Int J Clin Exp Pathol 2015;8(5):5336-5344
www.ijcep.com /ISSN:1936-2625/IJCEP0007215

Original Article

Clinical significance of up-regulated *ID1* expression in Chinese de novo acute myeloid leukemia

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Received February 19, 2015; Accepted April 13, 2015; Epub May 1, 2015; Published May 15, 2015

Abstract: To investigate the clinical significance of *ID1* expression in Chinese de novo AML patients. Real-time quantitative PCR was carried out to detect the status of *ID1* expression in 102 de novo AML patients and 28 controls. *ID1* transcript level was significantly increased in AML compared to normal controls (*P*=0.029). The age in the patients with high *ID1* expression is significantly older than in those with low *ID1* expression (*P*=0.044). *ID1* overexpression occurred with the highest frequency in the patients with poor karyotype (7/7, 100%), lower frequency in the patients with intermediate karyotype (28/60, 47%), and the lowest frequency in the patients with favorable karyotype (12/31, 39%). Both whole AML and non-M3 patients with high *ID1* expression had significantly lower rate of complete remission than those with low *ID1* expression (*P*=0.007 and 0.038). *ID1* high-expressed patients showed significantly shorter overall survival (OS) than *ID1* low-expressed patients in both whole AML and non-M3 according to Kaplan-Meier analysis (*P*=0.007 and 0.040). However, multivariate analysis indicated that *ID1* overexpression was not an independent risk factor in both whole AML and non-M3 patients. However, the adverse impact of *ID1* overexpression on outcome was revealed by both Kaplan-Meier analysis and multivariate analysis in the non-M3 patients less than 60 years old. Our study reveals that *ID1* overexpression may be associated with higher risk karyotype classification and act as an independent risk factor in young non-M3 patients.

Keywords: *ID1*, expression, prognosis, acute myeloid leukemia

Introduction

Acute myeloid leukemia (AML), a clonal hematological malignancy, is a biologically, clinically, and etiologically heterogeneous disease [1, 2]. Cytogenetic alterations and molecular biological changes play crucial roles in the pathogenesis and progression of AML. Despite the advancements in the treatment of leukemia, clinical outcome of AML remains unsatisfactory. Therefore, identifying genetic and epigenetic alterations which can recognize the patients who are at the risk of poor outcome is warranted to optimize treatment strategies. Over the past years, the prognosis of AML has been evaluated mainly based on cytogenetic analysis [3, 4]. Recently, numerous genetic changes including gene mutations, deletions, amplifications and gene expression abnormalities, have been identified [5-7]. These alterations contribute to further understanding of leukemogenesis and provide more prognostic markers in AML [8, 9].

ID (inhibitors of differentiation) gene encodes for a helix-loop-helix (HLH) protein, a group of dominant inhibitors of basic HLH transcriptional factors which promote cell differentiation [10, 11]. *ID1* (inhibitors of differentiation 1), a family member of *ID* genes, has been identified as a potential proto-oncogene for its role in inducing cell proliferation as well as invasion, and protecting cells against drug-induced apoptosis [11]. Overexpression of *ID1* has been found in a variety of solid tumors [12-22]. However, few studies investigated the clinical relevance of *ID1* expression in AML [23, 24]. Therefore, the current study was intended to investigate the clinical significance of *ID1* expression in Chinese de novo AML patients.

Table 1. Correlation between *ID1* expression and whole AML as well as CN-AML patients parameters

Patient's parameters	Status of *ID1* expression in whole AML			Status of *ID1* expression in CN-AML		
	Low (n=51)	High (n=51)	*P*	Low (n=27)	High (n=21)	*P*
Sex, male/female	29/22	32/19	0.687	15/12	16/5	0.224
Median age, years (range)	51 (10-93)	60 (17-87)	0.044	61 (15-86)	61 (17-85)	0.809
Median WBC, $\times 10^9$/L (range)	5.7 (0.3-528.0)	19.7 (1.1-185.4)	0.062	10.2 (0.8-528.0)	28.1 (1.2-136.1)	0.330
Median hemoglobin, g/L (range)	78 (32-131)	68 (40-138)	0.095	88.5 (32-131)	76.5 (40-138)	0.492
Median platelets, $\times 10^9$/L (range)	40 (6-140)	42 (4-264)	0.657	44.5 (6-140)	30 (4-124)	0.336
BM blasts, % (range)	44.0 (1.0-97.5)	53.3 (3.0-109.0)	0.132	51.8 (17.0-97.5)	65.0 (6.0-109.0)	0.778
FAB			0.450			0.542
M1	3 (6%)	5 (10%)		2 (7%)	1 (5%)	
M2	19 (37%)	19 (37%)		14 (52%)	9 (43%)	
M3	16 (31%)	8 (16%)		-	-	
M4	8 (16%)	15 (29%)		8 (30%)	8 (38%)	
M5	5 (10%)	2 (4%)		3 (11%)	1 (5%)	
M6	0 (0%)	2 (4%)		0 (0%)	2 (9%)	
WHO			0.100			0.542
AML with t(8;21)	3 (6%)	4 (8%)		-	-	
APL with t(15;17)	16 (31%)	8 (16%)		-	-	
AML without maturation	3 (6%)	5 (10%)		2 (7%)	1 (5%)	
AML with maturation	16 (31%)	15 (29%)		14 (52%)	9 (43%)	
Acute myelomonocytic leukemia	8 (16%)	16 (31%)		8 (30%)	8 (38%)	
Acute monoblastic and monocytic leukemia	5 (10%)	1 (2%)		3 (11%)	1 (5%)	
Acute erythroid leukemia	0 (0%)	2 (4%)		0 (0%)	2 (9%)	
Karyotype classification			0.011			-
Favorable	19 (37%)	12 (23%)		-	-	
Intermediate	32 (63%)	28 (55%)		-	-	
Poor	0 (0%)	7 (14%)		-	-	
No data	0 (0%)	4 (8%)		-	-	
Karyotype			0.033			-
normal	27 (53%)	21 (41%)		-	-	
T (8; 21)	3 (6%)	4 (8%)		-	-	
T (15; 17)	16 (31%)	8 (16%)		-	-	
complex	0 (0%)	6 (12%)		-	-	
others	5 (10%)	8 (16%)		-	-	
No data	0 (0%)	4 (8%)		-	-	
Gene mutation*						
C/EBPA (+/-)	6/42 (13%)	4/44 (8%)	0.740	4/23 (15%)	3/17 (15%)	1.000
NPM1 (+/-)	5/43 (10%)	3/45 (6%)	0.714	4/23 (15%)	1/19 (5%)	0.377
FLT3 ITD (+/-)	7/41 (15%)	7/41 (15%)	1.000	4/23 (15%)	2/18 (10%)	1.000
C-KIT (+/-)	0/48 (0%)	0/48 (0%)	-	0/27 (0%)	0/20 (0%)	-
N/K RAS (+/-)	4/44 (8%)	5/43 (10%)	1.000	4/23 (15%)	2/18 (10%)	1.000
IDH1/2 (+/-)	4/44 (8%)	1/47 (2%)	0.362	4/23 (15%)	0/20 (0%)	0.126
DNMT3A (+/-)	4/44 (8%)	3/45 (6%)	1.000	2/25 (7%)	2/18 (10%)	1.000
U2AF1 (+/-)	1/47 (2%)	4/44 (8%)	0.362	1/26 (4%)	1/19 (5%)	1.000
CR (+/-)	30/18 (63%)	15/30 (33%)	0.007	14/13 (52%)	8/11 (42%)	0.562

WBC, white blood cells; FAB, French-American-British classification; AML, acute myeloid leukemia; CR, complete remission; *, percentage was equal to the number of mutated patients divided by total cases in each group.

Materials and methods

Patients' samples

Bone marrow (BM) was collected from 102 patients with de novo AML treated at the Affiliated People's Hospital of Jiangsu University. The diagnosis and classification of AML patients were established according to the revised French-American-British (FAB) classification and the 2008 World Health Organization (WHO) criteria [25, 26]. Written informed consent was

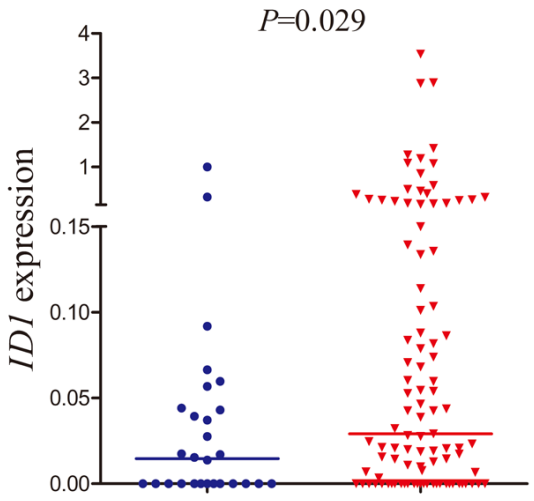

Figure 1. Relative expression levels of *ID1* expression in AML patients and controls.

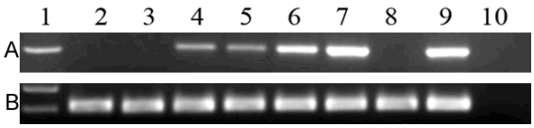

Figure 2. Electrophoresis results of RQ-PCR products in AML patients. 1: Gene Ruler™ 100bp DNA ladder; 2-3: normal controls; 4-8: AML samples; 9: positive control; 10: negative control. A: *ID1*; B: *ABL*.

obtained from all patients. The study was approved by the Institutional Review Board of the Affiliated People's Hospital of Jiangsu University. Karyotypes were analyzed by conventional R-banding method and karyotype risk was classified according to reported previously [27]. Treatment protocol was described previously [28]. The characteristics of AML patients were summarized in **Table 1**. 28 healthy donors were collected as controls. Bone marrow mononuclear cells (BMMNCs) were separated by Ficoll solution and washed twice with PBS.

RNA isolation, reverse transcription and RQ-PCR

Total RNA was isolated from the BMMNCs using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed on iCycler Thermal Cycler (Eppendorf, Hamburg, Germany). The reactions with final volume 40 μL contained 5× buffer 10 mM, dNTPs 10 mM, random hexamers 10 μM, RNAsin 80 units, and 200 units of MMLV reverse transcriptase (MBI Fermentas, Hanover, USA). The system of reverse transcription was incubated for 10 min at 25°C, 60 min at 42°C, and then stored at -20°C. Real-time quantitative PCR (RQ-PCR) was performed on a 7300 Thermo cycler (Applied Biosystems, CA, USA). The primer sequences of *ID1* expression were 5'-CTCAGCACCCTCAACGG-3' (forward) and 5'-GATCGGTCTTGTTCTCCCTC-3' (reverse) with expected product of 199 bp. Reaction system with a volume of 20 μL was consisted of cDNA 20 ng, 0.4 μM of primers, 10 μM of AceQ™ qPCR SYBR Green Master Mix (Vazyme Biotech Co., Piscataway, NJ, USA), and 0.4 μM of ROX Reference Dye 1 (Invitrogen, Carlsbad, CA, USA). RQ-PCR conditions were carried out at 95°C for 5 min, followed by 35 cycles at 95°C for 10 s, 62°C for 30 s, 72°C for 30 s, and 80°C for 30 s to collect fluorescence, finally followed by 95°C for 15 s, 60°C for 60 s, 99°C for 15 s, and 60°C for 15 s. Positive and negative controls were included in all assays. Housekeeping gene (*ABL*) was used to calculate the abundance of *ID1* mRNA. Relative *ID1* expression levels were calculated using the following equation: $N_{ID1} = (E_{ID1})^{\Delta CT\ ID1\ (control\text{-}sample)} \div (E_{ABL})^{\Delta CT\ ABL\ (control\text{-}sample)}$. The parameter efficiency (E) was derived from the formula $E=10^{(-1/slope)}$ (the slope referred to CT versus cDNA concentration plot). ΔCT reflected the disparity in CT value between control and target or reference sequences. We selected the bone marrow sample from one normal control that possessed the minimal ΔCT between *ID1* and *ABL* transcript as control and was defined as 100% expression for *ID1* transcript.

Gene mutation detection

IDH1/2, *DNMT3A*, *N/K-RAS*, *C-KIT, NPM1,* and *U2AF1* mutations were detected by high-resolution melting analysis (HRMA) as reported previously [29-32]. All positive samples were confirmed by DNA direct sequencing. *FLT3*-ITD and *C/EBPA* mutations were detected by direct DNA sequencing [33].

Statistical analysis

Statistical analyses were performed on SPSS 17.0 software package (SPSS, Chicago, IL). Mann-Whitney's *U* test was used to compare the difference of continuous variables in two groups. Pearson Chi-square analysis or Fisher exact test were employed to compare the difference of categorical variables. Receiver operating characteristic curve (ROC) and area under

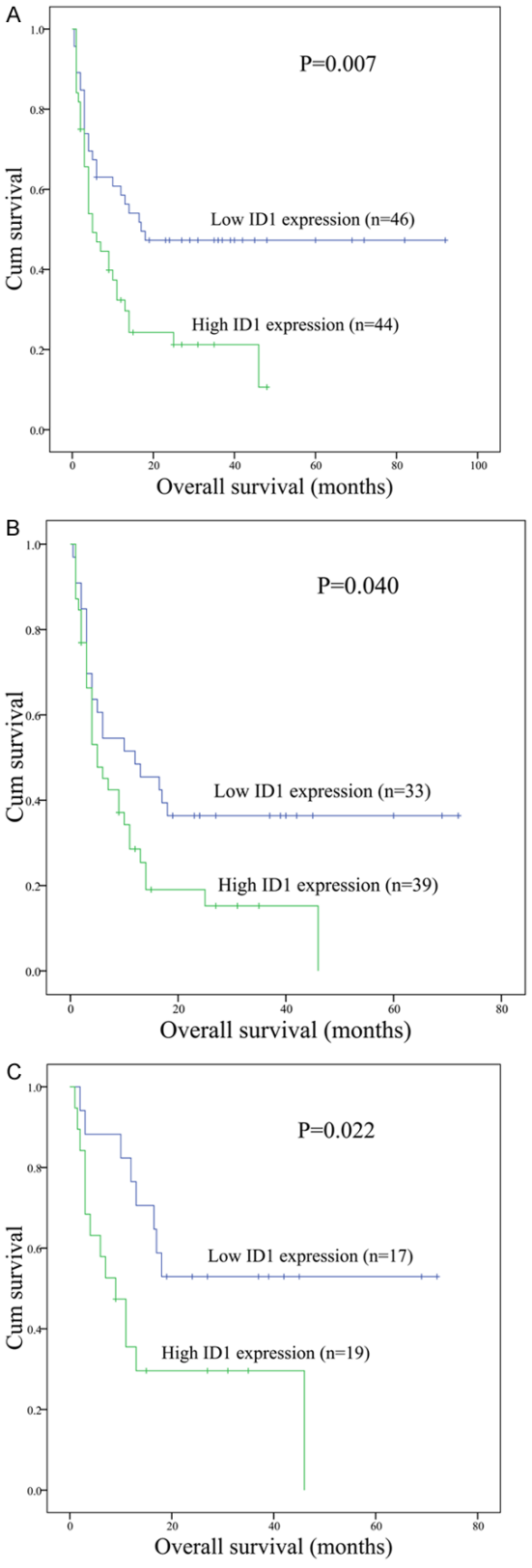

Figure 3. The impact of *ID1* expression on overall survival of AML patients. A: All patients; B: Non-M3 patients; C: Young (age <60 years old) non-M3 patients.

the ROC curve (AUC) were conducted to assess the value of *ID1* expression in distinguishing AML and cytogenetically normal AML (CN-AML) patients from normal controls. Kaplan-Meier curve done by log-rank test and Cox regression backward stepwise likelihood ratio were performed to analyze the impact of *ID1* expression on survival respectively. For all analyses, a two-tailed *P* value of 0.05 or less was determined as statistically significant.

Results

ID1 expression in normal controls and AML patients

ID1 transcript level in normal controls ranged from 0.000 to 1.000 with a median level of 0.015. The level of *ID1* expression (0.000-3.536, median 0.029) was significantly increased in AML compared to normal controls (*P*=0.029, **Figure 1**). The representative electrophoresis results of RQ-PCR products were shown in **Figure 2**.

Differentiating value of ID1 expression

ROC curve was applied to evaluate the differentiating value of *ID1* expression. It indicated that *ID1* level might serve as a biomarker for distinguishing AML from controls (AUC=0.633, 95% CI: 0.523-0.742, *P*=0.032).

Clinical and laboratory characteristics of AML patients

The whole cohort of AML patients were divided into two groups at the median level of *ID1* expression, and defined as low *ID1* expression ($ID1^{low}$) group (<0.029) and high *ID1* expression ($ID1^{high}$) group (>0.029). There were no significant differences in sex, hemoglobin (HB), platelets (PLT), and BM blasts between the $ID1^{high}$ and $ID1^{low}$ groups (*P*>0.05, **Table 1**). However, $ID1^{high}$ cases tended to have a higher white blood cell (WBC) than $ID1^{low}$ cases (*P*=0.062). $ID1^{high}$ patients had significantly older age than $ID1^{low}$ patients (*P*=0.044). No significant differences were found between the two groups in the distribution of both FAB and WHO subtypes. While, significant difference was observed in the distribution of karyotype classification between the $ID1^{high}$ and $ID1^{low}$ patients (*P*=0.011). *ID1* overexpression occurred with the highest frequency in the patients with poor karyotype (7/7, 100%), lower frequency in the

Table 2. Multivariate analyses of prognostic factors for overall survival in whole AML and non-M3 patients

	Whole AML		non-M3	
	hazard ratio (95% CI)	*P* value	hazard ratio (95% CI)	*P* value
Age	2.110 (1.141-3.901)	0.017	2.066 (1.129-3.782)	0.019
WBC	1.698 (0.929-3.102)	0.085	1.611 (0.844-3.076)	0.149
Karyotype classifications	2.561 (1.418-4.624)	0.002	2.521 (1.254-5.071)	0.009
ID1 expression	1.337 (0.725-2.468)	0.352	1.412 (0.750-2.659)	0.285
FLT3 mutation	0.551 (0.235-1.291)	0.170	0.715 (0.290-1.761)	0.466
NPM1 mutation	1.201 (0.338-4.266)	0.777	1.010 (0.270-3.777)	0.988
C/EBPA mutation	0.907 (0.310-2.653)	0.859	0.861 (0.290-2.557)	0.788

Table 3. Multivariate analyses of prognostic factors for overall survival in young (age <60 years old) whole AML and non-M3 patients

	Whole AML		non-M3	
	hazard ratio (95% CI)	*P* value	hazard ratio (95% CI)	*P* value
WBC	2.738 (1.137-6.597)	0.025	3.249 (1.178-8.956)	0.023
Karyotype classifications	3.845 (1.695-8.724)	0.001	3.835 (1.465-10.041)	0.006
ID1 expression	2.114 (0.850-5.259)	0.107	3.012 (1.105-8.213)	0.031
FLT3 mutation	0.902 (0.190-4.278)	0.897	1.553 (0.274-8.809)	0.619
NPM1 mutation	0.685 (0.089-5.303)	0.717	0.704 (0.084-5.877)	0.745
C/EBPA mutation	1.149 (0.334-3.958)	0.826	1.151 (0.319-4.158)	0.830

patients with intermediate karyotype (28/60, 47%), and the lowest frequency in the patients with favorable karyotype (12/31, 39%). No significant correlations were found between *ID1* expression and ten gene mutations ($P>0.05$, **Table 1**). In addition, among CN-AML patients, there were no significant differences in peripheral perameters, BM blasts, FAB subtypes, and gene mutations between the $ID1^{high}$ and $ID1^{low}$ patients ($P>0.05$, **Table 1**).

Correlation between ID1 expression and clinical outcome

Follow-up data was obtained for 93 AML patients. After induction therapy, $ID1^{high}$ patients had significantly lower rate of complete remission (CR) than $ID1^{low}$ patients (33% vs. 63%, respectively, $P=0.007$, **Table 1**). Among non-M3 patients, $ID1^{high}$ cases also showed significantly lower rate of CR than $ID1^{low}$ cases [30% (12/40) vs. 54% (19/35), respectively, $P=0.038$]. However, there was no significant difference among CN-AML patients (52% vs. 42%, respectively, $P=0.562$, **Table 1**). Moreover, significantly lower CR rate was observed in $ID1^{high}$ groups as compared with $ID1^{low}$ groups in both whole AML and non-M3 patients less than 60 years old [48% (11/23) vs. 84% (26/31) and 40% (8/20) vs. 84% (16/19); $P=0.007$ and 0.008, respectively], but not in whole AML and non-M3 patients more than 60 years old (data not shown). Survival analyses were performed in 90 patients with follow-up data ranged from 1 to 92 months (median 10 months). $ID1^{high}$ patients showed significantly shorter overall survival (OS) than $ID1^{low}$ patients (median 5 versus 17 months, respectively, $P=0.007$, **Figure 3A**). Significant difference was also observed in non-M3 patients. The median OS in $ID1^{high}$ and $ID1^{low}$ cases was 5 and 12 months, respectively ($P=0.040$, **Figure 3B**). However, significant difference was not found among CN-AML patients (median 6 versus 11 months, respectively, $P=0.339$). Multivariate analysis including age (≤60 y vs. >60 y), WBC ($\geq 30\times10^9$/L vs. $<30\times10^9$/L), karyotype classification (favorable vs. intermediate vs. poor), four gene mutations (mutant vs. wild-type), and *ID1* expression (high vs. low) variables disclosed that *ID1* overexpression was not an independent risk factor in both whole AML and non-M3 patients (**Table 2**). However, the adverse impact of *ID1* overexpression on outcome was revealed by both Kaplan-Meier analysis and multivariate analy-

sis in the non-M3 patients less than 60 years old (**Figure 3C**; **Table 3**), but not in the whole AML patients less than 60 years old (**Table 3**) as well as whole AML and non-M3 more than 60 years old (data not shown).

Discussion

The major biological effect of ID protein is the inhibition of differentiation and maintenance of self-renewal and multipotency of stem cells, which is coordinated with continuous cell cycling [11]. *ID1* proteins which could be activated by oncogenic factors are essential components of oncogenic pathways [11]. Deregulation of *ID1* proteins plays a direct role in cancer initiation, maintenance, progression, and drug resistance [11]. Additionally, *ID1* aberration may contribute to the initiation of myeloid malignancy [34]. Thus, *ID1* may represent a potential therapeutic target for tumors including hematopoietic malignancy.

The clinical significance of *ID1* aberration has been widely investigated. Although *ID1* overexpression predicts poor outcome in the majority of solid tumors [11], the impact of *ID1* aberration remains controversial in AML patients. Tang et al revealed that high *ID1* expression independently predicted lower CR rate and shorter disease-free survival (DFS) and OS in young (age <60 y) non-M3 or cytogenetically normal patients [23]. However, Damm et al disclosed that *ID1* overexpression was not an independent prognostic factor in young CN-AML patients [24]. Our results confirmed the adverse impact of high *ID1* expression on prognosis in non-M3 AML patients less than 60 years. The impact of *ID1* expression on outcome was not investigated in CN-AML patients less than 60 years due to the small size of case numbers.

Interestingly, our study further found the significant correlation between *ID1* expression and karyotype classification and indicated that the incidence of *ID1* overexpression was increased with the rising risk of karyotype. However, if M3 patients were excluded from analysis, we did not observe the significant association between *ID1* expression and karyotype classification, which was in accordance with the previous investigation [23]. An early study also observed the down-regulation of *ID1* expression in primary acute promyelocytic leukemia (APL) cells and NB4 cell lines, which could be rapidly induced upon all-trans retinoic acid (ATRA) treatment [35]. Moreover, *ID1* overexpression inhibited proliferation and induced a G0/G1 accumulation in NB4 cells [35]. However, a later study revealed that *ID1* overexpression enhanced the proliferation of primitive myeloid progenitor cells and immortalized bone marrow cells in vitro, and *ID1* silencing inhibited leukemic cell line growth [34]. These results indicated that the role of *ID1* in the process of leukemogenesis may be dependent on the context of different cytogenetics.

The association of *ID1* expression with gene mutations has been investigated. Damm et al revealed the significantly decreased incidences of *C/EBPA*^{mut} and *NPM1*^{mut}/*FLT3*-ITD^{neg} in *ID1*^{high} patients [24]. Moreover, *ID1*^{high} patients showed a significantly increased frequency of *FLT3*-ITD^{mut} [23, 24]. Our study did not observe the correlation between these gene mutations and *ID1* expression, probably due to the low frequency of these gene mutations in our cases. This difference may be attributed to the differences in ethnics and in AML subtype distribution. More cases of different races are needed to further determine the association of *ID1* expression with genetic mutations.

The underlying mechanism of regulating *ID1* expression was poorly studied. Although a large CpG island was identified at the 5' region of *ID1* promoter, *ID1* expression silencing was not associated with its promoter methylation [36, 37]. Our study further investigated the methylation status of *ID1* in both normal controls and leukemic cell lines using bisulfite sequencing and manifested that *ID1* promoter showed extremely low density in both normal controls and leukemic cell lines (data not shown). *ID1* expression was shown to be regulated by histone acetylation of its promoter in leukemic cell lines [37]. Recently, two microRNAs (*miR-29b* and *miR-381*) have been demonstrated to play important roles in the regulation of *ID1* expression in human lung adenocarcinoma [38, 39]. Garzon et al disclosed the decreased expression of *miR-29b* in AML [40]. Furthermore, ectopic *miR-29b* expression could induce apoptosis and reduce cell growth in primary AML cells and cell lines, and inhibit tumorigenicity in a Xenograft leukemia model [40]. Further studies are required to explore the role of these microRNAs in regulating *ID1* expression in AML patients.

In conclusion, our study suggests that *ID1* overexpression may correlate with higher risk karyotype classification and serve as an independent risk factor in young non-M3 patients.

Acknowledgements

This study was supported by National Natural Science foundation of China (81270630, 81172592), Science and Technology Special Project in Clinical Medicine of Jiangsu Province (BL2012056), 333 Project of Jiangsu Province (BRA2013136), Science and Technology Infrastructure Program of Zhenjiang (SS2012003), Medical Key Talent Project of Zhenjiang, Social Development Foundation of Zhenjiang (SH2013042, SH2013082, SH2014044, SH2014086), and Jiangsu Government Scholarship for Overseas Studies.

Disclosure of conflict of interest

None.

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References

[1] Estey E, Döhner H. Acute myeloid leukaemia. Lancet 2006; 368: 1894-1907.

[2] Smith M, Barnett M, Bassan R, Gatta G, Tondini C, Kern W. Adult acute myeloid leukaemia. Crit Rev Oncol Hematol 2004; 50: 197-222.

[3] Grimwade D. The clinical significance of cytogenetic abnormalities in acute myeloid leukaemia. Best Pract Res Clin Haematol 2001; 14: 497-529.

[4] Byrd JC, Mrózek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD; Cancer and Leukemia Group B (CALGB 8461). Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: Results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002; 100; 4325-4336.

[5] Look AT. Oncogenic transcription factors in the human acute leukemias. Science 1997; 278: 1059-1064.

[6] Krug U, Ganser A, Koeffler HP. Tumor suppressor genes in normal and malignant hematopoiesis. Oncogene 2002; 21: 3475-3495.

[7] Haferlach T. Molecular genetic pathways as therapeutic targets in acute myeloid leukemia. Hematol Am Soc Hematol Educ Program 2008: 400-411.

[8] Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A, Bloomfield CD. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009; 114: 937-951.

[9] Gulley ML, Shea TC, Fedoriw Y. Genetic tests to evaluate prognosis and predict therapeutic response in acute myeloid leukemia. J Mol Diagn 2010; 12: 3-16.

[10] Benezra R, Davis RL, Lockshon D, Turner DL, Weintraub H. The protein *ID*: a negative regulator of helix-loop-helix DNA binding proteins. Cell 1990; 61: 49-59.

[11] Lasorella A, Benezra R, Iavarone A. The *ID* proteins: master regulators of cancer stem cells and tumour aggressiveness. Nat Rev Cancer 2014; 14: 77-91.

[12] Kubelac MP, Fetica B, Vlad IC, Fulop A, Popa A, Achimas-Cadariu P. The role of inhibitor of DNA-binding 1 (ID-1) protein and angiogenesis in serous ovarian cancer. Anticancer Res 2014; 34: 413-416.

[13] Ponz-Sarvisé M, Castañón E, Panizo-Santos A, Redrado M, López I, Rosell D, Gil-Aldea I, Calvo A, Nguewa PA, Gil-Bazo I. Differential tumor expression of inhibitor of differentiation-1 in prostate cancer patients with extreme clinical phenotypes and prognostic implications. Clin Genitourin Cancer 2014; 12: 87-93.

[14] Wazir U, Jiang WG, Sharma AK, Newbold RF, Mokbel K. The mRNA expression of inhibitors of DNA binding-1 and -2 is associated with advanced tumour stage and adverse clinical outcome in human breast cancer. Anticancer Res 2013; 33: 2179-2183.

[15] Luo KJ, Wen J, Xie X, Fu JH, Luo RZ, Wu QL, Hu Y. Prognostic relevance of Id-1 expression in patients with resectable esophageal squamous cell carcinoma. Ann Thorac Surg 2012; 93: 1682-1688.

[16] Ponz-Sarvisé M, Nguewa PA, Pajares MJ, Agorreta J, Lozano MD, Redrado M, Pio R, Behrens C, Wistuba II, García-Franco CE, García-Foncillas J, Montuenga LM, Calvo A, Gil-Bazo I. Inhibitor of differentiation-1 as a novel prognostic factor in NSCLC patients with ade-

nocarcinoma histology and its potential contribution to therapy resistance. Clin Cancer Res 2011; 17: 4155-4166.

[17] Ding R, Han S, Lu Y, Guo C, Xie H, Zhang N, Song Z, Cai L, Liu J, Dou K. Overexpressed Id-1 is associated with patient prognosis and HBx expression in hepatitis B virus-related hepatocellular carcinoma. Cancer Biol Ther 2010; 10: 299-307.

[18] Yang HY, Liu HL, Liu GY, Zhu H, Meng QW, Qu LD, Liu LX, Jiang HC. Expression and prognostic values of Id-1 and Id-3 in gastric adenocarcinoma. J Surg Res 2011; 167: 258-266.

[19] Dong Z, Liu S, Zhou C, Sumida T, Hamakawa H, Chen Z, Liu P, Wei F. Overexpression of Id-1 is associated with tumor angiogenesis and poor clinical outcome in oral squamous cell carcinoma. Oral Oncol 2010; 46: 154-157.

[20] Straume O, Akslen LA. Strong expression of *ID1* protein is associated with decreased survival, increased expression of ephrin-A1/EPHA2, and reduced thrombospondin-1 in malignant melanoma. Br J Cancer 2005; 93: 933-938.

[21] Lee KT, Lee YW, Lee JK, Choi SH, Rhee JC, Paik SS, Kong G. Overexpression of Id-1 is significantly associated with tumour angiogenesis in human pancreas cancers. Br J Cancer 2004; 90: 1198-1203.

[22] Schindl M, Oberhuber G, Obermair A, Schoppmann SF, Karner B, Birner P. Overexpression of Id-1 protein is a marker for unfavorable prognosis in early-stage cervical cancer. Cancer Res 2001; 61: 5703-5706.

[23] Tang R, Hirsch P, Fava F, Lapusan S, Marzac C, Teyssandier I, Pardo J, Marie JP, Legrand O. High *ID1* expression is associated with poor prognosis in 237 patients with acute myeloid leukemia. Blood 2009; 114: 2993-3000.

[24] Damm F, Wagner K, Görlich K, Morgan M, Thol F, Yun H, Delwel R, Valk PJ, Löwenberg B, Heuser M, Ganser A, Krauter J. *ID1* expression associates with other molecular markers and is not an independent prognostic factor in cytogenetically normal acute myeloid leukaemia. Br J Haematol 2012; 158: 208-215.

[25] In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid tissues. Lyon, France: IARC Press; 2008.

[26] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985; 103: 620-625.

[27] Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, Wheatley K, Harrison CJ, Burnett AK; National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood 2010; 116: 354-365.

[28] Li Y, Lin J, Yang J, Qian J, Qian W, Yao DM, Deng ZQ, Liu Q, Chen XX, Xie D, An C, Tang CY. Overexpressed *let-7a-3* is associated with poor outcome in acute myeloid leukemia. Leuk Res 2013; 37: 1642-1647.

[29] Lin J, Yao DM, Qian J, Chen Q, Qian W, Li Y, Yang J, Wang CZ, Chai HY, Qian Z, Xiao GF, Xu WR. Recurrent *DNMT3A R882* muta-tions in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One 2011; 6: e26906.

[30] Lin J, Yao DM, Qian J, Chen Q, Qian W, Li Y, Yang J, Wang CZ, Chai HY, Qian Z, Xiao GF, Xu WR. *IDH1* and *IDH2* mutation analysis in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. Ann Hematol 2012; 91: 519-525.

[31] Yang X, Qian J, Sun A, Lin J, Xiao G, Yin J, Chen S, Wu D. *RAS* mutation analysis in a large cohort of Chinese patients with acute myeloid leukemia. Clin Biochem 2013; 46: 579-583.

[32] Qian J, Yao DM, Lin J, Qian W, Wang CZ, Chai HY, Yang J, Li Y, Deng ZQ, Ma JC, Chen XX. *U2AF1* Mutations in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One 2012; 7: e45760.

[33] Wen XM, Lin J, Yang J, Yao DM, Deng ZQ, Tang CY, Xiao GF, Yang L, Ma JC, Hu JB, Qian W, Qian J. Double CEBPA mutations are prognostically favorable in non-M3 acute myeloid leukemia patients with wild-type NPM1 and FLT3-ITD. Int J Clin Exp Pathol 2014; 7: 6832-40.

[34] Suh HC, Leeanansaksiri W, Ji M, Klarmann KD, Renn K, Gooya J, Smith D, McNiece I, Lugthart S, Valk PJ, Delwel R, Keller JR. *ID1* immortalizes hematopoietic progenitors in vitro and promotes a myeloproliferative disease in vivo. Oncogene 2008; 27: 5612-5623.

[35] Nigten J, Breems-de Ridder MC, Erpelinck-Verschueren CA, Nikoloski G, van der Reijden BA, van Wageningen S, van Hennik PB, de Witte T, Löwenberg B, Jansen JH. *ID1* and *ID2* are retinoic acid responsive genes and induce a G0/G1 accumulation in acute promyelocytic leukemia cells. Leukemia 2005; 19: 799-805.

[36] Ryu B, Kim DS, DeLuca AM, Healey MA, Dunlap S, Fackler MJ, Herman J, Alani RM. *ID1* expression is transcriptionally regulated in radial growth phase melanomas. Int J Cancer 2007; 121: 1705-1709.

[37] Yu WP, Scott SA, Dong WF. Induction of *ID1* expression and apoptosis by the histone deacetylase inhibitor (trichostatin A) in human acute myeloid leukaemic cells. Cell Prolif 2008; 41: 86-97.

[38] Rothschild SI, Tschan MP, Federzoni EA, Jaggi R, Fey MF, Gugger M, Gautschi O. MicroRNA-29b is involved in the Src-*ID1* signaling pathway and is dysregulated in human lung adenocarcinoma. Oncogene 2012; 31: 4221-4232.

[39] Rothschild SI, Tschan MP, Jaggi R, Fey MF, Gugger M, Gautschi O. MicroRNA-381 represses *ID1* and is deregulated in lung adenocarcinoma. J Thorac Oncol 2012; 7: 1069-1077.

[40] Garzon R, Heaphy CE, Havelange V, Fabbri M, Volinia S, Tsao T, Zanesi N, Kornblau SM, Marcucci G, Calin GA, Andreeff M, Croce CM. MicroRNA 29b functions in acute myeloid leukemia. Blood 2009; 114: 5331-5341.