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Research Paper

The prognostic value of the proteasome activator subunit gene family in skin cutaneous melanoma

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Abstract

Background: The functional significance of the proteasome activator subunit (*PSME*) gene family in the pathogenesis of skin cutaneous melanoma (SKCM) remains to be elucidated.

Materials and methods: Clinical data for patients with SKCM, including expression levels of *PSME* genes, were extracted from TCGA. GO term and KEGG pathway enrichment analyses were performed. Correlations between the expression levels of *PSME* genes in SKCM were evaluated with the Pearson correlation coefficient. Functional and enrichment analyses were conducted using DAVID. Univariate and multivariate survival analyses adjusted by Cox regression were used to construct a prognostic signature. The mechanisms underlying the association between *PSME* gene expression and overall survival (OS) were explored with gene set enrichment analysis. Joint-effects survival analysis was performed to evaluate the clinical value of the prognostic signature.

Results: The median expression levels of *PSME1*, *PSME2* and *PSME3* were significantly higher in SKCM than in normal skin. *PSME1*, *PSME2*, and *PSME3* were significantly enriched in several biological processes and pathways including cell adhesion, adherens junction organization, regulation of autophagy, cellular protein localization, the cell cycle, apoptosis, and the Wnt and NF-κB pathways. High expression levels of *PSME1* and *PSME2* combined with a low expression level of *PSME3* was associated with favorable OS.

Conclusion: Knowledge of the expression levels of the *PSME* gene family could provide a sensitive strategy for predicting prognosis in SKCM.

Key words: Proteasome activator subunit, melanoma, prognosis, nomogram, overall survival

Introduction

Skin cutaneous melanoma (SKCM) is considered one of the most aggressive and lethal cancers of the skin. In 2012, globally, there were an estimated 232,000 new cases of melanoma and 55,000 melanoma-related deaths.[1] In 2018, in the United States, there will be approximately 91,270 new cases of melanoma and 9,320 melanoma-related deaths.[2] Tumor stage is significantly associated with prognosis in melanoma, whereby early diagnosis and treatment results in favorable overall survival (OS) rates.[3]

Proteasome activator subunit 1 (PSME1), proteasome activator subunit 2 (PSME2), proteasome activator subunit 3 (PSME3) and proteasome activator subunit 4 (PSME4) are members of the proteasome

activator subunit (PSME) gene family. Proteasome activator 28 (PA28) consists of three subunits, PA28α, PA28β and PA28γ, encoded by PSME1, PSME2 and PSME3, respectively. Proteasome activators regulate proteasome function but have also been associated with several cancers and may have prognostic significance. Previous studies showed elevated expression of PSME1 in prostate cancer,[4] elevated expression of PSME2 in gastric cancer,[5] and elevated expression of PSME3 in breast cancer,[6-9] colorectal cancer,[10] and laryngeal carcinoma.[11] In some cancers, overexpression of PSME3 was associated with poor OS.[6, 12] Currently, the functional significance of PSME4 in the pathogenesis of cancer remains to be elucidated.

The objectives of the present study were to 1) identify associations between *PSME* gene expression levels in SKCM and 2) develop a risk score that includes clinical factors and the expression patterns of *PSME* genes to predict prognosis in patients with SKCM. In the present research, we were the first to analysis the prognosis value of PSME gene family in SKCM, made a nomogram model for predicting the prognosis of SKCM patients, and used whole-genome RNA-Seq dataset to explore prospective molecular mechanisms through gene set enrichment analysis (GSEA) approach.

Method and Materials

Data source

Clinical data for patients with SKCM, including gender, age, survival time, mortality, and expression levels of *PSME* genes, were extracted from The Cancer Genome Atlas (TCGA). Boxplots of expression profiles of the *PSME* genes in SKCM and healthy skin were created using Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/, accessed on June 20, 2018).[13] After exclusive the patients, which don't have gene expression data and complete prognostic information including survival status and days, 458 cases were included in ours research.

PSME gene family bioinformatics analysis and correlation analysis

Gene ontology (GO) term enrichment analysis, including molecular function (MF), cellular component (CC), and biological process (BP), as well as the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed. *PSME* gene co-expression networks and/or pathways were predicted with GeneMANIA (http://genemania.org/, accessed June 22, 2018).[14] Correlations between expression levels of *PSME* genes in SKCM were evaluated with the Pearson correlation coefficient. Functional and enrichment analyses were conducted using The Database for Annotation, Visualization, and Integrated Discovery (DAVID) v.6.8 (https://david.ncifcrf.gov/tools.jsp, accessed June 22, 2018).[15, 16]

Survival analysis

Prognosis of patients with SKCM was determined by OS. Correlations between expression levels of *PSME* genes in SKCM and patients' OS were evaluated using the Kaplan-Meier method and the log-rank test as well as Cox proportional hazards regression with adjustment for age and tumor stage; race was excluded as a variable due to small sample size (94% of the included patients were White). *PSME* genes were stratified by high or low expression around the median OS. The prognostic impact of high and low expression levels of each *PSME* gene was assessed.

Prognostic risk score

A prognostic risk score was developed based on the adjusted (age, tumor stage) expression levels of the *PSME1*, *PSME2* and *PSME3* genes in SKCM. Nomograms for predicting 1-, 3-, and 5-year survival were used to evaluate the association between the prognostic risk score and OS in patients with SKCM and its potential clinical application;[17] a high score was associated with poor prognosis.

Gene set enrichment analysis (GSEA)

The mechanisms underlying the association between *PSME* gene expression in SKCM and patients' OS were explored with GSEA. Pathway-based analysis in SKCM with high and low expression levels of each *PSME* gene was conducted using comparisons with the reference c5 (GO gene sets: c5.all.v6.1.symbols.gmt) and c2 (KEGG gene sets: c2.all.v6.1.symbols.gmt) gene sets from the Molecular Signatures Database (MSigDB) [18] using GSEA v.3.0 (http://software.broadinstitute.org/gsea/msigdb/index.jsp, accessed June 25, 2018). The number of permutations was set at 1,000. $P<0.05$ and a false discovery rate (FDR) <0.25 were considered statistically significant.

Joint-effects survival analysis

Associations between the expression levels of combinations of *PSME* genes in SKCM and patients' OS were assessed with joint-effects survival analysis. *PSME* genes with prognostic value on multivariate survival analysis were grouped as better OS, worse OS, or other. The prognostic value of the expression of combinations of *PSME* genes in each group was evaluated using the Kaplan-Meier method and the log-rank test.

Statistical analyses

Statistical analyses were performed with SPSS v.25.0 software (IBM, Chicago, IL, USA). Vertical scatter plots and survival curves were generated in GraphPad Prism v.7.0 (GraphPad Software, La Jolla, CA, USA) and R 3.5.1 (http://www.R-project.org). OS was calculated with the Kaplan-Meier method and the log-rank test. Multivariate survival analysis was evaluated with hazard ratios (HR), and 95% confidence intervals (CIs) were calculated using Cox proportional hazards regression with adjustment for influential clinical characteristics including age and tumor stage. $P<0.05$ was considered statistically significant.

Results

Patients' clinical characteristics

Demographic and clinical data obtained from TCGA for 458 patients with SKCM are summarized. The associations between demographic and clinical characteristics and OS in patients with SKCM are summarized in **Table 1**. Race, age and tumor stage were significantly associated with median survival time (MST; $P=0.004$, $P=0.001$, and $P=0.001$, respectively).

Table 1. Clinical data for included patients.

Variables	Patients (n=458)	No. of events (%)	MST (days)	HR (95% CI)	Log-rank *P*
Race					**0.004**
White	435	208 (47.8%)	2454	Ref.	
Others	13	8 (61.5%)	636	0.348 (0.171-0.709)	
Gender					0.278
Male	284	146 (51.4%)	2454	Ref.	
Female	174	72 (41.4%)	2030	0854 (0.642-1.136)	
Age (years)					**0.001**
≥60	219	102 (46.6%)	1860	Ref.	
<60	239	116 (48.3%)	3564	0.620 (0.470-2.136)	
Tumor stage					**0.001**
0+I+II+I/II nos	231	108 (46.8%)	3259	Ref.	
III+IV	191	91 (47.6%)	1960	0.600 (0.449-0.802)	
Missing	36				

Abbreviations: *PSME*, proteasome activator subunit; MST, median survival time; HR, hazard ratio; CI, confidence interval.

Boxplots showing the expression profiles of *PSME* genes in SKCM or healthy skin are presented in **Figure 1**. Findings showed that median expression levels of *PSME1*, *PSME2* and *PSME3* were significantly higher in SKCM than in healthy skin.

PSME gene family correlation analysis and bioinformatics analysis

GO term analysis and KEGG pathway enrichment analysis are shown in **Figure 2A.** The PSME gene family was involved in the MAPK cascade, NIF/NF-κB and Wnt signaling pathways and the cell cycle, which are tumor-related processes. The pathway and co-expression prediction among *PSME1*, *PSME2* and *PSME3* is shown in **Figure 2B**. Correlations between the expression levels of individual *PSME* genes in SKCM investigated with Pearson correlation coefficient are shown in **Figure 2C**. There were correlations between the expression levels of all *PSME* genes except for *PSME1* and *PSME3* and *PSME2* and *PSME3*.

Survival analysis

Scatter plots showing the expression levels of *PSME* genes in SKCM, stratified as high expression or low expression, are shown in **Figure 3**. Survival analysis is summarized in **Table 2** and shown in **Figure 4**. On univariate survival analysis, a high expression level of *PSME2* (log-rank $P=0.001$, HR=0.626, 95%CI=0.476-0.822; **Figure 4B**) and low expression level of *PSME3* (log-rank $P=0.001$, HR=0.638, 95%CI=0.488-0.817; **Figure 4C**) in SKCM were associated with better OS. On multivariate survival analysis, a high expression level of *PSME1* (log-rank $P=0.009$ HR=0.685 95%CI=0.516-0.910), high expression level of *PSME2* (log-rank $P=0.001$ HR=0.576 95%CI=0.431-0.769), and low expression level of *PSME3* (log-rank $P=0.002$ HR=0.634 95%CI=0.477-0.842) in SKCM were associated with better OS.

Table 2. Univariate and multivariate survival analyses.

Gene	Patients (n=458)	No. of events (%)	MST (days)	Crude HR (95% CI)	Crude *P*	Adjusted HR* (95% CI)	Adjusted *P**
PSME1					0.072		**0.009**
Low	229	124 (54.1%)	2030	Ref.		Ref.	
High	229	94 (41.0%)	3136	0.781 (0.596-1.023)		0.685 (0.516-0.910)	
PSME2					**0.001**		**0.001**
Low	229	133 (58.1%)	1917	Ref		Ref.	
High	229	85 (37.1%)	3379	0.626(0.476-0.822)		0.576 (0.431-0.769)	
PSME3					**0.001**		**0.002**
High	229	114 (49.8%)	1910	Ref.		Ref.	
Low	229	104 (45.4%)	3564	0.638 (0.488-0.817)		0.634 (0.477-0.842)	
PSME4					0.423		0.410
Low	229	100 (43.7%)	2028	Ref.		Ref.	
High	229	118 (51.5%)	2993	0.896 (0.686-1.172)		0.888(0.669-1.178)	

Notes: *, adjustment for age and tumor stage.
Abbreviations: *PSME*, proteasome activator subunit; MST, median survival time; HR, hazard ratio; CI, confidence interval.

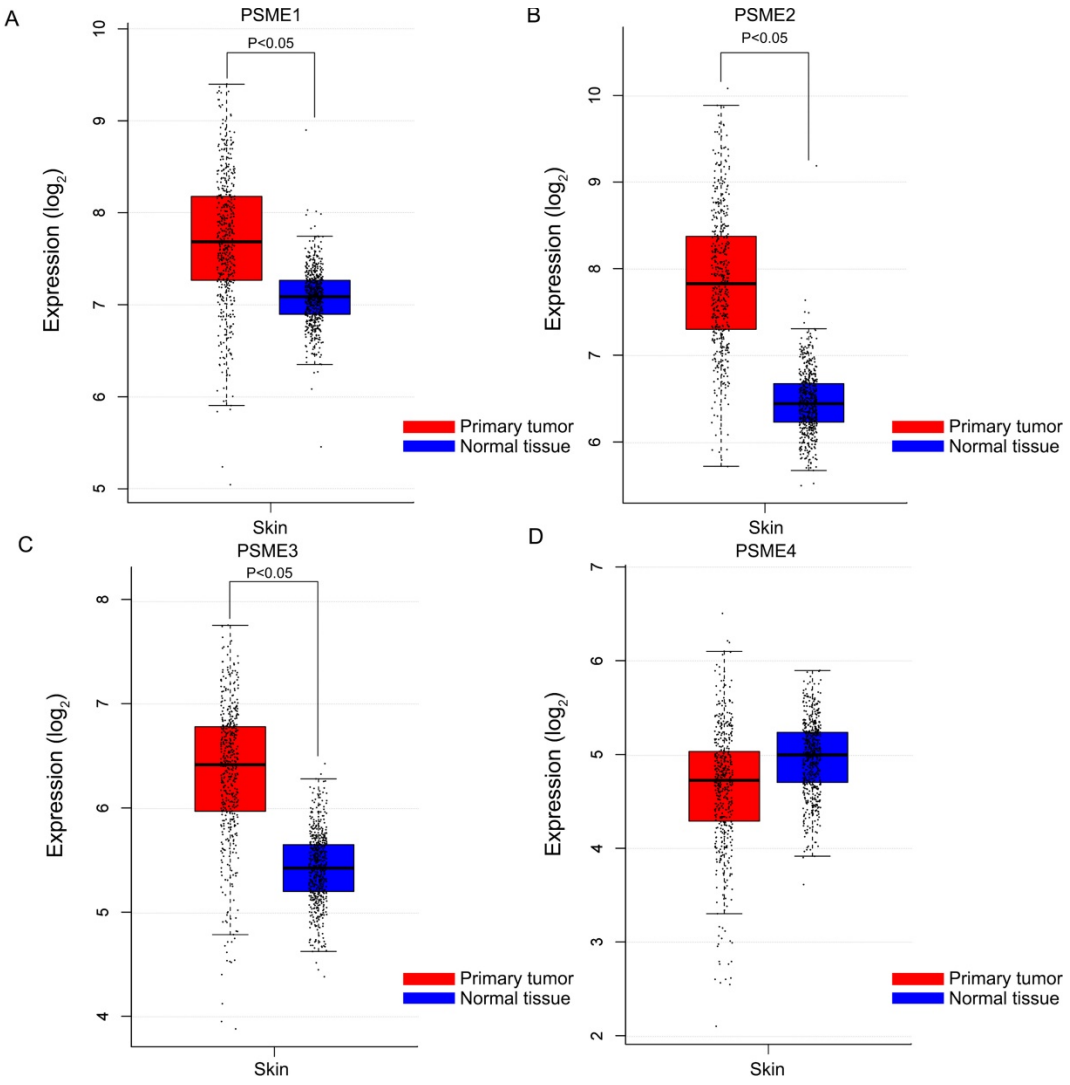

Figure 1. Boxplots showing *PSME* gene expression levels in SKCM and healthy skin. (A) *PSME1*; (B) *PSME2*; (C) *PSME3*; (D) *PSME4*. Abbreviations: *PSME*, proteasome activator subunit; GEPIA, gene expression profiling interactive analysis

Nomogram of SKCM risk score model

A nomogram substantiated that age, tumor stage, and *PSME2* and *PSME3* expression levels in SKCM created a prognostic signature that contributed the most risk (range 0–100 points) for poor OS. Each variable was assigned points based on the Cox regression coefficients. These points were summed, and the probability of survival was estimated by drawing a vertical line between the Total Points axis and the 1-year, 3-year and 5-year survival probability axes **(Figure 4E)**.

GSEA

Pathway-based analysis in SKCM with high and low expression levels of each *PSME* gene is shown in **Figure 5 (A-I)**, **Figure 6 (A-I)**, **Figure 7 (A-I)**, **Figure 8 (A-I)**, **Figure 9 (A-I)** and **Figure 10 (A-I)**. In the GO enrichment analysis, a high expression of *PSME1* was positively correlated with the apoptotic process (**Figure 5A**), cell adhesion (**Figure 5B**), and the NF-κB (**Figure 5C**) and Wnt signaling pathways (**Figure 5E, F**). High expression of *PSME2* was negatively correlated with *the apoptotic* process (**Figure 6B**), cell adhesion (**Figure 6C, F)**, and the NF-κB signaling pathway (**Figure 6D**). High expression of *PSME3* was positively correlated with the NF-κB (**Figure 7C**) and Wnt signaling pathways (**Figure 7E, F**). In the KEGG pathway, high expression of *PSME1* was positively correlated with cell adhesion (**Figure 8A**), apoptosis (**Figure 8 D, E**), the cell cycle (**Figure 8F**), metastasis (**Figure 8I**) and the Wnt and NF-κB signaling pathways (**Figure 8 B, C and G**). High expression of *PSME2* was negatively correlated with cell adhesion (**Figure 9B**), the cell cycle (**Figure 9E**), apoptosis (**Figure 9F**) and the Wnt signaling pathway (**Figure 9G**). High expression of *PSME3* was positively correlated with metastasis (**Figure 10A, D**), the

P53-induced cell cycle (**Figure 10F, G**), the cell cycle (**Figure 10H**), and the Wnt signaling pathway (**Figure 10C, I**). The remaining results were presented in Supplementary Table 1 and 2.

Joint-effects survival analysis

Based on the findings on multivariate survival analysis, a joint-effects survival analysis was performed to determine the combined effects of *PSME1*, *PSME2* and *PSME3* in SKCM on OS in patients grouped as summarized in **Table 3.** Results are summarized in **Table 4** and shown in **Figure 11**. High expression levels of *PSME1* and *PSME2* combined with low expression level of *PSME3* in SKCM in Groups I, IV, VII, and X was associated with better OS (all $P<0.05$). In contrast, low expression levels of *PSME1* and *PSME2* combined with a high expression level of *PSME3* in SKCM in Groups III, VI, IX and XII was associated with poor OS (all $P<0.05$).

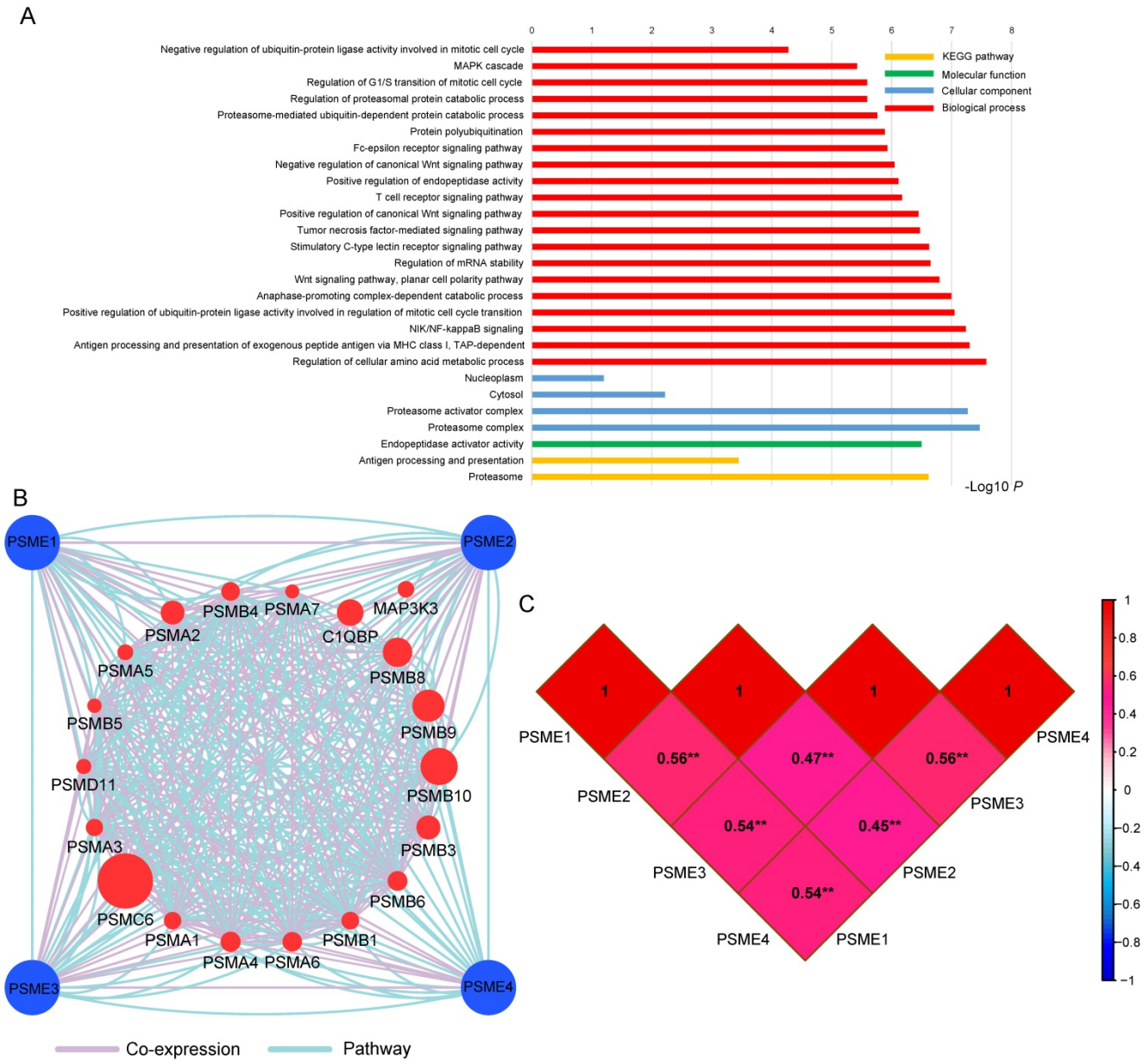

Figure 2. (A) GO enrichment and KEGG pathway analysis by DAVID; (B) Gene interaction networks among selected genes by GeneMANIA; (C) Pearson's correlation coefficients between *PSME1*, *PSME2* and *PSME3* expression levels; and **$P<0.001$. Abbreviations: *PSME*, proteasome activator subunit; TCGA, The Cancer Genome Atlas; GeneMANIA, gene multiple association network integration algorithm; DAVID, the database for annotation, visualization, and integrated discovery; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes.

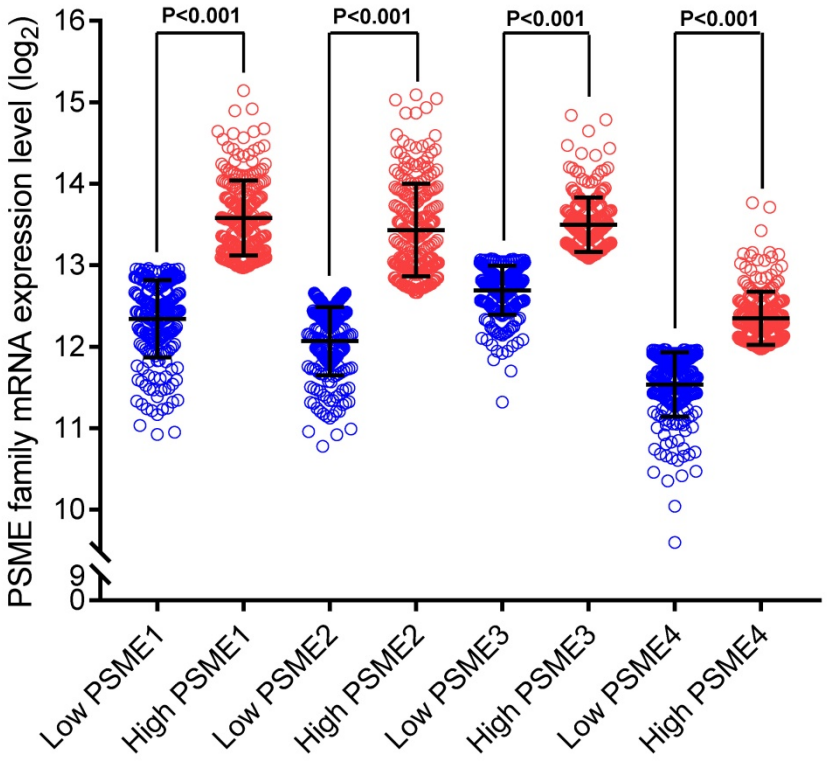

Figure 3. Scatter plots showing *PSME1*, *PSME2* and *PSME3* expression levels in SKCM Abbreviations: *PSME*, proteasome activator subunit; SKCM, skin cutaneous melanoma.

Table 3. Stratifications based on the expression levels of the *PSME1*, *PSME2* and *PSME3* genes.

Group	Composition	Group	Composition
I	high *PSME1*+high *PSME2*	**X**	high *PSME1*+high PSME2+low *PSME3*
II	low *PSME1*+high *PSME2* high *PSME1*+low *PSME2*	**XI**	
III	Low *PSME1*+low *PSME2*		high *PSME1*+low *PSME2*+high *PSME3*
IV	high *PSME1*+ low *PSME3*		low *PSME1*+high *PSME2*+high *PSME3*
V	low *PSME1*+low *PSME3* high *PSME1*+high *PSME3*		low *PSME1*+low *PSME2*+low *PSME3* high *PSME1*+high *PSME2*+high *PSME3*
VI	low *PSME1*+high *PSME3*		high *PSME1*+low *PSME2*+low *PSME3*
VII	high *PSME2*+low *PSME3*		low *PSME1*+high *PSME2*+low *PSME3*
VIII	low *PSME2*+low *PSME3* high *PSME2*+high *PSME3*		
IX	low *PSME2*+high *PSME5*	**XII**	Low *PSME1*+low *PSME2*+high *PSME3*

Abbreviation: *PSME*, proteasome activator subunit.

Table 4. Joint-effects survival analysis.

Group	Patients (n=458)	MST (days)	Crude *P*	Crude HR (95% CI)	Adjusted *P**	Adjusted HR* (95% CI)
I	196	3195	**0.005**	0.655 (0.487-0.881)	**0.004**	0.645 (0.479-0.867)
II	66	3869	**0.075**	0.702 (0.476-1.036)	**0.071**	0.697 (0.470-1.032)
III	196	1910	**0.012**	Ref.	**0.009**	Ref.
IV	111	4507	**<0001**	0.494 (0.334-0.730)	**<0.001**	0.486 (0.329-0.719)
V	236	2273	**0.070**	0.751 (0.552-1.023)	**0.036**	0.718 (0.527-0.979)
VI	111	1487	**0.002**	Ref.	**0.001**	Ref.
VII	120	4570	**<0.001**	0.430 (0.296-0.624)	**<0.001**	0.428 (0.295-0.622)
VIII	218	2454	**0.007**	0.660 (0.488-0.893)	**0.004**	0.643 (0.475-0.871)
IX	120	1478	**<0.001**	Ref.	**<0.001**	Ref.
X	98	4570	**<0.001**	0.048 (0.296-0.678)	**<0.001**	0.048 (0.290-0.667)
XI	260	2454	**0.011**	0.671 (0.492-0.914)	**0.006**	0.645 (0.472-0.881)
XII	100	1446	**0.001**	Ref.	**<0.001**	Ref.

Notes: *, adjustment for age and tumor stage. Bold type highlights statistically significant values ($P \leq 0.05$).

Abbreviations: *PSME*, proteasome activator subunit; MST, median survival time; HR, hazard ratio; CI, confidence interval.

Discussion

In this study, we used data from TGCA to investigate the associations between *PSME* gene expression levels in SKCM and developed a risk score that includes clinical factors and the expression patterns of *PSME* genes to predict prognosis in patients with SKCM. *PSME* genes, including *PSME1*, *PSME2* and *PSME3*, encode the PA28α, PA28β and PA28γ subunits, respectively, of PA28, which regulates function of the proteasomei.[19] In the present study, *PSME1*, *PSME2* and *PSME3* expression levels were significantly increased in SKCM compared to healthy skin. GO enrichment analysis showed that *PSME1* is a negative regulator of cell adhesion, *PSME2* is important for cell-cell adhesion and junction organization, and *PSME3* is associated with NF-κB signaling. Importantly, the activation of NF-κB can impart invasiveness and properties of cancer initiation on cells, and may act as a target for anti-cancer therapy.[20] GO term analysis also

showed that *PSME* was associated with MAPK cascade, which the pathway was found to be correlated with melanoma.[21, 22] High expression levels of *PSME1* and *PSME2* combined with a low expression level of *PSME3* in SKCM were associated with favorable prognosis. Pathway-based analysis revealed that *PSME1* is associated with KEGG and apoptosis pathways and that *PSME2* and *PSME3* are significantly enriched in the canonical and planar cell polarity Wnt signaling pathways, which have been associated with cancer.[23, 24] Taken together, the findings from the present study suggest that expression levels of the *PSME1*, *PSME2* and *PSME3* genes in SKCM, individually and in combination, may be used as potential biomarkers to predict prognosis.

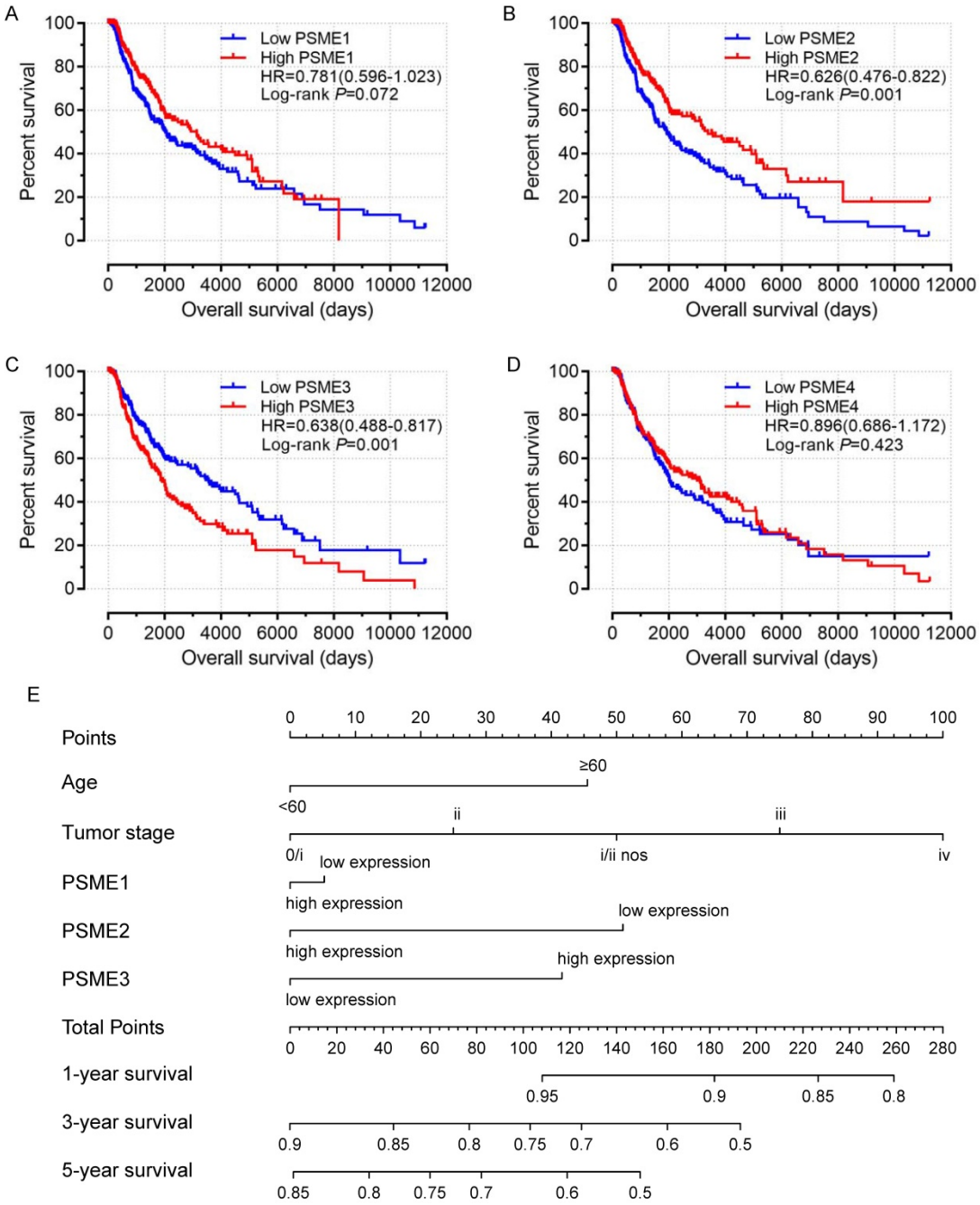

Figure 4. Univariate survival analysis and nomogram. (A) *PSME1*, (B) *PSME2*, (C) *PSME3*, (D) *PSME4*, (E) nomogram to predict survival in SKCM. Abbreviation: *PSME*, proteasome activator subunit; SKCM, skin cutaneous melanoma.

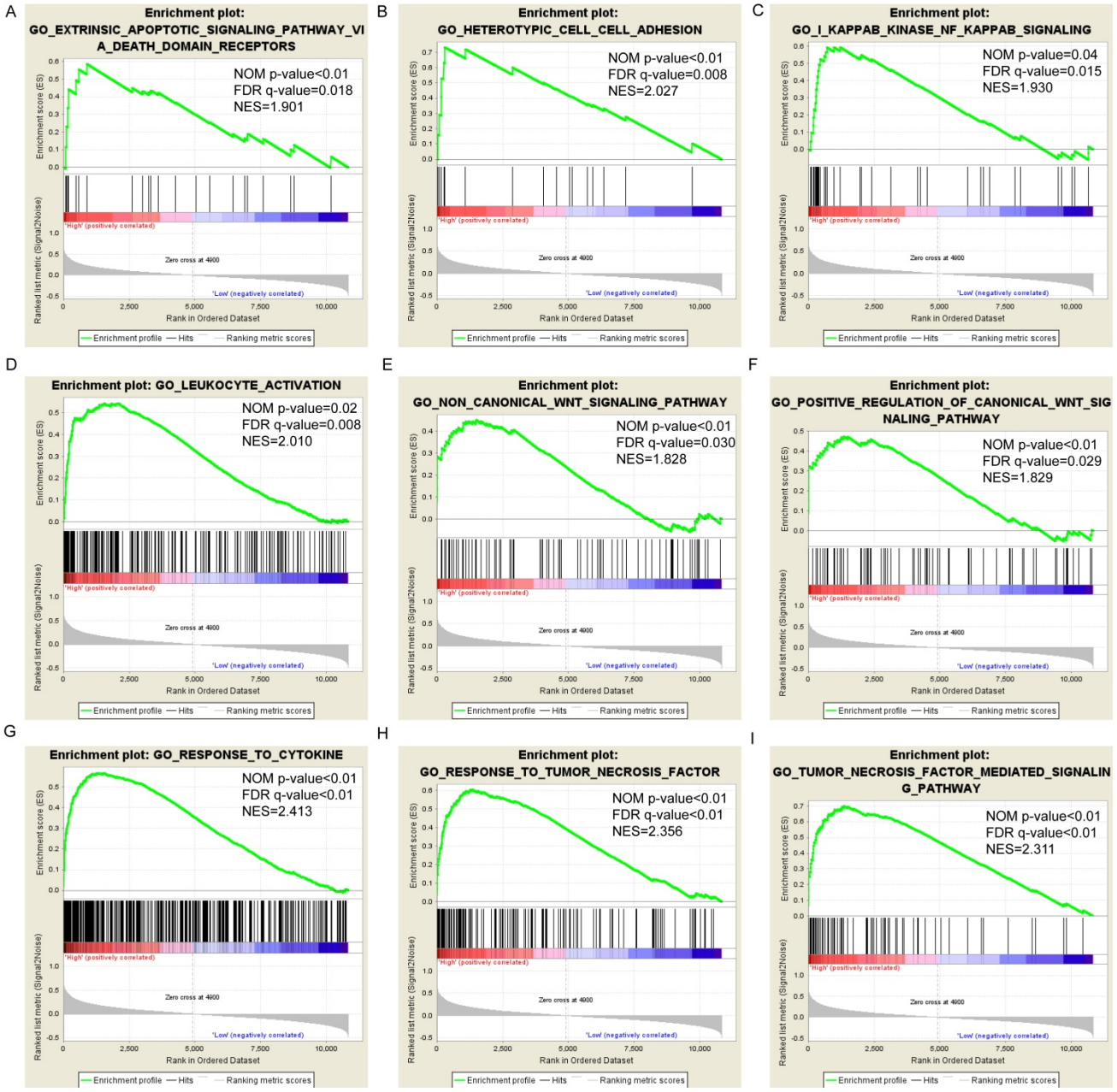

Figure 5. (A-I) GO enrichment analysis by low and high *PSME1* expression levels. Abbreviations: NES, Normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; GO, gene ontology.

For PSME1, the findings from the present study are in contrast to those from previous reports, which demonstrated that *PSME1* expression was increased in primary and metastatic human prostate cancer, PSME1 was a marker in mouse xenograft tumors,[4] and PA28α protein was downregulated in HBV-infected well-differentiated hepatocellular carcinoma.[25] The disparate findings between the present and some previous studies suggest that *PSME1* may play different roles in different types of cancer.

Previous reports on *PSME2* are in accordance with the results from the present study. Evidence suggests that PA28β protein regulates invasiveness and metastasis in gastric cancer, whereby the invasive abilities of gastric cancer cells were enhanced by the down-regulation of PA28β and inhibited when PA28β was overexpressed,[5] and that PA28β is physically associated with N-α-acetyltransferase 10 protein, which regulates various pathways associated with cancer cell proliferation, metastasis, apoptosis, and autophagy.[26]

The role of *PSME3* in cancer has been well characterized. *PSME3* knockout mice treated with dextran sodium sulfate to induce acute colitis showed decreased intestinal inflammation and colitis-associated cancer compared to wild-type mice.[27] In oral squamous cell carcinoma, high

expression of *PSME3* was correlated with worse OS, while *PSME3* silencing inhibited the growth, proliferation and mobility of oral squamous cell carcinoma cells *in vitro* and reduced tumor growth and angiogenesis in mice *in vivo* .[12] Similarly, *PSME3* silencing attenuated the cell proliferation, migration and invasive abilities of endometrial cancer cells. In a model of skin tumorigenesis, *PSME3* functioned as an oncogene, whereby the TPA-induced overexpression of *PSME3* was dependent on the activation of the MAPK-p38 signaling pathway.[28] In breast cancer, 5-year disease-free survival and OS in patients with undetectable or low *PSME3* expression were significantly higher than in patients with high *PSME3* expression.[6] In colorectal cancer, *PSME3* expression was higher in colorectal cancer tissue than in healthy tissues.[10] Other studies indicate that mutations in the *TP53* gene, which encodes the tumor suppressor protein p53, occur in various types of cancer, and that *PSME3* negatively regulates p53, whereby the elimination of endogenous *PSME3* in human cancer cells abrogates MDM2-mediated *p53* degradation, increases the activity of p53, and enhances apoptosis.[29] Notably, *p53* mutations show a positive correlation with *PSME3* expression in various cancer cell lines.[30] In normal endometrium, expression of *PSME3* was increased in p53-positive specimens compared to p53-negative specimens,[31] and in laryngeal carcinoma, the expression of *PSME3* was correlated with p53 and p21.[11, 32-34]

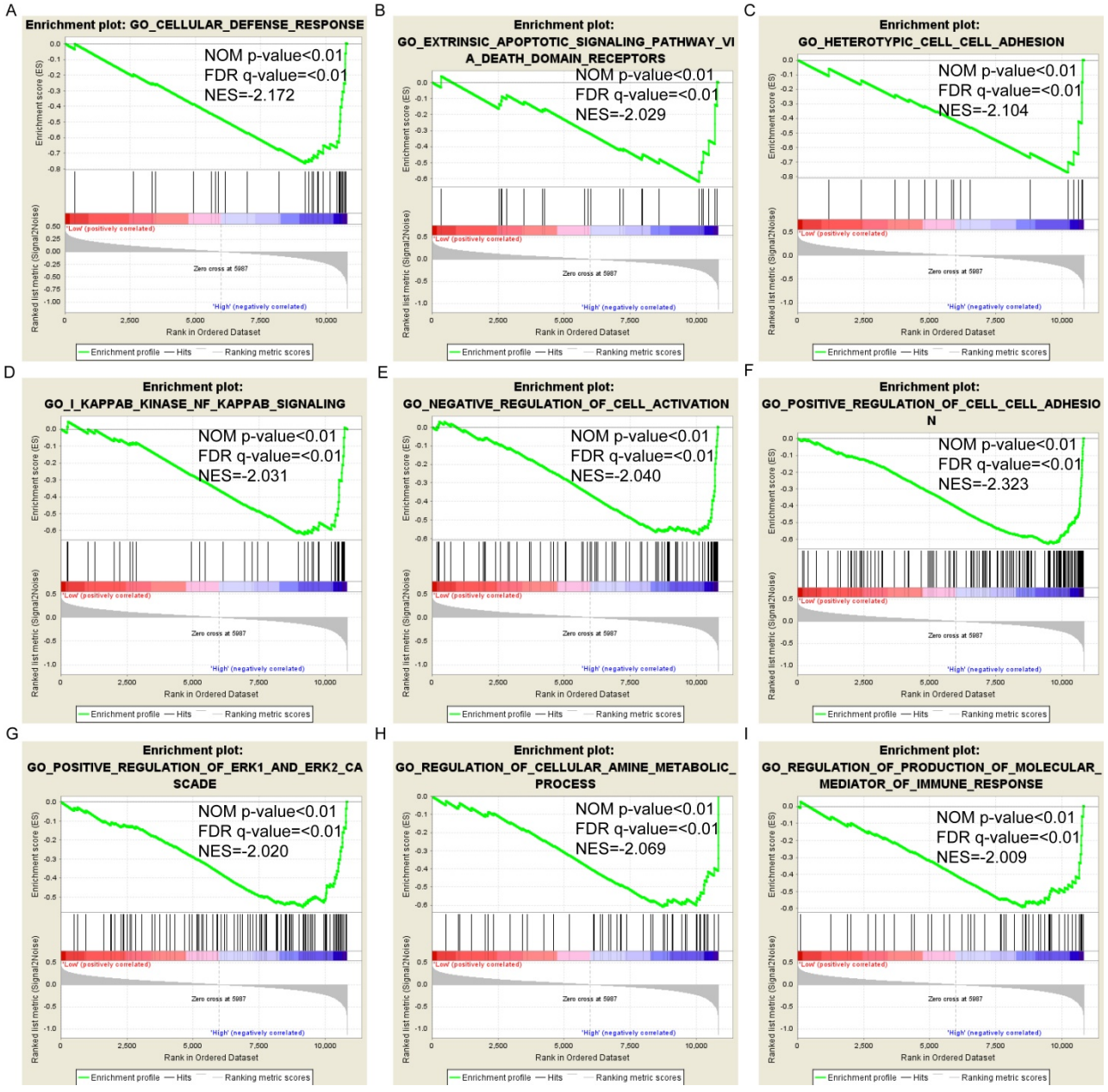

Figure 6. (A-I) GO enrichment analysis by low and high *PSME2* expression levels. Abbreviations: NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; GO, gene ontology.

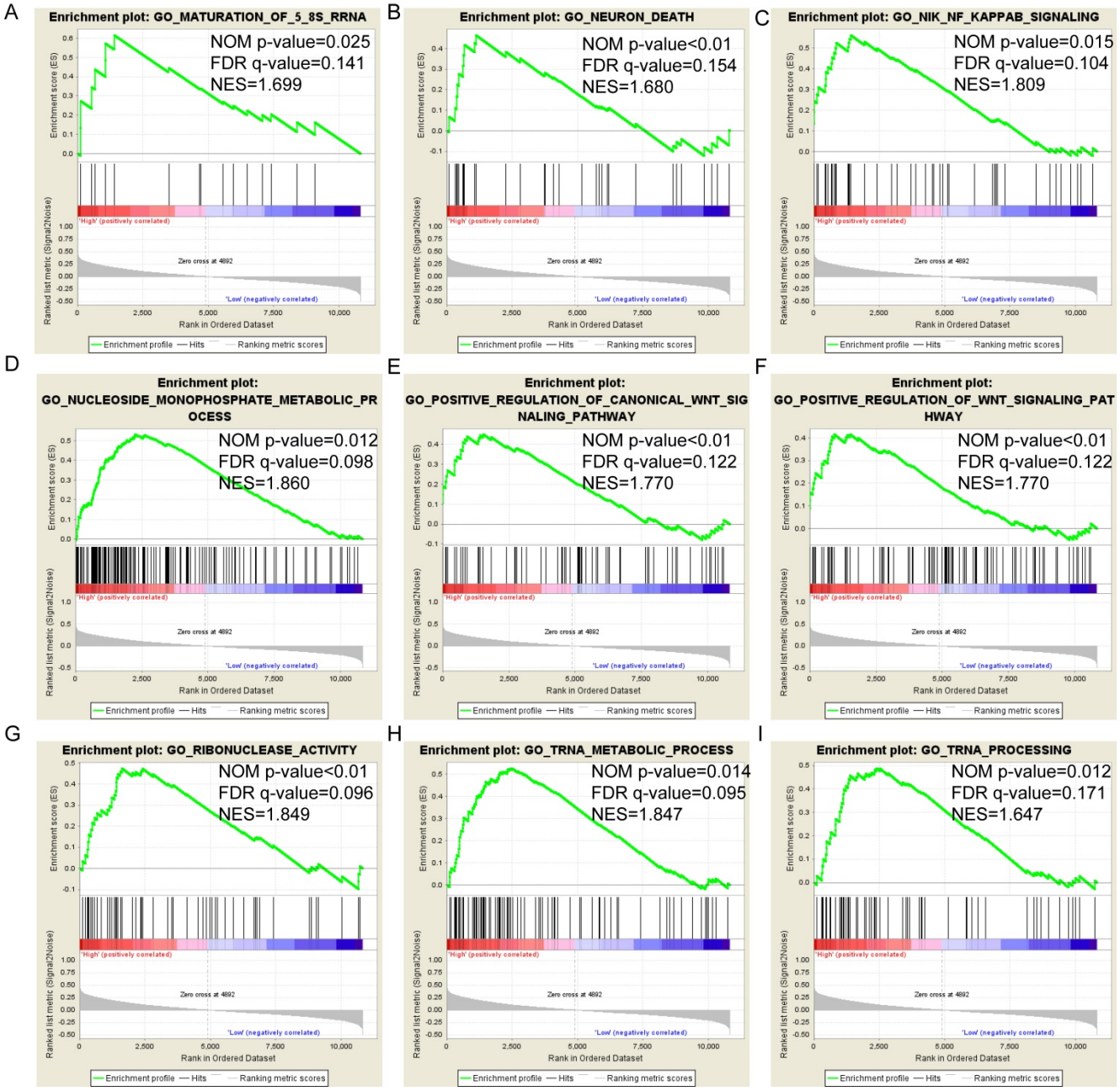

Figure 7. (A-I) GO enrichment analysis by low and high *PSME3* expression levels. Abbreviations: NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; GO, gene ontology.

Despite the wealth of literature on the role of *PSME* genes in cancer, to the authors' knowledge, the present study is the first to develop a risk score that includes clinical factors and the expression patterns of *PSME* genes to predict prognosis in patients with SKCM. The risk score can be used to stratify patients with SKCM into groups at high or low risk for poor prognosis. Univariate survival analysis showed that a high expression level of *PSME2* and low expression level of *PSME3* in SKCM were correlated with favorable OS. Multivariate survival analysis showed that high expression level of *PSME1*, adjusted by age and tumor stage, in SKCM was also correlated with favorable prognosis. Joint-effects survival analysis showed that high expression levels of *PSME1* and *PSME2* combined with a low expression level of *PSME3* in SKCM was associated with favorable OS. In contrast, low expression levels of *PSME1* and *PSME2* combined with a high expression level of *PSME3* was associated with poor OS.

This study had several limitations. First, the sample size was small. In particular, a more ethnically diverse study population is required. In the present study, the majority of subjects were White. Second, clinical information, including information on sun exposure and genetic factors, was lacking. Third, the

patients in the current study were from a single cohort, which may introduce bias. Findings from the present study should be verified in a larger and more diverse set of patients. Forth, our current study is a bioinformatics research and most of the findings were generated from public database and bioinformatics analysis, which lack of verification through in *vitro* and in *vivo* experiments. Finally, SKCM is the melanoma of skin is a fairly rare disease and the related resources are also rare, so this study lake of validation methods to confirm the results including independent cohort. Therefore, results of current study still need further verified.

Despite these limitations, to the authors' knowledge, this is the first study to demonstrate that high expression levels of *PSME1* and *PSME2* combined with a low expression level of *PSME3* is associated with favorable prognosis in SKCM. These findings may have prognostic significance in SKCM. The prognostic model constructed in this study may have value in clinical applications.

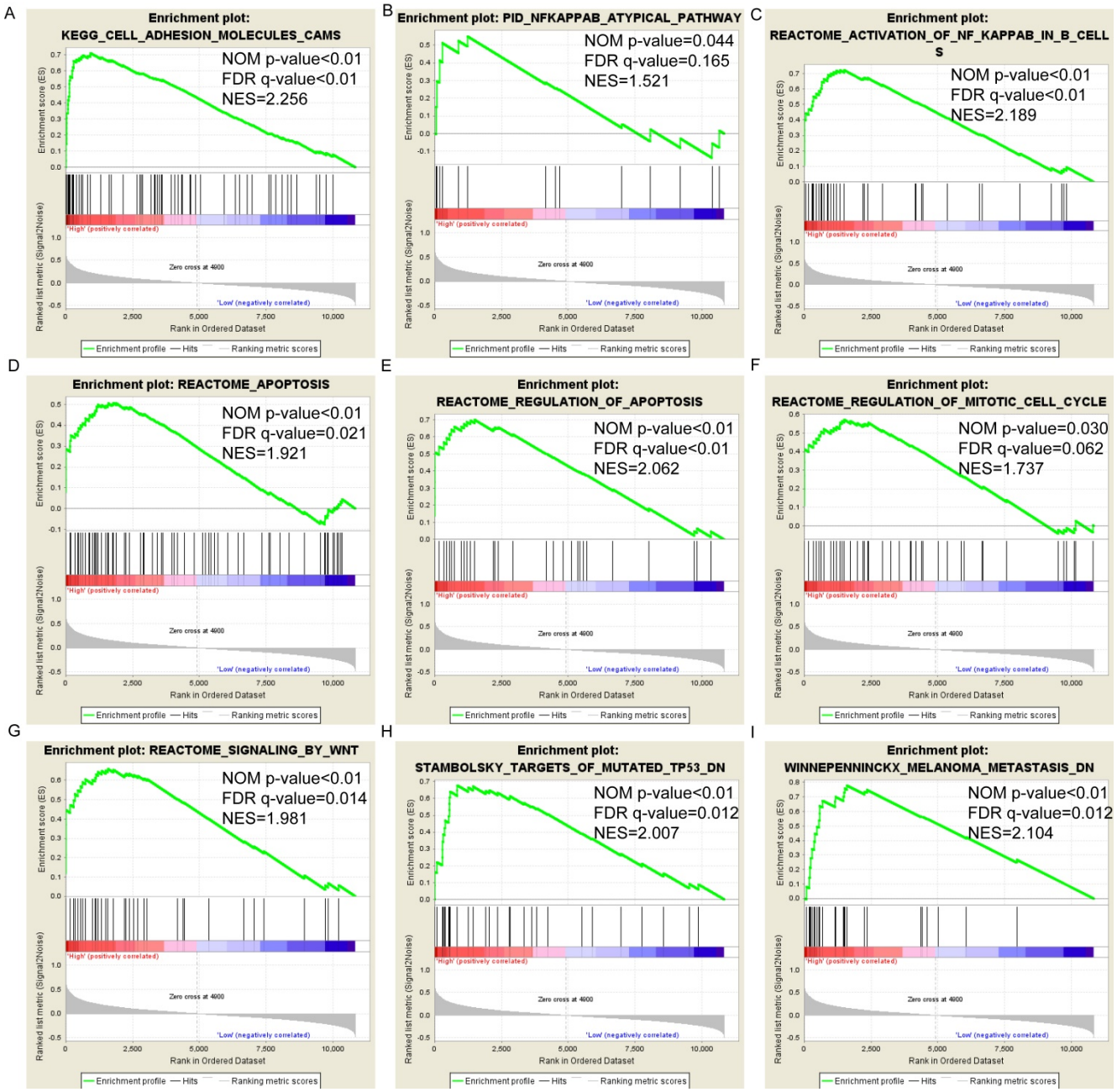

Figure 8. (A-I) KEGG pathway analysis by low and high *PSME1* expression levels. Abbreviations: NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; KEGG, Kyoto encyclopedia of genes and genomes.

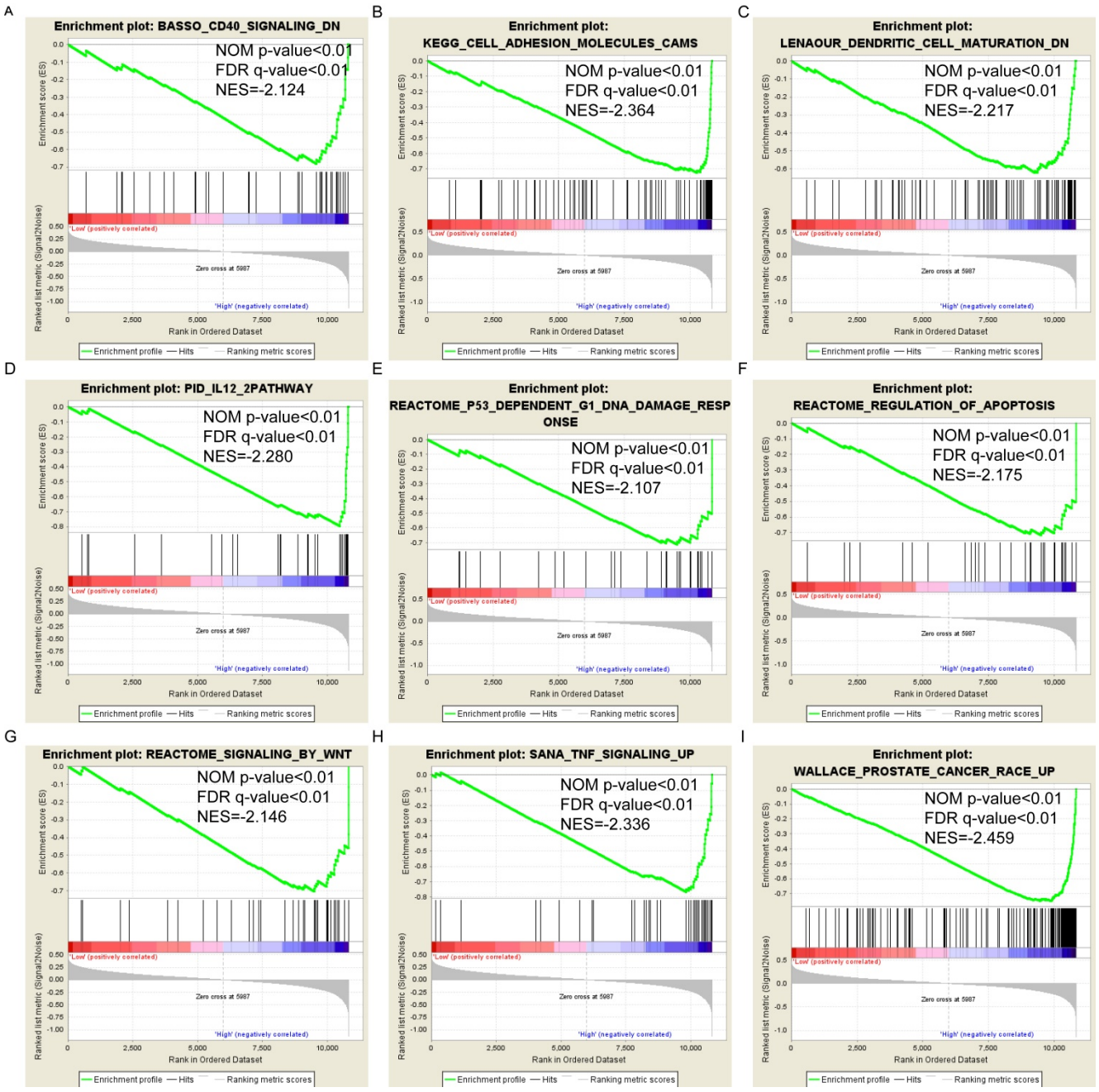

Figure 9. (A-I) KEGG pathway analysis by low and high *PSME2* expression levels. Abbreviations: NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; KEGG, Kyoto encyclopedia of genes and genomes.

Conclusion

Findings from the present study indicate that a high expression of *PSME1* and *PSME2* and low expression of *PSME3* are associated with favorable prognosis and may act as potential prognostic biomarkers in SKCM. The combined expression levels of these genes could provide a sensitive strategy for predicting prognosis in SKCM.

Supplementary Material

Supplementary table 1.
http://www.jcancer.org/v10p2205s1.xlsx

Supplementary table 2.
http://www.jcancer.org/v10p2205s2.xlsx

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Ethics approval and consent to participate

This article does not contain any studies with

human participants or animals performed by any of the authors. Since all datasets included in the present study were downloaded from TCGA, additional approval by an Ethics Committee was not needed. The procedures were in accordance with the Helsinki declaration of 1964 and its later amendments.

Competing Interests

The authors have declared that no competing interest exists.

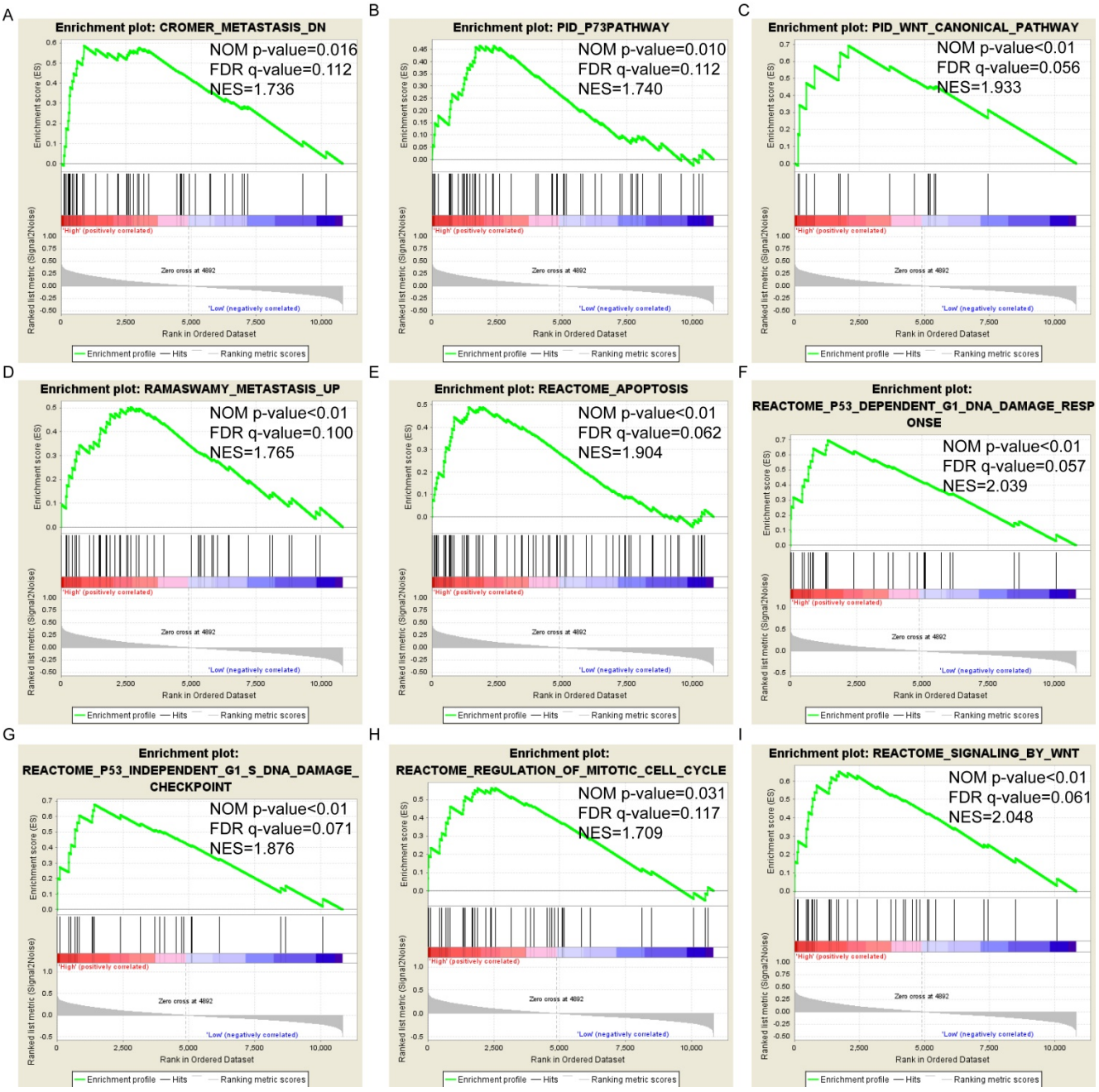

Figure 10. (A-I) KEGG pathway analysis by low and high *PSME3* expression levels. Abbreviations: NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; KEGG, Kyoto encyclopedia of genes and genomes.

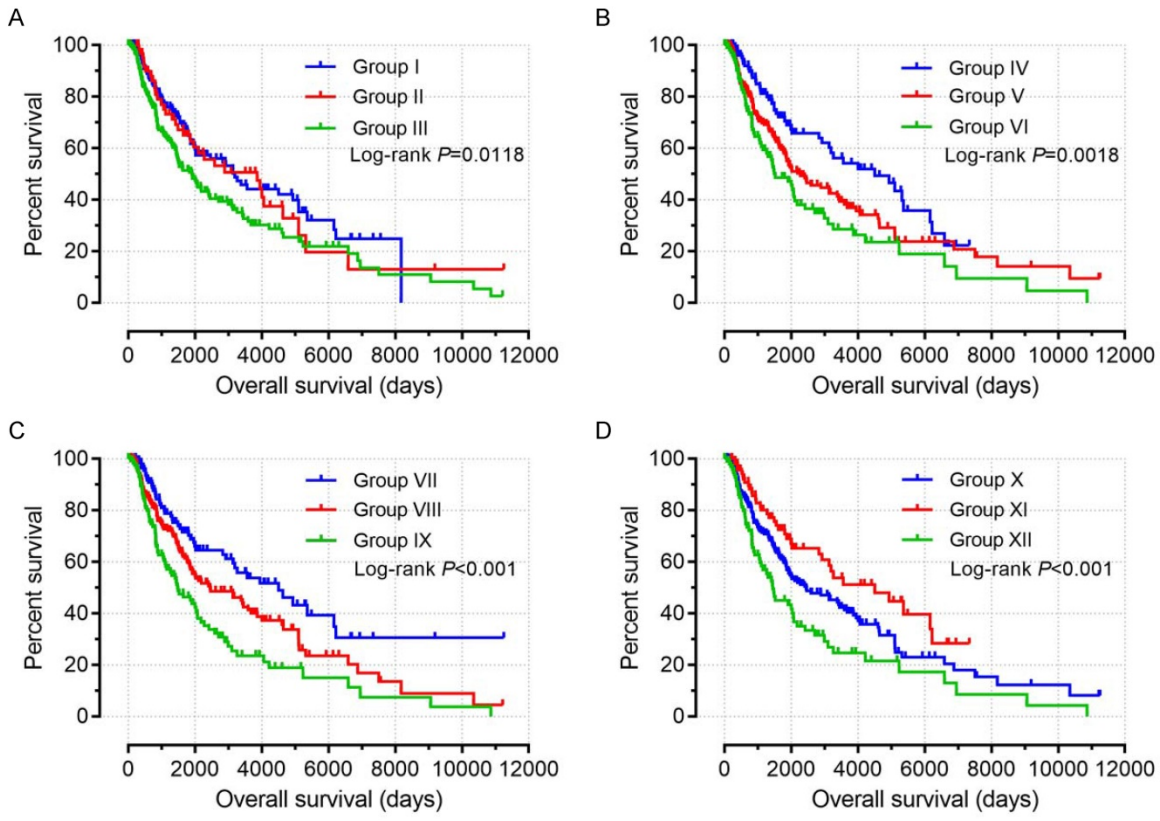

Figure 11. Joint-effects survival analysis of the influence of combined PSME gene expression on OS stratified for PSME1, PSME2 and PSME3 expression levels. (A) *PSME1* and *PSME2*; (B) *PSME1* and *PSME3*; (C) *PSME2* and *PSME3*; (D) *PSME1*, *PSME2* and *PSME3*. I, high *PSME1*+high *PSME2*; III, Low *PSME1*+low *PSME2*; IV, high *PSME1*+ low *PSME3*; VI, low *PSME1*+high *PSME3*; VII, high *PSME2*+low *PSME3*; IX, low *PSME2*+high *PSME5*; X, high *PSME1*+high PSME2+low *PSME3*; XII, Low *PSME1*+low *PSME2*+high *PSME3*. The combinations of genes and unlisted combinations are shown in Table 1. Abbreviation: *PSME*, proteasome activator subunit.

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