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Distinct Clinical and Biological Characteristics of Acute Myeloid Leukemia with Higher Expression of *PIWIL4*

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INTRODUCTION

PIWIL4, short for Piwi-like RNA-mediated gene silencing 4, is a member of the PIWI (P-element-induced wimpy testis) family of proteins. The PIWI family is known for its involvement in RNA silencing mechanisms, particularly in the regulation of germline development, maintenance of stem cells, and protection against transposable elements[1]. Recently, it has been shown to be overexpressed in individuals with acute myeloid leukemia (AML) and is deemed crucial for the proliferation of leukemia cells[2]. Its role extends to impeding the accumulation of R-loops and sustaining the expression of genes associated with leukemia stem cells (LSCs). Additionally, PIWIL4 is implicated in activating the ataxia telangiectasia and Rad3 (ATR) pathways within AML cells. PIWIL4 depletion in AML cells emerges as a potential and actionable strategy for therapeutic intervention in AML patients.

AIM

Since the clinical significance of *PIWIL4* expression in AML is not totally clear yet, the study aims to investigate its association with biological and clinical features in AML patients.

METHOD

We studied 552 de novo non-M3 AML patients from the National Taiwan University Hospital, who had available bone marrow (BM) samples for RNA-seq analysis at diagnosis and extensive clinical, cytogenetic and gene mutation data. RNA was extracted from fresh BM samples of these patients and sent for RNA sequencing. TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA) was used for library preparation. The *PIWIL4* expression levels (transcript per million, TPM) were calculated from RNAseq results and analyzed for their clinical significance. Targeted NGS was performed to detect myeloid-neoplasm relevant mutations in 54 genes and the library preparation and sequencing were performed per the manufacturer's instructions. We also validated the findings from this study in two public AML databases. For drug response analysis, we used single-sample gene set enrichment analysis (ssGSEA) to evaluate common chemotherapy agents

RESULTS

The 552 patients were divided into two groups based on the median TPM level of *PIWIL4* expression. The clinical and laboratory features including cytogenetics and mutations at diagnosis, and ELN risk groups of these patients are summarized in Table 1. Higher *PIWIL4* expression was associated with higher white blood cell count and lactate dehydrogenase level, indicating its more proliferative nature (Figure 1a). In addition, higher *PIWIL4* expression was correlated with t(8;21), *FLT3*-TKD and *NPM1* mutations while negatively associated with inv(16) and *RUNX1* mutation. Patients categorized to favorable risk subgroup by 2022 ELN risk classification more frequently had higher *PIWIL4* expression, while those categorized to adverse risk subgroup had significantly lower *PIWIL4* expression.

Moreover, patients with higher *PIWIL4* expression had a higher complete remission rate after receiving standard induction chemotherapy (77.9% vs. 63.4%, p=0.002) (Figure 1c), and longer overall survival (OS) (median, 52.8 months vs. 17.8 months, p<0.001) (Figure 1b) compared to those with lower expression. This prognostic significance could be validated in two external cohorts, TCGA-LAML and BEATAML. In line with the clinical observation, the ssGSEA analysis showed AML cells with higher *PIWIL4* were significantly chemo-sensitive to daunorubicin (p<0.01) in ours (Figure 1d) and the BEATAML cohort.

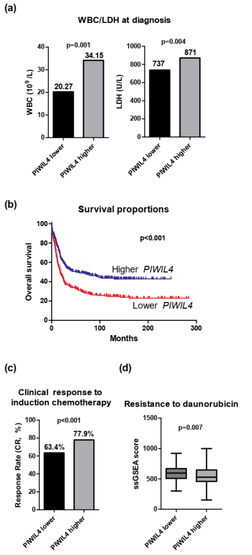
In multivariate analyses, higher expression of *PIWIL4* remained significantly favorable for OS independent of age, blast count and ELN 2022 risk categories.

Table 1. Comparison of clinical and laboratory features between patients with lower and higher *PIWIL4* expression

Clinical characters	Total (n=552)	Higher PIWIL4 (n=276)	Lower PIWIL4 (n=276)	p value
Age (year)[a]	47.3 (15.9-85.9)	47.8 (16.1-85.9)	47.2 (15.9-80.5)	0.915
Sex				0.495
Male	296 (53.6)	152 (55.1)	144 (52.2)	
Female	256 (46.4)	124 (44.9)	132 (47.8)	
Laboratory data[a]				
WBC (10^9/L)	27.80 (0.49-405.65)	34.15 (0.49-405.65)	20.27 (0.51-361.30)	0.001
Hb (g/dL)	8.1 (2.4-16.0)	8.1 (2.7-14.2)	8.1 (2.4-16.0)	0.357
Platelet (10^9/L)	44.5 (2-1017)	41 (3-712)	49 (2-1017)	0.016
BM blast (%)	62.1 (1.6-98.4)	58.3 (3.2-98.4)	64.7 (1.6-98.0)	0.001
LDH (U/L)	801.5 (137-8693)	871 (159-8693)	737 (137-7293)	0.004
Cytogenetics and mutations[b]				
t(8;21)	55 (10.1)	42 (15.5)	13 (4.7)	<0.001
inv(16)	26 (4.8)	6 (2.2)	20 (7.3)	0.006
NK	287 (52.6)	151 (55.7)	136 (49.5)	0.143
NPM1 mutation	110 (20.0)	72 (26.1)	38 (13.8)	<0.001
FLT3-ITD	128 (23.3)	66 (23.9)	62 (22.6)	0.721
FLT3-TKD	49 (8.9)	34 (12.3)	15 (5.5)	0.005
FLT3-ITD and/or TKD	166 (30.2)	93 (33.7)	73 (26.6)	0.072
CEBPA mutation	110 (20.0)	59 (21.4)	51 (18.5)	0.406
RUNX1 mutation	54 (9.8)	13 (4.7)	41 (15.0)	<0.001
TP53 mutation	18 (3.3)	8 (3.0)	10 (3.6)	0.654
Risk groups[c]				
ELN favorable	225 (40.8)	136 (49.3)	89 (32.2)	<0.001
ELN intermediate	159 (28.8)	73 (26.4)	86 (31.2)	0.222
ELN adverse	168 (30.4)	67 (24.3)	101 (36.6)	0.002
Induction CR	390 (70.7)	215 (77.9)	175 (63.4)	<0.001

* Statistically significant if p value <0.05. [a] Median (range). [b] Patients had available information: 546 for cytogenetics, 551 for *NPM1* and *CEBPA* mutations, 550 for *FLT3*-ITD and -TKD, 549 for *RUNX1* mutation, and 544 for *TP53* mutation. [c] Risk groups: ELN: European LeukemiaNet 2022 risk classification Abbreviations: WBC, white blood cells count; Hb, hemoglobin; BM, bone marrow; LDH, lactate dehydrogenase; NK, normal karyotype; CR, complete remission

Figure 1.



CONCLUSIONS

Higher expression of *PIWIL4* in AML patients was correlated with a more proliferative features, as well as t(8;21), *FLT3*-TKD and *NPM1* mutations, but negatively associated with inv(16) and *RUNX1* mutation. Counterintuitively, its high expression appears to be an independent favorable prognostic factor in our cohort, and could be validated externally by two independent cohorts. AML patients with higher *PIWIL4* expression had better response to the induction chemotherapy, in accordance with the ssGSEA results, may partly elucidate the clinical finding.

REFERENCES

1. Cox DN, Chao A, and Lin H, piwi encodes a nucleoplasmic factor whose activity modulates the number and division rate of germline stem cells. *Development*, 2000. **127**(3):503-514.
2. Bamezai S, Pulikkottil AJ, Yadav T, Vegi NM, Mueller J, Mark J, et al., A noncanonical enzymatic function of PIWIL4 maintains genomic integrity and leukemic growth in AML. *Blood*, 2023. **142**(1):90-105.

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