Prognostic implications of CDC45 expression in hepatocellular carcinoma

Chen Yang

1.Department of Clinical Medicine, Qingdao University;2. Molecular diagnosis laboratory, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang 310014, P. R. China

Shufang Xie

1.The Second Clinical Medical College, Zhejiang Chinese Medical University Hangzhou 310000, P. 2.Molecular diagnosis laboratory, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang 310014, P. R. China

Yi Wu

Department of Hematology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, P. R. China

Guoqing Ru

Department of Pathology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, P. R. China

Xianglei He

Department of Pathology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, P. R. China

Hongyin Pan

Department of Infectious diseases, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, P. R. China;

Shibing Wang (wangshibing@hmc.edu.cn)

1. Molecular diagnosis laboratory, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang 310014, P. R. China;2.The Key Laboratory of Tumor Molecular Diagnosis and Individualized Medicine of Zhejiang Provin https://orcid.org/0000-0002-7772-220X

Xiangmin Tong

Molecular diagnosis laboratory, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang 310014, P. R. China

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Research

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Abstract

Background

The outcomes of hepatocellular carcinoma (HCC) remain poor despite dramatic improvements in treatment, and novel prognostic biomarkers are urgently needed to ameliorate the situation. Cell division cycle protein 45 (CDC45) plays a key role in DNA replication, which is considered to be involved in tumorigenesis and has become a new prognostic marker. This study investigated CDC45 expression in tumour tissues and defined its prognostic value in HCC patients.

Methods

Differential transcriptional and proteomic expression profiles were obtained and validated using multiple datasets. We then used immunohistochemistry (IHC) staining to examine the expression of CDC45 in tumour tissue specimens and compare them with adjacent normal tissue specimens using a constructed tissue microarray (TMA) and analysed the relationship of clinical features and prognosis in HCC patients. Functional enrichment analyses were used to describe significantly involved hallmark pathways of differentially expressed genes (DEGs).

Results

Contrary to the transcriptome expression level in the database, our results showed that the proteome expression of CDC45 in was evidently downregulated in HCC compared with normal adjacent tissues ($P < 0.0001$). Although we did not find any differences in terms of vascular invasion, Edmondson grade, lymphatic infiltration, or metastasis between patients with high and low CDC45 expression in our limited number of HCC samples, low expression of CDC45 was significantly correlated with aggressive characteristics, including microvascular invasion ($P = 0.046$). In addition, a multivariate analysis indicated that CDC45 expression ($P = 0.035$) was an independent prognostic factor for the overall survival (OS) rate of patients with HCC. Furthermore, patients with low CDC45 expression levels were significantly correlated with inferior OS rates than patients with high CDC45 expression levels ($P < 0.05$). Functional annotations indicated that CDC45 is involved in the most significant pathways, including the cell cycle, DNA replication, drug metabolism – cytochrome P450, metabolism of xenobiotics by cytochrome P450, and chemical carcinogenesis pathways.

Conclusions

Our findings showed that a low protein level of CDC45 was associated with a poor prognosis in HCC patients, indicating that CDC45 might be a novel prognostic marker and help identify new therapeutic targets for HCC.

Background

Hepatocellular carcinoma (HCC) is the most prevalent type of primary liver cancer and ranks as the fourth leading cause of cancer mortality worldwide(1). In the last few years, its incidence has sharply increased(2). Although some curative therapies, including hepatic resection, liver transplantation and the application of targeted drugs, can prolong survival for HCC, the 5-year OS for HCC patients is quite disappointing(3, 4). In addition, the heterogeneity of liver cancer hinders choosing the optimal treatment for patients(5),(6). The biomarkers that can primely predict the prognosis may guide treatment in HCC patients and improve HCC clinical outcomes. However, the sensitivity and specificity of current biomarkers are not optimal(7). Therefore, it is particularly critical to identify effective biomarkers for the diagnosis and prognosis of HCC, as they might provide the opportunity to use targeted drugs and immune modulators earlier in the disease course.

Cell division cycle protein 45 (CDC45) is an integral component of the CDC45-MCM2-7-GINS (CMG) helicase complex, which plays a crucial role in DNA replication, especially in the initiation stage. Therefore, the existence of CDC45 effectively maintains the genome stability(8)(9). CDC45 expression is associated with tumorigenesis and is useful for tumour prognosis. Previous studies have shown high CDC45 expression in human cancer-derived cell lines, including breast carcinoma, cervix carcinoma and acute lymphoblastic leukaemia(10). Moreover, it was demonstrated that the upregulation of CDC45 in papillary thyroid cancer is correlated with a more advanced tumour stage(11). In non-small cell lung cancer, CDC45 can promote the growth of tumour cells and was defined as an oncogene(12). However, few studies have investigated the expression and predictive value of CDC45 in HCC.

In the present study, we first used a bioinformatics database to determine the expression and clinical significance of CDC45 in HCC. Next, we examined the protein expression level of CDC45 in HCC by comparing tumours with neighbouring healthy tissue specimens in tissue microarrays (TMAs). Then, a larger sample size was used to investigate the association of CDC45 expression and clinical characteristics of HCC patients. Additionally, we analysed the underlying biological interaction networks and their prognostic value. We hypothesized that the possible antioncogenic activity of CDC45 may impact the prognosis of HCC patients. Our findings may conceal a potential therapeutic target and provide insights into the molecular mechanisms of CDC45.

Methods

Gene expression profiling interactive analysis

To preliminarily analyse the transcriptional levels of CDC45 in HCC patients and its prognostic significance, we adopted the online database gene expression profiling interactive analysis (GEPIA) (http://gepia.cancer-pku.cn), which is an interactive website that includes 9,736 tumours and 8,587 normal samples from The Cancer Genome Atlas (TCGA) and the genotype-tissue expression (GTEx) projects(13).

Oncomine Database

Transcriptional expression profiles of CDC45 in HCC patients were also obtained from the Oncomine database (http://www.oncom ine.com)(14). The differences in the transcriptional expression were compared by Student's t-test. The cut-off values for the p-value and fold change were as follows: p-value = 0.05, fold change = 2, gene rank = 10%, and data type: mRNA.

Bioinformatics Analysis Of GSE76427

The gene expression omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database is an international public repository of high-throughput gene expression and other functional genomics datasets for the research community(15). We selected the GSE76427(16) dataset and downloaded the original CEL files as well as the platform files to explore CDC45 expression and its potential mechanisms using R software. Specifically, according to the median value of the CDC45 gene, samples were divided into high and low expression groups. Then, we used the adjusted p-value < 0.05 and |log2FC| >1.5 as cut-off criteria to identify differentially expressed genes (DEGs) between the two groups of samples. Subsequently, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were carried out to identify the DEGs.

In addition, a protein–protein interaction (PPI) network was constructed. The online search tool for the retrieval of interacting genes (STRING; http://string-db.org) database was used to predict the PPI network of DEGs and to analyse the functional interactions between proteins (an interaction with a combined score > 0.9 was considered statistically significant), which were then visualized using Cytoscape. Moreover, the molecular complex detection (MCODE) app plugin of Cytoscape was applied to verify important modules.

The Human Protein Atlas

In this study, the protein expression of CDC45 was detected in HCC and normal tissues from the human protein atlas (https://www.proteinatlas.org), which is a programme that contains immunohistochemistry (IHC) data that are from TMA-based analyses of different types of normal and cancer tissues(17). Staining intensity, quantity, location and clinical information from patients with respective cancer types are available online.

UALCAN Analysis

We evaluated the correlation between CDC45 expression and clinical relevance using the UALCAN (http://ualcan.path.uab.edu) online dataset, which is an effective website for the online analysis and mining of cancer data, mainly based on the relevant cancer data in the TCGA database(18).

Patient Characteristics And Construction Of The Tissue Microarray

TMAs from 321 HCC patients who underwent curative hepatectomy for primary HCC in Zhejiang Provincial People's Hospital between 2008 and 2015 were used for validation. None of the patients received radiotherapy or chemotherapy before the surgery. All of the patients were informed and signed informed consent forms. Total survival was calculated from the date of surgery to the end of follow-up (December 2016) or the date of death. In total, 321 HCC patients in our study presented with a mean age of 56.9 ± 11.4 years (range, 25.0–90.0 years), with 60 (18.7%) females and 261 (81.3%) males (Table 1). At the time of the primary diagnosis, 52.1% of the patients presented with a tumour diameter less than 5 cm, 47.9% with a tumour diameter greater than or equal to 5 cm. A total of 63.2% had an Edmondson grade of I/II, and 36.8% had grade III. In addition, the numbers of patients with or without metastasis were 288 (91.4%) and 27 (8.6%), respectively. The median OS was 23.0 months (range, 1.0–74.0). Other clinical characteristics are displayed in Table 1.

Table 1
Association between the expression of CDC45 expression and clinical characteristics of HCC patients.

Clinicopathological variables	n (%)	CDC45 expression		P-value
		Low ()	High ()	
Sex				0.213
Male	261 (81)	129	132	
Female	60 (19)	35	25	
Age (years)				0.544
< 55	124 (39)	66	58	
>=55	197 (61)	98	99	
Size (cm)				**0.034**
< 5	163 (52.1)	75	88	
>=5	150 (47.9)	87	63	
Tumour number				0.797
Single	264 (82)	134	130	
Multiple	57 (18)	30	27	
Edmondson grade				0.299
-	199 (63.2)	96	103	
	116 (36.8)	63	53	
Metastasis				0.306
M0	288 (91)	147	141	
M1	27 (9)	11	16	
Vessel invasion				0.169
Absence	120 (50)	62	58	
Presence	119 (50)	72	47	
Microvascular invasion				**0.046**
Negative	133 (57.6)	68	65	
Positive	98 (42.4)	63	35	
HBs antigen				0.570

Clinicopathological variables	n (%)	CDC45 expression		P-value
		Low ()	High ()	
Negative	59 (19)	28	31	
Positive	256 (81)	132	124	
Cirrhosis				0.971
Negative	106 (33)	54	52	
Positive	215 (67)	110	105	

Immunohistochemical Staining

IHC was performed using standard techniques. Briefly, 5-μm paraffin-embedded TMA sections were dewaxed in xylene and rehydrated in graded alcohols. Then, 3% hydrogen peroxide was used to block endogenous peroxidase. Antigen retrieval was accomplished by adding 10 mM citrate buffer (pH 6.0) to the TMA sections and putting them in a high-pressure cooker. Then, the TMA sections were incubated with 1% bovine serum albumin (BSA) for 20 min to reduce nonspecific protein binding. Next, the TMA sections were treated with recombinant rabbit monoclonal CDC45 antibody (1:50; HuaBio, Hangzhou, China) for 1 h at room temperature. They were then rinsed with phosphate-buffered saline (PBS) and incubated with biotinylated secondary antibody (MXB, Fuzhou, China) at room temperature for 30 min. Subsequently, the TMA sections were stained with DAB chromogen (Gene Tech, Shanghai, China), counterstained with Mayer's haematoxylin, dehydrated with gradient alcohol and xylene, and mounted on slides. Finally, the TMA sections were observed under a Nikon light microscope.

Assessment Of CDC45 Expression

The Densito Quant software in Quant Center was applied to automatically identify and set the areas on the tissue sections as follows: dark brown as strong positive, tan as moderate positive, light yellow as weak positive, and blue (nucleus) as negative. Then, each tissue point was identified and analysed to find the areas of strong positive, medium positive, weak positive and negative (unit: pixel); then, the percentage of positive staining and the histochemistry score (H-score) were calculated. The median H-score (median = 7.44) was selected as the cut-off value to classify the level of CDC45 expression. H-scores < 7.44 were used to define tumours with low CDC45 expression, and H-scores > = 7.44 were used to define tumours with high CDC45 expression.

Statistical analysis

All data were analysed with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). A Cox proportional hazards model was adopted to determine the univariate and multivariate hazards. Differences between

HCC and noncancerous tissues were examined by using the paired t-test. Kaplan–Meier analysis was used to estimate survival, and any differences in survival were evaluated with a stratified log-rank test. $P < 0.05$ was considered statistically significant.

Results

Our study consisted of 3 stages. We first compared the mRNA and protein expression levels of CDC45 in HCC tissues as well as normal tissues using various databases. We then investigated the prognostic value of CDC45 expression in HCC and found that patients with elevated CDC45 expression had advanced clinicopathological parameters and inferior survival. In the second stage, we assessed CDC45 expression at the protein level in our TMA and conducted a survival analysis based on a distinct comparison of the expression of CDC45. In the third stage, significantly involved DEGs of CDC45 were screened, and corresponding functional annotations were performed.

Transcriptional Levels Of CDC45 In Patients With HCC

We first compared the transcriptional levels of CDC45 expression between tissues from HCC and control (normal liver tissue) in three datasets. In the GEPIA database, 369 HCC and 160 normal tissues were used for the analysis, and the results showed that the CDC45 mRNA level was higher in HCC tissues than in normal tissues (Fig. 1a). CDC45 expression was also significantly higher in HCC tissues than in normal tissues in the Oncomine database (Fig. 1b). To further confirm the results, we analysed the expression of CDC45 in the GSE76427 dataset from the GEO database and obtained the results as above (Fig. 1c). In addition, the IHC results from the Human Protein Atlas showed that CDC45 staining was not detected in normal liver tissues(Fig. 1d,f), even if there were exsit tumour tissue that not detect CDC45 staining, a result showed that the medium levels of expression were observed in HCC tumour tissues (Fig. 1e,g).Taken together, these data indicated that CDC45 was upregulated at the transcriptional but unclear at proteomic levels in HCC tissues compared with normal tissues.

CDC45 mRNA expression correlated with advanced clinicopathological parameters and poor prognosis in HCC patients

Using the UALCAN analysis, we found significantly elevated CDC45 mRNA expression in HCC samples compared to normal samples. As shown in Fig. 2a, CDC45 mRNA expression in HCC samples was notably correlated with advanced clinical stage ($P < 0.01$). Similarly, the results shown in Fig. 2b suggested that CDC45 mRNA expression was significantly correlated with pathological grade ($P < 0.01$). Subsequently, the survival analysis using GEPIA showed that higher CDC45 expression was significantly correlated with shorter OS (Fig. 2c, $P < 0.001$) and disease-free survival (DFS) (Fig. 2d, $P < 0.01$) in 364 HCC patients. Overall, the upregulation of CDC45 expression levels was significantly associated with advanced clinicopathological parameters and poor prognosis in HCC patients.

Validation of CDC45 expression in HCC and adjacent normal tissues

After confirming the correlation between mRNA expression of CDC45 and prognosis of HCC patients in the bioinformatics database, we further detected the protein expression levels in our TMA. IHC staining was used to detect the CDC45 protein expression levels in HCC tumour samples and matched adjacent normal tissues from 56 HCC patients (Fig. 3a). CDC45 staining was observed mainly in the cytoplasm. Compared to adjacent normal liver tissues, the expression of CDC45 was significantly decreased in HCC tissues ($P < 0.0001$, Fig. 3b).

Association Of CDC45 Expression With Clinical Characteristics

We then explored the relationship between CDC45 expression levels and the clinicopathological features of HCC patients. Among the clinicopathological parameters, CDC45 expression was positively correlated with tumour size ($P = 0.034$) and microvascular invasion ($P = 0.046$). However, the correlation between CDC45 expression and other clinical parameters (including sex, age, tumour number, Edmondson grade, metastasis, vessel invasion, hepatitis B surface (HBs) antigen, and cirrhosis) was not significant ($P > 0.05$; Table 1). These findings suggested that low CDC45 expression is associated with worse overall disease condition.

Clinical significance of CDC45 expression in the prognosis of HCC

The Kaplan–Meier survival curve indicated that CDC45 expression was significantly associated with OS in HCC patients ($P = 0.019$; Fig. 3c). In all HCC patients, the OS was shorter in patients with low CDC45 expression than in patients with high CDC45 expression. Additionally, we used Cox regression to analyse the prognostic factors of HCC. A univariate Cox regression revealed that tumour size ($P = 0.007$), metastasis ($P < 0.001$), microvascular invasion ($P = 0.022$), Edmondson grade ($P < 0.001$) and vessel invasion ($P = 0.011$) were independent prognostic factors in patients with HCC. A multivariate Cox regression analysis revealed that distant metastases ($P < 0.001$) and Edmondson grade ($P = 0.013$) were independent prognostic factors for patients with HCC (Table 2). Consistent with the results of the Kaplan–Meier analysis, both the univariate ($P = 0.022$) and multivariate ($P = 0.035$) Cox regression analyses showed that CDC45 expression was significantly associated with the prognosis of HCC.

Table 2
Univariate and multivariate Cox regression survival analyses

Parameters	Univariate analyses		Multivariate analyses	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
CDC45 expression	0.558 (0.338–0.920)	**0.022**	0.509 (0.271–0.957)	**0.035**
Size	1.965 (1.201–3.213)	**0.007**	1.157 (0.614–2.177)	0.652
Metastasis	5.075 (2.673–9.636)	**0.000**	6.533 (2.916–14.635)	**0.000**
Microvascular invasion	1.879 (1.097–3.219)	**0.022**	1.015 (0.374–2.753)	0.977
Vessel invasion	2.035 (1.174–3.525)	**0.011**	1.636 (0.602–4.440)	0.334
Edmondson grade	2.696 (1.655–4.392)	**0.000**	2.082 (1.166–3.715)	**0.013**

CI, confidence interval.

Identification of DEGs and functional annotations as well as predicted signalling pathways

To further investigate the biological roles of CDC45 in HCC, the genes from the GSE76427 dataset were divided into two groups according to the median value of CDC45 expression to screen the DEGs between the low and high CDC45 expression groups. The screening criteria are discussed in the Materials and methods section). As shown in Fig. 4a, there were 233 upregulated and 167 downregulated DEGs. Specifically, the top 20 significant DEGs with positive and negative correlations are shown in the heat map in Fig. 4b. Subsequently, 400 involved DEGs were subjected to GO annotation and KEGG pathway analyses. The top 30 GO terms with the highest gene enrichment are shown in Fig. 4c. The related genes were significantly involved in eukaryote division, including organelle fission, nuclear division, chromosome segregation and mitotic nuclear division, and were markedly involved in the regulation of the cell cycle phase transition and DNA replication. Moreover, the KEGG analysis showed that most of the involved significant pathways included the cell cycle, DNA replication, drug metabolism and metabolism of xenobiotics by cytochrome P450, chemical carcinogenesis, meiosis and fatty acid degradation signalling (Fig. 4d). The detailed functional annotations, KEGG analysis information and percentage of each term are illustrated in additional file 1.

To further explore the interplay among the DEGs, we constructed a PPI network based on the STRING online database and Cytoscape software. As illustrated in Fig. 5a, the network contains 400 nodes and 1,622 edges. Clustering analysis of the PPI network was then carried out using Cytotype MCODE, and the top three significant modules were selected based on the degree of importance. Module 1 contains 43 nodes and 683 edges (Fig. 5b); module 2 contains 13 nodes and 39 edges (Fig. 5c); and module 3 contains 19 nodes and 58 edges (Fig. 5d). Submodule analysis information is shown in additional file 2.

Discussion

CDC45 is a key component of the CMG complex, is located on chromosome 22q11.21, and is required for the initiation and elongation of chromosomal DNA replication(8),(19). In recent years, several reports have shown that its expression is related to the occurrence and development of tumours. It has been reported that CDC45 overexpression was observed in lung cancer and was considered a novel tumour-associated antigen (TAA) that might be a useful target for lung cancer immunotherapy(20). Furthermore, Hu Y et al. verified that CDC45 expression was upregulated in colorectal cancer patients and associated with poor prognosis(21). A previous study also revealed that the CDC45 protein level is consistently higher in various kinds of human tumour cells than in primary human cells and was identified as a proliferation-associated antigen(10). Our preliminary analysis results from three databases showed that CDC45 expression is higher at both the mRNA and protein levels in HCC tissues than in normal tissues, and high mRNA levels of CDC45 indicated advanced clinicopathological parameters and a poor prognosis for HCC patients; these results are similar to those from previous studies.

However, in the present study, we found that CDC45 expression at the protein level was downregulated in HCC by detecting the expression of CDC45 in tumour tissues and matched adjacent normal tissues from 56 HCC patients. The processes of gene transcription and translation are very complex. It seems that it is not difficult to understand that the mRNA results from the database are inconsistent with those from our protein expression experiments. Although there are fewer samples in this study, this pairing analysis seems to be more convincing. Moreover, our results demonstrated that high protein expression of CDC45 negatively correlated with tumour size and metastasis, which revealed that decreased expression of CDC45 may promote cancer progression. Importantly, the Kaplan–Meier survival curve indicated that HCC patients with a low expression of CDC45 have an inferior prognosis than patients with a high expression of CDC45. To further analyse the possible causes, we screened for DEGs related to CDC45 expression from the GEO database and conducted GO and KEGG analyses. Our results showed that the genes mostly enriched in the GO terms included organelle fission, nuclear division, chromosome segregation, mitotic nuclear division, regulation of cell cycle phase transition, regulation of mitotic cell cycle phase transition, nuclear chromosome segregation, sister chromatid segregation, DNA replication, and cell cycle G1/S phase transition. Furthermore, the KEGG analysis showed that most of the involved significant pathways included the cell cycle, DNA replication, drug metabolism – cytochrome P450, metabolism of xenobiotics by cytochrome P450, and chemical carcinogenesis. Therefore, we speculated that the correlation between CDC45 and HCC is related to these functions.

In addition to the sustained proliferation, the escape from apoptosis, genomic instability, and DNA replication stress are also considered hallmarks of human cancers and are closely related to tumorigenesis and progression(22). Although it is not yet clear whether CDC45 plays any role in the repair pathways in mammalian cells, CDC45 plays an important biological role in maintaining the genomic stability(23),(24). Furthermore, it has been indicated that CDC45 is important for the response to replication stress by interacting with ssDNA(25) and recruiting Rad53(26). Recently, the Carsten group reported that CDC45 is limiting for replication initiation in humans, and inhibiting the degradation of

CDC45 might be a promising way to combat cancer(27). These findings might explain the poor prognosis of low CDC45 expression in HCC patients.

This study has several limitations. First, we failed to verify the expression level of CDC45 in the transcriptome due to the lack of relevant samples for qPCR and clinical prognosis information. Second, the underlying mechanisms of signalling pathways in HCC remain unclear, even though a series of functional annotations and enrichment analyses were performed. Future research is required to explore the detailed mechanism between CDC45 and carcinogenesis as well as reveal the mechanism of CDC45 in other carcinomas.

Conclusions

In conclusion, we revealed the crucial role of CDC45 in HCC patients. Decreased CDC45 protein expression correlated with the progression and poor prognosis in HCC, indicating that CDC45 is a valuable promising prognostic biomarker and might be a potential treatment target in HCC. Further studies are required to analyse its specific biological function in HCC.

Abbreviations

HCC: Hepatocellular carcinoma;CDC45:Cell division cycle protein 45;IHC: Immunohistochemistry; TMA: Tissue microarray; DEGs: Differentially expressed genes; OS: Overall survival;CMG:CDC45-MCM2-7-GINS;GEPIA:Gene expression profiling interactive analysis; TCGA: The Cancer Genome Atlas; GTEx: Genotype-tissue expression; GEO: gene expression omnibus; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein–protein interaction; MCODE: molecular complex detection; DFS: Disease-free survival. CI, Confidence interval.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The Ethics approval and consent to participate of the current study was approved and consented by the ethics committee of Zhejiang Provincial People's Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

All authors have significant contributions to the conception of the work. Chen Yang and Shufang Xie analyzed statistics and drafted the manuscript. Yi Wu, Guoqing Ru, Xianglei He provided the clinical data and revised manuscript. Hongyin Pan, Shibing Wang, Xiangmin Tong participated clarification of data and provided final approval of the submitted manuscript.All authors read and approved the final manuscript.

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Authors' information

1. Department of Clinical Medicine, Qingdao University, Qingdao, P. R. China
2. Molecular diagnosis laboratory, Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College, Hangzhou, Zhejiang 310014, P. R. China
3. The Second Clinical Medical College, Zhejiang Chinese Medical University Hangzhou 310000, P. R. China
4. Department of Hematology, Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College, Hangzhou, P. R. China
5. Department of Pathology, Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College, Hangzhou, P. R. China
6. Department of Infectious diseases, Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College, Hangzhou, P. R. China
7. The Key Laboratory of Tumor Molecular Diagnosis and Individualized Medicine of Zhejiang Province, Zhejiang Provincial People’s Hospital (People’s Hospital of Hangzhou Medical College), Hangzhou, 310014, P. R. China

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Supplementary Information

Additional file 1: KEGG and GO analysis information and percentage of each term are illustrate in KEGG.txt and GO.txt.

Additional file 2:PPI and submodule analysis information is shown in additional file 2.

Figures

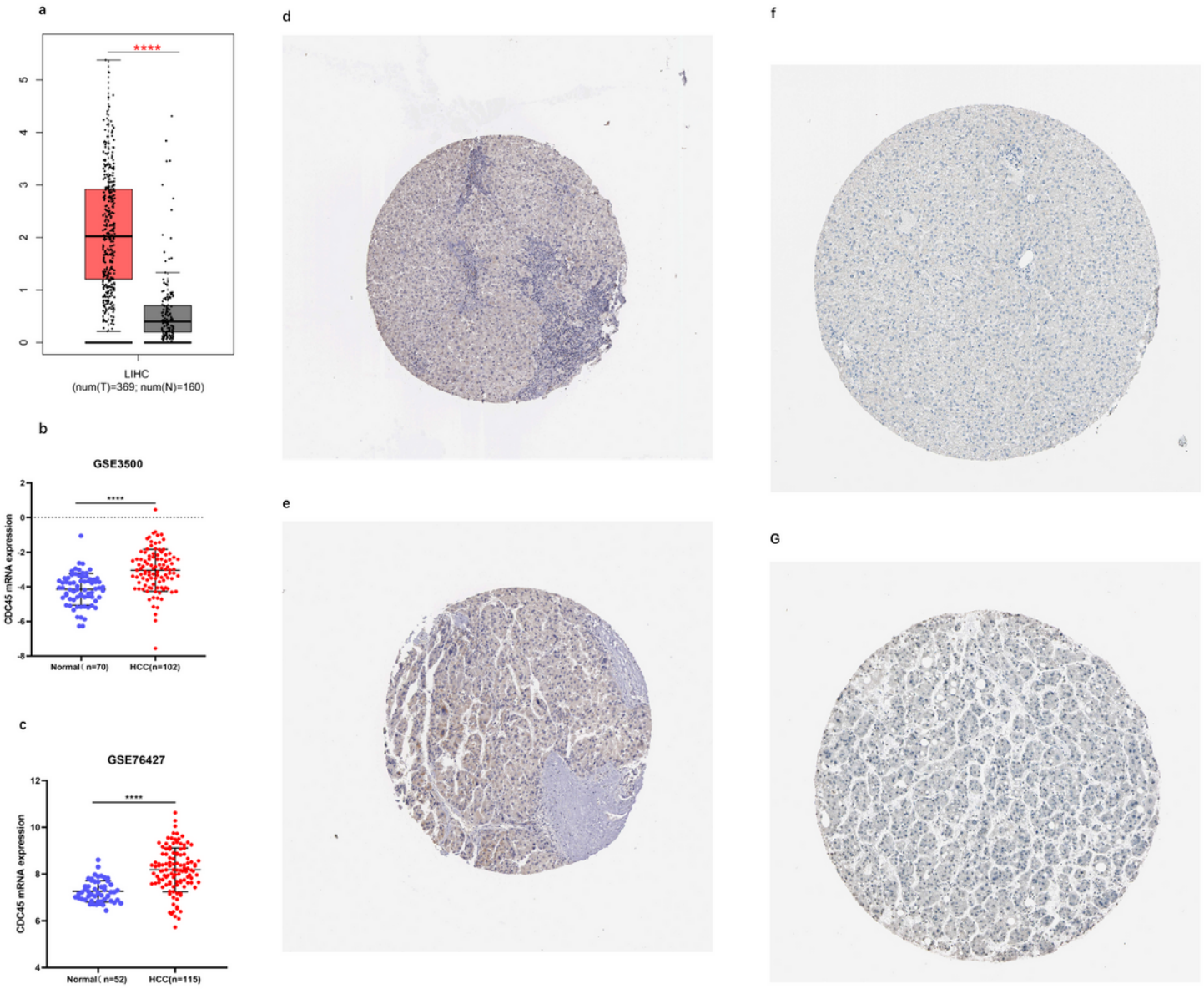

Figure 1

Differential CDC45 expression in HCC and normal liver tissues based on multiple databases. a Transcriptional level of CDC45 expression was found to be highly expressed in 369 HCC tissues compared with 160 normal tissues in the TCGA cohort (****p < 0.0001). b-c CDC45 expression was significantly higher in HCC primary tumour tissues than in normal liver tissues in GSE3500 (****p < 0.0001) and GSE76427 (****p < 0.0001). d-g CDC45 expression was detected in HCC tissues but not in normal tissues using an online database.

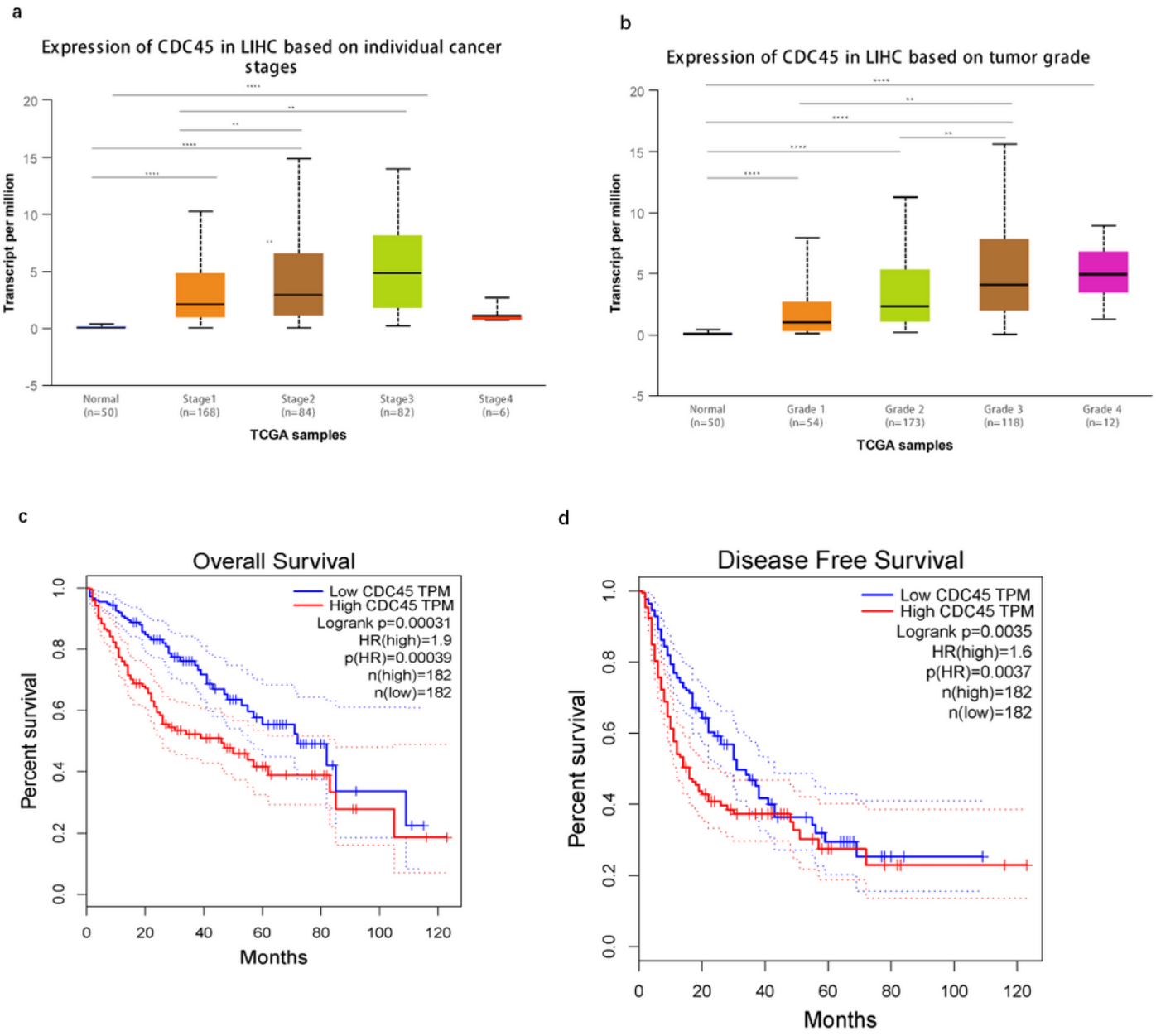

Figure 2

Transcriptional expression of CDC45 significantly correlated with advanced clinicopathological parameters and poor survival outcomes in HCC patients from the TCGA cohort. a Transcriptional expression of CDC45 was significantly correlated with American Joint Committee on Cancer (AJCC) stages, and patients who were in more advanced stages tended to express higher mRNA expression levels of CDC45. b Transcriptional expression of CDC45 was evidently correlated with pathological grade, and patients who had advanced grade scores tended to express elevated mRNA expression levels of CDC45. **p < 0.01, ***p < 0.001, **** p<0.0001. c Survival analysis using GEPIA indicated that CDC45 was significantly correlated with a shorter OS (p<0.001). d Survival curves suggested elevated CDC45 mRNA levels correlated with poorer DFS in the 364 included HCC patients (p < 0.001).

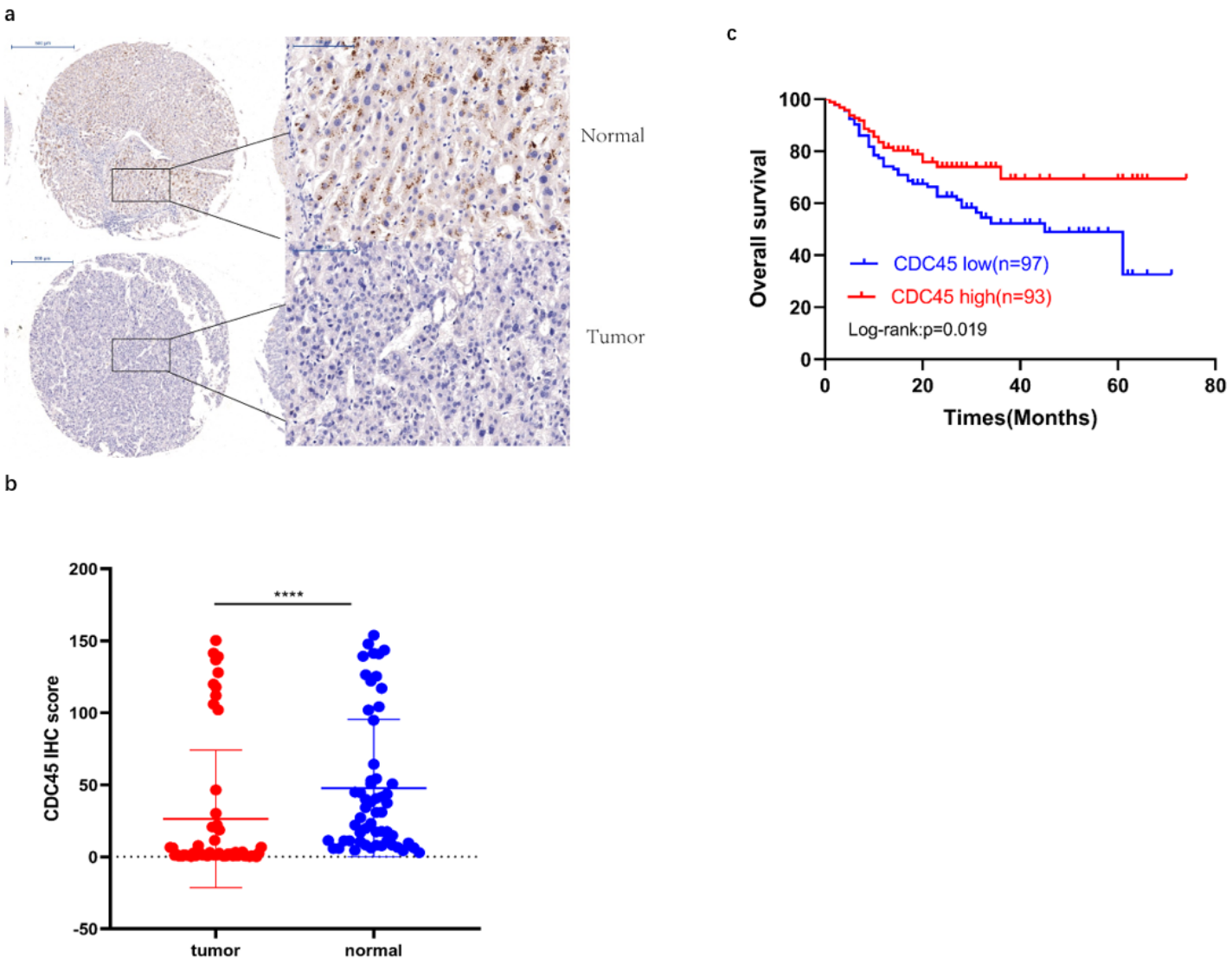

Figure 3

CDC45 protein expression and prognostic implications in the TMA of HCC. a IHC staining indicated significantly downregulated CDC45 expression in HCC tissues compared with adjacent normal liver tissues in the TMA. b The differential CDC45 protein expression in 56 paired tumour and normal liver tissues ($p < 0.0001$). c Survival analysis using the Kaplan–Meier method indicated that low CDC45 expression was significantly correlated with a shorter OS ($p = 0.019$).

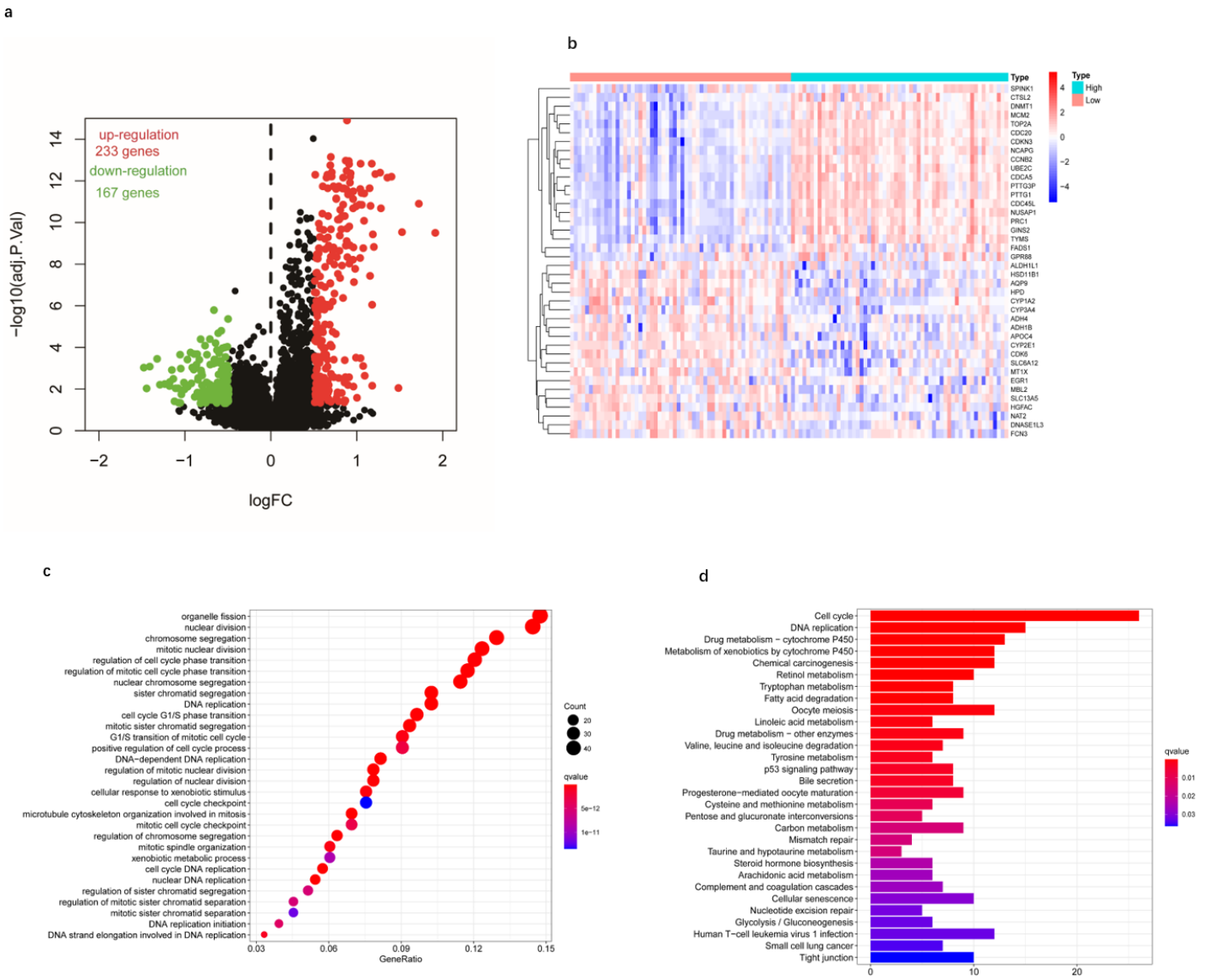

Figure 4

Enrichment analysis and PPI network construction among DEGs. a The volcano plot shows the DEG distributions in both up- and downregulated genes. The green colour represents the downregulated genes, while the red colour represents the upregulated genes. b The top 20 significant DEGs with positive and negative correlations are shown in a heat map. c The 30 most enriched GO terms of the DEGs were obtained in patients with HCC. d The 30 most enriched KEGG pathways of the DEGs are shown in the bar plot.

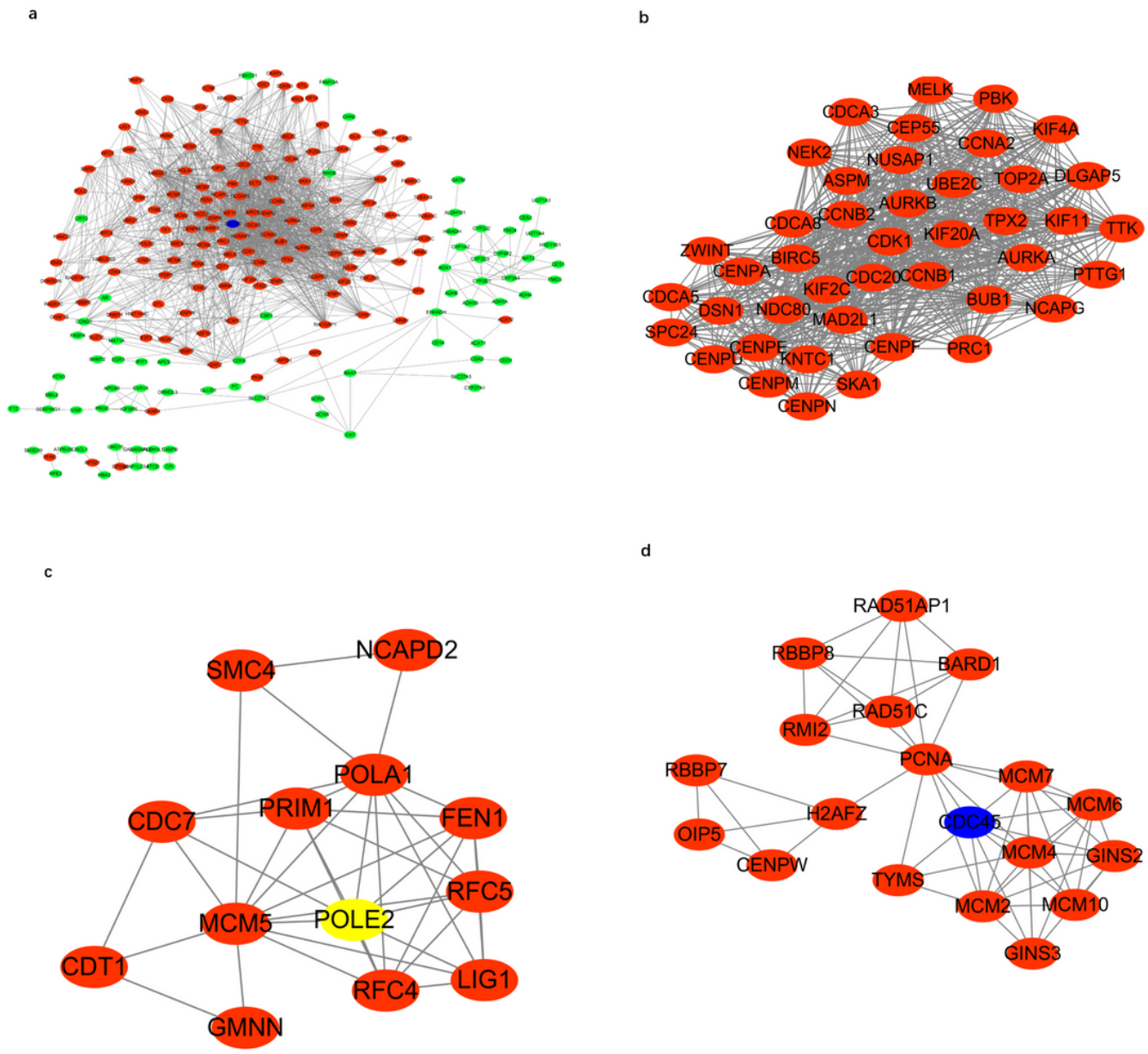

Figure 5

Construction of the PPI network and module analysis. a The PPI network has red nodes that represent upregulated genes, green nodes for downregulated genes, and blue nodes for CDC45. b Module 1 of the PPI network. c Module 2 of the PPI network. d Module 3 of the PPI network.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile2.rar
- Additionalfile1.rar