EBioMedicine 28 (2018) 90–96

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

# High Expression of *TET1* Predicts Poor Survival in Cytogenetically Normal Acute Myeloid Leukemia From Two Cohorts

Jinghan Wang [a,b,c], Fenglin Li [b], Zhixin Ma [b], Mengxia Yu [b], Qi Guo [d], Jiansong Huang [b,c], Wenjuan Yu [a,b,c], Yungui Wang [b], Jie Jin [a,b,c,*]

[a] Department of Hematology, The First Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, PR China
[b] Institute of Hematology, Zhejiang University, Hangzhou, PR China
[c] Key Laboratory of Hematologic Malignancies, Diagnosis and Treatment, Zhejiang, Hangzhou, PR China
[d] Department of Nephrology, The First Affiliated Hospital, Zhejiang University, Hangzhou, China

ARTICLE INFO

*Article history:*
Received 18 December 2017
Received in revised form 23 January 2018
Accepted 23 January 2018
Available online 31 January 2018

*Keywords:*
Prognosis
Acute myeloid leukemia
TET1
Survival

ABSTRACT

Ten-Eleven-Translocation 1 (*TET1*) plays a role in the DNA methylation process and gene activation. Recent reports suggest *TET1* acts as an oncogene in leukemia development. However, the clinical relevance and biological insight of *TET1* expression in cytogenetically normal acute myeloid leukemia (CN-AML) is unknown. In this study, quantification of *TET1* transcript by real-time quantitative PCR in bone marrow blasts was performed in 360 CN-AML patients. As a result, high *TET1* expression was more common in M0/M1 morphology and genes of *NPM1* mutations, and underrepresented in *CEBPA* double allele mutations in our AML patients. In addition, we found overexpression of *TET1* was associated with an inferior overall survival and event free survival in the two independent cohorts. Notably, mRNA and miRNA integrative analyses showed aberrant expression of several hub oncogenes appear to be regulated by some miRNAs like miR-127-5p, miR-494, miR-21 and miR-616 in high *TET1* expressers. In conclusion, the *TET1* gene expression might serve as a reliable predictor for patients survival in AML.



## 1. Introduction

Acute myeloid leukemia (AML) is a group of molecular and clinical heterogeneity of hematological malignances with adverse clinical outcome. The poor outcome may partly be explained by the lack of molecular markers to risk stratification for personalized therapy. Although chromosomal abnormalities have been validated as an effective tool for the risk stratification, chromosomal lesions are identified in approximately 50% of all AML patients. In contrast, about 50% of all AML cases are cytogenetically normal (CN) (Rowe, 2016). In order to refine classification for CN-AML patients, molecular diagnosis such as *NPM1*, *FLT3*-ITD, and *CEBPA* mutational analysis is crucial (Becker et al., 2010). Additionally, abnormal expression of some oncogenes like *MN1*, *ERG*, *EVI1*, the brain and acute leukemia cytoplasmic and isocitrate dehydrogenase 1 have been described previously in studies from other and our groups as prognostic biomarkers (Damiani et al., 2013; Ma et al., 2015; Haferlach et al., 2012).

Recently, with the extensive research of leukemogenesis and the development of new techniques, more and more novel biomarkers relevant to epigenetic alterations have emerged as candidates for disease detection, diagnosis and prognosis. For example, 5-hydroxymethylcytosine (5hmC), also called the "sixth DNA base", is catalyzed by the Ten-Eleven-Translocation (TET) family proteins, which are involved in the DNA demethylation process. Several studies demonstrated the expression of the TET1 protein and 5hmC is markedly reduced in a wide range of solid tumors like colon cancer (Neri et al., 2015) and gastric cancer (Frycz et al., 2014), suggesting that *TET1* functions as a tumor suppressor in these types of cancers. With respect to hematologic disease like leukemia, studies demonstrated an up-regulation of *TET1* was observed in MLL-rearranged leukemia and plays an indispensable oncogenic role in the development of MLL-rearranged leukemia in vitro and in vivo (Huang et al., 2016; Ittel et al., 2013; Huang et al., 2013a). Further studies revealed that several essential downstream direct target genes of MLL fusions, such as *HOXA9*, *MEIS1*, and *PBX3* have been shown to be critical for the development, maintenance and leukemia stem cells (LSCs) self-renewal of MLL-rearranged leukemia are also direct target genes of *TET1* (Huang et al., 2016; Wong et al., 2007; Zhu et al., 2016). Taken together, these results highlight *TET1* functions as a potent oncogenic role in MLL-arranged leukemia. However, it is not yet known about the clinical relevance and the upstream regulation mechanism of *TET1* expression in non-MLL-arranged leukemia, especially CN-AML. In this study, we found

* Corresponding author at: No.79 Qingchun Road, Hangzhou 310003, Zhejiang, PR China.
*E-mail address:* jiej0503@zju.edu.cn (J. Jin).

https://doi.org/10.1016/j.ebiom.2018.01.031

high *TET1* expression is associated with poor survival in the two independent cohort of AML patients, and provide the putative miRNA and mRNA regulation mechanism to decipher the biologic insights in high *TET1* expressers with CN-AML.

## 2. Methods

### 2.1. Patients

AML patients with age > 14 at diagnosis were included in this study. We collected the clinical data of 360 AML patients from medical records between January 2010 and July 2016. WHO classification, conventional cytogenetic banding assay, and molecular analyses were performed centrally in Zhejiang Institute of Hematology (ZIH), China as previously described in AML diagnosis (Wang et al., 2013). Patients were treated with intensive induction chemotherapy as previous reported (Jin et al., 2013). After the complete remission induction, younger patients were treated with a high-dose of cytarabine-based chemotherapy, whereas older patients were treated in an individualized manner decided by the physicians, as described previously (Wang et al., 2013). In the consolidation therapy, twenty-nine patients in this cohort received allogeneic transplantation. In addition, the published TCGA data was used as an external validation (https://tcga-data.nci.nih.gov/tcga/) (Cancer Genome Atlas Research N et al., 2013). All of the patients provided written informed consent to participate in the study. This study was approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

### 2.2. Cytogenetic and Gene Mutation Analysis

Chromosomal abnormalities and gene mutations of *NPM1*, *FLT3*-ITD, *CEBPA*, *DNMT3A*, *IDH1* and *IDH2* were analyzed by as previously described (Chen et al., 2014). The researchers conducted the above cytogenetics analyses blinded to both the levels of *TET1* expression and clinical outcome.

### 2.3. Quantitative Reverse Transcriptase-PCR

The researchers conducted PCR experiments without the knowledge of clinical outcome. The methods for RNA extraction, complementary DNA synthesis and quantitative PCR were reported previously (Yu et al., 2017). The commonly used housekeeping (HK) genes like *β-actin*, *α-tubulin*, *GUSB* and *GAPDH* were measured. In order to select the suitable HK genes in our cohort, we performed GeNorm analysis using R package "NormqPCR" (Perkins et al., 2012). As a result, the M values of *β-actin*, *α-tubulin*, *GUSB* and *GAPDH* were 0.11, 0.08, 0.08 and 0.07, respectively. Thus, *GAPDH* was selected as the most stable HK gene owing to the lowest M-value. PCR reactions were performed in a total volume of 25 μl containing of 1 μl of 100 ng/μl sample cDNA, 12.5 μl of 2 × PCR Mix, 1 μl of 0.5 μM of each primer, and 10.5 μl of ddH2O. mRNA levels were normalized to *GAPDH* housekeeping gene. The following primers were used for quantitative PCR:

*TET1* 5′- TTCGTCACTGCCAACCTTAG-3′ (sense) and 5′-ATGCCTCTTTC ACTGGGTG -3′ (antisense); *TET2* 5′- CCCTTCTCCGATGCTTTCTG-3′ (sense) and 5′-TGGGTTATGCTTGAGGTGTTC-3′ (antisense); *TET3* 5′-AAGACACCTCGCAAGTTCC-3′ (sense) and 5′-GTTGGTCACCTGGTTCTGA TAG-3′ (antisense); *GAPDH* (control), 5′-ATGGGGAAGGTGAAGGTCG-3′ (sense) and 5′-GGGTCATTGATGGCAACAATATC-3′ (antisense).

### 2.4. MicroRNA Experiments

For the miRNA measurement, total RNA was obtained using a miRNeasy Mini Kit (Cat # 217004,QIAGEN, GmbH, Germany) following the manufacturer's instructions and subsequently checked for a RNA integrity number (RIN) to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). The detailed methods for the miRNA experiments were described previously (Yu et al., 2017). We deposited the raw and processed data at the National Center for Biotechnology Information Gene Expression Omnibus (repository number GSE103431).

### 2.5. Gene Expression Arrays

Affymetrix Human Genome U133 Plus 2.0 Array GeneChip microarrays were used to assess the total RNA samples. The clinical annotation and microarray data of 12 and 10 samples were published previously (Shen et al., 2011; Wang et al., 2013b). GSE27187 raw data were downloaded from the GEO database (www.ncbi.nlm.nih.gov/geo). The raw data of 22 samples were pre-processed using functional normalization in the affy R package (Gautier et al., 2004). The Cancer Genome Atlas (TCGA) mRNA gene expression profiling data were generated by Affymetrix Human Genome U133Plus2.0 GeneChips. Gene expression of TCGA data was used for extended analyses. The relative expression of *TET1* in the TCGA cohort was calculated by the ratio of *TET1* to *GAPDH* transcript levels.

### 2.6. Definition of Clinical End Points and Statistical Analysis

Descriptive statistics including frequency counts, median and range were used to describe patient characteristics. The main objective of this study was to explore the prognostic value of *TET1* expression in AML patients. Overall survival (OS) was defined as time from date of diagnosis until death due to any cause or the last follow-up, and event-free survival (EFS) was defined as time from date of diagnosis until removal from study due to non-complete remission, relapse, or death. The adjusted variables like age, WBC, ELN favorable genotype, genes of *DNMT3A*, *IDH1* and *IDH2* mutations, BMT and treatment protocols are regarded as the well-established predictors for AML patients. Based on the rule of sample size calculation: 10 events per variable (Collins et al., 2015), we should enroll 300 patients with 90 deaths (the 3-year survival rate of Chinese patients is 30% (Wang et al., 2013)) to assess 9 variables in the Cox regression model. Because EFS estimated multiple clinical events including death, disease relapse, and treatment response, EFS was selected to estimate the cutoff value of *TET1* expression. To explore the patients with the best EFS, we subdivided the development cohort into four quartiles (Q1: <25%, Q2: 25%~50%, Q3: 50%~75%, Q4:>75%) according to the expression value of *TET1* (Fig. S1). The survival curves between Q1 and Q2 were comparable, while those in Q3 and Q4 had similarly poor EFS compared with those in Q1. Thus, we dichotomized these patients into high and low *TET1* groups based on the median value (Fig. 1). Comparison of survival curves were based on the Gehan-Wilcoxon and log-rank test. The proportional-hazards assumption was checked for each variable before fitting Cox models. A limma package (Ritchie et al., 2015) in R software was used to test for the difference of microRNA and mRNA signatures between high and low *TET1* expressers. Hierarchical clustering based on expression levels of these mRNAs was performed and visualized by heatmap. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were created with ClueGO v2.3.4 (Bindea et al., 2013) and visualizing by Cytoscape software (Shannon et al., 2003). The toppgene analysis was done on the platform (https://toppgene.cchmc.org/prioritization.jsp). Interaction of miRNA and mRNA integrative analyses in silico were using the mirtar platform (http://mirtar.mbc.nctu.edu.tw/human/index.php). All statistical analyses were conducted with R statistic packages, version 3.4.1 (www.r-project.org). The two-sided level of significance was set at $p$-value < 0.05.

## 3. Results

### 3.1. Characteristics of CN-AML Patients with High TET1 Expression

*TET1* expressions were measured in BM samples from 360 CN-AML patients at diagnosis. The median value of *TET1* transcript level was

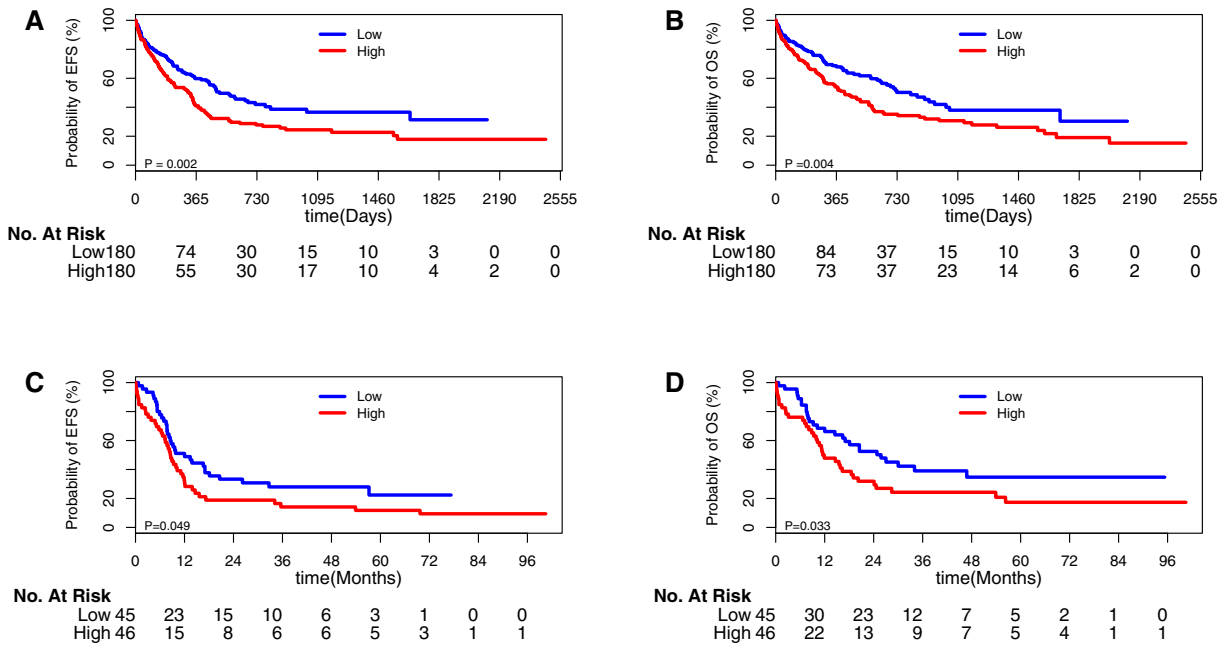


**Fig. 1.** Survival curves of CN-AML patients. Kaplan-Meier estimates of EFS (A) and OS (B) by high and low *TET1* expression for our patients, and EFS (C) and OS (D) for patients from the TCGA cohort, respectively.

0.73 expression value of *TET1/GAPDH* with the interquartile range from 0.39 to 1.37. We classified patients into high and low expression based on the median value. The median and interquantile range of *TET1* expressions were 1.38(0.97,2.21) and 0.39(0.26,0.53) for high and low expressers, respectively. Clinical characteristics of patients with high *TET1* expression are summarized in Table 1. High *TET1* expressers were more frequent in AML FAB subtype M0 (15.6% vs. 6.1%) and M1 (12.2% vs. 6.1%) morphology ($P = 0.010$). High expressers had the lower prevalence of *CEBPA* double allele mutations (9% vs. 20%, $P = 0.005$) than low expressers. Patients with high *TET1* expression had a tendency for higher frequency of *NPM1* mutations (32.5% vs. 23.3%, $P = 0.071$). Additionally, *TET1* expressions were positively correlated to *TET2* and *TET3* expressions. However, there was no statistically significant correlation between *TET1* expression and other variables including age, sex, white blood cell counts (WBC), hemoglobin levels, platelet counts, percentage of bone marrow blasts, genes of *FLT3*-ITD, *DNMT3A*, *IDH1* and *IDH2* mutations, and treatment protocols (Table 1).

### 3.2. Overexpression of TET1 Associated with Poor Clinical Outcome in CN-AML Patients

In our patients, three years overall survival (OS) rate and event free survival (EFS) rate were 34% and 31%, respectively. High *TET1* expressers ($n = 180$) had more adverse OS, EFS, and complete remission rate compared to low expressers (n = 180) (Table S1, Fig. 1A–B). However, there was no significant difference between high and low *TET2* or *TET3* expressers for OS and EFS (Figs. S2–3). Moreover, in the subgroup analyses we found *TET1* expressions can further stratify CN-AML patients with favorable ELN genotype into different subgroups with respect to OS and EFS (Fig. S4). In order to exclude the potential confounders factors, we conducted multivariate analyses. As shown in Table 2, *TET1* expression was still an independent prognostic factor after adjusting for age, WBC, ELN risk groups, and genes of *DNMT3A*, *IDH1* and *IDH2* mutations regardless of CR [OR (95%CI), 0.354(0.204, 0.602); $P < 0.001$], OS [HR (95%CI), 1.614(1.154, 2.256); $P = 0.005$] and EFS [HR (95%CI), 1.607(1.169, 2.21); $P = 0.004$]. Moreover, we also conducted landmark analysis by including patients whose survival was >30 days in order to ignore the cause of induction death by intense chemotherapy. As a result, high expression of *TET1* was still

**Table 1**
Characteristics of CN-AML patients by high and low *TET1* expression.

| Variables | Low expression | High expression | *P* value |
|---|---|---|---|
| Number(%) | 180(50%) | 180(50%) | |
| Male, n(%) | 107(59.4) | 103(57.2) | 0.748 |
| Age, median(IQR), years | 53.00(42.00,65.00) | 55.00(39.00,63.00) | 0.812 |
| WBC, median(IQR), $\times 10^9/L$[1] | 12.10(2.75,49.12) | 11.64(2.40,63.97) | 0.825 |
| HB, median(IQR), g/L[2] | 87.00(69.75,103.25) | 84.00(65.65,103.00) | 0.292 |
| PLT, median(IQR), $\times 10^9/L$[3] | 49.50(24.75,92.50) | 46.00(26.00,88.25) | 0.886 |
| BM blast, median(IQR),%[4] | 64.50(39.12,78.00) | 67.50(41.88,83.12) | 0.211 |
| FAB classification, n(%)[5] | | | 0.010 |
| M0 | 11(6.1) | 28(15.6) | |
| M1 | 11(6.1) | 22(12.2) | |
| M2 | 94(52.2) | 83(46.1) | |
| M4 | 12(6.7) | 7(3.9) | |
| M5 | 48(26.7) | 37(20.6) | |
| M6 | 4(2.2) | 3(1.7) | |
| Genes mutations, n(%) | | | |
| *FLT3*-ITD | 37(20.8) | 36(20.2) | 1.000 |
| *NPM1* | 41(23.3) | 55(32.5) | 0.071 |
| *CEBPA*[DM6] | 34(20.0) | 15(9.0) | 0.005 |
| *DNMT3A* | 26(15.7) | 19(11.9) | 0.340 |
| *IDH1* | 36(22.6) | 26(16.0) | 0.157 |
| *IDH2* | 22(13.9) | 26(16.7) | 0.533 |
| ELN favorable group, n(%) | 55(30.9) | 46(25.7) | 0.330 |
| Treatment, n(%)[7] | | | 0.060 |
| DA | 49(27.2) | 37(20.6) | |
| HAA | 21(11.7) | 36(20.0) | |
| IA | 110(61.1) | 107(59.4) | |
| BMT, n(%) | 11(6.1) | 18(10.0) | 0.245 |
| TET1, median(IQR) | 0.39(0.26,0.53) | 1.38(0.97,2.21) | <0.001 |
| TET2, median(IQR) | 2.17(1.68,2.93) | 3.01(2.19,4.21) | <0.001 |
| TET3, median(IQR) | 0.57(0.41,0.81) | 1.03(0.71,1.70) | <0.001 |

Abbreviations: [1]WBC, white blood cell; [2]HB, hemoglobin; [3]PLT, platelet counts; [4]BM, bone marrow; [5]FAB, French–American–British classification systems; [6]DM: Double-allele. [7]The protocols used for induction therapy in different groups including HAA, homoharringtonine-based treatment (homoharringtonine 2 mg/m$^2$/day for 3 days, cytarabine 75 mg/m$^2$ twice daily for 7 days, aclarubicin 12 mg/m$^2$ daily for 7 days) regiment; DA, daunorubicin 45 mg/m$^2$ daily for 3 days and cytarabine 100 mg/m$^2$ daily for 7 days; IA, idarubicin 6–8 mg/m$^2$ daily for 7 days and aclarubicin 20 mg/m$^2$ daily for 5 days. IQR, interquantile. BMT, bone marrow transplantation. ELN (European leukemia Net) favorable genotype represents *NPM1* mutant and *FLT3*-ITD negative or double allele *CEBPA* mutations.

**Table 2**
Multivariable analyses of clinical outcome for patients with CN-AML.

| Variables | Complete remission | | Overall survival | | Event free survival | |
|---|---|---|---|---|---|---|
| | *P* value | OR (95%CI) | *P* value | HR (95%CI) | *P* value | HR (95%CI) |
| *TET1* expression (High vs.Low) | <0.001 | 0.354(0.204, 0.602) | 0.005 | 1.614(1.154, 2.256) | 0.004 | 1.607(1.169, 2.210) |
| Age | 0.023 | 0.519(0.294, 0.912) | <0.001 | 2.132(1.506, 3.016) | <0.001 | 1.996(1.436, 2.776) |
| WBC | 0.062 | 0.600(0.349, 1.020) | <0.001 | 1.976(1.401, 2.787) | 0.001 | 1.724(1.242, 2.394) |
| ENL favorable group | 0.009 | 2.300(1.249, 4.355) | <0.001 | 0.401(0.260, 0.620) | <0.001 | 0.466(0.311, 0.696) |
| *DNMT3A* | 0.267 | 0.660(0.313, 1.372) | 0.001 | 2.087(1.362, 3.198) | <0.001 | 1.941(1.297, 2.904) |
| *IDH1* | 0.035 | 0.460(0.221, 0.943) | 0.042 | 1.596(1.016, 2.506) | 0.019 | 1.667(1.088, 2.554) |
| *IDH2* | 0.173 | 0.611(0.299, 1.239) | 0.658 | 0.901(0.569, 1.428) | 0.881 | 1.034(0.669, 1.599) |
| Treatment protocols | | | | | | |
| HAA vs. DA | 0.024 | 2.731(1.154, 6.643) | 0.06 | 0.601(0.354, 1.022) | 0.084 | 0.645(0.392, 1.060) |
| IA vs. DA | 0.001 | 3.098(1.652, 5.929) | 0.001 | 0.52(0.354, 0.766) | <0.001 | 0.565(0.389, 0.820) |
| BMT | 0.251 | 1.909(0.669, 6.349) | 0.014 | 0.368(0.166, 0.817) | 0.097 | 0.566(0.289, 1.108) |

Abbreviations: WBC, white blood cell; The treatment protocols used for induction therapy in different groups including HAA, homoharringtonine-based treatment (homoharringtonine 2 mg/m$^2$/day for 3 days, cytarabine 75 mg/m$^2$ twice daily for 7 days, aclarubicin 12 mg/m$^2$ daily for 7 days) regiment; DA, daunorubicin 45 mg/m$^2$ daily for 3 days and cytarabine 100 mg/m$^2$ daily for 7 days; IA, idarubicin 6–8 mg/m$^2$ daily for 7 days and aclarubicin 20 mg/m$^2$ daily for 5 days. BMT, bone marrow transplantation. ELN (European leukemia Net) favorable genotype represents *NPM1* mutant and *FLT3*-ITD negative or double allele *CEBPA* mutations.

independently associated with poor OS [HR (95%CI), 1.652(1.139, 2.396), $P = 0.010$], EFS [HR (95%CI), 1.629(1.148, 2.31), $P = 0.010$] and CR[OR (95%CI), 0.336(0.184, 0.598); $P < 0.001$] in the multivariate survival analyses (Table S2).

### 3.3. Validation of the Prognostic Impact of TET1 Expression in an Independent Cohort of CN-AML Patients

In this study, we firstly selected 91 CN-AML patients with normal karyotype based on cytogenetic classification from the TCGA cohort to validate our results. We defined the cutoff value using the same method based on the median value of *TET1* expression in the TCGA cohort. Consequently, poor survivals were still observed in patients with high *TET1* expressers (Fig. 1C–D). However, no significant differences of OS and EFS were observed in the TCGA cohort with respect to *TET2* or *TET3* expressions (Fig. S5). Secondly, in the 197 total AML patients, *TET1* expressions (continuous variable, log2 transformation) were associated with poor OS [HR(95%CI): 1.13(0.99,1.28), $P = 0.075$] and EFS[HR(95%CI): 1.15(1.02,1.29), $P = 0.024$], respectively. Thus, the expression of *TET1* was indeed a poor predictor in different populations.

### 3.4. mRNA Expression Profiling Associated with High TET1 Expressers

In order to further obtain biological insights of the aberrant *TET1* expressions on leukemia development, we conducted the metaanalysis in gene expression profiles from 91 CN-AML from the TCGA cohort (Cancer Genome Atlas Research N et al., 2013) and 22 CN-AML patients in our previously published researches (Shen et al., 2011; Wang et al., 2013b). As a result, a total of 1127 genes including 863 upregulated genes and 264 downregulated genes were identified in high expressers compared to low expressers using the false discovery rate (FDR) <0.05 as threshold values (Fig. S6 and Table S3). In the KEGG pathways enrichment analysis, we found *TET1* high expressers involved in the upregulated pathways such as cell cycle, purine metabolism, pyrimidine metabolism, RNA transport and ribosome biogenesis pathways (Fig. S7), and the downregulated pathways such as influenza A, endocytosis, MAPK singling pathway, proteoglycans in cancer and tuberculosis (Fig. S8). Notably, we found several upregulated hub genes involving at least 2 different pathways such as *RPP40*, *RPP38*, *POP1*, *POLD2*, *POLR1C*, *POLR3D*, *POLR2D*, *POLR1E*, *POLR2F* and *ZNRD1* (Fig. S7) and downregulated hub genes of *HSPA6*, *TLR4*, *MAPK13*, *MAP2K1*, *FGFR1*, *CD14* and *TLR2* in high *TET1* expressers (Fig. S8). We also found 4 genes like *FAM69B*, *NPR3*, *CD34*, *SLC37A1* in RNA-sequencing group 6 of the NEJM AML study (Cancer Genome Atlas Research N et al., 2013) were upregulated in high *TET1* expressers. Additionally, 230 *TET1* co-expressed genes in the TCGA cohort (Jamalpour et al., 2017) can be found as part of differently expressed genes in high *TET1* expressers (Table S3). Moreover, these differently expressed genes were enriched in 16 molecular functions: RNA and DNA binding, methyltransferase activity, DNA binding transcription factor activity, lipopolysaccharide receptor activity, etc. (Table S4).

### 3.5. Changes of miRNA Expression in High TET1 Expression

In order to exclude the effect of variables on the microRNA expression, we applied the propensity score analysis to match each CN-AML patient with high and low *TET1* expression based on variables are like age, WBC, and genes of *FLT3*-ITD, *NPM1, CEBPA, DNMT3A, IDH1* and *IDH2* mutations in our cohort (Table S5). Thus, we selected 12 paired samples with high and low *TET1* expression to assess the differences of microRNA (miR) expression. As a result, we identified 199 differently expressed miRs including 111 downregulated and 88 upregulated in high *TET1* expressers (*P*-value < 0.05, Fig. 2A, Table S6). Among these miRs, up-regulation of miR-127 and miR-494, down-regulation of miR-21 and miR-616 were seen significant changes in high *TET1* expressers from an independent cohort of TCGA patients with CN-AML (Fig. 2B–E).

### 3.6. Integrative Analysis of mRNA and miRNA Interaction

In order to further understand the regulated mechanism, we conducted the integrative analysis of mRNA and miRNA interaction between high and low expressers. Specifically, thirty-five genes upregulated in high *TET1* expressers were predicted to be targeted by miR-616 and miR-21 (Fig. 3). By contrast, we found 22 genes downregulated in high *TET1* expressers were predicted to be targeted by high expression of miR-127-5p and miR-494, respectively.

After combining the differentially expressed miRNAs and pathways together, we found miR-494 and miR-127-5p were upregulated in high *TET1* expressers (Table S5 and Fig. S8), and genes of *RAB11FIP5*, *RAB11FIP1* in the endocytosis pathway and *ITGAX* in the tuberculosis pathway were downregulated (Table S3). In silico analysis, miR-127-5p can regulate genes of *RAB11FIP1* in the endocytosis pathway and *ITGAX* in the tuberculosis pathway, and miR-494 can regulate *RAB11FIP5* in endocytosis pathway (Fig. S8), implicating some novel miRNA-mRNA regulated pathways occurring in high *TET1* expressers.

## 4. Discussion

In the MLL-rearranged leukemia, *TET1* acts as an oncogene. However, the clinical relevance and prognostic significance of *TET1* expression in non-*MLL*-rearranged leukemia, particularly in CN-AML are still

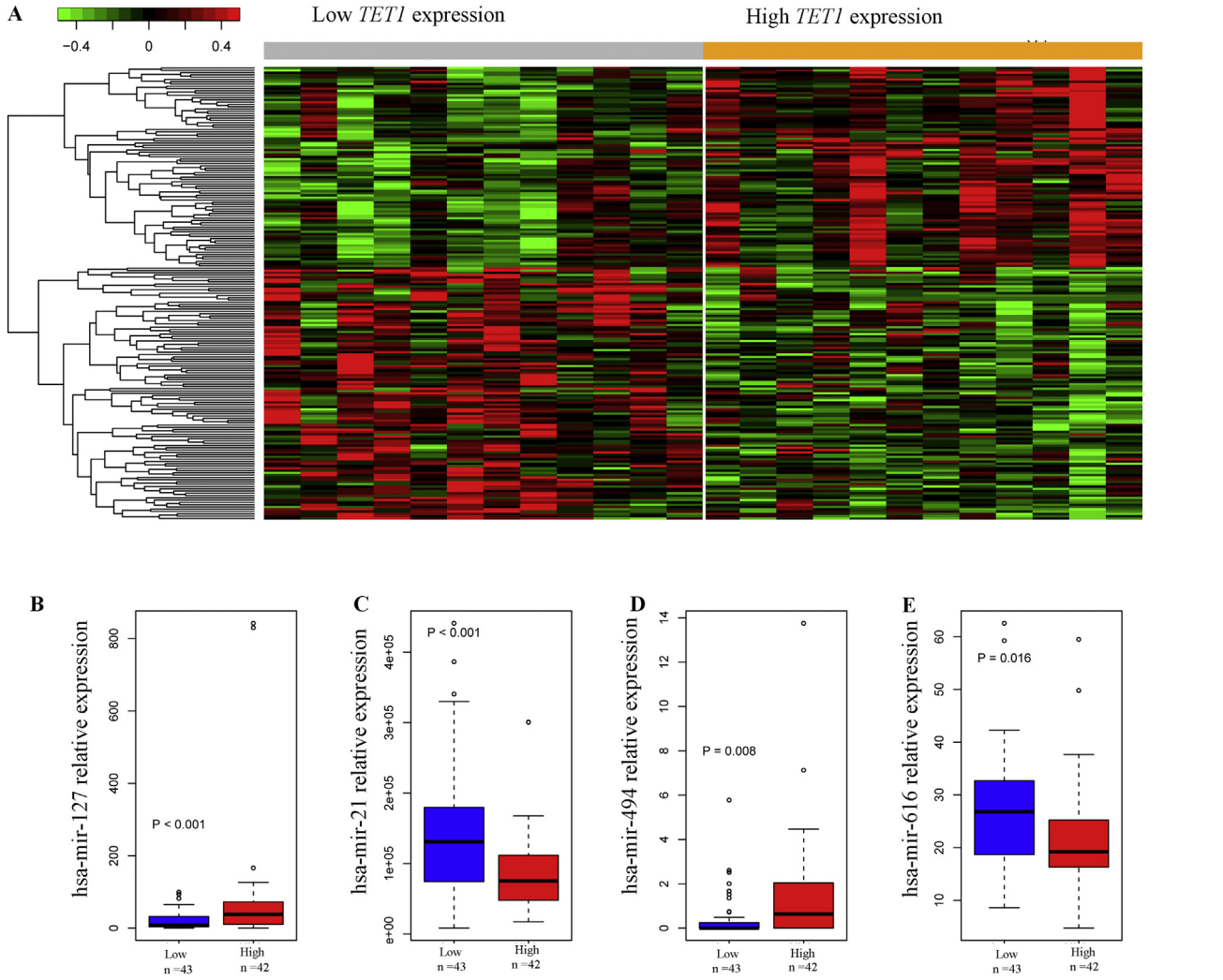


**Fig. 2.** Heatmap plot illustrating the microRNAs expression between high and low *TET1* expression(A). Validation of microRNAs expression (B-E) in patients with low vs high *TET1* expression in TCGA cohort.

unknown. In this study, we found CN-AML patients with high *TET1* expression obtained higher frequency of *NPM1* mutations and FAB M0/1 morphology. These results supported the hypothesis that increasing *TET1* expression might block hematopoietic stem cell differentiation. Taken together, the clinical characteristics of high *TET1* expressers implied *TET1* expression level might be associated with a poor outcome. As expected, we found that high *TET1* expressers harbored poor overall survival in the two independent cohorts of CN-AML patients. This result is also supported by the study that *TET1* acts as an oncogene in leukemia development (Huang et al., 2013a).

In order to further understand the underlying biological insights in high *TET1* CN-AML, we analyzed genes expression profiles in two independent cohorts of CN-AML patients. First, we found 1127 significantly expressed genes in high *TET1* expressers. These interesting genes were involved in 10 pathways together with several hub genes, which can be used as potential drug targets in the future. Specifically, in high *TET1* expressers the downregulated hub gene like *RAB11FIP1* has been described as tumor suppressors, and the downregulated pathways involving in innate immune reaction like endocytosis, tuberculosis, influenza A and MAPK signaling pathways might lead to leukemia development (Boulay et al., 2016). At the same time, high *TET1* expressers exhibit high activity of DNA and protein turnover, presenting with upregulation of purine and pyrimidine metabolism, RNA transport and ribosome biogenesis. These results might help us to explain the poor survival of the high *TET1* expression in CN-AML.

In order to further understand the biological links, we analyzed miRNAs profiles relevant to *TET1* expression. As a result, we found four miRNAs, miR-127-5p, miR-494, miR-616 and miR-21, with robust changes in high *TET1* expressers from the different cohorts of patients. The function of miR-494 and miR-127 is reported as an oncogene in the different context of tissues. For example, several authors have described them as oncomiRs in lung cancer (Shi et al., 2017), colorectal cancers (Sun et al., 2014), lymphoma and leukemia (Shi et al., 2017; Diakos et al., 2010). Here, we found miR-127-5p and miR-494 were positively correlated with the high *TET1* expression, supporting their function as oncogenes in CN-AML. Moreover, their oncogenic function can be deciphered by their putative targeting genes, which have been descripted as tumor suppressors like *ITGAX* (Ma et al., 2015) and *SLC46A2* (Kim et al., 2015). Notably, we also found downregulated *SGSH* expression was predicted to be targeted by miR-127 and miR-494 in high *TET1* expressers (Fig. 3). *SGSH* is one of enzymes in the lysosomal degradation, which often correlates with poor prognosis and increased recurrence in many cancers (Piao and Amaravadi, 2016). Recently, targeting lysosomes is reported as a novel therapeutic strategy to eradicate imatinib-resistant chronic myelogenous leukemia cells (Piao and Amaravadi, 2016; Puissant et al., 2010). These results implied miR-127 and miR-494 might subvert lysosomal proteases via downregulated expression of *SGSH* leading to leukemia development. Thus, the poor prognosis of high *TET1* expressers maybe explained by the aberrant expressions of miR-127 and miR-494 and their targeting genes.

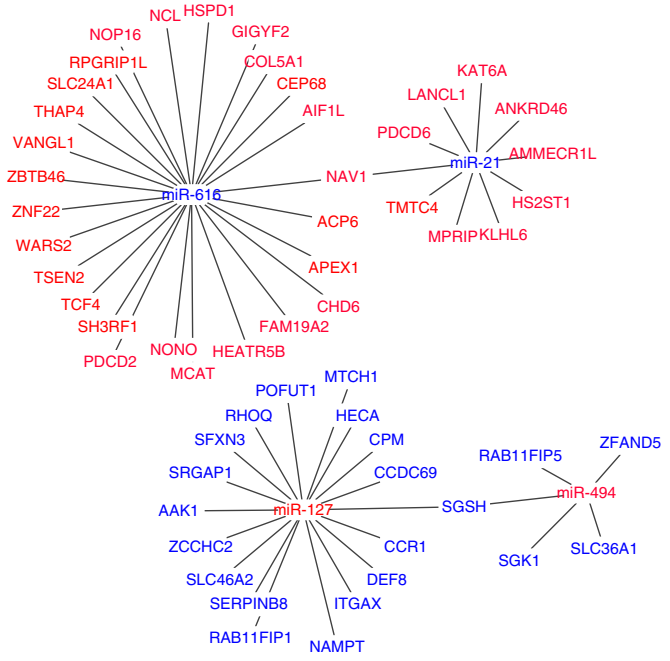


**Fig. 3.** MiRNAs and mRNAs interactive effects in the high *TET1* expressers. Red color labels represents overexpression of miRNAs and mRNAs and blue color represents downregulated expression in the high *TET1* expressers. The targeting relationships between miRNAs and mRNAs are shown by solid lines.

With respect to the function of miR-21, it has been reported miR-21 is required for maintaining the imatinib-resistant phenotype of CML stem cells (Wang et al., 2015), and upregulation of miR-21 is a poor prognostic marker in acute lymphoblastic leukemia (ALL) (Labib et al., 2017). These reports supported miR-21 as an oncogene in ALL and CML leukemia. However, downregulated expression of miR-21 in AML was also reported (Diaz-Beya et al., 2013) and seemed to act as a tumor suppressor. Here, we found miR-21 was significantly downregulated in high *TET1* expressers, supporting the function as a tumor suppressor. The exact function of miR-616 is not known in hematological disease, but it is thought to play a tumor suppressor role in CN-AML. In the current study, we found miR-616 was downregulated in high *TET1* expressers and negatively correlated to the expression of several targeting genes which have been demonstrated as a tumor promoter like *CHD6V* (Douet-Guilbert et al., 2015), *TCF4* (Ishiguro et al., 2016), *VANGL1* (Cetin et al., 2015), or associated with poor clinical outcome such as *WARS2* (Huang et al., 2013b) and *NOP16* (Zhang et al., 2014). With regards to the differentially expressed miRNAs and their targeting KEGG pathways, we found miR-494 and miR-127-5p was respectively involved in downregulation of the tuberculosis and endocytosis pathways by targeting genes of *RAB11FIP5, ITGAX* and *RAB11FIP1* in high *TET1* expressers (Fig. S8). Thus, targeting miR-494 expression and miR-127-5p may be a novel treatment strategy in CN-AML with high *TET1* expressers. These results also need more experimental clarification.

In conclusion, we uncovered high *TET1* expression could predict unfavorable survival in CN-AML patients. It is worthy to note that the prognostic value of the biomarker is dependent on the different treatment protocols. For example, the poor predictor of *DNMT3A* mutations might disappear when AML patients received high dose of daunorubicin induction therapy (Sehgal et al., 2015). Therefore, an analogous result might occur if patients with high *TET1* expression receive the strikingly intensive treatment. Additionally, we found four miRNAs and a group of targeting genes coexpressed with *TET1*. These biological links can help us answer the questions that high *TET1* expression contributes to poor clinical outcome. At the same time, these hub genes and the miRNA interaction uncover several important regulatory networks, but luciferase reporter assays are required to further study in the future. Thus, this study indicated routine measurement of *TET1* expression at diagnosis can add to risk classification and therapy decision making for CN-AML patients.

## Acknowledgement

We are very thankful to Ms. Jennifer Strong from University of Cincinnati College of Medicine for carefully reading and polishing the manuscript. We would like to thank the patients who donated leukemia specimens.

## Funding support

This work was supported by National Natural Science Foundation of China grants (81470305, 81670124, 81370643). The funders had no role in study design, data collection, data analysis, interpretation, writing of this report.

## Author Contributions

Jinghan Wang and Jie Jin: study design, methodology, data interpretation, writing and approval of the final manuscript. Fenglin Li, Zhixin Ma, Mengxia Yu, Qi Guo, Yungui Wang, Jiansong Huang and Wenjuan Yu: literature search, data collection, molecular genetic studies and approval of the final manuscript.

## Conflict of interest disclosures

The authors declare that they have no competing interests.

## Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.01.031.

## References

Becker, H., Marcucci, G., Maharry, K., et al., 2010. Favorable prognostic impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a cancer and leukemia group B study. J. Clin. Oncol. 28 (4), 596–604.
Bindea, G., Galon, J., Mlecnik, B., 2013. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. Bioinformatics 29 (5), 661–663.
Boulay, P.L., Mitchell, L., Turpin, J., et al., 2016. Rab11-FIP1C is a critical negative regulator in ErbB2-mediated mammary tumor progression. Cancer Res. 76 (9), 2662–2674.
Cancer Genome Atlas Research N, Ley, T.J., Miller, C., et al., 2013. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N. Engl. J. Med. 368 (22), 2059–2074.
Cetin, G.O., Toylu, A., Atabey, N., Sercan, Z., Sakizli, M., 2015. Downregulation of VANGL1 inhibits cellular invasion rather than cell motility in hepatocellular carcinoma cells without stimulation. Gen. Testing Mol. Biomarkers 19 (6), 283–287.
Chen, W.L., Wang, J.H., Zhao, A.H., et al., 2014. A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. Blood 124 (10), 1645–1654.
Collins, G.S., Reitsma, J.B., Altman, D.G., Moons, K.G., 2015. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. Ann. Intern. Med. 162 (1), 55–63.
Damiani, D., Tiribelli, M., Franzoni, A., et al., 2013. BAALC overexpression retains its negative prognostic role across all cytogenetic risk groups in acute myeloid leukemia patients. Am. J. Hematol. 88 (10), 848–852.
Diakos, C., Zhong, S., Xiao, Y., et al., 2010. TEL-AML1 regulation of survivin and apoptosis via miRNA-494 and miRNA-320a. Blood 116 (23), 4885–4893.
Diaz-Beya, M., Navarro, A., Ferrer, G., et al., 2013. Acute myeloid leukemia with translocation (8;16)(p11;p13) and MYST3-CREBBP rearrangement harbors a distinctive microRNA signature targeting RET proto-oncogene. Leukemia 27 (3), 595–603.
Douet-Guilbert, N., De Braekeleer, E., Tous, C., et al., 2015. A novel translocation (6;20)(q13;q12) in acute myeloid leukemia likely results in LMBRD1-CHD6 fusion. Leuk. Lymphoma 56 (2), 527–528.
Frycz, B.A., Murawa, D., Borejsza-Wysocki, M., et al., 2014. Decreased expression of ten-eleven translocation 1 protein is associated with some clinicopathological features in gastric cancer. Biomed. Pharmacother. Biomed. Pharmacother. 68 (2), 209–212.

Gautier, L., Cope, L., Bolstad, B.M., Irizarry, R.A., 2004. Affy—analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20 (3), 307–315.
Haferlach, C., Kern, W., Schindela, S., et al., 2012. Gene expression of BAALC, CDKN1B, ERG, and MN1 adds independent prognostic information to cytogenetics and molecular mutations in adult acute myeloid leukemia. Genes Chromosom. Cancer 51 (3), 257–265.
Huang, H., Jiang, X., Li, Z., et al., 2013a. TET1 plays an essential oncogenic role in MLL-rearranged leukemia. Proc. Natl. Acad. Sci. U. S. A. 110 (29), 11994–11999.
Huang, C.C., Tu, S.H., Lien, H.H., et al., 2013b. Concurrent gene signatures for han chinese breast cancers. PLoS One 8 (10), e76421.
Huang, H., Jiang, X., Wang, J., et al., 2016. Identification of MLL-fusion/MYC dash, verticalmiR-26 dash, verticalTET1 signaling circuit in MLL-rearranged leukemia. Cancer Lett. 372 (2), 157–165.
Ishiguro, H., Wakasugi, T., Terashita, Y., et al., 2016. Nuclear expression of TCF4/TCF7L2 is correlated with poor prognosis in patients with esophageal squamous cell carcinoma. Cell. Mol. Biol. Lett. 21, 5.
Ittel, A., Jeandidier, E., Helias, C., et al., 2013. First description of the t(10;11)(q22;q23)/MLL-TET1 translocation in a T-cell lymphoblastic lymphoma, with subsequent lineage switch to acute myelomonocytic myeloid leukemia. Haematologica 98 (12), e166–8.
Jamalpour, M., Li, X., Cavelier, L., et al., 2017. Tumor SHB gene expression affects disease characteristics in human acute myeloid leukemia. Tumour Biol. 39 (10), 1010428317720643.
Jin, J., Wang, J.X., Chen, F.F., et al., 2013. Homoharringtonine-based induction regimens for patients with de-novo acute myeloid leukaemia: a multicentre, open-label, randomised, controlled phase 3 trial. Lancet Oncol. 14 (7), 599–608.
Kim, K.Y., Lee, G., Yoon, M., Cho, E.H., Park, C.S., Kim, M.G., 2015. Expression analyses revealed thymic stromal co-transporter/Slc46A2 is in stem cell populations and is a putative tumor suppressor. Mol. Cell 38 (6), 548–561.
Labib, H.A., Elantouny, N.G., Ibrahim, N.F., Alnagar, A.A., 2017. Upregulation of microRNA-21 is a poor prognostic marker in patients with childhood B cell acute lymphoblastic leukemia. Hematology 22 (7), 392–397.
Ma, Q.L., Wang, J.H., Wang, Y.G., et al., 2015. High IDH1 expression is associated with a poor prognosis in cytogenetically normal acute myeloid leukemia. Int. J. Cancer 137 (5), 1058–1065.
Neri, F., Dettori, D., Incarnato, D., et al., 2015. TET1 is a tumour suppressor that inhibits colon cancer growth by derepressing inhibitors of the WNT pathway. Oncogene 34 (32), 4168–4176.
Perkins, J.R., Dawes, J.M., McMahon, S.B., Bennett, D.L., Orengo, C., Kohl, M., 2012. ReadqPCR and NormqPCR: R packages for the reading, quality checking and normalisation of RT-qPCR quantification cycle (Cq) data. BMC Genomics 13, 296.
Piao, S., Amaravadi, R.K., 2016. Targeting the lysosome in cancer. Ann. N. Y. Acad. Sci. 1371 (1), 45–54.
Puissant, A., Dufies, M., Raynaud, S., Cassuto, J.P., Auberger, P., 2010. Targeting lysosomes to eradicate imatinib-resistant chronic myelogenous leukemia cells. Leukemia 24 (5), 1099–1101.
Ritchie, M.E., Phipson, B., Wu, D., et al., 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43 (7), e47.
Rowe, J.M., 2016. AML in 2016: where we are now? Best Pract. Res. Clin. Haematol. 29 (4), 315–319.
Sehgal, A.R., Gimotty, P.A., Zhao, J., et al., 2015. DNMT3A mutational status affects the results of dose-escalated induction therapy in acute myelogenous leukemia. Clin. Cancer Res. 21 (7), 1614–1620.
Shannon, P., Markiel, A., Ozier, O., et al., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13 (11), 2498–2504.
Shen, Y., Zhu, Y.M., Fan, X., et al., 2011. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood 118 (20), 5593–5603.
Shi, L., Wang, Y., Lu, Z., et al., 2017. miR-127 promotes EMT and stem-like traits in lung cancer through a feed-forward regulatory loop. Oncogene 36 (12), 1631–1643.
Sun, H.B., Chen, X., Ji, H., et al., 2014. miR494 is an independent prognostic factor and promotes cell migration and invasion in colorectal cancer by directly targeting PTEN. Int. J. Oncol. 45 (6), 2486–2494.
Wang, J.H., Chen, W.L., Li, J.M., et al., 2013. Prognostic significance of 2-hydroxyglutarate levels in acute myeloid leukemia in China. Proc. Natl. Acad. Sci. U. S. A. 110 (42), 17017–17022.
Wang, W.Z., Pu, Q.H., Lin, X.H., et al., 2015. Silencing of miR-21 sensitizes CML CD34+ stem/progenitor cells to imatinib-induced apoptosis by blocking PI3K/AKT pathway. Leuk. Res. 39 (10), 1117–1124.
Wong, P., Iwasaki, M., Somervaille, T.C., So, C.W., Cleary, M.L., 2007. Meis1 is an essential and rate-limiting regulator of MLL leukemia stem cell potential. Genes Dev. 21 (21), 2762–2774.
Yu, M., Wang, J., Zhu, Z., et al., 2017. Prognostic impact of MYH9 expression on patients with acute myeloid leukemia. Oncotarget 8 (1), 156–163.
Zhang, C., Yin, C., Wang, L., et al., 2014. HSPC111 governs breast cancer growth by regulating ribosomal biogenesis. Mol. Cancer Res. 12 (4), 583–594.
Zhu, N., Chen, M., Eng, R., et al., 2016. MLL-AF9- and HOXA9-mediated acute myeloid leukemia stem cell self-renewal requires JMJD1C. J. Clin. Invest. 126 (3), 997–1011.