Distinct associations of NEDD4L expression with genetic abnormalities and prognosis in acute myeloid leukemia

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Abstract
Background: There is mounting evidence that demonstrated the association of aberrant NEDD4L expression with diverse human cancers. However, the expression pattern and clinical implication of NEDD4L in acute myeloid leukemia (AML) remains poorly defined.

Methods: We systemically determined NEDD4L expression with its clinical significance in AML by both public data and our research cohort. Moreover, biological functions of NEDD4L in leukemogenesis were further tested by in vitro experiments.

Results: By the public data, we identified that low NEDD4L expression was correlated with AML among diverse human cancers. Expression of NEDD4L was remarkably decreased in AML compared with controls, and was confirmed by our research cohort. Clinically, low expression of NEDD4L was correlated with greatly lower age, higher white blood cells, and higher bone marrow/peripheral blood blasts. Moreover, NEDD4L underexpression was positively correlated with normal karyotype, FLT3 and NPM1 mutations, but negatively associated with complex karyotype and TP53 mutations. Importantly, the association between NEDD4L expression and survival was also discovered in cytogenetically normal AML patients. Finally, a number of 1024 RNAs and 91 microRNAs were identified to be linked to NEDD4L expression in AML. Among the negatively correlated microRNAs, miR-10a was also discovered as a microRNA that may directly target NEDD4L. Further functional studies revealed that NEDD4L exhibited anti-proliferative and pro-apoptotic effects in leukemic cell line K562.

Conclusions: Our findings indicated that NEDD4L underexpression, as a frequent event in AML, was associated with genetic abnormalities and prognosis in AML. Moreover, NEDD4L expression may be involved in leukemogenesis with potential therapeutic target value.

Keywords: NEDD4L, Expression, Prognosis, Acute myeloid leukemia

Background
Acute myeloid leukemia (AML) is a heterogeneous clonal aggressive malignancy characterized by the uncontrolled proliferation and blocked differentiation of myeloid precursor cells [1]. Cytogenetic and genetic abnormalities in leukemic cells lead to a cascade of molecular events, which in turn cause cancer phenotype and inhibit normal hematopoiesis [2]. The genetic alterations emerging in AML has been linked to prognosis and play a crucial role in treatment strategy decision [3]. Moreover, gene
expression profiling has been widely used in AML, and was also helpful in evaluating the prognostic risk and disease recurrence [4]. At the same time, accumulating studies have reported that high transcript level of BAALC, MNI, ERG, and WT1 was significantly associated with poorer survival in AML [5]. Accordingly, screening and identifying additional AML-related prognostic biomarkers by high-throughput sequencing could precisely recognize higher risk AML, and finally improve the clinical outcome of AML.

The neural precursor cell expressed developmentally downregulated protein 4 (NEDD4) family comprises of nine members including NEDD4, NEDD4-2 (NEDD4L), ITCH, SMURF1, SMURF2, WWP1, WWP2, NEDL1, and NEDL2 in human, which are involved in the regulation of a variety of signaling pathways [6]. NEDD4L belongs to the evolutionarily conserved NEDD4 family of ubiquitin ligases characterized by a C2 domain, 2–4 WW domains, and a C-terminal HECT-type ubiquitin ligase domain [7, 8]. NEDD4L is originally discovered in identifying for downregulated genes during the development of the central nervous system [7, 8]. Recently, there is mounting evidence that showed the association of NEDD4L expression with prognosis in diverse human cancers [9–16].

Herein, as far as we known, it was the first time to report low expression of NEDD4L in AML. We identified and verified that NEDD4L was decreased in AML, and NEDD4L underexpression was correlated with specific cytogenetic/genetic abnormalities of AML. Moreover, low expression of NEDD4L was associated with clinical outcome in cytogenetically normal AML (CN-AML). Finally, a number of 1024 mRNAs and 91 microRNAs were identified to be linked to NEDD4L expression in AML. Among the negatively correlated microRNAs, miR-10a was also discovered as a microRNA that may directly target NEDD4L. Further functional studies revealed that NEDD4L exhibited anti-proliferative and pro-apoptotic effects in leukemic cell line K562.

Materials and methods

CCELE

The CCLE (Cancer Cell Line Encyclopedia) database (https://www.broadinstitute.org/ccle) focuses on the gene expression, methylation, and mutation data for over 1100 types of cancer cell lines [17]. NEDD4L expression in cancer cell lines was firstly identified by CCLE.

HPA

The HPA (Human Protein Atlas) database (https://www.proteinatlas.org/) focuses on proteins expression in cells, tissues, and organs [18]. NEDD4L expression in cancer cell lines was further identified by HPA.

GEPIA

The GEPIA (Gene Expression Profiling Interactive Analysis) database (http://gepi.a.cancer-pku.cn/) focuses on analyzing the RNA sequencing expression data of 9736 tumors and 8587 normal samples from the TCGA (The Cancer Genome Atlas) and the GTEx (Genotype-Tissue Expression) projects, using a standard processing pipeline [19]. NEDD4L expression in 33 types of cancer patients including AML and controls was analyzed by GEPIA.

BloodSpot

The Bloodspot (http://servers.binf.ku.dk/bloodspot/) provides a plot of gene expression in hematopoietic cells at different maturation stages based on curated microarray data [20]. NEDD4L expression between among AML subtypes and controls was identified by Bloodspot.

TCGA databases

TCGA is a landmark cancer genomics program, which molecularly characterized over 20,000 primary cancers and normal samples spanning 33 cancer types. The current study included a total of 173 AML patients with RNA-sequencing data (RNA Seq V2 RSEM) from the databases of TCGA (AML NEJM 2013) downloaded by cBioportal (http://www.cbioportal.org/) [21]. Expression and mutation data of these patients were also obtained by mRNA- and DNA-sequencing. Clinical features and treatment regimens for these patients were as reported [21].

GEO databases

Gene Expression Omnibus (GEO) is a public functional genomics data repository supporting MIAME-compliant data submissions. Three GEO datasets (GSE12417, GSE6891 and GSE10358) were used to evaluate the prognostic value of NEDD4L expression in AML. Firstly, the effect of NEDD4L expression on survival was analyzed in GSE12417 dataset which included 78 and 162 CN-AML patients through the online tool Genomicscape (http://genomicscape.com/microarray/survival.php) [22, 23]. Then, GSE6891 dataset consisted of 187 CN-AML patients as well as GSE10358 dataset comprised of 131 CN-AML patients were further used for validation.

Patients and samples

The validation cohort of 44 AML patients at newly diagnosis time (ND-AML, used ad cases) and 47 AML patients at complete remission (CR) time (CR-AML, used as controls) was also enrolled in this study. The
detailed information of 44 ND-AML patients was given in Additional file 1: Table S1. The age and sex between AML and controls presented no significant differences (P > 0.05). Bone marrow (BM) samples were collected from these patients. BM mononuclear cells (BMMNCs) separated from BM of these AML patients was used in this study. The current study protocol was approved by the Institutional Ethics Committee of The Affiliated People’s Hospital of Jiangsu University, and all the participants provided written informed consents.

RNA isolation and reverse transcription
Total RNA was isolated form BMMNCs by using Trizol reagent (Invitrogen, Carlsbad, CA) as our previous literature [24–26]. Reverse transcription was performed as reported [24–26]. The conditions performed as follows: 37 °C for 15 min, 85 °C for 5 s.

RT-qPCR
RT-qPCR (real-time quantitative PCR) analysis was performed to detect NEDD4L, CASP3 and CASP8 mRNA using AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co., Piscatway, NJ). The primers used for NEDD4L expression were 5'-CCCATAAGGTGTGGAAATGAA-3' (forward) and 5'-TAGTTGTCCGTGGCAAGTA-3' (reverse), primers for CASP3 expression were 5'-AATGGACCTGTGGACCT-3' (forward) and 5'-CTGTGTCCACCTTTTCG-3' (reverse), primers for CASP8 expression were 5'-GGAGCCAGGTTGGTTAT-3' (forward) and 5'-ACTTTGGGGAATGTAG-3' (reverse). Moreover, ABL1 (housekeeping gene) expression was also detected with the primers 5'-CGTCTCCAGCTGTATCTGGAAGA-3' (forward) and 5'-TTAACGAGCGGCTTACAC-3' (reverse). Relative target gene expression was calculated based on the 2^ΔΔCT target gene (control—sample)/2^ΔΔCT ABL1 (control—sample) (2^−ΔΔCT) formula.

Bioinformatics analysis
Analysis of differentially expressed genes (DEGs) and microRNAs associated with NEDD4L in AML, and the microRNAs-mRNAs network predictions could refer to our previous study [27].

Cell line and cell culture
Human leukemic cell lines HEL, HL60, K562, MOLM13, MV4-11, NB4, OCI, SHI-1, SKM-1, THP-1 and U937 as well as human bone marrow stromal cell line HS-5 was cultured in RPMI 1640 medium (BOSTER, Wuhan, China) containing 10% fetal calf serum (ExCell Bio, Shanghai, China) and grown at 37 °C in 5% CO₂ humidified atmosphere.

SiRNA transfection
Knockdown of NEDD4L expression used for loss-of-function experiments was done by siRNA. The siNEDD4L (sense strand: 5'-CCUCUGUAUGGAGGCAUUU-3' and antisense strand: 5'-AAAUGAUCCUAUUACAGAGG-3') [28] were purchased from GenePharma (Shanghai, China). SiRNA transfection was performed using the X-tremeGENE siRNA Transfection Reagent (Roche, Basel, Switzerland) according to the manufacturer’s instructions. Transfected cells were used for experiments in 48 h after siRNA transfection.

Cell proliferation assays
The tested cells (1 × 10^5 cells/mL) for 2 mL per well were seeded in a 6-well plate. After culturing for 0, 1 and 2 days, cells were counted in counting board for three times, respectively.

Cell apoptosis assays
The tested cells (2 × 10^5 cells/mL) for 2 mL per well were seeded in a 6-well plate. After culturing for 2 days, cells were used for apoptosis assays which were performed using Annexin V PE Apop Dtec Kit (BD Pharmingen, San Diego, CA) via flow cytometry. Each experiment was repeated three times.

Statistical analysis
Statistical analysis was accomplished by SPSS 22.0 software package. Pearson’s χ²/Fisher’s exact test and Mann–Whitney’s U/Kruskal–Wallis H test were used for the comparison of categorical and continuous variables, respectively. The impact of NEDD4L expression on leukemia-free survival (LFS)/event-free survival (EFS) and overall survival (OS) was analyzed using the Kaplan–Meier method. The receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) value were applied to determined NEDD4L expression in distinguishing AML from controls. The statistical P-values were two-sided and less than 0.05 in all analyses were considered as statistically significant differences.

Results
Low NEDD4L expression associated with AML
To investigate NEDD4L expression pattern in human cancers, we first used the CCLE databases. It was showed that NEDD4L was the lowest expression level in AML cell lines among 40 types of human cancer cell lines (Fig. 1a). Moreover, low NEDD4L expression was also closely correlated with myeloid cell lines, which was revealed by the HPA databases (Fig. 1b). Then, we
further explored NEDD4L expression in human cancer samples and normal controls by using the GEPIA databases. Among the 33 types of human cancers, significant differences of NEDD4L expression between patients and controls were observed in 10 kinds of human cancers. In detail, eight of them showed increased expression, whereas two of them presented decreased expression including AML (Fig. 1c, d). Moreover, reduced expression of NEDD4L in AML subtypes was also showed by BloodSpot online tool (Fig. 1e). In summary, low NEDD4L expression was closely associated with AML among the 40 types of human cancers.

Validation of NEDD4L expression in AML

To validate the expression pattern of NEDD4L expression in AML, we further detected NEDD4L mRNA expression in BMMNCs samples of another independent cohort of AML patients who were treated in our hospital. As expectedly, NEDD4L expression was significantly reduced in ND-AML (median 0.073, range 0.000–0.735) compared with CR-AML (median 0.140, range 0.003–1.000) ($P=0.017$, Fig. 2a). Moreover, ROC analysis revealed that NEDD4L expression may be served as a potential biomarker for distinguishing ND-AML from CR-AML with an AUC value of 0.645 (95% confidence interval: 0.532–0.758, $P=0.017$, Fig. 2b). These results further confirmed the low expression pattern of NEDD4L in AML and revealed that NEDD4L expression might serve as an underlying biological marker helpful for the diagnosis of AML.

Distinct association of NEDD4L expression with clinical features in AML

When analyzed the clinical implication of NEDD4L expression in AML, the whole-cohort cases were divided into two groups by the median level of NEDD4L expression. Comparison of clinic-pathologic characteristics between the two groups was presented in Table 1. AML cases with low NEDD4L expression exhibited markedly lower white blood cell (WBC) counts than those with high NEDD4L expression ($P<0.001$). Moreover, NEDD4L low-expressed patients presented quite higher BM and peripheral blood (PB) blasts than NEDD4L high-expressed patients ($P=0.002$ and 0.005, receptively). Moreover, significantly differences were found in the distribution of cytogenetics between low and high NEDD4L expressed groups ($P<0.001$). Low NEDD4L expression was appreciably associated with normal karyotype ($P=0.001$), hardly correlated with complex karyotypes ($P=0.001$, respectively). To further exhibit the associations of NEDD4L expression with cytogenetic classifications, NEDD4L expression level among different
karyotypes was further compared ($P < 0.001$, Fig. 3a). We further determined the significant associations of NEDD4L expression with common genetic mutations (Table 1). AML patients with low NEDD4L expression showed relatively higher incidence of FLT3, NPM1, and DNMT3A mutations ($P = 0.007, 0.001$, and $0.051$, respectively) but lower frequency of TP53, TET2, and U2AF1 mutations ($P = 0.005, 0.063$, and $0.064$, respectively) than those with high NEDD4L expression. Moreover, the level of NEDD4L expression between the mutant and wild-type groups of FLT3 ($P < 0.001$), NPM1 ($P < 0.001$), DNMT3A ($P = 0.033$), TET2 ($P = 0.088$), TP53 ($P < 0.001$), and U2AF1 ($P = 0.033$) genes was further exhibited (Fig. 3b–g). All these results suggested that aberrant NEDD4L expression was correlated with diverse genetic events in AML.

**Prognostic value of NEDD4L expression in AML**

We first determined the effect of NEDD4L expression on survival (OS and LFS) in AML from TCGA cohort. Although no remarkably differences of OS and LFS were observed between low- and high- NEDD4L expression groups among total AML ($P = 0.952$ and 0.972, respectively, Additional file 2: Fig. S1), patients with low NEDD4L expression tended to have shorter OS and LFS time than those with high NEDD4L expression among CN-AML ($P = 0.161$ and 0.122, respectively, Additional file 2: Fig. S1). Next, we analyzed the GEO datasets (GSE12417) including two cohorts of 78 and 162 CN-AML patients to evaluate the prognostic significance of NEDD4L expression in AML. The Genomicscape online tool through Kaplan–Meier analysis demonstrated that low NEDD4L expression was greatly correlated with shorter OS time in both 78 CN-AML (probe 212445_s_at: $P = 0.033$ and probe 241396_at: $P = 0.087$) and 162 CN-AML (probe 212445_s_at: $P = 0.0025$ and probe 241396_at: $P = 0.041$) cohorts (Fig. 4a). Moreover, the prognostic value of NEDD4L expression on EFS and OS was further confirmed in another two independent cohorts of CN-AML from GSE6891 (probe 212445_s_at: $P = 0.019$ and 0.005, respectively; probe 241396_at: $P < 0.001$ and 0.001, respectively) and GSE10358 (probe 212445_s_at: $P = 0.316$ and 0.076, respectively; probe 241396_at: $P = 0.005$ and 0.001, respectively) datasets (Fig. 4b, c). Taken together, low NEDD4L expression was correlated with unfavorable prognosis in CN-AML, and might serve as an underlying marker in AML prognosis prediction.

**Biological insights of aberrant NEDD4L expression in AML**

In order to take better understanding of biological insights correlated with aberrant NEDD4L expression in AML among TCGA databases, we first compared the transcriptomes between high and low NEDD4L expression groups in AML from TCGA cohorts. A number of 1024 DEGs including 933 upregulated and 91 downregulated (high vs low) were obtained between two groups (log2 FC $> 1.5$, FDR $< 0.05$ and $P < 0.05$) (Fig. 5a, b and Additional file 3: Table S2). The top 50 upregulated genes including CDH1 and the top 50 downregulated genes
such as H19 were significantly associated with prognosis in AML by our previous studies [29, 30]. In addition, the GO (Gene Ontology) analysis demonstrated that these DEGs involved in biologic processes, including multicellular organismal process, system development, multicellular organism development, and biological adhesion (Fig. 5c). Taken together, all the results supported the prognostic impact of low NEDD4L expression with potential role in AML.

We next determined the microRNA expression signature between low and high NEDD4L expression groups in AML among TCGA databases. We identified 39 differential expressed microRNAs including 27 upregulated and 12 downregulated between two groups (|log2 FC| > 1.0, FDR < 0.05 and \( P < 0.05 \)) (Fig. 5d, Additional file 3: Table S2). Downregulated microRNAs such as miR-375, miR-10a, and miR-100 were observed to be overexpressed in AML or have proto-leukemia effects in previous investigations [31–36]. These results together supported the anti-leukemia role and the prognostic effects of NEDD4L during leukemogenesis. Moreover, among these downregulated microRNAs, miR-10a was also discovered as a microRNA that could directly target NEDD4L (Fig. 5e, Additional file 4: Table S3), which indicated that NEDD4L may be seen as a directly target of miR-10a in AML.

Validation of the biological role of NEDD4L in AML
To validate the potential role of NEDD4L in AML development, we next performed in vitro experiments in leukemic cells. Since it is difficult to successfully transfect NEDD4L that has too long coding sequence (CDS > 2000 bp) into suspension cells, we conducted loss-of-function assays in the highest NEDD4L-expressed cells K562 (Fig. 6A). The successfully knockdown of NEDD4L expression in K562 cells by siRNAs was confirmed through RQ-PCR (Fig. 6B). Expectedly, K562-siNEDD4L cells presented markedly increased proliferation rate (Fig. 6C) and decreased apoptosis rate as compared with K562-siNC cells (Fig. 6D–F). Moreover, apoptosis-related markers CASP3 and CASP8 were remarkably reduced after NEDD4L Knockdown in K562 cells (Fig. 6G and H). All these results together suggested that NEDD4L may play a tumor suppressive role in AML biology.

Discussion
In the current investigation, we for the first time explored NEDD4L expression in AML, and demonstrated that low NEDD4L expression was a frequent event in AML.

Table 1 Correlation of NEDD4L expression with clinic-pathologic characteristics in AML

| Patient’s parameters | NEDD4L expression | \( P \) value |
|----------------------|-------------------|-------------|
|                      | Low (n = 87)      | High (n = 86) |
| Sex, male/female     | 48/39             | 44/42       | 0.353       |
| Median age, years (range) | 55 (21–77)    | 61 (18–88)  | 0.017       |
| Median WBC, \( \times 10^9 \) /L (range) | 31.5 (0.9–223.8) | 8.6 (0.4–297.4) | 0.000       |
| Median PB blasts, % (range) | 50 (0–97)     | 22 (0–98)  | 0.002       |
| Median BM blasts, % (range) | 76 (32–100)  | 62.5 (30–99) | 0.005       |
| FAB classifications   |                   |             |
| M0                   | 7                 | 9           | 0.611       |
| M1                   | 21                | 23          | 0.729       |
| M2                   | 22                | 16          | 0.359       |
| M3                   | 11                | 5           | 0.188       |
| M4                   | 13                | 21          | 0.130       |
| M5                   | 12                | 6           | 0.212       |
| M6                   | 0                 | 2           | 0.246       |
| M7                   | 0                 | 3           | 0.121       |
| No data              | 1                 | 0           | 1.000       |
| Cytogenetics         |                   |             |
| Normal               | 51                | 29          | 0.001       |
| t (15;17)            | 10                | 5           | 0.280       |
| t (8;21)             | 6                 | 1           | 0.117       |
| Inv (16)             | 3                 | 7           | 0.211       |
| +8                   | 3                 | 5           | 0.496       |
| Del (5)              | 0                 | 1           | 1.000       |
| −7/del (7)           | 2                 | 5           | 0.278       |
| 11q23                | 2                 | 1           | 1.000       |
| Others               | 3                 | 11          | 0.028       |
| Complex              | 5                 | 20          | 0.001       |
| No data              | 2                 | 1           | 1.000       |
| Gene mutation        |                   |             |
| FLT3(±)              | 33/54             | 16/70       | 0.007       |
| NPM1(±)              | 34/53             | 14/72       | 0.001       |
| DNMT3A(±)            | 27/60             | 15/71       | 0.051       |
| IDH2(±)              | 9/78              | 8/78        | 1.000       |
| IDH1(±)              | 8/79              | 8/78        | 1.000       |
| TET2(±)              | 4/83              | 11/75       | 0.063       |
| RUNX1(±)             | 10/77             | 14/72       | 0.388       |
| TPS3(±)              | 2/85              | 12/74       | 0.005       |
| NRAS(±)              | 5/82              | 7/79        | 0.566       |
| CEBPA(±)             | 7/80              | 6/80        | 1.000       |
| WT1(±)               | 7/80              | 3/83        | 0.329       |
| PTPN11(±)            | 4/83              | 4/82        | 1.000       |
| KIT(±)               | 3/84              | 4/82        | 0.720       |
| U2AF1(±)             | 1/86              | 6/80        | 0.064       |
| KRAS(±)              | 4/83              | 3/83        | 1.000       |

AML acute myeloid leukemia, WBC white blood cells, PB peripheral blood, BM bone marrow, FAB French-American-British
Moreover, *NEDD4L* expression was appreciably linked to the clinical outcome of CN-AML. Although it is the first report regarding the prognostic significance of *NEDD4L* expression in AML, several studies have shown the great correlations of *NEDD4L* expression with clinical outcome in solid tumors [9–16]. Reduced expression of *NEDD4L* correlated with adverse prognosis in non-small cell lung cancer, gastric cancer, hepatocellular carcinoma, ovarian cancer, and malignant glioma [9–16]. In addition, we also determined the potential role of *NEDD4L* correlated with adverse prognosis in non-small cell lung cancer, gastric cancer, hepatocellular carcinoma, ovarian cancer, and malignant glioma [9–16]. In addition, we also determined the potential role of *NEDD4L* in AML by further functional study validation, and showed the anti-proliferative and pro-apoptotic effects of *NEDD4L* in leukemic cell line K562, which suggested that *NEDD4L* may play a tumor suppressive role in AML biology. However, only a few studies determined the direct role of *NEDD4L* in tumorogenesis [10]. Accordingly, further clinical and functional studies are required to explore the potential role of *NEDD4L* in AML occurrence and development.

Additionally, we also observed a markedly correlation of *NEDD4L* expression with cytogenetic/genetic classifications in AML by our studies. Underexpression of *NEDD4L* was observed to be correlated with normal karyotype, FLT3 and NPM1 mutations, but negatively associated with complex karyotype and TP53 mutations. Notably, a recent study also showed that abnormal *NEDD9* expression, a member of *NEDD* family, was highly correlated with specific French-American-British (FAB) subtypes and karyotypes as well as genetic mutations, which was similar to our results [37]. These results together disclosed that *NEDD4L* underexpression play a key role in CN-AML biology caused by genetic mutations. Future studies are needed to determine the potential associations of aberrant *NEDD4L* expression with genetic abnormalities in CN-AML.

Accumulating studies have reported the expression of *NEDD4L* was regulated by microRNAs during biological processes including cancer development. For instance, *miR-98* by directly targeting *NEDD4L* played a key role in alleviating renal fibrosis in diabetic nephropathy [38]. *MiR-494* inhibited the TGF-beta1/Smads signaling pathway and prevented the development of hypospadias through targeting *NEDD4L* [39]. Chen et al. demonstrated that IGF-1-enhanced *miR-513a-5p* signaling desensitized glioma cells to temozolomide through targeting the *NEDD4L*-inhibited Wnt/beta-catenin pathway [40]. The *miR-106b-25* cluster through the direct repression of *NEDD4L* mediated breast tumor initiation by the activation of NOTCH1 signaling [41]. Moreover, Zhu et al. reported that the E3 ubiquitin ligase *NEDD4/NEDD4L* was directly regulated by *miR-1* [42].

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**Fig. 3** The associations of *NEDD4L* expression with cytogenetic/genetic abnormalities in AML. a *NEDD4L* expression among different cytogenetics of AML. *NEDD4L* expression only in normal karyotype, t(8;21), and complex karyotypes exhibited markedly difference when compared with the other karyotypes. *P* < 0.05; **P** < 0.01; ***P** < 0.001. b *NEDD4L* expression in AML patients with and without FLT3 mutations. c *NEDD4L* expression in AML patients with and without NPM1 mutations. d *NEDD4L* expression in AML patients with and without DNMT3A mutations. e *NEDD4L* expression in AML patients with and without TP53 mutations. f *NEDD4L* expression in AML patients with and without TET2 mutations. g *NEDD4L* expression in AML patients with and without U2AF1 mutations. The difference between two groups was compared by Mann–Whitney’s U test.
In this study, as far as we know, it is the first time to report the negative correlation of \textit{NEDD4L} expression with \textit{miR-10a} in AML. Although luciferase assays were not conducted to verify the direct link between \textit{miR-10a} and \textit{NEDD4L}, an increasingly number of studies revealed the oncogenic role of \textit{miR-10a} with prognostic value in AML \cite{32–34}. All the literatures in turn supported the association of \textit{NEDD4L} with \textit{miR-10a} together with prognostic value in AML.

**Conclusions**

In summary, our findings demonstrated that \textit{NEDD4L} underexpression, as a frequent event in AML, was associated with genetic abnormalities and prognosis in AML. Moreover, \textit{NEDD4L} expression may be involved in leukemogenesis with potential therapeutic target value.
Fig. 5 Biological insights of aberrant NEDD4L in AML. a Expression heatmap of differentially expressed genes between NEDD4L overexpression and underexpression groups in AML (|log2 FC|> 1.5, FDR < 0.05 and P < 0.05). b Volcano plot of differentially expressed genes between NEDD4L overexpression and underexpression groups in AML. c Gene Ontology analysis of differentially expressed genes conducted using online website of STRING (http://string-db.org). d Expression heatmap of differentially expressed microRNAs between NEDD4L overexpression and underexpression groups in AML. e Venn results of microRNAs which could target NEDD4L predicted by miRDB (http://mirdb.org/miRDB/), TargetScan (http://www.targetscan.org/vert_72/), starBase (http://starbase.sysu.edu.cn/) and miRWalk (http://mirwalk.umm.uni-heidelberg.de/)
Abbreviations
AML: Acute myeloid leukemia; NEDD4: Neural precursor cell expressed developmentally downregulated protein 4; FAB: French-American-British; CCLE: Cancer Cell Line Encyclopedia; HPA: Human Protein Atlas; GEPIA: Gene Expression Profiling Interactive Analysis; TCGA: The Cancer Genome Atlas; GTEx: Genotype-tissue expression; CN-AML: Cytogenetically normal AML; GEO: Gene Expression Omnibus; ND-AML: AML at newly diagnosis time; CR: Complete remission; CR-AML: AML at complete remission time; BM: Bone marrow; BMMNCs: BM mononuclear cells; RT-qPCR: Real-time quantitative PCR; DEGs: Differential expression genes; LFS: Leukemia-free survival; EFS: Event-free survival; GO: Gene ontology; CDS: Coding sequence.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12935-021-02327-7.

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

Additional file 2: Figure S1. The impact of NEDD4L expression on survival of AML patients from TCGA cohort. The effects of NEDD4L expression on leukemia-free survival and overall survival were determined by Kaplan–Meier methods using log-rank test in both total AML and CN-AML patients.

Additional file 3: Table S2. Differentially expressed RNAs and microRNAs between low and high NEDD4L expression groups.

Additional file 4: Table S3. Venn results of microRNAs targeting NEDD4L.

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None.

Authors’ contributions
J-dZ and T-jZ conceived and designed the experiments; M-qC performed the experiments; L-cZ analyzed the data; QY collected the clinical data; J-dZ wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials
All the data involved in this study had been included in the manuscript. The public data and the several datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethical approval and consent to participate
The present study approved by the Ethics Committee of the Affiliated People’s Hospital of Jiangsu University. Written informed consents were obtained from all enrolled individuals prior to their participation.

Consent for publication
All the co-authors agreed to publish the final version of this manuscript.

Competing interests
The authors declare that they have no competing interests.
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