Upregulated expression of SAC3D1 is associated with progression in gastric cancer

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Abstract. SAC3 domain containing 1 (SAC3D1) has been reported to be involved in numerous types of cancer. However, the role of SAC3D1 in GC has not yet been elucidated. In the present study, the mRNA expression level of SAC3D1 between GC and normal tissues were assessed with a continuous variable meta-analysis based on multiple datasets from public databases. The protein expression level of SAC3D1 in GC and normal tissues was assessed by an in-house immunohistochemistry (IHC). The association between SAC3D1 expression and some clinical parameters was assessed based on the TCGA and IHC data. Survival analysis was performed to assess the association between SAC3D1 expression and the survival of GC patients. The co-expressed genes of SAC3D1 were determined by integrating three online tools, and the enrichment analyses were performed to determine SAC3D1-related pathways and hub co-expressed genes. SAC3D1 was significantly upregulated in GC tumor tissues in comparison to normal tissues with the SMD being 0.45 (0.12, 0.79). The IHC results also indicated that SAC3D1 protein expression in GC tissues was markedly higher than in normal tissues. The SMD following the addition of the IHC data was 0.59 (0.11, 1.07). The protein levels of SAC3D1 were positively associated with the histological grade, T stage and N stage of GC (P<0.001). The TCGA data also revealed that the SAC3D1 mRNA level was significantly associated with the N stage (P<0.001). Moreover, prognosis analysis indicated that SAC3D1 was closely associated with the prognosis of patients with GC. Moreover, 410 co-expressed genes of SAC3D1 were determined, and these genes were mainly enriched in the cell cycle. In total, 4 genes (CDK1, CCNB1, CCNB2 and CDC20) were considered key co-expressed genes. On the whole, these findings demonstrate that SAC3D1 is highly expressed in GC and may be associated with the progression of GC.

Introduction

Gastric cancer (GC) is a common malignant tumor of the digestive system that originates in the gastric mucosal epithelium. GC is a frequently diagnosed type of cancer and is an important leading cause of cancer-related mortality according to the cancer statistics of 2019 (1). Currently, the majority of patients with early-stage GC have a relatively long-term survival time after selecting surgery as a principal treatment option (2-4). In recent years, a program combining immunotherapy, molecular targeted therapy and neoadjuvant chemoradiotherapy has been shown to be a promising treatment method for GC (5-9). However, the molecular mechanisms associated with the occurrence and progression of GC remain unclear. Therefore, the exploration of cancer-related genes and specific molecular targets for the effective treatment of GC is imperative.

SAC3 domain containing 1 (SAC3D1) is a protein-coding gene located on chromosome 11 and is widely found in the cytoplasm, cytoskeleton, microtubule tissue center, centrosome and spindle (10). SAC3D1 has been reported to be abnormally expressed in multiple types of cancer and may be associated with the occurrence or progression of numerous types of cancer. A previous study reported that SAC3D1 may serve as a prognostic biomarker in hepatocellular carcinoma by combining the data of Gene Expression Omnibus (GEO), The Cancer Genome Atlas and International Cancer Genome Consortium (11). The prognostic value of SAC3D1 has also been demonstrated in colon cancer (12). You *et al* reported that SAC3D1 was associated with SLC2A5-inhibited adjacent lung adenocarcinoma cytoplasmic pro-B cell progression (13). However, the role and molecular mechanisms of action of SAC3D1 in GC have not

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yet been reported. According to a preliminary calculation with TCGA RNA-seq data, SAC3D1 was found to be significantly abnormally expressed in GC. Thus, it was speculated that SAC3D1 may play a pivotal clinical role in GC.

In the present study, GC microarray data and RNA-seq data were integrated to assess the mRNA expression of SAC3D1 in GC, and an in-house immunohistochemistry (IHC) was performed to further validate the protein expression level of SAC3D1. The co-expressed genes of SAC3D1 in GC were also collected and the possible molecule molecular mechanisms of action of SAC3D1 were analyzed by bioinformatics methods (Fig. 1).

Materials and methods

Data sources and processing. GC microarray and RNA-seq data were screened from the Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) (14), Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) (15), ArrayExpress(http://www.ebi.ac.uk/arrayexpress/) (16) and Oncomine (https://www.oncomine.org/resource/main.html) (17) databases with the following keywords: ('gastric' OR 'stomach' OR 'gastrointestinal') AND ('cancer' OR 'carcinoma' OR 'tumor' OR 'adenocarcinoma'). The inclusion criteria were as follows: First, the experimental group and the control group should be human GC samples and healthy samples, respectively. Second, lymph node metastasis and distant metastasis tissues were also included in the present study. Third, the calculated mRNA expression data should be provided by all included datasets. The information of included GC microarray and RNA-seq data is presented in Table I. Besides, microarray and RNA-seq data with prognostic data were screened separately for prognostic-related analysis. The mRNA expression matrix data of each dataset were downloaded, and the mRNA expression data of SAC3D1 were extracted. The SAC3D1 expression data underwent a log2 transformation and were divided into cancer groups and normal groups. The GC RNA seq data of the TCGA database were downloaded from UCSC Xena (https://xena.ucsc.edu/), which included sequencing data of 373 GC and 32 normal tissues. The data were processed as microarray data. The GC-related clinical parameters, including sex, grade, age, TNM stage and survival data, were also acquired from UCSC Xena.

In-house IHC. The tissue array that included 179 cases of GC tissues and 147 normal tissues was purchased from Pantomics, Inc. and some clinical information for each sample, such as age, sex, tumor pathological grade and clinical stage, were also provided. In the IHC analysis, SAC3D1 was detected with anti-SAC3D1 antibody (at a 1/500 dilution; cat. no. ab122809, Abcam's RabMAb technology). The SAC3D1 expression intensity for each sample was evaluated based on the score, and the score was generated from the product of the proportion of stained cells among all cells (0, <5%; 1, 5-25%; 2, 25-50%; 3, 50-75%; 4, >75%) and the staining degree of the positive cells (0, no staining; 1, light yellow or yellow; 2, brown; 3, dark brown) (18). Images were captured using an optical microscope (Motic China Group Co., Ltd.). Moreover, to improve the accuracy of results, Image-Pro Plus version 6.0 software (Media Cybernetics, Inc.) was also used to evaluate the area and density of the dyed region and the integrated optical density (IOD) value of the IHC section. The mean densitometry of the digital image (magnification, x400) was regarded as representative SAC3D1 staining intensity (indicating the relative SAC3D1 expression level). The IOD values of the tissue areas from 179 cases of gastric cancer tissues and 147 normal tissues randomly selected fields were calculated counted in a blinded manner and subjected to statistical analysis.

Mutations of the SAC3D1 in GC. Genetic alterations of SAC3D1 in GC were investigated based on high throughput data in cBioPortal for Cancer Genomics (cBioportal) (http://cBioportal.org) and Catalogue Of Somatic Mutations In Cancer (COSMIC) (https://cancer.sanger.ac.uk/cosmic), including missense mutation, truncating mutation, deep deletion, and amplification.

Acquisition of co-expressed genes of SAC3D1 in GC. The co-expressed genes of SAC3D1 were obtained from the Multi Experiment Matrix (https://biit.cs.ut.ee/mem/index.cgi) (19) and COXPRESdb (http://coxpresdb.jp) (20). In the Multi Experiment Matrix, $P<0.05$ was regarded as statistically significant. In COXPRESdb, 2000 was set as the upper limit. In addition, GC-related differentially expressed genes were calculated with the edgeR package based on TCGA and GTEx data, and a log (fold change) equal to 1 and $P<0.05$ was defined as including condition. The overlapped genes of three parts were considered co-expressed genes of SAC3D1 in GC.

Enrichment and protein-protein interaction (PPI) analysis. The genes co-expressed with SAC3D1 were submitted to DAVID (https://david.ncifcrf.gov/) (21) for an enrichment analysis, including gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. STRING (https://string-db.org/) (22) was utilized to construct a PPI network, and based on the degree of nodes, hub co-expressed genes of SAC3D1 were identified.

Validation of hub co-expressed genes. The expression of hub co-expressed genes was further validated at the mRNA level based on the microarray and RNA-seq data via a meta-analysis and the protein expression levels of hub co-expressed genes were verified in Human Protein Atlas (HPA) (https://www.proteinatlas.org/) (23). The sensitivity and specificity of hub co-expressed genes on differentiating GC tissues and normal tissues were also calculated. Besides, genetic alterations of the hub co-expressed genes in GC were also investigated in cBioportal. A prognosis related meta-analysis was also conducted to assess the prognosis value of hub co-expressed gene, respectively. Moreover, the expression relationship between SAC3D1 and hub co-expressed genes was presented by correlation analysis.

Statistical analysis. Independent and paired sample t-tests were performed in SPSS 19.0 to calculate and evaluate the expression level of SAC3D1 in GC tissues and normal tissues based on the GC microarray data, RNA seq data and IHC data. Stata 12.0 was used to perform a continuous variable meta-analysis and calculate the value of SMD. One-way analysis of variance was used in the present study to compare the differences in the mean of three or more sets of data. Bonferroni and Tamhane's T2 were used as post hoc tests for equal variance assumed and equal variance not assumed, respectively. In addition, the sensitivity and specificity of SAC3D1 on differentiating GC tissues and

Table I. SAC3D1 expression profile based on immunohistochemistry data, GEO datasets and TCGA sequencing data.

				Patients			Normal				
Datasets	Country	Year	Platform	Number	Mean	SD	Number	Mean	SD	t-value	P-value
GSE103236	Romania	2017	GPL4133	10	10.127	0.70021	9	9.3167	0.423	-3.008	0.008
GSE81948	Italy	2017	GPL6244	15	7.5101	0.12937	5	7.5443	0.10822	0.53	0.603
GSE54129	China	2017	GPL570	111	6.9017	0.51905	21	6.9055	0.26462	0.05	0.96
GSE26942	USA	2016	GPL6947	205	8.9493	0.71607	12	9.0224	0.37734	0.61	0.551
GSE84787	China	2016	GPL17077	10	9.758	3.58558	10	9.7934	2.84105	0.024	0.981
GSE64951	USA	2015	GPL570	63	7.6095	1.74653	31	7.1908	2.02533	-1.036	0.303
GSE63089	China	2014	GPL5175	45	7.1186	0.52955	45	7.0702	0.53337	-0.432	0.667
GSE56807	China	2014	GPL5175	5	7.0561	0.24711	5	6.9774	0.32808	-0.428	0.68
GSE29272	USA	2013	GPL96	134	7.0223	0.52461	134	6.3874	0.29172	12.244	<0.001
GSE38940	Argentina	2012	GPL5936	34	0.0224	0.31734	31	0.0745	0.47533	0.515	0.609
GSE33429	China	2012	GPL5175, GPL9128	25	4.9522	0.14036	25	5.0153	0.11872	1.715	0.093
GSE20143	India	2010	GPL9365	5	-1.0585	0.60379	2	-0.8016	0.23093	0.559	0.601
GSE13911	Italy	2008	GPL80	38	9.3052	1.38313	31	7.1942	1.57059	-5.857	<0.001
GSE2685	Japan	2005	GPL571	22	7.0903	0.17473	8	7.0079	0.27967	-0.968	0.341
GSE109476	China	2018	GPL24530	5	11.5194	0.3444	5	11.1203	0.52596	-1.42	0.194
GSE112369	Japan	2018	GPL15207	37	9.0061	0.4449	25	8.6954	0.40925	-2.784	0.007
GSE26899	USA	2016	GPL6947	96	9.4018	0.6073	12	9.0224	0.37734	3.0272	0.007
GSE79973	China	2016	GPL570	8	9.3585	0.3251	9	8.5798	0.60777	3.229	0.0056
TCGA	-	-	-	373	17.3966	0.78827	32	16.8133	0.34279	-7.984	<0.001
IHC	-	-	-	179	10.1899	1.93074	147	3.2381	2.77793	26.57	<0.001

SAC3D1, SAC3 domain containing 1.

normal tissues were evaluated by drawing ROC curves in GraphPad Prism5 based on microarray data, RNA seq data, and IHC data. Stata 12.0 was also used to integrate the results of each ROC with a summary ROC. Finally, a Spearman's correlation analysis was used to examine the relationship between the expression of SAC3D1 and core co-expressed genes.

Results

Expression and clinical value of SAC3D1 in GC based on chips and RNA-seq data. First, a total of 18 eligible GEO chips and a section of TCGA sequencing data were collected, including 1,241 gastric cancer samples and 452 normal samples, from which the expression data of SAC3D1 was extracted. The expression of SAC3D1 in each chip or section of TCGA sequencing data was clarified through independent or paired sample t-tests. For the GEO chips, 5 chips (GSE103236, GSE29272, GSE13911, GSE112369 and GSE26899) exhibited a significantly upregulated trend of SAC3D1 in GC. For the TCGA sequencing data, SAC3D1 was found to be upregulated in 373 gastric cancer tissues (17.3966±0.78827) compared to 32 normal tissues (16.8133±0.34279, P<0.001) (Table I and Fig. 2). To further improve the accuracy of the results, the results of t-tests based on 18 eligible GEO chips and a section of TCGA sequencing data were integrated by a continuous variable meta-analysis. The results indicated that SAC3D1 was clearly upregulated in GC tissues with the SMD of the random effect model being 0.45 (0.12, 0.79), and the funnel plot indicated that there was no publication bias (Fig. 3A and B). The ROC of all chips and RNA-seq data was calculated (Table II and Fig. 4), and the AUC of sROC was 0.71 (0.67, 0.75), with pooled sensitivity and specificity being 0.68 (0.61, 0.74) and 0.66 (0.60, 0.72) (Fig. 5A and B). The prognosis-related meta-analysis indicated that the overexpression of SAC3D1 was closely associated with the poor prognosis of patients with GC [HR, 2.83 (2.25, 3.57); P<0.001] (Fig. 3E).

Expression and clinical value of SAC3D1 in GC based on chips, RNA seq data and IHC data. The protein expression of SAC3D1 was clearly high expressed in 179 GC tissues compared with 147 paracancerous tissues (Fig. 2T). The results of t-tests based on IHC data, 18 eligible GEO chips and a section of TCGA RNA-seq data were also merged by a meta-analysis. An upregulation of SAC3D1 was finally determined with the SMD of the random effect model being 0.59 (0.11, 1.07), and a corresponding funnel plot indicated that there was no publication bias (Fig. 3C and D). After constructing the sROC curve based on the IHC data, 18 eligible GEO chips and a section of TCGA RNA-seq data, it was found that SAC3D1 has a certain potential to be identified as a molecular indicator to identify GC tissues and normal tissues, and the sensitivity and specificity was 0.72 (0.63, 0.79) and 0.68 (0.62, 0.74), respectively (Fig. 5C and D). Moreover, it was found that the positive ratio of SAC3D1 staining was comparable with the original methods using Image-Pro Plus version 6.0 software (Fig. S1 and Table SI).

Table II. Potential of SAC3D1 to serve as a bio-marker on identifying gastric cancer tissues and normal tissue.

Datasets	Sensitivity	Specificity	TP	FP	FN	TN
GSE103236	80.00%	77.80%	8	2	2	7
GSE81948	53.33%	60.00%	8	2	7	3
GSE54129	53.15%	61.90%	59	8	52	13
GSE26942	51.71%	66.67%	106	4	99	8
GSE84787	60.00%	70.00%	6	3	4	7
GSE64951	58.73%	54.84%	37	14	26	17
GSE63089	57.78%	55.56%	26	20	19	25
GSE56807	80.00%	60.00%	4	2	1	3
GSE29272	82.09%	74.63%	110	34	24	100
GSE38940	64.71%	51.61%	22	15	12	16
GSE33429	64.00%	64.00%	16	9	9	16
GSE20143	80.00%	50.00%	4	1	1	1
GSE13911	86.84%	83.87%	33	5	5	26
GSE2685	63.64%	62.50%	14	3	8	5
GSE109476	80.00%	80.00%	4	1	1	4
GSE112369	62.16%	68.00%	23	8	14	17
GSE26899	62.50%	75.00%	60	3	36	9
GSE79973	100.00%	88.89%	8	1	0	8
TCGA	72.39%	65.63%	270	11	103	21
IHC	96.65%	86.39%	173	20	6	127

TP, true positive; FP, false positive; FN, false negative; TN, true negative; SAC3D1, SAC3 domain containing 1.

Table III. Association between SAC3D1 expression and some clinical pathological parameters based on immunohistochemistry data.

Clinicopathological parameters	Group	SAC3D1 expression		t-value	P-value
		Cases	Mean ± SD		
Tissue	GC tissue	179	10.1899±1.93074		
	Normal tissue	147	3.2381±2.77793	26.57	P<0.001
Age (years)	≤50	46	10.3043±2.22979		
	>50	128	10.1484±1.83616	0.466	0.642
Sex	Male	128	10.125±1.99606		
	Female	46	10.3696±1.7933	0.731	0.466
T	T1-T2	54	9.1111±1.9683		
	T3-T4	120	10.675±1.73041	-5.029	P<0.001
N	N0	65	9.1692±1.98879		
	N1	87	10.7356±1.69445		
	N2	22	11.0455±1.43019	17.277	P<0.001
Stage	IA-IB	38	8.7105±1.99875		
	IIA-IIB	117	10.5043±1.75491		
	IIIA	19	11.2105±1.35724	18.192	P<0.001
Histological grade	I	28	8.5714±2.1846		
	II	56	10.25±1.77098		
	III	63	10.8125±1.62202	F=15.261	P<0.001

SAC3D1, SAC3 domain containing 1.

Association of SAC3D1 expression with clinical parameters. According to the IHC data, the upregulation of SAC3D1 was statistically associated with the histological grade, clinical stage, T stage and N stage of GC. In a more advanced stage of

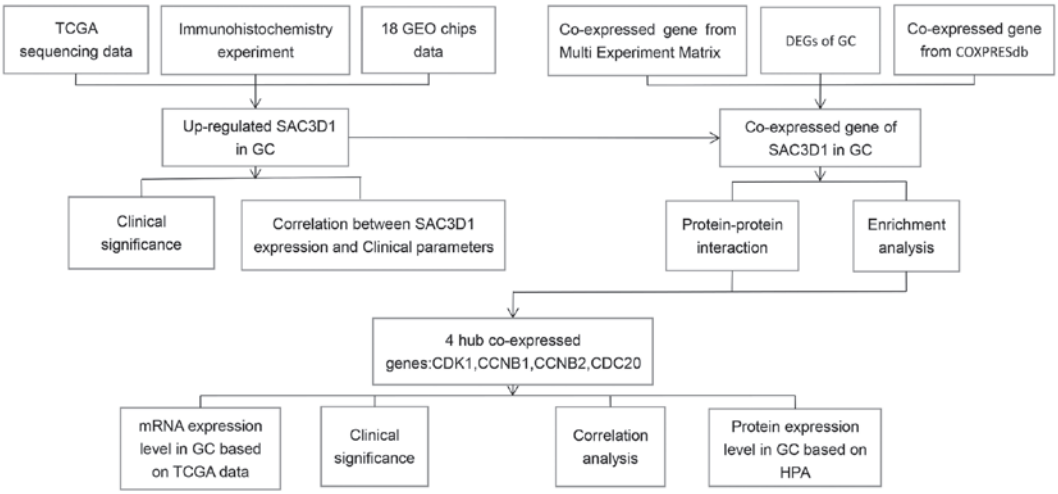

Figure 1. The main design of the present study. This study included the assessment of SAC3D1 expression in gastric cancer and the co-expressed genes of SAC3D1 in gastric cancer. SAC3D1, SAC3 domain containing 1.

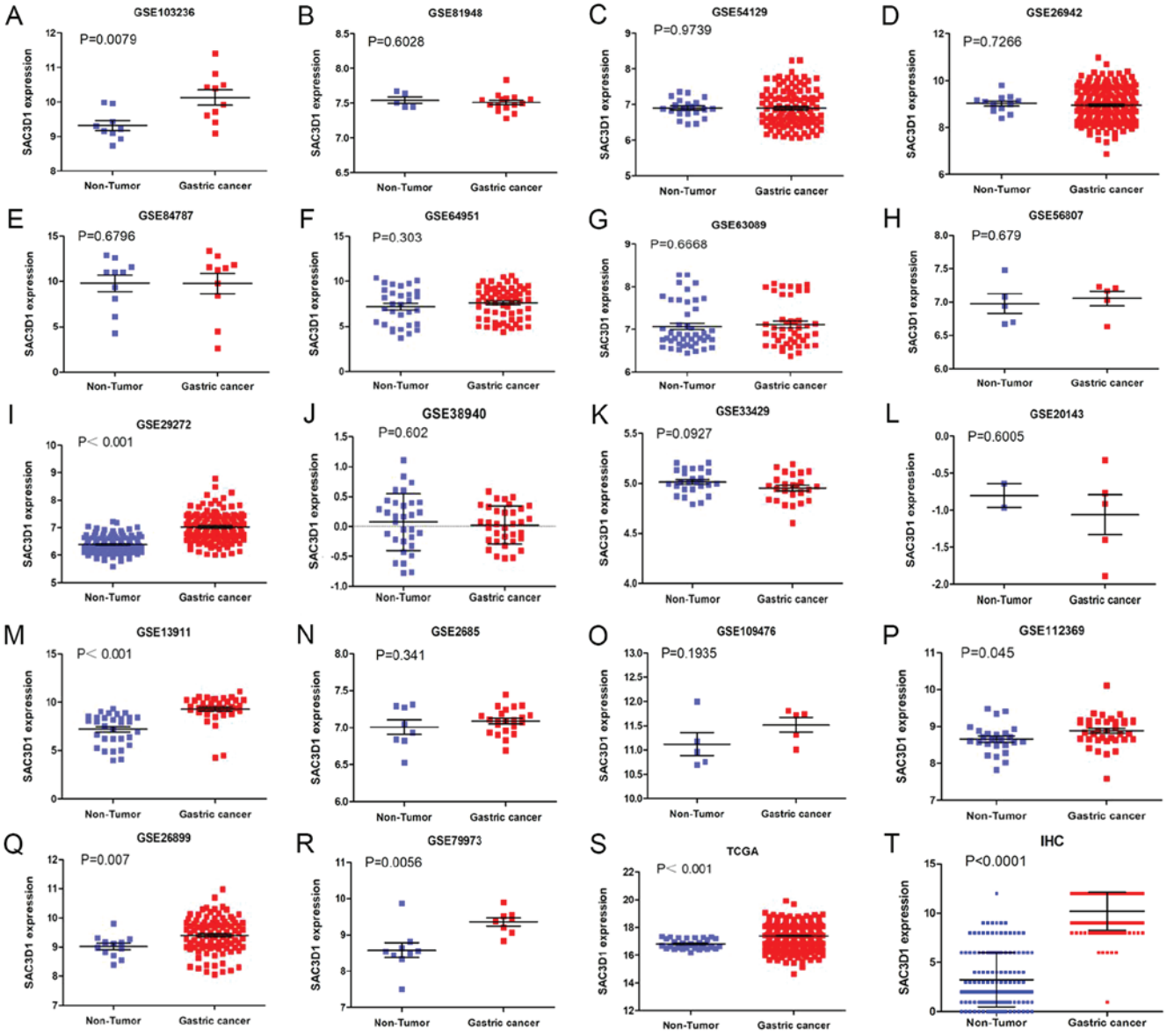

Figure 2. Scatterplots of SAC3D1 based on IHC data, GEO datasets and TCGA sequencing data. (A) GSE103236, (B) GSE81948, (C) GSE54129, (D) GSE26942, (E) GSE84787, (F) GSE64951, (G) GSE63089, (H) GSE56807, (I) GSE29272, (J) GSE38940, (K) GSE33429, (L) GSE20143, (M) GSE13911, (N) GSE2685, (O) GSE109476, (P) GSE112369, (Q) GSE26899, (R) GSE79973, (S) TCGA, (T) IHC. SAC3D1, SAC3 domain containing 1.

Table IV. Association between SAC3D1 expression and some clinical pathological parameters based on TCGA data.

	SAC3D1 expression			
Clinicopathological parameters	n	Mean ± SD	t-value	P-value
Tissue				
Non-tumor	32	16.8133±0.34279		
GC	373	17.3966±0.78827	-7.984	<0.001
Sex				
Male	258	17.3224±0.7312		
Female	143	17.4426±0.82768	1.504	0.133
Age (years)				
<60	124	17.3489±0.74119		
≥60	273	17.3752±0.78175	-0.316	0.752
Grade				
G1	11	17.094±1.12374		
G2	147	17.3488±0.7554		
G3	235	17.3891±0.76078		
Gx	8	17.3374±0.72202	F=0.557	0.644
TNM				
T1-T1b	25	17.2418±0.75401		
T2-T2b	88	17.3177±0.85172		
T3	179	17.452±0.72991		
T4-T4b	105	17.3393±0.71874	F=1.117	0.342
N0	121	17.4878±0.77501		
N1	104	17.3645±0.74107		
N2	85	17.361±0.63662		
N3-N3b	74	17.3808±0.79604		
Nx	16	16.3963±0.82501	F=7.596	<0.001
M0	352	17.393±0.73777		
M1	27	17.1293±1.08533		
Mx	22	17.2109±0.7571	F=1.957	0.143
I-IB	59	17.397±0.84514		
Stage				
II-IIB	124	17.4667±0.69269		
III-IIIC	156	17.4222±0.64408		
IV	42	17.2256±0.98701	F=1.142	0.332

SAC3D1, SAC3 domain containing 1.

the disease, or histological grade, the protein expression intensity of SAC3D1 was stronger than that in low-stage or grade. Thus, it was speculated that SAC3D1 may be involved in the development and progression of GC (Fig. 6 and Table III). In addition, the association between SAC3D1 and some clinical parameters was also calculated using the TCGA data, and the results indicated that the expression of SAC3D1 was associated with the N stage (Table IV, F=7.596, P<0.001).

Genetic alterations of the SAC3D1 in GC. From the online analysis of cBioPortal and COSMIC, it was found that SAC3D1 has a mutation in GC, although the genetic alteration rate was relatively low. Therefore, it was hypothesized that the role of highly expressed SAC3D1 in the development of GC may not be mutated, amplification-mediated (Fig. 7).

Enrichment and PPI analysis of co-expressed gene of SAC3D1. A total of 8,364 and 2,000 co-expressed genes of SAC3D1 were obtained in the Multi Experiment Matrix (https://biit.cs.ut.ee/mem/index.cgi) and COXPRESdb, respectively. In addition, 4,640 GC-related differentially expressed genes were acquired after TCGA and GTEx data calculations. Finally, 410 overlapping genes of 3 parts were considered co-expressed genes of SAC3D1 in GC (Fig. 8A). The GO-enriched analysis indicated that SAC3D1 and co-expressed genes were mainly enriched in mitotic sister chromatid segregation, nuclear chromosome and ATP binding (Table V and Fig. 8C-F). In the KEGG pathway analysis, the SAC3D1 and co-expressed genes were mainly enriched in DNA replication and the cell cycle (Table VI and Fig. 8B and G). The PPI network indicated that CDK1,

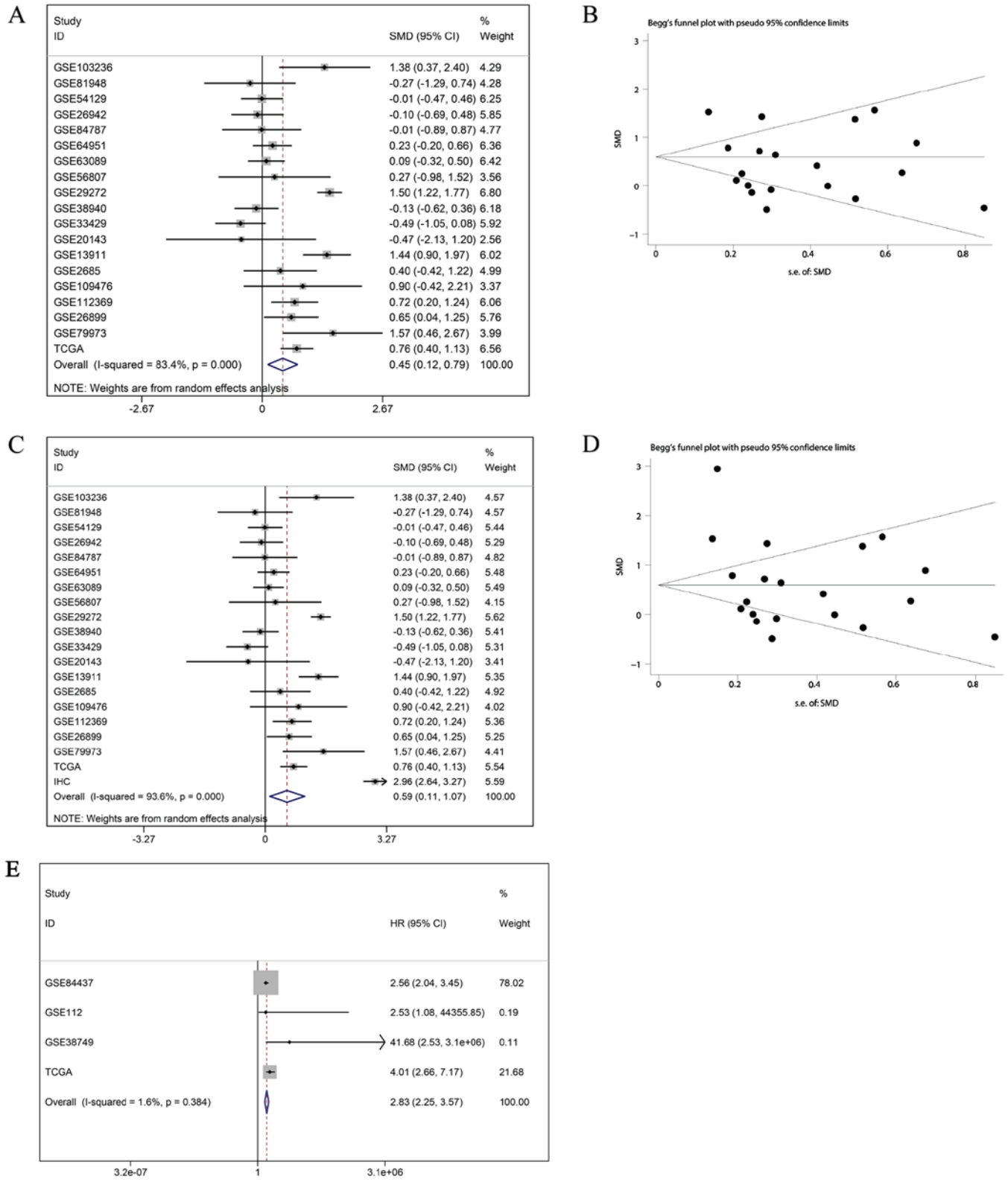

Figure 3. Expression level and survival analysis of SAC3D1 based on GEO datasets, TCGA RNA-seq data and IHC data. (A) Forest plot based on a random effect model, (B) funnel plot, (C) forest plot based on a random effect model after the adjunction of IHC data, (D) corresponding funnel plot after the adjunction of IHC data, (E) forest plot of prognosis related meta-analysis. SAC3D1, SAC3 domain containing 1.

CCNB1, CCNB2 and CDC20 were the hub co-expressed genes of SAC3D1 in GC (Fig. 9A and B).

Validation of hub co-expressed genes based on TCGA and HPA. Various types of mutations of the 4 hub co-expressed genes (CDK1, CCNB1, CCNB2 and CDC20) were observed in GC (Fig. 9C). CDK1, CCNB1, CCNB2 and CDC20 were evidently highly expressed in GC based on the microarray and RNA-seq data mRNA expression data (Fig. 10A-D) and CDK1, CCNB1, CCNB2 and CDC20 may also serve as

Table V. The top 10 GO items associated with SAC3D1 and its co-expressed genes.

Category	ID	Term	Count	P-value
BP	GO:0051301	Cell division	69	6.42E-44
BP	GO:0006260	DNA replication	48	5.22E-40
BP	GO:0007067	Mitotic nuclear division	49	6.14E-31
BP	GO:0000082	G1/S transition of mitotic cell cycle	33	4.98E-28
BP	GO:0007062	Sister chromatid cohesion	32	1.46E-26
BP	GO:0006270	DNA replication initiation	19	6.20E-22
BP	GO:0006281	DNA repair	35	5.10E-18
BP	GO:0000086	G2/M transition of mitotic cell cycle	26	6.69E-16
BP	GO:0000070	Mitotic sister chromatid segregation	14	1.42E-15
BP	GO:0000722	Telomere maintenance via recombination	14	8.38E-14
CC	GO:0005654	Nucleoplasm	189	7.71E-53
CC	GO:0005634	Nucleus	211	1.05E-22
CC	GO:0000776	Kinetochore	23	1.67E-18
CC	GO:0000777	Condensed chromosome kinetochore	23	9.02E-18
CC	GO:0000922	Spindle pole	25	9.22E-18
CC	GO:0000775	Chromosome, centromeric region	19	1.14E-16
CC	GO:0005829	Cytosol	141	2.46E-16
CC	GO:0005813	Centrosome	41	6.89E-15
CC	GO:0030496	Midbody	22	6.37E-13
CC	GO:0005819	Spindle	21	1.70E-12
MF	GO:0005515	Protein binding	305	5.16E-29
MF	GO:0005524	ATP binding	80	7.62E-13
MF	GO:0003682	Chromatin binding	35	2.18E-11
MF	GO:0019901	Protein kinase binding	34	3.39E-11
MF	GO:0043142	Single-stranded DNA-dependent ATPase activity	7	2.59E-08
MF	GO:0008017	Microtubule binding	21	6.22E-08
MF	GO:0003677	DNA binding	72	1.66E-07
MF	GO:0003697	Single-stranded DNA binding	14	1.79E-07
MF	GO:0003684	Damaged DNA binding	11	1.44E-06
MF	GO:0003777	Microtubule motor activity	12	1.88E-06

SAC3D1, SAC3 domain containing 1.

Table VI. The 10-most KEGG pathways associated with SAC3D1 and its co-expressed genes.

Category	ID	Term	P-value
KEGG	hsa04110	Cell cycle	1.05E-31
KEGG	hsa03030	DNA replication	2.24E-19
KEGG	hsa00240	Pyrimidine metabolism	1.25E-08
KEGG	hsa03430	Mismatch repair	1.01E-07
KEGG	hsa04115	p53 signaling pathway	1.78E-06
KEGG	hsa04114	Oocyte meiosis	1.87E-06
KEGG	hsa03460	Fanconi anemia pathway	1.20E-05
KEGG	hsa03410	Base excision repair	2.55E-05
KEGG	hsa03420	Nucleotide excision repair	2.71E-04
KEGG	hsa05203	Viral carcinogenesis	5.13E-04

SAC3D1, SAC3 domain containing 1.

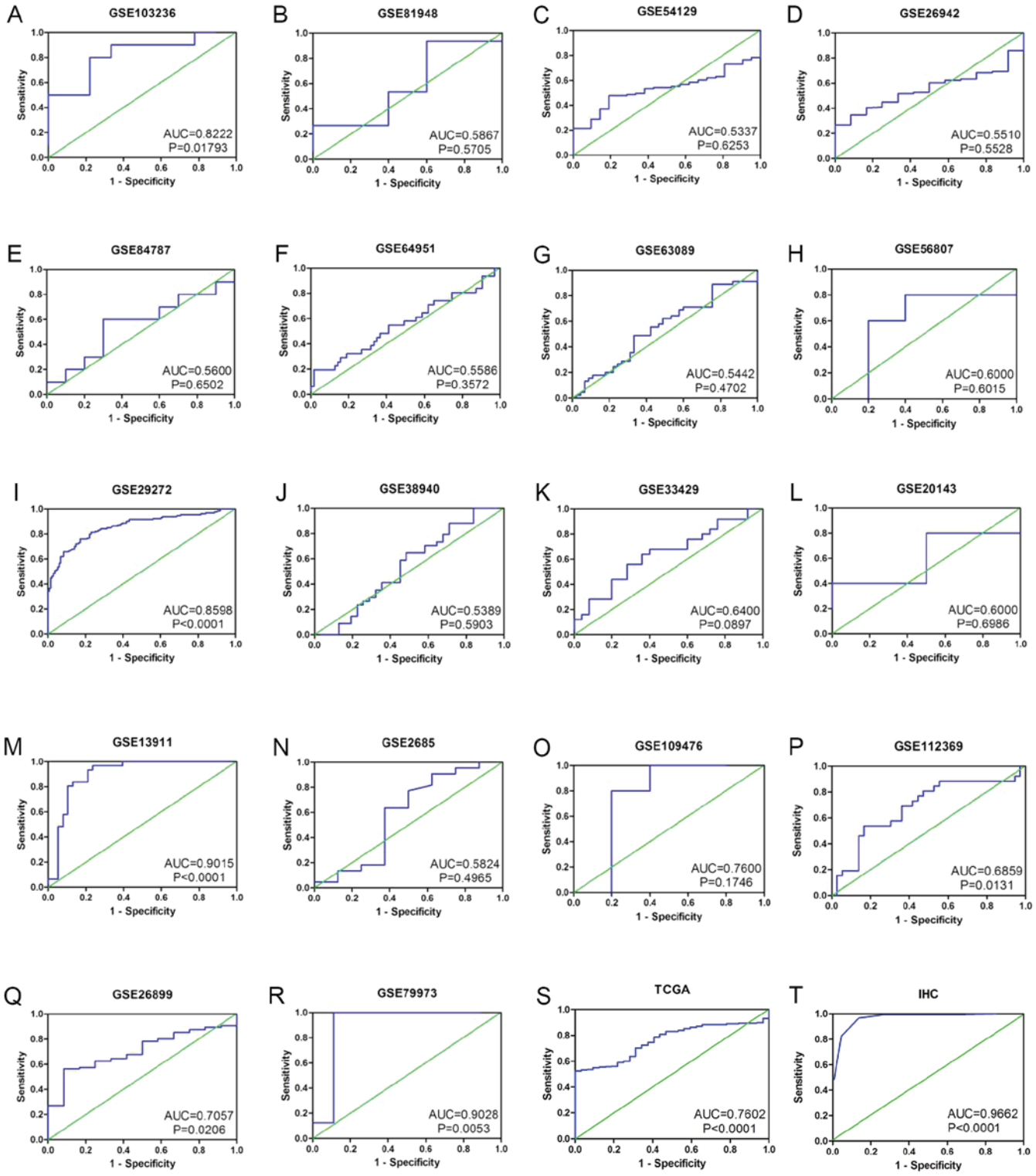

Figure 4. ROC curves of SAC3D1 based on GEO datasets, TCGA RNA seq data, and IHC data. (A) GSE103236, (B) GSE81948, (C) GSE54129, (D) GSE26942, (E) GSE84787, (F) GSE64951, (G) GSE63089, (H) GSE56807, (I) GSE29272, (J) GSE38940, (K) GSE33429, (L) GSE20143, (M) GSE13911, (N) GSE2685, (O) GSE109476, (P) GSE112369, (Q) GSE26899, (R) GSE79973, (S) TCGA, (T) IHC. SAC3D1, SAC3 domain containing 1.

biomarkers differentiating GC tissues and normal tissues with a relative high sensitivity and specificity (Fig. 10E-H). The high expression trends of these 4 genes were also observed in protein expression data based on the HPA database (Fig. 11). These genes were risk factors affecting the prognosis of gastric cancer (Fig. 12A-D). Moreover, Spearman's correlation analysis indicated that there were significant positive correlations between SAC3D1 and these core co-expressed genes (Fig. 12E-H).

Discussion

In the present study, the expression of SAC3D1 in GC was determined by integrated and thoroughly re-processed

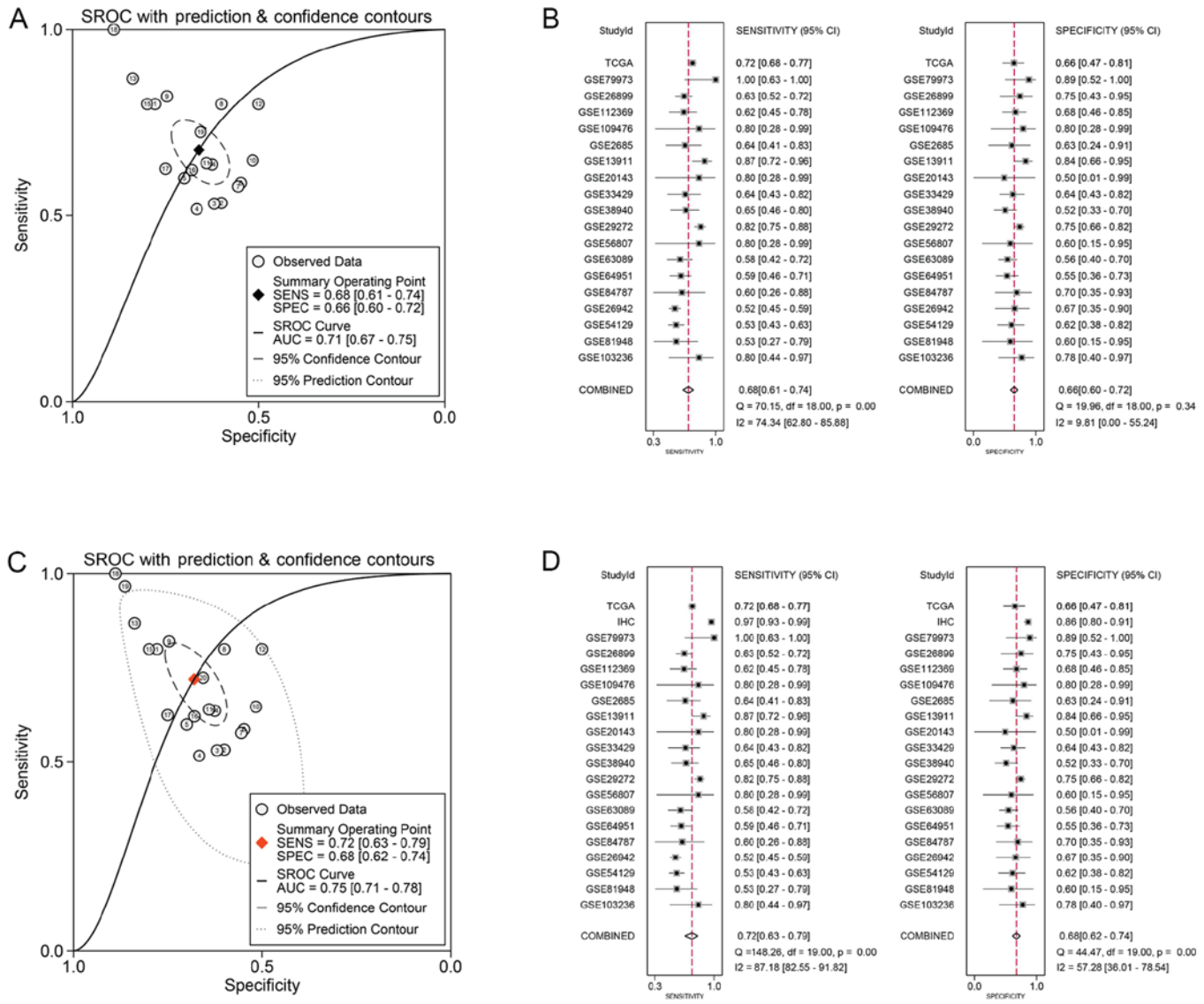

Figure 5. Validation of the ability of SAC3D1 to identify gastric cancer tissues and normal tissues. (A) sROC curve based on GEO datasets and TCGA RNA seq data, (B) Pooled sensitivity and specificity based on GEO datasets and TCGA RNA seq data, (C) sROC curve based on GEO datasets, TCGA RNA seq data, and IHC data, (D) pooled sensitivity and specificity based on GEO datasets, TCGA RNA-seq data, and IHC data. SAC3D1, SAC3 domain containing 1.

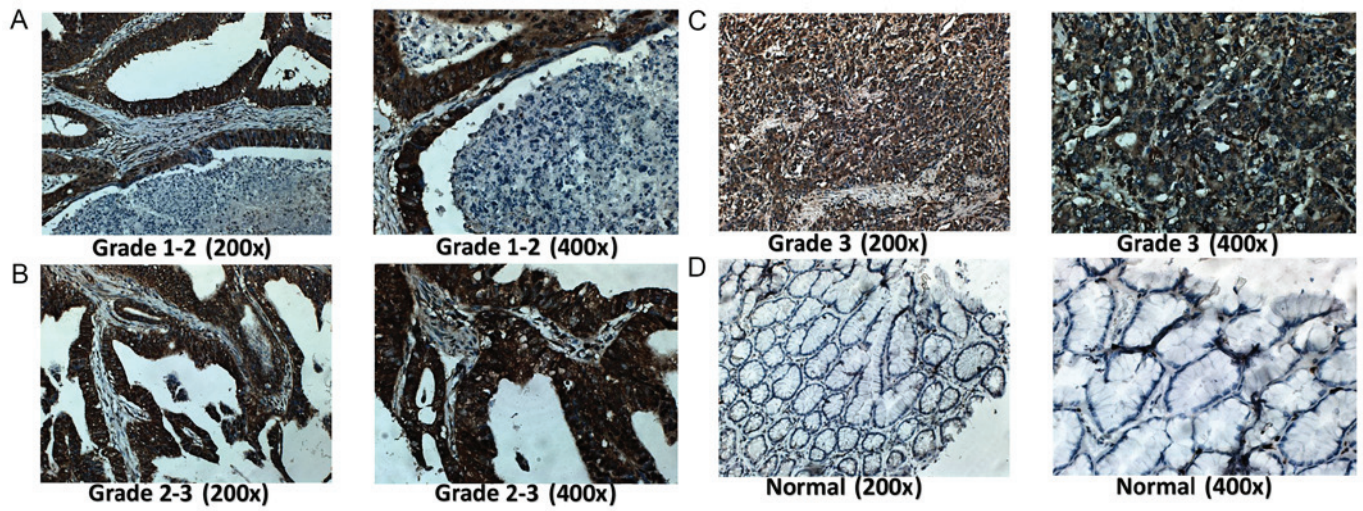

Figure 6. Expression of SAC3D1 protein in different grades of gastric cancer and normal tissues. (A) SAC3D1 protein expression in grades 1-2, (B) SAC3D1 protein expression in grades 2-3, (C) SAC3D1 protein expression in grade 3, (D) SAC3D1 protein expression in normal tissues. SAC3D1, SAC3 domain containing 1.

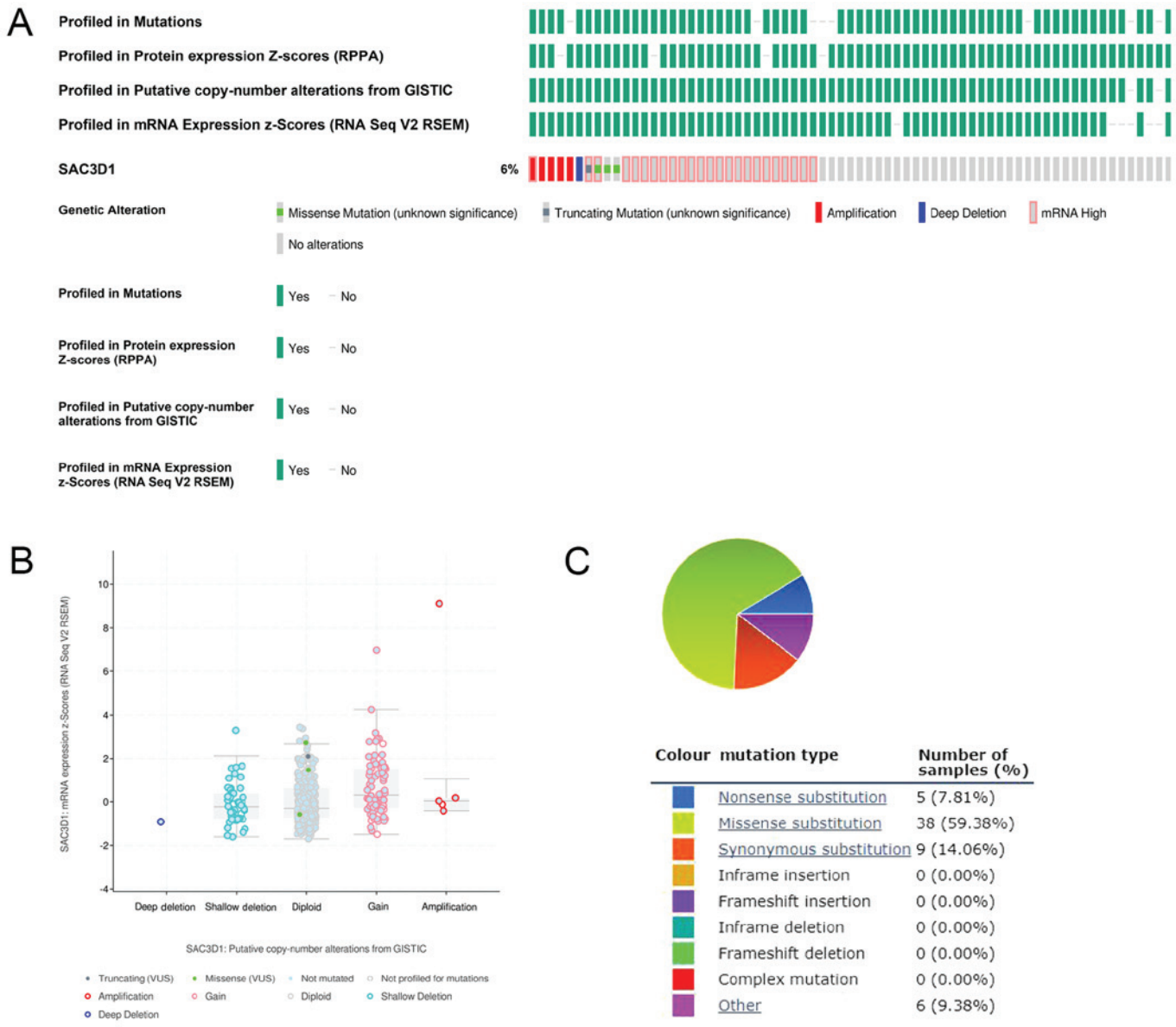

Figure 7. Genetic alterations of the SAC3D1 in gastric cancer. (A) Mutation rate of SAC3D1 in gastric cancer in cBioPortal; (B) putative copy number alterations of SAC3D1 in gastric cancer from cBioPortal; (C) the main mutation types of SAC3D1 in gastric cancer from COSMIC. SAC3D1, SAC3 domain containing 1.

18 GEO chips, TCGA RNA-seq data and IHC data, which included 1,420 GC tissues and 599 normal tissues. Notably, both SAC3D1 mRNA and protein levels were observed to be upregulated in GC tissues. The overexpression SAC3D1 was associated with the histological grade, clinical stage, T stage and N stage of GC, revealing that SAC3D1 may be involved in the development and progression of GC. Enrichment analysis revealed that SAC3D1 and 4 other SAC3D1-related genes (CDK1, CCNB1, CCNB2 and CDC20) are important for GC development via the cell cycle pathway.

Numerous studies have reported the overexpression of SAC3D1 in several types of cancer, including hepatocellular carcinoma (11), colon cancer (12) and lung adenocarcinoma (13). Recent studies have assessed the prognostic value of SAC3D1 using GEO, the Cancer Genome Atlas and International Cancer Genome Consortium and suggested that SAC3D1 may be a credible prognosis-related biomarker for hepatocellular carcinoma (11). In colon cancer, the upregulation of SAC3D1 was confirmed by a quantitative PCR (12). In lung adenocarcinoma, SAC3D1 may be involved in the inhibition of cytoplasmic pro-B cell developmental mechanisms in paracancerous tissue of lung adenocarcinoma by low glucose transporter SLC2A5 (13). However, to the best of our knowledge, no studies to date have clarified the expression of SAC3D1 in GC, and the expression of SAC3D1 in other cancers was only validated based on small sample sizes or a single method, which may decrease the reliability of their conclusion. Particularly, no research or clinical trials have specifically been done attempting to reveal the molecular mechanisms of SAC3D1 in cancers, including GC.

To explore the possible molecular mechanisms of actoin of SAC3D1 in GC, an enrichment analysis was performed for SAC3D1 and its co-expressed genes. The results indicated that SAC3D1 and co-expressed genes were positively associated with the cell cycle. Additionally, numerous studies have demonstrated that the cell cycle pathway plays an important role in cancer cells. Cao *et al* reported that the regulatory mechanism

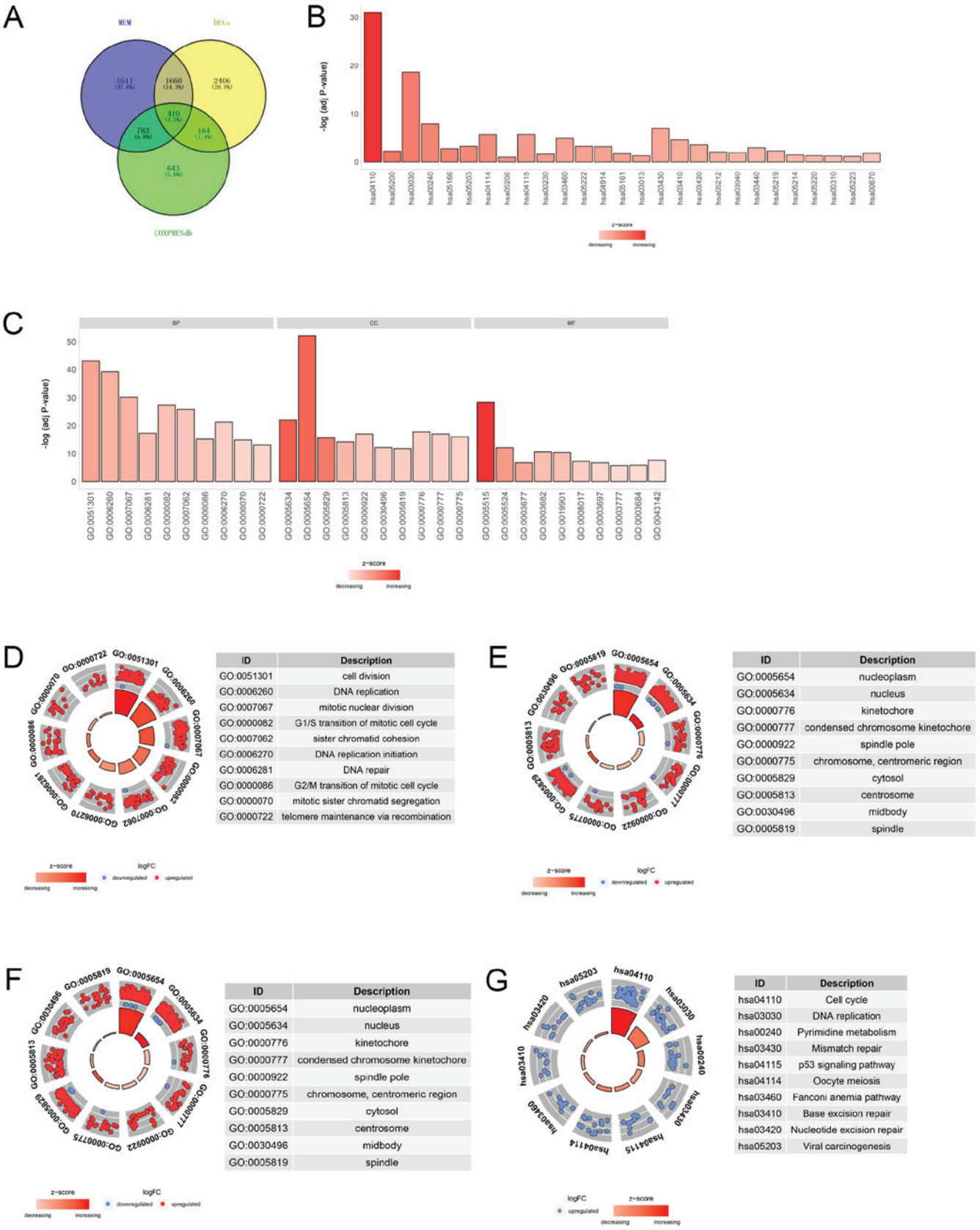

Figure 8. Co-expressed genes of SAC3D1 and enrichment analysis. (A) Venn diagram describes the co-expressed genes of SAC3D1 in gastric cancer; (B) bar chart of KEGG pathways; (C) bar chart of GO terms; (D) circular visualization of the biological process; (E) circular visualization of the cellular component; (F) circular visualization of the molecular functions, (G) circular visualization of the KEGG pathways. SAC3D1, SAC3 domain containing 1.

of BIRC5 and co-expressed genes in lung carcinoma may be closely related to the cell cycle (24). Liu *et al* reported that upregulated differentially expressed genes participated in regulating breast cancer cells by the cell cycle pathway (25).

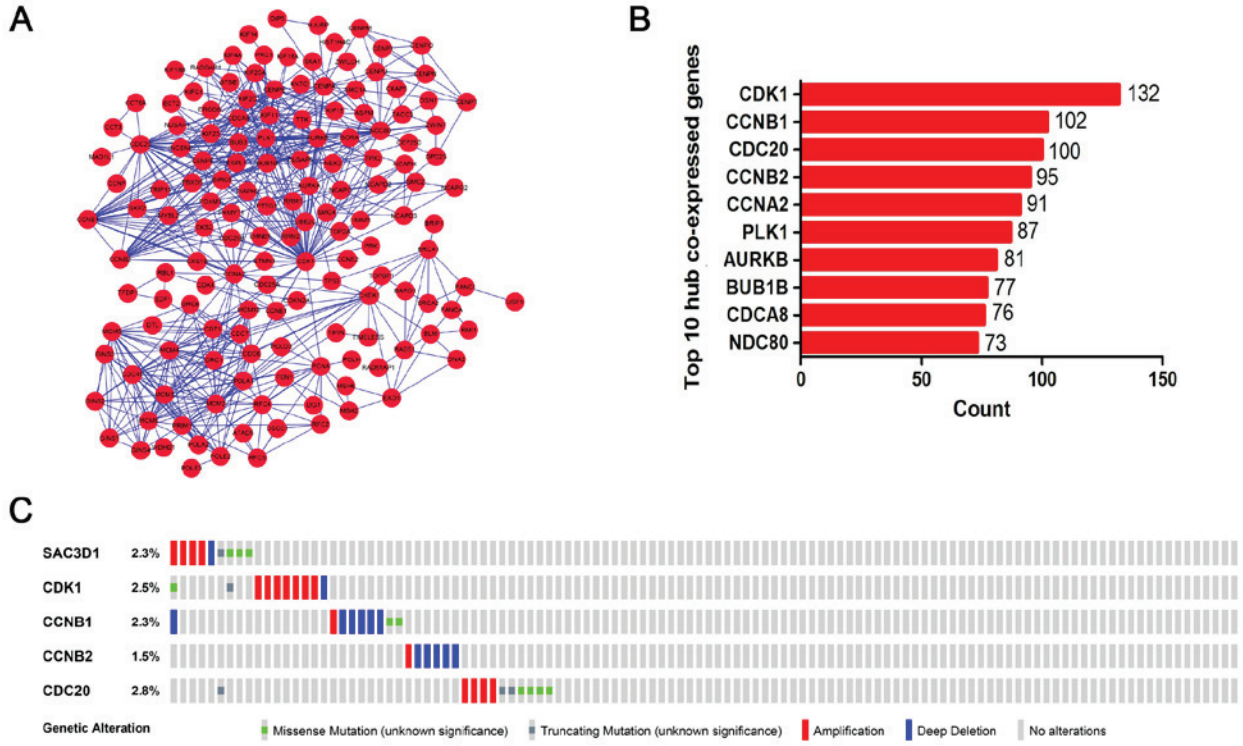

Figure 9. PPI of SAC3D1 and co-expressed genes in gastric cancer. (A) PPI network (combined score >0.99), (B) top 10 connection degree of hub co-expressed genes of SAC3D1, (C) mutation rate of SAC3D1 and hub co-expressed genes in gastric cancer based on TCGA data. SAC3D1, SAC3 domain containing 1.

Moreover, Qiu *et al* revealed that the modules and central genes associated with the development of breast cancer were significantly enriched in the cell cycle pathway (26). Feng *et al* investigated poor prognosis-related genes of ovarian cancer by bioinformatics analysis and found that these genes were mainly enriched in the cell cycle pathway (27). It has also been reported that the cell cycle pathway is the key signaling pathway for 8 target therapy of neuroblastomas (28). Zhang *et al* reported that LncRNA CASC11 promoted the proliferation, migration, and invasion of GC cells *in vitro* via the cell cycle pathway (29). A number of studies have documented that the cell cycle pathway may play a role in the regulation of multiple types of cancer, including GC and enrichment analysis revealed that SAC3D1 and its co-expressed genes were involved in the cell cycle pathway. This prompted the hypothesis that SAC3D1 may be related to the occurrence and progression of GC. A total of 4 genes (CDK1, CCNB1, CCNB2 and CDC20) were determined as the core co-expressed genes of SAC3D1 in GC, and it was speculated that SAC3D1 may cooperate with these genes to promote the progression of GC. Further *in vitro* experimental analyses are still required to verify the findings of the present study, such as SAC3D1 overexpression or interference.

CDK1 is a cell cycle-related gene that can be regulated by KIAA0101 and is involved in the occurrence and development of GC (30). CDK1 can also be regulated by LncRNA CASC11 and then participate in the proliferation, migration, and invasion of GC cells (29). Guo *et al* demonstrated that rhCNB may decrease the expression of cell cycle B1 and CDK1 proteins and participate in the mechanism of cell cycle arrest (31). CCNB1 is a cell cycle-related gene that can be regulated by ISL1 to promote the proliferation and tumor growth of GC cells (32). CCNB1 can be used as a biomarker to monitor prognosis and hormone therapy in ER breast cancer (33). It has also been reported that the overexpression of CCNB1 induced by chk1 can promote the proliferation and tumor growth of human colorectal cancer cells and inhibit the induction of apoptosis in some colorectal cancer cells (34). CCNB1 could also activate FOXM1 and promote the proliferation of human hepatocellular carcinoma cells (35). CCNB1 may serve as a promising diagnostic tool for determining the high risk of recurrence in patients with non-myenteric invasive bladder cancer (36). CCNB2 is a cell cycle-related gene that can be regulated by ISL1 to promote the proliferation and tumor growth of GC cells (32). In addition, the overexpression of CCNB2 protein is related to the clinical progress and poor prognosis of non-small cell lung cancer, and over-expressed CCNB2 is a biomarker of poor prognosis in Chinese patients with non-small cell lung cancer (37). The increased expression of the cell cycle-related gene CCNB2 is related to the advanced growth of prostate cancer cell subsets (38). Kim *et al* reported that the expression of CDC20 in early GC was significantly higher than that in normal mucous membranes (39). The upregulation of CDC20 was associated with invasive progress and poor prognosis in GC, and it was identified as an independent marker for predicting clinical outcomes in patients with GC (40). It has also been reported that CDC20 expression can be used as a biomarker for tumor prognosis or as a therapeutic target for other human cancers (41). In addition, CDC20 can mediate docetaxel resistance to castrated prostate cancer (42).

Microarray and RNA-seq data were combined to evaluate the prognostic value of 4 hub co-expressed genes via a prognostic-related meta-analysis. It was found that the upregulation of these genes were closely related to the poor prognosis of

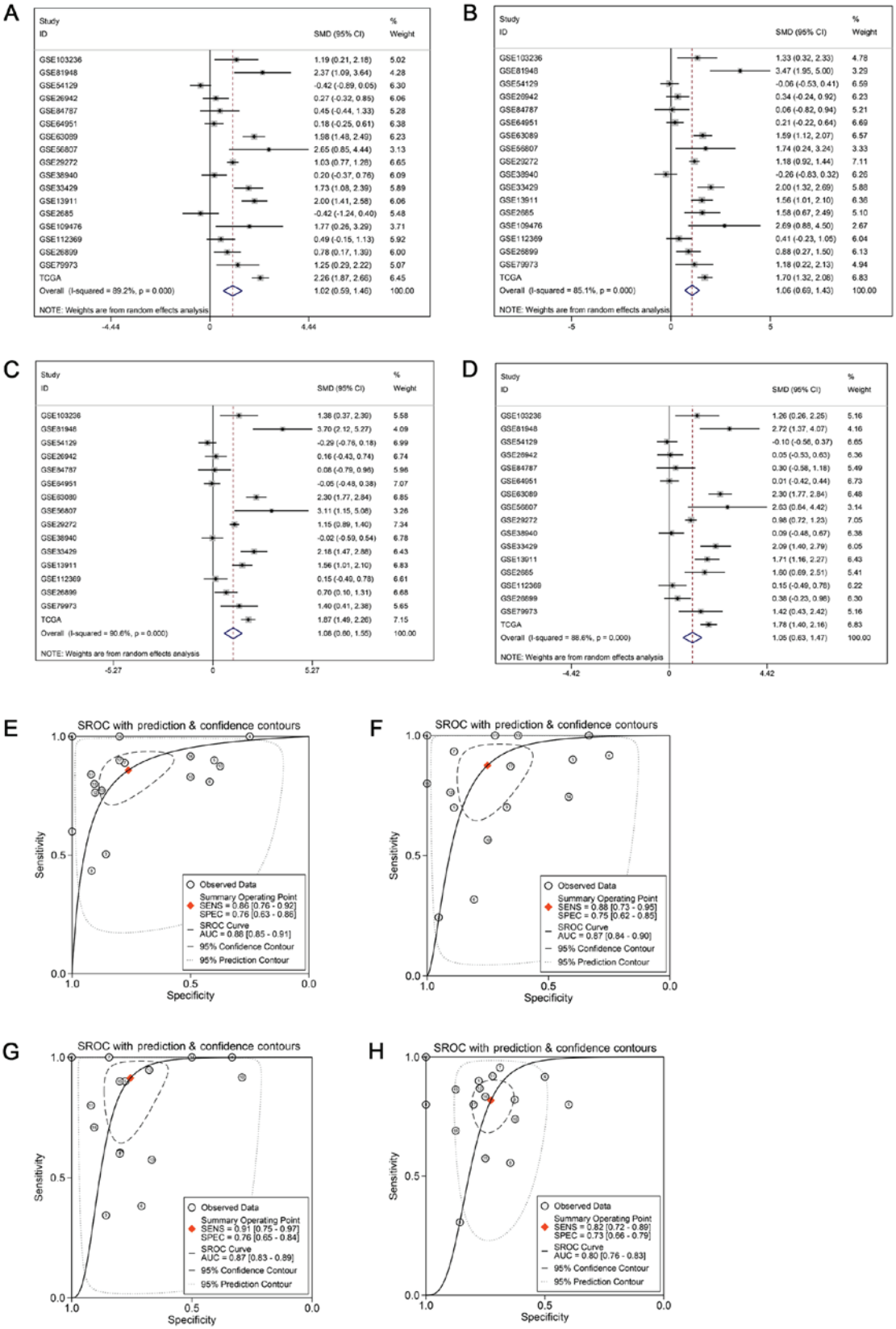

Figure 10. Validation of hub co-expressed genes on mRNA levels based on GEO and TCGA data. Expression level of hub co-expressed genes: (A) CDK1; (B) CCNB1; (C) CCNB2; (D) CDC20. SROC curve of hub co-expressed genes: (E) CDK1; (F) CCNB1; (G) CCNB2; (H) CDC20. SAC3D1, SAC3 domain containing 1.

patients with GC. From online analysis, it was found that the genetic alterations rate of SAC3D1 and its hub co-expressed genes in GC was relatively low. Therefore, it speculated that mutation and amplification may not be the main reasons for SAC3D1 to promote the development of GC. Further experimental analyses are warranted. In conclusion, the findings of the present study demonstrate that SAC3D1 is highly expressed in GC and may be associated with the progression of GC.

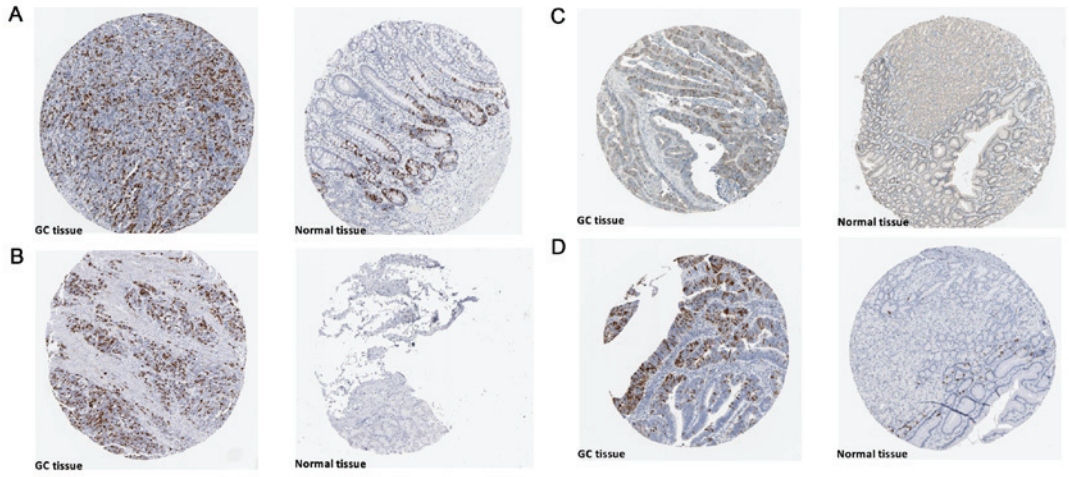

Figure 11. Validation of hub co-expressed genes on protein levels based on Human Protein Atlas. (A) CDK1, https://www.proteinatlas.org/ENSG000 00170312-CDK1/pathology/tissue/stomach+cancer#img, https://www.proteinatlas.org/ENSG00000170312-CDK1/tissue/stomach#img; (B) CCNB1, https:// www.proteinatlas.org/ENSG00000134057-CCNB1/pathology/tissue/stomach+ cancer#img, https://www.proteinatlas.org/ENSG00000134057-CCNB1/ tissue/stomach#img; (C) CCNB2, https://www.proteinatlas.org/ENSG000 00157456-CCNB2/pathology/tissue/stomach+cancer#img, https://www.protein-atlas.org/ENSG00000157456-CCNB2/tissue/stomach#img; (D) CDC20, https://www.proteinatlas.org/ENSG00000117399-CDC20/pathology/tissue/ stomach+ cancer#img, https://www.proteinatlas.org/ENSG00000117399-CDC20/tissue/stomach#img. SAC3D1, SAC3 domain containing 1.

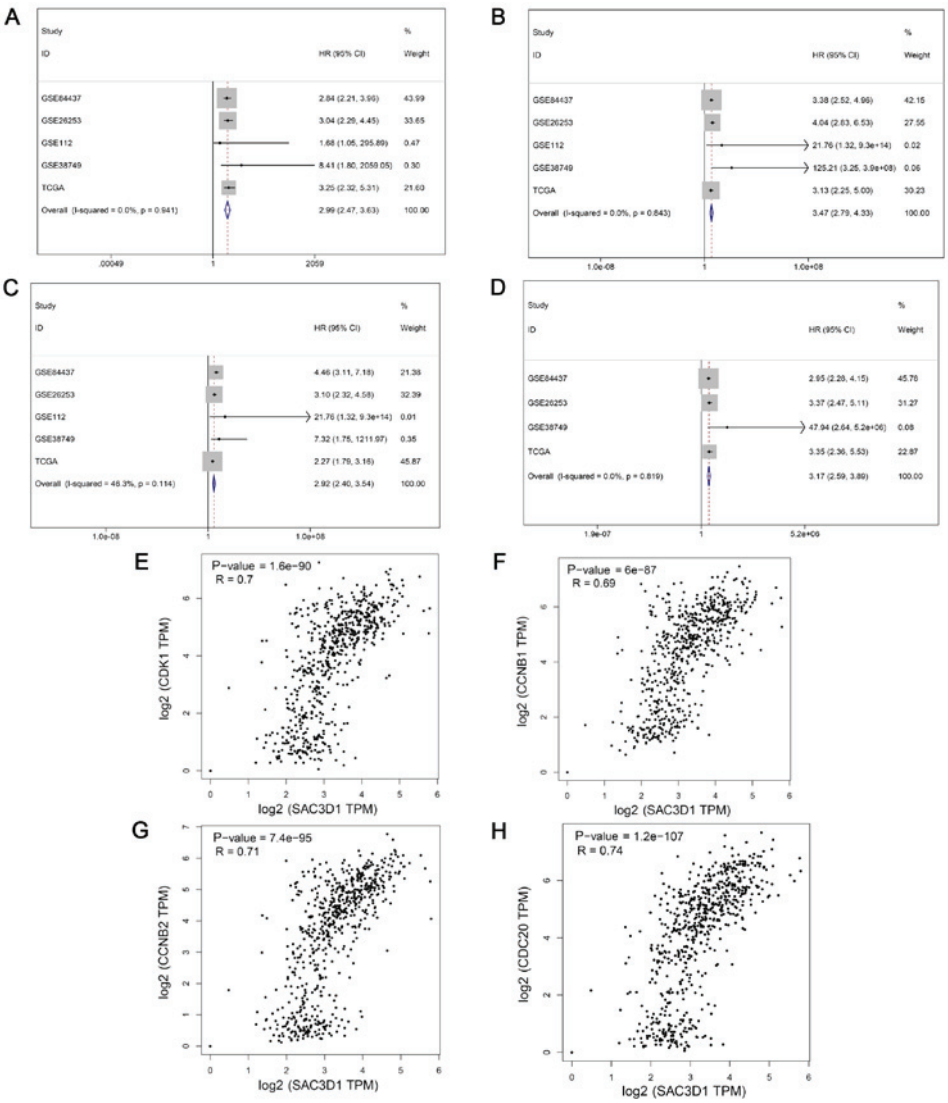

Figure 12. Prognostic value and correlation analysis of hub co-expressed genes based on GEO and TCGA data. Prognostic meta-analysis: (A) CDK1; (B) CCNB1; (C) CCNB2; (D) CDC20. Correlation between SAC3D1 and hub co-expressed genes: (E) CDK1; (F) CCNB1; (G) CCNB2; (H) CDC20. SAC3D1, SAC3 domain containing 1.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the TCGA (http://cancergenome.nih.gov/), the GEO (https://www.ncbi.nlm.nih.gov/geo/) and the SRA (https://www.ncbi.nlm.nih.gov/sra/) data portals. The in-house IHC data from the present study can be acquired from the correspondence author on reasonable request.

Authors' contributions

AGL and JCZ collected data from public datasets and analyzed the data and performed the statistical analysis. XGQ and WJM performed in-house IHC experiments. GC, RQH and JJL participated in the conception and design of the study and in language modification. AGL, JCZ and YQH drafted the manuscript and analyzed the GO and KEGG terms. JM, LHY, XJW and JTH conceived and designed the study and assisted in the drafting of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This research program was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. All participants signed informed consent forms as collected by Pantomics.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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