Comprehensive analysis of ID genes reveals the clinical and prognostic value of ID3 expression in acute myeloid leukemia using bioinformatics identification and experimental validation

Qi Zhao1,2,3,4,†, Yun Wang1,2,3,†, Di Yu1,2,3,†, Jia-Yan Leng1,2,3, Yangjing Zhao6, Mingqiang Chu2,3,4,5, Zijun Xu2,3,4, Hao Ding5*, Jingdong Zhou1,2,3,* and Tingjuan Zhang2,3,7*

Abstract

Background: Dysregulation of inhibitor of differentiation/DNA binding (ID) genes is linked to cancer growth, angiogenesis, invasiveness, metastasis and patient survival. Nevertheless, few investigations have systematically determined the expression and prognostic value of ID genes in acute myeloid leukemia (AML).

Methods: The expression and clinical prognostic value of ID genes in AML were first identified by public databases and further validated by our research cohort.

Results: Using public data, the expression of ID1/ID3 was markedly downregulated in AML, and the expression of ID2 was greatly upregulated in AML, whereas ID4 showed no significant difference. Among the ID genes, only ID3 expression may be the most valuable prognostic biomarker in both total AML and cytogenetically normal AML (CN-AML) and especially in CN-AML. Clinically, reduced ID3 expression was greatly associated with higher white blood cell counts, peripheral blood/bone marrow blasts, normal karyotypes and intermediate cytogenetic risk. In addition, low ID3 expression was markedly related to FLT3 and NPM1 mutations as well as wild-type TP53. Despite these associations, multivariate Cox regression analysis revealed that ID3 expression was an independent risk factor affecting overall survival (OS) and disease free survival (DFS) in CN-AML patients. Biologically, a total of 839 mRNAs/IncRNAs and 72 microRNAs were found to be associated with ID3 expression in AML. Importantly, the expression of ID3 with discriminative value in AML was further confirmed in our research cohort.

†Qi Zhao, Yun Wang and Di Yu contributed equally to this work.
*Correspondence: 18052853715@189.cn; zhoujingdong@ujs.edu.cn; zhangtingjuan1990@qq.com
1 Department of Hematology, Affiliated People's Hospital of Jiangsu University, 8 Dianli Rd, 212002 Zhenjiang, Jiangsu, P. R. China
5 Department of Respiratory Disease, Affiliated People's Hospital of Jiangsu University, 8 Dianli Rd, 212002 Zhenjiang, Jiangsu, P. R. China
7 Department of Oncology, Affiliated People's Hospital of Jiangsu University, 8 Dianli Rd, 212002 Zhenjiang, Jiangsu, P. R. China
Full list of author information is available at the end of the article

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Conclusion: The bioinformatics analysis and experimental verification demonstrate that low ID3 expression independently affects OS and DFS in patients with CN-AML, which might be seen as a potential prognostic indicator in CN-AML.

Keywords: AML, Expression, ID, ID3, Prognosis

Background

Acute myeloid leukemia (AML) is a clonal disease characterized by amplification of immature myeloid progenitors with differentiation arrest in the bone marrow (BM), finally resulting in hematopoietic failure [1]. AML is a clinically, cytogenetically and molecularly heterogeneous disease with variable clinical outcomes [1]. The features of morphology, immunology, cytogenetics and molecular biology (MICM) are the basis for AML diagnosis [1]. Cytogenetic abnormalities also provide the most important prognostic information of AML [2]. Molecular biological alterations, such as gene mutations and aberrant gene expression, also play important roles in leukemogenesis and predict treatment response and patient survival [2]. Therefore, the identification of biological markers to develop a better prognostic, diagnostic and therapeutic risk stratification for AML is of great importance.

The inhibitor of differentiation/DNA binding (ID) genes (ID1/ID2/ID3/ID4) encode ID proteins that are transcriptional regulators controlling the timing of cell fate determination and differentiation in stem and progenitor cells during physiological development [3, 4]. It was suggested that ID proteins could have key roles in cancer development [3, 4]. At the same time, dysregulated ID gene expression was linked to tumor growth, invasiveness, metastasis, angiogenesis and patient survival [3, 4]. ID1 and ID2 overexpression has been shown to correlate with enhanced malignant potential in various types of cancers including AML [3, 4]. Although increased ID1 expression was observed in AML patients, the prognostic value of ID1 overexpression remains controversial [5–7]. In addition, the prognostic effect of ID2 overexpression in AML was reported in our previous study [8]. In contrast, ID4 functioned as a tumor suppressor presenting a paradigm shift in the context of ID1 and ID2 during the process of tumorigenesis and leukemogenesis [3]. ID4 hypermethylation was an independent factor that affected clinical outcome and predicted leukemic transformation in patients with myelodysplastic syndrome (MDS) [9]. However, the function of ID3 and its expression pattern in AML are not completely understood. Herein, we systematically explored the expression and clinical implications of ID genes expression in AML.

Materials and methods

Patients from public datasets and our hospital

The identification cohort included 173 AML patients with ID gene (ID1/ID2/ID3/ID4) expression data (RNA-Seq V2 data) from public The Cancer Genome Atlas (TCGA) datasets [10]. All AML patients received standard chemotherapy as induction therapy. Following induction therapy, 100 patients underwent chemotherapy only, whereas the remaining 73 patients underwent auto/allogeneic hematopoietic stem cell transplantation as consolidation treatment. The ID gene expression in AML compared with controls was analyzed by GEPIA [11].

The validation cohort contained 107 AML patients treated at the Affiliated People’s Hospital of Jiangsu University. Patients with antecedent hematological diseases or therapy-related AML were excluded. The clinical characteristics of the AML patients are shown in Supplementary Table S1. BM samples were collected from AML patients once they were diagnosed. A total of 32 healthy donors served as normal controls. The age of the AML patients (median 57, range 18–87) showed no significant differences from that of the controls (median 52, range 20–66) (P>0.05). This current study was approved by the Ethics Committee of the Affiliated People’s Hospital of Jiangsu University, and all the individuals provided written informed consent.

RNA isolation and reverse transcription

BM mononuclear cells (BMMNCs) were separated through gradient centrifugation using Lymphocyte Separation Medium (Solarbio, Beijing, China), and then used for total RNA extraction by TRIzol reagent (Invitrogen, Carlsbad, CA). Reverse transcription was performed to synthesize cDNA as reported [12–14].

RT–qPCR

The detection of ID3 and ABL1 (housekeeping gene) mRNA was determined by real-time quantitative PCR (RT–qPCR) using AceQ qPCR SYBR Green Master Mix (Vazyme, Piscataway, NJ). The primers applied for ID3 expression detection were 5’-ACTCAGCTTAGCCAGGTGGA-3’ (forward) and 5’-AAGCTCCTTTTGTCTTGGAAGA-3’ (reverse), whereas those for ABL1 expression detection were 5’-TCTTCCAGCTTTATCTGGAGAAGTCCAC-3’ and 5’-TCAACACCTGGGCTCTCCAC-3’.
(reverse). The relative ID3 mRNA level was measured according to the $2^{-\Delta\Delta Ct}$ method [12–14].

Bioinformatics analysis
All procedures regarding the bioinformatics analysis were carried out as described in our previous studies [15, 16]. To obtain the differentially expressed genes/miRNAs (DEGs/DEmiRs), analysis of the RNA sequencing data was conducted based on the raw read counts with the R/Bioconductor package “edgeR”. All statistical analyses were controlled for the false discovery rate (FDR) by the Benjamini–Hochberg procedure.

Statistical analysis
Statistical analysis was carried out based on the SPSS 20.0 and GraphPad 5.0 software. Comparisons of continuous and categorical variables were conducted using the Mann–Whitney U test/Kruskal–Wallis test and Pearson’s χ2 test/Fisher’s exact test, respectively. Kaplan–Meier analysis (log-rank test) and Cox regression (proportional hazards model, backward method) were used to analyze the effect of ID1/ID2/ID3/ID4 expression on survival including disease-free survival (DFS) and overall survival (OS). The ability of ID3 expression to discriminate in AML patients from controls was evaluated by the receiver operating characteristic (ROC) curve and area under the ROC curve (AUC). A two-sided $P$ value less than 0.05 was considered statistically significant in all analyses.

Results
Identification of reduced ID3 expression among ID genes correlated with prognosis in AML from public TCGA datasets
We first searched GEPIA to determine the expression of ID genes (ID1/ID2/ID3/ID4) in AML. As presented in Fig. 1, the expression of ID1 and ID3 was markedly downregulated (both $P<0.001$), and the expression of ID2 was greatly upregulated in AML ($P<0.001$), whereas ID4 showed no dramatic difference in expression ($P>0.05$).

Next, to investigate the prognostic significance of the ID genes in AML, we evaluated the impact of ID gene expression on OS and DFS times by Kaplan–Meier analysis. When analyzing the prognostic value, the AML patients were divided into two groups by the median level of ID gene expression. In all the AML patients, only high ID2 expression was markedly associated with a shorter OS time ($P=0.023$), whereas the other ID members did not affect either OS or DFS time ($P>0.05$) (Fig. 2). In cytogenetically normal AML (CN-AML) patients, lower ID3 and ID4 expression was nearly or markedly correlated with shorter OS ($P=0.027$ and 0.034, respectively) and DFS ($P=0.037$ and 0.056, respectively), whereas the other ID members did not affect either OS or DFS times ($P>0.05$) (Fig. 2).

Finally, we further investigated the impact of ID gene expression on OS and DFS times in AML by Cox regression analysis. In all the AML patients, the expression of ID1, ID2 and ID3 independently affected the OS time ($P=0.016, 0.039$ and 0.028, respectively) (Table 1), whereas the expression of ID1 and ID3 independently affected the DFS time ($P=0.043$ and 0.022, respectively) (Supplementary Table S2). Among CN-AML patients, only ID3 expression independently affected both the OS and DFS times ($P=0.030$ and 0.041, respectively) (Table 1 and Supplementary Table S2).

Taken together, these results suggest that ID3 expression may be most valuable prognostic biomarker among the ID genes in AML, especially CN-AML, and it was selected for further analysis.

![Fig. 1](http://gepia.cancer-pku.cn/)
Clinical significance of ID3 expression and its correlation with gene mutations in AML

To further analyze the clinical relevance of ID3 expression in AML, the AML patients from TCGA dataset were divided into two groups by the median ID3 expression level. Comparisons of clinicopathological features, including age, sex, white blood cell (WBC) count, peripheral blood (PB)/BM blasts, French–American–British (FAB) classifications, cytogenetics and gene mutations, between the two groups (low and high ID3 expression) in both the total AML and the CN-AML cohort are shown in Table 2. In all AML patients, low ID3 expression was greatly correlated with higher WBC counts and PB/BM blasts ($P<0.001$, $=0.001$ and $=0.002$, respectively).
respectively). Moreover, low ID3 expression was markedly correlated with normal karyotype and intermediate cytogenetic risk ($P = 0.004$ and $0.014$, respectively). Based on the results, we further compared ID3 expression between AML patients with different cytogenetic risks, and confirmed the significant differences ($P = 0.036$, Fig. 3a). In addition, low ID3 expression was markedly associated with FLT3 and NPM1 mutations as well as wild-type TP53 ($P = 0.018$, 0.011 and 0.028, respectively). Similarly, we further determined ID3 expression between AML patients with and without these gene mutations. Expectedly, marked differences were observed in

### Table 1 Cox regression univariate and multivariate analysis of variables for overall survival in AML patients

| Variables       | Univariate analysis | Multivariate analysis |
|-----------------|---------------------|-----------------------|
|                 | Hazard ratio (95% CI) | $P$ value | Hazard ratio (95% CI) | $P$ value |
| **Total AML**   |                     |            |                     |            |
| ID1 expression  | 1.433 (0.991–2.073)  | 0.056      | 1.616 (1.094–2.387)  | 0.016      |
| ID2 expression  | 1.530 (1.056–2.216)  | 0.025      | 1.477 (1.020–2.141)  | 0.039      |
| ID3 expression  | 0.741 (0.512–1.072)  | 0.112      | 0.646 (0.437–0.953)  | 0.028      |
| ID4 expression  | 0.823 (0.569–1.189)  | 0.300      | -                     | -          |
| **CN-AML**      |                     |            |                     |            |
| ID1 expression  | 1.074 (0.627–1.840)  | 0.794      | -                     | -          |
| ID2 expression  | 1.224 (0.717–2.088)  | 0.459      | -                     | -          |
| ID3 expression  | 0.516 (0.284–0.939)  | 0.030      | 0.516 (0.284–0.939)  | 0.030      |
| ID4 expression  | 0.555 (0.319–0.964)  | 0.037      | 0.629 (0.356–1.109)  | 0.109      |

**Multivariate analysis includes variables with $P < 0.200$ in univariate analysis.**

AML Acute myeloid leukemia, CN-AML Cytogenetically normal AML, CI Confidence interval

**Fig. 3** The associations of ID3 expression with cytogenetic risks/genetic abnormalities in AML. a ID3 expression among different cytogenetic risks of AML. b ID3 expression in AML patients with and without FLT3 mutations. c ID3 expression in AML patients with and without NPM1 mutations. d ID3 expression in AML patients with and without CEBPA mutations. e ID3 expression in AML patients with and without TP53 mutations.
**Table 2** Correlation of ID3 expression with clinic-pathologic characteristics in AML patients

| Patient's parameters       | Total AML |                   | CN-AML |                   |
|----------------------------|-----------|-------------------|--------|-------------------|
|                            | Low (n=87) | High (n=86) | P      | Low (n=50) | High (n=30) | P      |
| Sex, male/female           | 44/43     | 49/37            | .447   | 23/27       | 14/16       | 1.000  |
| Median age, years (range)  | 57(18-82) | 59 (21-88)       | .727   | 57 (21-82)  | 59 (21-88)  | .970   |
| Median WBC, x 10^9/L (range)| 37 (1.4-297.4) | 5.5 (0.4-171.9) | .000   | 50.5 (1.4-297.4) | 8.6 (0.6-115.4) | .000   |
| Median PB blasts, % (range)| 49 (0-98) | 17 (0-97)        | .001   | 49 (0-98)   | 18.5 (0-97) | .137   |
| Median BM blasts, % (range)| 77 (32-100) | 65.5 (30-100)   | .002   | 76.5 (32-100) | 71 (30-100) | .161   |
| FAB classifications        |           |                   |        |             |             |        |
| M0                         | 6         | 10                | .307   | 1           | 2           | .553   |
| M1                         | 24        | 20                | .601   | 14          | 10          | .624   |
| M2                         | 19        | 19                | 1.000  | 14          | 6           | .595   |
| M3                         | 5         | 11                | .124   | 0           | 1           | .375   |
| M4                         | 20        | 14                | .339   | 11          | 5           | .774   |
| M5                         | 12        | 6                 | .212   | 10          | 4           | .552   |
| M6                         | 0         | 2                 | .246   | 0           | 0           | -      |
| M7                         | 0         | 3                 | .121   | 0           | 1           | .375   |
| No data                    | 1         | 1                 | 1.000  | 0           | 1           | 375    |
| Karyotypes                 |           |                   |        |             |             |        |
| normal                     | 50        | 30                | .004   | -           | -           | -      |
| t(15;17)                   | 5         | 10                | .188   | -           | -           | -      |
| t(8;21)                    | 3         | 4                 | .720   | -           | -           | -      |
| inv(16)                    | 4         | 6                 | .535   | -           | -           | -      |
| +8                         | 4         | 4                 | 1.000  | -           | -           | -      |
| del(5)                     | 0         | 1                 | .497   | -           | -           | -      |
| -7/del(7)                  | 2         | 5                 | .278   | -           | -           | -      |
| 11q23                      | 1         | 2                 | .621   | -           | -           | -      |
| others                     | 6         | 8                 | .590   | -           | -           | -      |
| complex                    | 11        | 14                | .524   | -           | -           | -      |
| No data                    | 1         | 2                 | .621   | -           | -           | -      |
| Risks (cytogenetic)        |           |                   |        |             |             |        |
| Good                       | 12        | 20                | .121   | -           | -           | -      |
| Intermediate               | 59        | 42                | .014   | -           | -           | -      |
| Poor                       | 15        | 22                | .199   | -           | -           | -      |
| No data                    | 1         | 2                 | .621   | -           | -           | -      |
| Risks (molecular)          |           |                   |        |             |             |        |
| Good                       | 12        | 21                | .084   | 0           | 1           | .497   |
| Intermediate               | 51        | 41                | .172   | 45          | 29          | .402   |
| Poor                       | 23        | 22                | 1.000  | 5           | 0           | .151   |
| No data                    | 1         | 2                 | .621   | 0           | 0           | -      |
| Gene mutations             |           |                   |        |             |             |        |
| FLT3 (+/-)                 | 32/55     | 17/69             | .018   | 22/28       | 9/21        | 244    |
| NPM1 (+/-)                 | 32/55     | 16/70             | .011   | 29/21       | 14/16       | 361    |
| DNMT3A (+/-)               | 24/63     | 18/68             | .376   | 18/32       | 11/19       | 1.000  |
| IDH2 (+/-)                 | 7/80      | 10/76             | .456   | 5/45        | 5/25        | 489    |
| IDH1 (+/-)                 | 6/81      | 10/76             | .307   | 3/47        | 6/24        | .073   |
| TET2 (+/-)                 | 8/79      | 7/79              | 1.000  | 5/45        | 4/26        | .722   |
| RUNX1 (+/-)                | 4/83      | 5/81              | .747   | 2/48        | 5/25        | .096   |
| TP53 (+/-)                 | 3/84      | 11/75             | .028   | 0/50        | 1/29        | 375    |
| NRAS (+/-)                 | 5/82      | 7/79              | .566   | 4/46        | 3/27        | 1.000  |
| CEBPA (+/-)                | 10/77     | 3/83              | .080   | 7/43        | 1/29        | 247    |
subgroups divided by FLT3 and NPM1 status ($P=0.005$ and 0.003, respectively, Fig. 3b-c), whereas a trend was observed in subgroups divided by TP53 and CEBPA status ($P=0.063$ and 0.088, respectively, Fig. 3d-e). In CN-AML, the above significant differences were not observed (Table 2).

### The independent prognostic value of ID3 expression in AML

Because a marked correlation was found between ID3 expression and common prognostic factors such as WBC, cytogenetics and gene mutations, we performed multivariate analysis by Cox regression to confirm the independent prognostic impact of ID3 expression in AML after adjusting for the prognosis-related factors. Multivariate Cox regression analysis indicated that ID3 expression was an independent risk factor affecting OS ($P=0.022$, Table 3) and DFS ($P=0.043$ and Supplementary Table S3) in CN-AML patients.

### Molecular signatures correlated with ID3 expression in AML

To investigate the biological network caused by aberrant ID3 expression in AML, we first analyzed the transcriptomes of the two groups of patients (low and high ID3 expression) from the TCGA dataset. Based on the conditions of $|\text{log}_2 \text{FC}|>1.5$, FDR < 0.05 and $P<0.05$, a total of 839 DEGs (706 downregulated and 133 upregulated) between the low and high ID3 expression groups were identified (Fig. 4a-b and Supplementary Table S4). The top 100 downregulated DEGs, such as SLIT3 and ID4, are reported to have antitumor activities in AML [9, 17, 18]. Moreover, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses [19–21] revealed that these DEGs are involved in multiple biological processes and the PI3K/AKT signaling pathway (Fig. 4c-d).

We next revealed 72 DEMiRs (38 downregulated and 34 upregulated) between the low and high ID3 expression groups according to the conditions of FDR < 0.05 and $P<0.05$ (Fig. 4e and Supplementary Table S4). The top 10 downregulated DEMiRs, including miR-139, miR-195, miR-203, miR-497 and miR-144, are reported to have antitumor effects in AML [22–26]. The top 10 upregulated DEMiRs, such as miR-196a, are reported to have protumor effects in AML [27]. Moreover, upregulated DEMiRs (potentially negatively associated with ID3 expression), such as miR-326, have been reported as potential microRNAs that directly target ID3 [28].

### Validation of ID3 expression and its discriminative capacity in AML patients from our hospital

Given the results above, we further validated the expression of ID3 in the BMMNC samples of 107 newly diagnosed AML patients and 32 healthy donors as normal controls from our hospital. The expression of ID3 was extremely decreased in AML patients compared with normal controls ($P=0.001$, Fig. 5a). Moreover, ROC analysis indicated that ID3 expression may serve as a prospective biomarker for discriminating AML patients from controls, with an AUC of 0.701 (95% CI: 0.598–0.805) ($P=0.001$, Fig. 5b). These results confirmed the low expression pattern of ID3 in AML and revealed that ID3 expression might serve as a latent biomarker that is helpful for the diagnosis of AML.

### Discussion

Dysregulation of ID gene expression has been revealed in various human cancers including AML, and was also associated with clinical outcome. Recently, Lu et al.

---

**Table 2** (continued)

| Patient’s parameters | Total AML | | CN-AML |
|----------------------|-----------|---|---------|
|                      | Low (n=87) | High (n=86) | $P$ | Low (n=50) | High (n=30) | $P$ |
| WTI (+/)             | 7/80       | 3/83       | 3.29 | 4/46       | 2/28       | 1.000 |
| PITPN11 (+/)         | 5/82       | 3/83       | 7.20 | 4/46       | 1/29       | 6.45  |
| KIT (+/)             | 2/85       | 5/81       | 0.005 | 0/50       | 0/30       | -     |
| U2AF1 (+/)           | 2/85       | 5/81       | 0.005 | 1/49       | 0/30       | 1.000 |
| KRAS (+/)            | 3/84       | 4/82       | 0.005 | 2/48       | 1/29       | 1.000 |
| ASXL1 (+/)           | 0/87       | 3/83       | 0.022 | 0/50       | 1/29       | 3.75  |

Cytogenetic and molecular risk classifications are based on the 2017 European LeukemiaNet (ELN) classification. Patients without required information for FAB subtypes, karyotypes and molecular/cytogenetic risks are considered as no data.

AML Acute myeloid leukemia, WBC White blood cells, PB Peripheral blood, BM Bone marrow, FAB French-American-British classification

a FLT3 mutation indicates both FLT3-ITD (high and low ratios) and FLT3-TKD mutations

b CEBPA mutation indicates both mono- and bi-allelic CEBPA mutation

---

Zhao et al. BMC Cancer (2022) 22:1229
using bioinformatics methods revealed that increased expression of ID1 and ID2 was correlated with poorer and better survival times, respectively, whereas ID3 and ID4 expression was not correlated with survival in lung adenocarcinoma patients [29]. Similarly, abnormal expression of ID genes may affect the occurrence and prognosis of lung cancer, and may be associated with cell metabolism and transcriptional regulation by using bioinformatics analysis [30]. These same results were further identified in breast cancer [31]. In the current study, by the bioinformatics analysis, we found that the expression of ID1 and ID3 was downregulated in AML, whereas the expression of ID2 was upregulated. Moreover, only abnormal ID3 expression may serve as an independent prognostic biomarker in AML and ID1/ID2 expression may independently affect clinical outcome in total AML. Previously, a few studies have reported the prognostic significance of ID gene expression in AML. Tang et al. revealed that high ID1 expression was correlated with adverse prognosis in AML [5]. However, a later study demonstrated that overexpression of ID1 was not an independent prognostic biomarker in young CN-AML patients [6]. Interestingly, our previous study indicated that overexpression of ID1 was correlated with higher karyotypic risk classification and served as an independent risk factor in young (age < 60 years) non-M3 patients [7]. Meanwhile, overexpression of ID2 was a frequent event in patients with AML and predicted poor chemotherapy response and clinical outcome [8]. Conversely, promoter hypermethylation-mediated ID4 repression was linked to disease progression in MDS and poor prognosis in AML. Altogether, these different results may be attributed to the differences in ethnicity and in AML subtype distribution. Accordingly, further studies are needed to validate the expression and clinical implications of the ID genes in AML.

In the present study, we mainly focused on ID3 expression in AML based on the bioinformatics identification and experimental validation. For the first time, we revealed that ID3 expression could serve as a prognostic predictor in AML. Notably, it is very interesting that ID3 could independently affect OS but not DFS by multivariate Cox regression analysis. We deduced that the role of aberrant ID3 expression in AML survival was not directly mediated by influencing leukemia development but could affect multiple factors that lead to all-cause death in AML. Previously, only May et al. revealed that ID2 and ID3 protein expression mirrored granulopoietic maturation and discriminated between acute leukemia subtypes [32]. However, numerous studies have investigated the expression and prognostic value of ID3 in human solid tumors. Xu et al. demonstrated that ID3 played a tumor

| Variables | Total AML | CN-AML |
|-----------|-----------|--------|
|           | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|           | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P |
| Age       | 1.040 (1.025–1.055) | < 0.001 | 1.028 (1.012–1.044) | < 0.001 | 1.023 (1.005–1.042) | 0.014 | 1.012 (0.991–1.034) | 0.272 |
| WBC       | 1.005 (1.001–1.008) | 0.018 | 1.007 (1.003–1.011) | 0.002 | 1.005 (1.000–1.010) | 0.032 | 1.003 (0.998–1.009) | 0.223 |
| Molecular risks | 1.921 (1.487–2.481) | < 0.001 | 2.184 (1.609–2.963) | < 0.001 | 1.817 (0.760–4.344) | 0.179 | 1.552 (0.573–2.401) | 0.387 |
| Treatment regimen | 0.519 (0.355–0.761) | < 0.001 | 0.026 (0.012–0.061) | < 0.001 | 0.525 (0.306–0.902) | 0.037 | 0.492 (0.263–0.922) | 0.027 |
| FLT3 mutation | 1.325 (0.885–1.984) | 0.171 | 1.642 (1.059–2.546) | 0.027 | 1.418 (0.818–2.458) | 0.214 | - | - |
| NPM1 mutation | 1.155 (0.770–1.732) | 0.486 | - | - | 1.059 (0.620–1.809) | 0.833 | - | - |
| TP53 mutation | 4.100 (2.291–7.339) | < 0.001 | 2.513 (1.288–4.903) | 0.007 | 2.614 (0.354–19.301) | 0.346 | - | - |
| CEBPA mutation | 0.928 (0.470–1.834) | 0.831 | - | - | 1.011 (0.432–2.365) | 0.980 | - | - |
| ASXL1 mutation | 2.365 (0.749–7.473) | 0.142 | 3.634 (1.091–12.102) | 0.036 | 10.795 (1.328–87.751) | 0.026 | 19.441 (2.200–171.761) | 0.008 |
| RUNX1 mutation | 1.069 (0.761–1.051) | 0.702 | - | - | 1.271 (0.502–2.314) | 0.613 | - | - |
| ID1 expression | 1.433 (0.991–2.073) | 0.056 | 1.301 (0.853–1.985) | 0.222 | 1.074 (0.627–1.840) | 0.794 | - | - |
| ID2 expression | 1.530 (1.056–2.216) | 0.025 | 1.050 (0.690–1.598) | 0.819 | 1.224 (0.717–2.088) | 0.459 | - | - |
| ID3 expression | 0.741 (0.512–1.072) | 0.112 | 0.712 (0.465–1.090) | 0.118 | 0.516 (0.284–0.939) | 0.030 | 0.502 (0.278–0.906) | 0.022 |
| ID4 expression | 0.823 (0.569–1.189) | 0.300 | - | - | 0.555 (0.319–0.964) | 0.037 | 0.492 (0.263–0.922) | 0.027 |

Variables including age (continuous variables), WBC (continuous variables), treatment regimen (with transplantation vs. without transplantation), molecular risks (good, intermediate, poor and unknown; classified by the 2017 European LeukemiaNet classification), FLT3/NPM1/TP53 mutation (wild type vs. mutant) and ID1/2/3/4 expression (low vs. high). Multivariate analysis includes variables with P < 0.200 in univariate analysis.

AML Acute myeloid leukemia, CN-AML Cytogenetically normal AML, HR Hazard ratio, CI Confidence interval, WBC White blood cells

a FLT3 mutation indicates both FLT3-ITD (high and low ratios) and FLT3-TKD mutations

b CEBPA mutation indicates both mono- and bi-allelic CEBPA mutation
suppressive role in papillary thyroid cancer and impeded metastasis by inhibiting E47-mediated epithelial to mesenchymal transition (EMT) [33]. Huang et al. indicated that ID3 could enhance the stemness of intrahepatic cholangiocarcinoma by gaining the transcriptional activity of β-catenin and could act as a potential biomarker in predicting response to adjuvant chemotherapeutics [34]. Moreover, ID3 overexpression was correlated with medulloblastoma seeding and is a poor prognostic factor in medulloblastoma patients [35]. Sharma et al. revealed that ID1 and ID3 overexpression alleviated all three cyclin-dependent kinase inhibitors (CDKN2B, -1 A, and −1B) resulting in a more aggressive prostate cancer phenotype [36]. Expression of ID1 and ID3 was increased in human invasive lobular carcinoma compared with invasive ductal carcinoma, associated with poor prognosis uniquely in patients with invasive lobular carcinoma and correlated with the upregulation of angiogenesis and matrisome-related genes [37]. In addition to the above results, several studies have also reported the value of the combination of ID3 expression with other members in cancer prognosis. For instance, Antonangelo et al. showed that ID1, ID2 and ID3 coexpression was associated with prognosis in stage I/II lung adenocarcinoma patients treated with surgery and adjuvant chemotherapeutics [38]. Additionally, ID1 and ID3 coexpression was correlated with a poor clinical outcome in patients with locally advanced non-small cell lung cancer treated with definitive chemoradiotherapy [39]. The combined expression of VPREB3 and ID3 was used to develop a new helpful tool for the routine diagnosis of mature aggressive B-cell lymphomas [40]. All these results suggested the prognostic value of ID3 expression in diverse human cancers.

The functional role of ID3 has also been widely investigated, and was reported to be associated with diverse biological processes such as angiogenesis, apoptosis, cell cycle regulation/proliferation, cell migration/invansion, epithelial-to-mesenchymal transition, stem cell renewal
and signaling [3]. Although we did not validate the direct role of ID3 in AML in this study, we identified the association of ID3 with PI3K/AKT signaling by bioinformatics methods. Moreover, the association of low ID3 expression with FLT3 mutation was also observed in AML patients. Similarly, Chen et al. demonstrated that miR-212-5p was involved in the progression of non-small cell lung cancer through the activation of PI3K/Akt signaling pathway by targeting ID3 [41]. Zhang et al. indicated that Per2 downregulated ID3 expression via the PTEN/AKT/Smad5 axis to inhibit glioma cell proliferation [42]. Moreover, ID3 was reported to play a significant role in reversing cisplatin resistance in human lung adenocarcinoma cells by regulating the PI3K/Akt pathway [43]. Accordingly, further functional studies are needed to confirm the direct role of ID3 in AML biology.

The regulatory mechanism of ID3 expression was preliminarily studied. Xu et al. demonstrated that hypermethylation of the CpG island at the promoter region of ID3 was the main contributor to the repression of this gene [33]. In addition, several studies also revealed the regulatory potential of miRNAs. Zhao et al. found that miR-326 could bind to ID3, which accelerated the development of medulloblastoma [28]. Moreover, high ID3 expression by silencing miR-212-5p expression suppressed the activity of the PI3K/Akt signaling pathway and consequently promoted apoptosis and inhibited proliferation in lung cancer cells [41]. Herein, we also observed the association of ID3 with several miRNAs such as miR-1259, miR-508, miR-9, miR-944, let-7b, miR-141, and miR-223. However, only miR-326 was confirmed by previous studies [28]. Accordingly, further studies are needed to confirm the direct association of ID3 with these miRNAs.

Conclusion

In summary, the bioinformatics analysis and experimental verification demonstrate that low ID3 expression independently affects OS and DFS in patients with CN-AML, which might be seen as a potential prognostic indicator in CN-AML.

Abbreviations

ID: Inhibitor of Differentiation/DNA binding; AML: Acute myeloid leukemia; CN-AML: Cytogenetically normal AML; BM: Bone marrow; MICM: Morphology, immunology, cytogenetics and molecular biology; TCGA: The Cancer Genome Atlas; BMMNCs: BM mononuclear cells; FDR: False discovery rate; DFS: Disease-free survival; OS: Overall survival; AUC: Area under the ROC curve; ROC: Receiver operating characteristic; WBC: White blood cell; PB: Peripheral blood; FAB: French–American–British; DEGs/DEmiRs: Differentially expressed genes/miRNAs; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; EMT: Epithelial to mesenchymal transition.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-022-10352-6.

Additional file 1: Table S1. Clinic-pathologic characteristics of AML in our research cohort.

Additional file 2: Table S2. Cox regression univariate and multivariate analysis of variables for disease free survival in AML patients.

Additional file 3: Table S3. Cox regression univariate and multivariate analysis of variables for disease free survival in AML patients.

Additional file 4: Table S4. Different expressed genes/miRNAs between low and high ID3 expression groups.
Acknowledgements

None.

Authors’ contributions

Jingdong Zhou and Tingjuan Zhang conceived and designed the experiments; Jingdong Zhou and Tingjuan Zhang performed the experiments; Qi Zhao, Yun Wang and Zijun Xu analyzed the data; Di Yu and Jiayen Leng collected the clinical data; Hao Ding and Yangjing Zhao provided the technical support; Jingdong Zhou and Tingjuan Zhang wrote the paper; All authors read and approved the final manuscript.

Funding

The work was supported by National Natural Science Foundation of China (81900166, 82101083), Natural Science Foundation of Jiangsu Province (BK20221287), Research Project of Jiangsu Commission of Health (MD2022123), Social Development Foundation of Zhenjiang (SM2020055), Medical Field of Zhenjiang “Jin Shan Ying Cai” Project, Medical Education Collaborative Innovation Fund of Jiangsu University (JDY202011).

Availability of data and materials

The datasets analyzed in this study are available in the following open access repositories: cBioPortal (http://www.cbioportal.org/); TCGA (https://portal.gdc.cancer.gov/) and GEPIA (http://geopia.cancer-pku.cn/).

Declarations

Ethics approval and consent to participate

The present study approved by the Ethics Committee of the Affiliated People’s Hospital of Jiangsu University, in compliance with the Declaration of Helsinki. Written informed consents were obtained from all enrolled individuals prior to their participation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1. Department of Hematology, Affiliated People’s Hospital of Jiangsu University, 8 Dianli Rd, 212002 Zhenjiang, Jiangsu, P. R. China. 2. Zhenjiang Clinical Research Center of Hematology, 212002 Zhenjiang, Jiangsu, P. R. China. 3. The Key Lab of Precision Diagnosis and Treatment of Zhenjiang City, Jiangsu 212002 Zhenjiang, P. R. China. 4. Laboratory Center, Affiliated People’s Hospital of Jiangsu University, 212002 Zhenjiang, Jiangsu, P. R. China. 5. Department of Respiratory Disease, Affiliated People’s Hospital of Jiangsu University, 8 Dianli Rd, 212002 Zhenjiang, Jiangsu, P. R. China. 6. Department of Laboratory Medicine, School of Medicine, Jiangsu University, 212013 Zhenjiang, Jiangsu, P. R. China. 7. Department of Oncology, Affiliated People’s Hospital of Jiangsu University, 8 Dianli Rd, 212002 Zhenjiang, Jiangsu, P. R. China.

Received: 3 August 2022   Accepted: 22 November 2022

Published online: 29 November 2022

References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136–52.
2. Döhner H, Estey E, Grimwade D, Armand S, Appelbaum FR, Büchner T, Domberth H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naeve T, Niederwieser D, Ossenkoppele GI, Sanz M, Siu EA, Tallman MS, Tien HF, Wei AH, Löwenberg B. Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.
3. Lasorella A, Benezra R, Lavarese A. The ID proteins: master regulators of cancer stem cells and tumour aggressiveness. Nat Rev Cancer. 2014;14(2):77–91.
4. Roschger C, Cabebre C. The Id-protein family in developmental and cancer-associated pathways. Cell Commun Signal. 2017;15(1):70.
5. Tang R, Hirsch P, Fava F, Lapusan S, Marzac C, Teysyrandier J, Paro J, Marie JP, Legrand O. High Id1 expression is associated with poor prognosis in 237 patients with acute myeloid leukemia. Blood. 2009;114(14):2993–3000.
6. Damm F, Wagner K, Görlich K, Morgan M, Thol F, Yun H, Delwel R, Volk P, 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 leukemia. Br J Haematol. 2012;158(2):208–15.
7. Zhou JD, Yang L, Zhu KW, Wen XM, Yang J, Guo H, Chen Q, Yao DM, Ma JC, Lin J, Qian J. Clinical significance of up-regulated ID1 expression in Chinese de novo acute myeloid leukemia. Int J Clin Exp Pathol. 2015;8(5):5336–44.
8. Zhou JD, Ma JC, Zhang TJ, Li XX, Zhang W, Wu DH, Wen XM, Xu ZJ, Lin J, Qian J. High bone marrow ID1 expression predicts poor chemotherapy response and prognosis in acute myeloid leukemia. Oncotarget. 2017;8(54):91979–89.
9. Zhou JD, Zhang TJ, Li XX, Ma JC, Guo H, Wen XM, Zhang W, Yang L, Yan Y, Lin J, Qian J. Epigenetic dysregulation of ID4 predicts disease progression and treatment outcome in myeloid malignancies. J Cell Mol Med. 2017;21(8):1468–81.
10. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, Hoadley K, Triche T, Lai J, Ladewig P, Baty JD, Fulton LL, Fulton R, Heath SE, Kalicki-Veizer J, Kandoth C, Klimo JM, Koboldt DC, Kanduri K, Lamprecht TL, Larson DE, Lin L, Lu C, McCallum MD, McMichael JF, Payton J, Schmidt H, Spencer DH, Tomasson MH, Walls JW, Wartman LD, Watson MA, Welch J, West DL, Alizadeh AA, Balasundaram M, Brot I, Butterfield Y, Chiu R, Chu A, Chuaah E, Chun HJ, Corbett R, Dhalla N, Guin R, He A, Hirst C, Hirst M, Holt RA, Jones S, Karsan A, Lee D, Li H, Marra MA, Mayo M, Moore RA, Mungall K, Parker J, Plass C, Plettenberg P, Schein J, Stoll D, Swanson L, Tam A, Thiessen N, Varhol R, Wye N, Zhao Y, Gabriel S, Getz G, Sougnez C, Zou L, Leiserson MD, Vandín F, Wu HT, Applebaum F, Baylin SB, Akbani R, Broom BM, Chen K, Motter TC, Nguyen K, Weinstein JN, Zhang N, Ferguson ML, Adams C, Black A, Bowen J, Gastier-Foster J, Grossman T, Lichtenberg T, Wise L, Davidsen T, Demchok JA, Shaw KR, Sheth N, Sofia H, Yang L, Downing JP, Eley G. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2035–74.
11. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45:W98–102.
12. Zhang TJ, Xu ZJ, Gu Y, Wen XM, Ma JC, Zhang W, Deng QZ, Leng JY, Qian J, Lin J, Zhou JD. Identification and validation of prognosis-related DLK5 methylation as an epigenetic driver in myeloid neoplasms. Clin Transl Med. 2020;10(2):e29.
13. Zhou JD, Zhang TJ, Xu ZJ, Deng QZ, Gu Y, Ma JC, Wen XM, Leng JY, Lin J, Chen SN, Qian J. Genome-wide methylation sequencing identifies progression-related epigenetic drivers in myelodysplastic syndromes. Cell Death Dis. 2020;11(11):996.
14. Zhang TJ, Xu ZJ, Gu Y, Ma JC, Wen XM, Zhang W, Deng QZ, Qian J, Lin J, Zhou JD. Identification and validation of obesity-related gene LEP methylation as a prognostic indicator in patients with acute myeloid leukemia. Clin Epigenetics. 2021;3:1(11):16.
15. Xu ZJ, Ma JC, Zhou JD, Wen XM, Yao DM, Zhang W, Ji RB, Wu DH, Tang LJ, Deng QZ, Qian J, Lin J. Reduced protocadherin17 expression as a prognostic and predictive biomarker in leukemia stem cells: the clinical and biological effect in acute myeloid leukemia. J Transl Med. 2019;17(1):102.
16. Gu Y, Chu MQ, Xu ZJ, Yuan Q, Zhang TJ, Lin J, Zhou JD. Comprehensive analysis of SPAG1 expression as a prognostic and predictive biomarker in acute myeloid leukemia by integrative bioinformatics and clinical validation. BMC Med Genomics. 2022;15(1):38.
17. Shi L, Huang Y, Huang X, Zhou W, Wei J, Deng D, Lai Y. Analyzing the key gene expression and prognostics values for acute myeloid leukemia. Transl Cancer Res. 2020;9(11):2784–98.
18. Zhou JD, Li XX, Zhang TJ, Xu ZJ, Zhang ZH, Gu Y, Wen XM, Zhang W, Ji RB, Deng QZ, Qian J, Lin J. MicroRNA-335/ID4 dysregulation predicts clinical outcome and facilitates leukemogenesis by activating PI3K/Akt signaling pathway in acute myeloid leukemia. Aging. 2019;11(10):3367–91.
19. Kanekihisa M, Goto S, KEGG, kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27–30.
20. Kanekihisa M. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 2019;28(11):1947–51.
21. Kanehisa M, Futymichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. KEGG: integrating viruses and cellular organisms. Nucleic Acids Res. 2021;49(D1):DS45–51.
22. Stavast C J, van Zuijen I, Karkoulia E, Özçelik A, van Hoven-Beijen A, Leon LG, Voerman J A, Jansen GMC, van Veelen PA, Bureczoza M, Brouwer RWW, van Ulden WJF, Maas A, Bindels EM, van der Velden VHJ, Schliche C, Katukis PD, Alberich-Jorda M, Erkland SJ. The tumor suppressor MIR139 is silenced by POLR2M to promote AML oncogenesis. Leukemia. 2022;36(3):867–700.
23. Cui L, Zeng T, Zhang L, Liu Y, Wu G, Fu L. High expression of miR-195 is related to favorable prognosis in cytogenetically normal acute myeloid leukemia. Int J Clin Oncol. 2021;26(10):1986–93.
24. Zhang Y, Zhou SY, Yang H, Xu DD, Chen HX, Wang XY, Wang X, Liu YT, Zhang L, Wang S, Zhou PJ, Fu WY, Ruan BB, Ma DL, Wang Y, Liu QY, Ren Z, Liu Z, Zhang R, Wang YF. miR-203 inhibits proliferation and self-renewal of leukemia stem cells by targeting survivin and Bmi-1. Sci Rep. 2016;6:19995.
25. Kang H, Tong C, Li C, Luo J. miR-497 plays a key role in Tanshinone IIA-related proliferation in OCI-AML3 cells via the MAPK/ERK1/2 pathway. Cytotechnology. 2020;72(3):427–32.
26. Sun X, Liu D, Yue X, Hu X. Enforced mir-144-3p expression as a non-invasive biomarker for the Acute myeloid leukemia patients mainly by targeting NRF2. Clin Lab. 2017;63:679–87.
27. Coskun E, van der Heide ER, Schlicht C, Kühnl A, Gökbuget N, Hoeller D, Hofmann WK, Thiel E, Baldus CD. The role of microRNA-196a and microRNA-196b as ERGs regulators in acute myeloid leukemia and acute T-lymphoblastic leukemia. Leuk Res. 2011;35:208–13.
28. Zhao X, Guan J, Luo M. Circ-SKA3 upregulates ID3 expression by decoy- miR-326 to accelerate the development of medulloblastoma. J Clin Neurosci. 2021;86:87–96.
29. Lu X, Shao L, Qian Y, Zhang Y, Wang Y, Miao L, Zhuang Z. Prognostic effects of the expression of inhibitor of DNA-binding family members on patients with lung adenocarcinoma. Oncol Lett. 2020;20(5):143.
30. Xu S, Wang Y, Li Y, Zhang L, Wang C, Wu X. Comprehensive analysis of inhibitor of differentiation/DNA-binding gene family in lung cancer using bioinformatics methods. Biocsi Rep. 2020;40(2):BSR20193075.
31. Zhou X, Zeng D, Ye YH, Sun SM, Lu XF, Liang WQ, Chen CF, Lin HY. Prognostic values of the inhibitor of DNA-binding family members in breast cancer. Oncol Rep. 2018;40(4):1897–906.
32. May AM, Frey AV, Bogatyreva L, Benkisser-Petersen M, Hauschke D, Lubbert M, Wasch R, Werner M, Hasskarl J, Lassmann S. ID2 and ID3 protein expression mirrors granulopoietic maturation and discriminates between acute leukemia subtypes. Histochem Cell Biol. 2014;141(4):431–40.
33. Xu S, Mo C, Lin J, Yan Y, Liu X, Wu K, Zhang H, Zhu Y, Chen L, Chen X. Loss of ID3 drives papillary thyroid cancer metastasis by targeting, E47-mediated epithelial to mesenchymal transition. Cell Death Discov. 2021;7(11):226.
34. Huang L, Cai J, Guo H, Gu J, Tong Y, Qiu B, Wang C, Li M, Xia L, Zhang J, Wu H, Kong X, Xia Q. ID3 promotes stem cell features and predicts chemotherapy response of Intrahepatic Cholangiocarcinoma. Hepatology. 2019;69(5):1995–2012.
35. Chen M, Huang J, Zhu Z, Zhang J, Li K. Systematic review and meta-analysis of tumor biomarkers in predicting prognosis in esophageal cancer. BMC Cancer. 2013;13:291.
36. Sharma P, Patel D, Chaudhary J. ID1 and ID3 expression is associated with increasing grade of prostate cancer. ID3 preferentially regulates CDKN1B. Cancer Med. 2012;1(2):187–97.
37. Tardemir N, Ding K, Savaria L, Levine KM, Du T, Elangovan A, Bossart EA, Lee AV, Davidson NE, Oesterreich S. Proteomic and transcriptomic profiling identifies mediators of anchorage-independent growth and roles of inhibitor of differentiation proteins in invasive lobular carcinoma. Sci Rep. 2020;10(1):11487.
38. Antonaragelo L, Turra T, Fabro A, Acenicio M, Terra R, Pane A, Vargas F, Taka- gaki T, Capelozzi V, Id-1, Id-2, and Id3 co-expression correlates with prognosis in stage I and II lung adenocarcinoma patients treated with surgery and adjuvant chemotherapy. Exp Biol Med. 2016;241(11):1159–68.
39. Castañon E, Bosch-Barrera J, López I, Collado V, Moreno M, López-Picazo JM, Arbea L, Lozano MD, Calvo A, Gil-Bazo I. ID1 and ID3 co-expression correlates with clinical outcome in stage III-N2 non-small cell lung cancer patients treated with definitive chemoradiotherapy. J Transl Med. 2013;11:13.
40. Soldini D, Georgis A, Montagna C, Schifferl PJ, Martin V, Curioni-Fontece- dro A, Martínez A, Tinguely M. The combined expression of VPREB3 and ID3 represents a new helpful tool for the routine diagnosis of mature aggressive B-cell lymphomas. Hematol Oncol. 2014;32(3):120–5.
41. Chen FF, Sun N, Wang Y, Xi HY, Yang Y, Yu BZ, Li XJ. Mir-212-5p exerts tumor promoter function by regulating the Id3/Pi3K/Akt axis in lung adenocarcinoma cells. J Cell Physiol. 2020;235(10):7273–82.
42. Zhang Y, Hu X, Li H, Yao J, Yang R, Lan Y, Xia H. Circadian period 2 (Per2) downregulates inhibitor of differentiation 3 (Id3) expression via PTEN/ AKT/Smad5 axis to inhibits glioma cell proliferation. Bioengineered. 2022;13(5):12350–64.
43. Chen FF, Lv X, Zhao QF, Xu YZ, Song SS, Yu W, Li XJ. Inhibitor of DNA-binding 3 reverses cisplatin resistance in human lung adenocarcinoma cells by regulating the PI3K/Akt pathway. Oncol Lett. 2018;16(2):1634–40.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

• Choose BMC and benefit from: