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High expression of *CPNE3* predicts adverse prognosis in acute myeloid leukemia

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CPNE3*, a member of a Ca^{2+}-dependent phospholipid-binding protein family, was identified as a ligand of ERBB2 and has a more general role in carcinogenesis. Here, we identified the prognostic significance of *CPNE3* expression in acute myeloid leukemia (AML) patients based on two datasets. In the first microarray dataset (n = 272), compared to low *CPNE3* expression (*CPNE3*^{low}), high *CPNE3* expression (*CPNE3*^{high}) was associated with adverse overall survival (OS, P < 0.001) and event-free survival (EFS, P < 0.001). In the second independent group of AML patients (TCGA dataset, n = 179), *CPNE3*^{high} was also associated with adverse OS and EFS (OS, P = 0.01; EFS, P = 0.036). Notably, among *CPNE3*^{high} patients, those received allogenic hematopoietic cell transplantation (HCT) had longer OS and EFS than those with chemotherapy alone (allogeneic HCT, n = 40 *vs* chemotherapy, n = 46), but treatment modules played an insignificant role in the survival of *CPNE3*^{low} patients (allogeneic HCT, n = 32 *vs* chemotherapy, n = 54). These results indicated that *CPNE3*^{high} is an independent, adverse prognostic factor in AML and might guide treatment decisions towards allogeneic HCT. To understand its inherent mechanisms, we investigated genome-wide gene/microRNA expression signatures and cell signaling pathways associated with *CPNE3* expression. In conclusion, *CPNE3*^{high} is an adverse prognostic biomarker for AML. Its effect may be attributed to the distinctive genome-wide gene/microRNA expression and related cell signaling pathways.

Acute myeloid leukemias (AML), which harbor mutations and aberrantly expressed genes,[1] microRNA,[2] lncRNA[3] 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 activity[9] 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*^{high} 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 AML patients.

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^{high} (n = 136)	*CPNE3*^{low} (n = 136)	*P*	*CPNE3*^{high} (n = 67)	*CPNE3*^{low} (n = 68)	*P*	*CPNE3*^{high} (n = 64)	*CPNE3*^{low} (n = 65)	*P*
Median age, years			0.11			0.12			0.15
Median	47	41		49	44		49	44	
Range	15–59	16–59		18–59	16–59		18–59	16–59	
Female sex	67	55	0.18	31	31	1	30	30	1
OS, months			<0.001			0.015			0.003
Median	15.7	41.74		18.2	47.72		10.2	53.88	
Range	0.07–214.5	0.43–210.9		0.3–214.5	0.43–210.9		0.07–214.5	0.43–190.3	
EFS, months			<0.001			0.021			0.015
Median	8.81	27.12		10.68	40.39		7.74	21.06	
Range	0.03–214.5	0.03–210.9		0.03–214.5	0.03–210.9		0.03–214.5	0.03–190.3	
FAB subtype, *n* (%)									
M0	5 (3.7)	6 (4.4)	1	4 (6)	4 (5.9)	1	1 (1.6)	1 (1.5)	1
M1	21 (15.4)	45 (33.1)	0.001	11 (16.4)	28 (41.2)	0.003	12 (18.8)	29 (44.7)	0.003
M2	17 (12.5)	44 (32.3)	<0.001	9 (13.4)	13 (19.1)	0.51	7 (10.9)	15 (23.1)	0.1
M4	43 (31.6)	13 (9.6)	<0.001	16 (23.9)	4 (5.9)	0.004	15 (23.4)	6 (9.2)	0.03
M5	41 (30.2)	21 (15.4)	0.006	20 (29.9)	15 (22.1)	0.4	24 (37.5)	11 (16.9)	0.02
M6	0	2 (1.5)	0.5	0	2 (2.9)	0.5	0	1 (1.5)	1
Others	9 (6.6)	5 (3.7)	0.41	7 (10.4)	2 (2.9)	0.1	5 (7.8)	2 (3.1)	0.27
Cytogenetics, *n* (%)									
CBF-AML	20 (14.7)	25 (18.4)	0.5	—	—	—	—	—	—
11q23/MLL	2 (1.5)	4 (2.9)	0.68	—	—	—	—	—	—
CN-AML	72 (52.9)	57 (41.9)	0.09	38 (56.7)	33 (48.5)	0.44	64	65	
Others	42 (30.9)	50 (36.8)	0.37	29 (43.3)	35 (51.5)	0.44	—	—	—
NPM1^{Mut}/FLT3^{WT}, *n* (%)	11 (8.1)	16 (11.8)	0.42	11 (16.4)	15 (22.1)	0.54	9 (14.1)	10 (15.6)	1
CEBPA, *n* (%)									
Single Mut	3 (2.2)	5 (3.7)	0.72	1 (1.5)	2 (2.9)	1	2 (3.1)	2 (3.1)	1
Double Mut	1 (0.7)	20 (14.7)	<0.001	1 (1.5)	17 (25)	<0.001	1 (1.6)	14 (21.5)	<0.001
Wild-type	132 (97.1)	111 (81.6)	<0.001	65 (97)	49 (72.1)	<0.001	61 (95.3)	49 (75.7)	<0.001
FLT3-ITD/NPM1^{WT} (%)	20 (14.7)	11 (8.1)	0.13	6 (9)	3 (4.4)	0.33	13 (20.3)	4 (6.2)	0.02
IDH1 mutation, *n* (%)	12 (8.9)	12 (8.9)	1	11 (16.4)	6 (8.8)	0.21	6 (9.4)	12 (18.5)	0.2
IDH2, Mut, (%)	7 (5.1)	17 (12.5)	0.05	4 (6)	15 (22.1)	0.01	3 (4.7)	9 (13.8)	0.13
NRAS, Mut, *n* (%)	14 (10.3)	12 (8.8)	0.84	6 (9)	5 (7.4)	0.76	5 (7.8)	4 (6.2)	0.74
KRAS, Mut, *n* (%)	3 (2.2)	1 (0.7)	0.62	2 (3)	0	0.24	1 (1.6)	0	0.5

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.

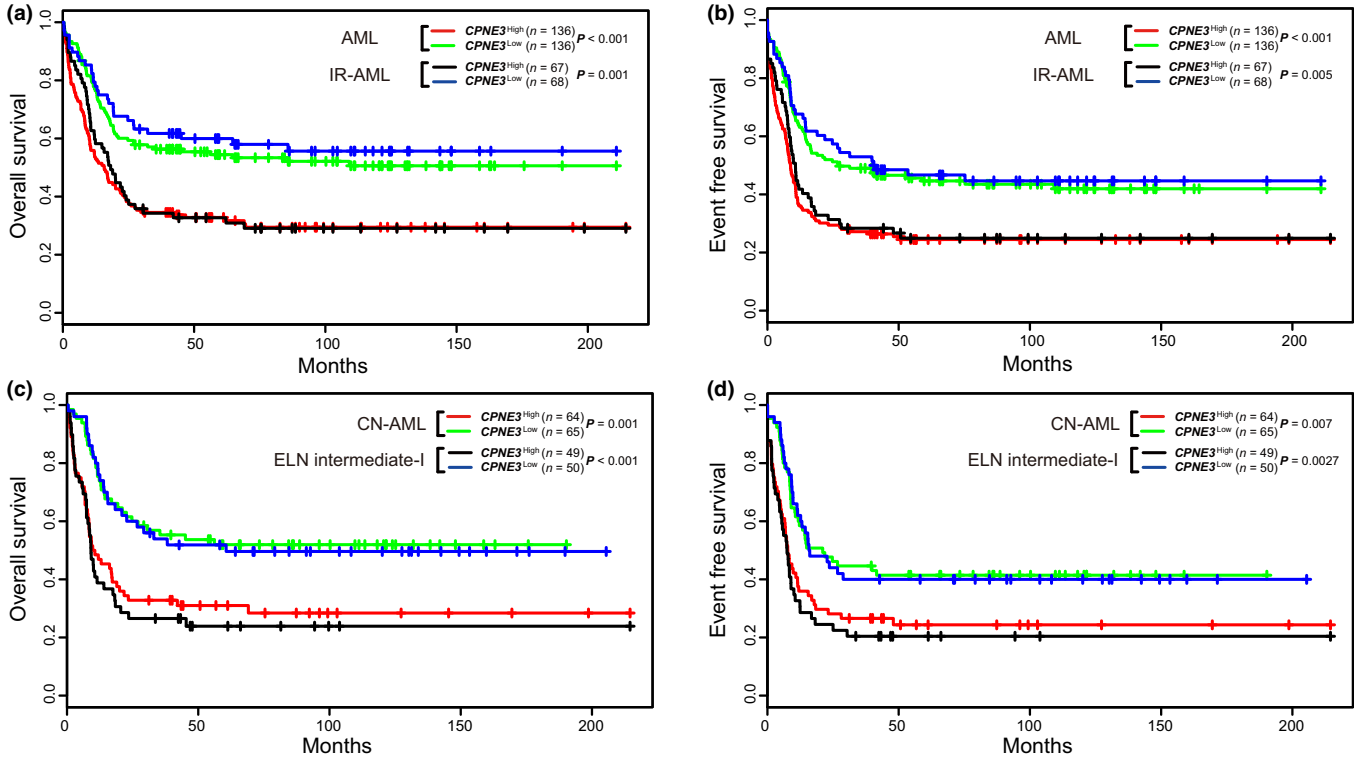

Fig. 1. The prognostic value of *CPNE3* expression in AML patients. (a) Overall survival (OS) and (b) event-free survival (EFS) of the entire cohort and the subgroup with NCCN intermediate risk. (c) OS and (d) EFS of the entire CN-AML patients and the ELN Intermediate-I category.

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 microRNA 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 and the lowest 50% *CPNE3* expressers as $CPNE3^{high}$ and $CPNE3^{low}$, 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/microRNA expression in $CPNE3^{high}$ and $CPNE3^{low}$ 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
NPM1^{Mut}/*FLT3*^{WT}, 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
NPM1^{Mut}/*FLT3*^{WT}, 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
NPM1^{Mut}/*FLT3*^{WT}, 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
NPM1^{Mut}/*FLT*^{WT}, 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
NPM1^{Mut}/*FLT3*^{WT}, 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
NPM1^{Mut}/*FLT3*^{WT}, 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 *CPNE3*^{high} and *CPNE3*^{low} 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 *CPNE3*^{low}, *CPNE3*^{high} 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 *CPNE3*^{high} group ($P < 0.001$), while more patients with AML-M1 and AML-M2 fell into the *CPNE3*^{low} group ($P = 0.001$ and $P < 0.001$, respectively). In the whole cohort of AML and CN-AML, more patients with AML-M5 fell into *CPNE3*^{high} groups ($P = 0.006$, $P = 0.02$). The fact that M4 and M5 subtypes would readily develop chemotherapy resistance, suggests that *CPNE3*^{high} might be an adverse prognostic factor of AML. Compared with *CPNE3*^{low} in the entire AML cohort, *CPNE3*^{high} 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 *CPNE3*^{high} was associated with *FLT3-ITD/NPM1*^{WT} in CN-AML ($P = 0.02$). Both wild type of *CEBPA* and *FLT3-ITD/NPM1*^{WT} represented poor molecular characteristics in AML patients.[15,16] These results indicated that *CPNE3*^{high} might be a useful prognosticator and a substitute for other molecular prognosticators.

***CPNE3*^{high} was associated with adverse outcomes.** We also analyzed the impact of *CPNE3* mRNA expression on clinical outcome in AML patients (Fig. 1). *CPNE3*^{high} 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, *CPNE3*^{high} 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 *NPM1*^{Mut}/*FLT3*^{WT} ($P = 0.0017$). Other factors associated with worse OS in IR-AML were: negative double *CEBPA* mutations and negative *NPM1*^{Mut}/*FLT3*^{WT} ($P = 0.05$ and $P = 0.04$, respectively). In the multivariable model for EFS, *CPNE3*^{high} 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 *NPM1*^{Mut}/*FLT3*^{WT} ($P = 0.02$ 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

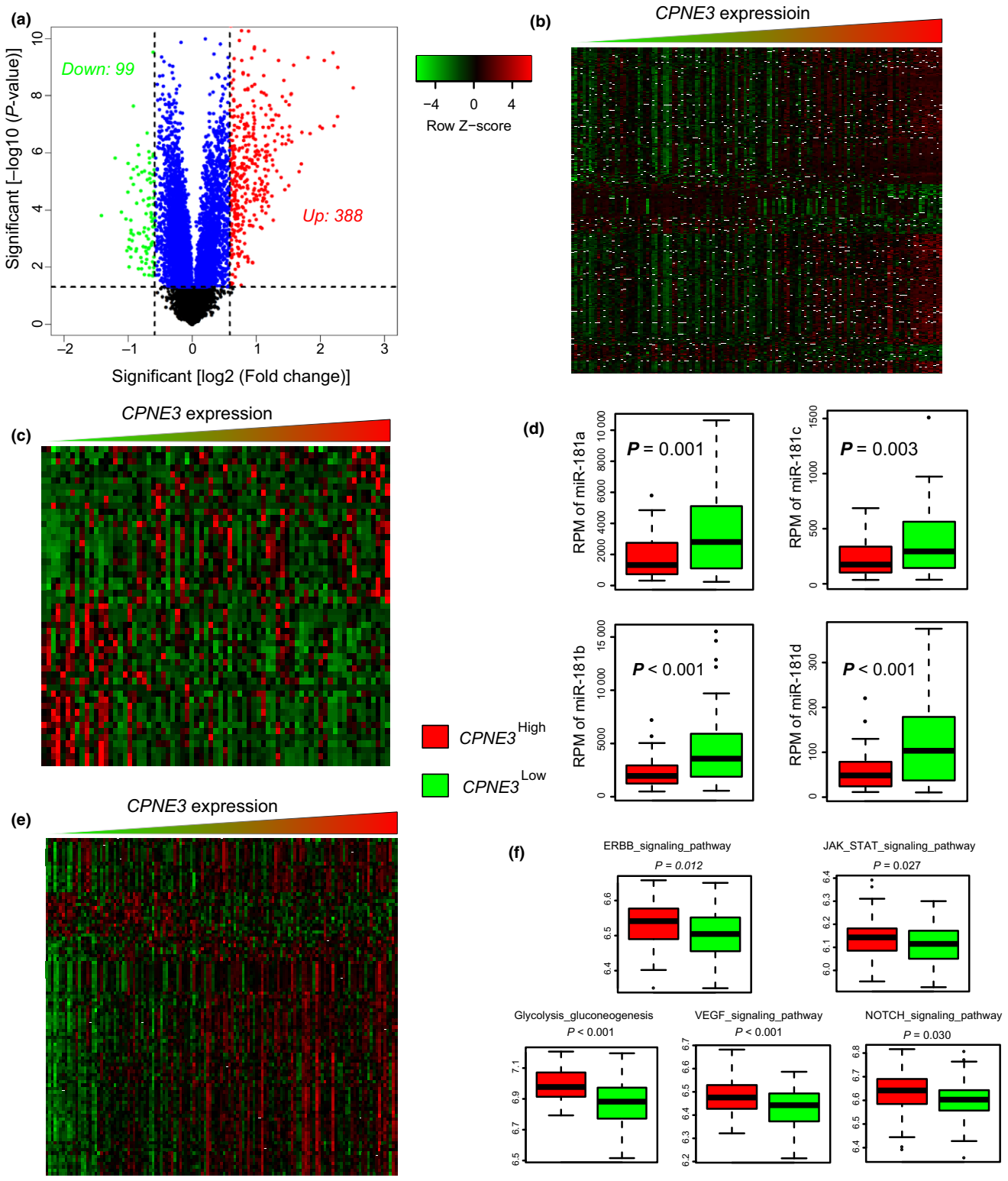

Fig. 2. Genome-wide gene/microRNA expression profile and cell signaling pathways associated with *CPNE3* expression. (a) Volcano plot of differential gene expression. *CPNE3*^{high} and *CPNE3*^{low} were marked by red and green circles, respectively. (b) Expression heatmap of associated genes. (c) Expression 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.

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).

Associations between genome-wide microRNA profiles and CPNE3 expression. Second, we analyzed TCGA-derived microRNA genome-wide profiles obtained by whole-genome high-

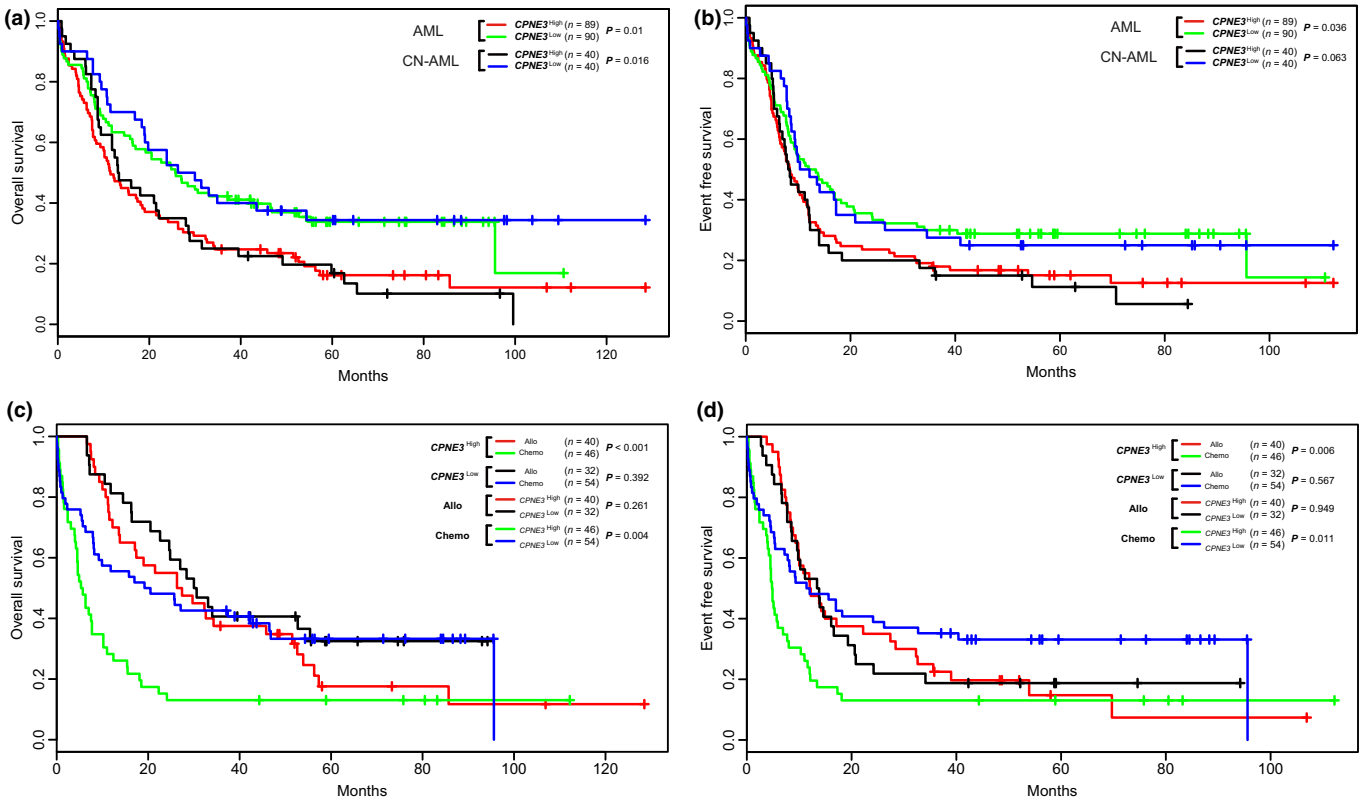

Fig. 3. The prognostic value of *CPNE3* expression in the second cohort. (a) Overall survival (OS) and (b) event-free survival (EFS) of the entire AML and CN-AML patients from TCGA data. (c) OS and (d) EFS of the AML patients of *CPNE3*^{high} group, *CPNE3*^{low} group, allogeneic HCT group and chemotherapy-only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

throughput sequencing. 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).

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 *CPNE3*^{high} ($P < 0.05$) (Fig. 2e). Of note, several important tumorigenic pathways were significantly upregulated, including "ERBB signaling pathway," "JAK/STAT signaling pathway,"[18] "glycolysis/gluconeogenesis,"[19] "VEGF signaling pathway"[20] and "Notch signaling pathway" (Fig. 2f).[21]

Association between CPNE3^{high} and adverse outcomes was confirmed by TCGA dataset. The prognostic value of *CPNE3*^{high} 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, *CPNE3*^{high} patients had significantly adverse OS and EFS compared to *CPNE3*^{low} 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 *CPNE3*^{high} and *CPNE3*^{low} groups (OS, $P = 0.261$; EFS, $P = 0.949$) (Fig. 3c, d). However, in the chemotherapy group, *CPNE3*^{high} had significantly worse OS and EFS than *CPNE3*^{low} patients (OS, $P = 0.004$; EFS, $P = 0.011$) (Fig. 3c,d). Moreover, *CPNE3*^{high} 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 *CPNE3*^{low} patients (allogeneic 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 *CPNE3*^{high} group, while more patients with AML-M1 and AML-M2 fell into the *CPNE3*^{low} group in the first cohort of AML patients. In the first cohort of AML patients, *CPNE3*^{high} 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 *CPNE3*^{high} 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-I 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.

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Disclosure Statement

The authors have no conflict of interest to declare.

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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.