

Identification of CD38 as a potential biomarker in skin cutaneous melanoma using bioinformatics analysis

XIANWANG WANG^{1,2*}, PENGLI WANG^{1*}, LEI GE^{2*}, JUAN WANG³,
SYED MANZAR ABBAS SHAH NAQVI² and SHUJUAN HU⁴

¹Department of Biochemistry and Molecular Biology, Health Science Center; ²Laboratory of Oncology, Center for Molecular Medicine, Health Science Center; ³Department of Pediatrics, The Second School of Clinical Medicine and Jingzhou Central Hospital; ⁴Department of Sports Medicine, School of Education and Physical Education, Yangtze University, Jingzhou, Hubei 434023, P.R. China

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Abstract. Skin cutaneous melanoma (SKCM) is the most aggressive type of skin cancer, with a high rate of metastasis and mortality; however, identification of biomarkers for the treatment of SKCM is required. Cluster of differentiation (CD)38 has emerged as an effective target for therapeutic drugs in several types of cancer, such as chronic lymphocytic leukemia and multiple myeloma. In the present study, to determine the contribution of CD38 to the diagnosis of SKCM, Gene Expression Profiling Interactive Analysis 2 and University of Alabama Cancer Database online tools were used to analyze The Cancer Genome Atlas-SKCM dataset. Moreover, Search Tool for the Retrieval of Interacting Genes/Proteins and GeneMANIA databases were used to determine protein-protein interaction networks and potential functions. To the best of our knowledge, the results of the present study indicated for the first time that high expression levels of CD38 were a favorable diagnostic factor for SKCM. Moreover, a correlation between CD38 expression levels and the survival probability of patients with SKCM was identified. Integrative analysis predicted that nine genes were correlated with CD38 in SKCM, and the similarity of these genes in SKCM expression and a survival heatmap was verified.

Gene ontology enrichment analysis using the Metascape tool revealed that CD38 and its correlated genes were significantly enriched in lymphocyte activation and T cell differentiation regulation. Collectively, the bioinformatics analysis revealed that CD38 might serve as a potential diagnostic predictor for SKCM.

Introduction

Skin cutaneous melanoma (SKCM) is the most aggressive type of skin cancer, with an increasing number of cases worldwide, potential for early metastasis and a high mortality rate (1). The number of treatment options available for human SKCM has increased, including radiotherapy, photodynamic therapy, immunotherapy, chemotherapy and biochemotherapy (2-6). Individualized treatment is difficult to administer to patients with SKCM, as locally advanced SKCM confers a major challenge in terms of surgical and medical management (2). Therefore, effective prognostic markers may provide an alternative therapeutic treatment strategy for patients with SKCM. With the establishment of The Cancer Genome Atlas (TCGA), bioinformatics research has enabled the identification of differentially expressed and mutated genes in different types of human cancer, which has led to the discovery of novel biomarkers from the analysis of TCGA-SKCM samples that display the potential to be used during the diagnosis, treatment and prognosis of SKCM (7-10). However, further investigation into the diagnostic and prognostic biomarkers identified in patients with SKCM is required to determine their suitability, as well as understand the molecular mechanisms and gene networks underlying the development and prognosis of SKCM.

Cluster of differentiation 38 (CD38) is a versatile membrane protein with various functions that was originally identified as a cell surface differentiation marker in B lymphocytes, and was subsequently characterized as a multifunctional enzyme that is expressed ubiquitously (11). Increasing evidence has supported the hypothesis that CD38 is involved during tumorigenesis, tumor growth and metastasis (12-17). In particular, CD38 has been used as a human multiple myeloma target for its combination therapy (12,15). Morandi *et al* (18) also confirmed that CD38 is expressed in human multiple myeloma

Correspondence to: Dr Xianwang Wang, Department of Biochemistry and Molecular Biology, Health Science Center, Yangtze University, 1 Nanhuan Road, Jingzhou, Hubei 434023, P.R. China

E-mail: 275379987@qq.com

Mrs Shujuan Hu, Department of Sports Medicine, School of Education and Physical Education, Yangtze University, 1 Nanhuan Road, Jingzhou, Hubei 434023, P.R. China

E-mail: 523254608@qq.com

*Contributed equally

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cells. Furthermore, several studies have revealed that CD38 serves an important role during primary human melanoma metastasis and T cell proliferation (13,18). The aforementioned studies suggested that CD38 is overexpressed in multiple tumor types, and therefore, may serve as a promising target for therapeutic antibodies and drugs.

To determine the diagnostic and prognostic value of CD38 in patients with SKCM, the publicly available TCGA-SKCM and healthy samples were analyzed using University of Alabama Cancer database (UALCAN) (19) and Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (20) online tools, as well as Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (21), GeneMANIA (22), cBioPortal for Cancer Genomics (23) and Metascape databases and resources (24). In the present study, the novel diagnostic and prognostic role of CD38 during SKCM was identified. Therefore, the results of the present study might advance the development of antagonist CD38 treatment strategies for patients with SKCM.

Materials and methods

CD38 expression and mutations analysis. To analyze the expression of CD38 in patients with SKCM, the online software tools UALCAN (ualcan.path.uab.edu/analysis.html) and GEPIA2 (gepia2.cancer-pku.cn/#index) were used. The UALCAN online tool analyzed CD38 expression levels in SKCM based on sample type, individual cancer stage, and the sex, weight, age and race of the patient. The cBioPortal for Cancer Genomics (www.cbioportal.org) was used to further evaluate the gene expression levels and mutations of CD38 in SKCM samples. All eight TCGA cutaneous melanoma categories were included. All patients with CD38-positive status were included in the present study, and both SKCM and healthy samples were analyzed.

Survival analysis. The Kaplan-Meier method was used to evaluate survival analysis based on the expression of CD38 between various groups. The primary endpoint was disease free survival (DFS), which was defined as the time interval between the initiation of curative treatment and the date of progression, the start date of a second-line treatment or the date of death, whichever occurred first. The secondary endpoint was overall survival (OS), which was defined as the length of time between the date of diagnosis or first therapy to the date of death or last follow-up. The follow-up duration was calculated and presented using the Kaplan-Meier method with 95% confidence intervals and the log-rank test was used to identify significant differences among the various groups. The effects of CD38 expression levels and the body weight, sex, race of the patient on patient survival were determined using UALCAN. The OS and DFS, based on the expression status of CD38 in patients with SKCM, were determined using GEPIA2. In the total number of patients with SKCM, 50% displayed high CD38 expression levels ($n=229$; $>$ median expression) and 50% displayed low expression levels of CD38 ($n=229$; $<$ median expression). Furthermore, the LOGpc database (bioinfo.henu.edu.cn/DatabaseList.jsp) was used to verify the identified OS, platinum-free interval (PFI) and disease-specific survival (DSS) results.

Correlation genes analysis. To identify genes that were correlated with CD38 during SKCM, a correlation analysis was performed. The top 50 genes that displayed a correlation with CD38 expression during SKCM were obtained using the GEPIA2 online tool. The SKCM healthy and tumor expression GDC TCGA Melanoma (SKCM) dataset (dataset ID: TCGA-SKCM.cnv.tsv, <http://gepia2.cancer-pku.cn/#dataset>) was analyzed from TCGA (<http://gepia2.cancer-pku.cn/#similar>). Pearson's correlation coefficient (r) was used to screen the top 50 positively correlated genes ($r \geq 0.75$) with a similar expression pattern to CD38 in SKCM tumor and normal tissues. Furthermore, the UALCAN tool was used to determine the correlation between nine highly correlated genes and CD38.

Expression and survival heatmap analysis. The heatmap profile of nine correlated genes with CD38 expression during SKCM and 32 additional TCGA cancer types, including adrenocortical carcinoma, bladder urothelial carcinoma, breast invasive carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, lymphoid neoplasm diffuse large B cell lymphoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, kidney chromophobe, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, acute myeloid leukemia, brain lower grade glioma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, mesothelioma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarcinoma, rectum adenocarcinoma, sarcoma, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine corpus endometrial carcinoma, uterine carcinosarcoma, uveal melanoma, were generated using an interactive heatmap and the multiple gene comparison tools in GEPIA2. Both healthy and tumor data from TCGA were selected for the expression heatmap analysis. To compare the survival contribution of the top nine correlated genes with CD38 expression during SKCM and additional TCGA cancer types, the survival map was calculated from TCGA-tumor specimens using the Mantel-Cox test.

Occurrence of CD38 isoforms and promoter methylation analysis. To verify the five structural isoforms of CD38 and to compare the occurrence in SKCM, the TCGA-SKCM dataset in the GEPIA2 online tool was used with the following parameters: Cancer, X and isoform, Y. To analyze the CD38 promoter methylation levels in patients with SKCM, the CD38 promoter methylation profile based on individual cancer stage, and the sex, weight and age of the patient was analyzed using the UALCAN online tool.

Protein-protein interaction (PPI) networks and Gene Ontology (GO) enrichment analysis. The STRING database (string-db.org) provides a critical evaluation and integration of PPI, including physical and functional relevance (21). The PPI network of CD38 was produced using STRING online tools (version 10.0). GeneMANIA (genemania.org) is a flexible user-friendly website for generating hypotheses regarding gene function, analyzing gene lists and prioritizing

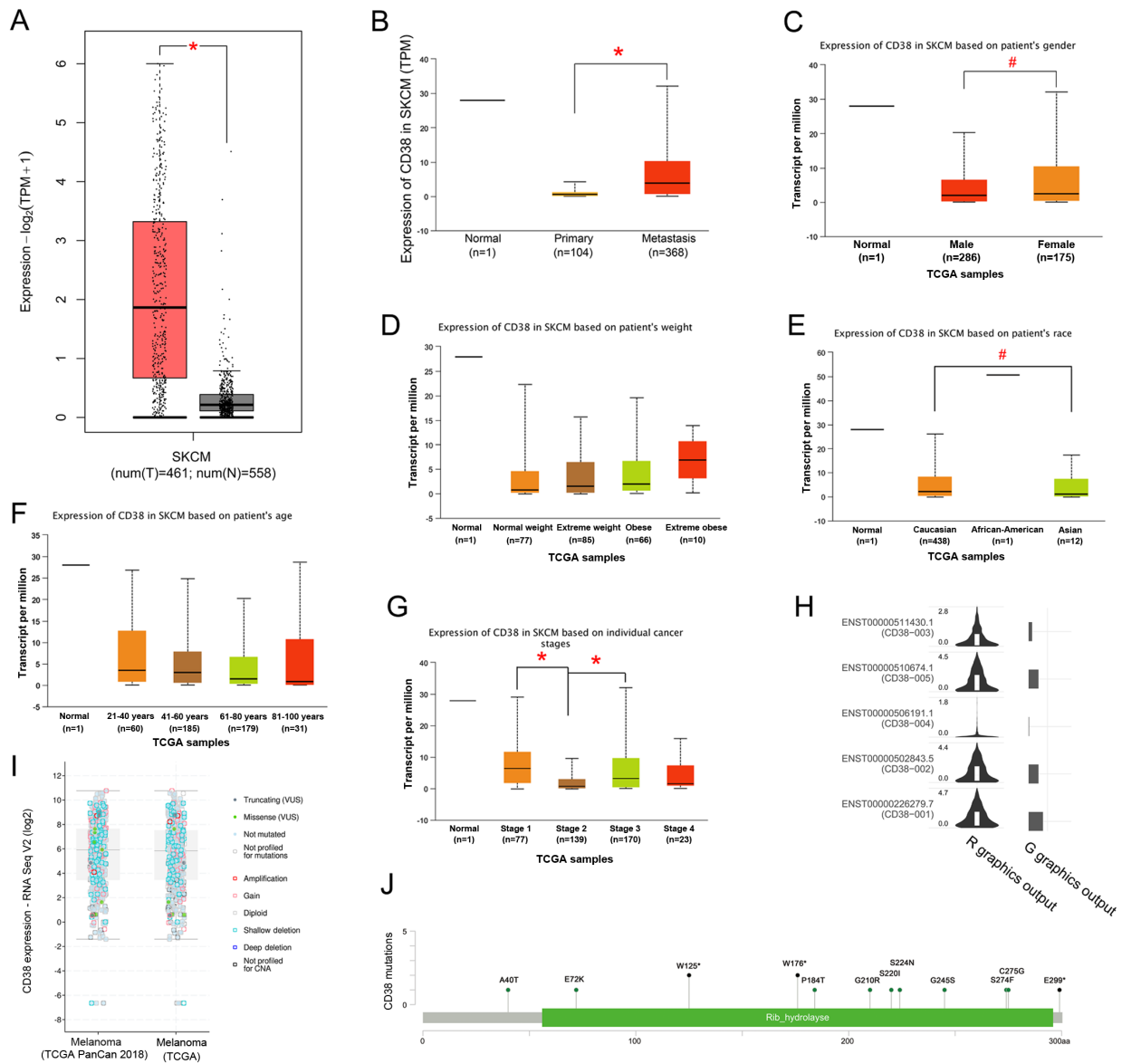


Figure 1. CD38 expression and mutations in patients with SKCM. (A) CD38 expression in SKCM using (A) the GEPIA2 online tool and (B) UALCAN. CD38 expression in SKCM based on (C) sex, (D) body weight, (E) race, (F) age and (G) individual cancer stage of the patient. (H) Occurrence of CD38 isoforms in patients from TCGA-SKCM dataset (X, cancer; Y, isoform). (I) CD38 expression and mutations analyzed using cBioPortal for Cancer Genomic. (J) Location of specific CD38 mutations in patients with SKCM. Green and black represent missense and truncating mutations, respectively. *P<0.05; *P<0.001. CD38, cluster of differentiation 38; SKCM, skin cutaneous melanoma; GEPIA2, Gene Expression Profiling Interactive Analysis 2; UALCAN, University of Alabama Cancer database; TCGA, The Cancer Genome Atlas; TPM, Transcripts Per Million; T, tumor; N, normal.

genes for functional assays. The Metascape tool (metascape.org) provides a resource for biologists for the analysis of systems-level datasets (24). Therefore, GeneMANIA and Metascape were used to further analyze the related genes and functional enrichment of CD38.

Results

Elevated expression level and specific mutations of CD38 in patients with SKCM from TCGA dataset. To determine the expression level of CD38 in patients with SKCM, the online analytical UALCAN and GEPIA2 tools were used. The expression level of CD38 was significantly higher in SKCM tissues (n=461) compared with healthy tissues (n=558; P<0.001; Fig. 1A). From the UALCAN analysis, CD38 expression levels

were significantly increased in SKCM metastatic tumors compared with primary tumors (P<0.001; Fig. 1B). In addition, there was a significant difference in CD38 expression levels between the sex (P<0.05; Fig. 1C), race (P<0.05; Caucasian vs. Asian; Fig. 1E) and individual cancer stage (P<0.001; stage 1 vs. stage 2 and stage 2 vs. stage 3; Fig. 1G) of the patient, but there were no significant differences in the expression levels of CD38 between the weight (Fig. 1D) and age (Fig. 1F) of the patient.

In addition, the GEPIA2 analysis indicated that the occurrence of the two major isoforms, CD38-001 and CD38-005, are more frequent in patients with SKCM compared with other isoforms (Fig. 1H). The cBioPortal for Cancer Genomics analysis suggested that there were a total of 22 mutations in CD38 in TCGA-SKCM samples, including 14 missenses and

Table I. Mutations in CD38 gene in TCGA-SKCM dataset.

Sample ID	Protein change	Mutation type	Copy number variation	Number of occurrences in COSMIC	Allele frequency (total)	Number of mutations in sample
MEL-JWCI-WGS-12	C275G	Missense	NA	NA	NA	2703
TCGA-FS-A1ZK-06	W176 ^a	Nonsense	Gain	2	0.16	1297
TCGA-EE-A2MR-06	S274F	Missense	Diploid	1	0.14	3944
TCGA-FW-A3R5-06	W125 ^a	Nonsense	Diploid	1	0.18	15939
TCGA-GF-A6C9-06	W125 ^a	Nonsense	ShallowDel	1	0.18	2762
TCGA-EB-A5UN-06	G245S	Missense	Diploid	1	0.19	625
TCGA-FW-A3R5-06	E72K	Missense	Diploid	1	0.37	15939
TCGA-BF-A5ER-01	A40T	Missense	NA	NA	0.38	165
TCGA-EB-A6QY-01	S224N	Missense	NA	NA	0.24	518
TCGA-FS-A1ZK-06	W176 ^a	Nonsense	Gain	2	0.16	1459
TCGA-EE-A2MR-06	S274F	Missense	Diploid	1	0.14	2921
TCGA-FW-A3R5-06	W125 ^a	Nonsense	Diploid	1	0.17	15832
TCGA-GF-A6C9-06	W125 ^a	Nonsense	ShallowDel	1	0.15	2318
TCGA-EB-A5UN-06	G245S	Missense	Diploid	1	0.19	627
TCGA-FW-A3R5-06	E72K	Missense	Diploid	1	0.38	15832
TCGA-BF-A5ER-01	A40T	Missense	NA	NA	0.37	159
TCGA-EB-A6QY-01	S224N	Missense	NA	NA	0.24	506
TCGA-D3-A2JL-06	P184T	Missense	Diploid	NA	0.11	1202
TCGA-EE-A183-06	S220I	Missense	Diploid	1	0.12	2156
TCGA-FS-A1Z4-06	E299 ^a	Nonsense	ShallowDel	NA	0.1	609
TCGA-Z2-A8RT-06	G210R	Missense	ShallowDel	NA	0.68	2422
YUKLAB	W176 ^a	Nonsense	NA	2	NA	3656

^aTermination codon; CD, cluster of differentiation; TCGA, The Cancer Genome Atlas; SKCM, skin cutaneous melanoma; COSMIC, Catalogue of Somatic Mutations in Cancer; NA, not available; Diploid, normal; ShallowDel, Shallow deletion; Gain, Low-level gain.

8 truncating mutations (Fig. II and J; Table I). Among them, the W125 mutation affects CD38 enzyme activity, and the single nucleotide polymorphism (SNP) at position (P184) is associated with autism spectrum disorder and type II diabetes, and C275 is a disulfide bond formation site for CD38 (11). Collectively, the results from the present study suggested that high expression levels and key mutations in CD38 might be involved in the development of SKCM.

Effect of CD38 expression level on patient survival in TCGA-SKCM dataset. To evaluate the prognostic value of CD38 in patients with SKCM, the effect of CD38 expression levels on survival time was analyzed. Higher CD38 expression levels resulted in a significantly higher survival probability compared with lower CD38 expression levels ($P < 0.0001$; Fig. 2A). In addition, survival probability was also associated with the body weight, sex and race of the patient. Low/medium CD38 expression levels in Asian patients resulted in a shorter survival time compared with patients from other ethnicities ($P < 0.0001$; Fig. 2B). CD38 expression levels and the sex ($P = 0.00029$) and body weight ($P = 0.021$) of the patients were also significantly associated with the survival of patients with SKCM (Fig. 2C and D). In particular, female patients diagnosed with SKCM displayed a longer survival time compared with male patients.

Moreover, the OS and DFS of CD38 expression levels in patients with SKCM were analyzed using the GEPIA2 online tool. Patients with high CD38 expression levels displayed a higher survival probability for both OS and DFS compared with patients with low CD38 expression levels (Fig. 2E and F). The contribution of the CD38 isoforms to the survival of patients with SKCM was determined. The survival map analysis verified that major isoform, CD38-001, affected the survival of patients with SKCM (Fig. 2G and H).

To verify the aforementioned results, the LOGpc database was used. Patients with high CD38 expression levels displayed higher OS ($P < 0.0001$), PFI ($P < 0.0001$) and DSS ($P < 0.005$) probabilities compared with patients with low CD38 expression levels (Fig. 2I-K).

Correlated genes with CD38 in patients with SKCM from TCGA dataset. To identify genes correlated with CD38 in SKCM, a correlation analysis was conducted. The top 50 correlated genes with CD38 expression in SKCM were investigated using GEPIA2 (Table II; Pearson $CC \geq 0.75$). A PPI network of these genes was created using the STRING tool (Fig. 3A). The identified genes were from diverse functional groups, such as cell adhesion molecules (CD80, CD86, CD8A, CD8B, CD2, ICOS, ITGAL, ITGB7 and TIGIT), chemokine signaling pathway molecules (CCL4, CCL25,

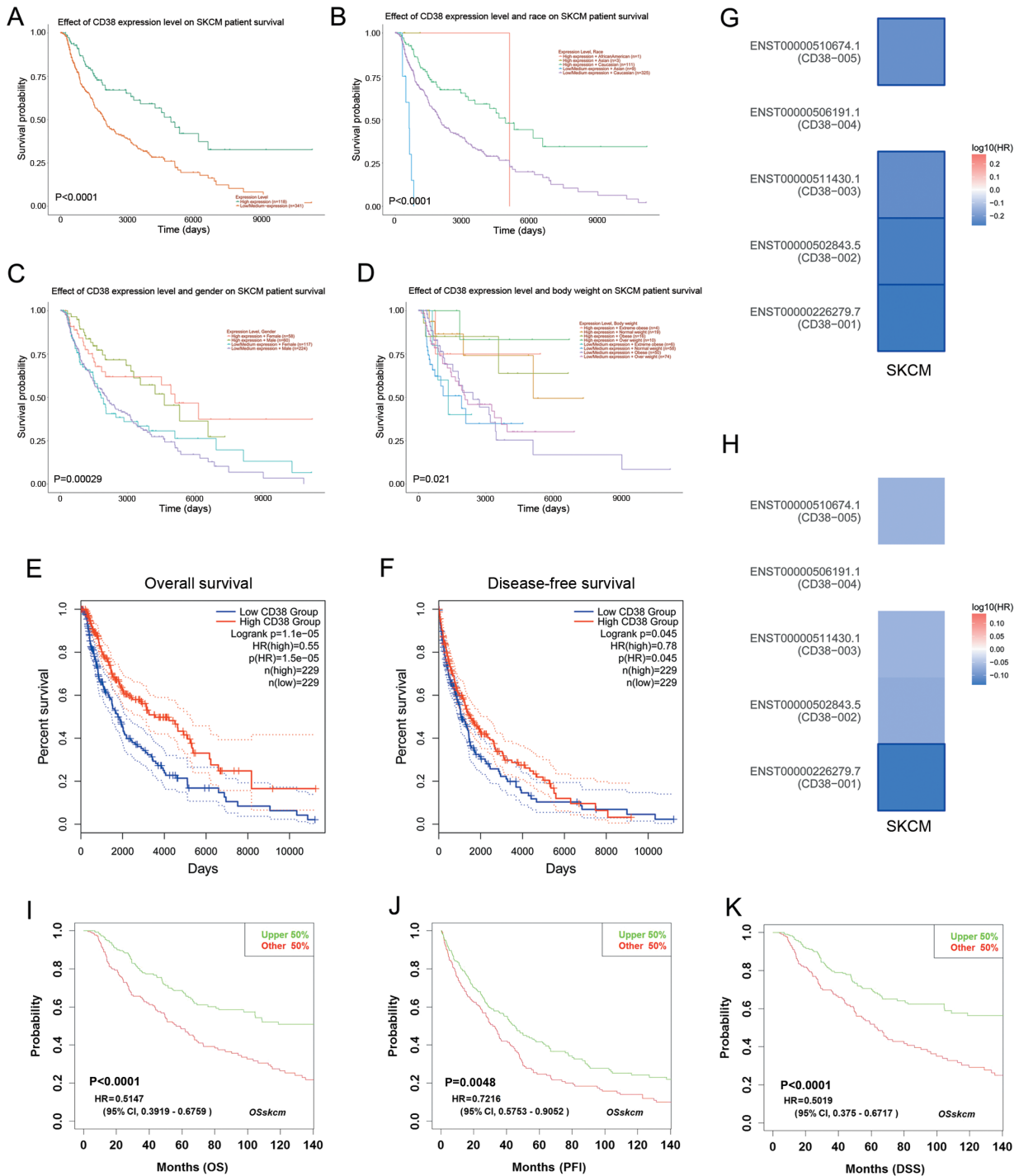


Figure 2. Effect of CD38 expression on overall survival in patients with SKCM. (A) Higher CD38 expression was associated with improved survival probability in patients with SKCM. Effect of CD38 expression and (B) race, (C) sex and (D) body weight on survival in patients with SKCM. (E) OS and (F) DFS were identified using the GEPIA2 online tool. (G) OS and (H) DFS heatmaps of five CD38 isoforms in patients with SKCM. (I) OS, (J) PFI and (K) DSS analysis using the LOGpc Database. CD38, cluster of differentiation 38; SKCM, skin cutaneous melanoma; OS, overall survival; DFS, disease-free survival; GEPIA2, Gene Expression Profiling Interactive Analysis 2; PFI, platinum-free interval; DSS, disease-specific survival; CI, confidence interval; HR, hazard ratio.

CXCR6, CXCL9, CCR2 and GNGT2) and intestinal immune network for IgA production molecules (CD80, CD86, ICOS, ITGB7 and CCL25). A total of nine correlated genes that interacted with CD38 were further predicted using the STRING database (Fig. 3B), which included CD86, CD80, CD27, CD2, IL21R, IL2RB, ITGAL, CCL4 and TBX21. Functional enrichment analysis from Metascape revealed that the nine correlated genes with CD38 in patients with

SKCM might be involved with lymphocyte activation and regulation of T cell differentiation (Fig. 3C).

In addition, the correlation between CD38 and the nine aforementioned correlated genes was further illustrated in Fig. 4A. To determine the similarity of the correlated genes, expression and survival heatmaps across TCGA tumors were generated. The survival heatmaps of the nine aforementioned CD38-correlated genes displayed a positive correlation with

Table II. Top 50 ranked CD38 correlated genes in TCGA-SKCM dataset.

Genenumber	Gene symbol	Gene ID	r
1	TIGIT	ENSG00000181847.11	0.82
2	TBX21	ENSG00000073861.2	0.81
3	PLEK	ENSG00000115956.9	0.81
4	ZBP1	ENSG00000124256.14	0.81
5	CCR2	ENSG00000121807.5	0.81
6	LAX1	ENSG00000122188.12	0.80
7	SLA	ENSG00000155926.13	0.80
8	SAMSN1	ENSG00000155307.17	0.80
9	CXCR6	ENSG00000172215.5	0.79
10	PIM2	ENSG00000102096.9	0.79
11	SIRPG	ENSG00000089012.14	0.79
12	GBP5	ENSG00000154451.14	0.78
13	GNGT2	ENSG00000167083.6	0.78
14	SLA2	ENSG00000101082.13	0.78
15	SLAMF6	ENSG00000162739.13	0.78
16	IL2RB	ENSG00000100385.13	0.78
17	SH2D1A	ENSG00000183918.14	0.78
18	AKAP5	ENSG00000179841.8	0.77
19	GCH1	ENSG00000131979.18	0.77
20	ITGAL	ENSG00000005844.17	0.77
21	CD2	ENSG00000116824.4	0.77
22	SCIMP	ENSG00000161929.14	0.76
23	USP30-AS1	ENSG00000256262.1	0.76
24	ICOS	ENSG00000163600.12	0.76
25	IL10RA	ENSG00000110324.9	0.76
26	CD86	ENSG00000114013.15	0.76
27	IL21R	ENSG00000103522.15	0.76
28	GIMAP5	ENSG00000196329.10	0.76
29	GIMAP4	ENSG00000133574.9	0.76
30	IGSF6	ENSG00000140749.8	0.76
31	NCKAP1L	ENSG00000123338.12	0.76
32	TRGV10	ENSG00000211694.2	0.76
33	CD8B	ENSG00000172116.21	0.76
34	CXCL9	ENSG00000138755.5	0.76
35	CXCR2P1	ENSG00000229754.1	0.76
36	TRGC2	ENSG00000227191.6	0.76
37	FAM26F	ENSG00000188820.12	0.75
38	ITGB7	ENSG00000139626.15	0.75
39	GPR171	ENSG00000174946.6	0.75
40	CD8A	ENSG00000153563.15	0.75
41	TRGV2	ENSG00000233306.2	0.75
42	CD80	ENSG00000121594.11	0.75
43	ABCD2	ENSG00000173208.3	0.75
44	CD27	ENSG00000139193.3	0.75
45	RP11-493L12.5	ENSG00000257924.1	0.75
46	DOK2	ENSG00000147443.12	0.75
47	LILRB1	ENSG00000104972.14	0.75
48	CCL4	ENSG00000275302.1	0.75
49	CCL25	ENSG00000131142.13	0.75
50	AC104820.2	ENSG00000234663.5	0.75

CD, cluster of differentiation; TCGA, The Cancer Genome Atlas; SKCM, skin cutaneous melanoma.

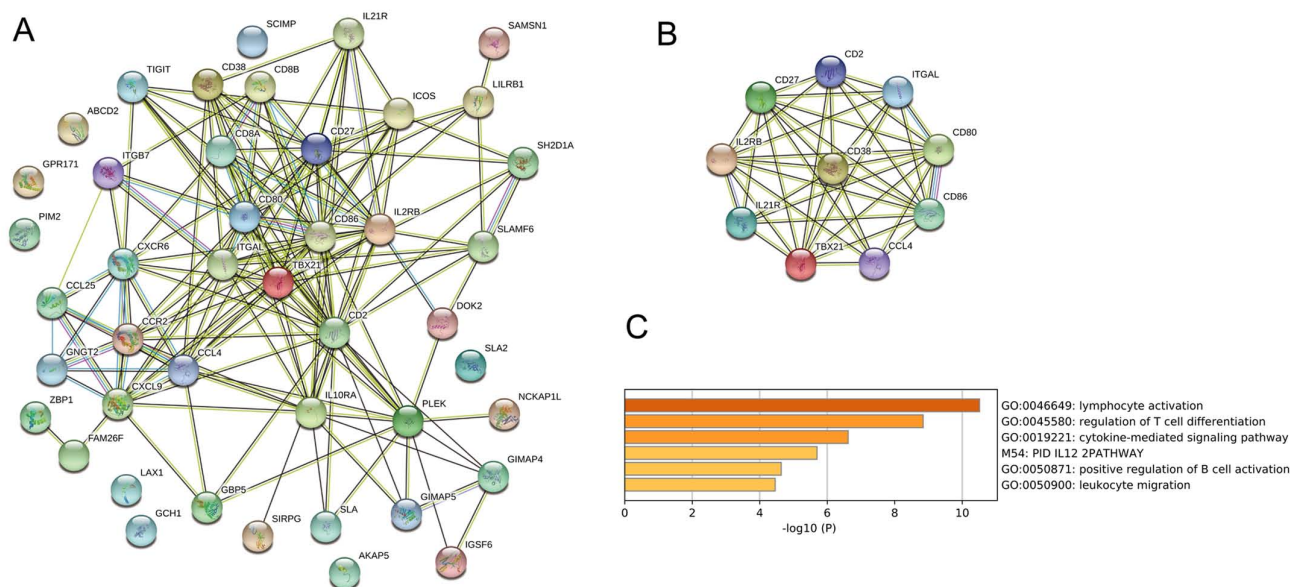


Figure 3. CD38-correlated genes and potential functions in patients with SKCM. Protein-protein interaction network of CD38 with (A) the top 50 ranked correlated genes and (B) nine correlated genes in SKCM. (C) Gene Ontology enrichment analysis of six potential functional processes of the nine correlated genes in SKCM. CD38, cluster of differentiation 38; SKCM, skin cutaneous melanoma.

survival in patients with SKCM, which was consistent with the results of CD38, but there was no correlation with other types of tumor. Moreover, the expression heatmaps in patients with SKCM and healthy tissues of the nine aforementioned genes was similar to the expression heatmap of CD38 (Fig. 4C). However, further studies are required to validate the correlated genes and to explore underlying functional mechanisms.

Promoter methylation levels of CD38 in patients with SKCM from TCGA dataset. To investigate the CD38 promoter methylation profile in patients with SKCM based on the cancer type, individual cancer stage, age, sex, race and weight of the patient, the UALCAN online tool was used. The results suggested that the individual cancer stage ($P < 0.05$; stage 2 vs. stage 3; Fig. 5B), age ($P < 0.05$; 41-60 vs. 21-40/61-80 years; Fig. 5C) and sex ($P < 0.05$; male vs. female; Fig. 5D) of the patient might contribute to the promoter methylation level of CD38 in patients with SKCM. However, there were no significant alterations in the promoter methylation levels of CD38 in the TCGA-SKCM dataset based on the cancer type (Fig. 5A), race (Fig. 5E) or weight (Fig. 5F) of the patient.

PPI network and GO enrichment analysis of CD38. The functional interactions between proteins can provide important information of the molecular mechanism involved. The PPI network of CD38 was determined using the STRING database (Fig. 6A). The results indicated that CD38 interacted with 10 functional genes, including NMNAT1, NMNAT2, NMNAT3, NAMPT, ENPP1, ENPP3, NADK, BST1, PNP and NNMT. From the STRING functional enrichments, the 10 associated genes were involved in the nucleotide metabolic process and nine of the genes were involved in the nicotinamide adenine dinucleotide (NAD) metabolic process. A further 20 genes interacting with CD38 were identified using GeneMANIA (Fig. 6B). Among them, LCK, CD3E, CD247, CD4 and FCGR3A were identified by physical interactions with CD38.

In particular, the functional enrichment analysis revealed that the proteins were involved in the immune response activating cell surface receptor signaling pathway. RYR1, RYR2 and RYR3 were also implicated in calcium release channel activity and intracellular ligand-gated calcium channel activity process. Consistent with the aforementioned correlation analysis, ITGAL belonged to the group of nine correlated genes with CD38 in patients with SKCM.

To further determine the potential function of CD38 in SKCM, GO enrichment analysis of CD38 and the genes it interacts with was performed using Metascape. The results suggested that CD38 and the 10 genes it interacted with were enriched in nicotinate and nicotinamide metabolism, metabolism of water-soluble vitamins and cofactors, and nicotinate metabolism processes ($-\log_{10}(P) > 20$; Fig. 6C). A total of 20 CD38-correlated genes identified using GeneMANIA were significantly enriched in translocation of ZAP-70 to immunological synapse, cellular response to caffeine, immunoregulatory interactions between a lymphoid and a non-lymphoid cell, and natural killer cell mediated cytotoxicity ($-\log_{10}(P) > 6$; Fig. 6D).

Discussion

Over the past few years, several reports have concentrated on differentially expressed genes and mutations in patients with SKCM, identifying diagnostic and prognostic biomarkers for SKCM (8,25-27). However, the current curative biomarkers identified for the therapy of patients with SKCM are not adequate (28). CD38 has emerged as an effective target for therapeutic antibodies and drugs in human multiple myeloma and neuroblastoma (14-16,29). The present study indicated that higher CD38 expression levels and specific CD38 mutations were favorable diagnostic factors in SKCM. Both UALCAN and GEPIA2 online tools revealed that the expression level of CD38 was significantly higher in patients with SKCM

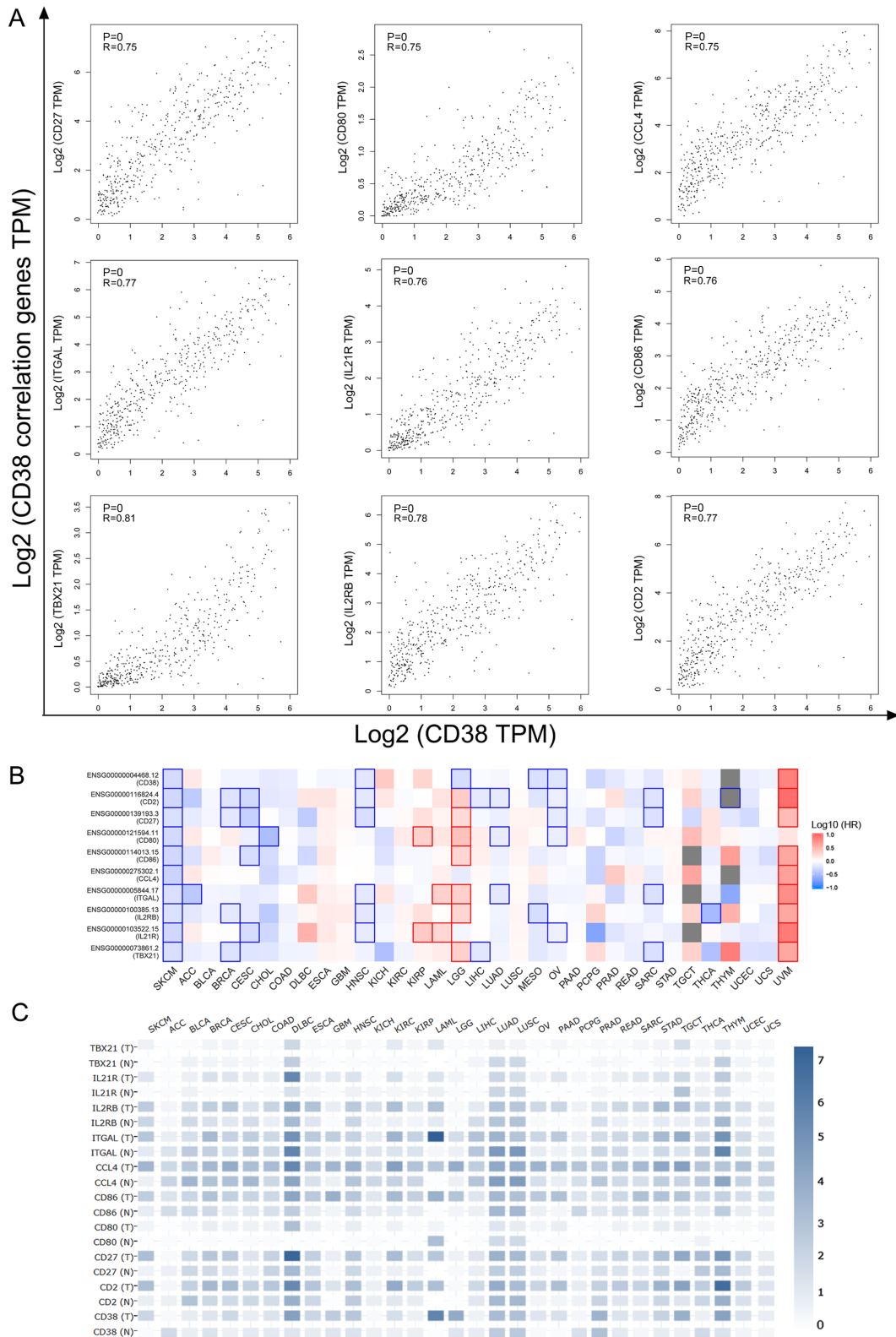


Figure 4. Correlation between CD38 and nine correlated genes. (A) Correlation analysis of CD86, CD80, CD27, CD2, IL21R, IL2RB, ITGAL, CCL4 and TBX21 with CD38 in patients with SKCM. (B) Survival heatmap of nine correlated genes and CD38 in SKCM and other TCGA tumors. (C) Expression heatmap of nine correlated genes and CD38 across TCGA tumors. The shades of blue represent the expression levels of these genes in different tissues (normal and tumor). $\text{Log}_2(\text{TPM} + 1)$ transformed expression data were chosen for plotting. CD38, cluster of differentiation 38; SKCM, skin cutaneous melanoma; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; TCGA, The Cancer Genome Atlas; TPM, Transcripts Per Million; HR, hazard ratio.

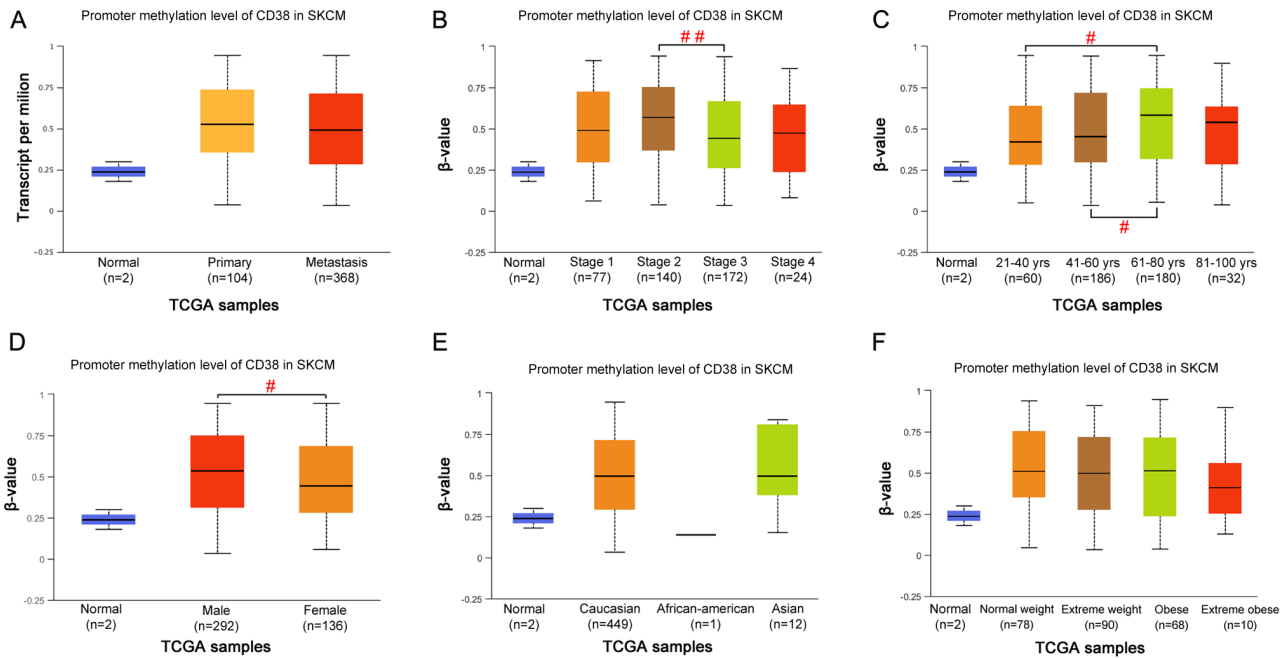


Figure 5. Promoter methylation levels of CD38 in patients with SKCM. Promoter methylation levels of CD38 in patients with SKCM based on the (A) cancer type, (B) individual cancer stages, (C) age, (D) sex, (E) race and (F) body weight of the patient. # $P < 0.05$ and ## $P < 0.005$. CD38, cluster of differentiation 38; SKCM, skin cutaneous melanoma; TCGA, The Cancer Genome Atlas.

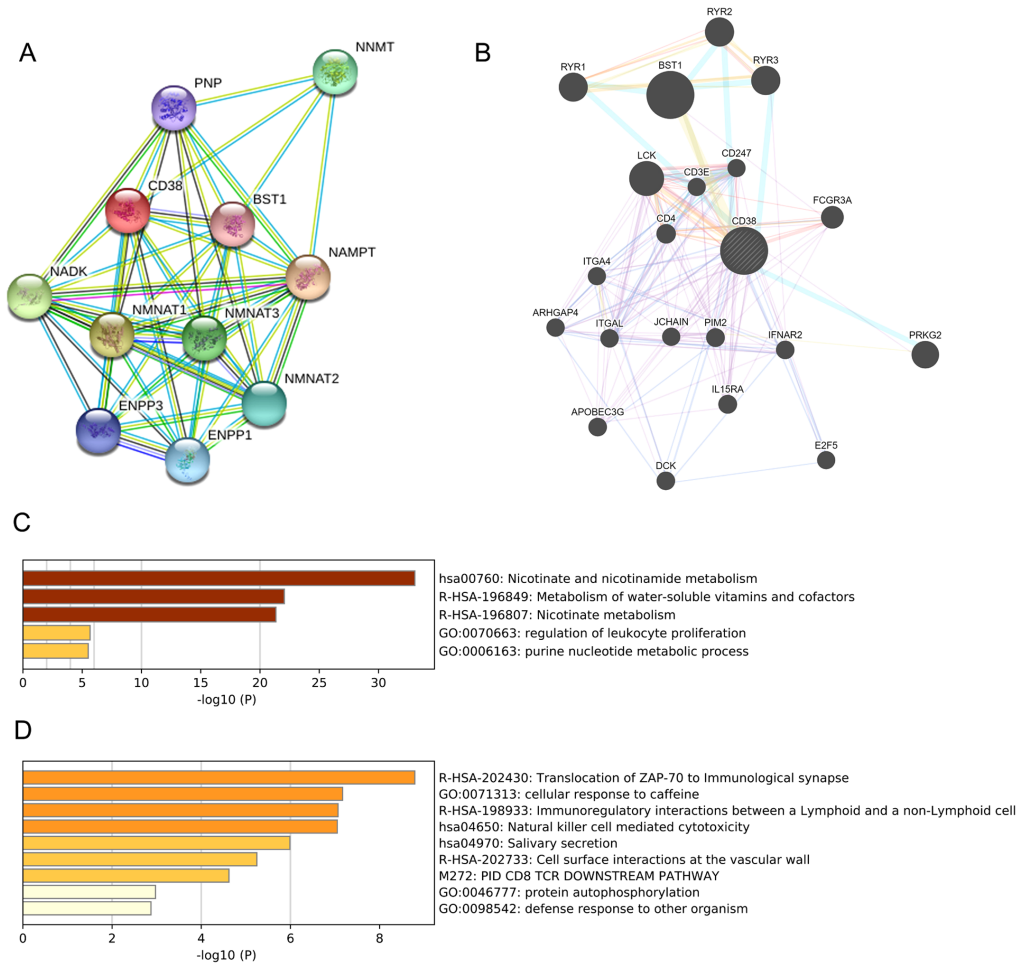


Figure 6. PPI networks and GO enrichment analysis of CD38. (A) PPI network of CD38 and its interactive genes. (B) PPI network of CD38 generated using GeneMANIA. (C) GO enrichment analysis of CD38 and the top 10 interactive genes obtained from the PPI network of CD38 and its interactive genes. (D) GO enrichment analysis of CD38 and the top 20 interactive genes obtained from the PPI network of CD38 generated using GeneMANIA. PPI, protein-protein interaction; GO, Gene Ontology; CD38, cluster of differentiation 38.

compared with healthy tissues. Furthermore, the results indicated that high expression of CD38 may serve as a biomarker for SKCM metastasis. In addition, several specific mutations of CD38 were identified in patients with SKCM. In particular, W125 is one of the key residues, which is responsible for nucleosidase activity of CD38 (30), P184 is a common SNP (11), and the cysteine residues between C275 and C254 form a disulfide bond within CD38 (11). The aforementioned mutations or polymorphisms might increase the risk of developing SKCM. In addition, a positive correlation between CD38 expression levels and survival probability in patients with SKCM was identified. The results suggested that higher CD38 expression levels resulted in improved survival probability, which could affect the sensitization of chemotherapy drugs or antibodies.

To the best of our knowledge, the present study also screened the top 50 correlated genes with CD38 in patients with SKCM for the first time, which might be implicated in the prognosis of SKCM. However, further studies are required to identify the molecular mechanism involved and the possible applications of the correlated genes in SKCM. The PPI network generated using the STRING online tool also identified nine CD38-interacting genes for correlation analysis in SKCM. The GO enrichment analysis revealed that the nine correlated genes (CD86, CD80, CD27, CD2, IL21R, IL2RB, ITGAL, CCL4 and TBX21) were involved in the regulation of T cell differentiation and lymphocyte activation. In particular, a previous study also identified CD2 as a correlated gene, indicating that CD2 might be an independent predictor of disease recurrence and OS in patients with primary cutaneous melanoma (27).

From the PPI network analysis, STRING and GeneMANIA identified 10- and 20-associated genes, respectively. Among the genes identified using STRING were genes from diverse functional groups, such as nicotinate-nucleotide adenylyltransferase activity (NMNAT1, NMNAT2 and NMNAT3), NAD⁺ nucleotidase and cyclic ADP-ribose generating [bone marrow stromal cell antigen-1 (BST1)] and pyrimidine metabolism (PNP, ENPP1 and ENPP3) genes. Several genes, such as NMNAT1, NMNAT2 and NMNAT3, have been previously identified to be involved in catalyzing NAD⁺ synthesis in the nicotinate and nicotinamide metabolism pathways (31). The genes predicted using GeneMANIA were from various functional enrichments, such as response to virus (LCK, CD247, CD4, PIM2 and IFNAR2) and cellular response to alkaloid (RYR1, RYR2 and RYR3). BST1 was predicted as a CD38-associated protein by both databases. It has been reported that some SNPs in BST1 serve as predictors for Parkinson's disease (32). Moreover, a previous study revealed that a deletion involving CD38 and BST1 facilitates a fusion transcript in a patient with autism and asthma (33). GeneMANIA also revealed that ITGAL was associated with CD38, which is consistent with the correlation analysis from GEPIA2, and further supports the findings of a previous study that indicated that ITGAL might serve as a prognostic factor in patients with R0 resected Dukes stage B and C colorectal cancer (34). A previous study also investigated ITGAL as a prognostic factor in the survival of men with castration-resistant prostate cancer (CRPC), and SNPs in ITGAL may be associated with the risk of death in men with CRPC (35).

Nevertheless, further investigation is required to determine the potential mechanism and application value of the

aforementioned CD38-associated genes in SKCM, including CD2, BST1 and ITGAL. In a previous study, photodynamic therapy was implemented for human melanoma (5,6). Further studies developed related photodynamic therapy schemes and identified possible mechanisms (36,37). Recent reports also demonstrate that targeting CD38 enhances antileukemic activity (38,39). Therefore, CD38-targeted photodynamic therapy for SKCM requires further investigation.

In summary, the present study identified that CD38 was highly expressed in patients with SKCM and was associated with SKCM metastasis. Moreover, elevated expression of CD38 was associated with OS in patients with SKCM. The present study might aid the identification of the mechanism underlying SKCM progression and advance the development of antagonist CD38 strategies for patients with SKCM. However, a key limitation of the present study was that the data obtained from TCGA-SKCM lacked analysis of patients with CD38 positive and negative expression.

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Availability of data and materials

The dataset GDC TCGA Melanoma (SKCM) generated and/or analyzed during the present study are available in The Cancer Genome Atlas repository (<https://portal.gdc.cancer.gov/>).

Authors' contributions

XW and SH designed the study. XW and PW drafted the manuscript. XW, PW, LG, JW and SMASN performed the research and analyzed the data. XW, PW and LG revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved and consented by the Ethics Committee of the Second Affiliated Hospital of Yangtze University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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