TG-interacting factor 1 improves risk stratification in patients with NPM1-mutated acute myeloid leukemia

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Abstract

Background. Acute myeloid leukemia (AML) is a heterogeneous disease characterized by diverse genetic abnormalities. The *NPM1* is the most commonly mutated gene in newly diagnosed patients. Optimizing risk stratification in this population could facilitate more rational clinical decision-making.

Objectives. To identify biomarkers that optimize risk stratification in AML patients with *NPM1* mutations.

Materials and methods. Acute myeloid leukemia patients from multiple centers were included in this study. Univariate, multivariate and Kaplan–Meier survival analyses were used to assess risk factors and clinical outcomes. The gene set enrichment analysis (GSEA) was conducted to identify the related enrichment of biological function.

Results. TG-interacting factor 1 (TGIF1) is a good prognostic indicator of disease progression in AML patients. It is closely related to *NPM1* mutation, in which age and TGIF1 expression are independent prognostic factors. Multicenter data sources have shown that high expression of TGIF1 is beneficial for AML, regardless of whether patients received bone marrow transplantation. In the *NPM1*-mutated AML group, age, *FLT3-ITD* and TGIF1 were independent prognostic factors. Moreover, the *NPM1*-mutated subgroup could be well dichotomized into 2 groups with distinct prognoses through TGIF1 combined with European LeukemiaNet (ELN) 2017 risk stratification.

Conclusions. The TGIF1 has an important value in the prognosis of AML. The *NPM1*-mutated patients were further subdivided into risk stratification groups based on TGIF1 expression, which could optimize the ELN 2017 to achieve individualized treatment.

Key words: risk stratification, NPM1, acute myeloid leukemia, TG-interacting factor 1

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Background

Acute myeloid leukemia (AML) is a highly aggressive hematologic malignancy of the myeloid lineage characterized by extramedullary malignant infiltration of various organs, portending a poor prognosis.[1] For patients with AML who underwent conventional chemotherapy, the 5-year overall survival (OS) rate was 40–45% compared with 10–20% OS rate of the elder ones.[2,3] Although the efficacy of the intensive frontline therapy is encouraging, 30% of those patients still relapse after treatment.[4] Furthermore, the pathophysiology of AML is tightly linked with distinct genetic and molecular abnormalities, and clinicians obtain prognostic information and classification due to the molecular changes occurring in AML. Despite the recent advances in the understanding of pathogenesis and therapeutic methods of AML, it is still intractable and its risk stratification and targeted therapy are complicated due to the cytogenetical, molecular and clinical heterogeneities. Since more and more abnormalities in gene expression have been reported in the occurrence and development of AML, it is apparent that finding more specific biomarkers for AML patients is conducive to patient classification, and serves as an essential prerequisite for individualized treatment.[5,6]

The *NPM1*-mutated AML is one of the monocytic subtypes of AML, which acts by translocating the *NPM1* mutation gene to partner genes such as anaplastic lymphoma kinase gene (NPM-ALK) and normal karyotype gene (NPM-NK), rendering heterodimers, producing fusion proteins and promoting cytoplasmic delocalization and cellular transformation.[7–9] About 30% of heterozygous *NPM1*-mutated AML cases occur in adults and 50–60% of them involve normal karyotype AML (NK-AML).[10] However, the complex interactions of this mutation with some prognosis-related accompanying mutations including *FLT3-ITD*, *NPM1/N-RAS* and *NPM1-RAD21*, as well as their joint effect on the clinical outcome remain unclear.[11–14] Novel molecular information is needed to identify subsets of patients and improve the diagnosis and outcome prediction.

The TG-interacting factor 1 (TGIF1) is a member of the three-amino-acid loop extension (TALE) family of atypical homeodomain-containing transcription factors.[15] It is known as a transcriptional repressor, which participates in various biological signaling pathways including TGF-β, retinoic acid signaling and others, in order to exert its suppressive effects by recruiting and interacting with other co-repressor complexes, such as mSin3/HDAC and CtBP.[16,17] As a critical suppressive factor of the important signaling pathway, the function of TGIF1 in tumorigenesis and tumor development has attracted considerable research attention. Therefore, in our study, we evaluated the expression of TGIF1 in patients with an initial diagnosis of AML, as well as explored the clinical and potential therapeutic value of TGIF1 in AML.

Objectives

Although several preclinical studies have confirmed that TGIF1 plays a key role in regulating hematopoietic stem cell self-renewal, and that a loss of TGIF1 can lead to the occurrence of AML, there is little evidence of clinical application of TGIF1 in guiding healthcare decisions. Therefore, in our study, we evaluated the expression of TGIF1 in patients with an initial AML diagnosis and demonstrated that TGIF1 plays an independent role in AML and *NPM1*-mutated AML in terms of the prediction of clinical outcomes, as well as provides useful insight for risk stratification and clinical individualized diagnosis and treatment.

Materials and methods

Data acquisition

The expression of TGIF1 was analyzed in the publicly available mRNA sequencing cohort from the Oregon Health and Science University (OHSU)-AML project (405 patients) that included detailed clinical data.[18] Another source of clinical data and gene expression for validation was The Cancer Genome Atlas (TCGA)-AML (151 patients).[19] Validation data analyses for survival outcomes were also obtained from the Therapeutically Applicable Research to Generate Effective Treatment (TARGET)-AML project (296 patients), and Gene Expression Omnibus (GEO) data that are accessible under accession No. GSE37642. We have downloaded the published data until April 28, 2022.

The patients were classified according to the French–American–British classification systems and the risk group stratification was as per the National Comprehensive Cancer Network (NCCN) guidelines. Patients included in the study were evaluated for somatic mutations as well as fusion genes that are common in AML. The datasets generated in this study are available as the Supplementary material. The patient data were obtained from publicly available datasets and did not require further ethical approval.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA, v. 4.0.1; http://www.gsea-msigdb.org/) is a widely used bioinformatics analysis tool that determines concordant differences between biological processes. The GSEA was used to verify the differences in molecular pathways between high-expression and low-expression TGIF1 groups. The input data matrix was derived from the OHSU cohort. The 'c2.cp.kegg.v7.1.symbols.gmt' were examined and 1000 permutations were run.

Statistical analyses

The univariate and multivariate Cox regression analyses were performed using the 'survival' R package, and

the main code was provided in Supplementary material. The Kaplan–Meier survival analysis with log-rank test was used to evaluate the prognostic role of TGIF1 cutoff value, and AML patients were grouped according to the optimal difference in OS. Results are expressed as hazard ratio (HR) and 95% confidence interval (95% CI). Statistical analysis and graphing were performed using GraphPad Prism v. 8.02 (GraphPad Software, San Diego, USA) and R software v. 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria). The Mann–Whitney test and χ^2 test were used to clarify the association between gene expression and clinicopathological characteristics, and when expected counts produced by IBM SPSS v. 26.0 software (IBM Corp., Armonk, USA) were less than 1, the Fisher's exact test was performed (Table 1).[20] The value of $p < 0.05$ was considered statistically significant.

Table 1. Relationship between clinical and molecular characteristics and TGIF1 expression in patients with AML

Characteristics		Total	TGIF1-low (n = 214)	TGIF1-high (n = 191)	p-value
Age [years]#			62 (46.25–71)	61 (45–71)	0.6380*
Age group (n (%))	<60 years ≥60 years	186 219	95 (51.1) 119 (54.3)	91 (48.9) 100 (45.7)	0.5122§
Gender (n (%))	male female	228 177	119 (52.2) 95 (53.7)	109 (47.8) 82 (46.3)	0.7674§
WBC [×10^9/L]#			20.8 (6.22–49.37)	16.66 (5.6–48.03)	0.0770*
PLT [×10^9/L]#			39.5 (22–74.75)	36 (23–73.5)	0.6480*
BM [blast/%]#			66 (33–86)	70 (33.75–85)	0.4660*
PB [blast/%]#			40.2 (12–77.6)	48.6 (12–77)	0.2130*
SCR [mg/dL]#			0.85 (0.66–1.03)	0.83 (0.66–1.04)	0.1170*
Gene fusions (n (%))					
Normal		298	161 (54)	137 (46)	0.4244§
Complex karyotype		2	2 (100)	0 (0)	0.1805§
CBFB-MYH11		22	5 (22.7)	17 (77.3)	**0.0036**§
DEK-NUP214		3	2 (66.7)	1 (33.3)	0.6301§
GATA2-MECOM		7	6 (85.7)	1 (14.3)	0.0788§
MLLT3-KMT2A		11	9 (81.8)	2 (18.2)	0.0509§
PML-RARA		12	2 (16.7)	10 (83.3)	**0.0108**§
RUNX1-RUNX1T1		11	3 (27.3)	8 (72.7)	0.0850§
Others		39	24 (61.5)	15 (38.5)	0.2523§
FLT3-ITD mutation (n (%))					
Positive		94	52 (55.3)	42 (44.7)	0.5826§
Negative		310	161 (51.9)	149 (48.1)	0.5103§
Others		1	1 (100)	0 (0)	0.3442§
NPM1 mutation (n (%))					
Positive		100	46 (46)	54 (54)	0.1144§
Negative		302	166 (55)	136 (45)	0.142§
Others		3	2 (66.7)	1 (33.3)	0.6301§
Risk_Cyto (n (%))					
Favorable		117	41 (35)	76 (65)	**0.0000**§
Intermediate		135	67 (49.6)	68 (50.4)	0.3602§
Adverse		152	105 (69.1)	47 (30.9)	**0.0000**§
Others		1	1 (100)	0 (0)	0.3442§
Response to treatment (n (%))					
Complete response		186	90 (48.4)	96 (51.6)	0.0981§
Refractory		115	69 (60)	46 (40)	0.0691§
Others		104	55 (52.9)	59 (56.7)	0.2464§

AML – acute myeloid leukemia; WBC – white blood cells; PLT – platelets; BM – bone marrow; PB – peripheral blood; SCR – serum creatinine; Risk_Cyto – cytogenetic risk stratification. Values in bold denote a statistically significant difference ($p < 0.05$). # continuous variable data shown as median (25th, 75th percentile) values; * Mann–Whitney U test; § χ^2 test. Complex karyotype is defined as more than or equal to 3 chromosomal abnormalities.

Results

Patient characteristics

A total of 405 AML cases were taken from the OHSU dataset and divided into 2 groups according to the expression level of TGIF1, for a further determination of the relevance between TGIF1 expression and other various clinical and molecular characteristics. The baseline characteristics of the patients are listed in Table 1. A considerable difference between TGIF1 high- and low-expression groups could be observed in cytogenetic risk stratification: the results showed that patients with high expression of TGIF1 tended to be allocated to favorable cytogenetic risk groups ($p < 0.0001$), whereas low TGIF1 expression was associated with adverse cytogenetic risk stratification ($p < 0.0001$). In addition, the TGIF1 high-expression group had a higher frequency of gene fusions *CBFB-MYH11* ($p = 0.0009$) and *PML-RARA* ($p = 0.0108$) when compared with the TGIF1 low-expression group. There was no statistical association between TGIF1 and age, gender, white blood cell (WBC) count, platelet (PLT) count, serum creatinine (SCr), peripheral blast (PB) count, complex karyotype, *DEK-NUP214*, *GATA2-MECOM*, *MLLT3-KMT2A*, *RUNX1-RUNX1T1*, *FLT3-ITD*, or *NPM1* mutations.

TGIF1 is a good indicator of AML disease progression

Due to the baseline association between TGIF1 and established prognosis-related cytogenetic risk stratification, it was important to analyze the clinical outcomes of patients with AML with different TGIF1 expression levels. Four databases were analyzed including OHSU, TCGA, TARGET, and GSE37642. The Kaplan–Meier survival analysis of OHSU cohort demonstrated that patients

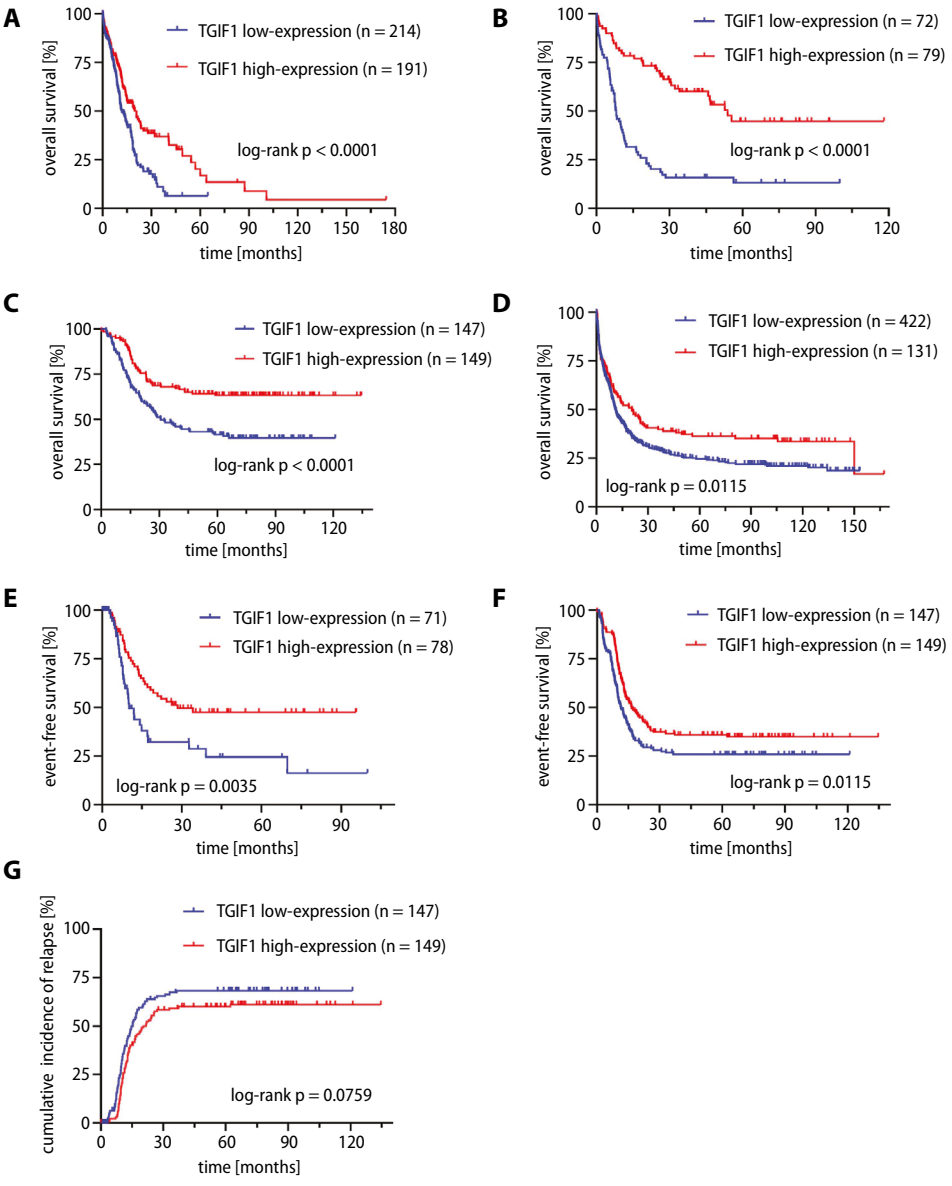

Fig. 1. TGIF1 expression level is a reliable prognostic indicator of disease progression in acute myeloid leukemia (AML) patients. A. Cumulative overall survival (OS) curves of 405 patients with the Oregon Health and Science University (OHSU)-AML divided into low-expression and high-expression subgroups ($p < 0.0001$); B. Cumulative OS curves of 151 patients with the Cancer Genome Atlas (TCGA)-AML divided into low-expression and high-expression subgroups ($p < 0.0001$); C. Cumulative OS curves of 296 patients with Therapeutically Applicable Research to Generate Effective Treatment (TARGET)-AML divided into low-expression and high-expression subgroups ($p < 0.0001$); D. Cumulative OS curves of 553 patients with GSE37642-AML divided into low-expression and high-expression subgroups ($p = 0.0115$); E,F. High TGIF1 expression was strongly associated with favorable event-free survival (TCGA-AML: $p = 0.0035$; TARGET-AML: $p = 0.0115$); G. The cumulative incidence of relapse with high TGIF1 expression was slightly lower (TARGET-AML: $p = 0.0759$)

with high TGIF1 levels had longer OS than those with low TGIF1 expression ($p < 0.0001$, Fig. 1A). Similar results were obtained from mutual validations over 3 other independent datasets ($p < 0.0001$ compared to $p < 0.0001$ compared to $p = 0.0115$; Fig. 1B–D). On the other hand, more favorable event-free survival (EFS) could be observed in the high TGIF1 expression group when compared to the patients with low TGIF1 expression (TCGA-AML cohort: HR = 0.5015, 95% CI: 0.2994–0.8399, $p = 0.0035$; TARGET-AML cohort: HR = 0.6997, 95% CI: 0.5304–0.9231, $p = 0.0115$; Fig. 1E,F). Figure 1G presents a slightly lower cumulative incidence of relapse in the high TGIF1 expression group (TARGET cohort: HR = 0.7576, 95% CI: 0.5551–0.1034, $p = 0.0759$; Fig. 1G).

Univariate and multivariate analyses for prognostic factors

Since the described results suggested the expression level of TGIF1 is closely correlated to a favorable AML prognosis, univariate and multivariate analyses were performed for further analysis. We evaluated clinical and molecular genetic factors of AML patients from the OHSU cohort, including age (≥60 years compared to <60 years), gender (male compared to female), *FLT3-ITD* (positive compared to negative), *NPM1* (mutated compared to wild-type), *CBFB-MYH11* (positive compared to negative), *RUNX1-RUNX1T1* (positive compared to negative), *MLLT3-KMT2A* (positive compared to negative), *PML-RARA* (positive compared to negative), cytogenetic risk stratification (adverse compared to intermediate/favorable), and expression levels of TGIF1 (high compared to low). After multivariable analyses, age <60 years (HR = 1.94, 95% CI: 1.48–2.55, $p < 0.0001$) and high expression level of TGIF1 (HR = 0.65, 95% CI: 0.50–0.86, $p = 0.0020$) remained significant independent predictors of a good prognosis in AML patients (Table 2).

Effect of TGIF1 expression on disease progression in AML patients with and without bone marrow transplantation

Currently, the standard treatment protocol for most AML patients is intensive induction chemotherapy, and once a complete remission is achieved, appropriate post-remission therapies are necessary, such as conventional chemotherapy and hematopoietic cell transplantation.[21] To verify the role of TGIF1 in different treatment groups, we compared Kaplan–Meier curves for the survival of the patients with and without bone marrow transplantation. The results presented in Fig. 2A,D (data from OHSU and TCGA cohort, respectively) show that bone marrow transplants can improve the OS rate by mutual validation. However, survival distribution curves of bone transplantation demonstrated a trend for shorter EFS than those of the standard chemotherapy group ($p = 0.0825$; Fig. 2G). Considering post-transplant complications such as graft-versus-host disease, the results were acceptable. In the chemotherapy group, the high-TGIF1-expression subgroup showed better OS (both $p < 0.0001$ in OHSU and TCGA cohorts) and EFS ($p = 0.0044$) than the low-expression subgroup (Fig. 2B,E,H). In the transplantation subgroup, high TGIF1 expression level was associated with better OS ($p = 0.0357$; Fig. 2C, and $p = 0.0051$; Fig. 2F), but no difference was found regarding EFS ($p = 0.2394$; Fig. 2I). The findings indicate that high TGIF1 might be a beneficial factor in AML patients undergoing conventional chemotherapy or bone marrow transplantation.

Risk stratification optimization based on AML cohort analysis

Patients from the OHSU cohort were classified according to the European LeukemiaNet (ELN) 2017 risk stratification systems[22] into favorable (n = 117), intermediate

Table 2. Influence of TGIF1 expression on overall survival (OS) analyzed with univariate and multivariate analysis in AML patients

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (≥60 years compared to <60 years)	2.23	1.71–2.92	<0.0001	1.94	1.48–2.55	<0.0001
Gender (male compared to female)	1.23	0.95–1.6	0.1116	–	–	–
FLT3-ITD (positive compared to negative)	1.27	0.95–1.71	0.1023	–	–	–
NPM1 (positive compared to negative)	0.99	0.74–1.34	0.9636	–	–	–
CBFB-MYH11 (positive compared to negative)	0.39	0.17–0.88	0.0232	0.63	0.27–1.48	0.2913
RUNX1-RUNX1T1 (positive compared to negative)	0.13	0.02–0.95	0.0439	0.23	0.03–1.63	0.1398
MLLT3-KMT2A (positive compared to negative)	0.66	0.27–1.6	0.3541	–	–	–
PML-RARA (positive compared to negative)	0.12	0.02–0.85	0.0340	0.17	0.02–1.28	0.0863
Risk_Cyto (adverse compared to intermediate/favorable)	2.07	1.48–2.91	<0.0001	1.37	0.96–1.97	0.0862
TGIF1 expression (high compared to low)	0.59	0.45–0.77	0.0001	0.65	0.50–0.86	0.0020

AML – acute myeloid leukemia; HR – hazard ratio; 95% CI – 95% confidence interval; Risk_Cyto – cytogenetic risk stratification. Values in bold denote a statistically significant difference ($p < 0.05$).

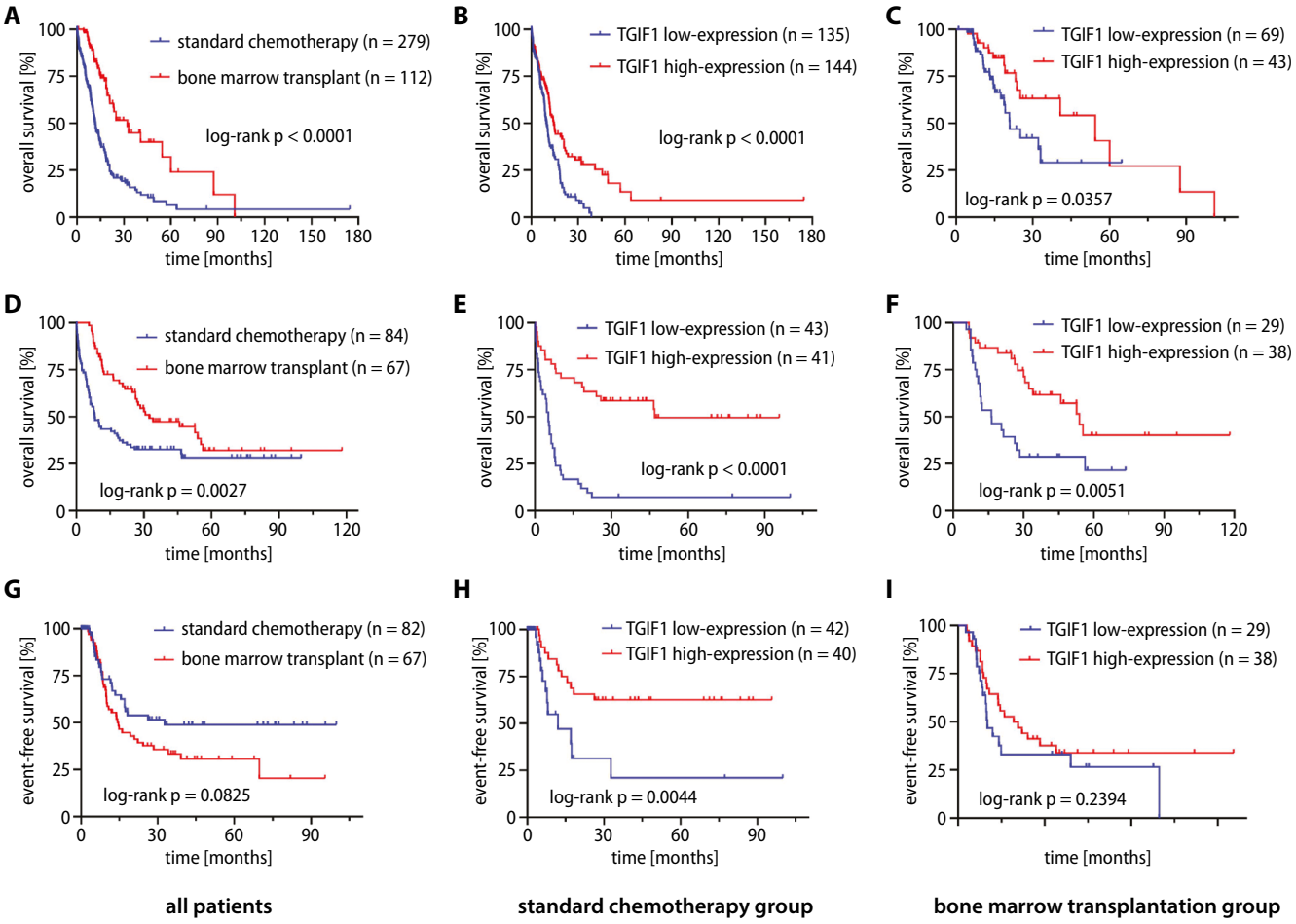

Fig. 2. Effect of TGIF1 expression on disease progression in acute myeloid leukemia (AML) patients with and without bone marrow transplantation. A–C. The Oregon Health and Science University (OHSU)-AML data suggest that bone marrow transplantation can improve the overall survival (OS) rate of AML patients ($p < 0.0001$), and high expression of TGIF1 is beneficial in both the standard chemotherapy group ($p < 0.0001$) and the bone marrow transplantation group ($p = 0.0357$); D–F. The Cancer Genome Atlas (TCGA)-AML data suggest that bone marrow transplantation can improve the OS rate of AML patients ($p = 0.0027$), and high expression of TGIF1 is beneficial in both the standard chemotherapy group ($p < 0.0001$) and the bone marrow transplantation group ($p = 0.0051$); G. TCGA data showed an increased incidence of events in AML patients who underwent bone marrow transplantation ($p = 0.0825$); H,I. TCGA data showed that low expression of TGIF1 was related to low event-free survival in the standard chemotherapy group ($p = 0.0044$), but not in the bone marrow transplantation group ($p = 0.2394$)

Table 3. Influence of TGIF1 expression on overall survival (OS) analyzed with univariate and multivariate analysis in *NPM1*-mutated AML patients

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (≥60 years compared to <60 years)	1.72	1.01–2.93	0.0460	2.05	1.19–3.52	0.0094
Gender (male compared to female)	1.30	0.77–2.21	0.3240	–	–	–
FLT3-ITD (positive compared to negative)	2.24	1.30–3.86	0.0037	2.35	1.13–4.89	0.0229
Risk_Cyto (adverse/intermediate compared to favorable)	1.79	1.05–3.05	0.0327	1.21	0.59–2.49	0.6041
TGIF1 expression (high compared to low)	0.44	0.26–0.76	0.0034	0.38	0.21–0.67	0.0008

AML – acute myeloid leukemia; HR – hazard ratio; 95% CI – 95% confidence interval; Risk_Cyto – cytogenetic risk stratification. Variables with $p < 0.1$ in the univariate analysis were included in the multivariate analysis. Values in bold denote a statistically significant difference ($p < 0.05$).

(n = 135) and adverse (n = 152) risk groups (Fig. 3A). Previous studies have suggested that there was a significant interaction effect between mutant *NPM1* and *FLT3-ITD*, leading to different treatment responses and survival prognoses.[23,24] Figure 3B demonstrates the effect on OS according to the interrelations between *NPM1* and *FLT3-ITD* mutations as per the survival curve, which was consistent with the current guidelines.[25,26] However, mutant/wild-type *NPM1* alone cannot independently predict OS in AML patients ($p = 0.9253$; Fig. 3C). Interestingly, TGIF1 was proved to be independently associated with favorable clinical outcomes in the *NPM1*-mutated subgroup of AML (Table 3). Therefore, attempts were made to reanalyze the role of TGIF1 played in the *NPM1*-mutated subgroup

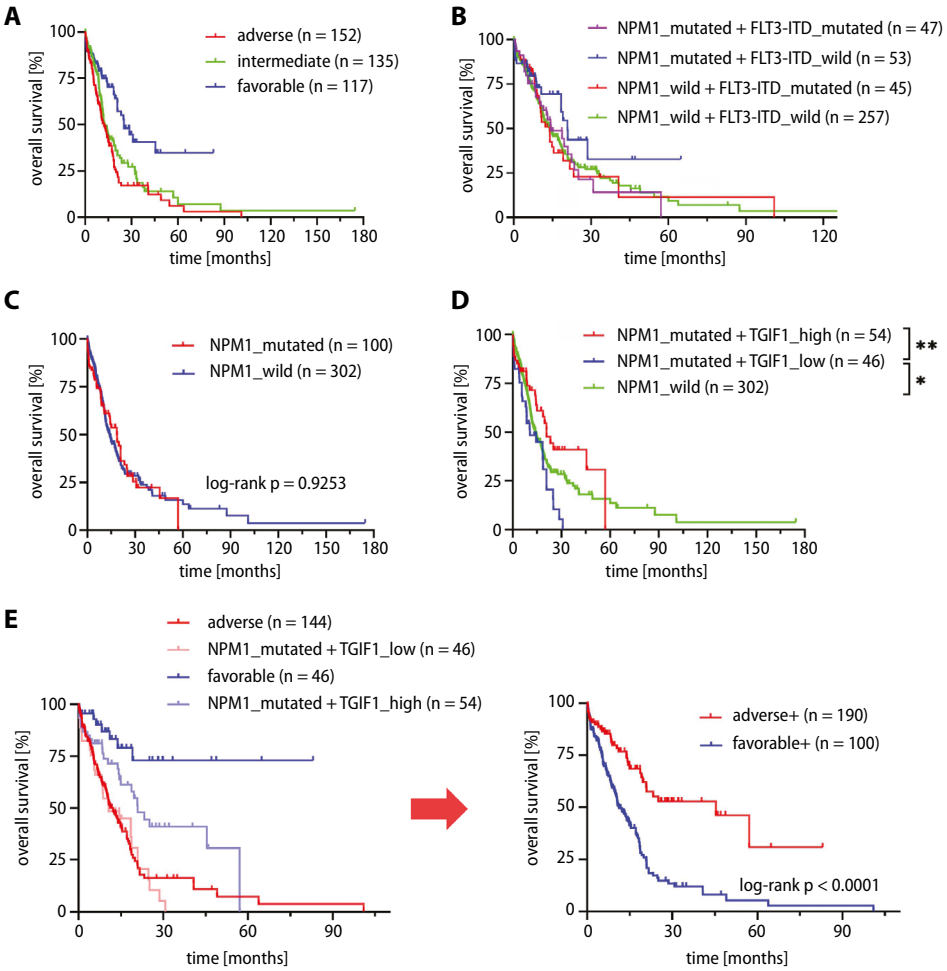

Fig. 3. Risk stratification of acute myeloid leukemia (AML) patients from the Oregon Health and Science University (OHSU)-AML data analysis. A. Prognostic impact of the European LeukemiaNet (ELN) 2017 risk classification in patients with AML; B. Frequent associations between *NPM1* and FLT3 mutations are known markers for predicting overall survival in AML; C. *NPM1* mutations and wild-type *NPM1* cannot independently predict overall survival in AML patients (p = 0.9253); D. Survival differences in *NPM1*-mutated AML patients were influenced by TGIF1 expression and distinguished from wild-type *NPM1*; E. The information on *NPM1* mutation combined with TGIF1 was integrated into the ELN classification schema to optimize the favorable and adverse group

from the OHSU cohort. The *NPM1*-mutated group with low TGIF1 levels had a shorter OS when compared with the *NPM1* wild-type group ($p < 0.05$; Fig. 3D). Besides, patients with high TGIF1 level had a longer OS than those with low TGIF1 in the *NPM1*-mutated subgroup ($p < 0.01$; Fig. 3D). These results prove the prognostic role TGIF1 plays in AML, especially in the *NPM1*-mutated subgroup. Furthermore, by integrating the information on AML prognosis relevant to *NPM1* mutation and TGIF1 expression level, improved risk stratification may be revealed ($p < 0.0001$; Fig. 3E), providing a theoretical basis and new ideas for treatment. For further validation, the same analyses were conducted with another TCGA dataset, and the results confirmed that TGIF1 expression significantly affected the prognosis of the *NPM1*-mutated group and supported risk stratification optimization (Fig. 4). Likewise, the TGIF1 expression remained a significant predictor of EFS in *NPM1*-mutated AML patients, which is consistent with our previous findings (Supplementary material).

GSEA delineates biological pathways correlated with TGIF1 expression

The GSEA is an important approach to identifying gene expression-related pathways. We performed GSEA between high and low TGIF1 levels data sets to elucidate biological pathways correlated with TGIF1. Some enrichment results showed that there was a significant correlation between high- and low-expression groups: multiple pathways, including galactose metabolism, biosynthesis of unsaturated fatty acids, glycolysis gluconeogenesis, peroxisome proliferator-activated receptor (PPAR) signaling pathway, and several others displayed the enrichment of low expression of TGIF1 and ribosome in the high-TGIF1-expression group (Fig. 5). The gene set pathways were listed in order of significance in Table 4. These results may provide a mechanistic explanation for the scientific value and clinical significance of TGIF1.

Discussion

Besides commonly reported cytogenetic changes and recurrent gene mutations mentioned in ELN risk classification,[22] numerous novel biomarkers also are related to AML leukemogenesis and were used to predict clinical outcomes,[26–28] providing insights to further disease mechanisms and therapeutic directions. The TGIF1 belongs to the family of TALE homeodomain, which regulates diverse cellular processes including proliferation, apoptosis and differentiation as a transcriptional repressor.

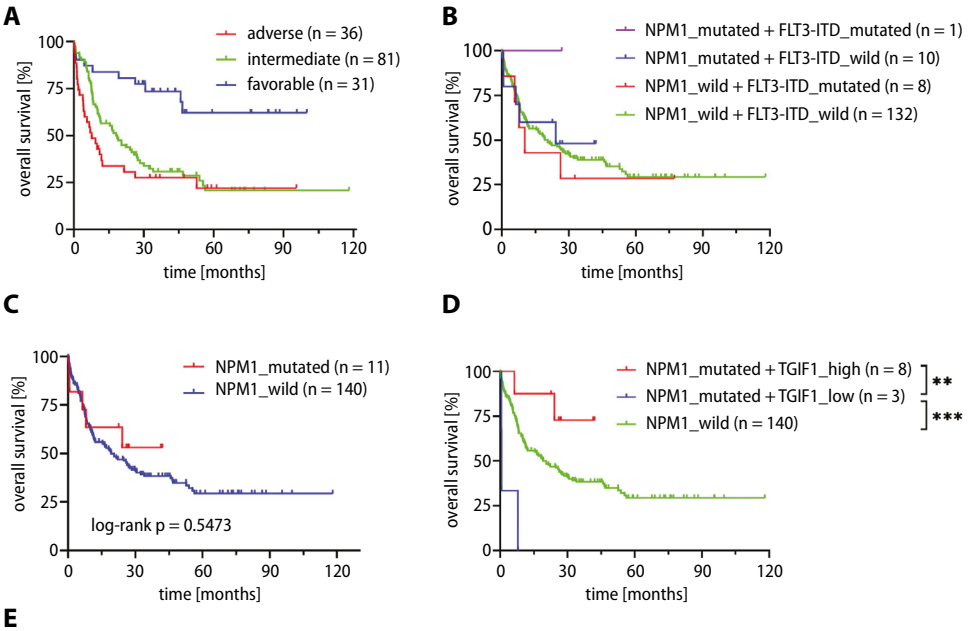

Fig. 4. The risk stratification of acute myeloid leukemia (AML) patients was analyzed using the Cancer Genome Atlas (TCGA)-AML data for validation. A. Overall survival (OS) probability over time based the European LeukemiaNet (ELN) 2017 risk group; B. Effect of *NPM1* and FLT3 on the prognosis of patients with AML; C. *NPM1* mutations and wild-type *NPM1* cannot independently predict OS in AML patients (p = 0.5473); D. High expression of TGIF1 is beneficial to the OS of AML patients with *NPM1* mutation; E. *NPM1* mutation combined with TGIF1 can optimize ELN 2017 risk stratification

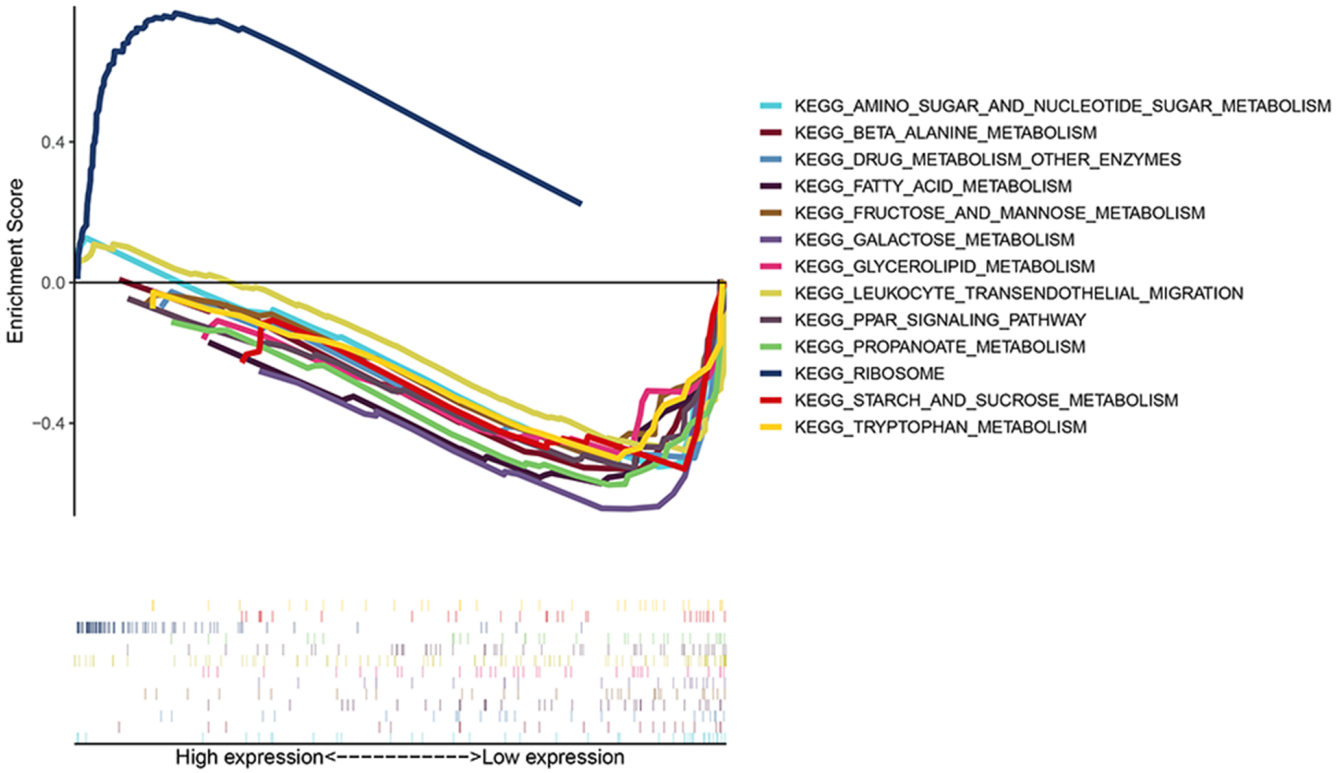

Fig. 5. Gene set enrichment analysis (GSEA) delineates biological pathways correlated with TGIF1 expression. Some enrichment results showed that there was a significant correlation between high and low TGIF1 expression groups

Table 4. Gene set enrichment analysis (GSEA) demonstrated the correlation between TGIF1_high and TGIF1_low phenotypes

Name	ES	NES	NOM p-value	FDR q-value	Leading edge
Ribosome	0.7657	1.6477	0.0393	0.7182	tags = 81%, list =16%, signal = 96%
Galactose metabolism	−0.6884	−1.9013	0.0032	0.2882	tags = 45%, list = 10%, signal = 51%
Biosynthesis of unsaturated fatty acids	−0.6983	−1.8904	0.0051	0.1622	tags = 67%, list = 19%, signal = 83%
Glycolysis gluconeogenesis	−0.6352	−1.8743	0.0062	0.1319	tags = 51%, list = 15%, signal = 60%
PPAR signaling pathway	−0.5521	−1.8445	0.0021	0.1366	tags = 35%, list = 14%, signal = 40%
Pentose phosphate pathway	−0.6633	−1.8314	0.0071	0.123	tags = 54%, list = 18%, signal = 66%
Leukocyte transendothelial migration	−0.4905	−1.8007	0.0058	0.132	tags = 28%, list = 6%, signal = 30%
Fc-gamma R-mediated phagocytosis	−0.5348	−1.797	0.0091	0.1175	tags = 39%, list = 10%, signal = 43%
Starch and sucrose metabolism	−0.5857	−1.7804	0.0141	0.1213	tags = 32%, list = 6%, signal = 34%
Amino sugar and nucleotide sugar metabolism	−0.5479	−1.7695	0.0102	0.1207	tags = 43%, list = 10%, signal = 47%
Lysosome	−0.5317	−1.7287	0.0326	0.1558	tags = 43%, list = 14%, signal = 50%
Drug metabolism other enzymes	−0.5791	−1.7214	0.0104	0.1505	tags = 26%, list = 5%, signal = 27%
Pathogenic *Escherichia coli* infection	−0.5274	−1.7129	0.0192	0.1473	tags = 30%, list = 6%, signal = 32%
Glycerolipid metabolism	−0.514	−1.7017	0.0135	0.1494	tags = 35%, list = 17%, signal = 42%
Fatty acid metabolism	−0.5976	−1.6919	0.0315	0.1497	tags = 45%, list = 19%, signal = 56%
Pantothenate and coa biosynthesis	−0.6258	−1.6918	0.0185	0.1399	tags = 53%, list = 14%, signal = 62%
Fructose and mannose metabolism	−0.5324	−1.6871	0.0235	0.1354	tags = 52%, list = 18%, signal = 63%
Systemic lupus erythematosus	−0.5401	−1.6828	0.0424	0.1324	tags = 50%, list = 24%, signal = 66%
Propanoate metabolism	−0.6005	−1.6664	0.0254	0.1426	tags = 45%, list = 16%, signal = 53%
β-alanine metabolism	−0.5775	−1.6543	0.0224	0.1475	tags = 45%, list = 13%, signal = 52%
Vibrio cholerae infection	−0.4992	−1.6506	0.0252	0.1444	tags = 36%, list = 10%, signal = 40%
Long-term potentiation	−0.4729	−1.6401	0.0292	0.1490	tags = 35%, list = 11%, signal = 39%
Alzheimer's disease	−0.4444	−1.633	0.0352	0.1487	tags = 38%, list = 16%, signal = 45%
Tryptophan metabolism	−0.5296	−1.6317	0.0262	0.1434	tags = 42%, list = 17%, signal = 50%
Regulation of actin cytoskeleton	−0.4169	−1.6314	0.0206	0.1379	tags = 22%, list = 6%, signal = 23%
Vascular smooth muscle contraction	−0.4232	−1.6191	0.0171	0.1437	tags = 26%, list = 11%, signal = 30%

ES – enrichment score; FDR – false discovery rate; NES – normalized enrichment score; NOM – nominal.

According to recent studies, TGIF1 functions as a tumor suppressor in pancreatic ductal adenocarcinoma by inhibiting Twist1 expression and activity.[29,30] The deletion of TGIF1 can induce the activation of the HAS2-CD44 signaling pathway and the upregulation of PD-L1, resulting in promoting the development of pancreatic ductal adenocarcinoma (PDAC). Given the essential role TGF-β and retinoic acid (RA) signaling play in hematopoiesis, some studies have been extended in recent years, attempting to find out the connections between TGIF1 and hematological diseases.[31] In myeloid lineage leukemias, TGIF1 seems to function as a vital regulator in normal and malignant hematopoiesis.[15,30] Hamid and Brandt[31] proposed that TGIF1 may regulate the balance between proliferation and differentiation during the myelopoiesis of human myeloid leukemia cells. Yan et al. showed that TGIF1 acts as a novel regulator of normal hematopoietic stem cell (HSC) function by suppressing HSC maintenance, self-renewal and quiescence in mice, resulting in the reduction of malignant transformation risk and leukemic stem cell or progenitor cell survival with chemotherapy.[15] Moreover, further exploration analyzed the specific role TGIF1 plays in leukemia initiation and progression.[32,33] For example, TGIF1 exerts antileukemic contributions by affecting TGF-β- and RA-driven functions.[31] Willer et al. showed that TGIF1 interferes with MLL-rearranged AML maintenance by competing with MEIS1 for chromatin-binding regions.[30] Collectively, these findings demonstrated that TGIF1 plays an important role in stem cell function regulation by maintaining the balance between proliferation and differentiation, and might possess potential prognostic value.

In the present study, clinical and molecular characteristics data of AML patients from the OHSU study cohort were analyzed to explore the relevance between TGIF1 expression and AML clinical features. A strong relationship between TGIF1 and cytogenetic risk stratification was confirmed. Considering the tight association between cytogenetics and the prognosis of the disease,[5] we examined TGIF1 expression compared to AML outcome, including OS, EFS and cumulative incidence of relapse (CIR) to assess the prognostic effects of TGIF1. Following

the identification of the relevance between higher TGIF1 expression and better prognosis, the same analyses were expanded on other independent datasets for confirmation, while univariate and multivariate analyses were performed for prognostic independence. Therefore, TGIF1 expression was regarded as the independent factor predicting better survival in patients with AML. Further analyses on TGIF1 should be performed to ensure the association between its expression and AML subtypes. For the OHSU cohort, Fig. 3D shows that AML patients with high TGIF1 level had a longer OS than those with low TGIF1 in the NPM1-mutated subgroup. We performed the Kaplan–Meier curve survival analysis, and the curve revealed a significant difference in survival between different expression levels of TGIF1 in the *NPM1*-mutated subgroup. This conclusion was congruent to TCGA cohort, which indicated that the favorable impact on prognosis that TGIF1 provided also applied for AML with *NPM1* mutations.

The *NPM1* and *FLT3-ITD* collectively determined AML prognosis when referring to the risk stratification systems by ELN 2017.[22] Therefore, based on the information above, we successfully constructed a more practical stratification system by integrating prognostic information of different TGIF1 expressions and *NPM1*-mutant AML into the original one. The reliability of this improvement was corroborated by a comprehensive analysis of a separate study cohort.

Limitations

The study was based on information obtained from mutual validation of publicly available data. The strengths of the trial include its strong eligibility criteria and a uniform treatment regimen according to standard guidelines. Despite the fact that our results provide a novel risk stratification option for *NPM1*-mutant AML, there are still certain limitations. Our analysis has a retrospective study design, and thus the accuracy rate may be lower in small sample cases. This work is based on the results of an RNA-sequencing dataset, and morphological insights remain to be explored. Further verification of the reliability is needed for the multivariate analysis to identify predictors after univariate analysis in statistical descriptions, similarly to previous studies.[33,34]

Conclusions

Our study proved that the upregulation of TGIF1 is closely associated with favorable prognosis in AML, and adding the expression level of TGIF1 examination can optimize risk stratification with *NPM1*-mutant AML, enhancing sensitivity and specificity in patient classification as well as providing reliable evidence for clinical decision-making. Further research is needed for new biomarkers and their combinations for personalized treatment.

Supplementary material

The supplementary files are available at https://doi.org/10.5281/zenodo.7414416. The package consists of the following files:

Supplementary Information 1. The difference analysis results of 2 groups of continuous variables involved in this study, including age, WBC, PLT, bone marrow (BM) blasts count, PB, and SCr.

Supplementary Information 2. Cox regression based on Schoenfeld residuals for testing proportional hazards assumptions.

Supplementary Information 3. The R code used in the proportional hazards assumption in this study.

Supplementary Information 4. The R code used in the univariate analysis in this study.

Supplementary Information 5. The R code used in the multivariate analysis in this study.

Supplementary Information 6. The R code used in the OS analysis in this study.

Supplementary Information 7. Event-free survival of AML patients was analyzed using TCGA-AML data.

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References

1. Papayannidis C, Sartor C, Marconi G, et al. Acute myeloid leukemia mutations: Therapeutic implications. *Int J Mol Sci*. 2019;20(11):2721. doi:10.3390/ijms20112721
2. Bose P, Vachhani P, Cortes JE. Treatment of relapsed/refractory acute myeloid leukemia. *Curr Treat Options Oncol*. 2017;18(3):17. doi:10.1007/s11864-017-0456-2
3. Edwards H, Ge Y. ONC201 shows promise in AML treatment. *Cell Cycle*. 2018;17(3):277–277. doi:10.1080/15384101.2017.1421035
4. Angenendt L, Wöste M, Mikesch JH, et al. Calcitonin receptor-like (CALCRL) is a marker of stemness and an independent predictor of outcome in pediatric AML. *Blood Adv*. 2021;5(21):4413–4421. doi:10.1182/bloodadvances.2021005236
5. Padmakumar D, Chandraprabha VR, Gopinath P, et al. A concise review on the molecular genetics of acute myeloid leukemia. *Leuk Res*. 2021;111:106727. doi:10.1016/j.leukres.2021.106727
6. Kantarjian HM, Short NJ, Fathi AT, et al. Acute myeloid leukemia: Historical perspective and progress in research and therapy over 5 decades. *Clin Lymphoma Myeloma Leuk*. 2021;21(9):580–597. doi:10.1016/j.clml.2021.05.016
7. Kumar D, Mehta A, Panigrahi MK, Nath S, Saikia KK. NPM1 mutation analysis in acute myeloid leukemia. Comparison of three techniques: Sanger sequencing, pyrosequencing, and real-time polymerase chain reaction. *Turk J Hematol*. 2018;35(1):49–53. doi:10.4274/tjh.2017.0095
8. Kelemen K. The role of nucleophosmin 1 (NPM1) mutation in the diagnosis and management of myeloid neoplasms. *Life*. 2022;12(1):109. doi:10.3390/life12010109
9. Balusu R, Fiskus W, Rao R, et al. Targeting levels or oligomerization of nucleophosmin 1 induces differentiation and loss of survival of human AML cells with mutant NPM1. *Blood*. 2011;118(11):3096–3106. doi:10.1182/blood-2010-09-309674

10. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352(3):254–266. doi:10.1056/NEJMoa041974
11. Falini B, Brunetti L, Sportoletti P, Martelli MP. *NPM1*-mutated acute myeloid leukemia: From bench to bedside. *Blood*. 2020;136(15): 1707–1721. doi:10.1182/blood.2019004226
12. Falini B, Martelli MP, Bolli N, et al. Acute myeloid leukemia with mutated nucleophosmin (NPM1): Is it a distinct entity? *Blood*. 2011;117(4): 1109–1120. doi:10.1182/blood-2010-08-299990
13. Eckardt JN, Middeke JM, Riechert S, et al. Deep learning detects acute myeloid leukemia and predicts NPM1 mutation status from bone marrow smears. *Leukemia*. 2022;36(1):111–118. doi:10.1038/s41375-021-01408-w
14. Fu W, Huang A, Xu L, et al. Cytogenetic abnormalities in *NPM1*-mutated acute myeloid leukemia. *Leuk Lymphoma*. 2022;63(8):1956–1963. doi:10.1080/10428194.2022.2045600
15. Yan L, Womack B, Wotton D, et al. *Tgif1* regulates quiescence and self-renewal of hematopoietic stem cells. *Mol Cell Biol*. 2013;33(24): 4824–4833. doi:10.1128/MCB.01076-13
16. Anderson AE, Taniguchi K, Hao Y, et al. *Tgif1* and *Tgif2* repress expression of the RabGAP Evi5l. *Mol Cell Biol*. 2017;37(5):e00527-16. doi:10.1128/MCB.00527-16
17. Kong L, Yu Y, Guan H, et al. TGIF1 plays a carcinogenic role in esophageal squamous cell carcinoma through the Wnt/β-catenin and Akt/mTOR signaling pathways. *Int J Mol Med*. 2021;47(5):77. doi:10.3892/ijmm.2021.4910
18. Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of acute myeloid leukaemia. *Nature*. 2018;562(7728):526–531. doi:10.1038/s41586-018-0623-z
19. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059–2074. doi:10.1056/NEJMoa1301689
20. Hess AS, Hess JR. Understanding tests of the association of categorical variables: The Pearson chi-square test and Fisher's exact test. *Transfusion*. 2017;57(4):877–879. doi:10.1111/trf.14057
21. Kadia TM, Wei AH. Evolution of therapy for older patients with acute myeloid leukemia: How should we use currently available agents? *Cancer J*. 2022;28(1):67–72. doi:10.1097/PPO.0000000000000574
22. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424–447. doi:10.1182/blood-2016-08-733196
23. Döhner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: Interaction with other gene mutations. *Blood*. 2005;106(12):3740–3746. doi:10.1182/blood-2005-05-2164
24. Verhaak RGW, Goudswaard CS, van Putten W, et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): Association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. *Blood*. 2005;106(12):3747–3754. doi:10.1182/blood-2005-05-2168
25. Ostronoff F, Othus M, Lazenby M, et al. Prognostic significance of *NPM1* mutations in the absence of *FLT3*-internal tandem duplication in older patients with acute myeloid leukemia: A SWOG and UK National Cancer Research Institute/Medical Research Council Report. *J Clin Oncol*. 2015;33(10):1157–1164. doi:10.1200/JCO.2014.58.0571
26. Pastore F, Dufour A, Benthaus T, et al. Combined molecular and clinical prognostic index for relapse and survival in cytogenetically normal acute myeloid leukemia. *J Clin Oncol*. 2014;32(15):1586–1594. doi:10.1200/JCO.2013.52.3480
27. Yi J, Zhou LY, Yi YY, et al. Low expression of pseudogene POU5F1B affects diagnosis and prognosis in acute myeloid leukemia (AML). *Med Sci Monit*. 2019;25:4952–4959. doi:10.12659/MSM.914352
28. Wu G, Ma Z, Hu W, et al. Molecular insights of Gas6/TAM in cancer development and therapy. *Cell Death Dis*. 2017;8(3):e2700. doi:10.1038/cddis.2017.113
29. Onishi-Seebacher M, Erikson G, Sawitzki Z, et al. Repeat to gene expression ratios in leukemic blast cells can stratify risk prediction in acute myeloid leukemia. *BMC Med Genomics*. 2021;14(1):166. doi:10.1186/s12920-021-01003-z
30. Willer A, Jakobsen JS, Ohlsson E, et al. TGIF1 is a negative regulator of MLL-rearranged acute myeloid leukemia. *Leukemia*. 2015;29(5): 1018–1031. doi:10.1038/leu.2014.307
31. Hamid R, Brandt SJ. Transforming growth-interacting factor (*TGIF*) regulates proliferation and differentiation of human myeloid leukemia cells. *Mol Oncol*. 2009;3(5–6):451–463. doi:10.1016/j.molonc.2009.07.004
32. Wang H, Peng J, Wang B, et al. Inconsistency between univariate and multiple logistic regressions. *Shanghai Arch Psychiatry*. 2017; 29(2):124–128. doi:10.11919/j.issn.1002-0829.217031
33. Lo SK, Li IT, Tsou TS, See L. Non-significant in univariate but significant in multivariate analysis: A discussion with examples [in Chinese]. *Changgeng Yi Xue Za Zhi*. 1995;18(2):95–101. PMID:7641117.
34. Yan L, Davé UP, Engel M, Brandt SJ, Hamid R. Loss of TG-interacting factor 1 decreases survival in mouse models of myeloid leukaemia. *J Cell Mol Med*. 2020;24(22):13472–13480. doi:10.1111/jcmm.15977