTNNBIP1* suppresses cancer migration, it has potential as a prognostic predictor for lung cancer.[13] These results confirmed our observation that *ICAT* can be used as a prognostic factor in AML.

In conclusion, this study showed that *ICAT* expression in AML patients is low and patients with lower levels of *ICAT* have a poorer prognosis. The *ICAT* level was also found to be correlated with clinical characteristics and subtypes in patients with AML. Despite this, there are limitations to this study, including the small sample size, the single-center study, and an analysis of only *ICAT* levels in PB. In addition, we did not examine the effect of gene-level factors on patient prognosis. Therefore, it is crucial to conduct a study with a larger sample size, a multicenter study, and also to clarify mechanisms by which *ICAT* affects AML in PB and BM in the future.

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Author contributions

Hui Han and Baofang Zhu collected the data, analyzed the data, and wrote the manuscript. Jinye Xie, Yunxiu Huang, and Yiyun Geng analyzed the data and revised the manuscript. Weijia Wang and Kang Chen designed the study, analyzed the data, and wrote the manuscript.

References

[1] Bullinger L, Döhner K, Bair E, et al. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. New Engl J Med. 2004;350:1605–16.
[2] Stanchina M, Soong D, Zheng-Lin B, et al. Advances in acute myeloid leukemia: recently approved therapies and drugs in development. Cancers. 2020;12:3225
[3] Shallis RM, Wang R, Davidoff A, et al. Epidemiology of acute myeloid leukemia: recent progress and enduring challenges. Blood Rev. 2019;36:70–87.
[4] Chen J, Odenike O, Rowley JD. Leukaemogenesis: more than mutant genes. Nat Rev Cancer. 2010;10:23–36.
[5] Xu ZJ, Ma JC, Zhou JD, et al. Reduced protocadherin17 expression in leukemia stem cells: the clinical and biological effect in acute myeloid leukemia. J Transl Med. 2019;17:102.

[6] Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon: IARC; 2017:141–5.
[7] Wang YH, Imai Y, Shiseki M,et al. Knockdown of the Wnt receptor Frizzled-1 (FZD1) reduces MDR1/P-glycoprotein expression in multidrug resistant leukemic cells and inhibits leukemic cell proliferation. Leukemia Res. 2018;67: 99–108.
[8] Wang W, Zhang X, Deng K, et al. ICAT as a potential enhancer of monocytic differentiation: implications from the comparative proteome analysis of the HL60 cell line stimulated by all-trans retinoic acid and NSC67657. Cell Biochem Function. 2009;27:329–37.
[9] Satoh K, Kasai M, Ishidao T, et al. Anteriorization of neural fate by inhibitor of beta-catenin and T cell factor (ICAT), a negative regulator of Wnt signaling. Proc Natl Acad Sci U S A 2004;101:8017–21.
[10] Hu J, Wang Z, Chen J, et al. Overexpression of ICAT inhibits the progression of colorectal cancer by binding with β-catenin in the cytoplasm. Technol Cancer Res Treat. 2021;20:15330338211041253.
[11] Chung EJ, Hwang SG, Nguyen P, et al. Regulation of leukemic cell adhesion, proliferation, and survival by beta- catenin. Blood. 2002;100:982–90.
[12] Kosari-Monfared M, Nikbakhsh N, Fattahi S, et al. CTNNBIP1downregulation is associated with tumor grade and viral infections in gastric adenocarcinoma. J Cell Physiol. 2019;234:2895–904.
[13] Chang JM, Tsai AC, Huang WR, et al. The alteration of CTNNBIP1 in lung cancer. Int J Mol Sci. 2019;20:5684.
[14] Jiang Y, Ren W, Wang W, et al. Inhibitor of β-catenin and TCF (ICAT) promotes cervical cancer growth and metastasis by disrupting E-cadherin/β-catenin complex. Oncol Rep. 2017;38:2597–606.
[15] Ji L, Lu B, Wang Z, et al. Identification of ICAT as an APC inhibitor, revealing Wnt-dependent inhibition of APC-axin interaction. Mol Cell. 2018;72:37–47.
[16] Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405.
[17] Shen Y, Zhao YQ. Diagnostic and Therapeutic Criteria for Hematological Diseases. 4th edn. Beijing: Science Press; 2018.
[18] Hwang SM. Classification of acute myeloid leukemia. Blood Res. 2020;55(S1):S1–S4.
[19] Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. New Engl J Med. 2016;374:2209–21.
[20] Marcucci G, Haferlach T, Döhner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol. 2011;29:475–86.
[21] Wang W, Zhang Y, Yuan Y, et al. Opposing roles of ICAT and Wnt/β-catenin signaling in NSC67657 induced monocytic differentiation. Oncotarget. 2017;8:69924–33.
[22] Zhang K, Zhu S, Liu Y, et al. ICAT inhibits glioblastoma cell proliferation by suppressing Wnt/beta-catenin activity. Cancer Lett. 2015;357:404–11.
[23] Mukherjee N, Dasgupta H, Bhattacharya R, et al. Frequent inactivation of MCC/CTNNBIP1 and overexpression of phospho-beta-catenin (Y654) are associated with breast carcinoma: clinical and prognostic significance. Biochim Biophys Acta 2016;1862:1472–84.
[24] Godley LA, Shimamura A. Genetic predisposition to hematologic malignancies: management and surveillance. Blood. 2017;130:424–2
[25] George B, Kantarjian H, Baran N, et al.TP53 in acute myeloid leukemia: molecular aspects and patterns of mutation. Int J Mol Sci. 2021;22:10782.
[26] Alshemmari SH, Pandita R, Ram M, et al. Prognosis and outcome of fit patients with acute myeloid leukemia in Kuwait. Clin Lymphoma Myeloma Leukemia 2021;21:e736–43.
[27] Ysebaert L, Chicanne G, Demur C, et al. Expression of beta-catenin by acute myeloid leukemia cells predicts enhanced clonogenic capacities and poor prognosis. Leukemia. 2006;20: 1211–6.
[28] Zhang Y, Wang X. Targeting the Wnt/beta-catenin signaling pathway in cancer. J Hematol Oncol. 2020;13:165.
[29] Duan L, Yang Q, Yang J, et al. Identification of serum β-catenin as a biomarker in patients with HBV-related liver diseases. J Transl Med. 2018;16:265.
[30] Galatola M, Miele E, Strisciuglio C, et al. Synergistic effect of interleukin-10-receptor variants in a case of early-onset ulcerative colitis. World J Gastroenterol. 2013;19:8659–70.