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SUCO as a Promising Diagnostic Biomarker of Hepatocellular Carcinoma: Integrated Analysis and Experimental Validation

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Background: Hepatocellular carcinoma (HCC) is not frequently diagnosed until the late stage due to its concealed symptoms. Therefore, the identification of biomarkers that have effective diagnostic performance and act as potential key therapeutic targets for HCC becomes urgent.

Material/Methods: Comprehensive analysis of accumulated data downloaded from the Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA) databases was used to obtain more reliable potential diagnostic biomarkers of HCC and to explore related molecular mechanisms. Meta-analysis and summary receiver operating characteristic (SROC) curve analysis were performed to evaluate the differential expression of *SUCO* gene in HCC and identify the capability of *SUCO* in distinguishing HCC-tissues from normal liver-tissues.

Results: *SUCO* was found to be upregulated in HCC-tissues and exhibited a favorable value in diagnosing HCC. Bioinformatics analysis showed that *SUCO* might play important roles in HCC progression, and was significantly related to cell cycle, cell metabolism, and proliferation.

Conclusions: This study was the first to demonstrate that *SUCO* was overexpressed in HCC-tissues, and that high expression of *SUCO* was significantly related to poor overall survival in HCC patients. *SUCO* might be a potential diagnostic biomarker for HCC patients, which promotes the tumorigenesis and progression of HCC.

MeSH Keywords: Biological Markers • Carcinoma, Hepatocellular • Tissue Array Analysis

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Background

Hepatocellular carcinoma (HCC) is one of the leading causes of death worldwide due to its concealed symptoms in early stage [1,2]. Numerous of studies have showed that aberrantly expressed genes often play key roles in HCC progression [3,4]. Serum alpha-fetoprotein (AFP) is by far the most reliable HCC biomarker even though it has limitations in sensitivity and specificity in the diagnosis of HCC [5,6]. Identification of novel biomarkers involved in HCC progression and revealing their roles in early detection and treatment of HCC have become urgent.

In recent years, a large number of high-throughput gene expression data, including microRNA (miRNA) expression, messenger RNA (mRNA) expression, and DNA methylation were collected in plenty of archives, such as the Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA) databases. Microarrays capable of rapidly detecting gene expression levels on a global scale are particularly useful for screening differentially expressed genes [7,8].

We found that *SUCO* was overexpressed in HCC-tissues and was significantly associated with the prognosis of HCC patients during our previous study [9]. The *SUCO* gene is located at 1q24.3 of humans and encodes the SUN domain-containing ossification factor that participates in protein synthesis, and promotes osteoblast proliferation. Studies showed that the lack of *SUCO* led to abnormal neuronal development and *SUCO* could be a generalized-onset epilepsy-related gene [10]. *SUCO* has also been reported to be a candidate disease gene with links to skeletal dysplasia [11].

However, according to our literature review, no previous studies have confirmed the relationship between *SUCO* expression and tumorigenesis. Due to its unknown biological function and potential diagnostic value, we decided to continue investigating *SUCO* expression level in HCC and exploring its potential molecular mechanism through experiments and comprehensive analysis.

Material and Methods

Public data and tools

The GEO (*https://www.ncbi.nlm.nih.gov/geo*) database and TCGA (*https://gdc-portal.nci.nih.gov*) database were used to extract the RNA sequencing (RNA-Seq) and clinical data. R software was used to screen out aberrantly expressed genes based on each dataset. Subsequently, the lists obtained from the differential expression analysis of each dataset were integrated using RobustRankAggreg (RRA) package (*http://cran.r-project.org*) [9].

Verification of *SUCO* expression based on multiple databases

Systematic literature searches were conducted in Web of Science, Embase, and PubMed. Both MeSH terms and free words were used to increase the sensitivity of the search. The literature search was conducted up to October 2018 and was limited to the English language. The search terms included ("HCC" OR "hepatocellular carcinoma" OR "hepatic" OR "liver") AND ("SUCO"). Besides, we searched gene expression data from GEO dataset. The following keywords were used: ("mRNA" OR "mRNAs") AND ("HCC" OR "hepatocellular carcinoma" OR "hepatic" OR "liver"). To reduce data source variability, we extracted only one platform (GPL570 platform) to minimize the impact on the heterogeneity in the analysis.

The inclusion criteria were as follows: the *SUCO* gene expression data in both HCC-tissues and adjacent liver-tissues can be calculated or provide. The exclusion criteria were: 1) non-human subject studies; 2) laboratory articles, letters, conference reports, case reports, and editorials; 3) expression of *SUCO* in adjacent liver-tissues cannot be calculated based on the data; 4) repeated studies.

Tissue samples and cell culture

Tissue samples in the study were collected at Beijing Tongren Hospital affiliated to Capital Medical University. Each patient signed a written informed consent that met the requirements of Declaration of the Helsinki. No patients underwent radiation therapy, chemotherapy, or other treatment prior to surgery. The research program was approved by the institutional review committee of Beijing Tongren Hospital affiliated to Capital Medical University.

HCC cell lines (BEL-7402, SMMC-7721, BEL-7404, Hep 3B, Hep G2, and Huh-7) and normal liver cell line (LO-2) were obtained from the Cell Bank of Shanghai Institute of Biochemistry & Cell Biology (Shanghai, China). All the cell lines were cultured by Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Grand Island, NY, USA), which contained 10% fetal bovine serum (FBS, Invitrogen, Camarillo, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from 66 HCC samples, 21 corresponding adjacent non-tumor tissues, and cell lines (LO-2, BEL-7402, BEL-7404, Hep 3B, Hep G2, Huh-7, and SMMC-7721) using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA), and cDNA was synthesized using a reverse transcription kit (Life Technologies, Carlsbad, CA, USA). Real-time PCR was repeated in triplicate using a PCR instrument (Thermo Fisher Scientific) according to

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the manufacturer's instructions. The primer sequences for *SUCO* were as follows: forward 5'-AGGGGGAAGAAGGAGGAGAA-3'; reverse 5'-GAGCACAGAAAGAGGCAGGA-3'. We used β-actin as the internal control, and the primers of β-actin were as follows: forward 5'-GAAGAGCTACGAGCTGCCTGA-3'; reverse 5'-CAGACAGCACTGTGTTGGCG-3'. The relative expression of each target gene was calculated by using a method of comparing Ct (2–ΔΔCT) values.

Statistical analysis

The Kaplan-Meier method and the log-rank test were used to analyze overall survival (OS) and disease-free survival (DFS). Log-rank $P<0.05$ was considered statistically significant. Curves and graphs were constructed using GraphPad Prism 7.0 software. Statistical analyses for the clinical implication of *SUCO* were performed using SPSS 21.0 statistical software. The pooled mean and standard deviation of *SUCO* expression in HCC-tissues and liver-tissues were calculated by STATA 15.0 software. Receiver operating characteristic (ROC) and summary ROC (SROC) curve analysis were conducted by SPSS 21.0 statistical software and MetaDiSc 1.4 software.

Results

SUCO was upregulated in various cancers including HCC

The differential expression analyses were performed after calibration, standardization, and log2 transformation for the downloaded data (TCGA-LIHC, GSE29721, GSE14520) using the R software. A total of 137 irregularly expressed genes were screened using the RRA analysis ($P<0.01$), including 96 upregulated genes and 41 downregulated genes. The heat map of the upregulated and downregulated genes are shown in Figure 1A.

SUCO was significantly overexpressed in HCC-tissues compared to adjacent liver-tissues, as shown in Figure 1B–1D. Besides, we checked the expression levels of *SUCO* in 31 types of cancers using data from TCGA and GTEx data using GEPIA online tools (*http://gepia.cancer-pku.cn/*). The results revealed that *SUCO* was found to be upregulated in 12 types of cancers, as shown in Figure 1E, including acute myeloid leukemia (LAML), breast cancer (BRCA), bile duct cancer (CHOL), esophageal cancer (ESCA), glioblastoma (GBM), lower grade glioma (LGG), large B-cell lymphoma (DLBC), lung adenocarcinoma (LUAD), liver cancer (LIHC), rectal cancer (READ), stomach cancer (STAD), and thymoma (THYM) (fold change >1.5 and *P* value <0.05).

High *SUCO* expression level indicated poor prognosis in patients with HCC

Kaplan-Meier curve analysis was performed based on the survival data of HCC patients and gene expression data downloaded from TCGA database. As shown in Figure 1F and 1G, *SUCO* was significantly associated HCC patient prognoses, and patients with high expression of *SUCO* gene had shorter DFS time and OS time.

Meta-analysis confirmed that *SUCO* was upregulated in HCC-tissues

The literature on the relationship between HCC and *SUCO* expression was not searched. A total of 6 studies (GSE17548, GSE19665, GSE29721, GSE55092, GSE62232, and GSE6764), which contained the expression data of *SUCO* gene and based on GPL570 platform were searched and downloaded from the GEO database. To draw a comprehensive conclusion, we integrated the data using a meta-analysis. Six GEO databases with 202 patients were included in the meta-analysis, as shown in Figure 2, and the SMD of *SUCO* expression was 2.01 (95% CI: 1.53 to 2.49; $I^2=62.7\%$, $P=0.02$) by the random-effects model. The aforementioned results certified that *SUCO* was evidently overexpressed in HCC-tissues.

SROC curve analysis

The ROC and SROC curve analysis were performed to further determine the ability of *SUCO* to distinguish between HCC and normal liver tissue [12]. Considering the small sample sizes, we omitted the TCGA, GSE60502-GPL96, and GSE14520-GPL571 databases. The ROC curve analysis was performed using SPSS 21.0 software. As shown in Figure 3A–3I, the AUC of *SUCO* from TCGA, GSE6764, GSE14520, GES17548, GSE19665, GSE29721, GSE55092, GSE60502, and GSE62232 data was 0.904 ($P<0.001$, cutoff value >9.930), 0.844 ($P<0.001$, cutoff value >8.652), 0.994 ($P<0.001$, cutoff value >6.056), 0.906 ($P<0.001$, cutoff value >8.770), 0.790 ($P=0.028$, cutoff value >8.080), 0.990 ($P<0.001$, cutoff value >7.799), 0.956 ($P<0.001$, cutoff value >7.856), 0.929 ($P<0.001$, cutoff value >9.050), 0.988 ($P<0.001$, cutoff value >7.066), respectively.

Then SROC curve analysis was performed, as shown in Figure 4A–4F, the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and the AUC of SROC of *SUCO* in these studies were 0.83 (95% CI: 0.80–0.86), 0.89 (95% CI: 0.85–0.93), 6.52 (95% CI: 4.14–10.28), 0.16 (95% CI: 0.10–0.25), 44.40 (95% CI: 22.46–87.77), and 0.9298, respectively. Based on the results of ROC and SROC curve analysis, *SUCO* could be used as a promising diagnostic biomarker in HCC.

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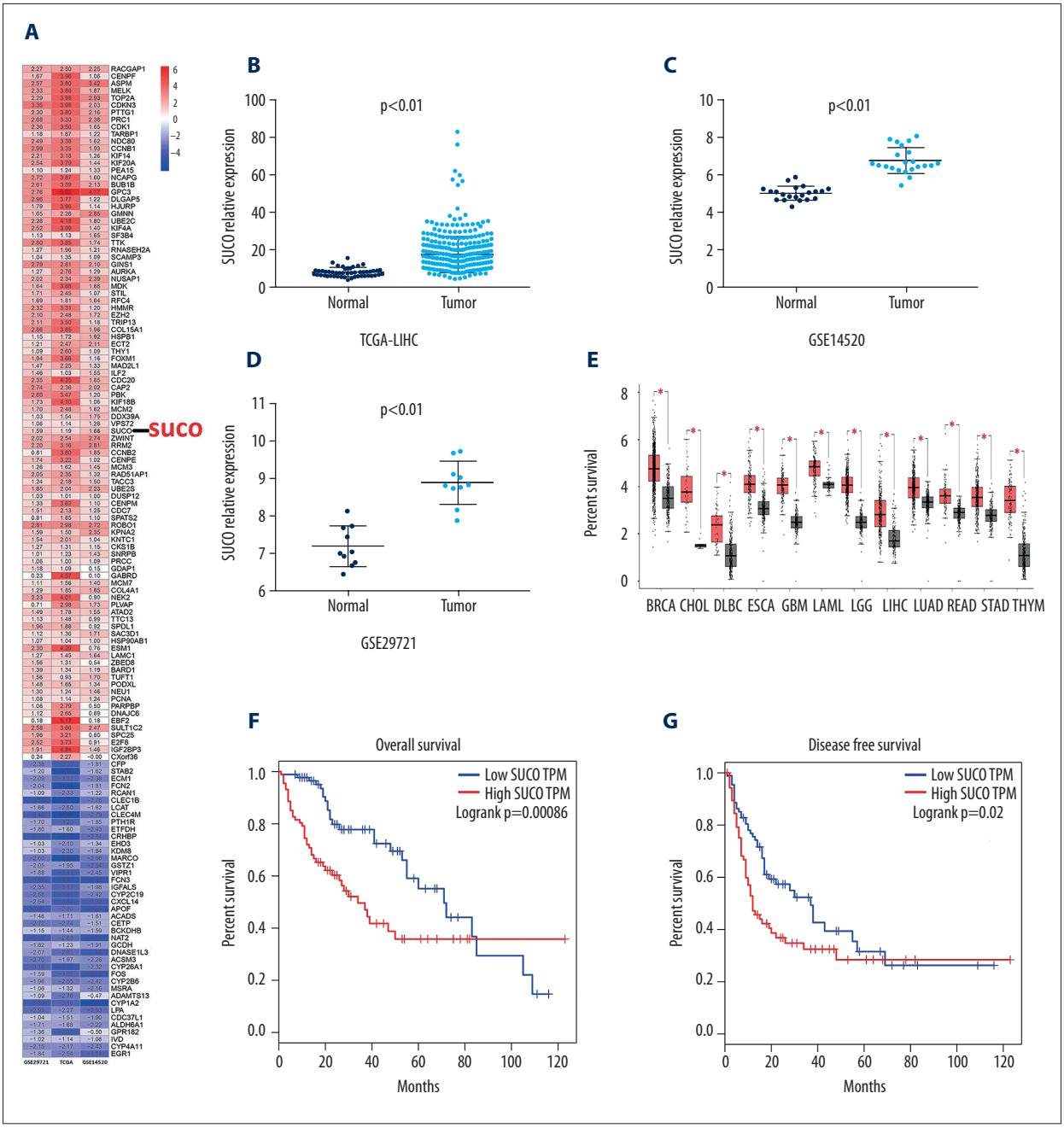

Figure 1. *SUCO* expression in multiple databases. (**A**) Log_2FC heatmap of the 96 upregulated and 41 downregulated genes which were identified based on GSE29721, TCGA and GSE14520 databases using the R software with limma and RobustRankAggreg packages. (**B–D**) *SUCO* expression levels in hepatocellular carcinoma (HCC) tissues compared with adjacent liver tissues of TCGA, GSE14520 and GSE29721 database, respectively. (**E**) *SUCO* expression levels in HCC tissues compared with adjacent liver tissues in multiple cancers ("*" indicates a fold change >1.5 and *P*-value <0.05 in TCGA cohort); (**F**) Overall survival plots of *SUCO* in TCGA cohort (log-rank *P*=0.00086). (**G**) Disease-free survival plots of *SUCO* in TCGA cohort (log-rank *P*=0.02).

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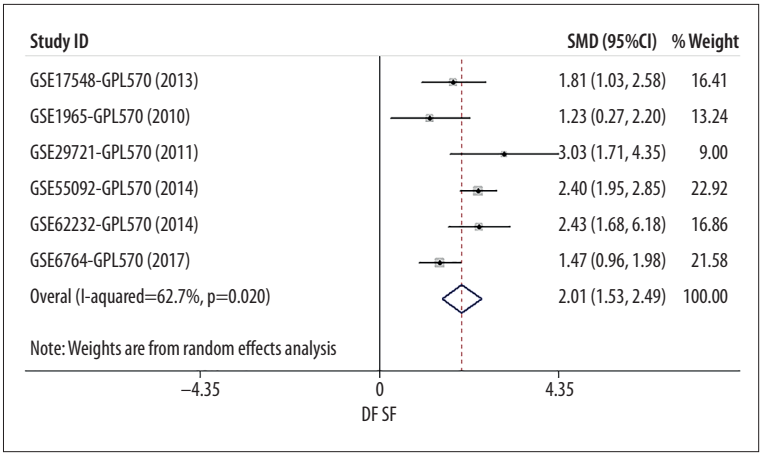

Figure 2. Forest plot of studies evaluating the *SUCO* expression level between hepatocellular carcinoma tissues and adjacent liver tissues.

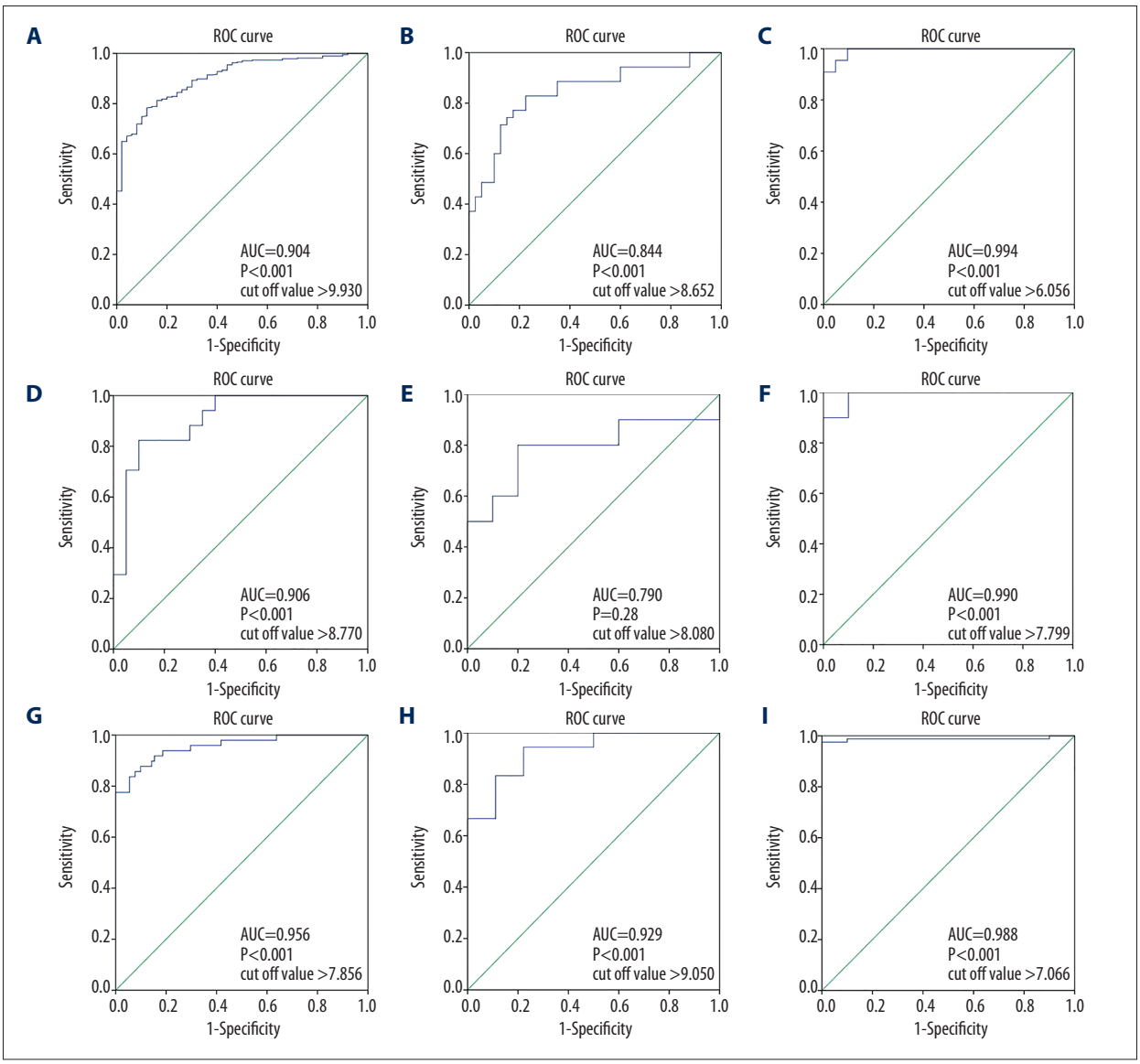

Figure 3. ROC curve of *SUCO* in hepatocellular carcinoma from (**A**) TCGA, (**B**) GSE6764, (**C**) GSE14520, (**D**) GES17548, (**E**) GSE19665, (**F**) GSE29721, (**G**) GSE55092, (**H**) GSE60502, (**I**) GSE62232.

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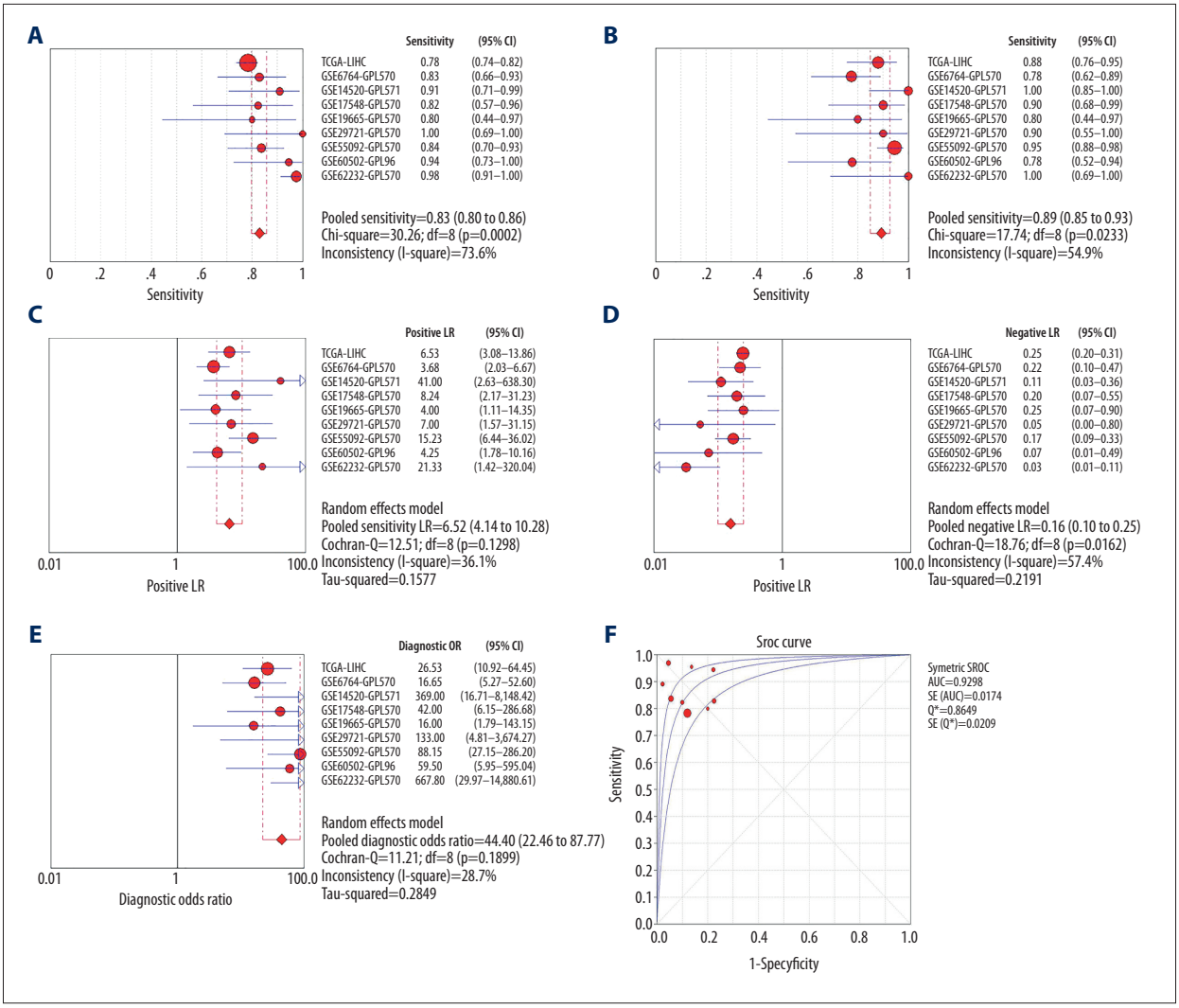

Figure 4. Forest plots exhibit diagnostic performance of *SUCO* in hepatocellular carcinoma.

Upregulated *SUCO* was significantly related to vascular invasion and high pathologic stage based on TCGA database

We analyzed the relationship between *SUCO* expression and clinicopathological based on the data downloaded from TCGA database. As shown in Table 1, significantly different expression values of *SUCO* were observed between negative and positive vascular invasion. Samples with vascular invasion versus without vascular invasion upregulated *SUCO* expression levels (10.697±0.761 versus 10.507±0.612, *P*=0.011). The obvious difference also occurred between high and low pathologic stages. *SUCO* expression levels were upregulated in samples with stage III+V versus stage I+II (10.651±0.732 versus 10.552±0.641, *P*=0.033). This result indicated that overexpression of SUCO might be associated with the metastasis and differentiation of HCC.

SUCO was overexpressed in HCC-tissue samples and HCC cell lines via qRT-PCR analysis

The *SUCO* expression levels in 66 HCC tissues and 21 adjacent liver tissues were compared using qRT-PCR analysis. *SUCO* gene was significantly upregulated in HCC-tissues ($P<0.05$), as shown in Figure 5A. Besides, *SUCO* was overexpressed in HCC cells (BEL-7402, BEL-7404, Hep 3B, Hep G2, Huh-7, and SMMC-7721) compared with normal liver cells (LO-2) (Figure 5C). These data certified that *SUCO* was evidently overexpressed in HCC-tissues.

In addition, we evaluated the relationship between *SUCO* expression levels and clinicopathological parameters of patients with HCC. As shown in Table 2, *SUCO* expression levels were upregulated in samples with stage III+IV versus stage I+II (18.5±5.791 versus 11.59±0.9068, *P*=0.0343). Kaplan-Meier survival analysis indicated that patients with high *SUCO* expression

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Table 1. Correlation between SUCO expression and clinicopathological characteristics in TCGA.

Characteristics	n	SUCO expression in TCGA database		
		M ±SD	t	*P*-values
Tissues				
HCC tissues	374	10.583±0.680	−10.633	0.000
Adjacent liver tissues	50	9.538±0.388		
Gender				
Male	253	10.545±0.679	−1.590	0.932
Female	121	10.664±0.678		
Age				
≥60	204	10.582±0.673	−0.014	0.597
<60	169	10.583±0.692		
Neoplasm stage				
G1+G2	233	10.492±0.657	−3.463	0.724
G3+G4	136	10.744±0.698		
Pathologic stage				
I–II	260	10.552±0.641	−1.215	0.033
III–IV	90	10.651±0.732		
Vascular invasion				
Negative	208	10.507±0.612	−2.414	0.011
Positive	110	10.697±0.761		
Person neoplasm cancer status				
Tumor free	234	10.546±0.658	−1.550	0.097
With tumor	113	10.668±0.753		

HCC – hepatocellular carcinoma.

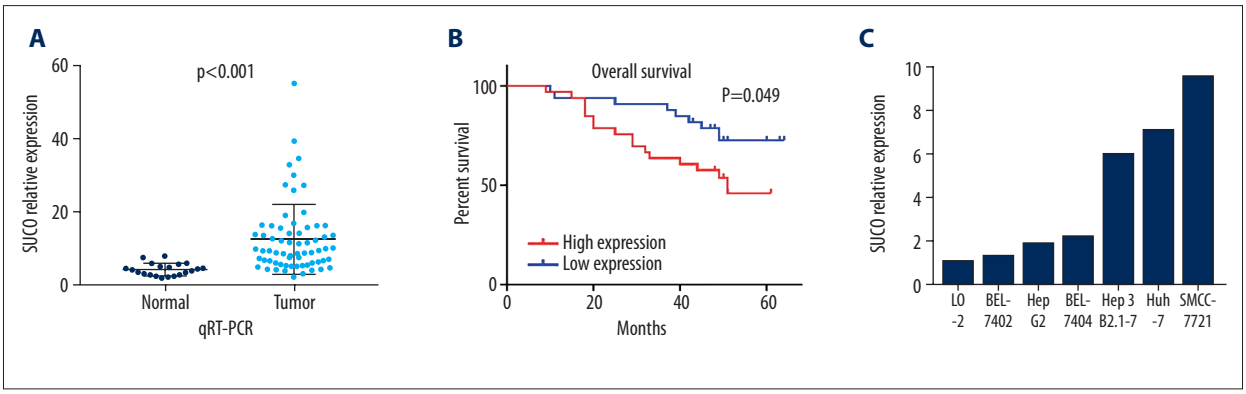

Figure 5. Verification of *SUCO* upregulation in hepatocellular carcinoma (HCC) via qRT-PCR analysis. (**A**) *SUCO* expression in 66 HCC tissue samples and 21 adjacent liver tissue samples measured by qRT-PCR. (**B**) Overall survival plots of *SUCO* based on the 66 HCC patients' data (log-rank *P*=0.049). (**C**) *SUCO* expression in different HCC cell lines and the normal liver cell line LO-2 measured by qRT-PCR. All detections were repeated 3 times and the mean values were used for comparison.

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Table 2. Correlation between SUCO expression and clinicopathological characteristics in qRT-PCR.

Characteristics	n	SUCO expression in TCGA database		
		M ±SD	t	P-values
Tissues				
HCC tissues	66	12.63±1.178	3.8760	0.0002
Adjacent liver tissues	21	4.468±0.3591		
Gender				
Male	57	12.83±1.314	0.4172	0.6779
Female	9	11.39±2.451		
Age				
≥60	26	13.93±1.956	0.8827	0.3807
<60	40	11.79±1.476		
Nodes				
Signal	46	11.93±1.18	0.3680	0.9066
Multi	20	14.26±2.81		
Status				
Alive	43	12.66±1.099	0.0348	0.9723
Death	23	12.58±2.731		
Diameter				
<5 cm	41	11.55±1.148	1.177	0.2437
≥5 cm	25	14.4±2.473		
Pathologic stage				
I–II	46	12.28±1.37	0.4544	0.6511
III–IV	20	13.45±2.325		
Clinical stage (TNM)				
I–II	56	11.59±0.9068	2.163	0.0343
III–IV	10	18.5±5.791		
Recurrence				
Yes	33	13.39±2.076	0.6374	0.5261
No	33	11.88±1.137		

HCC – hepatocellular carcinoma.

levels had worse postoperative outcomes (Figure 5B). However, *SUCO* expression was not significantly associated with tumor-free survival time in HCC patients, which might be due to insufficient cases in this study.

Identification of *SUCO*-related genes in HCC

To explore the potential mechanism of *SUCO* involved in HCC, *SUCO*-related genes in HCC were identified by R software package based on the gene expression matrix, which used two-sided Pearson correlation coefficients and the z-test, the genes positively or negatively correlated with the *SUCO* were considered as *SUCO*-related genes (|Pearson correlation| >0.40 and *P*-value <0.001). We examined the intersecting genes from TCGA and GSE62232 database to identify more reliable gene network and pathways. As shown in Figure 6A, expression of 1041 genes were significantly related to *SUCO* expression in both TCGA and GSE62232 database and were noted as *SUCO*-related genes in HCC.

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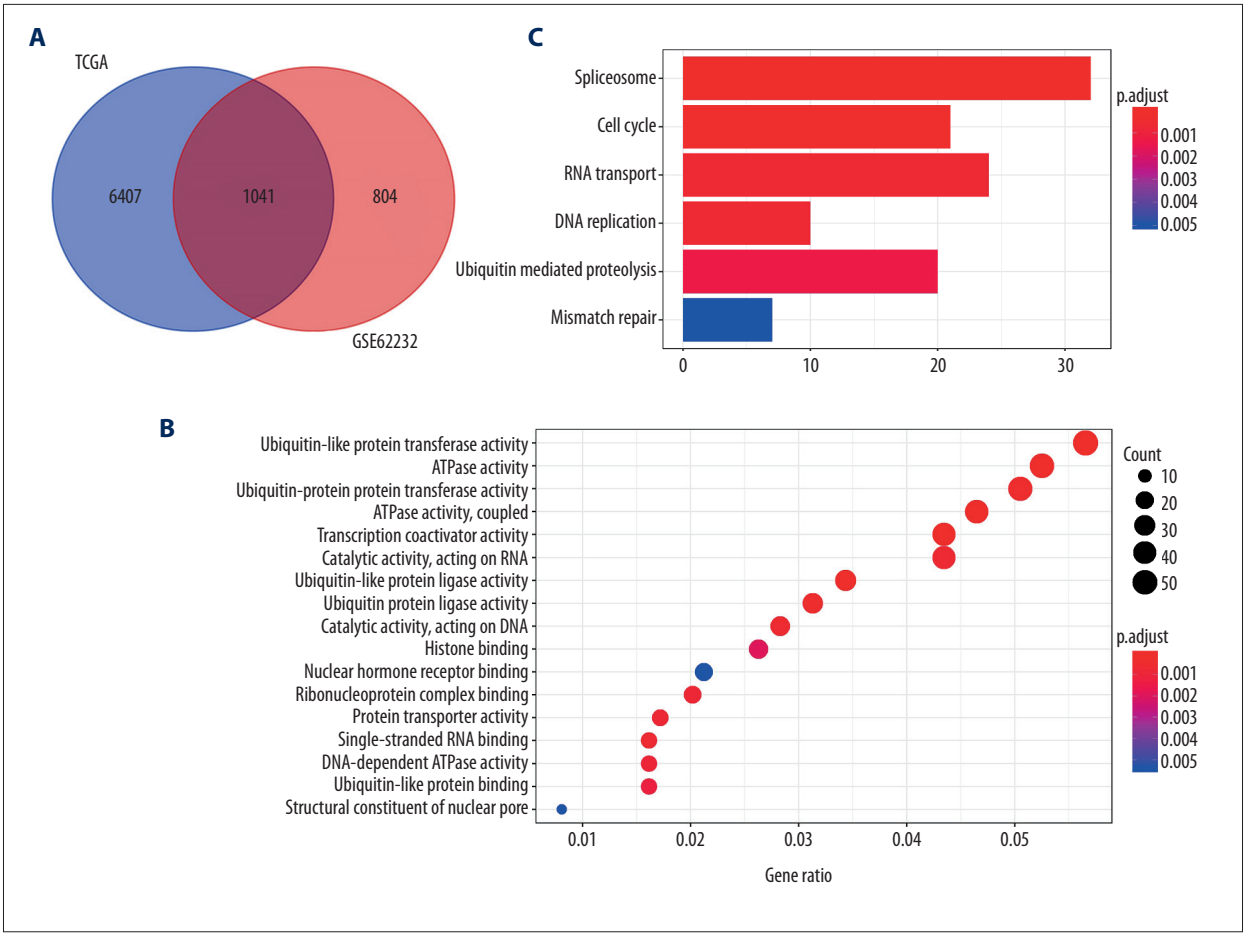

Figure 6. The enriched annotation pathways analysis of *SUCO* related genes in hepatocellular carcinoma. (**A**) Venn diagram of the overlap between the number of *SUCO* related genes using the TCGA and GSE62232 database. (**B**) The significantly enriched annotation of the Gene Ontology categories. (**C**) The significantly enriched annotation of the Kyoto Encyclopedia of Genes and Genomes pathway.

GO and KEGG pathway enrichment analysis

To further explore the potential mechanisms of *SUCO* in HCC, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using R program package and DAVID online tools (*https://david.ncifcrf.gov/*). $P<0.01$ was considered significant. The results were visualized using R software by clusterProfiler and pathview packages (*http://bioconductor.org/biocLite.R*).

GO analysis showed that 17 most significant biological processes were summarized, as shown in Figure 6B, and most of them were related to transcription, ATPase and DNA-related function. KEGG pathway analysis indicated that *SUCO*-related genes were significantly involved in 6 signaling pathways, including spliceosome, cell cycle, RNA transport, DNA replication, ubiquitin mediated proteolysis and mismatch repair, as shown in Figure 6C. Twenty-one *SUCO*-related genes (ANAPC4, BUB1B, BUB3, CCNA2, CCNB1, CCNB2, CDC23, CDC6, CDK1, CDK4, CDK7, CHEK1, GSK3B, MCM4, PCNA, PRKDC, PTTG1, SMAD3, SMC1A, SMC3, and TTK) participants in the cell cycle signaling pathway. The KEGG pathway of cell cycle and related genes were shown in Figure 7. Collectively, these results indicated that *SUCO* might be significantly related to cell cycle, cell metabolism, and proliferation in HCC.

Protein-protein interaction (PPI) network construction

The PPI network was created using the STRING online tool to reveal the interaction among *SUCO*-related genes, and 74 hub genes were identified by MCODE with Cytoscape 3.0 software, as shown in Figure 8A. Interestingly, 7 of the 74 hub genes were involved in the cell cycle signaling pathway, including CDK1, BUB3, SMC1A, SMC3, CCNB1, BUB1B, and CCNB2. The expression relationship between *SUCO* and these hub genes are shown in Figure 8B–8H.

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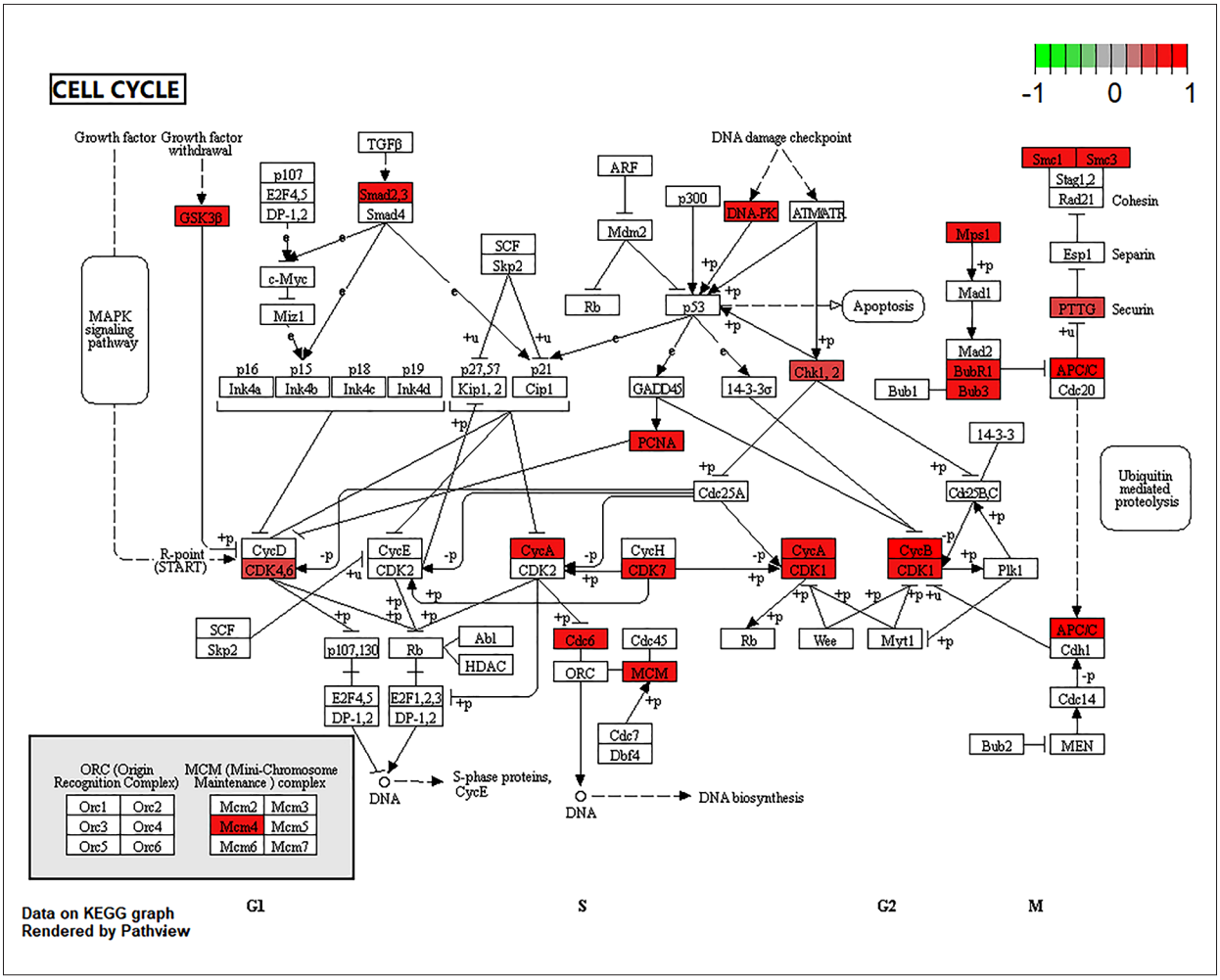

Figure 7. *SUCO* related genes involved in the cell cycle pathway. Red represents *SUCO* related genes identified based on TCGA and GSE62232 database.

Discussion

To the best of our knowledge, the current study was the first to investigate a novel gene *SUCO* in HCC, which was significantly overexpressed in HCC-tissue samples and was significantly associated with prognosis in HCC patients.

In this study, bioinformatics analysis based on TCGA and GEO databases revealed that *SUCO* expression was significantly upregulated in the HCC-tissues. Besides, patients with high expression of *SUCO* gene had shorter OS time and DFS time. Based on these results, we predicted that *SUCO* might be an effective biomarker and closely related to the occurrence of HCC. Then we validated *SUCO* expression level based on multiple databases using meta-analysis, which revealed that *SUCO* was overexpressed in HCC tissues compared with adjacent normal tissues. SROC curve analysis was performed to certificate the capability of *SUCO* in distinguishing HCC from normal liver-tissues. At last, we performed qRT-PCR experiment, which confirmed that *SUCO* was significantly overexpressed in HCC-tissue samples and HCC cell lines.

Although serum AFP is the most reliable biomarker for HCC, current data suggested that no single biomarker alone had optimal sensitivity and specificity for the detection of HCC, particularly at early stages of development [13]. Several genes have been identified as novel biomarkers for HCC diagnose. For instance, plasma miRNA-21 level was demonstrated as a promising biochemical marker for HCC base on ROC curve analysis [14]. Combinations of several biomarkers have been shown to improve the early diagnostic rate in several studies. Previous studies suggested that a panel of seven miRNAs, including miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801 has been shown to have high diagnostic accuracy in the diagnosis of HBV-related HCC based on ROC analysis [15].

Previous studies concerning HCC gene expression profiling have identified hundreds of differentially expressed genes [8,16].

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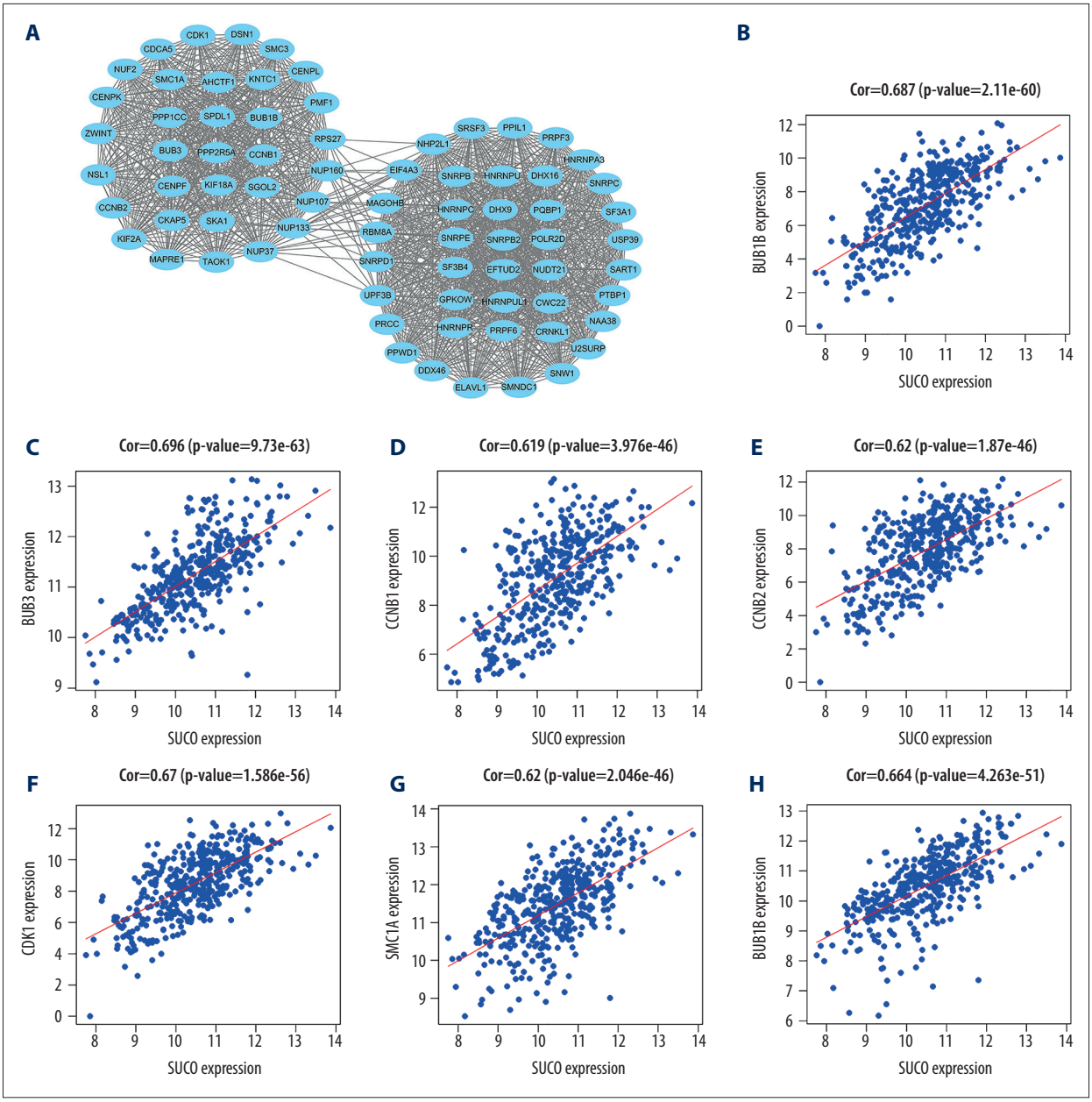

Figure 8. (**A**) Protein-protein interaction of *SUCO* related hub genes, and correlation analysis between *SUCO* and selected hub genes through Pearson's correlation in TCGA; (**B**) BUB1B; (**C**) BUB3; (**D**) CCNB1; (**E**) CCNB2; (**F**) CDK1; (**G**) SMC1A; and (**H**) SMC3.

However, the previous studies were mostly based on a single database [7,17]. In this study, *SUCO* was identified as promising diagnostic biomarker by integrated bioinformatics analysis, meta-analysis and SROC curve analysis based on the multiple data from the GEO and TCGA database, which could contribute to exploration of optimal combinations for successful detection of early stage HCC.

The clinical value of *SUCO* in HCC diagnosis and prognosis was investigated in this study. Surprisingly, we found that expression level of *SUCO* was significantly related to vascular invasion, pathologic stage, and clinical stage in HCC patients based on the qRT-PCR analysis data and the expression data downloaded from TCGA database. These observations suggested that *SUCO* might be closely related to the occurrence and development of HCC. The results of aforementioned analysis certified that *SUCO* was upregulated in HCC-tissues and could be used as a diagnostic biomarker in HCC patients.

We performed bioinformatics analysis to explore the potential mechanism of *SUCO* in HCC. First, we collected the co-expressed genes of *SUCO* through Pearson's correlation based on TCGA

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and GSE62232 database using R software, and then the GO and KEGG pathway enrichment analysis was performed. The results indicated that *SUCO* might be significantly related to cell cycle, cell metabolism, and proliferation in HCC.

Our study may be the first example of combining TCGA, GEO, meta-analysis, SROC analysis, experimental validation and bioinformatics to investigate the possible diagnostic biomarker *SUCO* and its potential molecular mechanisms in HCC. However, there are several limitations in this study. Western blot analysis on typical HCC tissues and *SUCO* knockdown experiments in HCC cells might provide strong support for the conclusion. However, the anti-SUCO antibodies we obtained were not able to perform satisfactory western blot experiments for some reasons. A mature anti-SUCO antibody is needed to support the further experimental validation. Besides, the prediction of *SUCO*-related genes was based on Pearson correlation analysis. More experimental validation will be needed to confirm *SUCO*-related genes, KEGG pathway analysis and GO enrichment results.

Conclusions

This is the first comprehensive analysis to explore the relationship between *SUCO* expression and tumorigenesis, and demonstrated that *SUCO* might be a promising diagnostic biomarker in HCC and illustrated the underlying mechanism of *SUCO* in HCC. However, furthermore investigations are required to fully elucidate the molecular mechanism of *SUCO* in HCC.

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