Original Paper

High Expression of AHSP, EPB42, GYPC and HEMGN Predicts Favorable Prognosis in FLT3-ITD-Negative Acute Myeloid Leukemia

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Key Words

AHSP • EPB42 • GYPC • HEMGN • FLT3-ITD • Acute myeloid leukemia

Abstract

Background/Aims: Acute myeloid leukemia (AML) is a heterogeneous clonal disease and patients with AML who harbor an FMS-like tyrosine kinase 3 (*FLT3*) mutation present several dilemmas for the clinician. This study aims to identify novel targets for explaining the dilemmas.

Methods: We analyzed four microarray gene expression profiles to investigate changes in whole genome expression associated with *FLT3*-ITD mutation. **Results:** We identified 22 differentially expressed genes which are commonly expressed among all four profiles. Kaplan-Meier analysis of the dataset GSE12417 revealed that low expression of *AHSP*, *EPB42*, *GYPC* and *HEMGN* predicted poor prognosis (*AHSP*: $P=0.0317$, $HR=1.894$; *EPB42*: $P=0.0382$, $HR=1.859$; *GYPC*: $P=0.0015$, $HR=2.051$; *HEMGN*: $P=0.0418$, $HR=1.838$ in GSE12417 test cohort; *AHSP*: $P=0.0279$, $HR=1.548$; *EPB42*: $P=0.0398$, $HR=1.505$; *GYPC*: $P=0.0408$, $HR=1.501$; *HEMGN*: $P=0.0143$, $HR=1.630$ in GSE12417 validation cohort). When patients were *FLT3*-ITD positive, the expression of *FLT3* was significantly increased (all $P<0.05$ in four profiles), and correlation analysis of four profiles revealed that the expression of the four candidate genes negatively correlated with *FLT3* expression. **Conclusions:** Our findings suggest that *AHSP*, *EPB42*, *GYPC* and *HEMGN* may be suitable biomarkers for diagnostic or therapeutic strategies for *FLT3*-ITD-positive AML patients.

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Introduction

Acute myeloid leukemia (AML) is a most common form of hematological malignancy characterized by uncontrolled proliferation of abnormal blast cells or hematopoietic myeloid progenitor cells [1, 2]. Cytarabine (also named as cytosine arabinoside, Ara-C) combined

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with anthracyclines are the most common chemotherapies in the treatment of AML for more than 40 years. Complete remission (CR) rate is about 70%, of which about 60% of patients relapse and die of their disease. There are many factors that contribute to the difference in chemotherapy efficacy and prognosis among patients with AML, among which genetic factors [3] and chemotherapy resistance [4, 5] are the most important factors.

As with other tumors, molecular and cytogenetic profiles are used to guide the clinical treatment of AML [6]. Presently, analysis of molecular abnormalities provides one of the most crucial prognostic information at diagnosis for AML patients [7, 8]. Numerous somatic mutations have been discerned in AML. Somatic mutations in gene encoding nucleophosmin (*NPM1*), CCAAT/enhancer binding protein alpha (*CEBPA*) and fms-related tyrosine kinase 3 (*FLT3*), DNA (cytosine-5-)-methyltransferase 3 alpha (*DNMT3A*) [9], tet methylcytosine dioxygenase 2 (*TET2*) [10], isocitrate dehydrogenase 1/2 (*IDH1/2*) [11, 12], additional sex combs like 1 (*ASXL1*) [13] and PHD finger protein 6 (*PHF6*) [14, 15] have been implicated as well-established genetic marker in AML [16, 17].

FLT3 gene, which plays a key role in normal hematopoiesis, is mutated in a third of AML cases [18]. There are two major mutations categories, in-frame duplications within the juxtamembrane region (*FLT3*-ITD) and point mutation in the tyrosine kinase domain (*FLT3*-TKD). Approximately 30% of AML patients harbor some form of *FLT3* mutation, 25% of AML patients carry *FLT3*-ITD mutation and 7% of AML patients carry *FLT3*-TKD mutation. These mutations lead to constitutive activation of the receptor, and then lead to distinctive prognostic effects. A mass of studies have confirmed that *FLT3*-ITD was an independent predictor of higher relapse rate and poorer overall survival (OS) [18]. However, the mechanism by which this mutation affects prognosis is still vague, suggesting that additional correlation factors reflecting differences in the spectra of associated cooperating mutations as well as differences in signaling pathways downstream of *FLT3* impacting disease biology need to be recognized.

In the present research, we analyzed four microarray gene expression profiles to investigate changes in gene expression associated with *FLT3*-ITD mutation and identify novel target for AML diagnosis and treatment. We identified 22 differentially expressed genes in AML that are commonly expressed among all four profiles. Kaplan-Meier analysis of GSE12417 dataset revealed that gene expression of *AHSP*, *EPB42*, *GYPC* and *HEMGN* were correlated with disease prognosis, which was coincident with the results in clinically applicable analysis. Correlation analysis of four profiles revealed that all four candidate genes expression negatively correlated with *FLT3* expression, suggesting that *AHSP*, *EPB42*, *GYPC* and *HEMGN* may be suitable biomarkers for diagnostic or therapeutic strategies for *FLT3*-ITD-positive AML patients.

Materials and Methods

Affymetrix Microarray Data

Four microarray datasets were included and three of them are available in the ArrayExpress database (<http://www.ebi.ac.uk/arrayexpress>) [19] under accession number E-TABM-1029, which included 38 *FLT3*-ITD-positive normal karyotype (NK) AML samples and 79 *FLT3*-ITD-negative NK-AML samples, and in the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) [20] under accession number GSE15434, including 90 *FLT3*-ITD-positive NK-AML samples and 161 *FLT3*-ITD-negative NK-AML samples, and GSE61804, which included 50 *FLT3*-ITD-positive AML samples and 236 *FLT3*-ITD-negative AML samples. Moreover, we also used public The Cancer Genome Atlas (TCGA, <http://cancergenome.nih.gov/>) data repositories as our source of samples [21], including 48 *FLT3*-ITD-positive AML samples and 122 *FLT3*-ITD-negative AML samples. A total of 831 AML samples were included in the study. The platform of four datasets is Affymetrix Human Genome U133 Plus 2.0 Array (HG-U133_Plus_2, GPL570). The original CEL files as well as the probe annotation were downloaded from the platform. For prognosis analysis, 242 NK-AML samples having prognosis information were included in survival analysis in GSE12417 dataset. There were two sample sets in GSE12417, which included test and training dataset. In test dataset,

analysis of 79 samples of bone marrow (BM) or peripheral blood mononuclear cells (PBMCs) from adult patients with untreated AML using GPL570 platforms. In training dataset, 163 samples of BM or PBMCs from adult patients with untreated AML were analyzed with two different platforms: Affymetrix Human Genome U133A Array (HG-U133A, GPL96) and Affymetrix Human Genome U133B Array (HG-U133B, GPL97). Detailed information used for these datasets were described in Table 1.

Analysis of Differently Expressed Probes (DEPs)

The probe-level data in CEL files were converted into expression profiles in R software (<https://www.r-project.org/>) and the robust multiarray average (RMA) algorithm with affy package was used to correct and normalize expression profiles data. Due to one gene has one or more corresponding probe sets, which have a plurality of expression values, the gene expression values reflect the averaged values of those probe sets [22]. All patients were classified into *FLT3*-ITD positive and negative groups. When meeting the criterion of $|\text{fold change}| \geq 2.0$ and $P \text{ value} < 0.05$, genes were considered differentially expressed. Thenceforth, DEPs among 4 datasets were merged and 28 overlap DEPs or 22 corresponding differentially expressed genes (DEGs) were taken out. Further validation of the association between these probes and prognosis was utilized in GSE12417 dataset. Finally, for the purpose to examine the relationship between DEGs and *FLT3* expression, correlation analysis was taken between 22 DEGs and *FLT3* in 4 datasets. Flow chart of the procedure for the literature search was described in Fig. 1.

Table 1. Characteristics of all datasets

Data sets	Platform	n	FLT3-ITD positive	FLT3-ITD negative	Sample
GSE15434	GPL570	251	90	161	NK-AML
GSE61804	GPL570	286	50	236	AML
E-TABM-1029	GPL570	117	38	79	NK-AML
TCGA	GPL570	170	48	122	AML
	GPL570	79	-	-	NK-AML
GSE12417	GPL96	163	-	-	NK-AML
	GPL97	163	-	-	NK-AML

Fig. 1. Flow chart of the procedure for the literature search. DEPs: differentially expressed probes; PPI: protein-protein interaction.

Flow chart of the procedure for the literature search was described in Fig. 1.

Gene ontology and pathway enrichment analysis of DEGs

Gene Ontology (GO, <http://www.geneontology.org>) is a useful method commonly used to annotate genes, gene products and sequences [23, 24]. Reactome (<http://www.reactome.org/>) is a free, open-source, curated and peer reviewed pathway database and is intuitive bioinformatics tool for the visualization, interpretation and analysis of pathway knowledge to support basic research, genome analysis, modeling, systems biology and education [25]. DAVID database (<https://david.ncicrf.gov/>) is a gene functional analysis tools aimed at systematically extracting biological meaning from large gene/user's gene [26, 27]. GO enrichment and Reactome pathway analysis were performed using DAVID online tool for analyzing the DEGs at the functional level. $P < 0.05$ was considered statistically significance.

Protein-protein interaction (PPI) network analysis

Search Tool for the Retrieval of Interacting Genes (STRING) is a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations; they stem from computational prediction, from knowledge transfer between organisms, and from interactions aggregated from other (primary) databases. To evaluate the interactive relationship between 22 DEGs

Table 2. Gene ontology and Reactome pathway analysis of differentially expressed genes associated with AML

Category	Term/Gene function	Gene count	%	P Value	Genes
GOTERM_BP_FAT	GO:0030097~hemopoiesis	7	31.82	9.26E-05	LTF, MEIS1, EPB42, HOXB3, ADD2, AHSP, ALAS2
GOTERM_BP_FAT	GO:0020027~hemoglobin metabolic process	3	13.64	1.15E-04	AHSP, EPB42, ALAS2
GOTERM_BP_FAT	GO:0048534~hematopoietic or lymphoid organ development	7	31.82	1.26E-04	LTF, MEIS1, EPB42, HOXB3, ADD2, AHSP, ALAS2
GOTERM_BP_FAT	GO:0002520~immune system development	7	31.82	1.74E-04	LTF, MEIS1, EPB42, HOXB3, ADD2, AHSP, ALAS2
GOTERM_BP_FAT	GO:0030099~myeloid cell differentiation	5	22.73	4.40E-04	LTF, MEIS1, EPB42, AHSP, ALAS2
GOTERM_BP_FAT	GO:0006811~ion transport	7	31.82	0.001	SLC4A1, RHD, CXCL12, SLC6A8, LTF, CA1, ADD2
GOTERM_BP_FAT	GO:0061515~myeloid cell development	3	13.64	0.002	LTF, MEIS1, EPB42
GOTERM_BP_FAT	GO:005072~iron ion homeostasis	3	13.64	0.003	EPB42, LTF, ALAS2
GOTERM_BP_FAT	GO:0055080~cation homeostasis	5	22.73	0.005	CXCL12, SLC4A1, LTF, EPB42, ALAS2
GOTERM_BP_FAT	GO:0030218~erythrocyte differentiation	3	13.64	0.005	AHSP, EPB42, ALAS2
GOTERM_CC_FAT	GO:0030863~cortical cytoskeleton	3	13.64	0.006	SLC4A1, GYPC, EPB42
GOTERM_CC_FAT	GO:0044448~cell cortex part	3	13.64	0.011	SLC4A1, GYPC, EPB42
GOTERM_CC_FAT	GO:0005938~cell cortex	3	13.64	0.038	SLC4A1, GYPC, EPB42
GOTERM_CC_FAT	GO:0005887~integral component of plasma membrane	5	22.73	0.044	RHD, GYPC, SLC4A1, SLC6A8, GYPA
GOTERM_MF_FAT	GO:0008519~ammonium transmembrane transporter activity	1	4.55	0.019	RHD
REACTOME_PATHWAY	R-HSA-1247673:Erythrocytes take up oxygen and release carbon dioxide	2	9.09	0.010	SLC4A1, CA1
REACTOME_PATHWAY	R-HSA-1237044:Erythrocytes take up carbon dioxide and release oxygen	2	9.09	0.015	SLC4A1, CA1

and *FLT3*, homeobox B4 (*HOXB4*), we uploaded these genes to STRING. When the experimental validation interactions score was greater than 0.4, the interaction was considered significant.

Statistical Analysis

Statistical analysis was carried out by the software SPSS version 23.0 (IBM corporation, Armonk, NY, USA) and GraphPad Prism 5.01 software (GraphPad Software Inc, CA, USA). Comparisons of continuous variables (*FLT3* expression) between *FLT3* positive and negative groups were performed using nonparametric tests (The Mann–Whitney U). Kaplan–Meier curves were depicted to estimate the profiles of survival, and the log-rank tests were carried out to compare the differences between survival curves. Spearman correlation analysis was performed between gene expression. Statistical significance was accepted when $P < 0.05$ in two sided.

Results

Identification of DEPs

Four gene expression profiles (E-TABM-1029, GSE15434, GSE61804 and TCGA) with *FLT3* mutation information were analyzed to authenticate DEPs between *FLT3*-ITD positive and negative AML samples. A total of 377 probes (126 up-regulated and 251 down-regulated probes, Fig. 2A) were identified to be differentially expressed in E-TABM-1029 dataset, 106 probes (60 up-regulated and 46 down-regulated probes, Fig. 2A) differentially expressed in GSE15434 dataset, 199 probes (98 up-regulated and 101 down-regulated probes, Fig. 2A) differentially expressed in GSE61804 dataset, 882 probes (85 up-regulated and 797 down-regulated probes, Fig. 2A) differentially expressed in TCGA dataset. Afterwards, 28 common overlap probes (all down-regulated), corresponding 22 common overlap genes, were taken out for subsequent analysis (Fig. 2B).

Gene ontology and pathway enrichment analysis of DEGs

We uploaded 22 DEGs to the DAVID online database for identifying overrepresented GO categories and Reactome pathways. GO analysis results showed that 22 DEGs were significantly enriched in hemopoiesis ($P=9.26e-5$), hemoglobin metabolic process ($P=1.15e-4$), and hematopoietic or lymphoid organ development biological processes ($P=1.26e-4$, Table 2), significantly enriched in cortical cytoskeleton ($P=0.006$), cell cortex part ($P=0.011$), and cell cortex cell component ($P=0.038$, Table 2), significantly enriched in ammonium transmembrane transporter activity molecular function ($P=0.019$, Table

2). Reactome pathways analysis results revealed that 22 DEGs significantly enriched in erythrocytes take up oxygen and release carbon dioxide (R-HSA-1247673, $P=0.010$) and erythrocytes take up carbon dioxide and release oxygen pathways (R-HSA-1237044, $P=0.015$, Table 2).

Survival value of 28 DEPs

To investigate the association between the expression of 28 DEPs and patients' survival, we analyzed the prognostic value of those probes using Kaplan-Meier method for expression profile GSE12417. The median probe expression value was used as the cut-off value to compare the differences between the two groups. 28 DEPs prognostic significance was shown in Table 3. Only 5 probes (alpha hemoglobin stabilizing protein (*AHSP*) 219672_at, erythrocyte membrane protein band 4.2 (*EPB42*) 210746_s_at, glycophorin C (*GYPC*) 202947_s_at and hemogen (*HEMGN*) 223669_at and 223670_s_at) expression was associated with survival in two platforms among NK-AML patients. According to platform annotation file, *AHSP*, *EPB42* and *GYPC* gene have only one probe, *HEMGN* has two probes. Therefore, *HEMGN* gene expression values reflect the averaged values of those probe sets in subsequent study. Prognostic analysis results suggested that high expression of four genes conferred a survival advantage to the low expression in NK-AML patients (*AHSP*: $P=0.0317$,

Table 3. 28 DEPs prognostic significance in GSE12417 dataset

Probe ID	GPL570(n=79)		GPL96&GPL97(n=163)	
	P value	HR	P value	HR
ADD2_205268_s_at	0.032	4.439	0.276	1.910
AHSP_219672_at	0.032	1.894	0.028	1.548
ALAS2_211560_s_at	0.038	0.313	0.190	1.334
ARG1_206177_s_at	0.738	1.657	0.059	0.868
C3orf80_236738_at	0.035	1.392	0.412	1.465
CA1_205950_s_at	0.038	2.813	0.329	1.130
COL4A5_213110_s_at	0.868	0.805	0.554	0.800
CXCL12_209687_at	0.073	0.620	0.036	0.991
EPB42_210746_s_at	0.038	1.859	0.040	1.505
FAM92A1_228011_at	-	-	-	-
GYPA_205837_s_at	0.035	0.267	0.015	1.620
GYPA_205838_at	0.065	0.383	0.045	0.613
GYPA_211820_x_at	0.035	0.859	0.065	0.963
GYPA_211821_x_at	0.035	2.220	0.024	0.566
GYPC_202947_s_at	0.015	2.051	0.041	1.501
HEMGN_223669_at	0.018	2.028	0.014	1.628
HEMGN_223670_s_at	0.049	0.879	0.047	0.453
HOXB-AS3_230743_at	0.321	0.664	0.942	0.761
HOXB-AS3_236892_s_at	0.260	1.870	0.960	0.885
HOXB-AS3_239791_at	0.244	0.362	0.970	1.178
HOXB3_228904_at	0.997	0.725	0.873	0.811
LTF_202018_s_at	0.670	0.790	0.675	1.093
MEIS1_1559477_s_at	0.560	0.973	-	-
RHD_210586_x_at	0.058	1.325	0.442	3.538
RHD_215819_s_at	0.058	1.137	0.145	0.478
SELENBP1_214433_s_at	0.203	2.201	0.065	1.363
SLC4A1_205592_at	0.084	1.207	0.032	1.216
SLC6A8_202219_at	0.008	2.059	0.102	1.319
SNURF_201522_x_at	0.528	0.939	0.991	0.911

Fig. 2. Volcano plots and Venn diagram of four datasets differential probes. A: E-TABM-1029 dataset (126 up-regulated and 251 down-regulated probes). GSE15434 dataset (60 up-regulated and 46 down-regulated probes). GSE61804 dataset (98 up-regulated and 101 down-regulated probes). TCGA dataset (85 up-regulated and 797 down-regulated probes). B: the venn diagram represents the share and unique probes and genes between 4 datasets.

Fig. 3. Associations of *AHSP*, *EPB42*, *GYPC* and *HEMGN* gene expression with disease survivals in NK-AML patients. A: Kaplan–Meier curves of OS according to four genes expression in GSE12417 test cohort. B: Kaplan–Meier curves of OS according to four genes expression in GSE12417 validation cohort.

Fig. 4. The relationship of *FLT3* mutation and its expression in four datasets. *represent $P < 0.05$, **represent $P < 0.01$, ***represent $P < 0.001$.

HR=1.894; *EPB42*: $P=0.0382$, HR=1.859; *GYPC*: $P=0.0015$, HR=2.051; *HEMGN*: $P=0.0418$, HR=1.838 in GSE12417 test cohort, Fig.3A; *AHSP*: $P=0.0279$, HR=1.548; *EPB42*: $P=0.0398$, HR=1.505; *GYPC*: $P=0.0408$, HR=1.501; *HEMGN*: $P=0.0143$, HR=1.630 in GSE12417 validation cohort, Fig.3B). Given the results of prognostic analysis, four prognostic genes were included as candidate gene in following analysis.

Influence of FLT3 mutation on its mRNA expression

To investigate the relationship between *FLT3* mutation status and its expression, we analyzed the difference in *FLT3* expression between *FLT3* genotypes using nonparametric tests in four datasets. When patients with *FLT3*-ITD positive, the expression of *FLT3* was significantly increased. The same results was obtained in the four datasets (Fig. 4).

Correlation analysis of four differently expressed genes and FLT3 expression

The above analysis demonstrated that four differently expressed genes were associated with prognosis, but the molecular mechanism remains unclear. Therefore, we analyzed the relationship between expression of four differently expressed genes and *FLT3*. There was a negative correlation between the expression of *FLT3* and four differently expressed genes in all the four datasets (*AHSP*, Fig. 5A; *EPB42*, Fig.5B; *GYPC*, Fig. 5C; *HEMGN*, Fig. 5D).

Correlation analysis of HEMGN and HOXB4 expression

Previous study has demonstrated that *HEMGN* is a direct transcriptional target of *HOXB4* and induces expansion of murine myeloid progenitor cells at the cellular level [28]. In contrast, it is reported that *HOXB4* can indirectly affects the expression of *HEMGN*

Fig. 5. Correlation analysis of *AHSP* (A), *EPB42* (B), *GYPC* (C), *HEMGN* (D) gene and *FLT3* gene in four datasets. Correlation analysis of *HEMGN* (E) gene and *HOXB4* gene in three datasets. Due to *HOXB4* gene expression was not found in E-TABM-1029 dataset, so E-TABM-1029 dataset was not included in this part.

using embryonic stem cell-derived hematopoietic stem/progenitor cells [29]. However, the relationship between expression of *HEMGN* and *HOXB4* is still unknown yet in human samples. Thus we analyzed the relationship between *HEMGN* and *HOXB4* expression. Given *HOXB4* gene expression was not found in E-TABM-1029 dataset, so this dataset was excluded from this part. In other three datasets, we found a negative correlation between *HEMGN* and *HOXB4* expression (GSE15434: Spearman $r = -0.3353$, $P < 0.0001$; TCGA: Spearman $r = -0.3532$, $P < 0.0001$; GSE61804: Spearman $r = -0.1369$, $P = 0.0205$, Fig. 5E).

PPI analysis

Based on STRING online network analysis website, we uploaded the 24 genes (22 DEGs, *FLT3* and *HOXB4*) to STRING database. The results showed that 13 genes were integrated into one module and the other genes were not (Fig. 6). In the module, *AHSP*, *EPB42*, *GYPC* and *HEMGN* protein were indirectly correlated with *FLT3* protein. Therefore, the results also showed that *HEMGN* and *HOXB4* were correlated and proved the above results to a certain degree.

A clinically applicable four prognostics genes signature

Due to the complexity of AML disease, single gene mutation or expression variation is insufficient to cause disease development, and so it is necessary to analyze the four genes together. The correlation analysis revealed that the four genes were highly significant in relation to each other (all Spearman $r > 0.6$, $P < 0.0001$, data not shown). For the purpose of better guiding treatment of individual patients, we therefore sought to establish a method to define four prognostics genes expression prospectively. In order to achieve this, we set thresholds for each four prognostics genes calculated by mean. The mean expression levels

Fig. 6. Protein-protein interaction network form STRING online analysis website. Network nodes represent proteins and edges represent protein-protein associations. Small nodes, protein of unknown 3D structure. Large nodes, some 3D structure is known or predicted. Colored nodes, query proteins and first shell of interactors. White nodes, second shell of interactors. Purple edges, the interactions from experimentally determined. Black edges, co-expression. Green edges, textmining.

Fig. 7. A scheme for a clinically applicable four prognostics genes signature. Upper panel, expression thresholds for each 4 prognostics genes to classify NK-AML patients as being called 'positive'. Examples of NK-AML patients and the positives numbers are shown. Lower panel, Kaplan-Meier survival curves for 4 prognostic genes-low and -high patients defined by the method above in GSE12417 test cohort (A), in GSE12417 validation cohort (B). 4 prognostic genes-high patients showed significantly longer OS compared to 4 prognostic genes-low patients in both datasets. P values are derived from the log-rank test.

of *AHSP*, *EPB42*, *GYPC*, and *HEMGN* were 10.723, 9.754, 10.655 and 8.991, respectively, and expression above these thresholds was identified as “positive” for each gene (Fig. 7). Afterwards, AML patients were classified into 4 prognostics genes low and high based on having zero to one or more than one expressed, respectively. Kaplan-Meier curves showed that the survival of patients with 4 prognostics genes low expression defined by this classification were statistically significantly poorer than those who were high expression in GSE12417 both datasets (HR=1.907, P=0.0365 in GSE12417 test cohort, Fig 7A; HR=1.524, P=0.0332 in GSE12417 validation cohort, Fig. 7B).

Discussion

Rational use of microarray data sets can not only be more rapid and simple analysis of a large number of genes, but also help to accurately identify the key molecular targets. In the present research, we analyzed four microarray gene expression profiles to investigate changes in gene expression associated with *FLT3*-ITD status and identified high expression of *AHSP*, *EPB42*, *GYPC* and *HEMGN* predicts favorable prognosis. Correlation analysis of four profiles all revealed that four genes negatively correlated with *FLT3* expression.

AHSP encodes a molecular chaperone, which binds reversibly to free alpha-globin and is involved in hemoglobin assembly [30]. Previous studies have reported that loss of *AHSP* expression results in globin precipitation in murine model and caused ineffective erythropoiesis, and more exacerbates β -thalassemia [31, 32] and *AHSP*- knockdown cells demonstrated an increased reactive oxygen species (ROS) production and increased rate of apoptosis [33]. Moreover, *AHSP* gene expression is controlled by some regulatory elements, such as GATA binding protein 1 (GATA-1) [30], Organic cation transporter 1 (Oct-1) [34] and Erythroid Kruppel-like factor (EKLF) [35-37]. GATA-1 is a transcription factor that is essential for the survival and maturation of lineage-committed erythroid precursors, Oct-1 and EKLF are two transcription factors that have a critical role in erythropoiesis. In our study, *FLT3*-ITD mutation resulted in down-regulation of *AHSP* expression through regulate the gene expression of *FLT3*, thus affecting the prognosis of NK-AML patients probably through transcription factor regulation or ROS production or the rate of apoptosis. These data suggest that there may exist new pathways influencing *AHSP* expression.

EPB42 is an ATP-binding protein which may regulate the association of protein 3 with ankyrin and plays an important role in maintaining the structural integrity of red cell membrane. Studies have shown that *EPB42* (protein 4.2) is expressed much earlier in progenitors, at around the basophilic erythroblast stage [38] and protein 4.2 deficiency results in overt haemolysis, anaemia and hereditary spherocytosis [39-41], but the exact role of protein 4.2 in red cells has not been elucidated. In this study, a high *EPB42* expression predicted a favorable prognosis in AML and may be related to the interactions with CD47 [42, 43] and cytoskeletal protein ankyrin [44].

GYPC is an integral membrane glycoprotein and is unique among the glycophorin genes in two respects: First, *GYPC* encodes two erythrocyte surface glycoproteins in human, glycophorin C (GPC) and glycophorin D (GPD) via initiation of translation at two separate start codons on a single transcript [45]. Second, its sequence is not homologous to any other gene, while glycophorin A (*GYPA*), glycophorin B (*GYPB*), and glycophorin E (*GYPE*) are all paralogous [46, 47]. Recent studies have shown that *GYPC* is a biomarker in breast cancer between high-risk and low-risk group using whole-genome methylation analysis [48] and is a major erythrocyte receptor for the rodent malaria parasite plasmodium berghei [49]. However, there is only a single study about its relationship with acute leukemia. The study shown that high *GYPC* gene expression was associated with an unfavorable outcome in acute lymphoblastic leukemia patients [50]. This is in contradiction to our findings which found high *GYPC* gene expression was associated with a favorable prognosis in AML and may be caused by the different disease type.

HEMGN gene maps to chromosome 9q22 and encodes a novel nuclear protein, which was first identified in 2001. K-F Wu's results showed that different expression patterns of *HEMGN* in various leukemia cell lines, and also found *HEMGN* gene significantly higher expression in de novo AML patients than that in the normal donors and lower *HEMGN* expression level was associated with a favorable therapy response [51]. However, knockdown of *HEMGN* gene by RNAi in leukemia cell lines showed that inhibition of *HEMGN* gene expression could inhibit the growth [52] and promote the differentiation of hematopoietic cells through increased p21 and reduced c-myc expression [51], or activation of nuclear factor- κ B [53]. To some extent, these explains the inverse relationship between *HEMGN* expression and the therapy response, but the precise mechanism needs further verification.

In view of the above analysis, *FLT3*-ITD was an independent predictor of higher relapse rate and poorer OS, but the mechanism by which this mutation affects prognosis is still vague. Thus we postulate that *FLT3*-ITD mutation may influence the disease prognosis of AML possibly by decreasing the expression of *AHSP*, *EPB42*, *GYPC* and *HEMGN* gene expression. Moreover, more experiments will be conducted to explore the exact molecule mechanism of the four genes in AML patients.

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Disclosure Statement

The authors declare that they have no conflict of interests.

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