GSG2 Promotes Tumor Growth Through Regulating Cell Proliferation in Hepatocellular Carcinoma

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Abstract

Background

Hepatocellular carcinoma (HCC) is one of the most commonly diagnosed malignant tumors in the world, and its recurrence and mortality rate are still in high level. In recent years, more and more inhibitors against gene targets have been found to be beneficial to survival. However, the function of homo-sapiens histone H3 associated protein kinase (GSG2) in HCC has not been completely understood.

Methods

The expression of GSG2 in HCC tissues was detected by immunohistochemical staining. The lentivirus-mediated short hairpin RNA (shRNA) was used to knockdown GSG2 expression in HCC cell lines Hep3B2.1-7 and SK-HEP-1. Cell proliferation and colony formation were detected by MTT assay and colony formation assay, respectively, and flow cytometry assay was used to investigate the cell apoptosis *in vitro*. Mice xenograft model was constructed to detect the functions of GSG2 on tumor growth *in vivo*. Human Apoptosis Antibody Array was conducted to find the possible mechanism.

Results

GSG2 was overexpressed in HCC tissues compared with adjacent normal tissues, which was positively related to the tumor pathological stage. The knockdown of GSG2 has the functions of inhibiting the progression of HCC, including inhibiting cell proliferation and colony formation and promoting cell apoptosis. Compared with shCtrl group, the shGSG2 group expressed higher apoptotic genes such as caspase 3, caspase 8, Fas and FasL, while lower IGF1, Bcl2 and Bcl-w.

Conclusions

Our study showed that knockdown of GSG2 suppresses the tumor growth *in vitro* and *vivo*. Therefore, GSG2 might play an oncogenic role in HCC.

Background

Hepatocellular carcinoma (HCC) is the major malignancy among three types of hepatic carcinoma. It has been recognized as the main cause of cancer-related death with a gradual rising trend across the world [1]. Presently, surgery radical resection is widely used and has been regarded as the curative method. However, in order to prevent the risk of postoperative liver failure, radical resection is limited to those patients who do not have liver cirrhosis or patients whose liver function meets the given standards. The low sensitivity to chemotherapy and increased drug resistance result in the poor clinical outcomes in HCC patients. For the purpose of getting rid of the dilemma, more attention has been focused on the potential mechanism of HCC and tried best to find specific genes that can influence the development of HCC.

GSG2 (also known as HASPIN: Homo-sapiens histone H3 associated protein kinase) is an unusual protein kinase with low sequence homologies with other members of eukaryotic protein kinase family. Studies have shown that GSG2 is a serine/threonine kinase whose protein can be expressed in somatic cells throughout the cell cycle at a low concentration, and its mRNA can be detected in all proliferating cell lines [2, 3]. Some studies have proved that CHR-6494 (GSG2 inhibitor) has displayed high efficacy in some types of cancers through suppressing the proliferation of cancer cells [4]. However, the role of GSG2 in HCC has not been investigated. In this study, clinical tissues were collected for further experiments, and it was found that GSG2 was highly expressed in HCC, and the measurements of cell functions showed that it was able to inhibit cell proliferation and promote cell apoptosis after being knocked down. This is the first report concerning the role of GSG2 in HCC, which indicates that it may act as a novel clinical therapeutic target of HCC.

Methods

Patients and tissue samples

A total of 180 HCC patients in The First Affiliated Hospital of Zhengzhou University were enrolled in this study, and all patients were diagnosed and confirmed by histopathological examination. The general clinical information and pathological characteristics of patients were summarized in Table 1. Written informed consent was collected from all patients whose tissue samples were used in this study, and this study was approved by the Ethics Committees of the First Affiliated Hospital of Zhengzhou University.

Table 1
Expression patterns in liver cancer tissues and para-carcinoma tissues revealed in immunohistochemistry analysis.

GSG2 expression	Tumor tissue		Para-carcinoma tissue		p value
	Cases	Percentage	Cases	Percentage	
Low	93	52. 0%	20	100%	0. 000***
High	86	48. 0%	-	-	

Immunohistochemistry

The expression patterns of GSG2 in tissues were detected by the immunohistochemistry. Selected tissues were made into slides and the subsequent operations were performed according to the manufacturer’s instructions. The slides were incubated with primary antibody (1:200, Cat. No. bs-15413R, BLOSS, Beijing, China) against GSG2 at 37°C for 1 h. The slides were washed with 1×PBST for 5 min. The secondary antibody was added to react with the anti-GSG2 antibody at 4°C overnight. The expression of GSG2 was detected by the staining of 3, 3'-diaminobenzidine (DAB) and the slides were kept away from light for 5 min. Finally, the tissue slides were scanned by Fluorescent inverted microscope (Olympus IX73) and were calculated to get the rates of positive cells which express GSG2 protein. The score standard of IHC (immunohistochemistry) were described as follows: positive cells score: 0, < 0% negative; 1, 10–25%

weak positive; 2, 25–50% moderate positive; 3, 50–75% strong positive; and 4, $\geq$ 75% stronger positive. Staining intensity score: 0, negative; 1, weak positive; 2, moderate positive; and 3, strong positive. Total scores: 0 (-); 1–4 (+); 5–8 (++); and 9–12 (+++).

Construction of lentivirus for shRNA

The RNAi specific targeting sequences were designed on the basis of GSG2 (NM_031965) in order to inhibit the expression of GSG2, and the sequence (5'-CCACAGGACAATGCTGAACTT-3') was chosen as the interference target. Then the shRNA was designed based on the above-mentioned sequence, and the sequences were as follows: positive chain: 5'-

CCGGCCACAGGACAATGCTGAACTTCTCGAGAAGTTCAGCATTGTCCTGTGGTTTTTG-3', inverse chain: 5'-AATTCAAAAACCACAGGACAATGCTGAACTTCTCGAGAAGTTCAGCATTGTCCTGTGG-3'. In searching human gene database, it was found that there was no homology to other known human genes. The linearized plasmid vectors (BR-V-108) were collected with the use of restriction enzyme *Age*I and *Eco*R, which were connected with the GSG2-shRNA. The connected vectors were transferred into the TOP10 *E. coli* competent cells (Cat. No. CB104-03, TIANGEN, Beijing, China). After confirming the accuracy of shRNA's sequence, plasmids were purified and packaged with the lentiviruses (LV). The recombinant lentiviruses expressing GSG2 shRNA and scrambled shRNA were named as LV-shGSG2 and LV-shCtrl, respectively.

Establishment of GSG2 knockdown cell lines

The packaged lentiviruses were used to infect the human HCC cell lines Hep3B2.1-7 and SK-HEP-1 (Supplementary Fig. 1). The cells were cultured with 1640 + 10% FBS in six-well plates. When the density of cells met the standard of 2×10^5 cells/well, 400 μ L lentiviruses (virus titer: 1×10^7 TU/mL) was added into the six-well plates with ENI. S + Polybrene. After 72 h, the cell lines of shGSG2 and shCtrl were observed under fluorescence microscope to find out the expression of GFP (green fluorescent protein), to further obtain the efficiency of cell infection.

Quantitative RT-PCR

Total RNA was collected by splitting cells with Trizol reagent according to the manufacturer's instructions, and cDNA was synthesized by using reverse transcription with Promega M-MLV kit (Promega, Beijing, China). The real-time PCR was performed with two-step method, and the whole procedure including an initial denaturation at 95°C for 30 s, then 40 cycles at 95°C for 5 s, and 60°C for 30 s. The PCR products from GAPDH and GSG2 were 121 bp and 251 bp respectively. The sequences of GAPDH and GSG2 were listed as follows: GAPDH-upstream: 5' –TGACTTCAACAGCGACACCCA-3', GAPDH-downstream: 5'-CACCTGTTGCTGTAGCCAAA-3'. GSG2-upstream: 5'-GGAAGGGGTGTTTGCGGAAGT-3', GSG2-downstream: 5'-TGAGGAGCAAGGGAGGGTAAG-3'. The mRNA expression level of GSG2 was normalized to GAPDH mRNA and the relative quantitative analysis was used to analyze the data.

Western blot

Total proteins were extracted from cells which were split completely on ice for 10–15 min by using 1× Lysis buffer (50 mM 1 M Tris-HCl (pH 6.8), 1% mercaptoethanol, 10% glycerin, 2% SDS, and 0.01% bromophenol blue). BCA protein assay kit (Cat. No. 23225, HyClone/Pierce, Guangzhou, China) was used to measure the concentration of extracted protein for adjusting the subsequent loading volume. After the protein was denatured due to high temperature, 10% SDS-PAGE gel was used to carry on electrophoresis (80 V 2 h) and transferred the protein in SDS-PAGE gel onto the PVDF membrane (300 mA 150 min) at 4°C. The PVDF membrane was stored in blocking solution (TBST with 5% skim milk) for 1 h at room temperature and incubated with primary anti-GSG2 (88/72 kDa, 1:1000, Cat. No. ab21686, Abcam, Cambridge, MA, USA) and anti-GAPDH (37 kDa, 1:3000) which was used as loading control at 4°C overnight. The membrane was washed with TBST for 3 times, each time lasting 10 min. After washing, the membrane was incubated with the secondary antibody (goat anti-rabbit IgG coupled to HRP) (1:3000, Beyotime Biotechnology, Shanghai, China) at room temperature for 2 h. Finally, the protein bands on the membrane were visualized with ECL + plus™ Western blotting system kit.

MTT assay

Cell lines transfected with LV-shGSG2 and LV-shCtrl were seeded in 96-well plates at the seeding density of 2000 cell/well in triplicate. The cell lines were cultured for 5 d altogether. 20 µL 5 mg/mL MTT (Cat. No. JT343, Genview, Craigieburn, Victoria, Australia) were added into each well and incubated for 14 h at 37°C from the second day on. The DMSO (100 µL) was used to dissolve the formazan crystals at the bottom. The tubes needed to be oscillated for 2–5 min before detecting the absorbance at 490 nm wavelength with microplate reader (Cat. No. M2009PR, Tecan infinite, Mannedorf, Switzerland).

Flow cytometry assay

The Annexin V-APC Apoptosis Detection Kit (Cat. No. 88-8007-74, eBioscience, CA, USA) was used to detect the apoptosis according to the manufacturer's instructions. Cultivated cell lines were trypsinized and resuspended in logarithmic growth phase. Cell sedimentation should be resuspended with 200 µL 1×binding buffer after washing the cells sedimentation with D-Hanks (pH = 7.2 ~ 7.4), which stored at 4°C previously. Then 10 µL Annexin V-APC was added into the cell suspension and kept the solution away from light for 10–15 min at room temperature. Finally, the cells were analyzed by flow cytometry system (Cat. No. Guava easyCyte HT, Millipore, MIT, USA).

Colony formation assay

The infected cells were trypsinized and resuspended in logarithmic growth phase, and the number of cells was counted. The cells in every experimental group needed to be reseeded into six-well plates at the density of 600 cells/well in triplicate. Cells were incubated for 14 d, and the medium was replaced at the interval of 3 d. 1 mL of 4% paraformaldehyde was added per well for 50 min. After washing the cells with PBS, 500 µL GIEMSA staining was added into each well for 20 min. Finally, we took the photograph of colony cells by fluorescence microscopy equipped with digital camera.

Mice xenograft model

We created mice xenograft model in 4-weeks nude mice (BALB/c, female, SLAC, Shanghai, China) according to the manufacturer's instructions and the experiments sustained for 33 d. The mice (amount to 12) were divided randomly into two groups, and cells (SK-HEP-1) were injected under their front legs. The general arrangements of the two group models were listed as follows: the mice (6) in shGSG2 group were injected 200 μ L cells (1×10^7 cells/mL) transfected with LV-shGSG2, and the mice (6) in shCtrl group were injected 200 μ L cells (1×10^7 cells/mL) transfected with LV-shCtrl. Following 4 weeks, the situation of tumors and fluorescence intensity were inspected under small animal living imaging system after being anesthetized by intraperitoneal injection of 0.7% sodium pentobarbital. Tumors were resected to detect the final weight and volume (calculated according to the formula: $\text{length} \times \text{width}^2 \times 3.14/6$) of tumors after being sacrificed with excessive sodium pentobarbital followed by cervical dislocation method. Finally, immunohistochemistry staining was performed to measure the expression of Ki-67. All the animal experimental protocols were approved by the Ethics Committee at the First Affiliated Hospital of Zhengzhou University.

Human apoptosis antibody array

The cultured SK-HEP-1 cells transfected with lentivirus were collected and detected with human apoptosis antibody array following the manufacturer's instructions (Cat. No. ab134001, Abcam, Cambridge, MA, USA). The proteins expressed differently in human apoptosis signal pathway were discovered and further relational proteins were predicted in this way.

Statistical analysis

Statistical analysis of data was performed by SPSS 22.0 software. Student's t-test, Mann-Whitney U test, and Pearson Correlation were performed to analyze the data from the experiment. All values in the text and figures were expressed as the mean $\pm$ standard deviation. It was considered to be statistically significant at the time that P value < 0.05 .

Results

GSG2 was overexpressed in tumor tissues

The expression levels of GSG2 were analyzed using immunohistochemistry which revealed that GSG2 was overexpressed in HCC tissues compared with para-carcinoma tissue (Table 1 and Fig. 1). Further statistical analysis between the expression of GSG2 and tumor characteristics in Table 2 showed that expression of GSG2 was significantly related to the pathological stage of tumors and T cell infiltrate ($P < 0.05$). It meant that the expression of GSG2 was related to the advanced tumor stage.

Table 2
Relationship between GSG2 expression and tumor characteristics in patients with liver cancer.

Features	No. of cases	GSG2 expression		p value
		low grade	high grade	
All cases	179	93	86	
Age (years)				0. 267
≤ 50	91	51	40	
>50	88	42	46	
Gender				0. 205
Male	145	72	73	
Female	34	21	13	
Grade				0. 453
1	21	7	14	
2	126	70	56	
3	22	10	12	
Stage				0. 000***
I	6	6	0	
II	79	51	28	
III	94	36	58	
T Infiltrate				0. 000***
T1	6	6	0	
T2	81	51	30	
T3	86	34	52	
T4	6	2	4	
lymphatic metastasis(N)				0. 140
N0	177	93	84	
N1	2	0	2	

Knockdown efficiency of GSG2 in HCC cells

LV-shGSG2 and LV-shCtrl were transfected into human HCC cell lines Hep3B2.1-7 and SK-HEP-1 in order to find out the effects of GSG2 knockdown on HCC cell functions. The fluorescence of cells, which were infected with shCtrl or shGSG2 for 72 h, observed by microscope demonstrates a >80% efficiency of infection (Fig. 2A). The results of qRT-PCR showed that, compared with the shCtrl group, the knockdown efficiencies of GSG2 in Hep3B2.1-7 and SK-HEP-1 cells were 40% ($P < 0.001$) and 83.4% ($P < 0.001$), respectively (Fig. 2B). Western blot analysis showed that compared with shCtrl group, the protein level of GSG2 in shGSG2 group was significantly down-regulated (Fig. 2C). Collectively, it could be concluded that the GSG2 knockdown cell models were constructed successfully.

Knockdown of GSG2 inhibits HCC cell proliferation and colony formation and promotes HCC cell apoptosis

For the purpose of investigating the effects of GSG2 knockdown on HCC cells proliferation, MTT assay was carried out. The cells in shGSG2 group showed slower proliferation rate compared with shCtrl group ($P < 0.001$) (Fig. 3A). The colony formation assay showed that the colony number in shGSG2 group was significantly decreased compared with shCtrl group ($P < 0.001$) (Fig. 3B). And wound-healing assay demonstrated that, the migration rate was obviously decreased in shGSG2 group (Fig. 4A,B). The transwell assay results showed that down expression of GSG2 significantly decreased the migration rate of HCC cells (**Supplementary Fig. 2**). In order to comprehend the relationship between GSG2 and cells apoptosis, flow cytometry was performed. The results demonstrated that cells apoptosis percentage increased in shGSG2 group compared with shCtrl group ($P < 0.001$) (Fig. 4C). And compared with shCtrl group, more cells were in stage G2 and less cells were in stage S in shGSG2 group (**Supplementary Fig. 3**). Therefore, it could be concluded that knockdown of GSG2 can suppress the cloning abilities and proliferation of HCC cells and promoted cell apoptosis *in vitro*.

Effects of GSG2 knockdown on tumor growth *in vivo*

The mice xenograft model was built with BALB/c nude mice to detect the effects of GSG2 knockdown on the tumorigenicity of SK-HEP-1 cells. After 4 weeks, the data about fluorescence intensity was measured under small animal living imaging system (Fig. 5D). The final weight and volume of tumors in mice was measured after the tumors were resected (Fig. 5E). The results were listed as follows: the volume and weight of tumor in shGSG2 group were significantly smaller than in shCtrl group ($P < 0.001$) (Fig. 5A, B). The fluorescence intensity in shGSG2 was significantly weaker than in the shCtrl group ($P < 0.001$) (Fig. 5C), which indicated the slower growth of HCC cells in shGSG2 group compared with that in shCtrl group. Expression level of Ki-67, which was detected by immunohistochemistry, was lower in shGSG2 than that in shCtrl (Fig. 5F).

Exploring the regulation mechanism of GSG2 in HCC

It was known that the knockdown of GSG2 promoted cell apoptosis, inhibited cells proliferation and colony formation according to the results of the experiments. However, the definite mechanism was

unclear; thus, Human Apoptosis Antibody Array was used to screen the affected signaling pathway in the SK-HEP-1 cell lines by the knockdown of GSG2(Fig. 6). The expressions of Caspase 3, Caspase 8, BID, CD40L, cytoC, DR6, Fas, and FasL were significantly up-regulated in shGSG2 compared with shCtrl ($P < 0.05$). The discrepancy of Caspase 3 and Fas was more than 20% in shGSG2 group (Fig. 6A,B). While IGF-1, Bcl-2 and Bcl-w were low-regulated in shGSG2 group(Fig. 6A,B). Caspase 3 is a member of the caspase family and it works a lot in the execution-phase of cell apoptosis after being activated by extrinsic and intrinsic pathways. IGF-1 is a member of the insulin-like growth factor (IGF) family and plays an important role in cell growth and migration. As we all know, PI3K/AKT signal pathway play important role in cell growth(Fig. 6C). Compared with shCtrl group, we found PI3K/AKT signal pathway was less activated in shGSG2 group. The conclusion is that Caspase 3 and IGF-1 and PI3K/AKT signaling pathway may play important roles in the knockdown of GSG2 in SK-HEP-1 cell lines. Further studies are needed to explain the specific mechanism of GSG2 in HCC clearly.

Discussion

Liver cancer, especially HCC, was predicted to be the sixth most commonly diagnosed cancer and the fourth death-related cancer throughout the world in 2018, with about 841, 000 new cases and 782, 000 deaths annually [5]. The main risk factors of HCC include chronic liver infection with hepatitis B virus (HBV), hepatitis C virus (HCV), and heavy alcohol intake, among many others. The major factors vary on the basis of geographical region[5]. Due to the resistance and low tolerance to chemotherapy drugs and the strict conditions for tumors to be resected completely, the recurrence and mortality rate are still high even though the medical technology has made great progress in the past decades[6, 7]. More and more researchers concentrate on finding new genes or signal pathways that are responsible for the occurrence, progression, and recurrence of HCC and they have made some achievements[8–12]. Bollard J, *et al.* [13] found that Palbociclib which inhibits the expression of CDK4/6 can restrict the growth of liver tumor by restraining the HCC cell proliferation and acquire good response in survival rate. Dawkins J, *et al.* [14] and Former A, *et al.* [15] found that the tyrosine-kinase inhibitors sorafenib, lenvatinib, and regorafenib, are proven to be beneficial on survival by acting on various gene targets in HCC. However, the recurrence and mortality rate remained uncontrolled. Therefore, further investigation on the molecular mechanism is important for the treatment of HCC.

Our study demonstrated the functions of GSG2 in HCC. HASPIN is an atypical protein kinase that was regarded as an inactive pseudokinase for a long time due to the low homology sequence compared with other members in the family of eukaryotic protein [2, 3]. HASPIN is a positive regulator of centromeric cohesion and it can catalyze histone H3 phosphorylation [16]. The histones phosphorylation appears to be critical in the histones PTMs (post-translational modifications), because of the important roles in DNA repair structure, transcription, and chromatin compaction during cell division and apoptosis. The PTMs in histone H3 is high and histone H3 phosphorylation has main effects on T3, T6, S10, T11, S28, S31, Y41 and T45 residues. Histone H3 phosphorylation results from JNK (Basal c-Jun N-terminal kinases) is critical for mitotic entry at G2/M phase and Aurora-B, PIM1, RSK2, MSK1/2, and IKK α can also result in

H3S10 phosphorylation^[17]. High H3S10 phosphorylation levels mediated by MSK1 through p38-MAPK pathway in both PRM (proximal surgical resection margin) and DRM (distal surgical resection margin) associated with clinical parameters and poor survival in gastric cancer^[18]. H3T3 phosphorylation by HASPIN in human is necessary for Aurora B accumulation on the centromere, and Aurora B kinase activation for accurate chromosome alignment and segregation^[16, 19, 20]. On the contrary, the reduction of H3T3 phosphorylation by HASPIN inhibitors (which have shown anti-tumor activity in a mouse xenograft model) causes Aurora B loss on the centromere, phosphorylation of centromere decreasing, and the reduced kinetochore Aurora B substrates. Metaphase chromosome alignment and spindle checkpoint signaling are compromised at the same time^[21–24]. In summary, HASPIN plays a key role in promoting accurate chromosome alignment, segregation, and spindle checkpoint signaling. Histone H3 phosphorylation is inhibited by the knockdown of GSG2 and low Histone H3 phosphorylation decreases the mitotic index. With further research, inhibiting HASPIN has the function of suppressing the cancer cells proliferation and it displays fine efficacy in several cancers by inhibiting HASPIN^[25]. Han L, *et al*^[26] showed that CHR-6494 (small molecule GSG2 inhibitor) alone had a dose dependent inhibition on the viability of several melanoma cell lines. The inhibitor exerts synergistic action with Trametinib on inhibiting cell growth of and promotes cell apoptosis. Jong- Eun Kim, *et al*^[27] showed that Coumestrol suppresses the activity of HASPIN to inhibit various cancer cell lines proliferation. However, the function of GSG2 in HCC has never been researched. This is the first report stating the functions and probable mechanism of GSG2 in HCC cell lines. We found that the knockdown of GSG2 inhibited HCC cell proliferation and colony formation, and the results were consistent with the previous research about the functions of GSG2 in other cancer cells^[2, 27, 28]. On the basis of above results, the fact that GSG2 regulates the HCC cell proliferation *in vitro* and *vivo* can be settled and it appears to be regarded as a novel gene target in HCC therapy.

The signal pathway was analyzed with Human Apoptosis Antibody Array and the results showed that Caspase-3 and Caspase-8 had significant up-regulation after the knockdown of GSG2. Caspase-3 induces cell apoptosis after transfecting insect Sf9 cells and can be blocked by Bcl-2. The extracting solution in apoptosis cells loses the ability to induce apoptosis after removing Caspase-3, and the addition of purified Caspase-3 restores the function of apoptosis. IGFs (Insulin-like growth factors) play key roles in cell growth, differentiation and metabolism. IGFBP-1 induction can restrict growth process and metabolism under stress conditions (liver diseases, amino acid depletion, hypoxia, dioxin, endoplasmic reticulum stress and proinflammatory cytokines) to save the energy for survival functions^[29]. IGF-1 is widely considered to be an important role in tumor growth, and shows a more chronic relationship to diet and energy balance^[30]. The PI3K/AKT signaling pathway is important for the regulation of cell apoptosis in different kinds of tumors^[31–33]. GSG2 knockdown appears to promote the expression of Caspase-3 and Caspase-8, as well as inhibit the expression of IGF-1, thus induces cell apoptosis and inhibits growth in tumor. And maybe PI3K/AKT signal pathway contribute to this. However, the mechanism that we found is just part of whole specific signal pathway and further studies would need to be performed.

Conclusions

In conclusion, this is the first study about the functions of GSG2 in HCC progression. Our study confirmed that GSG2 is overexpressed in hepatic carcinoma tissues, and that it regulates the progression of HCC by adjusting cell proliferation and apoptosis perhaps through PI3K/AKT signal pathway. The study has further proved that GSG2 play an important role in the progression of HCC and can be regarded as a new therapeutic target in HCC. Thus, further studies may accelerate our understanding about the functional role of GSG2 in HCC.

Abbreviations

Not applicable.

Declarations

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Availability of data and materials

The main datasets used and analyzed in the present study are available from the corresponding authors on reasonable request.

Authors' contributions

XD designed the research study. HW, QM, PW, and DS performed the experiments. CH1 (Luhao Li) and CH2 (Lin Li) performed the analysis of data and SL edited the final manuscript, and all authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was gained from all patients whose tissue samples were used in this study and this study was approved by Ethics committees of the First Affiliated Hospital of Zhengzhou University. All the animal experimental protocols were approved by the Ethics committee at the First Affiliated Hospital of Zhengzhou University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The expression of GSG2 was detected by immunohistochemistry and overexpressed in cancer tissues compared with normal tissues (Magnification x 200).

Figure 2

(A) The fluorescence of cells demonstrated a >80% efficiency of infection and the normal cell condition (Magnification x 100). (B) The expression of mRNA was significantly inhibited in shGSG2 group compared with shCtrl group. The knockdown efficiency of GSG2 in shGSG2 group is 40. 0% in Hep3B2.1-7 cells ($P<0.001$) and the knockdown efficiency of GSG2 in shGSG2 group is 83. 4% in SK-HEP-1 cells ($P<0.001$). (C) The results of Western blot show that, compared with shCtrl group, the protein level of GSG2 in shGSG2 group is down-regulated.

Figure 3

(A) MTT assay was performed to detect the cells proliferation rate and the OD490 in shGSG2 group was significantly lower than that in shCtrl group by t-test ($P < 0.0001$). (B) The colony formation assay showed that shGSG2 group showed decreased colony number.

Figure 4

(A,B) The results of wound-healing assay demonstrated that, the migration rate was decreased in shGSG2 group. (C) The results of flow cytometry demonstrated that, the apoptosis percentage was increased in shGSG2 group.

Figure 5

The effects of GSG2 were tested in vivo. The weight (A), volume (B) and radiant efficiency (C, D) of tumors were detected and the data were analyzed by t-test ($P < 0.0001$) and immunohistochemistry was performed to calculate the expression of Ki-67 (F). (E) Representative images of tumors in mice xenograft model.

Figure 6

The mechanism of GSG2 knockdown in HCC cells. (A,B)Down expression of GSG2 significantly increased the expression of Caspase3, BID, Caspase-8, CD40L, cytoC, DR6,Fas and FasL,while decreased the expression of Bcl-2,Bcl-w and IGF-1 (P <0.05).(C)Down expression of GSG2 significantly decreased PI3K/AKT signal pathway level.

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