HAUS5 is a novel prognostic biomarker for hepatocellular carcinoma that is associated with poor clinical outcomes

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Research Article

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Abstract

Background

The Augmin Like Complex Subunit 5 (HAUS5) is involved in microtubule generation and centrosome assembly. Loss of HAUS5 function leads to loss of centrosome integrity, ultimately promoting tumor formation by causing functional defects and chromosome dislocation in the bipolar spindle. However, the role of HAUS5 in the development and progression of hepatocellular carcinoma (HCC) remains unclear.

Methods and results

This research dealt with investigating the role of HAUS5 in HCC and reported that HAUS5 is over-expressed in HCC tissues and cells. It was also found that its high expression levels were a crucial risk factor that affected HCC patients’ survival status. Correlation analysis depicted that HAUS5 expression was linked to immune infiltration in HCC. A nomogram model with good predictive capability with an area under the curve (AUC) value of 0.969 was constructed by integrating the clinical features of HCC and HAUS5 expression levels. HAUS5 knockdown remarkably attenuated the migration abilities and invasiveness of HCC cells.

Conclusion

HAUS5 is over-expressed in HCC tissues and could be used as a novel prognostic biomarker for patients with HCC.

Introduction

Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer and the third highest cause of cancer-associated death globally[1]. Globally, sub-Saharan Africa and eastern Asia present the maximum numbers of HCC cases (80%) where the major risk factors are the development of chronic hepatitis B (CHB) and exposure to aflatoxin B1 (AFB1)[2, 3]. Accompanied by a poor prognosis, HCC patients face a greater fatality risk due to tumor invasion and metastasis since early diagnosis is difficult due to the asymptomatic presentations of early-stage patients[4, 5]. At present, treatment of HCC involves targeted therapy, immunotherapy, and radiation, all of which have limited efficacy[6, 7]; therefore, overall survival rates for patients with HCC are still poor. Consequently, the discovery of molecular markers and prognostic indicators for HCC diagnosis and treatment holds considerable significance[8, 9].

One of the eight subunits of the augmin complex, the Augmin Like Complex Subunit 5 (HAUS5), is also referred to as hDgt5 or KIAA0841[10]. It is involved in spindle formation, centrosome integrity, and division of the cytoplasm during cell division. Any abnormality in HAUS5 expression may result in the fragmentation of the centrosomal microtubule and functional defects in the bipolar spindle both of which
lead to chromosome dislocations that may result in tumor onset\cite{11,12}. Although HAUS5 was recently found to be overexpressed in some breast cancers\cite{13}, the expression levels and biological function of this gene in other tumors, especially HCC, are still unclear.

This research dealt with exploring the link between HAUS5 gene expression (via measurements of mRNA levels) levels and the clinical characteristics associated with HCC. This involved an examination of the prognosis- and immune characteristics-linked functions of HAUS5 in individuals with HCC. In vitro studies were used to understand the impact of HAUS5 knockdown on liver cancer cell lines. These results indicate that overexpression of HAUS5 may be potentially carcinogenic in liver cells and that targeting HAUS5 could be a promising strategy for HCC treatment.

**Materials And Methods**

**Datasets and data preprocessing**

The associated clinical data and RNA-sequencing (RNA-seq) data on samples of tumor and adjacent normal tissue from 33 types of cancer were retrieved from the databases Genotype-Tissue Expression (GTEX)\cite{14}, The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/)\cite{15} and Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo)\cite{16}. The RNA-seq data for 371 HCC and 50 normal tissue samples along with clinical information on patients were accessed at the Liver Hepatocellular Carcinoma (LIHC) Project of the GTEX and TCGA databases. GSE76427 and GSE17856 were downloaded from the GEO database. For further investigation, the RNA-seq data in the Fragments per kilobase per million (FPKM) format was converted into the transcripts per million (TPM) format.

**Bioinformatic analysis**

For the differentially expressed gene (DEG) analysis, the tissue samples were initially stratified as per the median HAUS5 expression levels values into high- and low-expression groups. These groups were comparatively analyzed concerning the expression levels of other genes utilizing the unpaired Student’s t-test function of the R package DESeq2 v3.6.3\cite{17}. The cutoff values for the DEGs were set to $|\log_{2}\text{fold change (FC)}| >2.0$ with P-values < 0.05. The databases UALCAN (http://ualcan.path.uab.edu/)\cite{18} and the Human Protein Atlas (https://www.proteinatlas.org/)\cite{19} were searched for analysis of the HAUS5 mRNA levels in the dataset TCGA and HAUS5 protein levels, respectively. The Kaplan-Meier Plotter (https://kmplot.com/analysis/)\cite{20} was employed to examine the associations between HAUS5 levels and patient survival rates. The database LinkedOmics (http://www.linkedomics.org/login.php)\cite{21} was utilized to conduct pathway analysis and functional annotation of genes, whereas the two expression groups were subjected to Gene set enrichment analysis (GSEA)\cite{22} concerning their differences in biological and functional pathways. The Tumor Immune Estimation Resource (TIMER2.0) (https://cistrome.shinyapps.io/timer/)\cite{23} determined the link between HAUS5 expression and tumor immune infiltration (TII). The KM plotter (http://kmplot.com/analysis/) was used to explore the prognostic values of HAUS5 in HCC.
Cell culture and treatment

The hepatocellular carcinoma cell lines (MHCC97H, Hep3B, HepG2, Huh7, PLC/PRF/5) and the human normal hepatocyte cell line (THLE-2) were purchased from SIBS, CAS (Shanghai, China). All cell lines were authenticated by Procell Life Science & Technology Co., Ltd. using STR analysis. These were grown in 6-well plates in RPMI 1640 medium containing 10% FBS (HyClone, USA), and maintained at 37°C and 5% CO₂.

Transfection with siRNA to knock down HAUS5 gene expression

Lipofectamine 2000 (Invitrogen, USA) was used to transfect siRNAs (Sangon Biotech, Shanghai, China) into Hep3B and HepG2 cells. The siRNA sequences were: siHAUS5-#1(si-1), CCACTTTGGCACTTCGTAC; siHAUS5-#2(si-2), CCAACGACGAGATGCGCAT; siNC, TTCTCCGAACGTGTCACGT. The efficiency of this technique in silencing the HAUS5 gene was evaluated through Western blotting assays and real-time quantitative PCRs (RT-qPCRs).

Reverse transcription quantitative (RT-qPCR) assays

TRIzol reagent (TakaraBio, Japan) was utilized to extract total RNA from cells. As per the instructions of the manufacturer, cDNA was obtained through the First Strand cDNA Synthesis kit (TakaraBio), and the LightCycler 480 v1.5 system (Roche, Penzberg, Germany) was employed for conducting RT-qPCR. The selected primers that were utilized in the study were as follows: HAUS5 Forward:

5’-TGCTCATCAAGGGAAACTCGG-3’, and Reverse: 5’-CTGTGGTGCCACTGCCTCAAA-3’; GAPDH Forward: 5’-TGACTTCAACAGCGACACCCA-3’, and Reverse: 5’-CACCATGTTGCTGTAGCCAAA-3’.

Western blotting

As Per the prior standard protocol, western blotting was conducted. This experiment utilized the following primary antibodies: Anti-HAUS5 (1:500 dilution, A15133, Abclonal, Woburn, MA, USA) and anti-GAPDH (1:2000 dilution, ab9485, Abcam, UK). The HRP-labeled secondary antibody utilized was an IgG anti-rabbit antibody (1:5000 dilution, 7074P2, Cell Signaling Technology, USA). The visualization of the membrane’s protein bands was done through the Femto ECL kit (#FD8030, FDbio, Shenzhen, China). Ultimately, ImageJ (NIH, MD, USA) was used to examine the blots.

Immunohistochemistry (IHC)

The diagnosed 20 HCC patients at Shunde Hospital, Southern Medical University (The First People's Hospital of Shunde), Foshan, China between 2017 and 2018 provided a total of 40 paraffin-embedded HCC tissue samples. The Institutional Research Ethics Committee granted its approval for the use of these tissue samples.
The procedures for IHC and scoring were executed as per the standard protocols. The antibodies and reagents employed were: anti-HAUS5 (1:200 dilution, Abclonal, Woburn, MA, USA), HRP-labeled IgG anti-rabbit secondary antibody (1:5000, 7074P2, Cell Signaling Technology, USA). In addition, Femto ECL Kit (#FD8030, FDbio, Shenzhen, China) was utilized for membrane visualization. Lastly, ImageJ (NIH, MD, USA) was used to examine protein blots.

**CCK8 assays**

Following the resuspension of the trypsinized logarithmic (growth phase), for cells in a complete medium, a 96-well plate was employed for overnight culturing of the cells in each experimental group. The cell counting kit-8 (CCK8) reagent (Abcam) was employed to examine cell proliferation on the second day following kit instructions. At 490nm the microplate reader (Molecular Devices, Rockford, IL, USA) was utilized to evaluate the optical density, from which cell numbers could be deduced.

**Migration assays**

Utilizing Transwell insert chambers (8-µm pore size; Corning, NY, USA), the migration assays were executed as per the manufacturer's instructions. The 10% Matrigel (Dow Corning) was utilized to pre-coat the upper chamber with a subsequent transfer of the cells (5×10⁴ cells/well) to the upper chamber and subsequent incubation at 37°C for 24 h. The cells that migrated to the lower chamber were then visualized using an inverted microscope.

**Wound healing assays**

The overnight-cultured Hep3B and HepG2 cells (cultured in 12-well plates (5×10⁴ cells/well)) at ~ 90% confluency were then utilized. A wound mimicking single line scratch was made using a sterile 10 µl pipette tip on the cell monolayer, which was incubated in a 1% FBS containing RPMI 1640 medium for 48 h. An inverted microscope was utilized to examine the scratches.

**Statistical analysis**

R (v 3.6.2) and SPSS (v 20.0; IBM, Chicago, IL, USA) were employed in this experiment for the analysis of the data. Mean ± SEM was utilized to present the data. Comparison between any two groups was performed employing the Student's t-test. Significance was set as P < 0.05.

**Results**

**Over-expression of HAUS5 in HCC tissues**

The results indicate that in comparison with adjacent normal tissues, the HAUS5 mRNA levels were elevated in tumor tissues in 19 of the 33 cancers investigated (Fig. 1A). The data indicated that in comparison with normal tissues, the levels of HAUS5 mRNA were remarkably elevated in HCC tissues (P < 0.001; Fig. 1B-E). The results from the UALCAN and Human Protein Atlas databases also suggest that HCC tissues have elevated HAUS5 protein levels in contrast with the normal tissues (Fig. 1F and G).
Altogether, these data indicate an upregulated HAUS5 expression in HCC tissues and that HAUS5 may play a central role in the onset and progression of HCC.

**Over-expression of HAUS5 was linked to adverse clinical parameters in HCC**

Increases in HAUS5 expression were remarkably linked to several adverse clinical features in HCC, namely, pathological stage, T stage, histological grade, tumor status, alpha-fetoprotein (AFP) levels, and vascular invasiveness (Fig. 2A-F and Table 1). The overall survival (OS) was influenced by factors that were determined through univariate regression analysis to be HAUS5 levels (P < 0.011), tumor status (P < 0.001) as well as pathologic (P < 0.001), T (P < 0.001), and M stages (P < 0.017) in HCC patients. These factors’ multivariate regression analysis depicted that the status of tumor (P < 0.004), and HAUS5 levels (P < 0.001) were major risk factors affecting HCC patients’ OS (Table 2).

**Gene function annotation and pathway analysis**

The results of the gene function annotation analysis using LinkedOmics indicate that there is a considerable positive link to HAUS5 gene expression levels. The heatmap of the results depicts a considerable positive or negative association of 50 gene sets with the expression levels of HAUS5 (Fig. 3A-C). The resulting data of the GO and KEGG analyses indicate that the genes associated with HAUS5 are involved in the nuclear division, organelle fission, segregation of chromosomes, mitotic nuclear division, and DNA replication (Fig. 3D). Changes in the biological processes (BP) and molecular functions (MF) of HAUS5 were correlated with changes in the chromosomal region, condensed chromosomes, chromosomes, centromeric region, spindle, acting on DNA, catalytic activity, as well as helicase, ATPase, and DNA-dependent ATPase activities (Fig. 3E and F). The KEGG molecular pathways identified were cell cycle, DNA replication, microRNAs in cancer, cellular senescence, and oocyte meiosis (Fig. 3G).

**HAUS5-related signaling pathways based on GSEA**

To determine the biological function of HAUS5, we analyzed the DEGs between the low and high HAUS5 expression groups based on the median expression value of HAUS5. GSEA pathway analysis results confirmed that HAUS5 is mainly involved in DNA replication, chromosome segregation, spindle organization, double-strand break repair, telomere organization, cell cycle G2/M phase transition, cell cycle checkpoint, DNA recombination, mitotic cell cycle phase transition, and DNA strand elongation (Fig. 4).

**Correlation between HAUS5 expression and methylation status of the HAUS5 gene**

To understand the mechanisms that lead to HAUS5 over-expression in HCC tissues, the link between the expression levels of the HAUS5 gene and its methylation status was investigated using tools that were
available online. In comparison with the normal liver tissues, the HCC tumor tissues exhibited considerably lowered DNA methylation levels in the HAUS5 gene promoter region (P < 0.001; Fig. 5A). In HCC tissues, a major proportion of the HAUS5 gene's methylation sites was hypomethylated, and the level of methylation was positively associated with the outcomes of patients (i.e., a poorer OS was depicted in patients with hypomethylated HAUS5 gene in tumor tissues than in patients with tumor tissues having higher levels of methylation at the HAUS5 gene) (Fig. 5B). Finally, two methylation sites were deduced to be (cg06791670 and cg25315287) indicative of poor prognosis (Fig. 5C and D).

**Correlation between HAUS5 expression levels and immune infiltration in HCC**

The expression levels of HAUS5 depicted a considerably negative correlation with the immune infiltration levels such as dendritic cells (DCs) (r = -0.373), neutrophils (r = -0.342), cytotoxic T cells (r = -0.314), and plasmacytoid dendritic cells (pDCs) (r = -0.297) (all P < 0.001; Fig. 6A). Additionally, considerably lowered enrichment scores of DCs, neutrophils, cytotoxic T cells, and pDCs were depicted in the HAUS5 group with enhanced expression than in the group with attenuated expression (all P < 0.001; Fig. 6B-I).

**Prognostic Value of HAUS5 in HCC**

The multivariate Cox analysis-based forest map for OS (Fig. 7A) indicates that HCC patients’ survival could be influenced by two major risk factors, namely, tumor status (P < 0.004) and HAUS5 expression levels (P < 0.001). The diagnostic role of HAUS5 expression levels in HCC was examined by a receiver operating characteristic (ROC) curve analysis using information from normal and HCC liver tissues obtained from TCGA. The area under the ROC curve (AUC) analysis indicates that HAUS5 expression levels were specific to and sensitive enough for diagnosing HCC (AUC = 0.969, CI = 0.953–0.984; Fig. 7B). The Kaplan-Meier method was utilized to determine the link between HAUS5 expression levels and breast cancer patients’ prognoses. The patients were classified into high and low HAUS5 expression groups as per the cutoff score that was determined by the median value of the HAUS5 expression levels. In contrast with the low- HAUS5 expression group, the OS, disease-free survival (DFS), progression-free survival (PFS) and recurrence-free survival (RFS) values of the group with higher expression of HAUS5 were considerably worse, indicating considerably worse prognosis (Fig. 7C-F).

**Construction and validation of a nomogram based on OS-influencing independent factors**

OS influencing independent factors were utilized to develop a nomogram for prognosis prediction of HCC patients. A worsening prognosis was linked to a greater score (Fig. 8A). Moreover, the efficacy of the nomogram concerning its prediction capabilities was assessed through calibration curves (Fig. 8B).
The expression profiles of HAUS5 in HCC tissues and cell lines

The experiment made use of liver cancer lines and 20 patients’ tissue samples for confirmation of the overexpression of HAUS5 in HCC using IHC, western blotting, and RT-qPCR. The former two procedures were executed to examine the levels of HAUS5 in HCC cell lines (MHCC97H, Hep3B, HepG2, Huh7, and PLC/PRF/5) and human normal hepatocyte cell line (THLE-2). The HAUS5 protein levels were considerably elevated in HCC tissues compared to normal tissues (Fig. 9A-D). The levels of HAUS5 mRNA and protein in the HCC cell lines were higher than those in the THLE-2 cell line (Fig. 9E-G). These results depict congruence with the bioinformatics data.

HAUS5 knockdown suppressed HCC cell lines' propagation and migration

We silenced the HAUS5 gene in Hep3B and HepG2 cell lines using si-RNA. After transfection with several siRNA constructs, the most efficient construct (si-1) was identified using RT-qPCR and western blotting (Fig. 10A and B); further experiments were conducted using the si-1 construct. The CCK8 results showed that the HAUS5 knockdown cells of both cell lines (Hep3B and HepG2) had lower proliferation rates than those of the control cells (P < 0.001; Fig. 10C). Plate cloning assays depicted that the HAUS5 knockdown cells from both cell lines had lower clone formation rates than those of control cells (P < 0.001; Fig. 11D). The effects of HAUS5 knockdown on the invasiveness and migration potential of the two cell lines were examined. The migration and wound healing assays depicted that HAUS5 knockdown attenuated the invasiveness and migration potential of both cell lines (P < 0.01; Fig. 10E and F).

Discussion

The abnormal expression of HAUS5 can induce the microtubule fragmentation of centrosome and the increase of centrosome size, resulting in the decrease of kinetochore fiber formation, chromosome dislocation and the functional defect of bipolar spindle, while abnormal bipolar spindles can induce tumor formation\(^{[24,25]}\). In previous studies, knockdown of HAUS5 was found to inhibit the proliferation of glioblastoma stem cells and their ability to form tumors\(^{[26,27]}\). At present, the HAUS5 gene has been studied in breast cancer, suggesting that it is involved in the onset and progression of the disease\(^{[13]}\). However, it remains unknown whether HAUS5 influences the development of HCC.

For the first time, HAUS5’s research concerning HCC was broadened by this experiment. We using datas retrieved from the TCGA, GTEX and GEO databases, it was demonstrated that the expression of HAUS5 is promoted in various human cancers, including HCC. In comparison with normal tissues, elevated levels of HAUS5 mRNA and protein were depicted in HCC tissues. Additionally, a positive link was observed between higher HCC levels and adverse clinically associated features in the histologic grade, tumor status, AFP levels, vascular invasion as well as pathological and T stages. The ROC curve analysis
indicated the potential of HAUS5 as an HCC diagnosis biomarker. The KM curves suggest that HAUS5 expression levels are negatively correlated with OS, DFS, PFS, and RFS, based on data from individuals with HCC in the TCGA datasets. Furthermore, the results of the assays in this research involving RT-qPCR, western blotting, and IHC have also demonstrated that HAUS5 is over-expressed in HCC cell lines and tissues. All of these results show that HAUS5 performs a key function in HCC progression.

Considering the data of prior studies, which documented the importance of HAUS5 for cell proliferation, division, and cell cycle progression in breast cancer\[13\], the possibility of HAUS5 having similar effects on the progression of HCC was investigated. GSEA enrichment confirmed that HAUS5 was significantly associated with the cell cycle, DNA replication, microRNAs in cancer, cellular senescence, and oocyte meiosis. A frequent epigenetic method of gene regulation is DNA methylation, which often inhibits gene expression\[28\]. Although data on the methylation status of the HAUS5 gene in solid tumors are scarce, this study successfully determines that the HAUS5 gene in HCC tumors is hypomethylated. Hence, it can be proposed that the hypomethylation contributes to the overexpression of the HAUS5 gene in HCC, especially since poor patient prognosis was linked to this methylation state.

A complicated microenvironment is utilized by the tumor cells for growth that is modulated by the cancer cells themselves, stromal, and immune cells\[29,30\]. Cancerous tumors, such as HCCs, usually contain large numbers of tumor-infiltrating immune cells\[31\]. Malignant solid tumors have revealed the prognosis-associated significance of the presence of the aforementioned cells. This significance is dependent on the location, density, and type of the infiltrating immune cells. The role of tumor immune infiltration (TII) cells in cancer onset and progression has recently been the focus of much research as TII cells have been depicted to affect the tumor cells’ responses to immune checkpoint inhibition (ICI) treatments and neoadjuvant chemotherapy\[32,33\]. Therefore, screening for TII cells in HCC could not only be useful in monitoring ICI treatment but may also aid in predicting the outcomes of ICI therapy. In this study, it was established that HAUS5 over-expression is negatively associated with inflammation in the HCC tumors as well as numbers of tumor-infiltrating DCs, neutrophils, cytotoxic T cells, and pDCs, all of which are known to arrest HCC cell growth. These results indicate that HAUS5 over-expression may affect HCC progression and patient prognosis by regulating the immune microenvironment in HCC tumors. Therefore, HAUS5 could potentially be used as an immune therapy-related biomarker for HCC.

Since the results from the GSEA enrichment analyses show that HAUS5 most likely plays a central role in the proliferation of cells and their migration, the potential biological effect of HAUS5 knockdown on cancer cells was examined using liver cancer-derived cell lines that over-express HAUS5. The data implied that the knockdown of HAUS5 in Hep3B and HepG2 cells attenuated cell proliferation, migration, and cell invasiveness in these cell lines. As per these resulting data, it can be assumed that HAUS5 performs an essential role in regulating tumor progression in HCC.

Although this work provides a deeper understanding of the relationships between HAUS5 expression levels and prognosis for patients with HCC, this work has some important limitations. First, since this study only used an online dataset, the results may be influenced by selection bias. Second, as a large
The proportion of the data utilized in this analysis came from online sources, certain crucial clinical data (for example, the chemotherapy regimens used) of the patients was not obtained. Moreover, further research and thorough experimental validations are needed for all of the in vitro and in vivo experiments of this study. The biological functions and underlying mechanisms of how HAUS5 expression levels impact progression and patient prognosis in patients with HCC remain unclear and require additional study.

This research concluded that HAUS5 over-expression may function as an independent prognostic factor in HCC progression and that it is strongly related to adverse effects such as adverse clinical features and tumor-promoting immune microenvironments. These findings imply the potential of HAUS5 expression levels as a novel biomarker for the prediction of HCC patients’ prognoses. The mechanisms via which HAUS5 affects tumorigenesis and progression in HCC require further study.

**Declarations**

**Acknowledgments**

Not applicable.

**Author contributions**

Yonggang Liu and Jiyun Liang designed the research; Yonggang Liu, Junyong Huang and Jiale Wang conducted the experiment; Xi Li and Yonggang Liu analyzed the data; all authors wrote the article together and agreed the submission.

**Funding**

None

**Availability of data and materials**

The datasets supporting the conclusions of this article are included within the article.

**Ethics approval and consent to participate**

All methods were carried out in accordance with the Declaration of Helsinki. No ethics approval was required for this work. All utilized public data sets were generated by others who obtained ethical approval.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.
Author details

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### Table I. Relation between HAUS5 expression and clinical characteristics

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<th>Characteristics</th>
<th>Total(N)</th>
<th>Odds Ratio(OR)</th>
<th>P value</th>
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<td>Age (&gt;60 vs. &lt;=60)</td>
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<td>0.686 (0.455-1.031)</td>
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<td>Gender (Male vs. Female)</td>
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<td>0.885 (0.573-1.365)</td>
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<td>T stage (T2&amp;T3 vs. T1)</td>
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<td>1.739 (1.155-2.629)</td>
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<td>N stage (N1 vs. N0)</td>
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<td>2.687 (0.339-54.709)</td>
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<td>M stage (M1 vs. M0)</td>
<td>272</td>
<td>0.314 (0.015-2.487)</td>
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<tr>
<td>Pathologic stage (Stage II &amp; Stage III vs. Stage I)</td>
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<td>1.697 (1.114-2.596)</td>
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<td>Histologic grade (G3&amp;G4 vs. G1&amp;G2)</td>
<td>369</td>
<td>1.770 (0.991-3.226)</td>
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<td>Tumor status (With tumor vs. Tumor free)</td>
<td>355</td>
<td>1.576 (1.034-2.412)</td>
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<td>AFP(ng/ml) (&gt;400 vs. &lt;=400)</td>
<td>280</td>
<td>2.801 (1.576-5.110)</td>
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<td>Child-Pugh grade (B&amp;C vs. A)</td>
<td>241</td>
<td>0.497 (0.184-1.228)</td>
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<td>Vascular invasion (Yes vs. No)</td>
<td>318</td>
<td>1.690 (1.062-2.704)</td>
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### Table II. Univariate and multivariate analyses of clinicopathological parameters in patients with hepatocellular carcinoma in TCGA

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<th>Multivariate analysis</th>
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<td>Hazard ratio (95% CI)</td>
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<tr>
<td>Gender</td>
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<td>T stage</td>
<td>2.126 (1.481-3.052)</td>
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<td>N stage</td>
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<td>Pathologic stage</td>
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<td>Histologic grade</td>
<td>1.188 (0.721-1.958)</td>
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<td>Tumor status</td>
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<td>HAUS5</td>
<td>1.570 (1.108-2.225)</td>
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Figure 1

HAUS5 mRNA and protein expression in HCC. (A) Expression of HAUS5 in different types of tumors compared with normal tissues in TCGA and GTEx databases. (B-E) HAUS5 mRNA expression levels in HCC patients and matched adjacent normal samples in TCGA, GTEx and GEO databases (GSE76427 and GSE17856). (F-G) HAUS5 protein expression level based on CPTAC and Human Protein Atlas. *P<0.05, **P<0.01, ***P<0.001.
Figure 2

Correlation between HAUS5 expression and clinicopathological features. (A) Pathologic stage. (B) T stage. (C) Histologic grade. (D) Tumor status. (E) AFP. (F) Vascular invasion. *P<0.05, ***P<0.001.
Figure 3

Functional enrichment analysis of HAUS5 in HCC. (A-C) The correlation analysis of HAUS5 expression and its top 50 co-expressed gene network. (D-G) GO and KEGG enrichment analysis of co-expressed genes.
Figure 4

HAUS5-related signaling pathways based on GSEA. HAUS5, Augmin like complex subunit 5; GSEA, Gene set enrichment analysis.
Figure 5

DNA methylation level of HAUS5 and its effect on prognosis of patients with HCC. (A) The promoter methylation level of HAUS5 in HCC was obtained from the UALCAN database. (B) Correlation between HAUS5 mRNA expression level and methylation level. (C-D) Kaplan-Meier survival curves for two methylation sites of HAUS5. ***P<0.001.
**Figure 6**

Correlation of HAUS5 expression with immune infiltration level in HCC. (A) Correlation between HAUS5 expression and relative abundance of 24 types of immune cell. The size of dot corresponds to the absolute Spearman’s correlation coefficient values. (B-E) Comparison of immune infiltration levels of immune cells (including DCs, Neutrophils, Cytotoxic cells, and pDCs) between the high- and low-HAUS5 expression groups. (F-I) Correlations between the relative enrichment scores of immune cells (including DCs, Neutrophils, Cytotoxic cells, and pDCs) and the expression of HAUS5. ***P<0.001.
Figure 7

Prognostic value of HAUS5 in HCC. (A) Forest map based on multivariate Cox analysis for overall survival. (B) ROC curves for classifying HCC versus normal liver tissues in the TCGA-LIHC database. (C-F) Kaplan-Meier survival curves showed that HCC patients with high HAUS5 expression exhibited poor OS, DFS, PFS and RFS of HAUS5 in HCC determined by the TCGA-LIHC database.

Figure 8
A nomogram and calibration curves for prediction of one year overall survival rates of patients with HCC. (A) A nomogram for prediction of one year overall survival rates of patients with HCC. (B) Calibration curves of the nomogram prediction of one year overall survival rates of patients with HCC.

Figure 9

The expression level of HAUS5 in HCC tissue and cell lines. (A-D) IHC and western blotting assay examines the protein expression level of HAUS5 in HCC tissues and their matched paracancerous tissues.
(E-G) qPCR and western blotting assay examines the expression level of HAUS5 in HCC cell lines, including MHCC97H, Hep3B, HepG2, Huh7 and PLC/PRF/5, compared to human normal hepatocyte cell line (THLE-2). ***P<0.001.

Figure 10
Knockdown of HAUS5 inhibited the proliferation and migration of HCC cell lines. (A-B) The efficiency of si-HAUS5 measured with RT-qPCR and Western blot. (C-D) Effect of HAUS5 knockdown on cells proliferation in vitro. (E-F) Migration assay and Wound healing assay within 48h of incubation displayed inhibited migration and scratch repair of HAUS5-silenced HCC cells. **P<0.01, ***P<0.001.