

Downregulated long non-coding RNA DREH promotes cell proliferation in hepatitis B virus-associated hepatocellular carcinoma

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Received March 27, 2016; Accepted March 23, 2017

DOI: 10.3892/ol.2017.6436

Abstract. The hepatitis B virus X (HBx) protein has been characterized as an oncogene involved in epigenetic modifications during hepatocarcinogenesis; however, the underlying mechanisms are not entirely clear. Long non-coding RNAs (lncRNAs), a type of epigenetic regulator molecules, have also been demonstrated to serve crucial roles in carcinogenesis, including hepatocellular carcinoma (HCC). In the present study, a human lncRNA DREH was identified, which inhibits cell proliferation *in vitro* and *in vivo*, and acts as a tumor suppressor in HBx-mediated hepatocarcinogenesis. The study revealed that the expression of DREH was frequently downregulated in hepatitis B virus (HBV)-associated HCC tissues in comparison with adjacent non-cancerous hepatic tissues, and was inversely correlated with HBx mRNA expression in HBV-associated HCC. In addition, the levels of DREH were inversely correlated with hepatitis B surface antigen and tumor size in HCC tissues. The forced expression of HBx in liver cell lines resulted in a significant decrease in the expression of DREH. Furthermore, suppression of DREH expression promotes the proliferation of HCC cells *in vitro* and *in vivo*. In conclusion, the present findings support the role of HBx-downregulated lncRNA DREH in tumor suppression in HBV-associated HCC patients. This contributes to a better understanding of epigenetic aberration of

deregulated lncRNAs by HBx and the potential development of lncRNA-based targeted approaches for the treatment of HBV-associated HCC.

Introduction

As one of the most common cancer types in the world, hepatocellular carcinoma (HCC) has an extremely high morbidity and mortality rate, particularly in Asia and Africa (1). Overall, 50 to 55% of HCC cases are attributable to persistent hepatitis B virus (HBV) infections, which may result in end-stage liver disease, including liver cirrhosis and HCC (2). As the smallest open reading frame of the HBV genome, HBx encodes the hepatitis B virus X (HBx) protein which has been implicated in HBV-associated HCC pathogenesis, acting as a weak oncogene or a cofactor in hepatocarcinogenesis (3-5). However, the molecular mechanisms underlying HBx protein-mediated tumorigenesis are not entirely clear. Previous studies have demonstrated that genetic alterations alone do not account for the complexity of HBx-induced hepatocarcinogenesis, but that epigenetic changes, including DNA methylation (6), histone modifications (7) and non-coding RNA expression (6,8), are also involved in this process.

Long non-coding RNAs (lncRNAs) are a type of non-coding RNAs which are longer than 200 nucleotide transcripts and have little or no protein-coding capacity (9,10). Previous studies have demonstrated that lncRNAs are involved in diverse biological functions and pathological processes (10,11), and that altered lncRNA levels may result in aberrant gene expression through a variety of mechanisms, including transcription, post-transcriptional processing (12), chromatin modification, genomic imprinting and the regulation of protein function (13). Increasing evidence demonstrates that altered expression levels of lncRNAs contribute to a wide range of cancer types, including breast, lung, prostate and liver cancer (14-17). Therefore, lncRNAs may potentially be used as diagnostic markers or therapeutic targets for cancer in the clinic.

Using lncRNA microarrays and gene sequencing technology, a large number of lncRNAs have been observed to be aberrantly expressed in HCC tissues and involved in hepatocarcinogenesis. These include highly upregulated in liver

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Abbreviations: HBV, hepatitis B virus; HBx, hepatitis B virus X; HCC, hepatocellular carcinoma; lncRNA, long non-coding RNA; HBsAg, hepatitis B surface antigen; AFP, α -fetoprotein; ALT, alanine aminotransferase; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; siRNA, small interfering RNA; CCK-8, Cell Counting Kit-8

Key words: hepatocellular carcinoma, hepatitis B virus X protein, long non-coding RNA, cell proliferation, DREH

cancer (HULC), high expression in HCC (HEIH), activated by TGF- β (ATB) and HOX transcript antisense RNA (HOTAIR), which serve a role in diverse biological processes including cell proliferation, apoptosis and metastasis (17-20). Several lncRNAs have been identified to be associated with the HBx protein (17,21). Huang *et al* (22) examined the lncRNA expression profiles in the livers of HBx transgenic and wild-type mice, and observed that certain lncRNAs are dysregulated and associated with HBx in HBx transgenic mice. These authors further investigated the biological function of the lncRNA DREH, which may be downregulated by HBx protein, in mice. It was observed to inhibit HCC growth and metastasis, acting as a tumor suppressor in the development of HBV-HCC. The same authors also identified a human ortholog of DREH, which was termed DREH, and observed that its expression level was frequently downregulated in HBV-associated HCC tissues. This decrement was significantly correlated with poor survival in HCC patients. However, the specific role of lncRNA DREH in HCC remains largely unknown.

In the present study, the expression levels of lncRNA DREH in 30 pairs of human HBV-positive HCC tissues and 30 pairs of HBV-negative HCC tissues and their pair-matched normal liver tissues were assessed. The results revealed that the expression level of DREH was significantly downregulated in HBV-HCC tissues compared with their adjacent non-cancerous hepatic tissues, and was inversely correlated with HBx mRNA expression in HBV-associated HCCs. Further investigation of the biological function of DREH *in vivo* and *in vitro* revealed that inhibition of DREH promotes cell proliferation in HBx-induced hepatocarcinogenesis. Together, these results suggest that DREH exerts an impact as a potential tumor repressor gene and may provide new insight into the role of HBx-associated lncRNAs in the development of HCC.

Materials and methods

Animal and patient samples. The four-week-old male BALB/c nude mice used in this study were purchased from the Experimental Animal Center of the Chinese Academy of Medical Sciences (Beijing, China). All mice were bred and maintained in a pathogen-free facility and were used in accordance with the institutional guidelines for animal care. The animal studies were approved by the Institutional Animal Care and Use Committee of the Capital Medical University, Beijing, China.

The 30 HBV-associated HCC tissues and 30 HBV-negative HCC tissues and corresponding adjacent non-cancerous liver tissues used in this study were obtained with informed consent from patients who underwent radical resection in the Peking University People's Hospital (Beijing, China). Studies using human tissues were reviewed and approved by the Committees for Ethical Review of Research Involving Human Subjects of the Capital Medical University. The clinicopathological characteristics of the 60 patients are summarized in Table I.

Construction of vectors. To construct HBx-expressing vectors, complementary DNA encoding HBx was PCR-amplified and sub-cloned into the pcDNA3.1 vector (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). All vectors were constructed according to standard methods and verified by

Table I. Clinicopathological characteristics of 60 HCC patients.

Characteristic	Number (n=60)	Percentage
Age (years)		
≤ 55	39	65.00
> 55	21	35.00
Sex		
Male	51	85.00
Female	9	15.00
Tumor differentiation		
I-II	21	35.00
III-IV	39	65.00
TNM stage		
I	25	41.67
II-III	35	58.33
Tumor size (cm)		
≤ 5	31	51.67
> 5	29	48.33
Tumor number		
Single	52	86.67
Multiple	8	13.33
AFP ($\mu\text{g/l}$)		
≤ 20	12	20.00
> 20	48	80.00
Encapsulation		
Absent	29	48.33
Complete	31	51.67
Microvascular invasion		
Absent	51	85.00
Present	9	15.00
Macrovascular invasion		
Absent	54	90.00
Present	6	10.00
Liver cirrhosis		
Absent	13	21.67
Present	47	78.33
HBs antigen		
Negative	30	50.00
Positive	30	50.00
HBe antigen		
Negative	40	66.67
Positive	20	33.33
ALT (U/l)		
≤ 40	26	43.33
> 40	34	56.67

HCC, hepatocellular carcinoma; TNM, tumor-node-metastasis; AFP, α -fetoprotein; HBs antigen, hepatitis B surface antigen; HBe antigen, hepatitis B envelope antigen; ALT, alanine aminotransferase.

sequencing. The polymerase chain reaction (PCR) primers used are presented in Table II.

Table II. Sequences of primers and siRNAs used in study.

Name	Sequences	
qPCR primers		
HBx	Sense	5'-CCCTTCTTCATCTACCGTTCC-3'
	Anti-sense	5'-CGTTGACATTGCTGCGAGT-3'
β -actin	Sense	5'-TGTGTTGGCGTACAGGTCTTTG
	Anti-sense	5'-GGGAAATCGTGCGTGACATTAAG
DREH	Sense	5'-CATTTGGCGGGACTACTTATT-3'
	Anti-sense	5'-TTCAATCTGGCTTTGTTTCGTT-3'
Primers for vector construction		
DREH clone	Sense	5'-GGGGTACCCCATGGCTGCTAGGGTGTG-3'
	Anti-sense	5'-CGGGATCCCGTCAGGCAGAGGTGAAAAAG-3'
siRNA sequences		
DREH siRNA	Sense	5'-UCAUUUGGCGGGACUACUUTT-3'
	Anti-sense	5'-AAGUAGUCCCGCCAAAUGATT-3'
siRNA NC	Sense	5'-UUCUCCGAACGUGUCACGUTT-3'
	Anti-sense	5'-ACGUGACACGUUCGGAGAATT-3'
HBx siRNA	Sense	5'-CCCACCAAUAUUGCCCAATT-3'
	Anti-sense	5'-UUGGGCAAUAUUGGUGGGTT-3'

siRNA, small interfering RNA; qPCR, quantitative polymerase chain reaction; HBx, hepatitis B virus X; NC, negative control.

Cell culture and transfection. The liver cell lines HepG2, HepG2.2.15, Hep3B, Huh-7 and SMMC-7721 were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were grown in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (Gibco) and were maintained in a humidified 37°C incubator with an atmosphere of 5% CO₂. The different plasmids and small interfering RNA (siRNA) sequences were transfected into cells using a Lipofectamine® 3000 kit (Invitrogen) according to the manufacturer's protocol. The siRNAs were synthesized by GenePharma (Shanghai, China). The siRNA sequences are provided in Table II.

Reverse transcription and quantitative PCR (RT-qPCR). Total RNA was extracted using TRIzol reagent (Invitrogen). First-strand cDNA was generated using the Reverse Transcription system kit (Stratagene, La Jolla, CA, USA). Random primers (6mer; Takara Bio, Inc., Otsu, Japan) were used for RT-PCR for lncRNAs. Real-time PCR was performed using a standard SYBR-Green PCR kit protocol on a StepOne Plus system (Applied Biosystems, Thermo Fisher Scientific, Inc.). β -actin was employed as an endogenous control to normalize for the amount of total mRNA in each sample. The qPCR reactions were performed in triplicate, including no-template controls. The relative RNA expression was calculated using the comparative Cq method. The primer sequences are presented in Table II.

Cell Counting Kit-8 (CCK-8) assay. HepG2 or Huh-7 cells (2x10³ cells/well) transfected with DREH siRNA or negative control were dispensed in 100- μ l aliquots into 96-well plates. At the indicated time points, CCK-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to the cells

for 2 h and then the optical density was read using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All of the experiments were performed in triplicate.

Colony formation assay. For colony formation assay, cells were seeded at a density of 100 cells per well in a 12-well culture plate and cultured for 2 weeks, then cells were washed twice with phosphate-buffered saline (PBS), fixed with methanol, and the colonies were stained with 1% crystal violet and counted.

In vivo assay for tumor growth. Lentivirus-based short hairpin RNA (shRNA) constructs (GenePharma) were used to stably knock down DREH gene expression according to the manufacturer's protocol. HepG2 cells were stably transduced with DREH shRNA lentivirus. Cells transfected with DREH shRNA or control shRNA (1.0x10⁷) were suspended in 100 μ l PBS and implanted subcutaneously into the bilateral armpit of BALB/c nude mice (five in each group). The tumors were measured every three days after implantation, and the volume of each tumor was calculated as: Length x width² x 0.4. All mice were sacrificed four weeks later.

Statistical analysis. The expression of DREH in HCC patients was compared using the paired samples t-test. The association between DREH and HBx mRNA expression was analyzed by Pearson's correlation. The correlations between DREH and clinicopathological characteristics in the 60 HCC patients were analyzed by the χ^2 test or Fisher's exact probability test. Others comparisons were determined by Student's t-test. All P-values were two-sided and obtained using the SPSS 18.0 software package (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

LncRNA DREH is significantly downregulated in HBV-associated HCC tissues. To confirm the role of lncRNA DREH in HCC, DREH expression levels were first examined in 30 pairs of human HBV-associated HCC tissues and 30 pairs of HBV-negative HCC tissues and their pair-matched normal liver tissues by qPCR. The results revealed that the expression levels of DREH were significantly downregulated in HBV-HCC tissues in comparison with adjacent non-cancerous hepatic tissues from the same patient ($P < 0.0001$, paired samples t-test); however, no significant difference was observed in the expression levels between the HBV-negative HCC tissues and the adjacent non-cancerous hepatic tissues. In addition, the expression of DREH was significantly higher in HBV-negative HCC tissues compared with HBV-positive HCC tissues (Fig. 1A).

DREH expression was further compared with clinicopathological characteristics in these 60 HCC patients, and statistical analysis revealed that lower DREH expression levels in HCC tissues were significantly positively correlated with tumor size ($\chi^2 = 5.406$, $P = 0.020$, Table III) and hepatitis B surface antigen (HBsAg) ($\chi^2 = 4.267$, $P = 0.039$, Table III). However, no direct correlation was identified between the expression of lncRNA DREH and other clinical characteristics, including age, sex, tumor differentiation, tumor-node-metastasis stage, tumor number, α -fetoprotein (AFP), encapsulation, microvascular invasion, macrovascular invasion, liver cirrhosis, hepatitis B envelope antigen and alanine aminotransferase (ALT) (Table III). These results indicate that DREH may be involved in HCC tumor growth and potentially associated with HBV infection.

DREH and HBx mRNA levels are inversely correlated in human HBV-associated HCC tissues. Next, it was assessed whether decreased DREH expression was correlated with the levels of HBx expression in human HBV-associated HCC tissues. The expression levels of HBx were further analyzed in the aforementioned 30 HCC tissues. A statistically significant inverse correlation was observed between DREH and HBx mRNA ($n = 30$, $r = -0.531$, $P = 0.0033$, Pearson's correlation; Fig. 1B). These data reveal the potential reciprocal regulation of DREH expression induced by HBx in human HCCs, and suggest that DREH may be involved in HCC pathogenesis as a tumor suppressor subsequent to HBx overexpression in chronic hepatitis B patients.

DREH is significantly downregulated in human HCC cell lines expressing HBx. To investigate the correlation between HBx and DREH expression, it was first determined whether DREH was differentially expressed in human HCC cells. The expression levels of DREH were assessed by RT-qPCR. The results revealed that the expression of DREH was markedly lower in HepG2.2.15 (a derivative of the human hepatoma cell line HepG2 that has been stably transformed with a head-to-tail dimer of HBV DNA) and Hep3B (a cell line containing the integrated hepatitis B viral genome) cell lines compared with HepG2, Huh-7 and SMMC-7721 cells, which do not express HBx (Fig. 2A).

Enforced HBx expression downregulates DREH in human HCC cells. In order to verify whether this downregulation was

Table III. Correlation between lncRNA DREH expression and clinicopathological characteristics in 60 HCC patients.

Characteristic	LncRNA DREH expression		P-value
	Low (n=30)	High (n=30)	
Age (years)			0.787
≤55	20	19	
>55	10	11	
Sex			0.278
Male	24	27	
Female	6	3	
Tumor differentiation			0.787
I-II	10	11	
III-IV	20	19	
TNM stage			0.190
I	10	15	
II-III	20	15	
Tumor size (cm)			0.020 ^a
≤5	20	11	
>5	10	19	
Tumor number			0.254 ^b
Single	24	28	
Multiple	6	2	
AFP ($\mu\text{g/l}$)			0.333 ^b
≤20	4	8	
>20	26	22	
Encapsulation			0.196
Absent	12	17	
Complete	18	13	
Microvascular invasion			1.000 ^b
Absent	25	26	
Present	5	4	
Macrovascular invasion			0.671 ^b
Absent	26	28	
Present	4	2	
Liver cirrhosis			0.754
Absent	6	7	
Present	24	23	
HBs antigen			0.039 ^a
Negative	11	19	
Positive	19	11	
HBe antigen			0.273
Negative	18	22	
Positive	12	8	
ALT (U/l)			0.297
≤40	11	15	
>40	19	15	

^a $P < 0.05$; ^bFisher's exact test; χ^2 tests for all other analyses. The median expression level of lncRNA DREH was used as the cutoff. Patients with HCC were divided into an lncRNA DREH 'low' group (whose expression was lower than the median) and 'high' group (whose expression was higher than the median). lncRNA, long non-coding RNA; HCC, hepatocellular carcinoma; AFP, α -fetoprotein; HBs antigen, hepatitis B surface antigen; HBe antigen, hepatitis B envelope antigen; ALT, alanine aminotransferase.

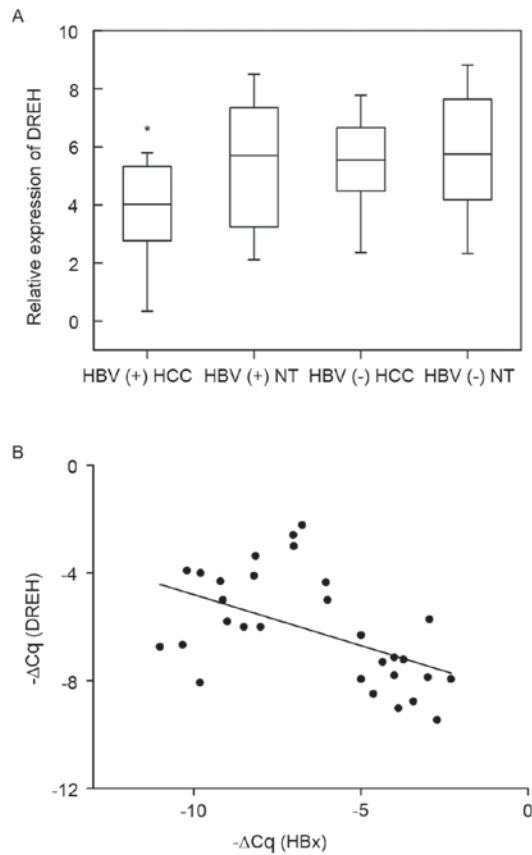


Figure 1. LncRNA DREH is significantly downregulated in HBV-associated HCC tissues. (A) LncRNA DREH expression in HCC tissues vs. paired adjacent non-cancerous hepatic tissues by qPCR (from 30 pairs of HBV-associated HCC patients and 30 pairs of HBV-negative HCC patients). Statistical differences between HBV-HCC tissues and paired adjacent non-cancerous hepatic tissues were analyzed with the paired samples t-test ($P < 0.0001$). (B) LncRNA DREH and HBx mRNA expression levels were inversely correlated in 30 HBV-associated HCC samples. DREH and HBx expression levels in these samples were measured by qPCR, and respective ΔCq values normalized to β -actin were subjected to a Pearson correlation analysis ($n=30$, $r=-0.531$, $P=0.0033$, Pearson's correlation). LncRNA, long non-coding RNA; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; qPCR, quantitative polymerase chain reaction; HBx, hepatitis B virus X; NT, normal tissues.

correlated with HBx expression, HepG2 and Huh-7 cells were transiently transfected with HBx expression vector pc-HBx and control vector pcDNA3.1. The levels of DREH were measured 72 h after transient transfection. The mRNA expression of HBx following infection is shown in the left panel of Fig. 2B. The results reveal that DREH was downregulated in pc-HBx-transfected cells in comparison with the pcDNA3.1 control groups (Fig. 2B).

Conversely, HBx expression was also repressed by siRNA; the knockdown efficacy of HBx siRNA is shown in the left panel of Fig. 2C. The inhibition of HBx by siRNA was observed to increase DREH expression in HepG2.2.15 and Hep3B cells which express HBx (Fig. 2C).

Inhibition of DREH promotes cell proliferation of HCC cells *in vitro*. The frequent downregulation of lncRNA DREH by HBx and the inverse correlation between DREH expression and tumor size in HBV-HCC patients implies that DREH may have a role in cell proliferation in HBV-associated hepatocarcinogenesis. To prove this, the effects of reduced expression of

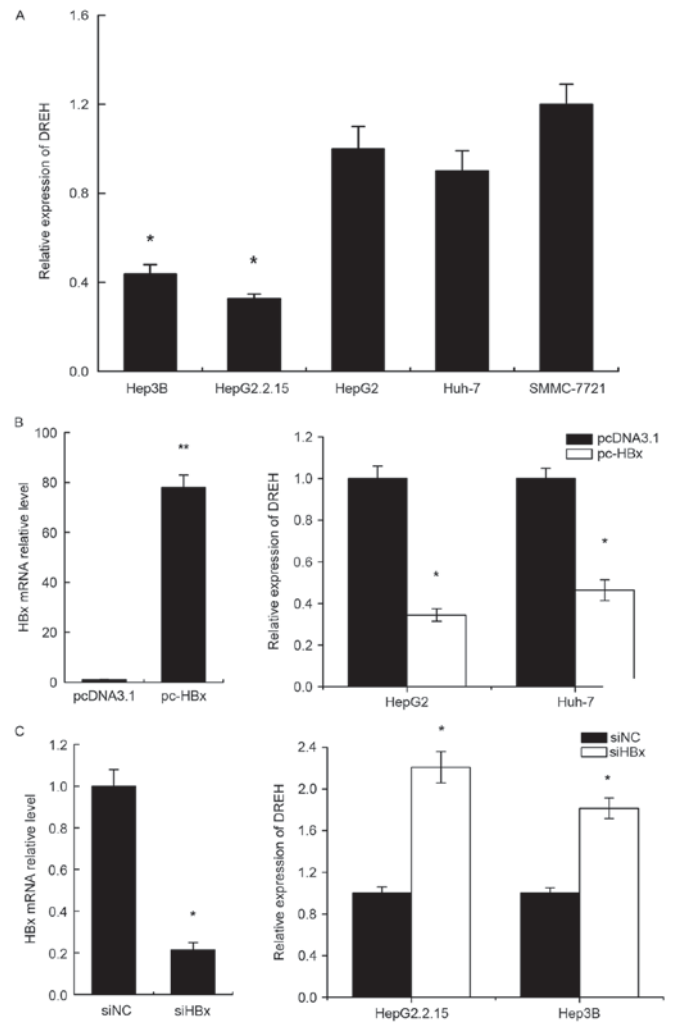


Figure 2. DREH is significantly downregulated in human HCC cell lines expressing HBx. (A) Relative expression of DREH in human liver cell lines Hep3B, HepG2.2.15, HepG2, Huh-7 and SMMC-7721 cells. The expression of DREH was normalized to β -actin with respect to HepG2 cells. Data are shown as the means \pm standard deviation from triplicate experiments. $*P < 0.05$ vs. HepG2 group. (B) HBx mRNA (left panel) and lncRNA DREH (right panel) expression levels following transfection of pc-HBx or control pcDNA3.1 plasmid in HepG2 and Huh-7 cells. Data are shown as the means \pm standard deviation from at least three independent experiments. $*P < 0.05$; $**P < 0.01$ vs. respective pcDNA3.1 groups. (C) HBx mRNA (left panel) and DREH (right panel) expression levels following transfection of HBx-specific siRNA (siHBx) or control siRNA (siNC) in Hep3B and HepG2.2.15 cells. Data are shown as the means \pm standard deviation from at least three independent experiments. $*P < 0.05$ vs. respective siNC groups. HBx, hepatitis B virus X; NC, negative control.

DREH on cell proliferation were investigated in two HCC cell lines. DREH expression was repressed by RNA interference, and the relative expression levels of DREH following infection of DREH siRNA or control siRNA are shown in the left panel of Fig. 3A and B. Cell-Counting Kit-8 assays demonstrated that suppression of cellular DREH enhanced the cell proliferation index compared with the control siRNA group in HepG2 and Huh-7 cells. The negative control siRNAs did not affect the cell proliferation index compared with the mock cells with no treatment (Fig. 3A and B).

Further colony formation assays also revealed that downregulation of DREH significantly enhanced the colony formation ability in HepG2 and Huh-7 cells compared with the

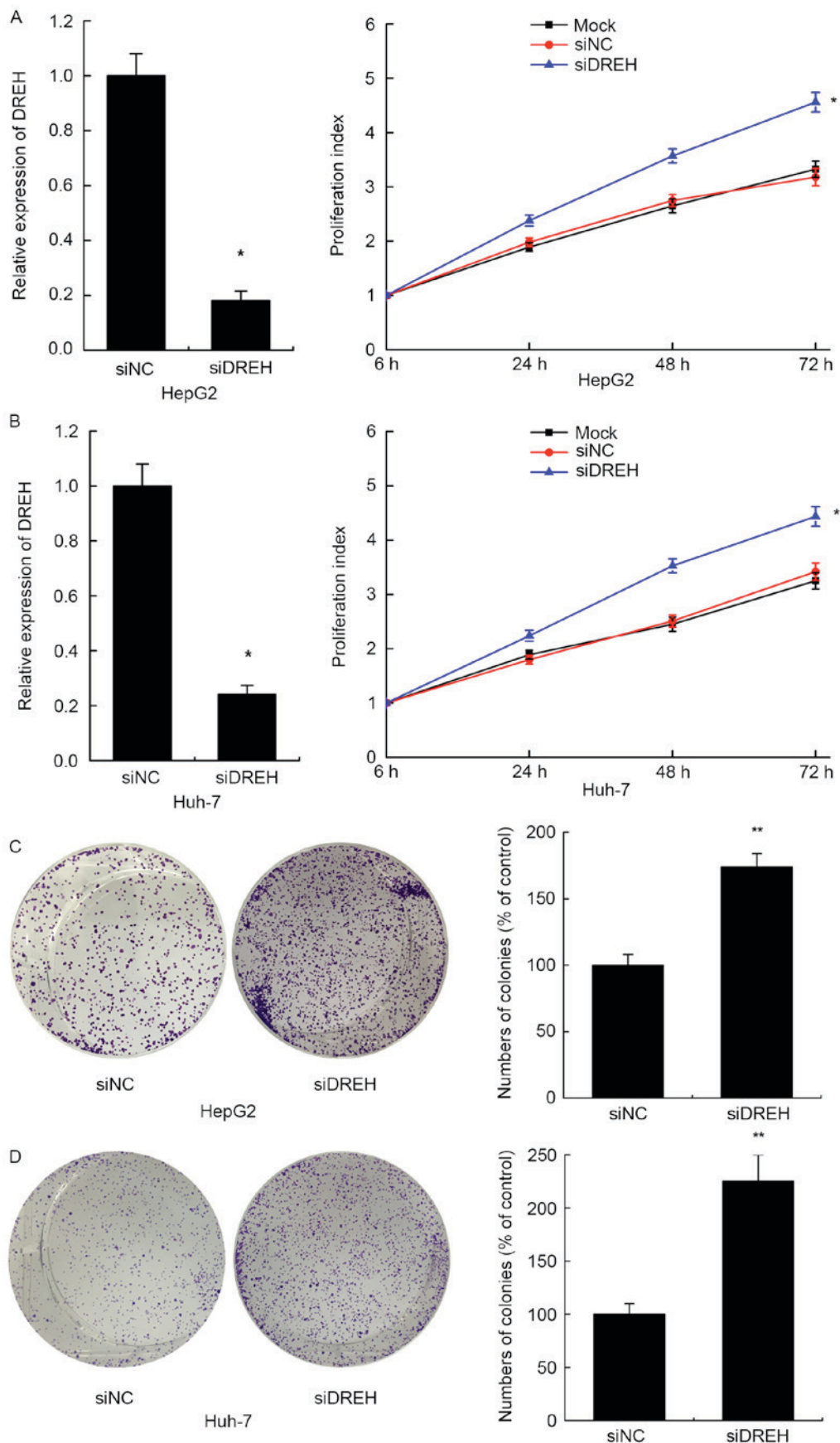


Figure 3. Inhibition of DREH promotes cell proliferation of HCC *in vitro*. (A) and (B) HepG2 and Huh-7 cells were infected with siRNA against DREH (siDREH) or respective control siRNA (siNC) and control cells without treatment (mock), and the cell proliferation activity was assessed by Cell Counting Kit-8 assay. Following transfection, cells were seeded in 96-well plates, and optical density at 450 nm was assessed 6, 24, 48 and 72 h after cells were adherent. (C) and (D) Colony formation assays were performed on HepG2 and Huh-7 cells following transfection of siRNA against DREH (siDREH) or respective control siRNA (siNC) Cells were stained with 1% crystal violet and counted. Data are shown as the means \pm standard deviation based on at least three independent experiments. * $P < 0.05$; ** $P < 0.01$ vs. siNC group.

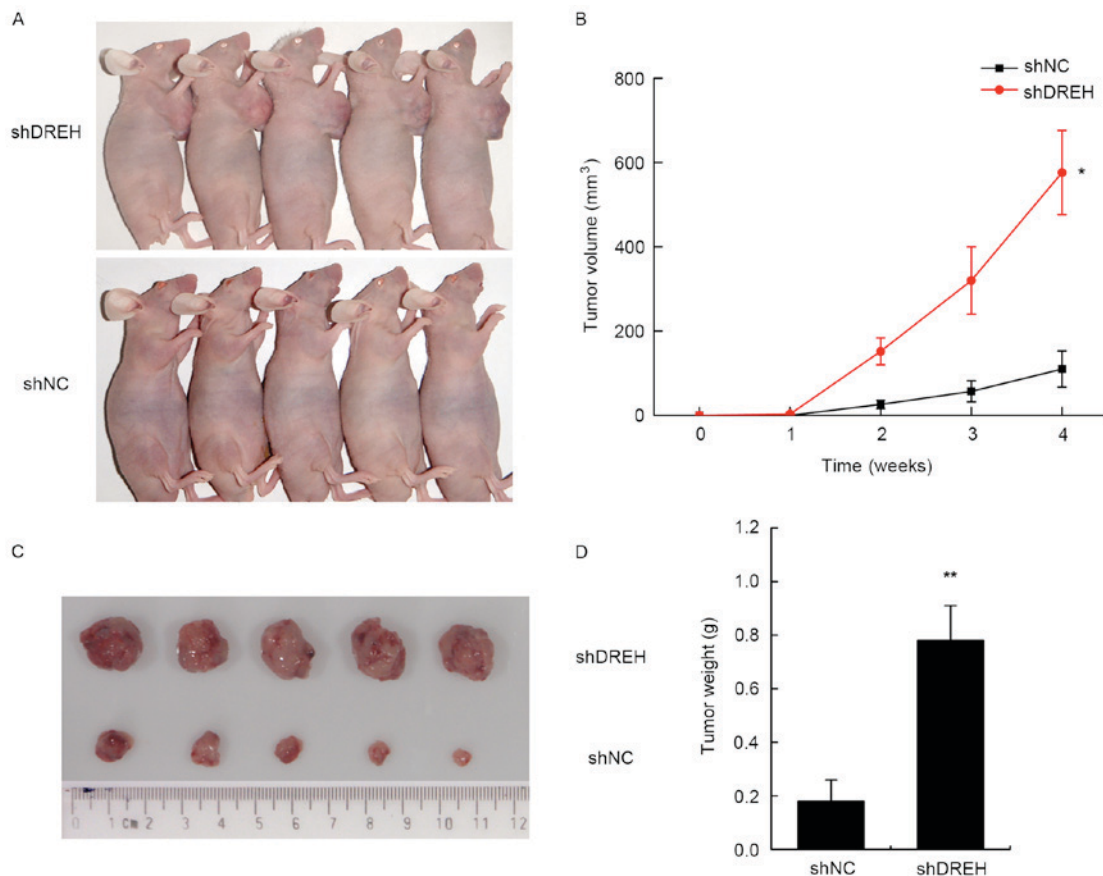


Figure 4. Inhibition of DREH promotes tumor growth *in vivo*. (A) Images of tumors that developed in xenograft-transplanted nude mice four weeks after cell injection [HepG2 with shRNA against DREH (shDREH) or control shRNA (shNC)]. (B) Tumor volume in the two groups of nude mice following ectopic subcutaneous implantation of HepG2 cells transfected with shDREH or shNC. (C) Images of tumor xenografts 4 weeks after ectopic subcutaneous implantation in nude mice. (D) Effect of shDREH or shNC on hepatocellular carcinoma tumor growth was described by tumor weight in the two groups. Data are shown as the means \pm standard deviation. * $P < 0.05$; ** $P < 0.01$ vs. shRNA NC group.

control cells, consistent with the above results (Fig. 3C and D). Thus, these results suggest that DREH may serve a key role in HBx-induced hepatocellular proliferation.

Inhibition of DREH promotes tumor growth in vivo. To determine the effects of DREH on tumorigenesis *in vivo*, DREH-downregulated or control cells (HepG2 cells stably transfected with either shRNA-DREH or control shRNA) were subcutaneously injected into nude mice for xenopplantation. Mice injected with cells transfected with shRNA-DREH demonstrated significantly increased tumor growth compared with those injected with cells transfected with control shRNA (Fig. 4A and C).

As assessed by measurements of tumor volume, tumor weight and tumor weight/body weight ratio, the inhibition of DREH expression significantly promoted overall tumor growth 4 weeks after ectopic subcutaneous implantation in nude mice (Fig. 4B and D). These results further indicated that DREH was involved in the biological function of cell proliferation in HBV-associated HCC.

Discussion

HCC is a leading cause of cancer-associated mortality worldwide (23). Current guidelines recommend different therapeutic measures for the treatment of HCC patients with different

stages, including surgery, chemotherapy, radiation therapy, and sorafenib and transarterial chemoembolization (24,25). Despite several recent advances and technical refinements, the long-term survival outcome of patients remains unsatisfactory (26). Therefore, it is necessary to thoroughly investigate the pathogenetic mechanism of HCC and develop new targeted treatments. The majority of recent investigations into cancer etiology have identified that epigenetics serves a critical role in cancer (27,28). Alterations in epigenetic modifications regulate all DNA-based processes, including transcription, DNA repair and replication, and are considered to be early events in tumorigenesis. There are also potential targets for therapeutic intervention using epigenetic drugs (29,30).

LncRNAs are a type of epigenetic regulator and are becoming one of the hot topics in genome research. Previous studies have revealed various functions and molecular mechanisms of these enigmatic molecules in biological processes of human health and diseases (31,32). With the development of high-throughput detection technologies including lncRNA microarray, RNA sequencing and the recent application of next-generation sequencing, thousands of lncRNAs have been observed to be aberrantly expressed and associated with various cancer types (33). The HBx protein has been reported to promote malignant transformation by epigenetic modifications and genetic regulation during hepatocarcinogenesis (34,35). HBx also alters the expression profiles of

lncRNAs, and these cancer-associated lncRNAs may serve key roles in gene regulation and thus affect various aspects of cellular homeostasis (21,22).

In this study, a human lncRNA DREH was identified, which was downregulated by HBx protein. The suppression of DREH expression promotes the proliferation of HCC cells *in vitro* and *in vivo*, acting as a tumor suppressor in HBx-mediated hepatocarcinogenesis. The expression levels of DREH were examined in 30 pairs of human HBV-positive HCC tissues and 30 pairs of HBV-negative HCC tissues and their pair-matched normal liver tissues. The results revealed that the expression of DREH was frequently downregulated in HBV-associated HCC tissues compared with their adjacent non-cancerous hepatic tissues and was inversely correlated with HBx mRNA expression in HBV-associated HCCs. Clinical correlation analysis demonstrated that the levels of DREH were inversely correlated with HBsAg and tumor size in HCC tissues.

In summary, these findings suggest that lncRNA DREH exerts an impact as a potential tumor repressor gene in the development of human HBV-associated HCC. The modulation of cell proliferation by DREH may be used as a potential target for the prevention and treatment of HBV-associated HCC.

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