Prognostic value of key genes of the JAK-STAT signaling pathway in patients with cutaneous melanoma

FUQIANG PAN[1*], QIAOQI WANG[1*], SIZHU LI[2], RUI HUANG[3], XIANGKUN WANG[4], XIWEN LIAO[4], HAIYAN MO[1], LIMING ZHANG[1] and XIANG ZHOU[1]

[1]Department of Medical Cosmetology, The Second Affiliated Hospital of Guangxi Medical University; Departments of [2]Medical Oncology, [3]Hematology and [4]Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region 530000, P.R. China

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Abstract. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway is involved in cell immunity, division and death, as well as in tumor formation. The expression of key genes in the JAK-STAT signaling pathway in different types of cancer serves different roles. However, few reports are available on the prognostic value of the genes of the JAK-STAT signaling pathway in skin cutaneous melanoma (SKCM). The potential prognostic value of gene expression in the JAK-STAT signaling pathway in patients with SKCM was analyzed in the present study using data obtained from The Cancer Genome Atlas. To predict the potential functions and mechanisms of these genes in SKCM, gene set enrichment analysis (GSEA) and bioinformatics analysis were performed. A nomogram model including gene expression level and high risk factors was used to predict the risk level of prognostic. High expression levels of *STAT1*, *STAT3*, *STAT4* and *STAT5B*, and low expression levels of *STAT6* were associated with favorable prognosis [adjusted P<0.001; hazard ratio (HR), 0.595; 95% confidence interval (CI), 0.455-0.778; adjusted P=0.018; HR, 0.725; 95% CI, 0.555-0.947; adjusted P<0.001; HR, 0.590; 95% CI, 0.450-0.773; adjusted P=0.007; HR, 0.690; 95% CI, 0.526-0.940; and adjusted P=0.026; HR, 0.737, 95% CI, 0.563-0.964, respectively]. GSEA results demonstrated that these genes were involved in cell differentiation, invasion, adhesion, migration, cycle, colony formation and mitogen-activated protein kinase signaling. The combination of genes with favorable prognosis had a better effect on the overall survival (univariate survival analysis, P<0.05). The results of the present study suggest that *STAT1*, *STAT3*, *STAT4*, *STAT5B* and *STAT6* gene expression may be used as a potential prognostic biomarker of SKCM, and the combined outcomes may exhibit a stronger interaction and higher survival time for SKCM.

Correspondence to: Dr Xiang Zhou, Department of Medical Cosmetology, The Second Affiliated Hospital of Guangxi Medical University, 166 Daxue East Road, Nanning, Guangxi Zhuang Autonomous Region 530000, P.R. China
E-mail: zx_gxmu@hotmail.com

*Contributed equally

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Introduction

Skin cutaneous melanoma (SKCM) is a highly aggressive skin cancer, which arises from the malignant transformation of melanocytes in the basal layer of the epidermis (1,2) and has a poor prognosis, with a 5-year overall survival (OS) of 91.8% worldwide (3). The number of new cases of SKCM that will emerge in 2019 was estimated to be 96,480, with the mortality estimated to be 7,230 (4,5). Therefore, there is an urgent need to identify novel biomarkers and prognostic predictive indicators for the detection and management of SKCM.

The JAK-STAT signaling pathway serves a crucial role in cell immunity, division and death, and in tumor formation (6). The two key components involved in this pathway are Janus kinases (JAKs) and signal transducer and activator of transcription proteins (STATs), which are encoded by the genes *JAK* (*JAK1, JAK2, JAK3* and *TYK2*) and *STAT* (*STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B* and *STAT6*), respectively (6).

A previous study has demonstrated that the key genes involved in the JAK-STAT signaling pathway are associated with several types of cancer, including breast (7,8), ovarian, lung, brain (9) and colorectal (10) cancer, and that their differential expression may result in different prognosis outcomes in different types of cancer. However, few reports about the association between these genes and SKCM are available, and further investigation analyzing the prognostic value of gene expression in the JAK-STAT signaling pathway in SKCM is needed. The aim of the present study was to identify the prognostic values of the expression of genes involved in the JAK-STAT signaling pathway in patients with SKCM based on data derived from public databases and bioinformatics analysis, and to explore the underlying mechanism that may affect the outcome in SKCM prognosis.

Materials and methods

Data preparation. The Cancer Genome Atlas (TCGA; https://cancergenome.nih.gov; accessed on September 10th 2018 was used to obtain the gene expression and clinical data of patients with SKCM, including sex, age and tumor stage. A total of 458 SKCM cases were selected after removing the cases with missing mRNA expression or clinical data and 0-day survival time.

Functional analysis of key genes in the JAK-STAT signaling pathway. The Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway map was generated using the KEGG website (https://www.kegg.jp; accessed on September 10th 2018 (11-13). Gene ontology (GO) term analysis, including biological function (BP), molecular function (MF) and cellular component (CC), as well as KEGG enrichment analysis for JAK and STAT gene families, were performed using the Database for Annotation, Visualization and Integrated Discovery version 6.8 (DAVID; https://david.ncifcrf.gov/tools.jsp; accessed on September 13th 2018. The official gene symbol was used as the identifier; the species was *Homo sapiens* (14,15).

Gene interaction and association analysis. Gene-gene interaction analysis was performed using gene multiple association network integration algorithm (GeneMANIA; http://genemania.org; accessed on September 15th 2018 using the default parameters (16,17). The correlation between JAK and STAT pathway gene expression in SKCM was evaluated using the Pearson's correlation coefficient; $P<0.01$ was considered to indicate a significant correlation. Protein-protein interaction analysis was performed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org; accessed on September 25th 2018. The minimum required interaction score was 0.400 (18,19).

Patient grouping based on gene expression level. Patients with SKCM were divided into high or low expression groups using the median value, and the vertical scatter plots were generated.

Survival analysis. Kaplan-Meier survival plots with log-rank test were used to evaluate the OS for the high and low-expression groups of each gene and clinicopathological characteristics. In addition, the Cox proportional hazard regression model was used for univariate and multivariate survival analyses, and 95% confidence intervals (CIs) and hazard ratios (HRs) were calculated. Joint effects analysis was performed for the combination of genes identified as significant by the survival analysis.

Nomogram. A nomogram was used to evaluate the association between JAK and STAT gene expression and clinical information in SKCM. In addition, the risk rank for each gene and clinicopathological characteristic, including age, sex and tumor stage, was evaluated by the risk points and total points. Survival rates (1-, 3- and 5-year) were also scored. A high score was associated with a low survival rate.

Gene set enrichment analysis (GSEA). To investigate the potential underlying mechanism of the differential expression of *STAT1*, *STAT3*, *STAT4*, *STAT5B* and *STAT6* in SKCM, GSEA version 3.0 software (http://software.broadinstitute.org/gsea/msigdb/index.jsp; accessed on September 20th 2018 (20) was used, and the difference in the expression levels in the low and high-expression groups for each gene was analyzed with reference gene sets, which were based on the Molecular Signatures Database sets c2 (KEGG gene sets, c2.all.v6.2.symbols.gmt), c5 [Gene Ontology (GO) gene sets, c5.all.v6.2.symbols.gmt] and c6 (oncogenic signatures gene sets, c6.all.v6.2.symbols.gmt) (21). The permutation number was set to 1,000. Those enrichment gene sets revealed by GSEA as exhibiting a nominal $P<0.05$ and a false discovery rate (FDR) <0.25 were considered to indicate a statistically significant difference. The default parameters were used in GSEA software.

Statistical analysis. Statistical analysis was performed using SPSS v.25.0 software (IBM Corp.) and R 3.3.5 (https://www.r-project.org/). Kaplan-Meier survival analysis and the log-rank test were used to calculate the OS and P-values for all associations. The Cox proportional hazards regression model was used for univariate and multivariate survival analyses. HRs and 95% CIs were calculated using the Cox proportional hazards regression model with adjustment for influential clinical characteristics such as race, sex, age, Tumor-Node-Metastasis stage (22) and body mass index. FDRs in the GSEA were adjusted for multiple testing with the Benjamini-Hochberg procedure to control the FDR (23,24). $P<0.05$ was considered to indicate a statistically significant difference. Vertical scatter plots and survival curves were generated in GraphPad Prism v.7.0 (GraphPad Software, Inc.).

Results

Clinicopathological characteristics of patients with SKCM. The clinicopathological data of the 458 patients included in the present study are presented in Table I. Race, age and tumor stage were significantly associated with median survival time ($P=0.003$, $P<0.001$ and $P<0.001$, respectively; Table I). After normalization, only *JAK1*, *STAT1*, *STAT3*, *STAT4*, *STAT5B* and *STAT6* genes mRNA expression were observed.

Bioinformatics analysis of JAK and STAT genes. The KEGG pathway map of the JAK-STAT signaling pathway is represented in Fig. 1 (KEGG map no. 04630; https://www.genome.jp/dbget-bin/www_bget?pathway:map04630). The GO term and KEGG enrichment analysis for JAK and STAT genes included BP (Figs. S1A and S2A), CC (Figs. S1B and S2B), MF (Figs. S1C and S2C) and KEGG (Figs. S1D and S2D). The DAVID result for the combination of JAK and STAT gene families suggested that the JAK and STAT signaling pathways were associated with cell migration, the mitogen-activated protein kinase (MAPK) cascade, cell differentiation (Fig. 2A), cytosol, cytoplasm (Fig. 2B), DNA binding, ATP binding, signal transducer activity (Fig. 2C), the PI3K-AKT signaling pathway and the pancreatic cancer signaling pathway (Fig. 2D).

Table I. Survival analysis based on clinical information.

Characteristic	Patients (n=458)	No. of events (%)	MST (days)	HR (95% CI)	Crude P-value
Race					0.003[a]
Caucasian	435	210 (48.3)	2,454	0.337 (0.166-0.687) Ref.	
Other	13	8 (61.5)	636		
Unknown	10				
Sex					0.345
Male	284	146 (51.4)	2,421	Ref. 0.872 (0.657-1.158)	
Female	174	73 (42.0)	2,367		
Age (years)					<0.001[a]
≤60	239	115 (48.1)	3,196	0.587 (0.445-0.773) Ref.	
>60	219	104 (47.5)	1,864		
Tumor stage[b]					<0.001[a]
Early	231	108 (46.8)	3,195	0.547 (0.411-0.728) Ref.	
Advanced	191	96 (50.3)	1,927		
Unknown	36				

[a]P<0.05. [b]Due to the small sample size of certain tumor stages, two groups were used to reduce the probability of statistical errors. MST, median survival time; HR, hazard ratio; CI, confidence interval; Ref., reference group.

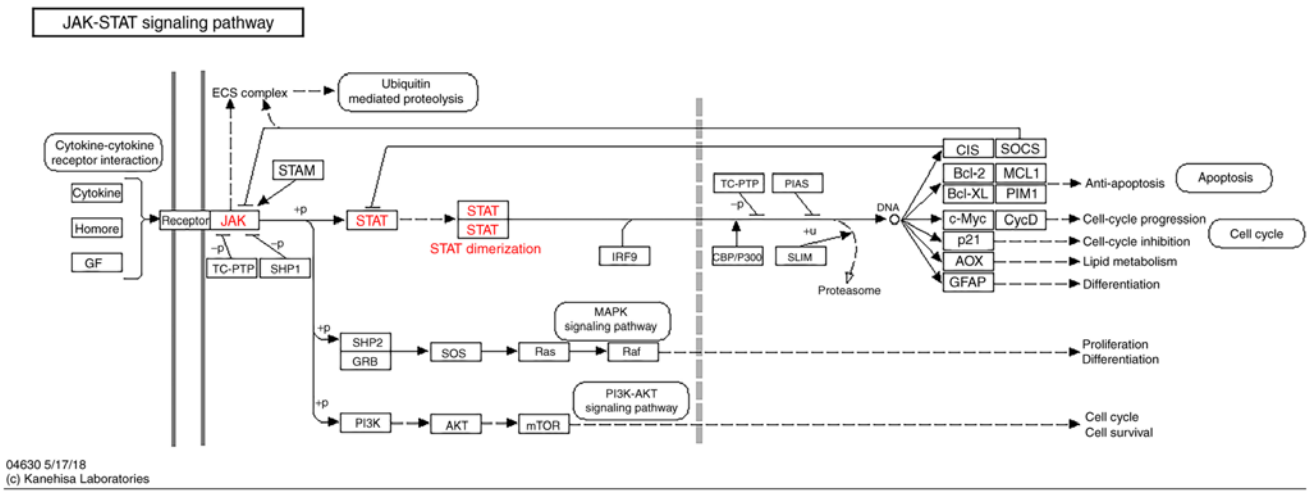

Figure 1. KEGG pathway map of the JAK-STAT signaling pathway obtained from the KEGG website. KEGG, Kyoto Encyclopedia of Genes and Genomes; JAK, Janus kinase; STAT, signal transducer and activator of transcription.

Gene interaction and association analysis. The gene-gene interaction network was analyzed separately in the *JAK* family (Fig. S3) and in the *STAT* family (Fig. S4), as well as in their combination (Fig. 3A). The protein-protein interaction network is presented in Fig. 3B.

Pearson's correlation coefficient was used to analyze the correlation between JAK and STAT genes in SKCM tissues based on the TCGA dataset (Fig. 3C). The results suggested that, with the exception of *STAT6*, the expression of STAT genes (*STAT1*, *STAT3*, *STAT4* and *STAT5B*) in SKCM strongly correlated with each other.

Scatter plots of the expression of all JAK and STAT genes in TCGA dataset (using the median as the cut-off value) are presented in Fig. 3D. The difference between the high and low-expression groups was statistically significant (P<0.001).

Survival analysis. The results of univariate survival analysis of JAK and STAT genes are presented in Fig. 4 and Table II. High expression levels of *STAT1*, *STAT3*, *STAT4* and *STAT5B*, and low expression levels of *STAT6* were associated with a favorable prognosis (P<0.05). Multivariate Cox proportional hazards regression analysis identified that sex, race, age and tumor stage were associated with the prognosis of patients with SKCM. Multivariate survival analysis, in agreement with univariate survival analysis, demonstrated that high expression of *STAT1*, *STAT3*, *STAT4* and *STAT5B*, and low expression of *STAT6* was associated with a favorable prognosis (adjusted P<0.001; HR, 0.595; 95% CI, 0.455-0.778; adjusted P=0.018; HR, 0.725; 95% CI, 0.555-0.947; adjusted P<0.001; HR, 0.590; 95% CI, 0.450-0.773; adjusted P=0.007; HR, 0.690; 95% CI, 0.526-0.940; and adjusted P=0.026; HR, 0.737; 95% CI, 0.563-0.964, respectively; Table II).

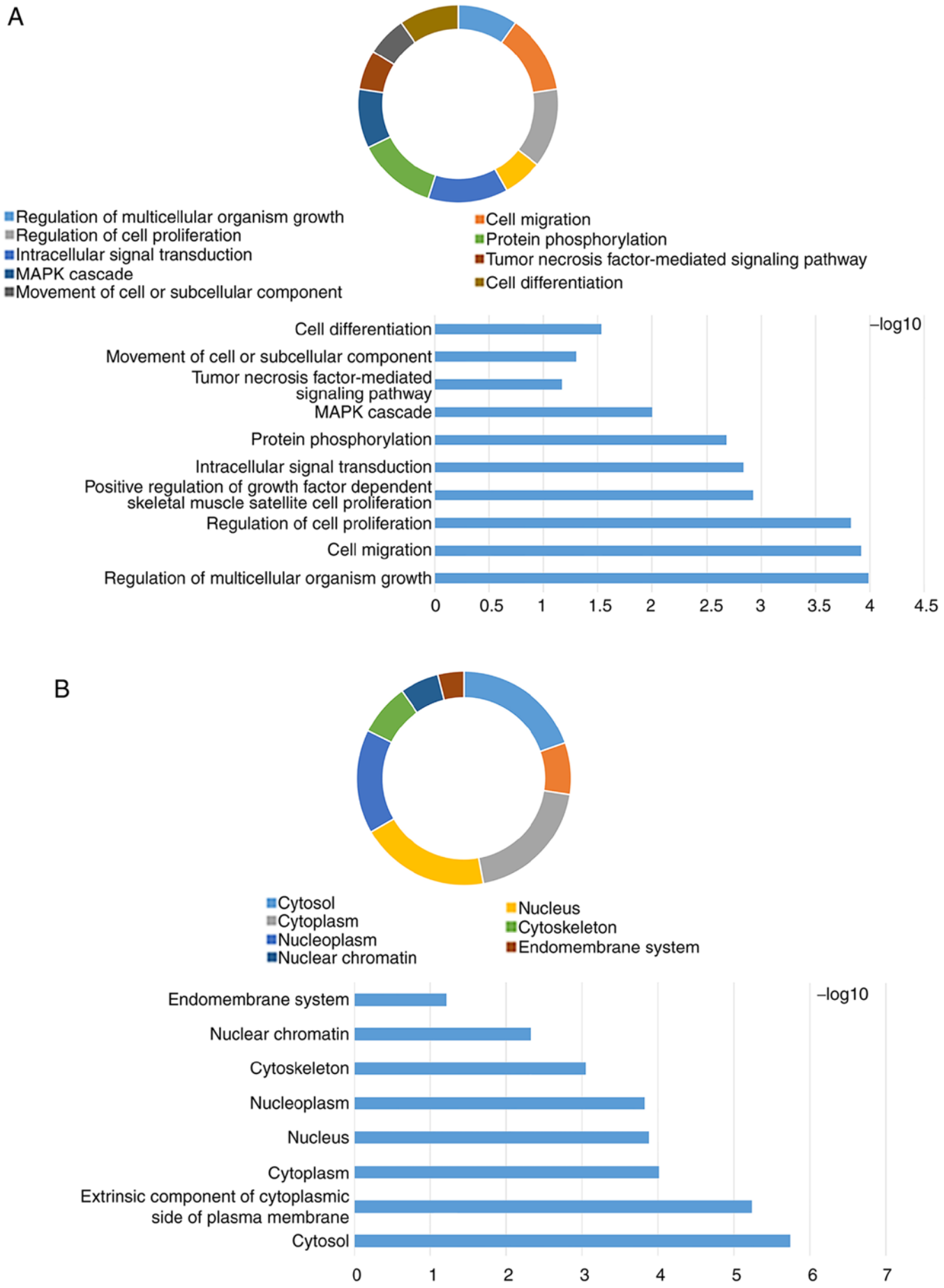

Figure 2. Analysis of enriched GO terms and KEGG pathways for JAK and STAT genes obtained using Database for Annotation, Visualization and Integrated Discovery. (A) Biological process results of GO functional enrichment analysis. (B) Cellular component results of GO functional enrichment analysis.

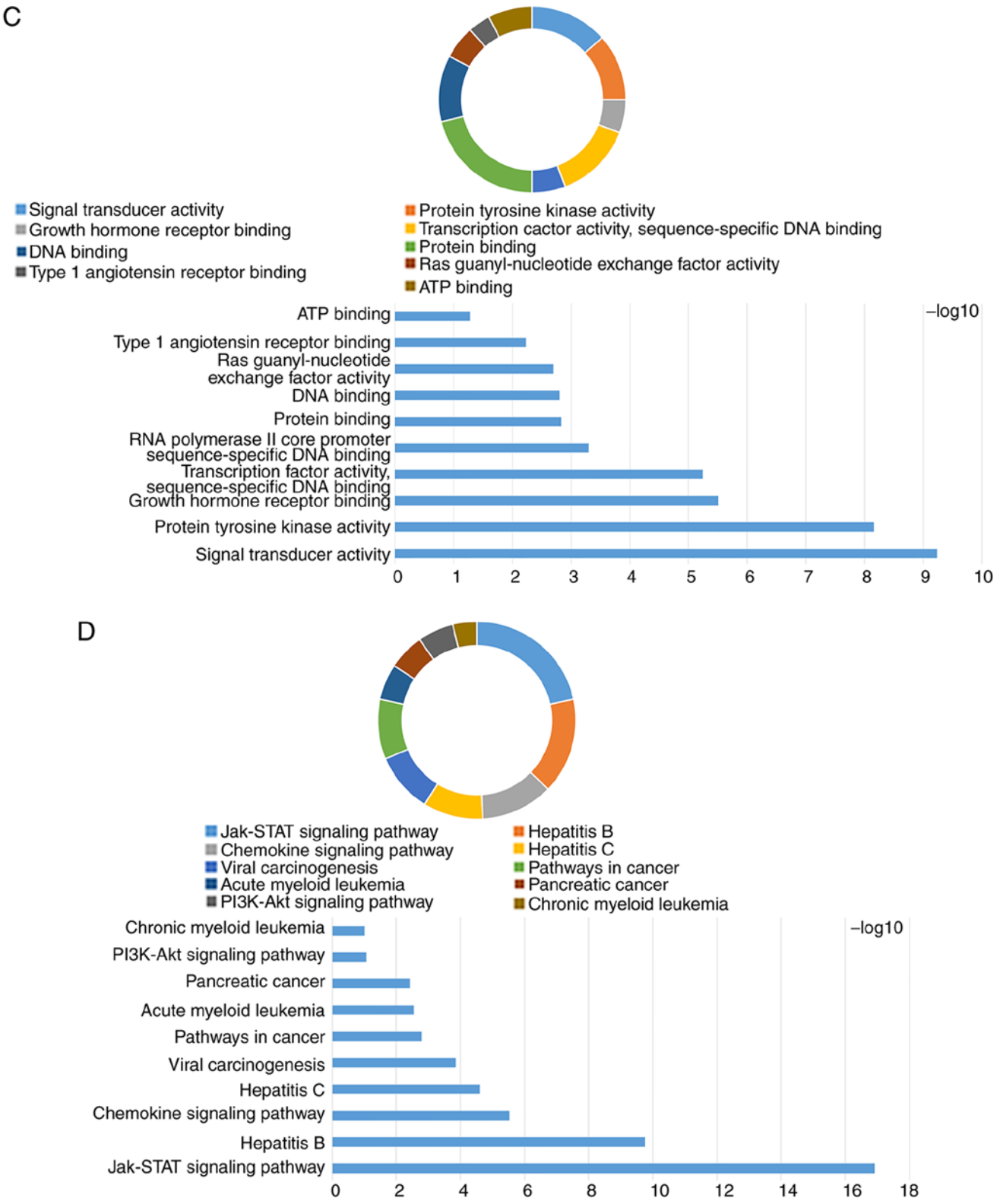

Figure 2. Continued. (C) Molecular function results of GO functional enrichment analysis. (D) KEGG pathway analysis results. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; *JAK*, Janus kinase; *STAT*, signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase.

Predictive nomogram and joint effects analysis. Independent factors, including age, sex, tumor stage and mRNA expression, were integrated into the prognostic nomogram to predict the clinical outcomes of patients with SKCM (Fig. 5A). Age, tumor stage, and *JAK1*, *STAT1*, *STAT4*, *STAT5B* and *STAT6* expression levels exhibited major contributions as prognostic signatures in the risk scores (range, 0-100).

All survival related genes, including *STAT1*, *STAT3*, *STAT4*, *STAT5B* and *STAT6*, were selected and grouped to perform joint effects analysis. The grouping information of joint effects analysis

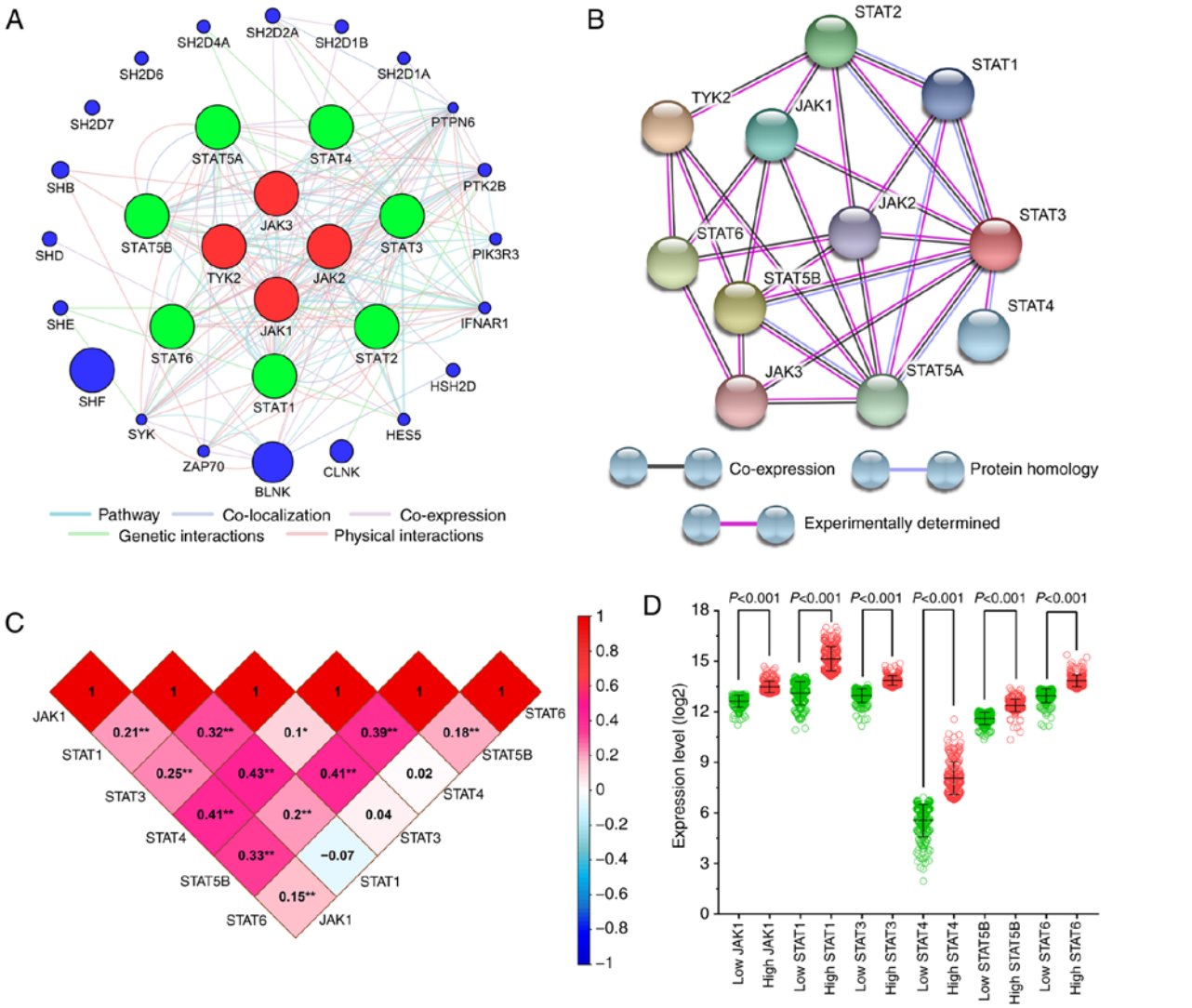

Figure 3. Correlation and association analysis for JAK and STAT genes in SKCM (A) Gene interaction network of JAK and STAT genes generated by GeneMANIA. (B) Protein-protein interaction network generated using the STRING database. (C) Pearson's correlation coefficients for *JAK1, STAT1, STAT3, STAT4, STAT5B* and *STAT6* gene expression levels. (D) Scatter plots of *JAK1, STAT1, STAT3, STAT4, STAT5B* and *STAT6* gene expression level in The Cancer Genome Atlas database. GeneMANIA, gene multiple association network integration algorithm; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; *STAT*, signal transducer and activator of transcription; SKCM, skin cutaneous melanoma; error bars in the scatter plots, mean ± SD.

is presented in Tables III-VI. The group number with simple Arabic numerals represented all gene selected groups (Table III); Arab numerals with brackets are 4 selected genes (Table IV); Lowercase Roman numerals are 3 selected genes (Table V); Capitalized Roman numerals are 2 selected genes (Table VI). The combination of genes with favorable prognosis had a better effect on the OS (univariate survival analysis; P<0.05; Table VII and Figs. 5B-G, 6A-J and 7A-J).

GSEA. The detailed GSEA results, including KEGG, GO and oncogenic signatures, are shown in Tables SI, SII and SIII, respectively, and in Fig. 8. High expression of *STAT1* was significantly associated with immune response (normalized P=0.002; FDR=0.243; Fig. 8A), cell adhesion (normalized P=0.002; FDR=0.206; Fig. 8B; normalized P=0.010; FDR=0.241; Fig. 8D) and WNT protein binging (normalized P=0.006; FDR=0.261; Fig. 8C). By contrast, low expression of *STAT4* was associated with WNT protein binding (normalized P=0.001; FDR=-0.234; Fig. 8E), whereas high expression of *STAT5B* was associated with gene silencing (normalized P=0.002; FDR=0.249; Fig. 8F).

Discussion

In the Surveillance, Epidemiology, and End Results Program database, the 5-year relative survival is 98% for prostate cancer, 89.9% for breast cancer and 19.4% for lung cancer (3,4). For melanoma, a 91.8% 5-year relative survival rate appears satisfactory; however, the 5-year relative survival for patients with tumor stage IV is only 3% (22,25).

The JAK-STAT signaling pathway serves a crucial role in functions such as cell proliferation, differentiation, migration and apoptosis, cell survival in hematopoiesis, immune cell development, stem cell maintenance and organismal growth processes (26-28). Dysfunction in the JAK-STAT signaling pathway is associated with diseases such as cancer and immune disorders (6,27). In the JAK-STAT signaling pathway, the *JAK* family comprises *JAK1*, *JAK2*, *JAK3* and *TYK2*, and the *STAT*

Table II. Prognostic survival analysis based on high or low expression of JAK and STAT family genes

Gene	Patients (n=458)	No. of events (%)	MST (days)	Crude HR (95% CI)	Crude P-value	Adjusted HR[b] (95% CI)	Adjusted P-value[b]
JAK1							
Low	229	99 (43.2)	2,588	Ref. 1.056 (0.808-1.379)	0.690	Ref. 0.950 (0.720-1.253)	0.716
High	229	120 (52.4)	2,365				
STAT1							
Low	229	119 (52.0)	1,910	Ref 0.587(0.449-0.767)	<0.001[a]	Ref. 0.595 (0.455-0.778)	<0.001[a]
High	229	100 (47.3)	3,259				
STAT3							
High	229	113 (49.3)	2,030	Ref. 0.701 (0.537-0.915)	0.009[a]	Ref. 0.725 (0.555-0.947)	0.018[a]
Low	229	106 (46.3)	3,080				
STAT4							
Low	229	121 (52.8)	1,785	Ref. 0.562 (0.430-0.735)	<0.001[a]	Ref. 0.590 (0.450-0.773)	<0.001[a]
High	229	98 (42.8)	3,176				
STAT5B							
Low	229	117 (48.1)	2,022	Ref. 0.675 (0.516-0.884)	0.004[a]	Ref. 0.690 (0.526-0.940)	0.007[a]
High	229	102 (47.4)	3,139				
STAT6							
High	229	115 (50.4)	2,184	Ref. 0.731 (0.559-0.956)	0.022[a]	Ref. 0.737 (0.563-0.964)	0.026[a]
Low	229	104 (47.2)	3,139				

[a]P<0.05. [b]Adjusted for age and tumor stage. *JAK1*, Janus kinase 1; *STAT*, signal transducer and activator of transcription; MST, median survival time; HR, hazard ratio; CI, confidence interval; Ref., reference.

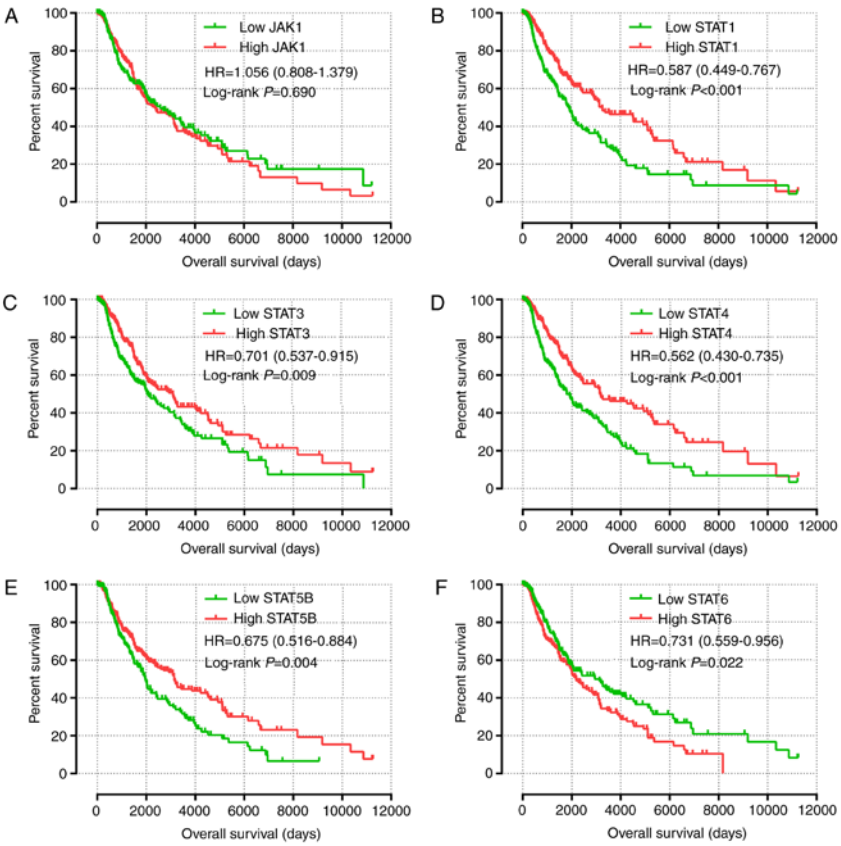

Figure 4. Prognostic value of the expression of key genes of the JAK-STAT signaling pathway for overall survival. (A-F) Kaplan-Meier survival plots for all patients with skin cutaneous melanoma according to (A) *JAK1*, (B) *STAT1*, (C) *STAT3*, (D) *STAT4*, (E) *STAT5B* and (F) *STAT6* expression. *JAK*, Janus kinase; *STAT*, signal transducer and activator of transcription.

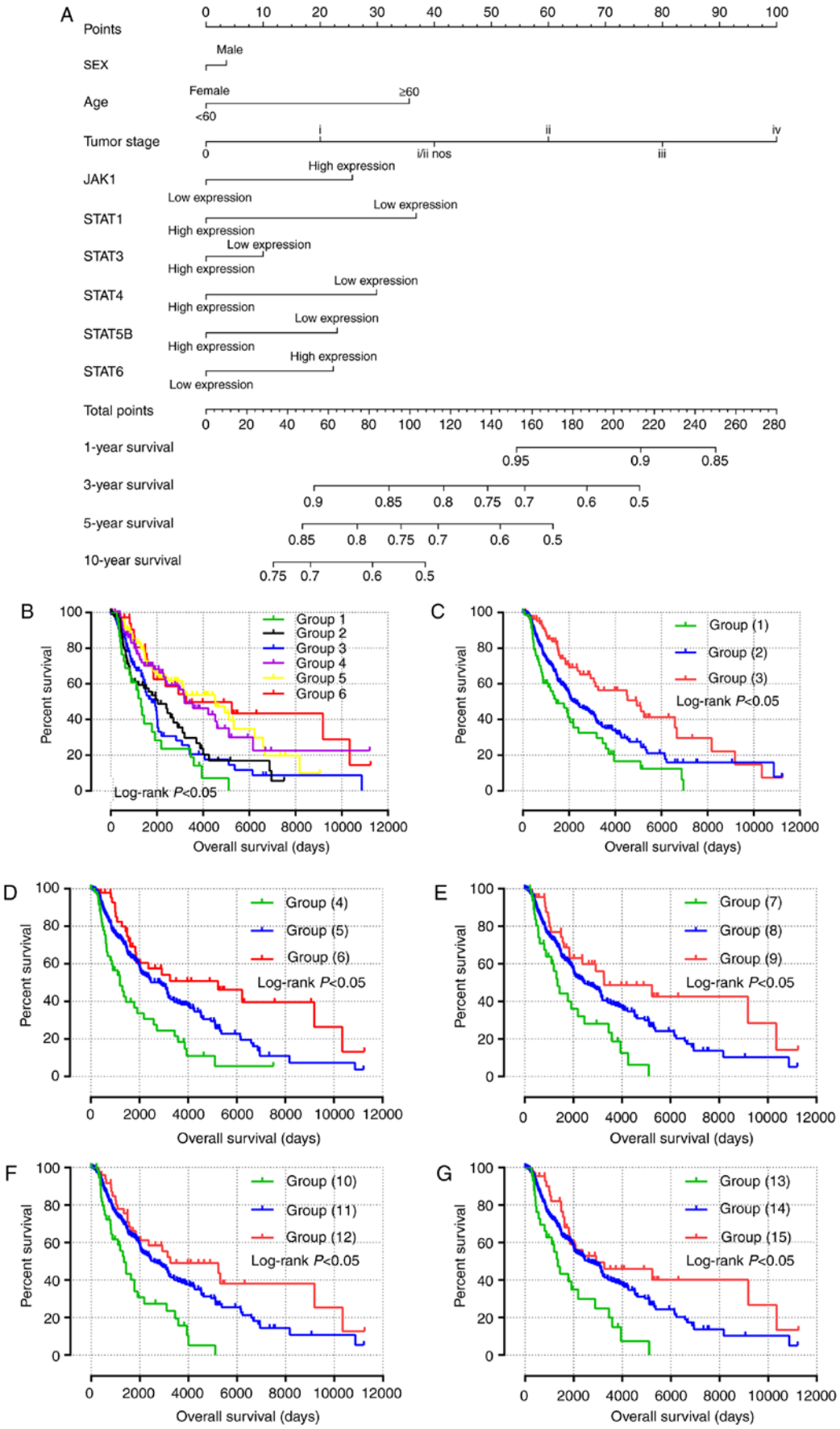

Figure 5. Nomogram and joint-effects survival analysis of JAK and STAT genes. (A) Nomogram for predicting the 1-, 3- and 5-year event (death) based on risk scores and clinical information. (B) Joint effects analysis of the influence of combined gene expression on overall survival in patients with skin cutaneous carcinoma. (C-G) Joint effects analysis of the influence of combined expression of four selected genes on overall survival in patients with skin cutaneous melanoma according to (C) group (1)-(3), (D) group (4)-(6), (E) group (7)-(9), (F) group (10)-(12) and (G) group (13)-(15). *JAK1*, Janus kinase 1; *STAT*, signal transducer and activator of transcription. NOS, non-specific.

Table III. Grouping information for joint effects analysis of all *STAT* genes.

	Composition				
Group	*STAT1*	*STAT3*	*STAT4*	*STAT5B*	*STAT6*
1	Low	Low	Low	Low	High
2	**High**	Low	Low	Low	High
	Low	**High**	Low	Low	High
	Low	Low	**High**	Low	High
	Low	Low	Low	**High**	High
	Low	Low	Low	Low	**Low**
3	**High**	**High**	Low	Low	High
	High	Low	**High**	Low	High
	High	Low	Low	**High**	High
	High	Low	Low	Low	**Low**
	Low	**High**	**High**	Low	High
	Low	**High**	Low	**High**	High
	Low	**High**	Low	Low	**Low**
	Low	Low	**High**	**High**	High
	Low	Low	**High**	Low	**Low**
	Low	Low	Low	**High**	**Low**
4	**High**	**High**	**High**	Low	High
	High	**High**	Low	**High**	High
	High	Low	**High**	**High**	High
	Low	**High**	**High**	**High**	High
	High	**High**	Low	Low	**Low**
	High	Low	**High**	Low	**Low**
	Low	**High**	**High**	Low	**Low**
	High	Low	Low	**High**	**Low**
	Low	**High**	Low	**High**	**Low**
	Low	Low	**High**	**High**	**Low**
5	**High**	**High**	**High**	**High**	High
	High	**High**	**High**	Low	**Low**
	High	**High**	Low	**High**	**Low**
	High	Low	**High**	**High**	**Low**
	Low	**High**	**High**	**High**	**Low**
6	**High**	**High**	**High**	**High**	**Low**

Bold, favorable prognosis groups in univariate survival analysis. *STAT*, signal transducer and activator of transcription.

Table IV. Grouping information for joint effects analysis of four selected *STAT* genes.

	Composition				
Group	*STAT1*	*STAT3*	*STAT4*	*STAT5B*	*STAT6*
(1)	Low	Low	Low	Low	-
(2)	**High**	Low	Low	Low	-
	Low	**High**	Low	Low	-
	Low	Low	**High**	Low	-
	Low	Low	Low	**High**	-
	High	**High**	Low	Low	-
	High	Low	**High**	Low	-
	High	Low	Low	**High**	-
	Low	**High**	**High**	Low	-
	Low	**High**	Low	**High**	-
	Low	Low	**High**	**High**	-
	High	**High**	**High**	Low	-
	High	**High**	Low	**High**	-
	High	Low	**High**	**High**	-
	Low	**High**	**High**	**High**	-
(3)	**High**	**High**	**High**	**High**	-
(4)	Low	Low	Low	-	High
(5)	**High**	Low	Low	-	High
	Low	**High**	Low	-	High
	Low	Low	**High**	-	High
	Low	Low	Low	-	**Low**
	High	**High**	Low	-	High
	High	Low	**High**	-	High
	High	Low	Low	-	**Low**
	Low	**High**	**High**	-	High
	Low	**High**	Low	-	**Low**
	Low	Low	**High**	-	**Low**
	High	**High**	**High**	-	High
	High	**High**	Low	-	**Low**
	High	Low	**High**	-	**Low**
	Low	**High**	**High**	-	**Low**
(6)	**High**	**High**	**High**	-	**Low**
(7)	Low	Low	-	Low	High
(8)	**High**	Low	-	Low	High
	Low	**High**	-	Low	High
	Low	Low	-	**High**	High
	Low	Low	-	Low	**Low**
	High	**High**	-	Low	High
	High	Low	-	**High**	High
	High	Low	-	Low	**Low**
	Low	**High**	-	**High**	High
	Low	**High**	-	Low	**Low**
	Low	Low	-	**High**	**Low**
	High	**High**	-	**High**	High
	High	**High**	-	Low	**Low**
	High	Low	-	**High**	**Low**
	Low	**High**	-	**High**	**Low**
(9)	**High**	**High**	-	**High**	**Low**
(10)	Low	-	Low	Low	High
(11)	**High**	-	Low	Low	High

family comprises *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5A*, *STAT5B* and *STAT6* (6,29). A number of diseases are associated with the expression levels of genes of the JAK-STAT signaling pathway. A previous study has demonstrated that increased expression of *STAT3* or *STAT1*, but not of both, was present in melanoma compared with that in benign nevi or skin melanocytes (30). In addition, active *STAT3* was present in melanoma (30). By contrast, overexpression of microRNA (miR)-214 in a lung cancer cell line reduced the expression of *JAK1*, which inhibited cell proliferation and colony formation, and suppressed cell migration and invasion (31). In papillary thyroid cancer, downregulation of *JAK1* by miR-520a-3p inactivated the JAK-STAT signaling pathway (32).

Table IV. Continued.

Group	Composition				
	STAT1	*STAT3*	*STAT4*	*STAT5B*	*STAT6*
	Low	-	**High**	Low	High
	Low	-	Low	**High**	High
	Low	-	Low	Low	**Low**
	High	-	**High**	Low	High
	High	-	Low	**High**	High
	High	-	Low	Low	**Low**
	Low	-	**High**	**High**	High
	Low	-	**High**	Low	**Low**
	Low	-	Low	**High**	**Low**
	High	-	**High**	**High**	High
	High	-	**High**	Low	**Low**
	High	-	Low	**High**	**Low**
	Low	-	**High**	**High**	**Low**
(12)	**High**	-	**High**	**High**	**Low**
(13)	-	Low	Low	Low	High
(14)	-	**High**	Low	Low	High
	-	Low	**High**	Low	High
	-	Low	Low	**High**	High
	-	Low	Low	Low	**Low**
	-	**High**	**High**	Low	High
	-	**High**	Low	**High**	High
	-	**High**	Low	Low	**Low**
	-	Low	**High**	**High**	High
	-	Low	**High**	Low	**Low**
	-	Low	Low	**High**	**Low**
	-	**High**	**High**	**High**	High
	-	**High**	**High**	Low	**Low**
	-	**High**	Low	**High**	**Low**
	-	Low	**High**	**High**	**Low**
(15)	-	**High**	**High**	**High**	**Low**

'-', gene not selected. Bold, favorable prognosis groups in univariate survival analysis. *STAT*, signal transducer and activator of transcription.

Table V. Grouping information for joint effects analysis of three selected *STAT* genes.

Group	Composition				
	STAT1	*STAT3*	*STAT4*	*STAT5B*	*STAT6*
i	Low	Low	Low	-	-
ii	**High**	Low	Low	-	-
	Low	High	Low	-	-
	Low	Low	**High**	-	-
	High	**High**	Low	-	-
	High	Low	**High**	-	-
	Low	**High**	**High**	-	-
iii	**High**	**High**	**High**	-	-
iv	Low	Low	-	Low	-
v	Low	Low	-	Low	-
	High	High	-	Low	-
	High	**High**	-	**High**	-
	Low	**High**	-	Low	-
	Low	Low	-	**High**	-
	High	**High**	-	**High**	-
vi	**High**	**High**	-	**High**	-
vii	Low	Low	-	-	High
viii	**High**	Low	-	-	High
	Low	**High**	-	-	High
	Low	Low	-	-	**Low**
	High	**High**	-	-	High
	High	Low	-	-	**Low**
	Low	**High**	-	-	**Low**
ix	**High**	**High**	-	-	**Low**
x	Low	-	Low	Low	-
xi	**High**	-	Low	Low	-
	Low	-	**High**	Low	-
	Low	-	Low	**High**	-
	High	-	**High**	Low	-
	High	-	Low	**High**	-
	Low	-	**High**	**High**	-
xii	**High**	-	**High**	**High**	-
xiii	Low	-	Low	-	High
xiv	**High**	-	Low	-	High
	Low	-	**High**	-	High
	Low	-	Low	-	**Low**
	High	-	**High**	-	High
	High	-	Low	-	**Low**
	Low	-	**High**	-	**Low**
xv	**High**	-	**High**	-	**Low**
xvi	Low	-	-	Low	High
xvii	**High**	-	-	Low	High
	Low	-	-	**High**	High
	Low	-	-	Low	Low
	High	-	-	**High**	High
	High	-	-	Low	**Low**
	Low	-	-	**High**	**Low**
xviii	**High**	-	-	**High**	**Low**
xiv	-	Low	Low	Low	-
xx	-	**High**	Low	Low	-

However, studies that explored the association between the prognosis of melanoma and the JAK-STAT signaling pathway are limited. In the present study, the expression of *JAK* and *STAT* family genes in melanoma was investigated based on TCGA data. The observation that JAK-STAT gene expression is associated with the MAPK signaling pathway is in agreement with a previous study, which demonstrated that the JAK-STAT signaling pathway is integrated with the MAPK signaling pathway (33-35) and is associated with melanoma (36). Expression of *JAK2*, *JAK3*, *TYK2*, *STAT2* and *STAT5B* was not observed after normalizing mRNA expression in the present study, suggesting that these genes were expressed at a low level. High expression of *STAT1*, *STAT3*, *STAT4* and *STAT5B*, as well as low expression of *STAT6*, were associated with a favorable prognosis of patients with SKCM. These results are consistent with other studies, in which high

Table V. Continued.

	Composition				
Group	*STAT1*	*STAT3*	*STAT4*	*STAT5B*	*STAT6*
	-	Low	**High**	**High**	-
	-	Low	Low	Low	-
	-	**High**	**High**	**High**	-
	-	**High**	Low	Low	-
	-	Low	**High**	**High**	-
xxi	-	**High**	**High**	**High**	-
xxii	-	Low	Low	-	High
xxiii	-	**High**	Low	-	High
	-	Low	**High**	-	High
	-	Low	Low	-	**Low**
	-	**High**	**High**	-	High
	-	**High**	Low	-	**Low**
	-	Low	**High**	-	**Low**
xxiv	-	**High**	**High**	-	**Low**
xxv	-	Low	-	Low	High
xxvi	-	**High**	-	**High**	High
	-	Low	-	Low	High
	-	Low	-	Low	Low
	-	**High**	-	**High**	High
	-	**High**	-	**High**	**Low**
	-	Low	-	Low	**Low**
xxvii	-	**High**	-	**High**	**Low**
xxviii	-	-	Low	Low	High
xxix	-	-	**High**	**High**	High
	-	-	Low	Low	High
	-	-	Low	Low	**Low**
	-	-	**High**	**High**	High
	-	-	**High**	**High**	**Low**
	-	-	Low	Low	**Low**
xxx	-	-	**High**	**High**	**Low**

'-', gene not selected. Bold, favorable prognosis groups in univariate survival analysis. STAT, signal transducer and activator of transcription.

Table VI. Grouping information for joint effects analysis of two selected *STAT* genes

	Composition				
Group	*STAT1*	*STAT3*	*STAT4*	*STAT5B*	*STAT6*
I	Low	Low	-	-	-
II	**High**	Low	-	-	-
	Low	**High**	-	-	-
III	**High**	**High**	-	-	-
IV	Low	-	Low	-	-
V	**High**	-	Low	-	-
	Low	-	**High**	-	-
VI	**High**	-	**High**	-	-
VII	Low	-	-	Low	-
VIII	Low	-	-	**High**	-
	High	-	-	Low	-
IX	**High**	-	-	**High**	-
X	Low	-	-	-	High
XI	Low	-	-	-	**Low**
	High	-	-	-	High
XII	**High**	-	-	-	**Low**
XIII	-	Low	Low	-	-
XIV	-	**High**	Low	-	-
	-	Low	**High**	-	-
XV	-	**High**	**High**	-	-
XVI	-	Low	-	Low	-
XVII	-	Low	-	High	-
	-	**High**	-	Low	-
XVIII	-	**High**	-	**High**	-
XIX	-	Low	-	-	High
XX	-	Low	-	-	**Low**
	-	**High**	-	-	High
XXI	-	**High**	-	-	**Low**
XXII	-	-	Low	Low	-
XXIII	-	-	Low	**High**	-
	-	-	**High**	Low	-
XXIV	-	-	**High**	**High**	-
XXV	-	-	Low	-	High
XXVI	-	-	Low	-	**Low**
	-	-	**High**	-	High
XXVII	-	-	**High**	-	**Low**
XXVIII	-	-	-	Low	High
XXIV	-	-	-	Low	**Low**
	-	-	-	**High**	High
XXX	-	-	-	**High**	**Low**

'-', gene not selected. Bold, favorable prognosis groups in univariate survival analysis. STAT, signal transducer and activator of transcription.

expression of *STAT1* was associated with favorable prognosis in high-grade serous ovarian cancer (HGSC) (37), colorectal cancer (38) and esophageal squamous cell carcinoma (39). In addition, high expression of *STAT1* in HGSC was significantly associated with the recruitment of intraepithelial $CD8^+$ T cells, which enhanced the prognostic and predictive value of intratumoral $CD8^+$ T cells in HGSC (40), potentially due to tumors with high *STAT1* mRNA expression exhibiting elevated expression of genes specific for tumor-associated macrophages and immunosuppressive T lymphocytes (7). The results of the enrichment analysis in the present study also revealed that *STAT1* was associated with the immune response, which suggested that *STAT1* may accelerate the immune cell response to cancer (41). However, high *STAT1* expression was associated with poor prognosis in glioblastoma (42), and breast (7,8), ovarian, lung, blood and brain (9) cancer.

In contrast to that of *STAT1*, *STAT3* expression is downregulated in malignant pleural mesothelioma (43); however, the prognostic value of this association has not been reported. The majority of studies on *STAT3* and cancer prognosis have

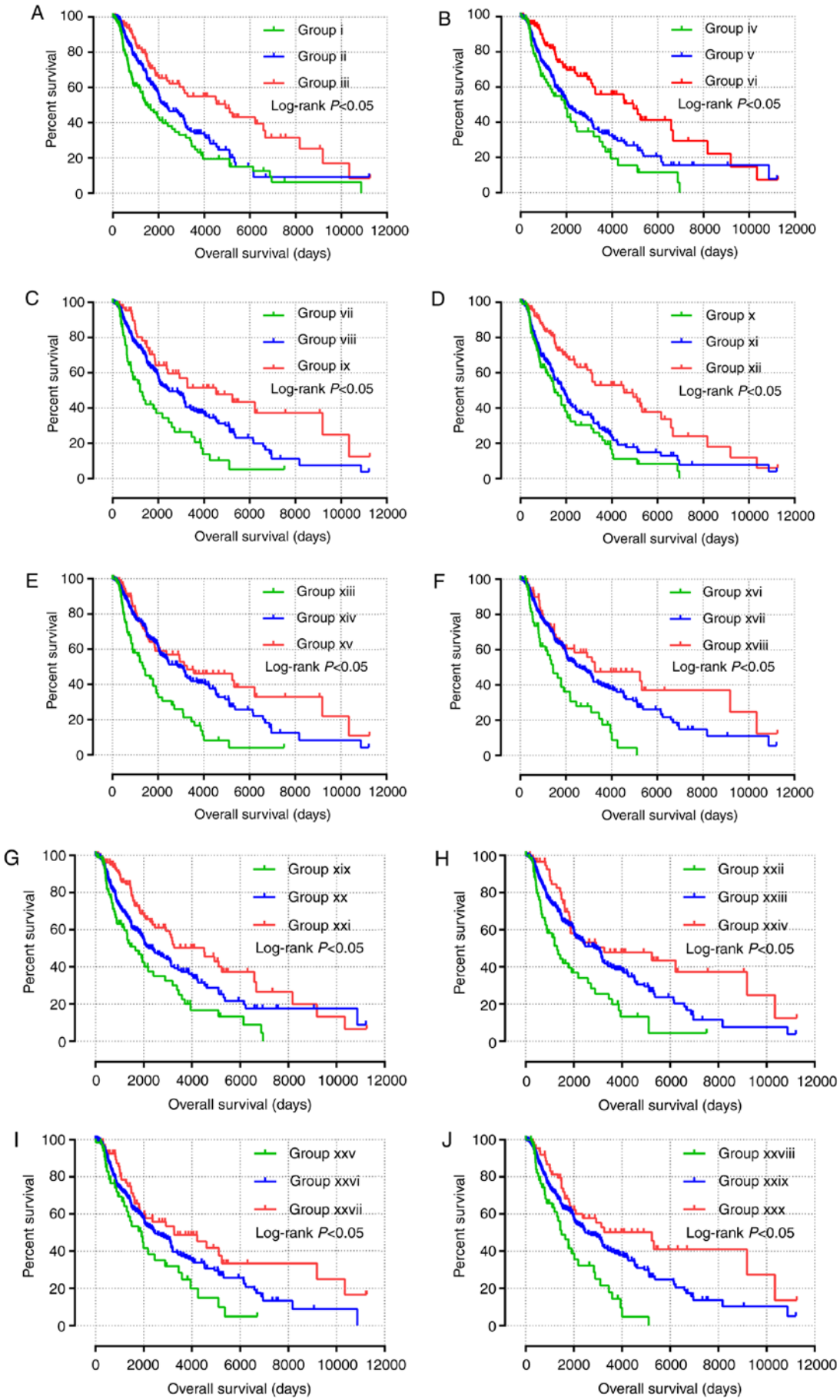

Figure 6. (A-J) Joint effects analysis of the effects of combined expression of three selected genes on overall survival in patients with skin cutaneous melanoma according to (A) group i-iii, (B) group iv-vi, (C) group vi-ix, (D) group x-xii, (E) group xiii-xv, (F) group xvi-xvii, (G) group xix-xxi, (H) group xxii-xxiv, (I) group xxv-xxvii and (J) group xxviii-xxx.

Table VII. Joint effects analysis of the prognostic value of combinations of gene expression in skin cutaneous melanoma.

Group	No. of genes	Patients (n=458)	No. of events (%)	MST (days)	Log-rank P-value	HR (95% CI)
1	5	38	24 (63.1)	1,301	Ref.	Ref.
2	5	87	46 (52.9)	2,030	0.073	0.636 (0.487-1.043)
3	5	106	53 (50.0)	1,766	0.094	0.661 (0.407-1.073)
4	5	98	37 (37.8)	3,196	<0.001[a]	0.352 (0.210-0.590)
5	5	93	42 (45.2)	4,533	<0.001[a]	0.322 (0.194-0.535)
6	5	36	17 (47.2)	3,259	<0.001[a]	0.280 (0.149-0.528)
(1)	4	71	42 (59.2)	1,441	Ref.	Ref.
(2)	4	301	140 (46.4)	2,192	0.014[a]	0.648 (0.458-0.915)
(3)	4	86	37 (43.0)	4,930	<0.001[a]	0.376 (0.241-0588)
(4)	4	61	37 (60.7)	1,197	Ref.	Ref.
(5)	4	351	160 (45.6)	2,829	<0.001[a]	0.515 (0.360-0.738)
(6)	4	46	22 (47.8)	5,237	<0.001[a]	0.312 (0.182-0.535)
(7)	4	47	27 (57.4)	1,354	Ref.	Ref.
(8)	4	365	171 (46.8)	2,470	0.002[a]	0.530 (0.352-0.798)
(9)	4	46	21 (45.7)	3,266	<0.001[a]	0.370 (0.189-0.601)
(10)	4	56	34 (60.7)	1,354	Ref.	Ref.
(11)	4	351	160 (45.6)	2,588	<0.001[a]	0.484 (0.333-0.704)
(12)	4	51	25 (49.0)	3,259	<0.001[a]	0.338 (0.200-0.573)
(13)	4	42	26 (61.9)	1,354	Ref.	Ref.
(14)	4	372	171 (46.0)	2,588	0.001[a]	0.498 (0.328-0.755)
(15)	4	44	22 (50.0)	2,927	<0.001[a]	0.344 (0.193-0.612)
i	3	130	75 (57.7)	1,441	Ref.	Ref.
ii	3	250	112 (44.8)	2,273	0.022[a]	0.710 (0.529-0.952)
iii	3	103	46 (44.7)	4,930	<0.001[a]	0.418 (0.289-0.605)
iv	3	91	47 (51.6)	1,949	Ref.	Ref.
v	3	267	131 (49.1)	2,071	0.086	0.746 (0.534-1.042)
vi	3	125	51 (40.8)	4,930	<0.001[a]	0.415 (0.278-0.620)
vii	3	78	44 (56.4)	1,197	Ref.	Ref.
viii	3	312	146 (46.8)	2,470	<0.001[a]	0.530 (0.377-0.744)
ix	3	93	39 (41.9)	4,526	<0.001[a]	0.328 (0.212-0.508)
x	3	103	61 (59.2)	1,487	Ref.	Ref.
xi	3	245	127 (51.8)	1,864	0.127	0.788 (0.580-1.070)
xii	3	135	60 (44.4)	4,533	<0.001[a]	0.388 (0.270-0.557)
xiii	3	95	55 (57.9)	1,354	Ref.	Ref.
xiv	3	276	152 (551)	2,889	<0.001[a]	0.480 (0.348-0.661)
xv	3	112	50 (44.6)	3,259	<0.001[a]	0.367 (0.249-0.542)
xvi	3	69	40 (58.0)	1,429	Ref.	Ref.
xvii	3	324	148 (45.7)	2,588	<0.001[a]	0.502 (0.352-0.715)
xviii	3	90	40 (44.4)	3,266	<0.001[a]	0.358 (0.229-0.559)
xix	3	87	51 (58.6)	1,655	Ref.	Ref.
xx	3	268	121 (45.1)	2,367	0.012[a]	0.658 (0.474-0.913)
xxi	3	128	57 (44.5)	4,507	<0.001[a]	0.419 (0.286-0.613)
xxii	3	70	43 (61.4)	1,301	Ref.	Ref.
xxiii	3	328	148 (45.1)	2,948	<0.001[a]	0.530 (0.377-0.745)
xxiv	3	85	38 (44.7)	3,259	<0.001[a]	0.348 (0.223-0.542)
xxv	3	66	35 (53.0)	1,910	Ref.	Ref.
xxvi	3	323	150 (46.4)	2,470	0.043[a]	0.683 (0.472-0.988)
xxvii	3	94	44 (46.8)	3,266	0.001[a]	0.462 (0.294-0.724)
xxviii	3	70	40 (57.1)	1,486	Ref.	Ref.
xxix	3	323	149 (46.1)	2,470	0.001[a]	0.536 (0.376-0.764)
xxx	3	90	40 (44.4)	5,237	<0.001[a]	0.349 (0.223-0.545)

Table VII. Continued.

Group	No. of genes	Patients (n=458)	No. of events (%)	MST (days)	Log-rank P-value	HR (95% CI)
I	2	139	70 (50.4)	1,949	Ref.	Ref.
II	2	180	92 (51.1)	2,005	0.519	0.902 (0.660-1.233)
III	2	164	67 (40.9)	4,526	<0.001[a]	0.485 (0.346-0.678)
IV	2	168	96 (57.1)	1,487	Ref.	Ref.
V	2	122	48 (39.3)	2,454	0.017[a]	0.655 (0.462-0.928)
VI	2	193	85 (44.0)	3,564	<0.001[a]	0.477 (0.356-0.641)
VII	2	133	70 (52.6)	1,780	Ref.	Ref.
VIII	2	192	90 (46.9)	2,273	0.008[a]	0.654 (0.477-0.897)
IX	2	133	59 (44.4)	4,507	<0.001[a]	0.449 (0.316-0.638)
X	2	125	70 (56.0)	1,438	Ref.	Ref.
XI	2	208	94 (45.2)	3,080	<0.001[a]	0.483 (0.353-0.662)
XII	2	125	55 (44.0)	3,259	<0.001[a]	0.435 (0.304-0.623)
XIII	2	130	75 (57.7)	1,441	Ref.	Ref.
XIV	2	198	84 (42.4)	2,545	0.011[a]	0.677 (0.488-0.912)
XV	2	130	60 (46.2)	3,259	<0.001[a]	0.466 (0.332-0.656)
XVI	2	148	72 (48.6)	1,992	Ref.	Ref.
XVII	2	162	80 (49.4)	2,071	0.297	0.843 (0.612-1.162)
XVIII	2	148	67 (45.3)	3,259	0.001[a]	0.564 (0.403-0.791)
XIX	2	111	61 (54.9)	1,910	Ref.	Ref.
XX	2	236	106 (44.9)	3,080	0.008[a]	0.652 (0.476-0.894)
XXI	2	111	52 (46.8)	3,259	<0.001[a]	0.508 (0.349-0.739)
XXII	2	141	77 (54.6)	1,780	Ref.	Ref.
XXIII	2	176	78 (44.3)	2,192	0.015[a]	0.674 (0.490-0.926)
XXIV	2	141	64 (45.4)	3,259	<0.001[a]	0.466 (0.333-0.653)
XXV	2	118	65 (55.1)	1,544	Ref.	Ref.
XXVI	2	222	106 (47.4)	3,136	<0.001[a]	0.548 (0.400-0.750)
XXVII	2	118	48 (40.7)	3,259	<0.001[a]	0.399 (0.273-0.583)
XXVIII	2	105	56 (53.3)	1,780	Ref.	Ref.
XXIX	2	248	114 (46.0)	2,470	0.013[a]	0.667 (0.484-0.920)
XXX	2	105	49 (46.7)	3,424	<0.001[a]	0.466 (0.314-0.692)

[a]P<0.05. MST, median survival time; HR, hazard ratio; CI, confidence interval; Ref., reference.

demonstrated that phosphorylated *STAT3* is associated with a poor outcome in colorectal cancer (10), multiple myeloma (44) and urothelial carcinoma (45). In addition, *STAT4* has been detected in several types of cancer, including prostate (46), breast (47), gastric (48) and ovarian (49) cancer. The upregulation of *STAT4* in hepatocellular carcinoma is associated with favorable prognosis, possibly due to the expression of *STAT4* in the immune cells; however, the function and mechanism of *STAT4* in non-immune cells remains unknown (50). High expression of *STAT5B* in astrocytoma is associated with poor prognosis (51), whereas high expression of *STAT6* is associated with poor prognosis in colon cancer (52).

The potential effects of these associations require further exploration. For instance, colorectal carcinoma cell lines exhibiting low *STAT1* and high *STAT3* expression levels are associated with enhanced tumor growth in xenografts; by contrast, xenograft cell lines with high *STAT1* and low *STAT3* levels grew slowly (53). Thus, gene interactions may influence the cancer outcome. The different expression level of these two genes in different types of cancer may serve different roles. Joint effects analysis in the present study suggested that any combination of the tested markers may have a higher prognostic value compared with that of any individual biomarker.

The STAT gene family affects cell differentiation (54), invasion (55-57), adhesion (58) and migration (59), as well as cell cycle (60-62) and colony formation (39,63) through the JAK-STAT signaling pathway; these processes are associated with the occurrence, development and outcome of cancer. The potential mechanism of how these genes affect prognosis should be further studied.

The present study investigated the prognostic value of the key genes in the JAK-STAT signaling pathway; only STAT genes were demonstrated to affect the prognosis of SKCM. In melanoma research, no golden standard exists for diagnosis or prognosis that would serve a similar role as the hairy-related 2 gene in breast cancer or prostate specific antigen in prostate

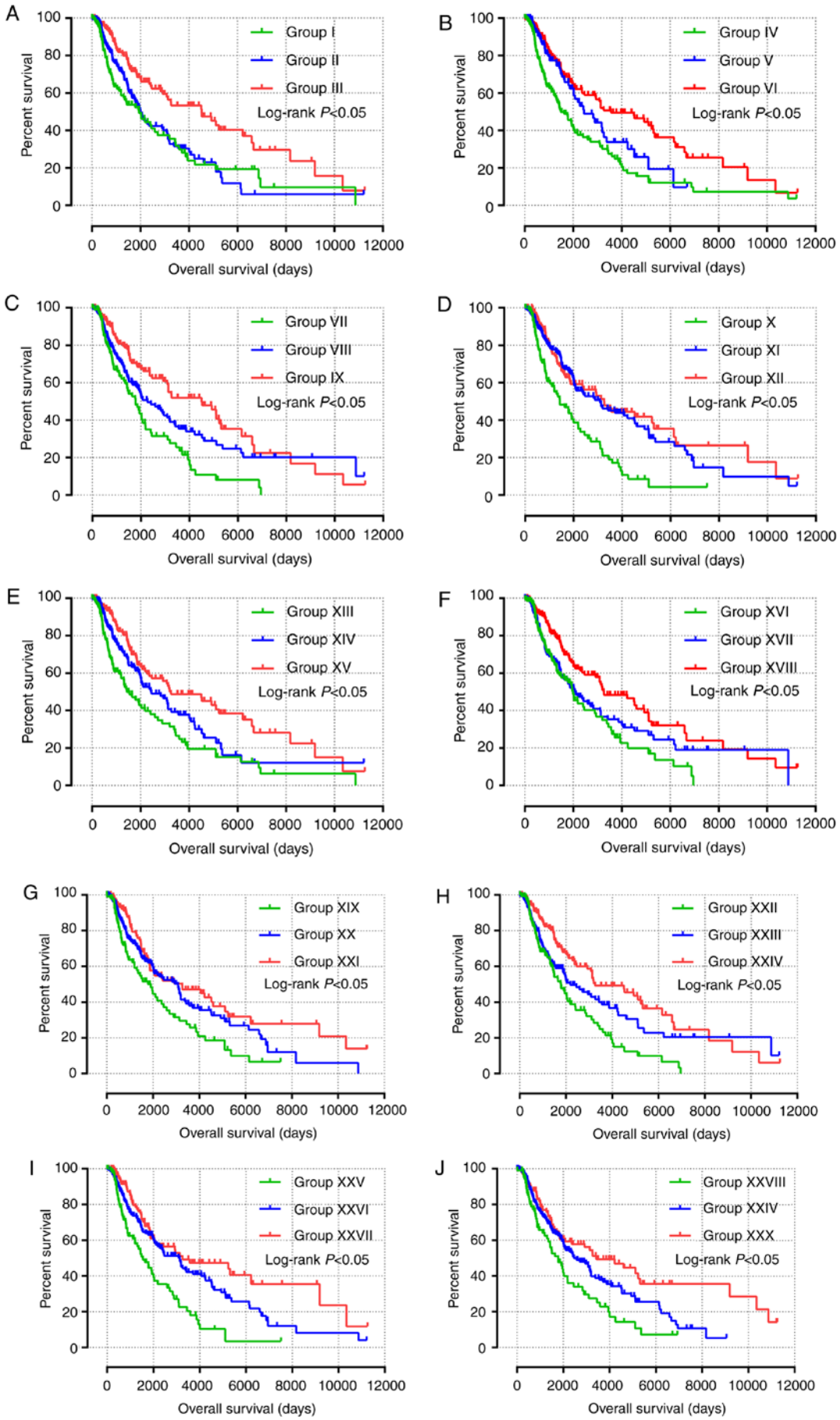

Figure 7. (A-J) Joint effects analysis of the influence of combined expression of two selected genes on overall survival in patients with skin cutaneous melanoma according to (A) group I-III, (B) group IV-VI, (C) group VI-IX, (D) group X-XII, (E) group XIII-XV, (F) group XVI-XVII, (G) group XIX-XXI, (H) group XXII-XXIV, (I) group XXV-XXVII and (J) group XXVIII-XXX.

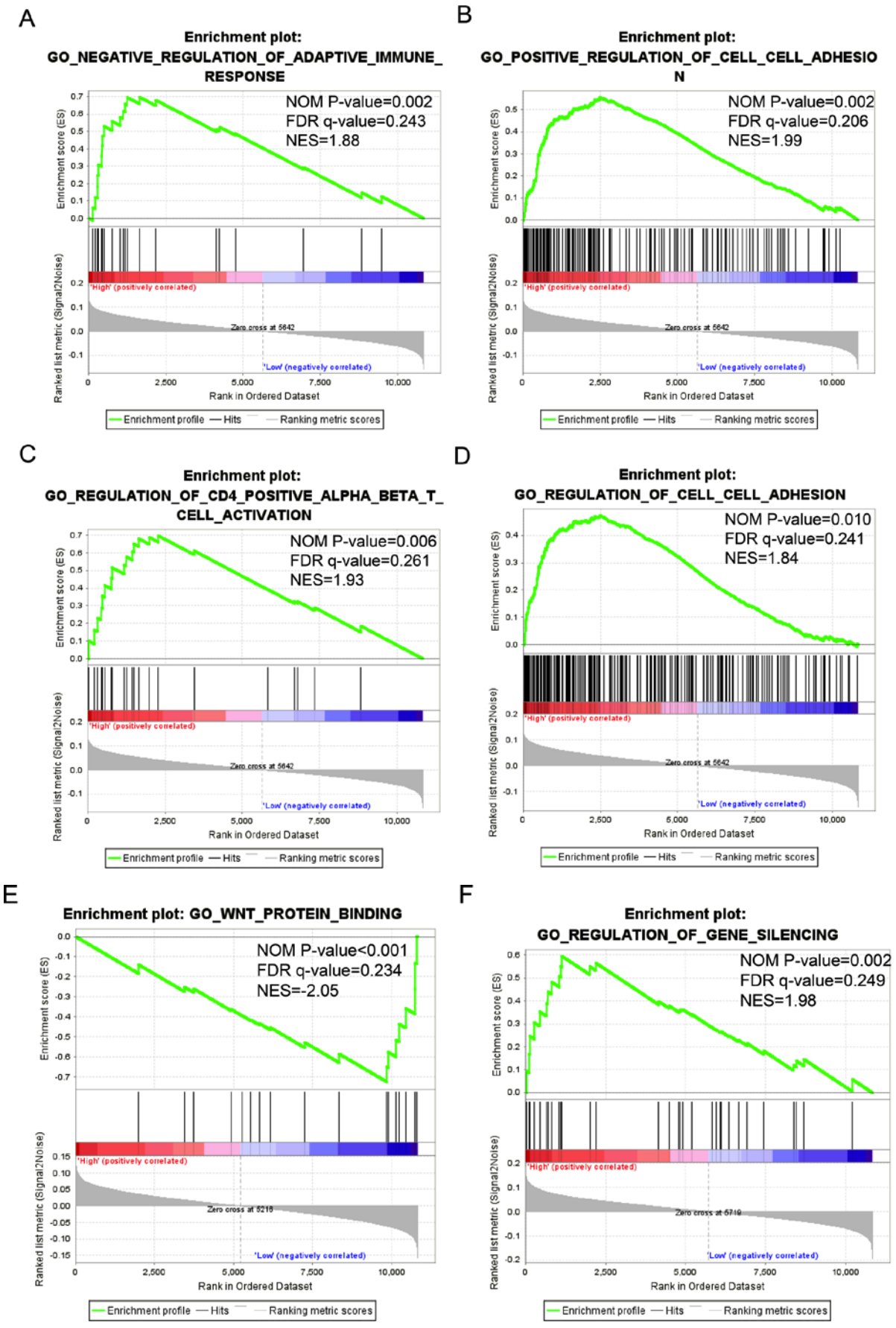

Figure 8. GSEA of genes expressed in patients with skin cutaneous melanoma. (A-D) GSEA of the c5 reference gene sets for the high *STAT1* expression group. (A) c5 item negative regulation of adaptive immune response, (B) c5 item positive regulation of cell-cell adhesion, (C) c5 item regulation of CD4 positive alpha beta T cell activation and (D) c5 item regulation of cell-cell adhesion. (E) GSEA of the c5 reference gene sets for the low *STAT4* expression group. (F) GSEA of the c5 reference gene sets for the high *STAT5B* expression group. *STAT*, signal transducer and activator of transcription; GSEA, gene set enrichment analysis; NES, normalized enrichment score; FDR, false discovery rate; NOM, nominal.

cancer. Certain genes may act as biomarkers to predict the prognosis and mechanism of SKCM, including RAF (Raf proto-oncogene), MEK (MAP/ERK kinase), MAPK, RAS, myelocytomatosis oncogene and S100 calcium binding protein (64-69). However, further research is required to identify a golden standard for predicting melanoma.

There were certain limitations in the present study. Firstly, only *JAK* expression data were reported following normalization; additional mRNA expression data are needed to further confirm these observations. Secondly, this is an association study. Further research is needed to explore the function and mechanism of the genes of the JAK-STAT signaling pathway identified in the present study in patients with SKCM. Another limitation of the current study is the limited sample size. Improved design and larger sample size studies are necessary to validate these results. Finally, there were no expression standards to measure whether the gene expression was high or low.

To the best of our knowledge, the present study is the first to evaluate the association between the expression of genes of the JAK-STAT signaling pathway and OS in patients with SKCM, and to identify the joint effects of prognostic values among the five identified genes. Overall, the results of the present study provided a novel insight into the function of these genes in SKCM clinical outcomes, and may be further utilized in the clinic for predicting the prognosis of SKCM.

In conclusion, the combination of the highly expressed *STAT1*, *STAT3*, *STAT4* and *STAT5B* genes, and the lowly expressed *STAT6* gene is associated with a favorable prognosis in patients with SKCM, and may be used as a novel biomarker for predicting the prognosis of patients with SKCM. The expression of the genes in the *STAT* family may affect the prognosis of SKCM by accelerating the immune response and immune cell activity, and by involving the MAPK signaling pathway. Further studies are required to validate these findings.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the TCGA repository, https://portal.gdc.cancer.gov/.

Authors' contributions

XZ and FP conceived and designed the study. QW, HM and SL processed the data and performed the statistical analysis. LZ, RH, XW and XL wrote and revised the manuscript and helped to perform the analysis and interpretation of data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study did not involve human or animal subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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