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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 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 $P = .005$, $P = .022$, $P = .002$, and $P = .024$; 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 = 0.310-0.776, 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 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

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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.[1] The main treatments for AML include chemotherapy, radiation therapy, molecular therapy, and allogeneic hematopoietic stem cell transplantation.[2,3] 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*,[4] *RUNX1*, *TP53*,[5] *MLL-TD*, *CBFB/MYH11*, *TET2*, *DNMT3A*, *JAK-STAT*, and *CXCR4*,[6] 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. *MAP4K*s reported so far include *MAP4K1/HPK1*, *MAP4K2/GCK*, *MAP4K3/GLK*, *MAP4K4/HGK*, *MAP4K5/KHS*, and *MAP4K6/MINK1*.[7] Through the activation of the *MAP3K–MAP2K* cascade, MAP4Ks can induce Janus kinase (JNK) activation, which is vital for medullary differentiation of hematopoietic tissue.[7-10] 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 downregulated,[8] *MAP4K3* is upregulated in systemic lupus erythematosus (SLE).[11] Upregulation of *MAP4K3* and *MAP4K4* promotes metastasis of breast cancer cells[12,13] and liver cancer cells.[14,15] Elevated expression of *MAP4K2* boosted tumor proliferation in diffuse large B-cell lymphoma[16] and UV resistance in melanoma cells.[17,18] While downregulation of *MAP4K5* promoted the progression of pancreatic cancer,[19] it inhibited the activity of *BCR-ABL* in CML.[20] *MINK1* was reported to be involved in cell division and dendritic structure integrity and synaptic transmission.[21,22] 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, *RUNX1*, 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).[23] Transcript expression of the *MAP4K* family in AML tissues was obtained from OncoLnc (http://www.oncolnc.org/, accessed by January 15, 2019).[24] 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).[25] 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.[26,27] The GO annotations included molecular function (MF), cellular component (CC), and biological process (BP). GeneMANIA (http://genemania.org/; accessed February 11, 2019)[28] was used to reveal interactions among *MAP4K* family members, and correlations were identified by Pearson correlation coefficient analysis.

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
			High MAP4K1 + high MAP4K3		
D	Low MAP4K1 + high MAP4K4	E	Low MAP4K1 + low MAP4K4	F	High MAP4K1 + low MAP4K4
			High MAP4K1 + high MAP4K4		
G	Low MAP4K1 + high MAP4K5	H	Low MAP4K1 + low MAP4K5	I	High MAP4K1 + low MAP4K5
			High MAP4K1 + high MAP4K5		
J	Low MAP4K3 + high MAP4K4	K	Low MAP4K3 + high MAP4K4	L	Low MAP4K3 + low MAP4K4
			High MAP4K3 + low MAP4K4		
M	Low MAP4K3 + high MAP4K5	N	Low MAP4K3 + high MAP4K5	O	Low MAP4K3 + low MAP4K5
			High MAP4K3 + low MAP4K5		
P	Low MAP4K4 + high MAP4K5	Q	Low MAP4K4 + high MAP4K5	R	Low MAP4K4 + low MAP4K5
			High MAP4K4 + low MAP4K5		

Abbreviation: MAP4K, mitogen-activated protein kinase 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
	Low MAP4K1 + low MAP4K3 + high MAP4K4		Low MAP4K1 + low MAP4K3 + high MAP4K5
	Low MAP4K1 + high MAP4K3 + low MAP4K4		Low MAP4K1 + high MAP4K3 + low MAP4K5
	High MAP4K1 + high MAP4K3 + high MAP4K4		High MAP4K1 + high MAP4K3 + high MAP4K5
	High MAP4K1 + high MAP4K3 + low MAP4K4		High MAP4K1 + high MAP4K3 + low MAP4K5
	High MAP4K1 + low MAP4K3 + high MAP4K4		High MAP4K1 + low MAP4K3 + high MAP4K5
iii	High MAP4K1 + low MAP4K3 + low MAP4K4	vi	High MAP4K1 + low MAP4K3 + low MAP4K5
vii	Low MAP4K1 + high MAP4K4 + high MAP4K5	x	High MAP4K3 + high MAP4K4 + high MAP4K5
viii	Low MAP4K1 + low MAP4K4 + low MAP4K5	xi	High MAP4K3 + high MAP4K4 + low MAP4K5
	Low MAP4K1 + low MAP4K4 + high MAP4K5		High MAP4K3 + low MAP4K4 + high MAP4K5
	Low MAP4K1 + high MAP4K4 + low MAP4K5		High MAP4K3 + low MAP4K4 + high MAP4K5
	High MAP4K1 + high MAP4K4 + high MAP4K5		Low MAP4K3 + high MAP4K4 + high MAP4K5
	High MAP4K1 + high MAP4K4 + low MAP4K5		Low MAP4K3 + high MAP4K4 + low MAP4K5
	High MAP4K1 + low MAP4K4 + high MAP4K5		High MAP4K3 + low MAP4K4 + low MAP4K5
ix	High MAP4K1 + low MAP4K4 + low MAP4K5	xii	Low MAP4K3 + low MAP4K4 + low MAP4K5

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

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 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
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	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
	High MAP4K1 + high MAP4K3 + low MAP4K4 + low MAP4K5
	Low MAP4K1 + low MAP4K3 + low MAP4K4 + low MAP4K5
3	High MAP4K1 + low MAP4K3 + low MAP4K4 + low MAP4K5

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

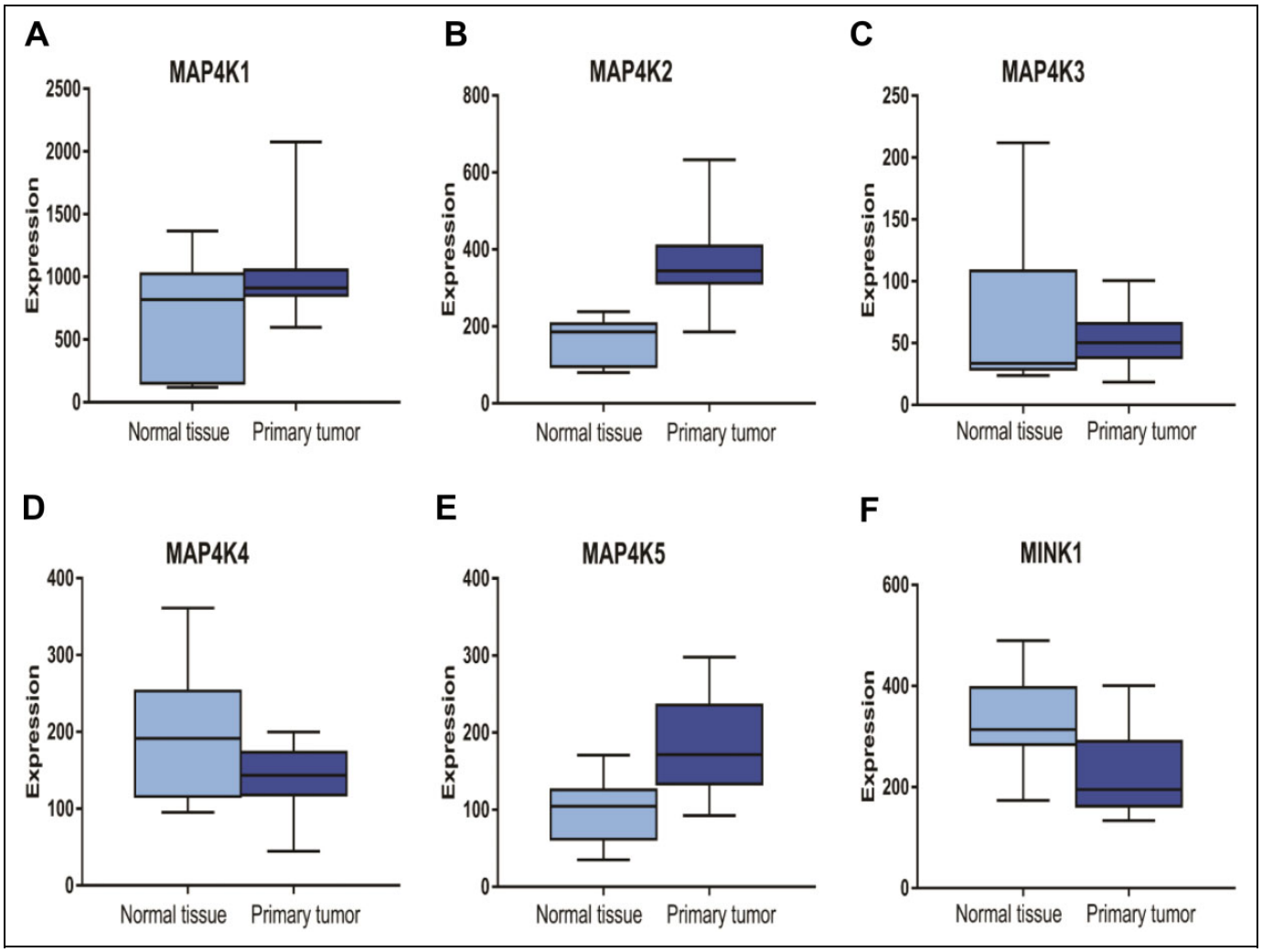

Figure 1. Metabolic gEne RApid Visualizer boxplots for *MAP4K* gene expression in normal hematopoietic and lymphoid tissue and primary AML tissue: (A) MAP4K1; (B) MAP4K2; (C) MAP4K3; (D) MAP4K4; (E) MAP4K5; and (F) MINK1. AML indicates acute myeloid leukemia; MAP4K, MAP kinase kinase kinase kinase.

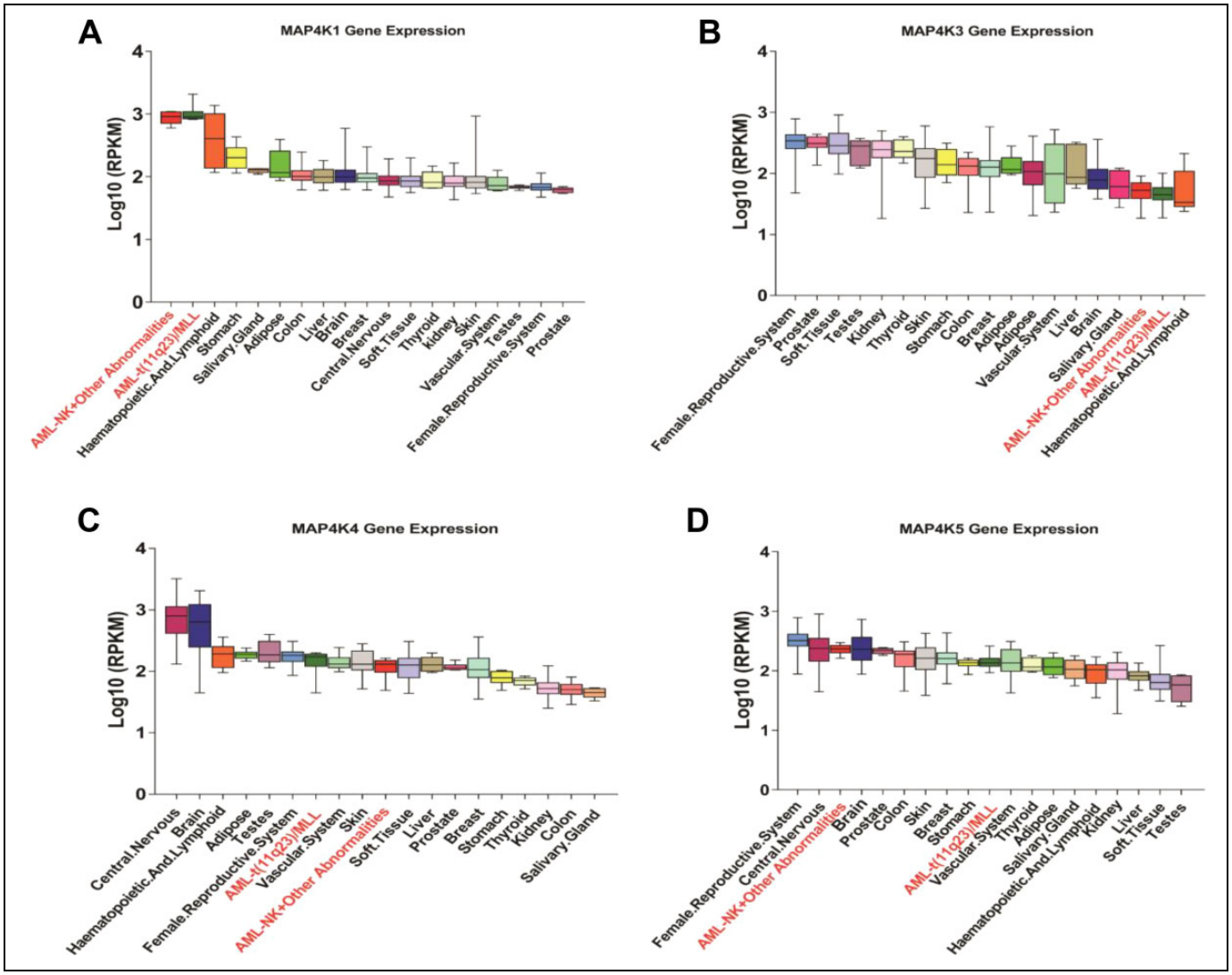

Figure 2. Transcript expression of *MAP4K* genes in multiple normal tissues and AML. The expression of *MAP4K* genes in AML is highlighted in red. (A) MAP4K1; (B) MAP4K3; (C) MAP4K4; (D) MAP4K5. AML indicates acute myeloid leukemia; MAP4K, MAP kinase kinase kinase kinase.

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 ($P < .001$, $P = .787$, $P = .005$, $P = .925$, $P = .254$, $P = .039$, $P = .065$, 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

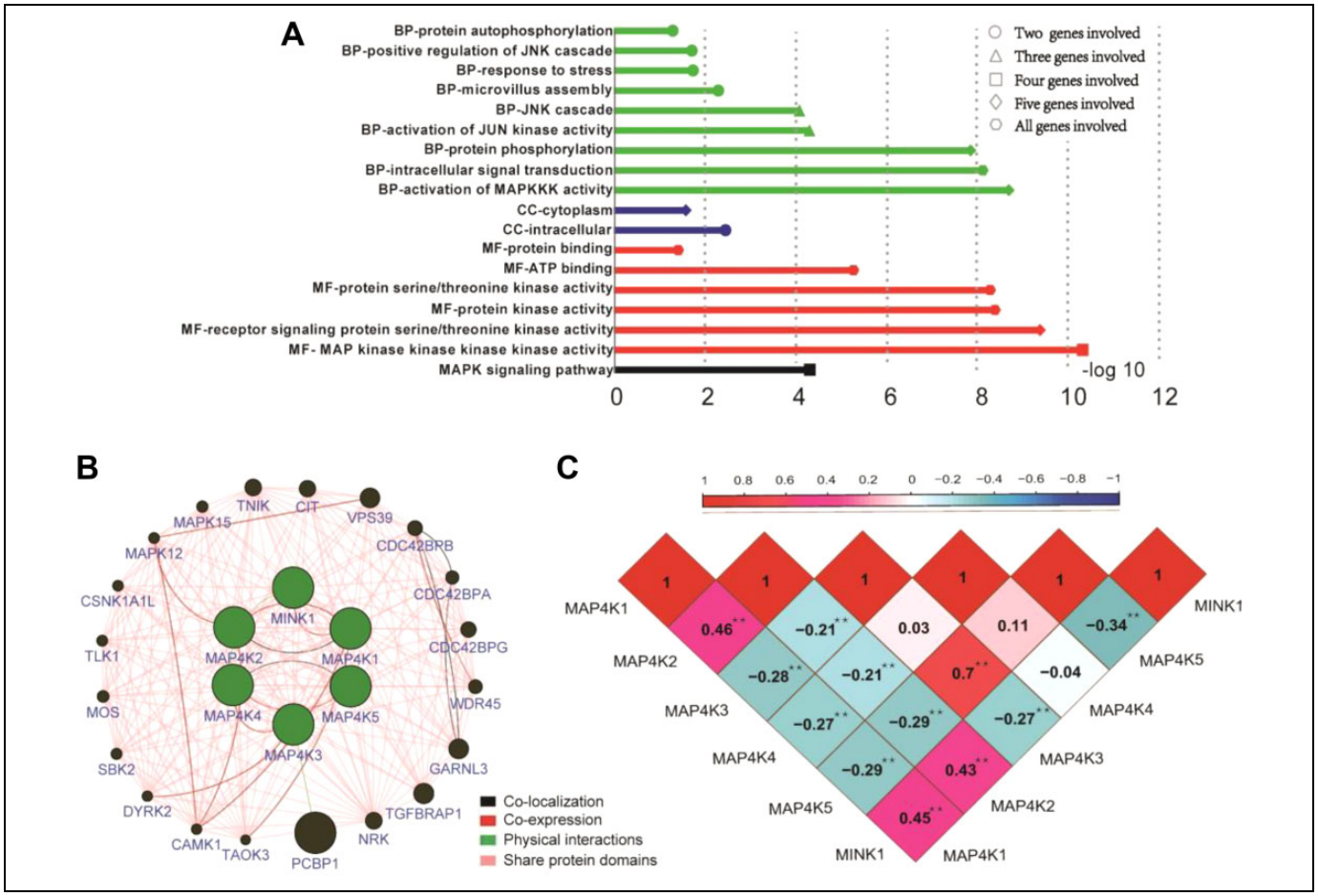

Figure 3. A, Analysis of enriched GO terms and KEGG pathways for *MAP4K* genes by using Database for Annotation, Visualization, and Integrated Discovery. B, Gene interaction networks according to selected gene expression levels in GeneMANIA. C, Pearson correlation coefficients for MAP4K1, MAP4K2, MAP4K3, 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					<.001
≤60	88	47 (53.4%)	915	Ref	
>60	63	12 (19.0%)	275	0.262 (0.160-0.427)	
Gender					.787
Male	81	32 (39.4%)	518	Ref	
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)	
Poor	27	9 (33.3%)	365	1.152 (0.596-2.227)	
Missing	2				
Leukocyte, 10^9/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)	

(continued)

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					.065
Mutation negative	136	56 (41.2%)	580	Ref	
Mutation positive	15	3 (20.0%)	335	1.765 (0.954-3.266)	
TP53					<.001
Mutation negative	141	59 (41.8%)	609	Ref	
Mutation positive	10	0 (0.00%)	214	3.573 (1.813-7.039)	

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

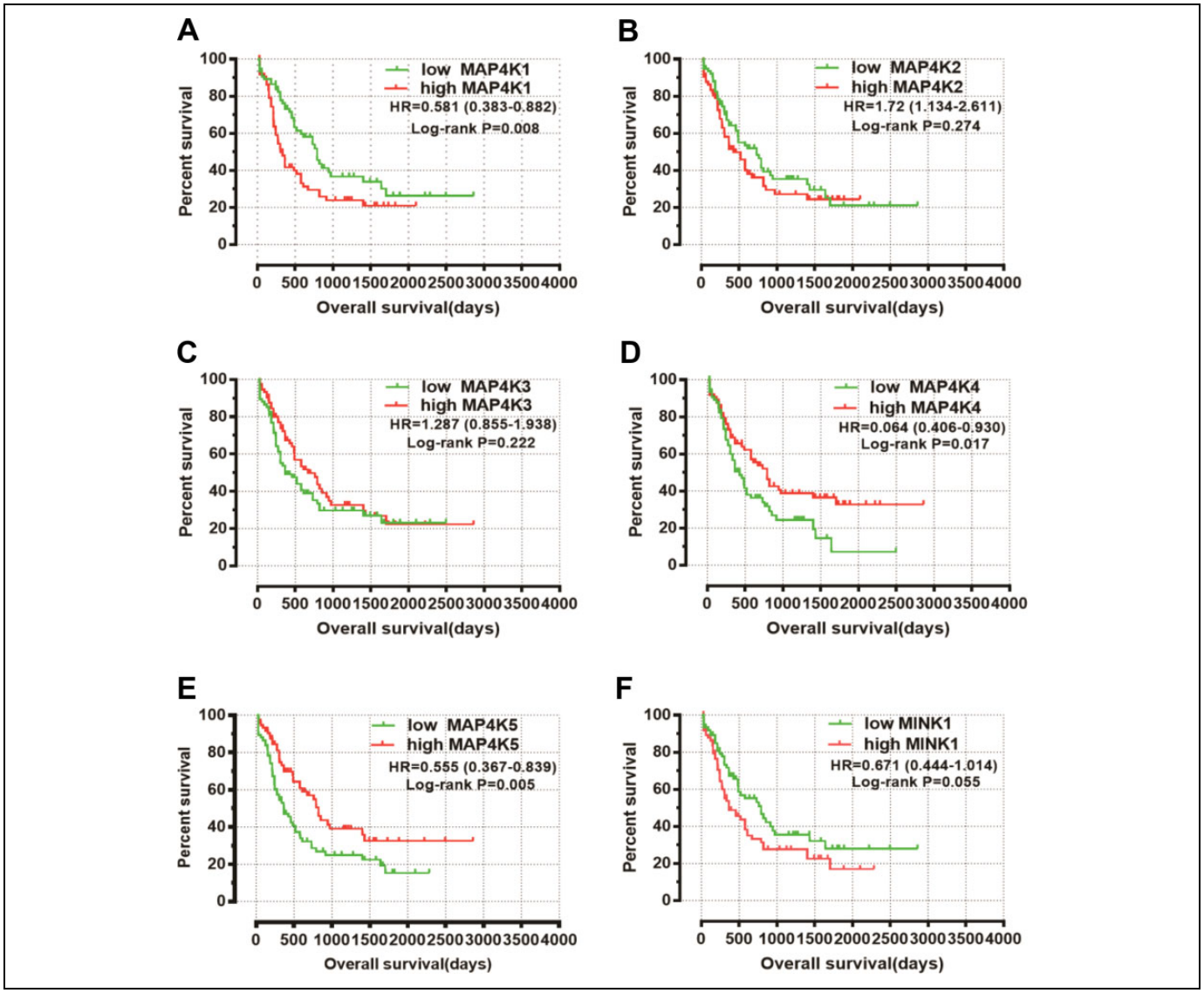

Figure 4. Prognostic value of *MAP4K* genes expression. A-F, Kaplan-Meier survival curves for all patients with AML according to MAP4K1 (A), MAP4K2 (B), MAP4K3 (C), MAP4K4 (D), MAP4K5 (E), and MINK1 (F) expression. AML indicates acute myeloid leukemia; MAP4K, MAP kinase kinase kinase kinase.

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*[a]
MAP4K1					.008		.024
Low	75	34 (45.3%)	792	Ref.		Ref.	
High	75	25 (33.3%)	305	0.581		0.615	
Missing	1			(0.383-0.882)		(0.403-0.938)	
MAP4K2					.274		.804
Low	75	28 (37.3%)	731	Ref.		Ref.	
High	75	30 (38.7%)	427	0.798		0.947	
Missing	1			(0.529-1.206)		(0.618-1.452)	
MAP4K3					.222		.005
Low	75	26 (34.7%)	366	Ref.		Ref.	
High	75	32 (42.7%)	671	1.287		0.490	
Missing	1			(0.855-1.938)		(0.297-0.809)	
MAP4K4					.017		.022
Low	75	25 (33.3%)	427	Ref.		Ref.	
High	75	34 (45.3%)	792	0.614		0598	
Missing	1			(0.406-0.930)		(0.385-0.928)	
MAP4K5					.005		.002
Low	75	21 (28.0%)	365	Ref.		Ref.	
High	75	38 (50.7%)	822	0.555		0.490	
Missing	1			(0.367-0.839)		(0.310-0.776)	
MINK1					.055		.179
Low	75	34 (45.3%)	792	Ref.		Ref.	
High	75	25 (33.3%)	366	0.671		1.375	
Missing	1			(0.444-1.014)		(0.864-2.189)	

Abbreviations: CI, confidence interval; HR, hazard ratio; MAP4K, MAP kinase kinase kinase kinase; MST, median survival time.
[a]Adjustment 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-kB.[18] *MAP4K3* also regulates T-cell function via NF-kB. *MAP4K1*, on the other hand, inhibited NF-kB 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

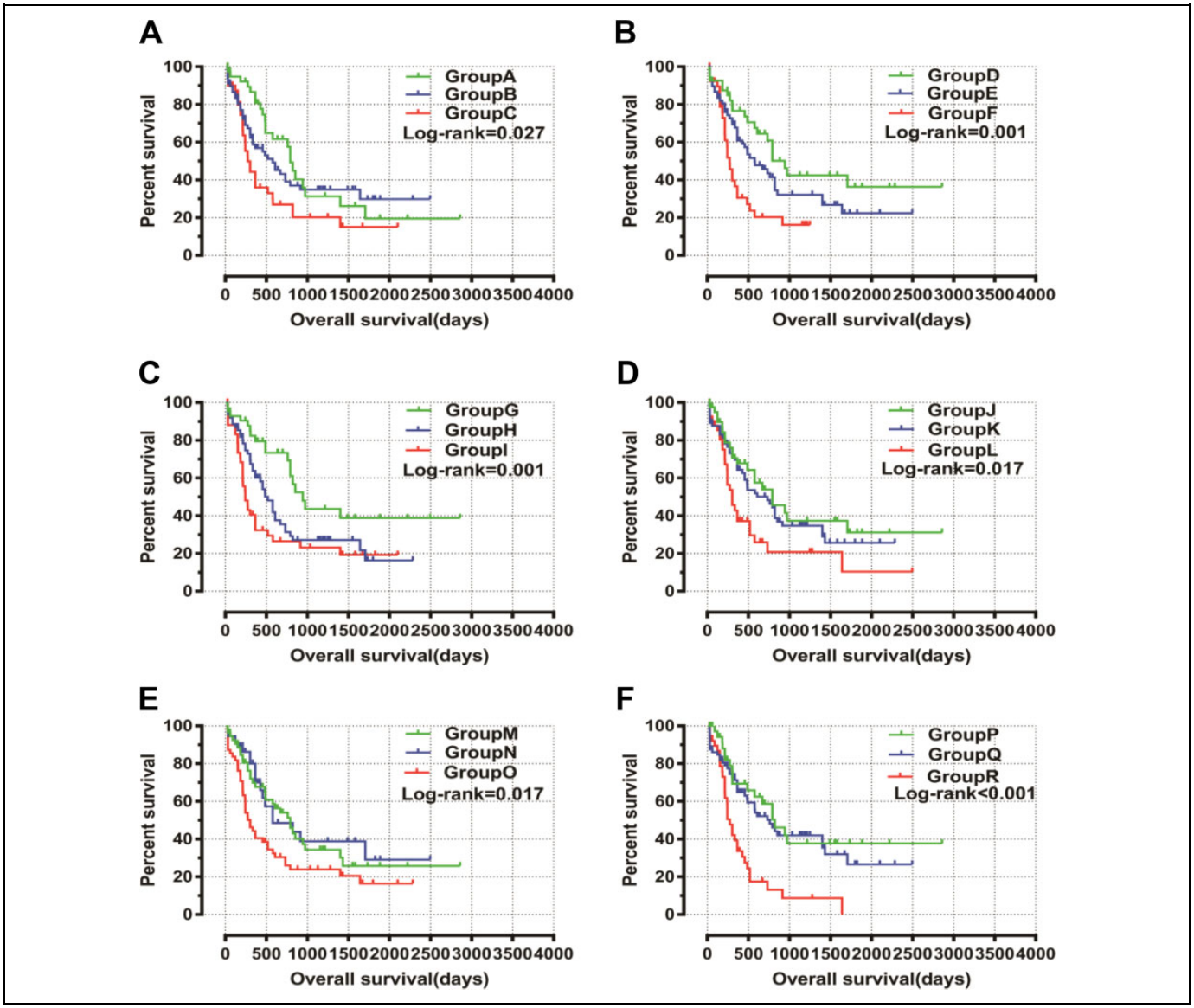

Figure 5. Joint effect analysis for *MAP4K* genes expression with stratified OS according to 2 selected *MAP4K* genes among MAP4K1, MAP4K3, MAP4K4, and MAP4K5. (A) MAP4K1 and MAP4K3, (B) MAP4K1 and MAP4K4, (C) MAP4K1 and MAP4K5, (D) MAP4K3 and MAP4K4, (E) MAP4K3 and MAP4K5, and (F) MAP4K4 and MAP4K5. 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.

found to be an inhibitor of JNK pathway.[37] Coexpression of *MAP4K3* and *DYRK2* (Figure 3B) was a significant finding as both are known to play vital roles in breast cancer and metastasis.[38]

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.[5] Thus, *MAP4K1*, *MAP4K3*, and *MAP4K4* 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.[8-10] The expression of *MAP4K1* is increased in AML but decreased in autoimmune diseases. Inhibition of *HPK1* expression is beneficial to the survival of

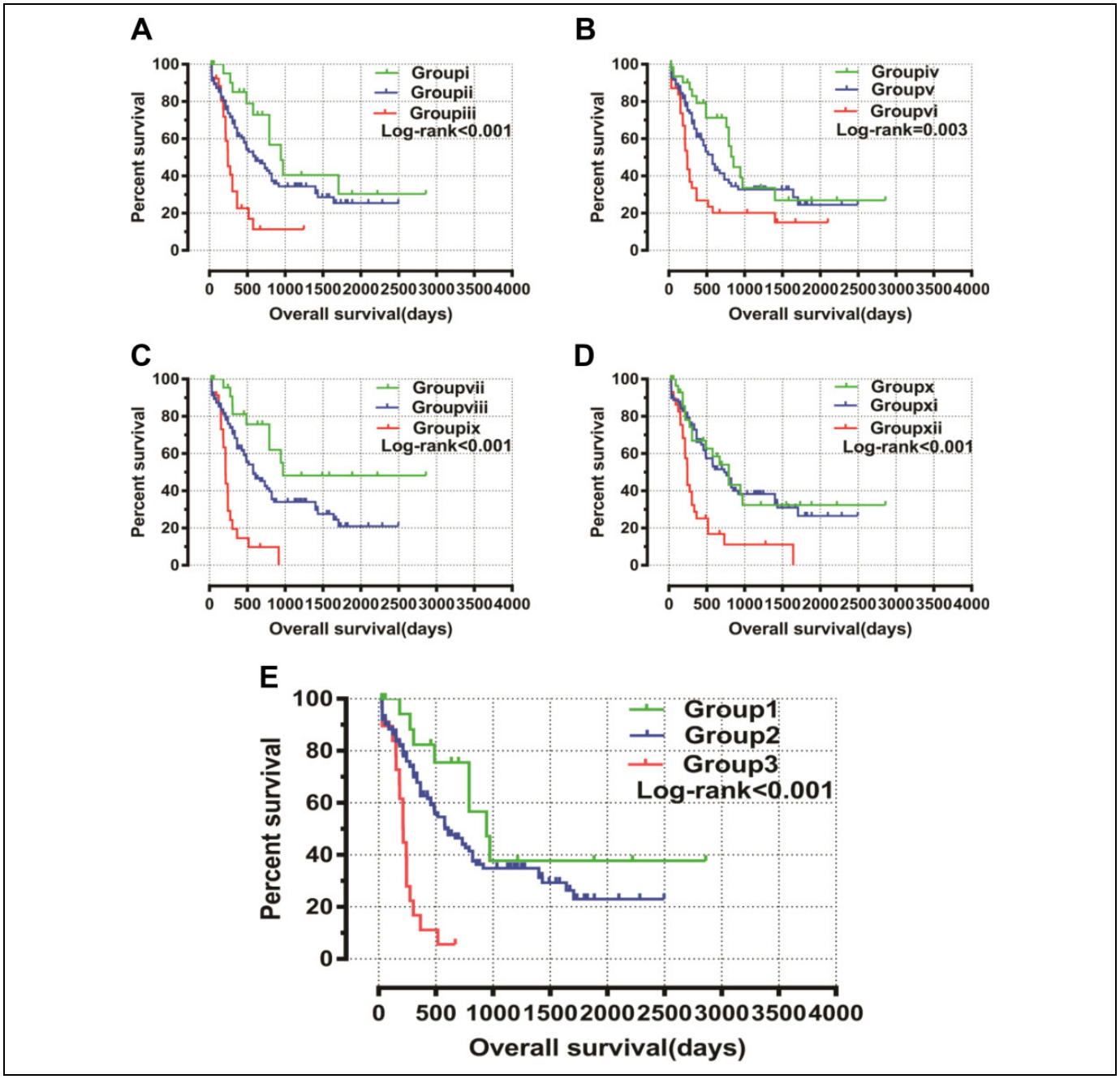

Figure 6. Joint effect analysis for *MAP4K* genes expression with stratified OS according to 3 or 4 selected *MAP4K* genes among MAP4K1, MAP4K3, MAP4K4, and MAP4K5. (A) MAP4K1, MAP4K3, and MAP4K4; (B) MAP4K1, MAP4K3, and MAP4K5; (C) MAP4K1, MAP4K4, and MAP4K5; (D) MAP4K3, MAP4K4, and MAP4K5; (E) MAP4K1, 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.

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.

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*[a]	Adjust HR (95% CI)[a]
MAP4K1 and MAP4K3	151	518	.027		.003	
A	40	792	Ref.	Ref.	Ref.	Ref.
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)
Missing	2					
MAP4K1 and MAP4K4	151	518	.001		.002	
D	41	945	Ref.	Ref.	Ref.	Ref.
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 (1.576-5.333)
Missing	2					
MAP4K1 and MAP4K5	151	518	.001		.003	
G	43	945	Ref.	Ref.	Ref.	Ref.
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)
Missing	2					
MAP4K3 and MAP4K4	151	518	.017		<.001	
J	42	792	Ref.	Ref.	Ref.	Ref.
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)
Missing	2					
MAP4K3 and MAP4K5	151	518	.017		.002	
M	56	792	Ref.	Ref.	Ref.	Ref.
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)
Missing	2					
MAP4K4 and MAP4K5	151	518	<.001		<.001	
P	38	822	Ref.	Ref.	Ref.	Ref.
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.
[a]Adjustment of *MAP4K* genes for age, cytogenetic, FAB stage, RUNX1, and TP53.

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 implicated 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.[39] Downregulation of *MAP4K4* expression in cancer cells promoted apoptosis[40] and inhibited migration and invasion.[41] 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. *MAP4K*1, 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 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*[a]	Adjust HR[a] (95% CI)
MAP4K1 + MAP4K3 + MAP4K4	151	518	.001		<.001	
I	22	945	Ref.	Ref.	Ref.	Ref.
Ii	102	608	.133	1.668 (0.854-3.257)	.334	1.416 (0.700-2.866)
Iii	27	243	<.001	4.059 (1.866-8.830)	<.001	4.228 (1.880-9.511)
Missing	2					
MAP4K1 + MAP4K3 + MAP4K5	151	518	.003		.004	
Iv	32	854	Ref.	Ref.	Ref.	Ref.
V	84	577	.183	1.446 (0.821-2.545)	.174	1.524 (0.830-2.799)
vi	32	243	.002	2.697 (1.434-5.074)	.002	3.032 (1.511-6.083)
Missing	3					
MAP4K1 + MAP4K4 + MAP4K5	151	518	<.001		<.001	
vii	24	973	Ref.	Ref.	Ref.	Ref.
viii	103	580	.031	2.123 (1.054-4.273)	.104	1.801 (0.887-3.659)
ix	24	214	<.001	6.769 (3.012-15.211)	<.001	6.364 (2.780-14.570)
Missing	2					
MAP4K3 + MAP4K4 + MAP4K5	151	518	<.001		<.001	
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	2.908 (1.538-5.499)	<.001	4.260 (2.127-8.530)
Missing	1					
MAP4K1 + MAP4K3 + MAP4K4 + MAP4K5	151	518	<.001		<.001	
1	19	945	Ref.	Ref.	Ref.	Ref.
2	112	580	.135	1.739 (0.835-3.623)	.300	1.494 (0.699-3.194)
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 kinase.
[a]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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Declaration of Conflicting Interests

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