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The mitochondrial transcription machinery genes are upregulated in acute myeloid leukemia and associated with poor clinical outcome

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ABSTRACT

Background: Acute myeloid leukemia (AML) is characterized by rapid growth of abnormal blasts that overcrowd normal hematopoiesis. Defective mitochondrial biogenesis has been implicated in AML, which we believe is partly due to the deregulation of the mitochondrial transcription machinery (MTM) genes influencing the expression of mitochondrial genes. Here, we aim to characterize MTM gene upregulation in AML.

Methods: Molecular and clinical patient data were retrieved from several public AML datasets. Kaplan-Meier survival curves were used to compare overall survival between patients, while Mann–Whitney U's non-parametric and Fisher's exact test were used for comparing continuous and categorical variables, respectively.

Results: The MTM genes *TFB1M, TFB2M, TFAM,* and *POLRMT* were upregulated in patients with AML compared with healthy donors. Upregulation of one or more of these genes was associated with higher percentage of peripheral blood blasts ($P = 0.002$), normal cytogenetic status ($P = 0.027$) and *NPM1* mutations ($P = 0.009$). Additionally, patients with high expression of MTM genes ($Z \geq 1$) had shorter median overall survival compared with low MTM gene expression ($Z < 1$) (months: 11.8 vs 24.1, $P = 0.027$; multivariate survival analysis Cox Proportional Hazards model, HR: 1.82 (1.22–2.70); p-value: 0.003).

Conclusion: The mitochondrial transcriptional machinery is upregulated and associated with worse clinical outcome in patients with AML and may present a viable therapeutic target.

1. Background

Acute myeloid leukemia (AML) is a myeloproliferative disease of hematopoietic cells characterized by the production of excess immature and abnormal myeloid blasts [1]. One of the difficulties in the treatment of AML lies in the heterogeneous nature of this disease with several subtypes diverging into different cytogenetic abnormalities, clinical features, and molecular risk [1]. While a specific mitochondrial genetic cause of AML has not been identified, dysfunctions including mutations in mitochondrially encoded genes have been implicated in the disease [2–4].

Mitochondria contain their own mitochondrial genome (mtDNA). However, mtDNA does not contain sufficient information for the mitochondria to live independently. Although mitochondria contain the requisite genetic instructions for the oxidative phosphorylation (OXPHOS) pathway involved in the provision of cellular energy, they lack the means to organize mtDNA and regulate gene transcription [5]. The components of the mitochondrial transcription machinery (MTM) are encoded in the nuclear genome [6]. Approximately 1500 mitochondrial proteins are encoded in the nucleus, including mitochondrial transcription genes *POLRMT, TFB1M, TFB2M, TFAM* and several *MTERF* isoforms [7,8]. The transcription of the MTM genes is regulated by nuclear respiratory factors (*NRF-1* and *NRF-2*) that induce mitochondrial biogenesis [9].

During the initiation of mtDNA transcription, transcriptional machinery components begin assembly once heavy or light-strand transcripts are produced [8]. The heavy or light-strand promoter regions contain upstream binding sites for mitochondrial transcription factor A (*TFAM)* to bind and regulate transcription initiation [7]. Transcription factors B1 and/or B2 (*TFB1/2M*) interact with

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Abbreviations

AML	Acute Myeloid Leukemia
MTM	Mitochondrial transcriptional machinery
ETC	Electron Transport Chain
OXPHOS	Oxidative Phosphorylation
MtDNA	Mitochondrial DNA
MtRNA	Mitochondrial RNA
CN-AML	Cytogenetically Normal Acute Myeloid Leukemia
CA-AML	Cytogenetically Abnormal Acute Myeloid Leukemia

mtRNA polymerase (*POLRMT*) in the presence of mtDNA and then bind to promoter-bound transcription factor A at the C-terminal tail [10]. Transcription factor A is critical for promoter recognition *in vitro* as is the simultaneous presence of B1/2 with mtRNA polymerase at the promoter site for transcription initiation to occur [11]. Once the transcript is produced, termination factor isoforms (*mTERF1-3*) collectively facilitate transcriptional termination through an unknown inhibitory mechanism [7]. Reports on the structure and function of the MTM system have advanced our understanding of the steps during mitochondrial transcription, with several studies highlighting transcription factors A, B2, and mtRNA polymerase as basal transcription regulators [7,8,12]. However, it is unknown what downstream effects develop when MTM factors are deregulated and if these alterations are associated with clinical effects in hematological malignancies. Previously, the expression of *NRF-1* and *TFAM* were found to be significantly increased in breast cancer tissues and associated with worse clinical outcome [13]. Likewise, the expression levels of *TFB1M* and *TFB2M* were associated with higher tumor grades in all astrocytomas [12], but their roles in other malignancies have yet to be explained.

Recently, we found mutations in mitochondrial-encoded ETC genes were associated with worse overall survival in patients with AML [2]. It has also been reported that mitochondria are preferentially dependent on the translation of respiratory components that, when inhibited, were specifically cytotoxic to AML cells [14]. AML cells have high reactive oxygen species due to defective OXPHOS activity, increased mitochondrial mass and low spare reserve capacity [15]. Larger mitochondrial mass suggests an increased cellular requirement for ATP synthesis, which also necessitates enhanced transcription and translation of the nuclear genes involved in mtDNA regulation. Metabolism is also deregulated in leukemic stem cells [16]. This makes a subset of AML cells with higher mitochondrial biogenesis susceptible to agents that target mitochondrial function. Presumably, the inhibition of mitochondrial transcription genes may present a therapeutic target in AML when overexpressed in order to satisfy the augmented energy demands from the cell. Therefore, we speculate that genes involved in the mitochondrial transcription machinery are upregulated in AML compared with normal cells. Here we aim to test this hypothesis and characterize the upregulation of these genes and their association with patients' clinical and molecular characteristics and clinical outcome.

2. Methods

2.1. Patient data from public datasets

Molecular and clinical patient data were retrieved from the Cancer Genome Atlas (TCGA) provisional dataset of AML from cBioPortal. We identified 173 patients with AML (median age 58 years; range 18–88). These patients were diagnosed and received treatment according to National Comprehensive Cancer Network (NCCN) guidelines between November 2001 and March 2010. The diagnosis of AML as well as risk group stratification were according to NCCN guidelines. Patient's classifications according to the French–American–British (FAB) classifications were also available. The patients included in the study were assessed for gene expression as well as somatic mutations frequently found in AML, such as *FLT3*, *NPM1*, *IDH1/2*, *TET*, etc. Expression values (mRNA and Z-scores) were used to dichotomize patients into two groups based on MTM genes (*TFB1M*, *TFB2M*, *TFAM* and *POLRMT*) expression data $Z \geq 1$ and $Z < 1$. Other datasets were downloaded from Oncomine.

To analyze differential expression for the MTM genes in normal tissue vs AML we utilized the Andersson Leukemia dataset (GSE7186) [17] with 6 samples from healthy bone marrow and 23 samples from patients with AML, the Haferlach Leukemia dataset (GSE13159) [18] with 74 samples from healthy donor peripheral blood mononuclear cells (PBMCs) and 542 samples from patients with AML and the Valk Leukemia dataset (GSE1159) [19] with 5 samples from healthy donor bone marrow, 3 samples from $CD34^+$ PBMC and 285 samples from patients with AML. To validate the association between upregulation of the MTM genes and overall survival in other datasets we used two Metzeler datasets (GSE12417 [20] $n = 79$ and $n = 163$), Bullinger (GSE425 [21] $n = 119$), Raponi (GSE8970 [22] $n = 34$) and Heuser (GSE4137 [23] $n = 35$) Leukemia datasets. All datasets were downloaded from Oncomine.

2.2. Statistical analyses

The time between diagnosis and removal from study due to lack of complete remission, relapse, or death was defined as disease free survival (DFS). The time between diagnosis and death due to any reason was defined as overall survival (OS). Kaplan–Meier survival curves were generated for the comparison of overall and disease-free survival between patients with $Z \geq 1$ and $Z < 1$ for *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression. To determine associations between *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression levels and patient clinical/molecular characteristics, Mann–Whitney U's non-parametric and Fisher's exact test were used for continuous and categorical variables, respectively, using STATA 12.0 SE. Figures were generated using GraphPad Prism software package (ver. 5.0; GraphPad Software Inc., La Jolla, CA, USA). The Cox Proportional Hazards Model was used to assess the association between *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression and OS after adjusting for other clinical factors and excluding M3 patients. For survival analysis, 173 patients total and 157 excluding M3 patients with complete clinical and molecular data were included in this analysis. A statistical cut-off of $p < 0.05$ was used for inclusion of variables from univariate analysis to multivariate analysis.

3. Results

3.1. Genes of the mitochondrial transcription machinery (MTM) are upregulated in AML

To assess whether *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* are upregulated in AML blasts compared with healthy cells, we compared the median expression levels of these genes in AML and healthy cells from three leukemia datasets (Andersson, Haferlach and Valk). We observed significant upregulation of *TFB1M* in AML compared with healthy cells in the Valk Leukemia dataset (Reporter: 219169_s_at; 1.31-fold increase; p-value: 0.0493; Fig. 1A) but not the Haferlach dataset (Fig. S1A); additionally, the Andersson dataset did not include expression data for *TFB1M*. Significant

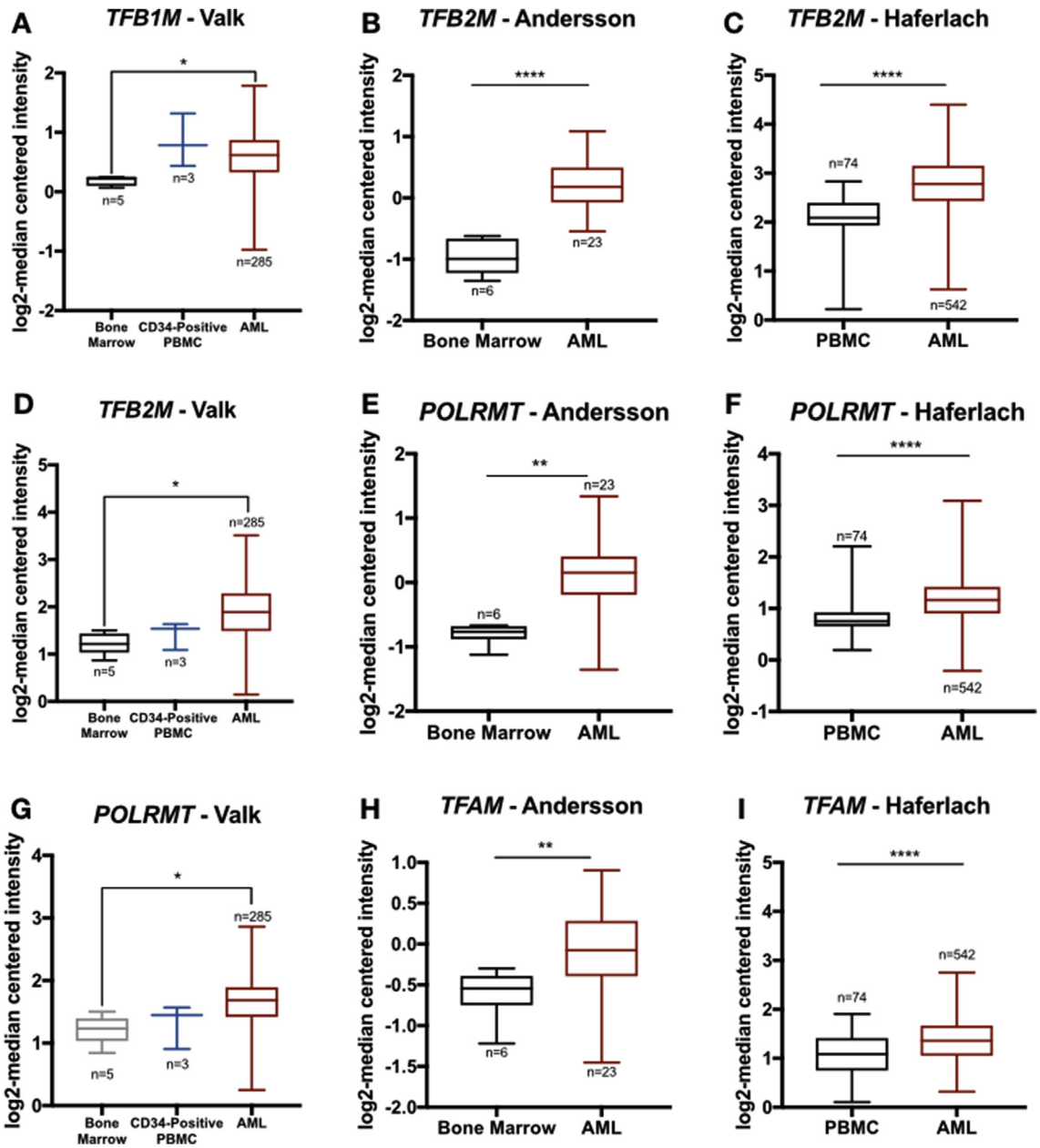

Fig. 1. MTM genes are upregulated in AML. We compared expression of *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* in three datasets: Andersson (GSE7186 - Bone marrow samples n = 6, AML samples n = 23), Haferlach (GSE13159 - PBMC samples n = 74, AML samples n = 542) and Valk (GSE1159 - Bone marrow samples n = 5, $CD34^+$ PBMC samples n = 3 and AML samples n = 285) Leukemia. Expression of *TFB1M* in the (A) Valk Leukemia dataset. Expression of *TFB2M* in the (B) Andersson, (C) Haferlach and (D) Valk, *POLRMT* in the (E) Andersson, (F) Haferlach and (G) Valk and *TFAM* in the (H) Andersson and (I) Haferlach Leukemia datasets. Mann-Whitney U's non-parametric t-test was used. $^*p < 0.05$, $^{**}p < 0.01$ and $^{****}p < 0.0001$.

upregulation of *TFB2M* in AML compared with healthy cells was observed across all three datasets: Andersson (Reporter: 218605_s_at; 2.26-fold increase; p-value: <0.0001; Fig. 1B), Haferlach (Reporter: 897576; 1.61-fold increase; p-value: <0.0001; Fig. 1C) and Valk (Reporter: 218605_s_at; 1.60-fold increase; p-value: 0.0287; Fig. 1D). Similarly, we also observed significant upregulation of *POLRMT* in all three datasets: Andersson (Reporter: 134269; 1.89-fold increase; p-value: 0.0016; Fig. 1E), Haferlach (Reporter: 203782_s_at; 1.33-fold increase; p-value: <0.0001; Fig. 1F) and Valk (Reporter: 203782_s_at;1.37-fold increase; p-value: 0.0219; Fig. 1G). *TFAM* on the other hand was upregulated in AML patients compared with healthy donors in two datasets: Andersson (Reporter: 504826; 1.38-fold increase; p-value: 0.0064; Fig. 1H) and Haferlach (Reporter: 203176_s_at; 1.21-fold increase; p-value: <0.0001; Fig. 1I) but not the Valk Leukemia dataset (Fig. S1B).

Nuclear respiratory factors *NRF-1* and *NRF-2* are known to regulate the MTM genes, thus, we also assessed the differential expression of these genes in healthy donors vs patients with AML in the same three datasets. In general, there was a trend towards decreased median *NRF-1* expression in patients with AML compared with that in healthy donors: Andersson (1.40-fold decrease; p-value: 0.0225) (Fig. S2a), Haferlach (Reporter: 204651_at | 1.24-fold decrease; p-value: <0.0001) (Fig. S2b) and Valk (1.34-fold decrease; p-value: 0.043) (Fig. S2c). Whereas, there was a trend towards increased *NRF-2* expression in patients with

AML compared with that in healthy donors: Andersson (Reporter: 260325 | 1.23-fold increase; p-value: 0.142) (Fig. S2d), Haferlach (Reporter: 210188_at | mean: 1.08-fold increase; p-value: 0.0003) (Fig. S2E) and Valk (mean: 1.19-fold increase; p-value: 0.0571) (Fig. S2F).

We also explored the expression levels of the MTM genes in the TCGA dataset. We dichotomized patients into high and low expression based on Z-score. Dotplots depicting the distribution of the Z-score data are shown in Figs. S3A–D. Based on the distribution of the Z-scores, cut-offs of $Z \geq 1$ and $Z \geq 2$ were used for further analysis.

3.2. Characterization of the expression of MTM genes according to patients' clinical characteristics

We assessed the association between upregulation of MTM genes and primary patient characteristics in 173 patients with AML from the TCGA dataset. High expression ($Z \geq 1$) of any or all of the four MTM genes was significantly associated with higher median percentage of peripheral blood blasts (median %: 52.5 vs 22; p-value: 0.002) and normal cytogenetic status (%: 56.5 vs 39.4; p-value: 0.027) but reversely with good molecular risk (%: 10.1 vs 25.0; p-value: 0.018) compared with MTM genes low expression (Z<1 for all MTM genes) (Table 1).

When each gene was analyzed individually, we found that high *TFB1M* expression ($Z \geq 1$) was significantly associated with intermediate risk status (%: 72.4 vs 49.3; p-value: 0.021) and normal cytogenetic status (%: 72.4 vs 41.0; p-value: 0.002) but reversely with good risk status (%: 0 vs 22.9: p-value: 0.003) (Table S1). High *TFB2M* expression ($Z \geq 1$) was significantly associated with FAB M1 classification (%: 47.1 vs 23.1; p-value: 0.032), high white blood cell count (median: 45 vs 15.2; p-value: 0.004), higher percentage of peripheral blood blasts (median %: 50 vs 34; p-value: <0.001) and normal cytogenetic status (%: 70.6 vs 43.6; p-value: 0.033, Table S2). Conversely, high *TFAM* expression ($Z \geq 1$) was not significantly associated with any primary patient characteristics (Table S3). High *POLRMT* expression ($Z \geq 1$) was significantly associated with poor molecular risk (%: 45.5 vs 23.2; p-value: 0.039) (Table S4). Upregulation of the MTM genes was not associated with age.

Table 1
Clinical characteristics of patients with AML in the TCGA dataset with available information on *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression ($Z \geq 1$). *P-values calculated using non-parametric Mann-Whitney U or Fisher's Exact test.*

Characteristic	Z < 1 (n = 104)	Z ≥ 1 (n = 69)	*p-value*
Age, median (years)	60	56	0.193
Young (<60)	50 (48.1%)	41 (59.4%)	0.163
Old (≥60)	54 (51.9%)	28 (40.6%)	
Sex			>0.999
Female *(n, %)*	49 (47.1%)	32 (46.4%)	
Male *(n, %)*	55 (52.9)	37 (53.6%)	
FAB			
M0 (n, %)	8 (7.7%)	8 (11.6%)	0.427
M1 *(n, %)*	23 (22.1%)	21 (30.4%)	0.217
M2 *(n, %)*	19 (18.3%)	19 (27.5%)	0.188
M3 *(n, %)*	13 (12.5%)	3 (4.30%)	0.106
M4 *(n, %)*	25 (24.0%)	9 (13.0%)	0.082
M5 *(n, %)*	12 (11.5%)	6 (8.70%)	0.619
M6 *(n, %)*	2 (1.9%)	0	0.518
M7 *(n, %)*	1 (1.0%)	2 (2.90%)	0.564
WB Count, median	13.95	27.6	0.240
% BM Blast, median	72	74	0.120
% PB Blast, median	22	52.5	0.002
Molecular Risk Status			
Poor (n, %)	26 (25.0%)	19 (27.5%)	0.723
Intermediate (n, %)	51 (49.0%)	41 (59.4%)	0.158
Good (n, %)	26 (25.0%)	7 (10.1%)	0.018
Cytogenetic Status			0.027
Normal (n, %)	41 (39.4%)	39 (56.5%)	
Abnormal (n, %)	62 (59.6%)	28 (40.6%)	
Transplant (Y/N)			0.271
No (n, %)	64 (61.5%)	36 (52.2%)	
Yes (n, %)	40 (38.5%)	33 (47.8%)	

3.3. Characterization of the expression of MTM genes according to patients' molecular characteristics

Upregulation of *TFB1M*, *TFB2M*, *TFAM* and/or *POLRMT* ($Z \geq 1$) was significantly associated with the presence of *NPM1* mutation (%: 39.1 vs 20.2; p-value: 0.009; Table 2). Frequencies of *NPM1* mutation dichotomized by high expression of *TFB1M*, *TFB2M*, *TFAM* and/or *POLRMT* ($Z \geq 1$) are displayed in Fig. 2a. When each gene was analyzed individually, we found that upregulation of *TFB1M* ($Z \geq 1$) was significantly associated with increased frequencies of *FLT3* mutation (%: 55.2 vs 22.9; p-value: 0.001), *DNMT3A* mutation (%: 44.8 vs 20.8; p-value: 0.010) and *NPM1* mutation (%: 58.6 vs 21.5; p-value: <0.001) (Table S5). Upregulation of *TFB2M* was significantly associated with higher frequency of *NPM1* mutation (%: 64.7 vs 23.7; p-value: 0.001, Table S6). But upregulation of *TFAM* and *POLRMT* were not significantly associated with any molecular characteristics (Tables S7-8).

We compared log2-transformed median-centered mRNA expression of *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* individually, in patients with and without *NPM1* mutations. Median mRNA expression of *TFB1M* (median: 7.54 vs 7.30; p-value: 0.023) and *TFB2M* (median: 8.80 vs 8.48; p-value: 0.010) were significantly higher in patients with *NPM1* mutation than in patients with *NPM1* wild-type (Fig. 2B–C). However, there was no difference in *TFAM* or *POLRMT* expression in patients with or without *NPM1* mutation (Fig. 2D–E).

Additionally, we assessed if mRNA expression levels of the MTM genes were correlated with the expression of *NPM1*. We found a significant and positive correlation between *NPM1* and *TFB1M* (Spearman: 0.155 (p-value: 0.0499) (Fig. S4A), *TFB2M* (Spearman: 0.115; p-value: 0.042) (Fig. S4B) and *TFAM* (Spearman: 0.181; p-value: 0.011) (Fig. S4C). The correlation between *NPM1* and *POLRMT* was not statistically significant (Spearman: 0.019; p-value: 0.816) (Fig. S4D). We also assessed differential *NPM1* expression in patients with high MTM gene expression ($Z \geq 1$) and low MTM gene expression ($Z < 1$) in patients with and without *NPM1* mutation. We found a trend towards increased log2-transformed median-centered mRNA expression of *NPM1* in patients with or without *NPM1* mutation that have higher MTM gene expression ($Z \geq 1$), but this increase was not statistically significant (Figs. S4E–F).

Table 2
Molecular characteristics of patients with AML in the TCGA data set with available information on *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression ($Z \geq 1$). *P-values calculated using Fisher's Exact test.*

Gene	Z < 1 (n = 104)	Z ≥ 1 (n = 69)	*p-value*
FLT3	27 (26.0%)	22 (31.9%)	0.491
TP53	8 (7.69%)	6 (8.70%)	>0.999
DNMT3A	21 (20.2%)	22 (31.9%)	0.106
CEBPA	5 (4.81%)	8 (11.6%)	0.140
NRAS	9 (8.65%)	3 (4.35%)	0.367
TET2	10 (9.62%)	5 (7.25%)	0.784
IDH1	10 (9.62%)	6 (8.70%)	>0.999
IDH2	14 (13.5%)	3 (4.35%)	0.067
RUNX1	12 (11.5%)	4 (5.80%	0.285
NPM1	21 (20.2%)	27 (39.1%)	0.009
WT1	4 (3.85%)	6 (8.70%)	0.200
mtDNA ETC	10 (9.62%)	5 (7.25%)	0.784

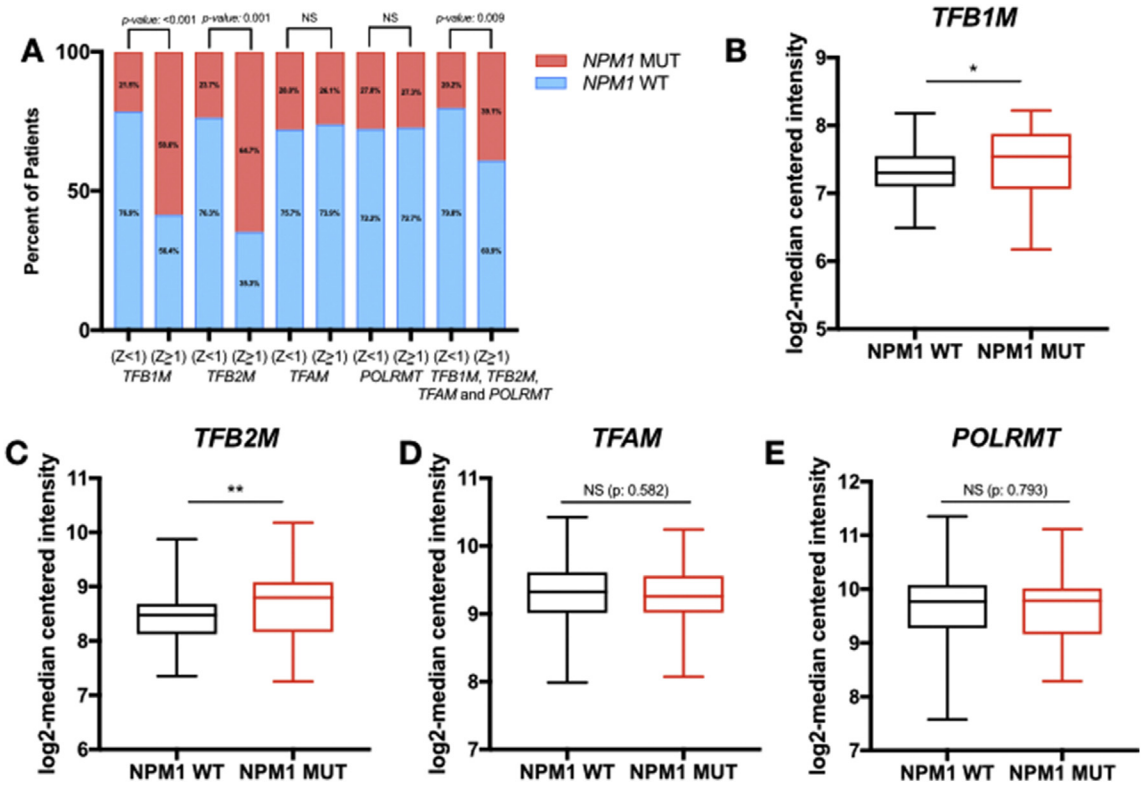

Fig. 2. MTM genes uprgulation is associated with *NPM1* mutations. (A) Distribution of *NPM1* in AML patients stratified by Z-score. The percentage of patients with $Z \geq 1$ of *TFB1M*, *TFB2M*, *TFAM* and/or *POLRMT* that have *NPM1* mutations. mRNA expression levels of (B) *TFB1M*, (C) *TFB2M*, (D) *TFAM* and (E) *POLRMT* in *NPM1* wildtype and mutated patients. Fisher Exact and Mann-Whitney U non-parametric test. *p-value < 0.05 **p-value < 0.01.

3.4. Upregulation of MTM genes is associated with worse clinical outcome

Patients with upregulation of *TFB1M*, *TFB2M, TFAM and POLRMT* ($Z \geq 1$) had significantly worse overall survival (median: 11.8 vs 24.1 months; p-value: 0.027) than patients with $Z < 1$ (Fig. 3A). There was also a trend towards worse disease-free survival in patients with MTM gene upregulation ($Z \geq 1$) that did not reach statistical significance (Fig. 3B). Because patients with FAB M3 classification or t15; 17 translocation have better clinical outcome and receive different treatment, we excluded these patients and reanalyzed the cohort for overall survival. Consistently, we found that patients with high expression of *TFB1M*, *TFB2M*, *TFAM* and/or *POLRMT* ($Z \geq 1$) had significantly shorter median overall survival (median: 11.5 vs 20.5 months; p-value: 0.044) than patients with $Z < 1$ (Fig. 3C). There was a similar trend in disease-free survival that did not reach statistical significance (Fig. 3D).

Since high *TFB1M*, *TFB2M, TFAM* and *POLRMT* expression ($Z \geq 1$) was associated with cytogenetic status, we performed overall survival analysis in cytogenetically normal (CN-AML) patients and found that upregulation ($Z \geq 1$) had a trend towards worse overall survival (median: 14.5 vs 24.1 months; p-value: 0.057) (Fig. 4A). But there was no observed trend towards worse overall survival in cytogenetically abnormal patients (CA-AML) (Fig. 4B). Due to the association between high expression of MTM genes and the presence of *NPM1* mutation, we also assessed the association between MTM gene upregulation with overall survival among patients with *NPM1* mutations. We found that among patients with *NPM1* mutation (who generally have better outcome), patients with high expression of *TFB1M*, *TFB2M, TFAM* and/or *POLRMT* ($Z \geq 1$) had significantly worse overall survival (median: 10.2 vs 24.8 months; p-value: 0.036) (Fig. 5).

When we considered a $Z \geq 2$ for any of the MTM genes, we found that patients with increased expression ($Z \geq 2$) had worse overall survival compared with patients with all MTM genes ($Z < 2$) (median OS including M3 patients: 8.2 vs 21.5 months; p-value: 0.037; median OS excluding M3: 8.2 vs 18.5 months; p-value: 0.097; Figs. S5A and C). However, there was no significant association with disease-free survival (Figs. S5B and D).

When considering each gene individually, upregulation of *TFB1M* expression ($Z \geq 1$) was associated with worse overall survival (median: 8.1 vs 18.1; p-value: 0.033) and worse disease-free survival (median: 8.5 vs 16.6 months; p-value: 0.020) (Figs. S6A–B). Neither *TFB2M* ($Z \geq 1$), *TFAM* ($Z \geq 1$) nor *POLRMT* ($Z \geq 1$) showed an association with overall or disease-free survival (Figs. S6C–H).

In multivariable survival analysis using the Cox Proportional Hazards model, we adjusted for various risk factors such as age, molecular risk status and transplant status and found that upregulation of *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* ($Z \geq 1$) was significantly associated with worse overall survival after exclusion of M3 patients ($n = 153$, HR: 1.82(1.22–2.70); p-value: 0.003) (Table 3). Upregulation of *TFB1M*, *TFB2M*, *TFAM* and/or *POLRMT* expression ($Z \geq 2$) was also associated with worse overall survival after exclusion of M3 patients but did not reach statistical significance ($n = 153$, HR: 1.55(0.94–2.55); p-value: 0.083) (Table S9).

Additionally, we also analyzed survival outcome in four other data sets downloaded from Oncomine: two Metzeler, Heuser and Bullinger. We did not find an association between high *TFB1M*, *TFB2M*, *TFAM* and/or *POLRMT* expression—regardless of our cut-offs for mRNA expression—and worse overall survival. However, after stratification by quartiles (top 25% vs bottom 75%) for *POLRMT*

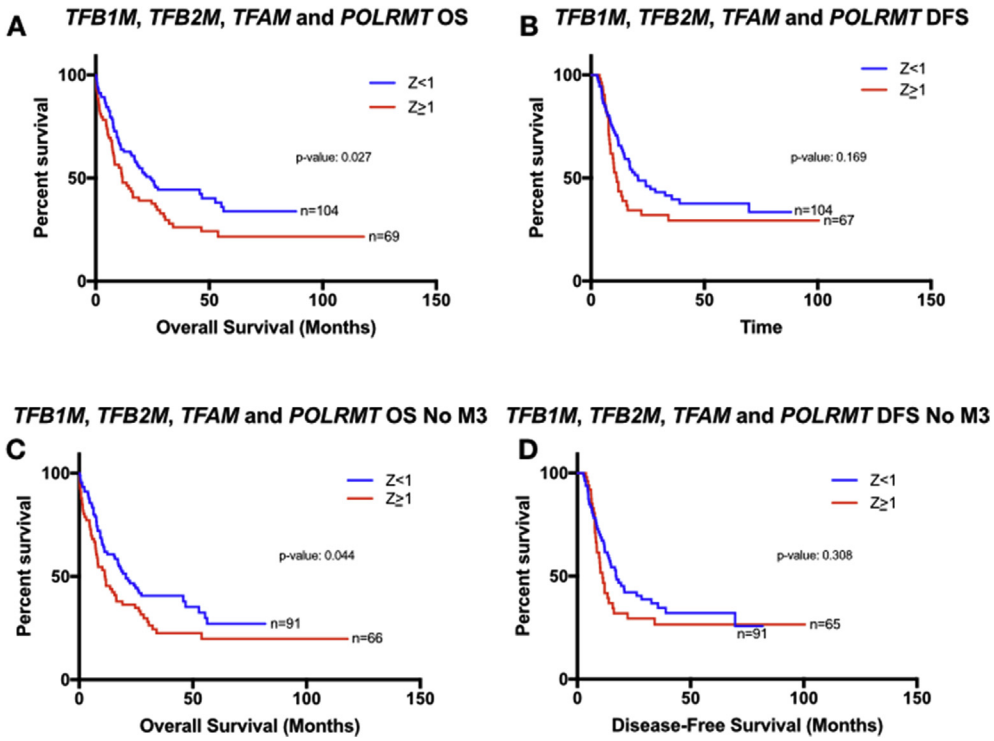

Fig. 3. MTM genes upregulation is associated with shorter overall survival. Kaplan-Meier Survival Curves. (A) Overall Survival (OS) and (B) Disease-Free Survival (DFS) in 173 AML patients stratified by *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression ($Z \geq 1$). (C) Overall Survival and Disease-Free Survival in 157 AML patients (excluding M3 FAB classification PML-RARA patients) stratified by *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression ($Z \geq 1$).

expression, we found a worse overall survival in patients with high expression (top 25%) of *POLRMT* in the Metzeler (median: 240 vs 432 days; p-value: 0.022) and the Bullinger (median: 291 vs 570; p-value: 0.016) datasets compared with the rest of the patients in each data set (Figs. S7A–B).

4. Discussion

Aberrant mitochondrial biogenesis and gene mutations are common features of malignant cells, and there have been recent advancements linking altered mitochondrial metabolism to AML [16]. In this study, we report significant upregulation of mitochondrial transcription machinery genes *TFAM, TFB1M*, *TFB2M* and *POLRMT* in AML. We also establish that this overexpression is associated with shorter overall survival. Together, these findings highlight the importance of mitochondrial metabolic regulation and its association with clinical outcome in leukemia.

The transcriptional machinery in the mitochondria has several important basal regulators, *TFAM, POLRMT* and *TFB1M/TFB2M,* for transcription initiation [7]. POLRMT has no effect on nuclear gene expression, but rather, remains exclusive to the mitochondria for the transcription of mtDNA [24]. Conditional knockdown of *POLRMT* was found to be lethal in OCI-AML2 cells, decreasing OXPHOS, assembly of the ETC complex, and cell growth and viability [25]. We found that the deregulation of the transcription factors, particularly *TFB1M,* contributed significantly more than *POLRMT* to overall survival of patients with AML in the TCGA data. *TFB1M* was not, however, significantly upregulated in AML compared with normal healthy hematopoietic cells in all datasets we analyzed. Consistent with our results, a previous study outlined an ~6-fold excess of *POLRMT* to mtDNA and ~3-fold more *TFB2M* than *TFB1M* at steady state in HeLa cells [26]. This observation suggests a relative abundance of the human mitochondrial transcription machinery and distinct roles for *TFB1M* and *TFB2M* in mitochondrial biogenesis and gene expression [26].

NRF-1 activates the expression of select genes in maintaining mitochondrial respiratory function and has recognition sites on TFAM promoter [27]. This observation describes an ability to communicate changes in nuclear gene expression from the mitochondria, especially when transcriptional machinery is upregulated [28]. We observed lower levels of NRF-1 but not NRF-2 in AML compared with healthy cells, further suggesting the dysfunctional communication signaling between the mitochondria and the nucleus in AML.

NPM1 mutations found in 45–60% of CN-AML patients are associated with a greater chance of complete remission and overall survival outcomes, especially in the absence of *FLT3-ITD* mutations [29]. Interestingly, we found an association between upregulated MTM genes and the presence of *NPM1* mutations. Mitochondrial proteins encoded within nuclear DNA reach their destination in the mitochondria to perform their roles in transcription and translation. About 99% of mitochondrial proteins are synthesized on cytosolic ribosomes and require transport mechanisms to translocate through the outer and inner mitochondrial membrane [30]. NPM1 was found to prevent p53 localization to the mitochondria to initiate apoptosis [31]. Whether NPM1 may play a role in the transport of MTM proteins to the mitochondria remains to be examined. Future studies are necessary to better elucidate the role of mutant *NPM1* on the regulation of MTM proteins and to assess whether higher levels of translocated MTM genes to the mitochondria is present in *NPM1* mutants compared with wildtype cells.

Mitochondrial gene expression requires proper ribosomal biogenesis. Several post-transcriptional RNA modifications must occur for ribosome assembly, such as the methylation of two adenine residues in a highly conserved stem-loop region of small

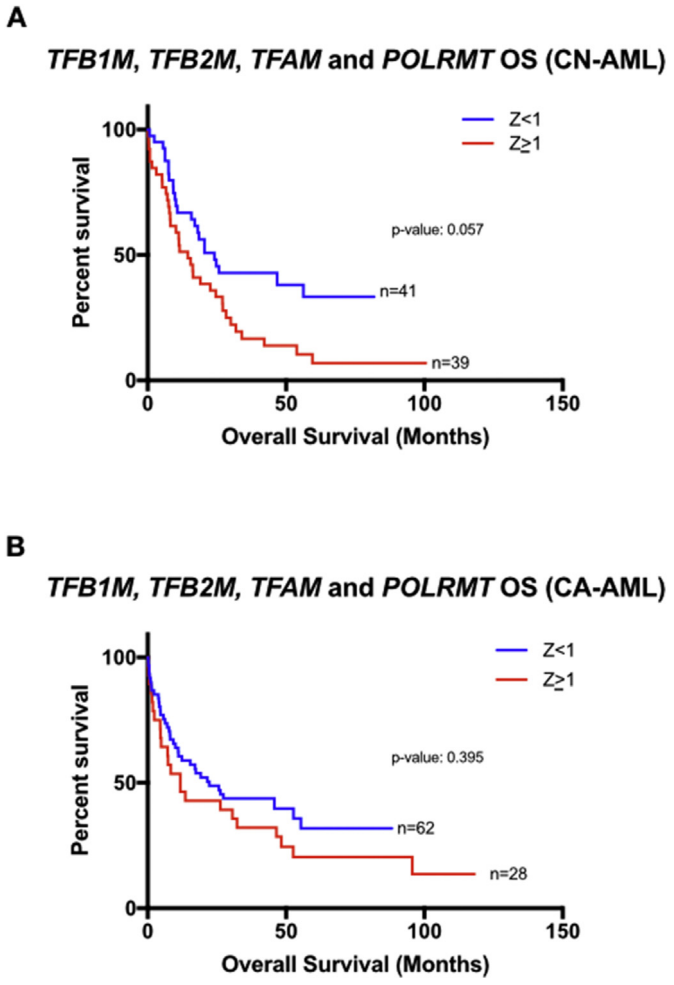

Fig. 4. Survival Analysis of patients with MTM gene upregulation in patients with CN-AML and CA-AML. Kaplan-Meier Survival Curves. Overall Survival (OS) in (A) 79 CN-AML and (B) 75 CA-AML patients stratified by *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression ($Z \geq 1$ and Z<1).

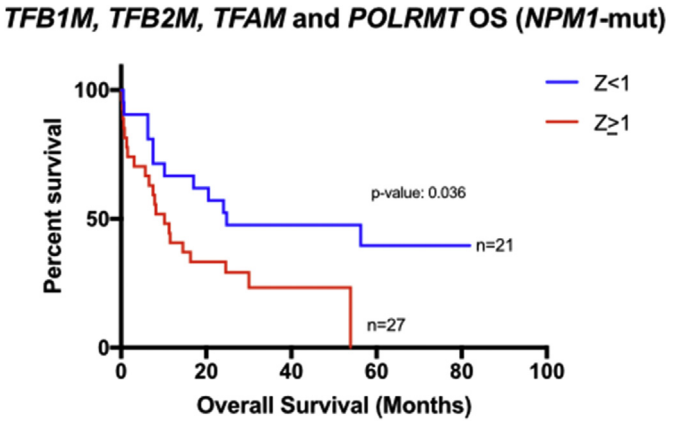

Fig. 5. Association of high MTM gene expression (Z≥1) with overall survival in *NPM1*-mutated patients. Overall Survival (OS) in 48 patients with *NPM1* mutation stratified by *TFB1M, TFB2M, TFAM* and *POLRMT* expression ($Z \geq 1$ and Z<1).

subunit rRNAs [26]. Transcription factors B1 and B2 are dual-function proteins in that they are both involved in mitochondrial transcription and methylation of rRNA, yet these roles have been reported to be independent of each other [26]. A point mutation in *TFB1M* did not affect its ability to stimulate transcription but did eliminate its ability to stimulate rRNA methyltransferase activity [11]. In this context, it is important to consider the different functionalities of the B1/2 proteins to determine if the methyltransferase function explains the role of their upregulation in AML.

Notably, *TFB2M* was associated with the highest rate of overexpression and the strongest association with poor outcome, which could market the gene in AML as a possible therapeutic target. Transcription factor B2 is responsible for transcription initiation and forming a stable complex during mtRNA transcript formation [8] and was found upregulated in renal cancers, lymphomas, and astrocytomas [12,32].

Table 3
Cox Proportional Hazards modeling for overall survival in patients stratified by high ($Z \geq 1$) and low (Z<1) *TFB1M*, *TFB2M*, *TFAM* and *POLRMT* expression excluding M3 patients (n = 153).

Variables	Hazard Ratio	95% CI		p-value
Age	1.02	1.00	1.04	0.013
Molecular Risk Status				
Intermediate	3.23	1.36	7.66	0.008
Poor	7.19	2.88	18.0	<0.001
Transplant Status	0.37	0.23	0.59	<0.001
MTM genes	1.82	1.22	2.70	0.003

5. Conclusions

In sum, the mitochondrial transcriptional machinery is upregulated in AML and associated with poor clinical outcome and the presence of *NPM1* mutations. Altogether, our study highlights the potentially important role of this system in AML and suggests the need to further investigate each component of the MTM to establish whether a therapeutic approach in targeting MTM genes is viable.

Declarations

Ethics approval and consent to participate

The data used in this study is publicly available online from previously published work. Therefore, additional ethical approval and consent to participate was not needed.

Availability of data and materials

All data can be found online at cBioPortal and Oncomine.

Conflicts of interest

The authors declare that they have no competing interests.

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Authors' contributions

H.A., S.W. and N.F. conceived and designed the research project and wrote the manuscript. H.A. supervised the analysis. S.W. and N.F. conducted the data analyses. All authors reviewed the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2019.100009.

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