High expression of CPNE3 predicts adverse prognosis in acute myeloid leukemia

Lin Fu,1,4,5,10 Huaping Fu,4,10 Jianlin Qiao,4,10 Yifan Pang,8 Keman Xu,9 Lei Zhou,7 Qingyun Wu,4 Zhenyu Li,4 Xiaoyan Ke,1 Kailin Xu4 and Jinlong Shi2,3,5

© 2017 The Authors. Cancer Science published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.

Abstract: Acute myeloid leukemias (AML), which harbor mutations and aberrantly expressed genes,1 microRNA,2 lncRNA3 and changes in DNA methylation that are potential prognostic markers,4 are a group of myeloid malignancies with remarkably heterogeneous outcomes.5 The need to find effective prognostic biomarkers is pressing and has become a research hotspot.

ERBB signaling pathway, a paradigm for oncogene addiction,6 promotes AML growth.7 ERBB2, which can promote breast cancer growth, metastasis and drug-resistance, is an important factor of ERBB signaling pathway.8 CPNE3, as a phosphoprotein with associated kinase activity9 and a novel metastasis-promoting gene in non-small-cell lung cancer,10 has been identified as a ligand of ERBB2 and has a more general role in carcinogenesis.11 Jun activation domain-binding protein 1 (Jab1) can enhance the ERBB2-binding ability of CPNE3, further activating the ERBB signaling pathways involved in breast cancer cell pathogenesis.12

According to the role of ERBB2 in the pathogenesis of carcinogenesis, it was speculated that the expression of CPNE3 might be related to prognosis in patients with AML. Here, we demonstrate CPNE3 as an adverse prognostic biomarker for AML based on analysis of two separate datasets. We also explore the distinctive gene/microRNA patterns and cell signaling pathways associated with CPNE3 expression. In conclusion, CPNE3 is an independent, adverse prognostic factor in AML and might guide treatment decisions towards allogeneic HCT.

Key words
Acute myeloid leukemia, CPNE3, expression, predicts, prognosis

Correspondence
Jinlong Shi, Department of BioMedical Engineering, Department of Medical Big Data, Chinese PLA General Hospital, Beijing, China. Tel: +86-10-66936921; Fax: +86-10-66936921; E-mail: jinlong_301@163.com and Xiaoan Ke, Department of Hematology and Lymphoma Research Center, Third Hospital, Peking University, Beijing, China. Tel: +86-10-82266699; Fax: +86-10-82266699; E-mail: xiaoyanx@yahoo.com and Kailin Xu, Department of Hematology, the Affiliated Hospital of Xuzhou Medical University, Xuzhou, China. Tel: +86-516-85601527; Fax: +86-516-85601527; E-mail: lihmd@163.com

10 These authors contributed equally to this work.

Funding information
This work was supported by grants from the National Natural Science Foundation of China (81500118, 61501519), the China Postdoctoral Science Foundation (through project No. 2016M600443) and the PLAGH Project of Medical Big Data (2016-MBD-025).

Received May 4, 2017; Revised June 27, 2017; Accepted June 28, 2017

Cancer Sci 108 (2017) 1850–1857
doi: 10.1111/cas.13311
Table 1. Comparison of clinical and molecular characteristics of 272 acute myeloid leukemia (AML) patients according to CPNE3 expression

| Variable                  | AML | IR-AML | CN-AML |
|---------------------------|-----|--------|--------|
|                           | CPNE3\textsuperscript{high} (n = 136) | CPNE3\textsuperscript{low} (n = 136) | P |
|                           | CPNE3\textsuperscript{high} (n = 67) | CPNE3\textsuperscript{low} (n = 68) | P |
|                           | CPNE3\textsuperscript{high} (n = 64) | CPNE3\textsuperscript{low} (n = 65) | P |
| Median age, years         | 0.11 | 0.12   | 0.15   |
| Median                    | 47   | 41     | 49     |
| Range                     | 15–59| 16–59  | 18–59  |
| Female sex                | 0.18 | 1      | 0.015  |
| Median                    | 15.7 | 41.74  | 18.2   |
| Range                     | 0.07–214.5 | 0.43–210.9 | 0.07–214.5 | 0.43–210.9 | 0.07–214.5 | 0.43–210.9 |
| EFS, months               | 0.15 | 0.003  | 0.003  |
| Median                    | 8.81 | 27.12  | 10.68  |
| Range                     | 0.03–214.5 | 0.03–210.9 | 0.03–214.5 | 0.03–210.9 | 0.03–214.5 | 0.03–210.9 |
| FAB subtype, n (%)        |      |        |        |
| M0                        | 5 (3.7) | 6 (4.4) | 1 |
| M1                        | 21 (15.4) | 45 (33.1) | 0.001  |
| M2                        | 17 (12.5) | 44 (32.3) | <0.001 |
| M4                        | 43 (31.6) | 13 (9.6) | <0.001 |
| M5                        | 41 (30.2) | 21 (15.4) | 0.006  |
| M6                        | 0     | 2 (1.5) | 0.5 |
| Others                    | 9 (6.6) | 5 (3.7) | 0.41  |
| Cytogenetics, n (%)       |      |        |        |
| CBF-AML                   | 20 (14.7) | 25 (18.4) | 0.5  |
| t1q23/MLL                 | 2 (1.5) | 4 (2.9) | 0.68  |
| CN-AML                    | 72 (52.9) | 57 (41.9) | 0.09  |
| Others                    | 42 (30.9) | 50 (36.8) | 0.37  |
| NPM1\textsuperscript{mut}/FLT3\textsuperscript{WT}, n (%) | 11 (8.1) | 16 (11.8) | 0.42  |
| CEBPA, n (%)              |      |        |        |
| Single Mut                | 3 (2.2) | 5 (3.7) | 0.72  |
| Double Mut                | 1 (0.7) | 20 (14.7) | <0.001 |
| Wild-type                 | 132 (97.1) | 111 (81.6) | <0.001 |
| FLT3-ITD/NPM1\textsuperscript{WT} (%) | 20 (14.7) | 11 (8.1) | 0.13  |
| IDH1 mutation, n (%)      | 12 (8.9) | 12 (8.9) | 1 |
| IDH2, Mut, (%)            | 7 (5.1) | 17 (12.5) | 0.05  |
| NRAS, Mut, n (%)          | 14 (10.3) | 12 (8.8) | 0.84  |
| KRAS, Mut, n (%)          | 3 (2.2) | 1 (0.7) | 0.62  |

EFS, event-free survival; FAB, French-American-British classification; ITD, internal tandem duplication; Mut: mutated; WT, wild type; OS, overall survival; CBF-AML, AML1-ETO and CBFB-MYH11.
Methods

Patients. The first cohort was derived from a whole AML cohort (n = 272, aged <60 years) diagnosed and collected at Erasmus University Medical Center (Rotterdam) between 1990 and 2008, approved by the institutional review boards at Weill Cornell Medical College and Erasmus University Center, and all subjects provided written informed consent in accordance with the Declaration of Helsinki. All patients were uniformly treated under the study protocols of the Dutch–Belgian Cooperative Trial Group for Hematology Oncology (HOVON; details of the therapeutic protocol are available from http://www.hovon.nl). All samples were collected at diagnosis containing 80%–100% blast cells after thawing. Total RNA from mononuclear cells was extracted by lysis with guanidium isothiocyanate followed by cesium chloride gradient purification. CPNE3 expression values were measured by Affymetrix HGU133 plus 2.0 arrays. All clinical, cytogenetic and molecular information as well as microarray data of these patients were publicly accessible at the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo, GSE6891). (13)

The second cohort was derived from The Cancer Genome Atlas (TCGA) dataset, including 200 clinically annotated adult de novo AML samples. In this cohort, RNA sequencing for 179 samples and miRNA sequencing for 194 samples has been reported previously. (14) These sequencing data provided exact measures for expression levels. Detailed descriptions of clinical and molecular characteristics were also provided. All these data were publicly accessible from the TCGA website. Written informed consent was obtained from all patients, and was approved by the human studies committee at Washington University.

Statistical analyses. Overall survival (OS) was defined as the time from the date of diagnosis to death due to any cause. Event-free survival (EFS) was defined as the time from the date of diagnosis to removal from the study due to the absence of complete remission, relapse or death from any cause. Statistical distribution and quartiles of CPNE3 expressions were used to define the optimal cut-off. First, CPNE3 expression was found to be normally distributed in AML patients (Fig. S1a). Second, all the AML patients were divided into four subgroups (Q1: <25%, Q2: 25%–50%, Q3: 50%–75%, Q4: >75%) based on the quartile of CPNE3 expression value; however, no significant difference was observed between Q1 and Q2 (OS: Q12, P = 0.169), just as for the result for Q23 and Q34 (OS: Q23, P = 0.132, Q34, P = 0.128, respectively). (Fig. S1b). Thus, we chose median value of CPNE3 expression as the cut-off, and defined the highest 50% CPNE3 expressers as CPNE3high and the lowest 50% CPNE3 expressers as CPNE3low, respectively. In the first cohort, microarray expression profiles were obtained by Affymetrix Human Genome 133 plus 2.0 and U133A Gene Chips from GSE6891 data. All experiments’ design, quality control and data normalization were in line with the standard Affymetrix protocols. To investigate the associations between CPNE3 expression levels and clinical, molecular characteristics, the Fisher exact and Wilcoxon rank-sum tests were used for hypothesis testing with categorical and continuous variables, respectively. Multivariate Cox proportional hazard models were employed to study the associations between CPNE3 expression levels and OS and EFS in the presence of other known risk factors. The Kaplan–Meier method and the log-rank test were utilized to estimate the association between OS, EFS and CPNE3 expression. Student’s t-test and multiple hypothesis correction (false discovery rate, FDR) were used to identify differences in gene/miRNA expression in CPNE3high and CPNE3low groups. The statistical cutoff values were an absolute fold-change (FC)
Table 2. Multivariable analysis with OS and EFS in the primary cohort of 272 AML patients

| Variables in final model by end points | HR/OR  | 95% CI | P-value |
|----------------------------------------|--------|--------|---------|
| OS (AML, n = 272)                       |        |        |         |
| CPNE3 expression, high versus low       | 1.71   | 1.23-2.38 | 0.001  |
| CBF-AML, yes versus no                  | 0.54   | 0.33-0.89 | 0.017  |
| Single CEBPA mutation versus wild       | 1.46   | 0.59-3.60 | 0.412  |
| Double CEBPA mutation versus wild       | 0.38   | 0.16-0.89 | 0.025  |
| NPM1mut/FLT3wt, presented versus others | 0.45   | 0.23-0.87 | 0.017  |
| FLT3-ITD, presented versus others       | 1.26   | 0.89-1.79 | 0.194  |
| EFS (AML, n = 272)                      |        |        |         |
| CPNE3 expression, high versus low       | 1.73   | 1.26-2.36 | 0.0007 |
| CBF-AML, yes versus no                  | 0.59   | 0.37-0.93 | 0.02   |
| Single CEBPA mutation versus wild       | 1.64   | 0.66-4.07 | 0.29   |
| Double CEBPA mutation versus wild       | 0.52   | 0.25-1.04 | 0.066  |
| NPM1mut/FLT3wt, presented versus others | 0.52   | 0.29-0.93 | 0.028  |
| FLT3-ITD, presented versus others       | 1.16   | 0.83-1.63 | 0.37   |
| OS (IR-AML, n = 135)                    |        |        |         |
| CPNE3 expression, high versus low       | 1.71   | 1.04-2.79 | 0.03   |
| Single CEBPA mutation versus wild       | 0.66   | 0.09-4.84 | 0.69   |
| Double CEBPA mutation versus wild       | 0.38   | 0.15-1.01 | 0.05   |
| NPM1mut/FLT3wt, presented versus others | 0.49   | 0.25-0.97 | 0.04   |
| FLT3-ITD, presented versus others       | 1.31   | 0.69-2.53 | 0.41   |
| EFS (IR-AML, n = 135)                   |        |        |         |
| CPNE3 expression, high versus low       | 1.59   | 1.00-2.52 | 0.049  |
| Single CEBPA mutation versus wild       | 0.64   | 0.09-4.70 | 0.660  |
| Double CEBPA mutation versus wild       | 0.57   | 0.26-1.25 | 0.157  |
| NPM1mut/FLT3wt, presented versus others | 0.59   | 0.32-1.09 | 0.091  |
| FLT3-ITD, presented versus others       | 1.14   | 0.60-2.17 | 0.692  |
| OS (CN-AML, n = 129)                    |        |        |         |
| CPNE3 expression, high versus low       | 2.06   | 1.26-3.35 | 0.004  |
| Single CEBPA mutation versus wild       | 2.13   | 0.65-7.05 | 0.214  |
| Double CEBPA mutation versus wild       | 0.66   | 0.27-1.64 | 0.372  |
| NPM1mut/FLT3wt, presented versus others | 0.50   | 0.22-1.16 | 0.105  |
| FLT3-ITD, presented versus others       | 1.28   | 0.77-2.11 | 0.342  |
| EFS (CN-AML, n = 129)                   |        |        |         |
| CPNE3 expression, high versus low       | 1.76   | 1.11-2.79 | 0.02   |
| Single CEBPA mutation versus wild       | 2.44   | 0.73-8.11 | 0.15   |
| Double CEBPA mutation versus wild       | 0.79   | 0.35-1.76 | 0.56   |
| NPM1mut/FLT3wt, presented versus others | 0.71   | 0.34-1.46 | 0.35   |
| FLT3-ITD, presented versus others       | 1.32   | 0.82-2.12 | 0.26   |

AML, acute myeloid leukemia; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival.

≥1.5 and an adjusted P-value ≤0.05. In the second cohort, expression data was obtained with whole-genome high-throughput sequencing. The associations between CPNE3 expression and the OS, EFS and RFS were analyzed using the Kaplan–Meier method and the log-rank test. All analyses were performed using the R 3.1.1 software packages.

Results

Differences in clinical and molecular characteristics between CPNE3high and CPNE3low groups. We analyzed the impact of CPNE3 mRNA expression on clinical and molecular characteristics and clinical outcome in AML patients (Table 1). Based on Student’s test of continuous variables, compared with CPNE3low, CPNE3high have a significantly shorter survival time in the entire AML (AML: OS, P < 0.001; EFS, P < 0.001, n = 272), National Comprehensive Cancer Network (NCCN) criteria of intermediate risk AML (IR-AML) (IR-AML: OS, P = 0.015; EFS, P = 0.021, n = 135) and cytogenetically normal AML (CN-AML) (CN-AML: OS, P = 0.003; EFS, P = 0.015, n = 129). CPNE3 expression showed significant associations with FAB classifications of AML. More patients with AML-M4 fell into the CPNE3low group (P < 0.001), while more patients with AML-M1 and AML-M2 fell into the CPNE3high group (P = 0.006, P = 0.02). The fact that M4 and M5 subtypes would readily develop chemotherapy resistance, suggests that CPNE3high might be an adverse prognostic factor of AML. Compared with CPNE3low in the entire AML cohort, CPNE3high carried more wild-type CEBPA (P < 0.001) and fewer double CEBPA mutations (P < 0.001), which were also shown after risk stratification by IR-AML and CN-AML. In addition, we found that CPNE3high was associated with FLT3-ITD/NPM1WT in CN-AML (P = 0.02). Both wild type of CEBPA and FLT3-ITD/NPM1WT were confirmed to represent poor molecular characteristics in AML patients. These results indicated that CPNE3high might be a useful prognosticator and a substitute for other molecular prognosticators.

CPNE3high was associated with adverse outcomes. We also analyzed the impact of CPNE3 mRNA expression on clinical outcome in AML patients (Fig. 1). CPNE3high was confirmed as an adverse prognosticator not only for the entire AML (AML: OS, P < 0.001; EFS, P < 0.001) and IR-AML (IR-AML: OS, P = 0.001; EFS, P = 0.005), but also for CN-AML (CN-AML: OS, P = 0.001; EFS, P = 0.007) and the European LeukemiaNet (ELN) Intermediate-I category (ELN Intermediate-I: OS, P < 0.001; EFS, P = 0.0027, n = 99).

CPNE3 expression was associated with shorter overall survival and event-free survival in multivariate analyses. To further assess the prognostic significance of CPNE3 expression, multivariable OS/EFS models were constructed after adjusting for established prognostic factors (Table 2). For OS, CPNE3high was proved to be a high-risk factor not only in the entire cohort of AML (HR = 1.71, P = 0.001), but also in the refined risk classifications, IR-AML (HR = 1.71, P = 0.03) and CN-AML (HR = 2.06, P = 0.004) sub-categories. Other factors associated with worse OS in the entire cohort of AML were: negative CBF (AML1-ETO and CBFB-MYH11, P = 0.017), negative double CEBPA mutations (P = 0.025) and negative NPM1mut/FLT3WT (P = 0.0017). Other factors associated with worse OS in IR-AML were: negative double CEBPA mutations and negative NPM1mut/FLT3WT (P = 0.05 and P = 0.04, respectively). In the multivariable model for EFS, CPNE3high was also proved as a high-risk factor in the cohorts of entire AML, IR-AML and CN-AML (P = 0.0007, P = 0.049 and P = 0.02, respectively). Other factors associated with poorer EFS in the cohort of AML were negative CBF and negative NPM1mut/FLT3WT (P = 0.02 and P = 0.028, respectively).

Associations between genome-wide gene-expression profiles and CPNE3 expression. First, to further explore the role of CPNE3 in leukemogenesis, we derived CPNE3-associated gene-expression profiles in the cohort CN-AML patients who had relatively uniform cytogenetical backgrounds. A total of 388 upregulated and 99 downregulated genes that were
significantly associated with CPNE3 expression ($P < 0.05$, fold change $= 1.5$) were identified (Fig. 2a). These genes are presented in the aberrant expression heat map (Fig. 2b).

Fig. 2. Genome-wide gene/microRNA expression profile and cell signaling pathways associated with CPNE3 expression. (a) Volcano plot of differential gene expression. CPNE3$^{\text{High}}$ and CPNE3$^{\text{Low}}$ were marked by red and green circles, respectively. (b) Expression heatmap of associated genes. (c) Expression heatmap of associated microRNA. (d) Boxplots of miR-181a, miR-181b, miR-181c and miR-181d expression associated with CPNE3 expression. (e) Expression heatmap of associated cell signaling pathways. (f) Boxplots of classic cell signaling pathways associated with CPNE3 expression.

Associations between genome-wide microRNA profiles and CPNE3 expression. Second, we analyzed TCGA-derived microRNA genome-wide profiles obtained by whole-genome high-
of note, several important tumorigenic pathways found to be significantly associated with CPNE3 expression. Using mean expression of all genes in a pathway to quantify its expression level, 14 downregulated and 38 upregulated pathways were significantly associated with CPNE3high (P < 0.05) (Fig. 2e). Of note, several important tumorigenic processes associated with CPNE3 expression, including “ERBB signaling pathway,” “JAK/STAT signaling pathway,” “glycolysis/gluconeogenesis,” “VEGF signaling pathway” and “Notch signaling pathway” (Fig. 2f).

Third, dysregulation of cell signaling pathways in the Molecular Signatures Database (MSigDB)(17) were used to assess the leukemogenic processes associated with CPNE3. Third, dysregulation of cell signaling pathways in the Molecular Signatures Database (MSigDB)(17) were used to assess the leukemogenic processes associated with CPNE3expression. Using mean expression of all genes in a pathway to quantify its expression level, 14 downregulated and 38 upregulated pathways were significantly associated with CPNE3high (P < 0.05) (Fig. 2e). Of note, several important tumorigenic processes associated with CPNE3 expression, including “ERBB signaling pathway,” “JAK/STAT signaling pathway,” “glycolysis/gluconeogenesis,” “VEGF signaling pathway” and “Notch signaling pathway” (Fig. 2f).

Association between CPNE3high and adverse outcomes was confirmed by TCGA dataset. The prognostic value of CPNE3high in AML was also found in another independent cohort obtained from The Cancer Genome Atlas (TCGA) database (n = 179, RNA-Seq data obtained through high throughput sequencing). A total of 145 microRNA were identified. A total of 145 microRNA were strongly in association with CPNE3 expression (P < 0.05) (Fig. 2c), including the downregulation of miR-181 family (miR-181a, P = 0.001; miR-181b, P = 0.003; miR-181c, P < 0.001; miR-181d, P < 0.001) (Fig. 2d).

The identification of prognostic factors in AML is important for the development of new targeted therapies and risk stratification treatment strategies for AML patients. CPNE3 was identified as a ligand of ERBB2, which is an important factor of ERBB signaling pathway that promotes AML growth. We found that CPNE3 showed higher expression in myelocyte, metamyelocytes and monocytes, while lower expression in early promyelocyte (Fig. S2) using publicly available expression data (http://servers.binf.ku.dk/bloodspot/), which may explain why more patients with AML-M4 and AML-M5 fell into the CPNE3high group, while more patients with AML-M1 and AML-M2 fell into the CPNE3low group in the first cohort of AML patients. In the first cohort of AML patients, CPNE3high also acted as an independent adverse prognostic factor in the entire cohort, the NCCN intermediate risk subgroup, the CN-AML subgroup, as well as the ELN Intermediate-I subgroup. Those results indicated that CPNE3high is an adverse prognostic biomarker for AML and could be used to refine the risk stratification for NCCN IR-AML and ELN Intermediate-I AML sub-groups.

Discussion

Fig. 3. The prognostic value of CPNE3 expression in the second cohort. (a) Overall survival (OS) and (b) event-free survival (EFS) of the entire AML and CN-AML patients from TCGA data. (c) OS and (d) EFS of the AML patients of CPNE3high group, CPNE3low group, allogeneic HCT group and chemotherapy-only group.

CPNE3-associated cell signaling pathways. Third, dysregulation of cell signaling pathways in the Molecular Signatures Database (MSigDB)(17) were used to assess the leukemogenic processes associated with CPNE3 expression. Using mean expression of all genes in a pathway to quantify its expression level, 14 downregulated and 38 upregulated pathways were found to be significantly associated with CPNE3high (P < 0.05) (Fig. 2c). Of note, several important tumorigenic pathways were significantly upregulated, including “ERBB signaling pathway,” “JAK/STAT signaling pathway,” “glycolysis/gluconeogenesis,” “VEGF signaling pathway” and “Notch signaling pathway” (Fig. 2f).

Association between CPNE3high and adverse outcomes was confirmed by TCGA dataset. The prognostic value of CPNE3high in AML was also found in another independent cohort obtained from The Cancer Genome Atlas (TCGA) database (n = 179, RNA-Seq data obtained through high throughput sequencing). Among the AML and CN-AML patients, CPNE3high patients had significantly adverse OS and EFS compared to CPNE3low patients (AML: OS, P = 0.01; EFS, P = 0.036). (CN-AML: OS, P = 0.018; EFS, P = 0.063) (Fig. 3a,b). In the allogeneic HCT group, there were no significant differences in OS and EFS between CPNE3high and CPNE3low groups (OS, P = 0.261; EFS, P = 0.949) (Fig. 3c,d). However, in the chemotherapy group, CPNE3high had significantly worse OS and EFS than CPNE3low patients (OS, P = 0.004; EFS, P = 0.011) (Fig. 3c,d). Moreover, CPNE3high patients had longer OS and EFS after allogeneic HCT than those receiving only chemotherapy (OS, P < 0.001; EFS, P = 0.006, respectively), but treatment modules play an insignificant role in the survival of CPNE3low patients (allygenetic HCT versus chemotherapy-only; OS, P = 0.392; EFS, P = 0.567) (Fig. 3c,d).

Discussion

The identification of prognostic factors in AML is important for the development of new targeted therapies and risk-stratified treatment strategies for AML patients. CPNE3 was identified as a ligand of ERBB2, which is an important factor of ERBB signaling pathway that promotes AML growth. We found that CPNE3 showed higher expression in myelocyte, metamyelocytes and monocytes, while lower expression in early promyelocyte (Fig. S2) using publicly available expression data (http://servers.binf.ku.dk/bloodspot/), which may explain why more patients with AML-M4 and AML-M5 fell into the CPNE3high group, while more patients with AML-M1 and AML-M2 fell into the CPNE3low group in the first cohort of AML patients. In the first cohort of AML patients, CPNE3high also acted as an independent adverse prognostic factor in the entire cohort, the NCCN intermediate risk subgroup, the CN-AML subgroup, as well as the ELN Intermediate-I subgroup. Those results indicated that CPNE3high is an adverse prognostic biomarker for AML and could be used to refine the risk stratification for NCCN IR-AML and ELN Intermediate-I AML sub-groups.
To further confirm the prognostic significance of CPNE3, we have demonstrated that CPNE3-high was associated with shorter OS and EFS in the second cohort of AML patients (TCGA database). Notably, CPNE3-high patients had longer OS and EFS after receiving allogeneic HCT than chemotherapy-only patients, but similar differences between treatment modules were not observed in CPNE3-low patients. These results confirmed that CPNE3-high is an independent, adverse prognostic factor in AML and indicated that the expression of CPNE3 may guide treatment decisions towards allogeneic HCT.

The mechanisms underlying the association between CPNE3-high and adverse treatment outcomes are unclear. In the present study, we analyzed gene and microRNA expression, and cell signaling pathways to identify biological pathways that are associated with CPNE3 expression in AML. First, it was determined that the distinctive genome-wide gene expression patterns are significantly associated with CPNE3 expression. Second, the CPNE3-associated microRNA profile was found to be associated with CPNE3 expression, as it included miR-181 family, which were proposed as tumor suppressors; in addition, their downregulation predicts adverse prognosis in AML. (22–25) HOXA9, as well as PBX3 downstream of HOXA9, could block apoptosis and promote cell growth of AML cells. (26) HOXA9 and PBX3 are direct targets of miR-181. (24) Accumulating evidence indicates that miR-181 family acts as a diagnostic marker and a potential therapeutic target for AML. (27) MiR-181a/b-enhanced drug sensitivity in chronic lymphocytic leukemia cells (28) and miR-181a could also enhance the chemotherapeutic sensitivity of chronic myeloid leukemia to imatinib. (29) Third, the distinctive cell signaling pathways were found to be associated with CPNE3 expression. These three major findings supported that CPNE3 was possibly involved in the leukemogenesis of AML and might contribute to an adverse outcome.

In summary, CPNE3-high is an independent prognostic factor for adverse prognosis in AML patients and its presence should favor allogeneic HCT in AML. Our results also indicate that CPNE3 expression can be used to refine the risk stratification for IR-AML and ELN intermediate-1 AML sub-groups. Considering the high accuracy of high-throughput sequencing just as for real-time quantitative PCR (qPCR), (30) AML patients from the TCGA database further confirmed our results regarding the prognosis of CPNE3. In CN-AML patients, distinctive gene/microRNA expression profiles and cell signaling pathways associated with CPNE3 expression provide further insights into CPNE3-related leukemogenic processes.

Acknowledgments
This work was supported by grants from the National Natural Science Foundation of China (81500118, 61501519), the China Postdoctoral Science Foundation (through project No. 2016M600443) and the PLAGH Project of Medical Big Data (2016-MBD-025).

Disclosure Statement
The authors have no conflict of interest to declare.

References
1. Mrozek K, Marucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood 2007; 109: 431–48.
2. Marucci G, Mrozek K, Radmacher MD, Garzon R, Bloomfield CD. The prognostic and functional role of microRNAs in acute myeloid leukemia. Blood 2011; 117: 1121–9.
3. Garzon R, Volin S, Pampananou D et al. Expression and prognostic impact of lncRNAs in acute myeloid leukemia. Proc Nat Acad USA 2014; 111: 18679–84.
4. Marucci G, Yan P, Maharry K et al. Epigenetics meets genetics in acute myeloid leukemia: clinical impact of a novel seven-gene score. J Clin Oncol 2014; 32: 548–56.
5. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. New Engl J Med 2015; 373: 1136–52.
6. Jacobi N, Seeboeck R, Hofmann E, Eger A. ErbB family signalling: a paradigm for oncogene addiction and personalized oncology. Cancers 2017; 9: 33.
7. Ufkin ML, Peterson S, Yang X, Driscoll H, Duarte C, Sathyanarayana P. miR-125a regulates cell cycle, proliferation, and apoptosis by targeting the ErbB pathway in acute myeloid leukemia. Leuk Res 2014; 38: 402–10.
8. Elizalde PV, Cordo Russo RI, Cervio MF, Schillaci R, ErbB-2 nuclear function in breast cancer growth, metastasis and resistance to therapy. Endor Relat Cancer 2016; 23: T243–57.
9. Caudell EG, Caudell JJ, Tang CH, Yu TK, Frederick MJ, Grimm EA. Characterization of human copine III as a phosphoprotein with associated kinase activity. Biochemistry 2000; 39: 13034–43.
10. Lin HC, Zhang FL, Geng Q et al. Quantitative proteomic analysis identifies CPNE3 as a novel metastasis-promoting gene in NSCLC. J Proteome Res 2013; 12: 3423–33.
11. Heinrich C, Keller C, Boulay A et al. Copine-III interacts with ErbB2 and promotes tumor cell migration. Oncogene 2010; 29: 1598–610.
12. Cho HY, Park N, Na JB, Ko ES, Park JY, Yoo JC. Direct binding of Copine3 with Jab1 activates downstream ErbB2 signaling and motility in SKBr 3 breast cancer cells. Oncol Rep 2016; 35: 1147–52.
13. Verhaak RG, Wouters BJ, Erpelinck CA et al. Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling. Haematologica 2009; 94: 131–4.
14. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. New Engl J Med 2013; 368: 2059–74.
15. Li HY, Deng DH, Huang Y et al. Favorable prognosis of biallelic CEBPA gene mutations in acute myeloid leukemia patients: a meta-analysis. Eur J Haematol 2015; 94: 439–48.
16. Santos FP, Jones D, Qiao W et al. Prognostic value of FLT3 mutations among different cytogenetic subgroups in acute myeloid leukemia. Cancer 2011; 117: 2145–55.
17. Subramanian A, Tamayo P, Mootha VK et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Nat Acad USA 2005; 102: 15545–50.
18. Vainchenker W, Constantinescu SN, JAK/STAT signaling in hematological malignancies. Oncogene 2013; 32: 2601–13.
19. Chen WL, Wang JH, Zhao AH et al. A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. Blood 2014; 124: 1645–54.
20. Song G, Li Y, Jiang G. Role of VEGF/VEGFR in the pathogenesis of leukaemias and as treatment targets (Review). Oncol Rep 2012; 28: 1935–44.
21. Gu Y, Maisero M, Banham AH. Notch signaling: its roles and therapeutic potential in hematological malignancies. Oncotarget 2016; 7: 29804–23.
22. Marucci G, Maharry K, Radmacher MD et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study. J Clin Oncol 2008; 26: 5078–87.
23. Marucci G, Radmacher MD, Maharry K et al. MicroRNA expression in cytogenetically normal acute myeloid leukemia. New Engl J Med 2008; 358: 1910–28.
24. Li Z, Huang H, Li Y et al. Up-regulation of a HOXA-PBX3 homeobox-gene signature following down-regulation of miR-181 is associated with adverse prognosis in patients with cytogenetically abnormal AML. Blood 2012; 119: 2314–24.
25. Liu S, Pan L, Guo S et al. Prognostic role of microRNA-181a/b in hematological malignancies: a meta-analysis. PLoS ONE 2013; 8: e59532.
26. Faber J, Krivtsov AV, Stubbs MC et al. HOXA9 is required for survival in human MLL-rearranged acute leukemias. Blood 2009; 113: 2375–85.
27. Weng H, Lal K, Yang FF, Chen J. The pathological role and prognostic impact of miR-181 in acute myeloid leukemia. Cancer Genet 2015; 208: 225–9.
Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Median value of CPNE3 expression as the cut-off. (a) CPNE3 expression is normally distributed. (b) The overall survival (OS) of acute myeloid leukemia (AML) patients were subdivided into four quartiles based on the quartile of CPNE3 expression.

**Fig. S2.** The hierarchical differentiation tree of relationship between CPNE3 expression level and hematopoietic cell differentiation.