Expression and prognosis analysis of TET family in acute myeloid leukemia

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ABSTRACT

TET family members (TETs) encode proteins that represent crucial factors in the active DNA demethylation pathway. Evidence has proved that TET2 mutation is associated with leukemogenesis, drug response, and prognosis in acute myeloid leukemia (AML). However, few studies revealed the TETs expression and its clinical significance in AML. We conducted a detailed expression and prognosis analysis of TETs expression in human AML cell lines and patients by using public databases. We observed that TETs expression especially TET2 and TET3 was closely associated with AML among various human cancers. TET1 expression was significantly reduced in AML patients, whereas TET2 and TET3 expression was significantly increased. Kaplan-Meier analysis showed that only TET3 expression was associated with overall survival (OS) and disease-free survival (DFS) among both total AML as well as non-M3 AML, and was confirmed by another independent cohort. Moreover, Cox regression analysis revealed that TET3 expression may act as an independent prognostic factor for OS and DFS in total AML. Interestingly, patients that received hematopoietic stem cell transplantation (HSCT) did not show significantly longer OS and DFS than those who did not receive HSCT in TET3 high-expressed groups; whereas, in TET3 low-expressed groups, patients that accepted HSCT showed significantly longer OS and DFS than those who did not accept HSCT. By bioinformatics analysis, TET3 expression was found positively correlated with tumor suppressor gene including CDKN2B, ZIC2, miR-196a, and negatively correlated with oncogenes such as PAX2 and IL2RA. Our study demonstrated that TETs showed significant expression differences in AML, and TET3 expression acted as a potential prognostic biomarker in AML, which may guide treatment choice between chemotherapy and HSCT.
INTRODUCTION

DNA methylation has contributed to the understanding of the complexities of genomic instability and gene regulation without altering the DNA sequence [1]. Aberrations in DNA methylation status are closely associated with tumor progression and prognosis of patients especially in blood cancers including acute myeloid leukemia (AML) [1, 2]. During malignant transformation, CpG islands in the promoter region of numerous genes become hypermethylated, silencing the expression of suppressor genes, and leading to a loss in the control of cell apoptosis, proliferation, and differentiation [1]. Conversely, hypomethylation of oncogenes enhances the tumorigenic potential of normal cells [1]. The process of DNA methylation controlled by several molecules such as DNA methyltransferases (DNMTs) has been well characterized [3, 4], but the underlying mechanism of demethylation remains to be elucidated. In recent years, Ten-eleven translocation (TET) proteins have been identified and expand the understanding about mechanisms of DNA demethylation [5].

The TET protein family includes TET1, TET2 and TET3, which can modify 5-methylcytosine (5-mC) by oxidation to 5-hydroxymethylcytosine (5-hmC) and further 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC) [6–8]. TET family members (TETs) were dysregulated in multiple malignances, and loss-of-function mutations or decreased expression of TETs inhibited the DNA demethylation pathway, which prevents the removal of 5mC from genomic DNA [5]. Functional studies have revealed the direct role of TET2 in blood cancers especially in AML. Cimmino et al reported that restoration of TET2 reversed aberrant hematopoietic stem and progenitor cell self-renewal in vitro and in vivo, and suppressed human leukemic colony formation and leukemia progression of primary human leukemia patient-derived xenografts [9]. Rasmussen et al indicated that loss of TET2 in hematopoietic cells lead to DNA hypermethylation of active enhancers and induction of leukemogenesis [10]. TET2 mutations frequently occur in AML, myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML), whereas TET1 and TET3 mutations rarely happen [11, 12]. Moreover, TET2 mutations were important prognostic factors in AML and also predicted response to hypomethylating agents in MDS patients [13]. However, few studies investigated TETs expression and its clinical significance in AML [14, 15]. Herein, we determined the clinical significance of TETs expression in AML among The Cancer Genome Atlas (TCGA) databases.

RESULTS

TETs expression associated with AML among human cancer cell lines

By assembling the Cancer Cell Line Encyclopedia (CCLE), we found that TETs expression especially TET2 and TET3 was highly expressed in AML cell lines among 40 types of human cancer cell lines (Figure 1A–1C). Moreover, The Human Protein Atlas (HPA) also presented that TET2 and TET3 expression was also highly associated with myeloid cell lines (Figure 1D–1F). The detailed comparison of TETs expression in AML cell lines was assessed by using the European Bioinformatics Institute (EMBL-EBI) website (Figure 1G–1I). In addition, TET1/2/3 mutations in human cancer cell lines were given in Supplementary Table 1.

TETs expression associated with AML patients among human cancers

We further evaluated TETs expression in AML patients by using the Gene Expression Profiling Interactive Analysis (GEPIA) dataset including TCGA and the Genotype-Tissue Expression (GTEx) projects. Aberrant expression of all TETs members was only observed in AML patients among 33 types of human cancers (Figure 2A–2C). TET1 expression was significantly reduced in AML patients, whereas TET2 and TET3 expression was significantly increased in AML patients (Figure 2D–2F). Moreover, TET1 expression did not show a significant correlation with TET2/TET3 expression in AML patients, whereas TET2 expression was positively correlated with TET3 expression in AML patients (Figure 2G–2I). In addition, TET1 and TET3 mutations were identified in none of these AML patients, whereas TET2 mutation was identified in 8.5% (17/200) of these AML patients.

Prognostic value of TETs expression in AML

In order to evaluate the prognostic value of TETs expression in AML, we further divided these patients into two groups based on median level of TET1/2/3 transcript respectively (TET1low vs. TET1high, TET2low vs. TET2high; TET3low vs. TET3high). Based on Kaplan-Meier analysis, we did not observe the significant associations of TET1 and TET2 expression with overall survival (OS) and disease-free survival (DFS) among both total AML and non-M3 AML (Figure 3). However, TET3high patients showed markedly longer OS and DFS than TET3low patients among total AML (Figure 3, P=0.018 and 0.019, respectively). Moreover, if French-American-British
(FAB)-M3 patients were excluded, patients with high expression of TET3 also had significantly longer OS and DFS than those with low expression of TET3 (Figure 3, \(P=0.006\) and 0.007, respectively). We next determined the prognostic effect of TET3 expression in AML by using Cox regression analysis. Both univariate and multivariate analysis showed that TET3 expression may act as an independent prognostic factor for OS and DFS in total AML (Table 1, \(P=0.011\) and 0.026, respectively) and non-M3 AML (Table 2, \(P=0.038\) and 0.026, respectively).

In addition, the positive impact of high TET3 expression on OS in cytogenetically normal AML (CN-AML) patients was also validated by Gene Expression Omnibus (GEO) data (GSE12417) via online web tool Genomicscape (Figure 4A–4D).

**Association between TET3 expression and clinical/molecular characteristics**

Due to the significant association of TET3 expression with AML prognosis, we next analyzed the clinical relevance of TET3 expression with clinical/molecular characteristics in AML. As presented in Table 3. There were no significant differences between TET3\textsuperscript{high} and TET3\textsuperscript{low} groups in sex, age, white blood cells (WBC), bone marrow (BM)/peripheral blood (PB) blasts, and the distributions of cytogenetics (\(P>0.05\)). Significant difference was observed between two groups in the distribution of FAB subtypes (\(P=0.009\)). TET3\textsuperscript{high} patients was frequently occurred in FAB-M1/M4 (\(P=0.083\) and 0.022, respectively), and less frequently occurred in FAB-M0 (\(P=0.016\)). Among common gene mutations, high expression of TET3 was associated with FLT3 wild-type and NRAS mutation (\(P=0.018\) and 0.018, respectively). No significant differences were found between TET3 expression with other gene mutations (\(P>0.05\)). Since TET2 mutation is frequent molecular event in AML, we further analyzed the relationship between TET2 mutation and TET1/2/3 expression in AML patients. As presented in Supplementary Figure 1, no significant differences were found between TET2 mutation (TET2\textsuperscript{mut}) and TET2 wild-type (TET2\textsuperscript{wt}) regarding TET1/2/3 expression (\(P>0.05\)).

**TET3 expression may guide treatment choice between chemotherapy and HSCT**

Because low expression of TET3 predicted poor clinical outcome in AML, we intended to investigate whether patients with low expression of TET3 could benefit from hematopoietic stem cell transplantation (HSCT). We compared OS and DFS between patients with and without HSCT among both TET3\textsuperscript{high} and TET3\textsuperscript{low} groups. In TET3\textsuperscript{high} groups, although patients who received HSCT presented longer OS and DFS compared with patients who did not receive HSCT among both total AML (Figure 5A and 5B, \(P=0.052\)

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**Figure 1. The expression of TETs in human cancer cell lines including AML cell lines.** (A–C) The expression of TETs in human cancer cell lines, analyzing by the Cancer Cell Line Encyclopedia (CCLE) dataset (https://www.broadinstitute.org/ccle). (D–F) The expression of TETs in human cancer cell lines, analyzing by The Human Protein Atlas (HPA) dataset (https://www.proteinatlas.org/). (G–I) The expression of TETs in leukemia cell lines, analyzed by the European Bioinformatics Institute (EMBL-EBI) dataset (https://www.ebi.ac.uk).
Figure 2. The expression of TETs in human cancers including AML patients. (A–C) The expression of TETs in pan-cancer analyzed by the Gene Expression Profiling Interactive Analysis (GEPIA) dataset (http://gepia.cancer-pku.cn/). Tumor abbreviations: ACC: Adrenocortical carcinoma; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute Myeloid Leukemia; LGG: Brain Lower Grade Glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma; UCS: Uterine Carcinosarcoma; UVM: Uveal Melanoma. Tumor abbreviations showed in black indicated no TETs over- or under-expression, in red color indicated TETs overexpression, whereas in green color indicated TETs underexpression. (D–F) The expression of TETs in AML analyzed by the GEPIA dataset (http://gepia.cancer-pku.cn/). (G–I) The correction between TETs in AML analyzed by the GEPIA dataset (http://gepia.cancer-pku.cn/).
and 0.221, respectively) and non-M3-AML (Figure 5C and 5D, \( P = 0.021 \) and 0.128, respectively), the \( P \) did not attach statistical significance especially for DFS. However, in \( TET3^{\text{low}} \) groups, patients who accepted HSCT showed significantly longer OS and DFS than patients who did not accept HSCT among both total AML (Figure 5E and 5F, \( P = 0.003 \) and 0.005, respectively) and non-M3-AML (Figure 5G and 5H, \( P < 0.001 \) and 0.001, respectively).

**Correlations between \( TET3 \) expression and molecular signature**

To gain insights into the biological function of \( TET3 \) in AML, we first compared the transcriptomes of \( TET3^{\text{high}} \) and \( TET3^{\text{low}} \) groups. A total of 464 differentially expressed genes were identified (FDR<0.05, |log2 FC|>1.5; Figure 6A and 6B; Supplementary Table 2), in which 300 genes were positively correlated with \( TET3 \) expression, and 164 were negatively correlated. Positively correlated genes such as \( \text{CDKN2B} \) and \( \text{ZIC2} \) were reported to have anti-leukemia effects [16, 17]. Among the negatively associated genes, several genes including \( \text{PAX2}, \text{IL2RA}, \text{SOX11} \), and \( \text{PAK7} \) played as oncogenes in leukemia [18–21]. Furthermore, the Gene Ontology analysis was also showed in Figure 6C.

We next derived microRNA expression signatures associated with \( TET3 \) expression, and only 5

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**Figure 3. The impact of \( TETs \) expression on survival of AML patients.** Kaplan–Meier survival curves of \( TETs \) expression on overall survival and disease free survival in both chemotherapy and hematopoietic stem cell transplantation groups.
Table 1. Cox regression analyses of variables for OS and DFS in total AML patients.

| Variables            | OS (Univariate) | OS (Multivariate) | DFS (Univariate) | DFS (Multivariate) |
|----------------------|-----------------|-------------------|------------------|-------------------|
|                      | HR (95% CI)     | P                 | HR (95% CI)      | P                 |
| TET3 expression      | 0.644 (0.445-0.932) | 0.020             | 0.610 (0.416-0.895) | 0.011             |
|                      |                 |                   | 0.647 (0.447-0.936) | 0.021             |
|                      |                 |                   | 0.647 (0.441-0.950) | 0.026             |
| Age                  | 1.040 (1.027-1.054) | 0.000             | 1.023 (1.007-1.039) | 0.005             |
|                      |                 |                   | 1.035 (1.022-1.048) | 0.000             |
|                      |                 |                   | 1.022 (1.007-1.038) | 0.005             |
| WBC                  | 1.003 (0.999-1.006) | 0.119             | 1.008 (1.004-1.012) | 0.000             |
|                      |                 |                   | 1.003 (1.000-1.006) | 0.091             |
|                      |                 |                   | 1.008 (1.004-1.012) | 0.000             |
| Karyotype risk       | 1.854 (1.465-2.346) | 0.000             | 1.687 (1.236-2.303) | 0.001             |
|                      |                 |                   | 1.829 (1.448-2.311) | 0.000             |
|                      |                 |                   | 1.853 (1.398-2.455) | 0.000             |
| Treatment regimen    | 0.551 (0.389-0.780) | 0.001             | 0.398 (0.254-0.623) | 0.000             |
|                      |                 |                   | 0.615 (0.434-0.871) | 0.006             |
|                      |                 |                   | 0.476 (0.308-0.734) | 0.001             |
| FLT3 mutations       | 1.269 (0.869-1.852) | 0.217             |                 |                   |
|                      |                 |                   | 1.254 (0.859-1.829) | 0.241             |
| NPM1 mutations       | 1.220 (0.837-1.778) | 0.301             |                 |                   |
|                      |                 |                   | 1.268 (0.869-1.848) | 0.218             |
| CEBPA mutations      | 0.913 (0.464-1.796) | 0.792             |                 |                   |
|                      |                 |                   | 1.053 (0.535-2.073) | 0.881             |
| DNMT3A mutations     | 1.615 (1.104-2.362) | 0.014             | 1.433 (0.919-2.234) | 0.113             |
|                      |                 |                   | 1.511 (1.035-2.206) | 0.033             |
|                      |                 |                   | 1.308 (0.839-2.040) | 0.236             |
| IDH1 mutations       | 0.843 (0.466-1.527) | 0.574             |                 |                   |
|                      |                 |                   | 0.890 (0.492-1.611) | 0.700             |
| IDH2 mutations       | 1.113 (0.649-1.910) | 0.697             |                 |                   |
|                      |                 |                   | 0.987 (0.576-1.691) | 0.963             |
| TET2 mutations       | 0.953 (0.514-1.767) | 0.879             |                 |                   |
|                      |                 |                   | 0.945 (0.510-1.751) | 0.857             |
| RUNXI mutations      | 1.853 (1.077-3.186) | 0.026             | 2.169 (1.157-4.064) | 0.016             |
|                      |                 |                   | 1.644 (0.959-2.817) | 0.071             |
|                      |                 |                   | 1.742 (0.937-3.240) | 0.079             |
| TP53 mutations       | 3.687 (2.144-6.339) | 0.000             | 2.311 (1.187-4.947) | 0.014             |
|                      |                 |                   | 3.257 (1.912-5.549) | 0.000             |
|                      |                 |                   | 2.174 (1.128-4.189) | 0.020             |

OS: overall survival; DFS: disease-free survival; HR: hazard ratio; CI: confidence interval; WBC: white blood cells. Variables in multivariate analysis including TET3 expression (Low vs. High), age, WBC, karyotype (favorable vs. intermediate vs. poor), treatment regimen (with transplantation vs. without transplantation) and gene mutations (mutant vs. wild-type).

miRNAs were significantly correlated (FDR<0.05, |log2 FC|>1.5; Supplementary Table 3). MiR-196a-2 and miR-1269 were positively correlated with TET3 expression. Previous studies showed the anti-leukemia role of miR-196a as ERG regulators contributed to AML biology [22]. Negatively correlated miRNAs included miR-1247, miR-205, and miR-935. Interestingly, of these miRNAs, none of them were identified as predicted miRNAs that direct target TET3 (Figure 6D, Supplementary Table 4).

**DISCUSSION**

Aberrant promoter methylation, an important hallmark of cancer cells, is considered as a major mechanism underlying the activation/inactivation of tumor-related
Table 2. Cox regression analyses of variables for OS and DFS in non-M3 AML patients.

| Variables                  | OS Univariate analysis | DFS Univariate analysis | OS Multivariate analysis | DFS Multivariate analysis |
|----------------------------|------------------------|-------------------------|--------------------------|---------------------------|
|                            | HR (95% CI)            | P                       | HR (95% CI)              | P                         |
| TET3 expression            | 0.589 (0.403-0.862)    | 0.006                   | 0.644 (0.425-0.975)      | 0.038                     |
|                            |                        |                         | 0.597 (0.408-0.873)      | 0.008                     |
| Age                       | 1.033 (1.019-1.047)    | 0.000                   | 1.011 (0.994-1.027)      | 0.203                     |
|                            |                        |                         | 1.027 (1.014-1.041)      | 0.000                     |
| WBC                       | 1.001 (0.997-1.005)    | 0.609                   | 1.001 (0.998-1.005)      | 0.450                     |
| Karyotype risk            | 1.698 (1.308-2.205)    | 0.000                   | 1.674 (1.292-2.169)      | 0.000                     |
| Treatment regimen          | 0.445 (0.311-0.636)    | 0.000                   | 0.518 (0.363-0.740)      | 0.000                     |
| FLT3 mutations            | 1.334 (0.903-1.969)    | 0.148                   | 1.534 (0.953-2.469)      | 0.078                     |
| NPM1 mutations            | 1.049 (0.717-1.535)    | 0.804                   | 1.099 (0.751-1.608)      | 0.628                     |
| CEBPA mutations           | 0.802 (0.407-1.581)    | 0.523                   | 0.940 (0.477-1.852)      | 0.857                     |
| DNMT3A mutations          | 1.414 (0.964-2.074)    | 0.077                   | 1.520 (0.970-2.382)      | 0.068                     |
| IDH1 mutations            | 0.735 (0.405-1.333)    | 0.311                   | 0.778 (0.429-1.410)      | 0.408                     |
| IDH2 mutations            | 0.972 (0.566-1.671)    | 0.918                   | 0.857 (0.499-1.471)      | 0.575                     |
| TET2 mutations            | 0.837 (0.451-1.554)    | 0.573                   | 0.830 (0.447-1.542)      | 0.556                     |
| RUNX1 mutations           | 1.661 (0.965-2.860)    | 0.067                   | 2.955 (1.580-5.678)      | 0.001                     |
| TP53 mutations            | 3.214 (1.840-5.614)    | 0.000                   | 2.578 (1.317-5.045)      | 0.006                     |

OS: overall survival; DFS: disease-free survival; HR: hazard ratio; CI: confidence interval; WBC: white blood cells. Variables in multivariate analysis including TET3 expression (Low vs. High), age, WBC, karyotype (favorable vs. intermediate vs. poor), treatment regimen (with transplantation vs. without transplantation) and gene mutations (mutant vs. wild-type).

In addition to DNMTs, TET gene family encodes proteins that represent crucial factors in the active DNA demethylation pathway [3–5]. A loss-of-function mutation in the TET2 gene is associated with leukemogenesis, drug response, and treatment outcome [11]. However, few studies investigated TETs expression and its clinical significance in AML [14, 15]. Herein, we systemically explored the TETs expression and its clinical significance in AML, and we hope that our findings could provide new insight into AML biology, improve treatment designs, and enhance the accuracy of prognosis for patients with AML. In this study, we showed that TETs expression showed differentially expressed in AML, which indicated different role of TETs during AML pathogenesis. In solid tumors, a number of studies showed the direct role of TETs in cancer biology. For example, two studies have showed that TET1 was a tumor suppressor gene that inhibited colon cancer growth by derepressing inhibitors of the WNT pathway [23, 24]. Xu et al
Table 3. Correlation of TET3 expression with clinic-pathologic characteristics in AML.

| Patient's parameters                     | TET3 expression |   |   |
|-----------------------------------------|-----------------|---|---|
|                                         | Low (n=87)      | High (n=86) | P  |
| Sex, male/female                        | 44/43           | 48/38       | 0.543 |
| Median age, years (range)               | 60 (21-88)      | 57 (18-82)  | 0.113 |
| Median WBC, ×10⁹/L (range)              | 15.1 (0.5-297.4)| 17 (0.4-223.8) | 0.678 |
| Median PB blasts, % (range)             | 45 (0-98)       | 29 (0-97)   | 0.370 |
| Median BM blasts, % (range)             | 75 (32-100)     | 72 (30-100) | 0.294 |
| FAB classifications                      |                 |             | 0.009 |
| M0                                      | 13              | 3           |     |
| M1                                      | 17              | 27          |     |
| M2                                      | 21              | 17          |     |
| M3                                      | 11              | 5           |     |
| M4                                      | 11              | 23          |     |
| M5                                      | 9               | 9           |     |
| M6                                      | 1               | 1           |     |
| M7                                      | 3               | 0           |     |
| No data                                 | 1               | 1           |     |
| Cytogenetics                            | 0.637           |             |     |
| normal                                  | 39              | 41          |     |
| t(15;17)                                | 10              | 5           |     |
| t(8;21)                                 | 3               | 4           |     |
| inv(16)                                 | 3               | 7           |     |
| +8                                      | 5               | 3           |     |
| del(5)                                  | 1               | 0           |     |
| -7/del(7)                               | 3               | 4           |     |
| 11q23                                   | 1               | 2           |     |
| others                                  | 7               | 7           |     |
| complex                                 | 12              | 13          |     |
| No data                                 | 3               | 0           |     |
| Gene mutation                           |                 | 0.018       |     |
| FLT3 (+/-)                              | 32/55           | 17/69       |     |
| NPM1 (+/-)                              | 22/65           | 25/61       | 0.611 |
| DNMT3A (+/-)                            | 24/63           | 18/68       | 0.376 |
| IDH2 (+/-)                              | 6/81            | 11/75       | 0.212 |
| IDH1 (+/-)                              | 7/80            | 9/77        | 0.611 |
| TET2 (+/-)                              | 8/79            | 7/79        | 1.000 |
| RUNX1 (+/-)                             | 7/80            | 8/78        | 0.794 |
| TP53 (+/-)                              | 8/79            | 6/80        | 0.782 |
| NRAS (+/-)                              | 2/85            | 10/76       | 0.018 |
| CEBPA (+/-)                             | 5/82            | 8/78        | 0.404 |
| WT1 (+/-)                               | 4/83            | 6/80        | 0.535 |
| PTPN11 (+/-)                            | 2/85            | 6/80        | 0.168 |
| KIT (+/-)                               | 3/84            | 4/82        | 0.720 |
| U2AF1 (+/-)                             | 2/85            | 5/81        | 0.278 |
| KRAS (+/-)                              | 3/84            | 4/82        | 0.720 |
SMC1A (+/-) 4/83 3/83 1.000
SMC3 (+/-) 3/84 4/82 0.720
PHF6 (+/-) 2/85 3/83 0.682
STAG2 (+/-) 2/85 3/83 0.682
RAD21 (+/-) 2/85 2/84 1.000

AML, acute myeloid leukemia; WBC, white blood cells; PB, peripheral blood; BM, bone marrow; FAB, French-American-British classification.

Figure 4. The impact of TET3 expression on overall survival of AML patients. (A–D) Two independent cohorts of 162 and 78 cytogenetically normal AML (CN-AML) patients were obtained from Gene Expression Omnibus (GEO) data (http://www.ncbi.nlm.nih.gov/geo/; accession number GSE12417). Survival analysis was performed through the online web tool Genomicscape (http://genomicscape.com/microarray/survival.php). (A) probe 214754_at (TET3) in 78 CN-AML patients; (B) probe 235542_at (TET3) in 78 CN-AML patients; (C) probe 214754_at (TET3) in 162 CN-AML patients; (D) probe 235542_at (TET3) in 162 CN-AML patients.
disclosed that tumor suppressive role of TET2 promoted cancer immunity and immunotherapy efficacy [25]. Moreover, TET2 controlled chemoresistant slow-cycling cancer cell survival and tumor recurrence [26]. Cui et al demonstrated that TET3 as a potential tumor suppressor induced by the nuclear receptor TLX to regulate the growth and self-renewal in glioblastoma stem cells [27]. Moreover, several tumor suppressors, including BTG2, TUSC1, BAK1, LATS2, FZD6 and PPP2R1B, were regarded as common targets of TET3 [27]. Additionally, TET3 expression was decreased in ovarian cancer tissues, acted as a suppressor of ovarian cancer by demethylating miR-30d precursor gene promoter to block TGF-β1-induced epithelial-mesenchymal transition [28]. In our study, we showed that TET1 expression was significantly decreased in AML, whereas TET2 and TET3 expression was significantly increased in AML. Notably, we did not observe the direct association of TET3 with these factors, and found that several tumor suppressor genes (CDKN2B, ZIC2, and miR-196a) and oncogenes (PAX2, IL2RA, SOX11, and PAK7) were associated with TET3 in AML biology [16–22]. Moreover, these genes were important factors as cellular component or involving in many crucial biological processes contributing to cancer development.

Lastly, TET3 was differently expressed among the distributions of FAB subtypes in AML. These results suggested that the biological network of TETs in cancer was dependent on cancer type and stage specific.

Although previous studies showed the significant associations of TET1 and TET2 expression with AML prognosis [14, 15], herein, we only observed that TET3 expression acted as an independent prognostic factor in AML, and could be overcome by HSCT. It was very interesting that TET3 expression was increased in AML, and its high expression showed a positive effect in AML. Possible reason was that TET3 expression may play a different role between cancer occurrence and development, and further functional studies are needed to explore the underlying mechanism in AML development. The expression pattern and clinical significance of TET3 have been determined in several human cancers. Several studies revealed that high expression of TET3 was revealed in renal cell carcinoma as well as endometrial cancers, and high mRNA levels of TET3 were independent predictors of poor outcome in renal cell carcinoma patients [29, 30]; whereas, several other investigations reported that TET3 was low-expressed in diverse human cancers. For

Figure 5. The effect of hematopoietic stem cell transplantation on survival of AML patients among different TET3 expression groups. (A–D) Kaplan–Meier survival curves of overall survival and disease free survival in low TET3 expression group. (E–H) Kaplan–Meier survival curves of overall survival and disease free survival in high TET3 expression group.
instance, Bronowicka-Kłys et al showed that TET3 transcript levels were lower in stage III samples of cervical cancer [31]. Moreover, TET3 mRNA was decreased in chronic lymphocytic leukemia cells compared with healthy B cells [32]. In colorectal cancer, reduced transcript level of TET3 was observed in cancerous tissue compared with their histopathologically unchanged counterparts [33]. In addition, Misawa et al reported that TET3 methylation was highly associated with poor survival in T1 and T2 tumor stages of oropharyngeal cancer and oral cancer patients [34]. All these results further indicated that the role of TET3 in diverse human cancers was specific among different cancer types.

Figure 6. Molecular signatures associated with TET3 in AML. (A) Expression heatmap of differentially expressed genes between TET3\textsuperscript{low} and TET3\textsuperscript{high} AML patients (FDR<0.05, P<0.05 and |log2 FC|>1.5). (B) Volcano plot of differentially expressed genes between TET3\textsuperscript{low} and TET3\textsuperscript{high} AML patients. (C) Gene Ontology analysis of DEGs conducted using online website of STRING (http://string-db.org). (D) Venn results of microRNAs which could target TET3 predicted by DIANA (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index), miRDB (http://mirdb.org/miRDB/), mirDIP (http://ophid.utoronto.ca/mirDIP/), TargetScan (http://www.targetscan.org/vert_72/), and miRWalk (http://mirwalk.umm.uni-heidelberg.de/).
In summary, our study demonstrated that TETs showed significant expression differences in AML, and TET3 expression acted as a potential prognostic biomarker in AML, which may guide treatment choice between chemotherapy and HSCT.

MATERIALS AND METHODS

CCLE, HPA, and EMBL-EBI dataset

Firstly, TETs expression in human cancer cell lines is assessed by the CCLE dataset (https://www.broadinstitute.org/ccle), which provides public access to genomic data, analysis, and visualization for about 1000 cell lines [35]. Secondly, we also used The HPA dataset (https://www.proteinatlas.org/) to verify TETs expression in human cancer cell lines [36]. Lastly, TETs expression in AML cell lines is verified by the EMBL-EBI dataset (https://www.ebi.ac.uk), which has provided free and open access to a range of bioinformatics applications for sequence analysis since 1998 [37].

GEPIA dataset

TETs expression in AML patients and normal controls was analyzed by the GEPIA web (http://gepia.cancer-pku.cn/), whose data from TCGA and the GTEx projects [38].

Patients from TCGA and GEO

A total of 173 AML patients with available TETs expression data from TCGA (https://cancergenome.nih.gov/ and http://www.cbioportal.org/) were identified and included in this study [39]. Clinical and molecular characteristics were obtained, including, age, sex, WBC counts, PB blasts, BM blasts, FAB subtypes, and the frequencies of genetic mutations as presented in Table 3. After induction chemotherapy, consolidation treatment included chemotherapy (100 patients received) and HSCT (73 patients accepted).

In addition, two cohorts of 162 and 78 CN-AML patients from GEO data (GSE12417) were also included. The online web tool Genomicscape (http://genomicscape.com/microarray/survival.php) was applied to validate the prognostic value of TETs expression among CN-AML patients.

Bioinformatics analysis

The details for the identification of microRNAs targeting TET3 were reported as our previous study [40].

Statistical analysis

Statistical analysis and figures creation were performed on SPSS 22.0 software. Mann-Whitney’s U test was used for the comparison of continuous variables, whereas Pearson Chi-square analysis or Fisher exact test was applied for the comparison of categorical variables. The prognostic effect of TETs expression on DFS and OS was evaluated analyzed though Kaplan-Meier analysis and Cox regression analysis. The two-tailed P value < 0.05 in all statistical analysis was defined as statistically significant.

Ethical approval

All procedures performed in studies involving human participants were approved by the Ethics Committee of the Affiliated People’s Hospital of Jiangsu University and the Washington University Human Studies Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all patients included in this study.

Abbreviations

AML: acute myeloid leukemia; DNMTs: DNA methyltransferases; TET: Ten-eleven translocation; MDS: myelodysplastic syndromes; CMML: chronic myelomonocytic leukemia; CN-AML: cytogenetically normal AML; TCGA: The Cancer Genome Atlas; CCLE: Cancer Cell Line Encyclopedia; HPA: The Human Protein Atlas; EMBL-EBI: European Bioinformatics Institute; GEPIA: Gene Expression Profiling Interactive Analysis; GTEx: Genotype-Tissue Expression; WBC: white blood cell; PB: peripheral blood; BM: bone marrow; FAB: French-American-British; HSCT: hematopoietic stem cell transplantation; DFS: disease-free survival; OS: overall survival; CN-AML: cytogenetically normal AML.

AUTHOR CONTRIBUTIONS

Jingdong Zhou conceived and designed the study; Tingjuan Zhang, Yangli Zhao and Yangjing Zhao analyzed the data; Jingdong Zhou wrote the paper. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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Supplementary Figure 1. The expression of TETs in AML patients with and without TET2 mutation. (A) For TET1 expression; (B) For TET2 expression; (C) For TET3 expression.
Supplementary Tables

Please browse Full Text version to see the data of Supplementary Tables 1–4

Supplementary Table 1. TETs mutations in human cancer cell lines. The mutation of TETs in human cancer cell lines, analyzing by the Cancer Cell Line Encyclopedia (CCLE) dataset (https://www.broadinstitute.org/ccle).

Supplementary Table 2. Different expressed genes of RNA for \( TET3^{\text{high}} \) and \( TET3^{\text{low}} \).

Supplementary Table 3. Different expressed genes of microRNA for \( TET3^{\text{high}} \) and \( TET3^{\text{low}} \).

Supplementary Table 4. Venn results of microRNAs targeting \( TET3 \).