Prognostic Value of mRNA Expression of MAP4K Family in Acute Myeloid Leukemia

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Abstract

Background: Despite diverse functions in diseases, the prognostic potential of the family of mitogen-activated protein kinase kinase kinase genes in acute myeloid leukemia remains unknown. Methods: The messenger RNA expression of the MAP4K family members in 151 patients with acute myeloid leukemia was extracted from the OncoLnc database. Data for gender, age, cytogenetic, leukocyte count, CD34, FAB classification, RUNX1, and TP53 were provided by the University of California–Santa Cruz Xena platform. Kaplan-Meier analysis and Cox regression model provided an estimate of the hazard ratio with 95% confidence intervals for overall survival. Results: Analysis demonstrated favorable overall survival in patients with acute myeloid leukemia attributing to high expression of MAP4K3, MAP4K4, and MAP4K5 and low expression of MAP4K1 (adjusted \( P = 0.005 \), \( P = 0.022 \), \( P = 0.002 \), and \( P = 0.024 \); adjusted hazard ratio = 0.490, 95% confidence interval = 0.297-0.809, hazard ratio = 0.598, 95% confidence interval = 0.385-0.928, hazard ratio = 0.490, 95% confidence interval = 0.310-0.938, and hazard ratio = 0.615, 95% confidence interval = 0.403-0.938, respectively). Combining the high-expressing MAP4K3, MAP4K4, and MAP4K5 with the low-expressing MAP4K1 in a joint effect analysis predicted a favorable prognosis of overall survival in acute myeloid leukemia. Conclusion: High expression of MAP4K3, MAP4K4, and MAP4K5 combined with low expression of MAP4K1 can serve as a sensitive tool to predict favorable overall survival in patients with acute myeloid leukemia.

Keywords
MAP4K, acute myeloid leukemia, prognosis

Abbreviations

AML, acute myeloid leukemia; BP, biological process; CC, cellular component; CI, confidence interval; JNK, Janus kinase; KEGG, Kyoto Encyclopedia of Genes; HR, hazard ratio; MAP4K, mitogen-activated protein kinase kinase kinase; MF, molecular function; mRNA, messenger RNA; GO, Gene Ontology; NF-κB, nuclear factor κB; HPK1, hematopoietic progenitor kinase 1; OS, overall survival; SLE, systemic lupus erythematosus

Introduction

Acute myeloid leukemia (AML) is a malignant clonal disease of the hematopoietic stem cells. Among the malignant tumor mortality rates in China, leukemia ranks sixth in men and seventh in women. Acute myeloid leukemia is the most common form of leukemia in adults accounting for 32.4% of new cases with leukemia and 43.8% of leukemia-related deaths. The International Classification of Childhood Cancer reported that leukemia accounts for 29% of all childhood cancers. The US 2007 to 2013 report on the common childhood cancers predicts that the 5-year survival rate of patients with AML diagnosed

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between 0 and 14 years is the lowest at 65.1%, posing a serious threat to the health of children. The main treatments for AML include chemotherapy, radiation therapy, molecular therapy, and allogeneic hematopoietic stem cell transplantation. Prognosis and treatment options for AML are determined by the effective detection of genetic markers. Studies have shown that genes, including NPM1, FLT3, C-KIT, AML1-ETO, TET2, RUNX1, TP53, MLL-TD, CBFB/MYH11, TET2, DNMT3A, JAK-STAT, and CXCR4, are associated with prognosis of AML. However, there exists a gap in our understanding of the prognostic value of family of MAP4K genes in AML.

Mitogen-activated protein kinase kinase kinase kinase (MAP4Ks) belong to the family of mammalian ste20-like serine/threonine kinases. MAP4Ks reported so far include MAP4K1/HPK1, MAP4K2/GCK, MAP4K3/GLK, MAP4K4/HGK, MAP4K5/KHS, and MAP4K6/MINK1. Through the activation of the MAP3K–MAP2K cascade, MAP4Ks can induce Janus kinase (JNK) activation, which is vital for medullary differentiation of hematopoietic tissue. The MAP4K1 is reported to be involved with various adapter proteins, such as CARD11, HSI, HIP-55, GRB2, LAT, SLP-76, CRK, and BAM32, and plays important roles in autoimmune diseases, tumorigenesis, apoptosis, inhibition of TCR/BCR signaling, and T/B/dendritic cell-mediated immune responses. Both MAP4K1 and MAP4K3 are involved in the progression of immune diseases. While MAP4K1 is down-regulated, MAP4K3 is upregulated in systemic lupus erythematosus (SLE). Uregulation of MAP4K3 and MAP4K4 promotes metastasis of breast cancer cells and liver cancer cells. Elevated expression of MAP4K2 boosted tumor proliferation in diffuse large B-cell lymphoma and UV resistance in melanoma cells. While downregulation of MAP4K5 promoted the progression of pancreatic cancer, it inhibited the activity of BCR-ABL in CML. MINK1 was reported to be involved in cell division and dendritic structure integrity and synaptic transmission. Nevertheless, the relationship between MAP4K family and patients with AML remains understudied. In this study, the prognostic value of individual MAP4K messenger RNA (mRNA) expression was evaluated by combined effect analysis using data from the OncoLnc database and the University of California, Santa Cruz Xena.

Materials and Methods

Data

Clinical information including events, survival time, death status, age, gender, cytogenetic, leukocyte count, CD34, FAB classification, RUNXI, and TP53 from 151 patients with AML was extracted from the University of California, Santa Cruz Xena (https://xenabrowser.net/datapages/, accessed by January 15, 2019). Transcript expression of the MAP4K family in AML tissues was obtained from OncoLnc (http://www.oncolnc.org/, accessed by January 15, 2019). The expression of MAP4K subunits in clinical patients was downloaded from the Metabolic gEne RAPid Visualizer (http://merav.wi.mit.edu/SearchByGenes.html, accessed by January 30, 2019). Boxplots were created on GraphPad Prism v.7.0 (La Jolla, California).

Survival and Joint Effect Analyses

For each MAP4K mRNA, patients were divided into high- and low-expression groups according to a 50th percentile cutoff. Correlation between the 6 MAP4K genes and survival of patients with AML was determined by Kaplan-Meier analysis and a log-rank test. Cox proportional hazard regression model was used to adjusting the P values, hazard ratios (HRs), and 95% confidence intervals (CIs) of the clinical information. Significant genes from the joint effect analysis were grouped into the better OS, the worse OS, and the other groups (Tables 1-3).

Statistical Analyses

Kaplan-Meier survival analysis and the log-rank test were used to calculate OS and P values for all associations. A Cox proportional hazards regression model was used for univariate and multivariate survival analyses. Hazard ratios and 95% CIs were calculated with the Cox proportional hazards regression model, which was used to adjust for age, cytogenetic, FAB classification, RUNX1, and TP53. P values of OS were calculated with the Cox proportional hazards regression model, which indicates whether there is a difference in OS rate. The best prognostic group in each combination was used as a reference, comparing with the poor prognosis group and the moderate prognosis group, respectively, and 2 P values were obtained. Statistical analyses were performed on SPSS v.22.0 software (IBM, Chicago, Illinois). Vertical scatter plot of MAP4K mRNA expression and survival curves for MAP4K family were generated in GraphPad Prism v.7.0 (La Jolla, California).

Analysis of Functional Enrichment and Pearson Correlation

The Database for Annotation, Visualization, and Integrated Discovery V6.8 (https://david.ncifcrf.gov/tools.jsp, accessed February 10, 2019), Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used to reveal functional enrichment. The GO annotations included molecular function (MF), cellular component (CC), and biological process (BP). GeneMANIA (http://genemania.org/; accessed February 11, 2019) was used to reveal interactions among MAP4K family members, and correlations were identified by Pearson correlation coefficient analysis.
Ethics Statement

All data used in this study were obtained from public databases; therefore, approval by an ethics committee was not required.

Results

Differential Expression of MAP4K in Normal Hematopoietic and Lymphoid Primary Tumor and Normal Tissue

Figure 1 depicts boxplots of the differential expression of the 6 MAP4K genes extracted from the MERAV database. The median expression of MAP4K4 and MINK1 was higher in normal hematopoietic and lymphoid tumors than AML tumors. MAP4K1, 3, 4, and 5 were expressed both in human normal and in AML tissues. The AML tissues showed higher MAP4K1 expression compared to MAP4K3 and moderate expression of MAP4K4 and 5 (Figure 2).

Analysis of Coexpression and Functions of the MAP4K Family

Gene Ontology functional analysis revealed that the MAP4K genes were overrepresented in the BP, MF, and CC

| Table 1. Group of 2 Selected Genes. |
|------------------------------------|
| Group | Ingredient | Group | Ingredient | Group | Ingredient |
| A     | Low MAP4K1 + high MAP4K3 | B     | Low MAP4K1 + low MAP4K3 | C     | High MAP4K1 + low MAP4K3 |
| D     | Low MAP4K1 + high MAP4K4 | E     | Low MAP4K1 + low MAP4K4 | F     | High MAP4K1 + low MAP4K4 |
| G     | Low MAP4K1 + high MAP4K5 | H     | Low MAP4K1 + low MAP4K5 | I     | High MAP4K1 + low MAP4K5 |

Abbreviation: MAP4K, mitogen-activated protein kinase kinase kinase

| Table 2. Group of 3 Selected Genes. |
|------------------------------------|
| Group | Ingredient | Group | Ingredient |
| i     | Low MAP4K1 + high MAP4K3 + high MAP4K4 | iv    | Low MAP4K1 + high MAP4K3 + high MAP4K5 |
| ii    | Low MAP4K1 + low MAP4K3 + low MAP4K4 | v     | Low MAP4K1 + low MAP4K3 + low MAP4K5 |
| iii   | High MAP4K1 + low MAP4K3 + high MAP4K4 | vi    | High MAP4K1 + low MAP4K3 + high MAP4K5 |
| viii  | Low MAP4K1 + high MAP4K3 + high MAP4K5 | x     | High MAP4K3 + high MAP4K4 + high MAP4K5 |
| ix    | High MAP4K1 + low MAP4K3 + low MAP4K4 | xi    | High MAP4K3 + high MAP4K4 + low MAP4K5 |
| xii   | Low MAP4K1 + low MAP4K4 + low MAP4K5 | xii   | Low MAP4K3 + high MAP4K4 + low MAP4K5 |

Abbreviation: MAP4K, mitogen-activated protein kinase kinase kinase kinases.
Table 3. Group of 4 Selected Genes.

| Group | Ingredient                                                                 |
|-------|-----------------------------------------------------------------------------|
| 1     | Low MAP4K1 + high MAP4K3 + high MAP4K4 + high MAP4K5                        |
| 2     | High MAP4K1 + high MAP4K3 + high MAP4K4 + high MAP4K5                      |
|       | Low MAP4K1 + low MAP4K3 + high MAP4K4 + high MAP4K5                        |
|       | Low MAP4K1 + high MAP4K3 + low MAP4K4 + high MAP4K5                        |
|       | Low MAP4K1 + high MAP4K3 + high MAP4K4 + low MAP4K5                        |
|       | Low MAP4K1 + low MAP4K3 + low MAP4K4 + high MAP4K5                         |
|       | Low MAP4K1 + low MAP4K3 + high MAP4K4 + low MAP4K5                         |
|       | Low MAP4K1 + high MAP4K3 + low MAP4K4 + low MAP4K5                         |
| 3     | High MAP4K1 + low MAP4K3 + high MAP4K4 + high MAP4K5                        |
|       | High MAP4K1 + high MAP4K3 + low MAP4K4 + high MAP4K5                       |
|       | High MAP4K1 + high MAP4K3 + high MAP4K4 + low MAP4K5                       |
|       | High MAP4K1 + low MAP4K3 + low MAP4K4 + high MAP4K5                        |
|       | High MAP4K1 + low MAP4K3 + high MAP4K4 + low MAP4K5                        |
|       | Low MAP4K1 + low MAP4K3 + low MAP4K4 + low MAP4K5                          |
|       | Low MAP4K1 + low MAP4K3 + high MAP4K4 + low MAP4K5                         |
|       | Low MAP4K1 + high MAP4K3 + low MAP4K4 + low MAP4K5                         |

Abbreviation: MAP4K, mitogen-activated protein kinase kinase kinase kinase.

Figure 1. Metabolic gEne RApid Visualizer boxplots for MAP4K gene expression in normal hematopoietic and lymphoid tissue and primary AML tissue: (A) MAP4K1; (B) MAP4K2; (C) MAP4K3; (D) MAP4K4; (E) MAP4K5; and (F) MINK1. AML indicates acute myeloid leukemia; MAP4K, MAP kinase kinase kinase kinase.
categories (Figure 3A). The result of KEGG pathway is exhibited in Figure 3A. Interactions among MAP4K1, MAP4K2, MAP4K3, MAP4K4, MAP4K5, and MINK1 are shown in Figure 3B.

**Pearson Correlation Coefficients**

Pearson correlation coefficient analysis revealed that while there was a collinearity between MAP4K1 and MAP4K2 expression, MAP4K4 expression correlated only with MAP4K1 and MAP4K2. MAP4K3 expression, which was similar to MAP4K5 and MINK1, strongly correlated with the other members except for MAP4K4 ($P < .05$; Figure 3C).

**Clinical Information**

Table 4 shows clinical data of the selected cohort. Age, gender, cytogenetic, leukocyte count, CD34, FAB classification, RUNX1, and TP53 predominantly associated with median survival time ($P < .001$, $P = .787$, $P = .005$, $P = .925$, $P = .254$, $P = .039$, $P = .925$, and $P < .001$, respectively; Table 4). Age, cytogenetic and FAB classification, RUNX1, and TP53 were used as adjusting factors in the Cox proportional hazards regression model.

**Survival Influence of Differential MAP4K Gene Expression**

Univariate survival analysis of the MAP4K family revealed that low expression of MAP4K1 ($P = .008$; Figure 4A) and high expression of MAP4K4 and MAP4K5 ($P = .017$ and $P = .005$, respectively; Figure 4D and E) contributed to a favorable OS in patients with AML. A higher or lower expression of MAP4K2, MAP4K3, and MINK1 did not impact survival ($P = .274$, $P = .222$, and $P = .055$, respectively; Figure 4B, C, and F). A multivariate Cox proportion hazards regression analysis
Figure 3. A, Analysis of enriched GO terms and KEGG pathways for MAP4K genes by using Database for Annotation, Visualization, and Integrated Discovery. B, Gene interaction networks according to selected gene expression levels in GeneMANIA. C, Pearson correlation coefficients for MAP4K1, MAP4K2, MAP4K3, MAP4K4, MAP4K5, and MINK1 gene expression levels. **P < .05. GO indicates Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAP4K, MAP kinase kinase kinase kinase.

Table 4. Clinical Data for 151 Patients With AML.

| Elements          | Cases, n = 151 | No. of Events (%) | MST, days | HR (95% CI)       | P Value |
|-------------------|----------------|-------------------|-----------|-------------------|---------|
| Age, years        |                |                   |           |                   |         |
| ≤60               | 88             | 47 (53.4%)        | 915       | Ref               | <.001   |
| >60               | 63             | 12 (19.0%)        | 275       | 0.262 (0.160-0.427) | .262    |
| Gender            |                |                   |           |                   |         |
| Male              | 81             | 32 (39.4%)        | 518       | Ref               | .787    |
| Female            | 70             | 27 (38.6%)        | 609       | 1.065 (0.670-1.690) |       |
| Cytogenetic       |                |                   |           |                   |         |
| Favorable         | 31             | 20 (64.5%)        | 1402      | Ref               | .005    |
| Middle            | 91             | 30 (33.0%)        | 489       | 0.617 (0.243-1.569) | .039    |
| Poor              | 27             | 9 (33.3%)         | 365       | 1.152 (0.596-2.227) |       |
| Missing           | 2              |                   |           |                   |         |
| Leukocyte, 10⁹/L  |                |                   |           |                   |         |
| <100              | 136            | 53 (39.0%)        | 577       | Ref               | .925    |
| ≥100              | 15             | 6 (40.0%)         | 731       | 1.160 (0.511-2.632) |       |
| CD34              |                |                   |           |                   |         |
| Negative          | 58             | 23 (39.7%)        | 731       | Ref               | .254    |
| Positive          | 93             | 36 (38.7%)        | 489       | 0.755 (0.420-1.357) |       |
| FAB               |                |                   |           |                   |         |
| M0                | 13             | 5 (38.5%)         | 577       | Ref               | .039    |
| M1                | 37             | 15 (40.5%)        | 731       | 0.196 (0.047-2.806) |       |
Table 4. (continued)

| Elements | Cases, n = 151 | No. of Events (%) | MST, days | HR (95% CI) | P Value |
|----------|---------------|-------------------|-----------|-------------|---------|
| M2       | 33            | 13 (39.4%)        | 366       | 0.488 (0.136-1.758) |         |
| M3       | 15            | 11 (73.3%)        | 563       | 0.510 (0.144-1.803) |         |
| M4       | 33            | 9 (27.3%)         | 577       | 0.169 (0.030-0.973) |         |
| M5       | 14            | 5 (35.7%)         | 243       | 0.448 (0.128-1.561) |         |
| M6       | 2             | 0 (0.00%)         | 215       | 0.757 (0.187-3.073) |         |
| M7       | 3             | 0 (0.00%)         | 304       | 1.159 (0.191-7.031) |         |
| Missing  | 1             |                   |           |             |         |

RUNX1
- Mutation negative: 136 cases, 56 (41.2%) events, MST = 580 days, HR = 1.765 (0.954-3.266), P = .065
- Mutation positive: 15 cases, 3 (20.0%) events, MST = 335 days, HR = 3.573 (1.813-7.039), P < .001

TP53
- Mutation negative: 141 cases, 59 (41.8%) events, MST = 609 days, HR = 1.765 (0.954-3.266), P = .065
- Mutation positive: 10 cases, 0 (0.00%) events, MST = 214 days, HR = 3.573 (1.813-7.039), P = .065

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; HR, hazard ratio; MST, median survival time.

Figure 4. Prognostic value of MAP4K genes expression. A-F, Kaplan-Meier survival curves for all patients with AML according to MAP4K1 (A), MAP4K2 (B), MAP4K3 (C), MAP4K4 (D), MAP4K5 (E), and MINK1 (F) expression. AML indicates acute myeloid leukemia; MAP4K, MAP kinase kinase kinase kinase.
Table 5. Prognostic Survival Analysis for High or Low Expression of MAP4K Family Genes.

| Gene   | Cases, n = 151 | No. of Events (%) | MST, days | Raw HR (95% CI) | Raw P  | Adjust HR (95% CI)a | Adjust P
|--------|----------------|-------------------|-----------|-----------------|--------|---------------------|--------
| MAP4K1 |                |                   |           |                 |        |                     |        |
| Low    | 75             | 34 (45.3%)        | 792       | Ref.            | .008   | Ref.                | .024   |
| High   | 75             | 25 (33.3%)        | 305       | 0.581           | (0.383-0.882) | 0.615 | (0.403-0.938) |        |
| Missing| 1              |                   |           |                 |        |                     |        |
| MAP4K2 |                |                   |           |                 | .274   | Ref.                | .804   |
| Low    | 75             | 28 (37.3%)        | 731       | Ref.            |        | Ref.                |        |
| High   | 75             | 30 (38.7%)        | 427       | 0.798           | (0.529-1.206) | 0.947 | (0.618-1.452) |        |
| Missing| 1              |                   |           |                 |        |                     |        |
| MAP4K3 |                |                   |           |                 | .222   | Ref.                | .005   |
| Low    | 75             | 26 (34.7%)        | 366       | Ref.            |        | Ref.                |        |
| High   | 75             | 32 (42.7%)        | 671       | 1.287           | (0.855-1.938) | 0.490 | (0.297-0.809) |        |
| Missing| 1              |                   |           |                 |        |                     |        |
| MAP4K4 |                |                   |           |                 | .017   | Ref.                | .022   |
| Low    | 75             | 25 (33.3%)        | 427       | Ref.            |        | Ref.                |        |
| High   | 75             | 34 (45.3%)        | 792       | 0.614           | (0.406-0.930) | 0.598 | (0.385-0.928) |        |
| Missing| 1              |                   |           |                 |        |                     |        |
| MAP4K5 |                |                   |           |                 | .005   | Ref.                | .002   |
| Low    | 75             | 21 (28.0%)        | 365       | Ref.            |        | Ref.                |        |
| High   | 75             | 38 (50.7%)        | 822       | 0.555           | (0.367-0.839) | 0.490 | (0.310-0.776) |        |
| Missing| 1              |                   |           |                 |        |                     |        |
| MINK1  |                |                   |           |                 | .055   | Ref.                | .179   |
| Low    | 75             | 34 (45.3%)        | 792       | Ref.            |        | Ref.                |        |
| High   | 75             | 25 (33.3%)        | 366       | 0.671           | (0.444-1.014) | 1.375 | (0.864-2.189) |        |
| Missing| 1              |                   |           |                 |        |                     |        |

Abbreviations: CI, confidence interval; HR, hazard ratio; MAP4K, MAP kinase kinase kinase kinase; MST, median survival time.

aAdjustment of MAP4K genes for age, cytogenetic, FAB stage, RUNX1, and TP53.

revealed that age, cytogenetic and FAB classification, RUNX1, and TP53 in association with high expression of MAP4K3, MAP4K4, and MAP4K5 and low expression MAP4K1 predicted favorable OS (adjusted P = .005, P = .022, P = .002, and P = .024; adjusted HR = 0.490, 95% CI = 0.297-0.809; HR = 0.598, 95% CI = 0.385-0.928; HR = 0.490, 95% CI = 0.310-0.776; and HR = 0.615, 95% CI = 0.403-0.938, respectively; Table 5). MAP4K2 (P = .804) or MINK1 (P = .179) expression did not impact overall survival in patients with AML (both adjusted P > .05; Table 5).

**Joint Effect Analysis**

The effect of MAP4K genes on OS of patients with AML were determined by a joint effects analysis. Prognostic value of different gene groups based on the expression of MAP4K1, MAP4K3, MAP4K4, and MAP4K5 was evaluated by the Kaplan-Meier analysis and a log-rank test (Tables 1-3; Figures 5 and 6). Groups with low expression of MAP4K1 and high expression of MAP4K3, MAP4K4, and MAP4K5, A, D, G, J, M, P, i, iv, vii, x, and 1 were highly correlated with favorable OS (all P < .05; Table 6-7). On the other hand, groups formed with high MAP4K1 expression and low MAP4K3, MAP4K4, and MAP4K5 expression, including C, F, I, L, O, R, iii, vi, ix, xii, and 3, were predictive of poor OS (all P < .05; Tables 6 and 7).

**Discussion**

The threonine/serine mitogen-activated protein kinase is a key regulator of cell signaling in eukaryotes. The MAP4K family of 6 genes plays essential roles in immune response and signaling. The MAP4K1, also known as hematopoietic progenitor kinase 1 (HPK1), is vital to the hematopoietic tissue and positively regulates neutrophil adhesion in contrast to its function in lymphocytes.29

In the present study, MAP4K1, MAP4K2, MAP4K3, and MAP4K4 were linked with nuclear factor κB (NF-κB) signaling, which plays a critical role in cancer.30,31 MAP4K2 combined with TNF receptor associated factor 2 was reported to mediate cell resistance to UV irradiation by regulating NF-κB. MAP4K1, on the other hand, inhibited NF-κB activation via its regulatory C terminus.29 Upregulation of MAP4K3 expression directly affects the severity of SLE. We found that the expression level of MAP4K3 was significant enhanced in autoimmune diseases, whereas the MAP4K1 expression was decreased. MAP4K1 may be an inhibitor of MAP4K3 to regulate immunity. It was reported that the MAP4K1 deficiency in mice resulted in high levels of IgM and IgG.32 MAP4K3, MAP4K4, and MAP4K5 were recognized to affect the development of type 2 diabetes.33-36

In this study, MAP4K1 and TAOK3 were coexpressed (Figure 3B). While JNK was activated by MAP4K1, TAOK3 was
found to be an inhibitor of JNK pathway. Coexpression of MAP4K3 and DYRK2 (Figure 3B) was a significant finding as both are known to play vital roles in breast cancer and metastasis.

In the MAPK signaling cascade, MAP4K1 together with MAP3K3 and MAP4K5 activates MAP3K11 and MAP3K1. Subsequently, MAP3K11 along with MAP3K1 activate MAP2K7 and MAP2K4, respectively, which together activate the JNK signaling pathway. ATF2, ELK1, and TP53 were activated by JNK signaling pathway, further activating the P53 signaling pathway in apoptosis, growth inhibition, and inhibition of cell cycle progression. In recent years, TP53 has been directly linked to the prognosis of AML. Thus, MAP4K1, MAP4K3, and MAP4K5 can influence the prognosis of AML through the P53 signaling pathway.

Most of the available literature on the MAP4K genes is related to progression of cancer, especially, MAP4K1, which was closely linked to AML. The expression of MAP4K1 is increased in AML but decreased in autoimmune diseases. Inhibition of HPK1 expression is beneficial to the survival of

Figure 5. Joint effect analysis for MAP4K genes expression with stratified OS according to 2 selected MAP4K genes among MAP4K1, MAP4K3, MAP4K4, and MAP4K5. (A) MAP4K1 and MAP4K3, (B) MAP4K1 and MAP4K4, (C) MAP4K1 and MAP4K5, (D) MAP4K3 and MAP4K4, (E) MAP4K3 and MAP4K5, and (F) MAP4K4 and MAP4K5. Group A, low MAP4K1 + high MAP4K3; group C, high MAP4K1 + low MAP4K3; group D, low MAP4K1 + high MAP4K4; group F, high MAP4K1 + low MAP4K4; group G, low MAP4K1 + high MAP4K5; group I, high MAP4K1 + low MAP4K5; group J, high MAP4K3 + high MAP4K4; group L, low MAP4K3 + low MAP4K4; group M, high MAP4K3 + high MAP4K5; group O, low MAP4K3 + low MAP4K5; group P, high MAP4K4 + high MAP4K5; group R, low MAP4K4 + low MAP4K5; groups B, E, H, K, N, and Q correspond to other combinations of genes as detailed in Table 1. MAP4K indicates MAP kinase kinase kinase kinase; OS, overall survival.
patients with cancer, which may be caused by the immune response of downregulated MAP4K1. Nevertheless, relationship between MAP4K mRNA and the prognosis of AML remains unexplored. Combined with clinical data, we assessed the association of every MAP4K gene, individually as well as in combination with the prognosis of AML. Furthermore, we investigated whether the MAP4K genes could be hallmarks of prognosis in AML.

Figure 6. Joint effect analysis for MAP4K genes expression with stratified OS according to 3 or 4 selected MAP4K genes among MAP4K1, MAP4K3, MAP4K4, and MAP4K5. (A) MAP4K1, MAP4K3, and MAP4K4; (B) MAP4K1, MAP4K3, and MAP4K5; (C) MAP4K1, MAP4K4, and MAP4K5; (D) MAP4K3, MAP4K4, and MAP4K5; (E) MAP4K1, MAP4K3, MAP4K4, and MAP4K5. Group i, Low MAP4K1 + high MAP4K3 + high MAP4K4; group iii, High MAP4K1 + low MAP4K3 + low MAP4K4; group iv, Low MAP4K1 + high MAP4K3 + high MAP4K5; group vi, High MAP4K1 + low MAP4K3 + low MAP4K5; group vii, Low MAP4K1 + high MAP4K4 + high MAP4K5; group ix, High MAP4K1 + low MAP4K4 + low MAP4K5; group x, High MAP4K3 + high MAP4K4 + high MAP4K5; group xii, Low MAP4K3 + low MAP4K4 + low MAP4K5. Group ii, v, viii, and xi correspond to other combinations of genes as detailed in Table 2. Group 1, low MAP4K1 + high MAP4K3 + high MAP4K4 + high MAP4K5; group 3, High MAP4K1 + low MAP4K3 + low MAP4K4 + low MAP4K5. 2 correspond to other combinations of genes as detailed in Table 3. MAP4K indicates MAP kinase kinase kinase kinase; OS, overall survival.
Contrary to their low abundance in normal tissue, MAP4K1 and MAP4K5 were found to be elevated in primary tumor, and a lower MAP4K1 expression was favorable to OS in AML, suggesting that MAP4K1 may be an oncogene. In contrast, a higher expression of MAP4K5 predicted favorable OS, which implied that MAP4K5 may be a tumor suppressor. On the other hand, MAP4K5 was downregulated in pancreatic cancer and upregulated in AML and CML indicating dichotomous pathological roles. Many studies have shown negative prognostic value of MAP4K4 in a variety of cancers. Downregulation of MAP4K4 expression in cancer cells promoted apoptosis and inhibited migration and invasion. However, in this study, MAP4K4 was found to be highly expressed in normal tissue and predictive of favorable OS in AML. It can be speculated that higher expression of MAP4K4 may inhibit AML. The MAP4K3 expression failed to correlate with favorable OS in the univariate survival analysis. However, in an adjusted Cox proportional hazards regression model, multivariate survival analysis showed MAP4K3 expression to be a prognostic biomarker for AML. MAP4K1, 3, 4, and 5 may play divergent roles in different cancers, including promoting tumor cell growth, inhibiting tumor development, and progression. This study is only a preliminary exploration of the role of these genes on the prognosis of AML, and further research is required to promote the MAP4K gene family as a targeted therapy.

In joint effects analysis, a combination of high expression of MAP4K3, MAP4K4, and MAP4K5 and low expression of MAP4K1 were predictive of a favorable OS, while a combination of expression of MAP4K3, MAP4K4, and MAP4K5 at low levels and expression of MAP4K1 at a high level were predictive of poor OS.

There were some limitations in our study: a small sample size, and several clinical characteristics, such as smoking history, family history, radiation, and chemical exposure, were not included in the analyses; this study included data from a single source. ASXL1, as an important gene for the prognosis of AML, is almost absent from the database. Since NPM1 and FLT3 cannot be applied to the grouping method for analysis according to the 2017 European LeukemiaNet recommendations, they were excluded from our study. There is also a need to expand the sample and include more detailed gene groupings. Generation of data from another source is necessary to

### Table 6. The Prognostic Value According to Association of MAP4K1, MAP4K3, MAP4K4, and MAP4K5 Expression in AML.

| Group          | Cases | MST, days | Raw P | Raw HR (95% CI) | Adjust P<sup>a</sup> | Adjust HR (95% CI)<sup>a</sup> |
|----------------|-------|-----------|-------|----------------|----------------------|----------------------------------|
| MAP4K1 and MAP4K3 | 151   | 518       | .027  | Ref.           | Rep.                 | Rep.                             |
| A              | 40    | 792       | Ref.  | Rep.           | Rep.                 | Rep.                             |
| B              | 68    | 577       | .368  | 1.280 (0.758-2.160) | .198              | 1.456 (0.822-2.581)               |
| C              | 41    | 275       | .007  | 2.032 (1.169-3.532) | .001              | 2.755 (1.498-5.068)               |
| MAP4K1 and MAP4K4 | 151   | 518       | .001  | Rep.           | Rep.                 | Rep.                             |
| D              | 41    | 945       | Ref.  | Rep.           | Rep.                 | Rep.                             |
| E              | 68    | 577       | .100  | 1.552 (0.910-2.649) | .267               | 1.356 (0.792-2.323)               |
| F              | 40    | 273       | <.001 | 2.825 (1.570-5.082) | .001              | 2.891 (1.576-5.323)               |
| MAP4K1 and MAP4K5 | 151   | 518       | .001  | Rep.           | Rep.                 | Rep.                             |
| G              | 43    | 945       | Ref.  | Rep.           | Rep.                 | Rep.                             |
| H              | 63    | 518       | .009  | 2.005 (1.150-3.494) | .037               | 1.827 (1.038-3.218)               |
| I              | 43    | 243       | <.001 | 2.826 (1.576-5.069) | .001              | 2.967 (1.593-5.527)               |
| MAP4K3 and MAP4K4 | 151   | 518       | .017  | <.001          | <.001                | <.001                            |
| J              | 42    | 792       | Ref.  | Rep.           | Rep.                 | Rep.                             |
| K              | 65    | 731       | .462  | 1.223 (0.722-2.072) | .505               | 1.212 (0.688-2.133)               |
| L              | 42    | 304       | .010  | 2.118 (1.200-3.740) | <.001             | 3.340 (1.784-6.255)               |
| MAP4K3 and MAP4K5 | 151   | 518       | .017  | Rep.           | Rep.                 | Rep.                             |
| M              | 56    | 792       | Ref.  | Rep.           | Rep.                 | Rep.                             |
| N              | 37    | 577       | .730  | 0.887 (0.495-1.588) | .606               | 1.190 (0614-2.307)               |
| O              | 56    | 303       | .024  | 1.716 (1.077-2.732) | .001             | 2.493 (1.436-4.327)               |
| MAP4K4 and MAP4K5 | 151   | 518       | <.001 | <.001          | <.001                | <.001                            |
| P              | 38    | 822       | Ref.  | Rep.           | Rep.                 | Rep.                             |
| Q              | 72    | 761       | .467  | 1.227 (0.703-2.142) | .419               | 1.266 (0.714-2.247)               |
| R              | 39    | 275       | <.001 | 3.090 (1.711-5.581) | <.001             | 3.823 (1.993-7.331)               |
| Missing        | 2     |           |       |                |                      |                                  |

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; HR, hazard ratio; MAP4K, mitogen-activated protein kinase kinase kinase kinase; MST, median survival time.

<sup>a</sup>Adjustment of MAP4K genes for age, cytogenetic, FAB stage, RUNX1, and TP53.
validate the present findings. Despite these limitations, this is the first study to report the favorable prognostic value of the association between downregulated MAP4K1 and upregulated MAP4K3, MAP4K4, and MAP4K5 in AML. This 4-gene signature has the potential to be a prognostic biomarker in patients with AML.

**Conclusion**

Low expression of MAP4K1 concomitant with high expression of MAP4K3, MAP4K4, and MAP4K5, either individually or in combination, are associated with favorable OS in AML. This 4-gene signature may be a potential prognostic biomarker for patients with AML. However, these findings need further validation in a large cohort study.

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**Table 7. The Prognostic Value According to Association of MAP4K1, MAP4K3, MAP4K4, and MAP4K5 Expression in AML.**

| Group | Cases | MST, days | Raw P | Raw HR (95% CI) | Adjust P* | Adjust HR* (95% CI) |
|-------|-------|-----------|-------|------------------|-----------|---------------------|
| MAP4K1 + MAP4K3 + MAP4K4 | 151 | 518 | .001 | 1.668 (0.854-3.257) | <.001 | 1.416 (0.700-2.866) |
| I | 22 | 945 | Ref. | Ref. | Ref. | Ref. |
| li | 102 | 608 | .133 | 4.059 (1.866-8.830) | <.001 | 4.228 (1.880-9.511) |
| lii | 27 | 243 | <.001 | 2.697 (1.434-5.074) | .002 | 3.032 (1.511-6.083) |
| Missing | 2 | | | | |
| MAP4K1 + MAP4K3 + MAP4K5 | 151 | 518 | .003 | 1.446 (0.821-2.545) | .174 | 1.524 (0.830-2.799) |
| Iv | 32 | 854 | Ref. | Ref. | Ref. | Ref. |
| V | 84 | 577 | .183 | 6.769 (3.012-15.211) | <.001 | 6.364 (2.780-14.570) |
| vi | 32 | 243 | <.001 | 2.123 (1.054-4.273) | .104 | 1.801 (0.887-3.659) |
| Missing | 3 | | | | |
| MAP4K1 + MAP4K4 + MAP4K5 | 151 | 518 | <.001 | 2.908 (1.538-5.499) | <.001 | 4.260 (2.127-8.530) |
| vii | 24 | 973 | Ref. | Ref. | Ref. | Ref. |
| viii | 103 | 580 | .031 | 6.425 (2.699-15.296) | <.001 | 5.489 (2.242-13.437) |
| ix | 24 | 214 | <.001 | 4.059 (1.866-8.830) | <.001 | 4.228 (1.880-9.511) |
| Missing | 3 | | | | |
| MAP4K3 + MAP4K4 + MAP4K5 | 151 | 518 | <.001 | 2.123 (1.054-4.273) | <.001 | 1.801 (0.887-3.659) |
| X | 32 | 792 | Ref. | Ref. | Ref. | Ref. |
| xi | 88 | 731 | .755 | 1.089 (0.620-1.914) | .541 | 1.201 (0.667-2.164) |
| xii | 30 | 243 | <.001 | 2.697 (1.434-5.074) | .002 | 3.032 (1.511-6.083) |
| Missing | 1 | | | | |
| MAP4K1 + MAP4K3 + MAP4K4 + MAP4K5 | 151 | 518 | <.001 | 1.739 (0.835-3.623) | .300 | 1.494 (0.699-3.194) |
| 1 | 19 | 945 | Ref. | Ref. | Ref. | Ref. |
| 2 | 112 | 580 | .135 | 1.469 (0.853-2.527) | <.001 | 5.489 (2.242-13.437) |
| 3 | 20 | 215 | <.001 | 6.425 (2.699-15.296) | <.001 | 5.489 (2.242-13.437) |
| Missing | 0 | | | | |

Abbreviations: AML, acute myeloid leukemia; MST, median survival time; HR, hazard ratio; CI, confidence interval; MAP4K, mitogen-activated protein kinase kinase kinase.  
*Adjustment of MAP4K genes for age, cytogenetic, FAB stage, RUNX1, and TP53.

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