Identification of a novel gene *HEPT3* that is overexpressed in human hepatocellular carcinoma and may function through its noncoding RNA

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Abstract. Genetic alterations have been defined as the hallmark of cancers as they are responsible for the differences between normal and malignant phenotypes. A widely accepted approach to study genetic instability is to identify cancer-related genes, in particular, the two major groups of growth regulatory genes - oncogenes and tumour suppressor genes. Using the technique of suppression subtractive hybridisation, we identified a novel gene transcript, designated as *HEPT3*. RT-PCR demonstrated that *HEPT3* was overexpressed in 87% (20/23) of HCC patients and in 4/5 HCC cell lines tested. Sequence analyses performed on the full-length cDNA revealed that *HEPT3* is an intronless gene mapped to human chromosome 6q13-14. The gene transcript lacks an extensive open reading frame and contains an Alu sequence near the 5' terminus, indicating that *HEPT3* encodes a noncoding RNA. Antisense studies on the HCC cell line HepG2 showed that, when *HEPT3* expression level was reduced, cell proliferation rate was inhibited by ~5-fold and cell colony formation was reduced by at least 50%. Our data suggest that the novel gene *HEPT3* may function through its noncoding RNA and its over-expression may play a role in hepatocarcinogenesis.

Introduction

Despite some advances in diagnosis and treatment, the prognosis of hepatocellular carcinoma (HCC) remains extremely poor accounting for almost one million deaths annually (1). The need of improving the overall outlook of HCC has urged scientists to delineate the yet mysterious mechanisms of hepatocarcinogenesis. Genetic alterations have been defined as the hallmark of cancers because they are responsible for the differences between normal and malignant phenotypes. A widely accepted approach to study genetic instability is to identify cancer-related genes, in particular, the two major groups of growth regulatory genes - oncogenes and tumour suppressor genes. The activation of oncogenes and inactivation of tumour suppressor genes play critical roles in the mechanisms of cancer development. These genes can induce malignant transformation when inappropriately expressed as a result of mutation, deletion, amplification or rearrangement. Studies on the genetic alterations in HCC have recently increased, reporting genes either up- or down-regulated or lost in hepatocellular carcinoma (2-8). A considerable number of novel genes that are involved in hepatocarcinogenesis have hence emerged.

Exploring genes associated with HCC, we identified a novel gene, *HEPT3*, which is frequently overexpressed in HCC. *HEPT3* is intronless and may function through its noncoding RNA. In this study, we report the identification and characterisation of the novel gene *HEPT3* as well as the potential role of *HEPT3* in liver tumorigenesis.

Materials and methods

Specimens and cell culture. A total of 23 pairs (tumour and non-tumour) of liver specimens and 2 pairs of colon specimens were collected after surgical resection from the No. 3 Hospital of Chongqing in China. Each sample was snap-frozen and stored in liquid nitrogen before experiment. The final diagnosis of HCC and colon cancer was confirmed by histological examination. The cell lines used in the study were mainly purchased from the American Type Culture Collection and maintained in the recommended media and culture conditions.

Suppression subtractive hybridisation. The technique of suppression subtractive hybridisation (SSH) was used to compare the mRNA profiles of HCC and its adjacent non-tumourous liver tissues of a randomly chosen HCC patient. SSH was performed by using the PCR Select cDNA subtraction kit (Clontech, Palo Alto, CA, USA). The manufacturer's instructions were strictly followed. The SSH products, cDNA fragments representing the gene transcripts upregulated in

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Table I. Sequence information and applications of the primers.

Primer	Sequence (from 5' to 3')	PCR product (bp)	Application
T3			
Forward	CAGTGAGAGTAGGCTTGTTTTAC	281	RT-PCR and real-time RT-PCR;
Reverse	GGCCTCAAGACAACATAAGATAG		ß-actin or GAPDH as internal control
GAPDH			
Forward	CGGATTTGGTCGTATTGGGC	340	
Reverse	GGCAGAGATGATGACCCTTTG		
ß-actin			
Forward	CTCTTCCAGCCTTCCTTCCT	698	
Reverse	TGTGGACTTGGGAGAGGACT		
T3			
5'RACE	GTCCGACAGAAGCCTCTACCGAGAACGT	1641	5' RACE
nest5'RACE	CCTCATAGGGAGAAATCTTGCCAAGGGAG		
T3			
3'RACE	CAGTGAGAGTAGGCTTGTTTTAC	710	3' RACE
nest3'RACE	CTATTTCTAGCCAGTGAGTTGTG		
T3full			
Forward	ATTAGGAAACACTAGAATGGAA	2613	Cloning of the full-length
Reverse	TCCTTATTTCAACAGTGTTCTTCTC		cDNA into the pGEM-T vector
AS			
Forward	Gctcgag[a]AAGGAACATAAAGGGTTAG	1455	Cloning of the HEPT3 antisense
Reverse	Cgaattc[a]TACTCCCAGTGTAACATAAG		sequence into the pcDNA3.1 vector

[a]The letters in lower case are the sequences of the restriction enzyme cutting sites *XhoI* and *EcoRI* incorporated into the forward and the reverse primers, respectively.

HCC, were cloned into pGEM-T vector (Promega, Madison, WI, USA) and sequenced.

Rapid amplification of cDNA ends. Rapid amplification of cDNA ends (RACE) was employed to isolate the full-length cDNA of *HEPT3*. RACE was performed with the Human Liver Marathon-Ready cDNA kit (Clontech) using the *HEPT3*-specific primers (Table I) in combination with the provided adapter primer AP1 or nested adapter primer AP2 to amplify the 5' and 3' portions of the *HEPT3* transcript. The RACE products were cloned into pGEM-T vector and sequenced. Primers T3full-forward and T3full-reverse (Table I) at the 5'- and 3'-end of *HEPT3* cDNA were then designed to generate the full-length cDNA by end-to-end PCR. The full-length cDNA was cloned into pGEM-T and sequenced.

Reverse transcription-polymerase chain reaction (RT-PCR). Semi-quantitative RT-PCR was performed with the OneStep RT-PCR kit (Qiagen, Hilden, Germany) while real-time RT-PCR was performed with the LightCycler RNA amplification kit SYBR Green I (Roche, Basel, Switzerland). To determine the mRNA level of *HEPT3*, primers T3-forward and T3-reverse (Table I) were used to generate a *HEPT3* fragment from 0.2 μg of DNase-treated total RNA. The OneStep RT-PCR conditions were: (1) 50˚C for 30 min; (2) 95˚C for 15 min; and (3) 35 cycles of 30 sec at 94˚C, 30 sec at 55˚C, and 1 min at 72˚C;

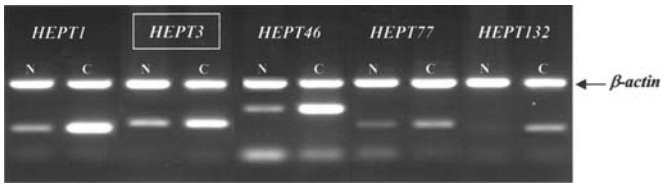

Figure 1. Evaluation of novel genes upregulated in an HCC patient by semi-quantitative RT-PCR. HCC patient No. 8 was randomly chosen for SSH studies. The 5 novel genes identified by SSH were subjected to RT-PCR to confirm their differential expression in the same HCC patient. The RT-PCR products were analysed by gel electrophoresis. N, non-tumourous liver tissue adjacent to the HCC; C, HCC tissue; ß-actin, internal control. The designated name of each novel gene is indicated in the upper panel on the gel image. HEPT3 is highlighted in the box.

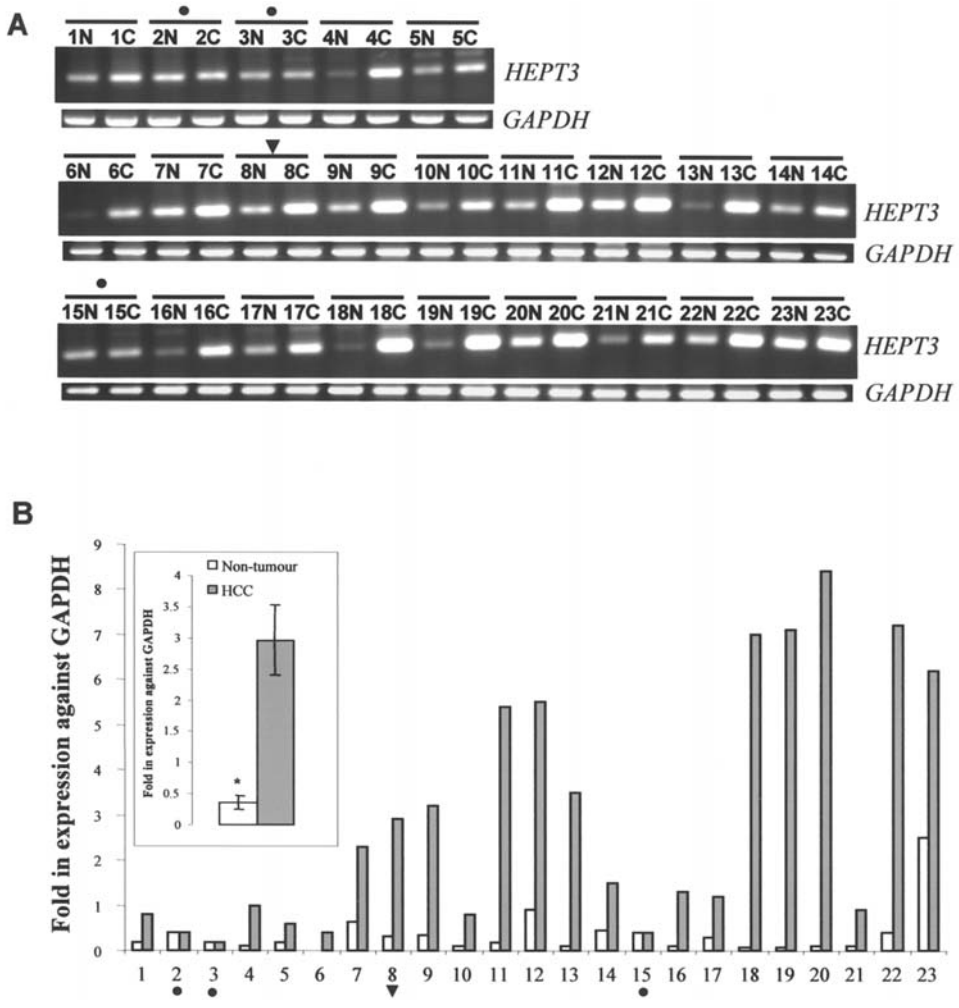

Figure 2. Overexpression of *HEPT3* in HCC. (A) Screen of *HEPT3* expression in 23 HCC patients. Semi-quantitative RT-PCR was performed to examine the differential expression of *HEPT3* in paired liver specimens. The RT-PCR products were analysed by gel electrophoresis. N, non-tumourous liver tissue adjacent to the HCC; C, HCC tissue; samples marked by '●' did not show clear difference; '▼', sample chosen for SSH studies; GAPDH, internal control. (B) Quantitation of *HEPT3* expression in 23 HCC patients. The expression level of *HEPT3* in each sample was determined by real-time RT-PCR. Each result was normalised and presented as a fold increase in expression computed against the expression level of GAPDH in the same sample. The average expression level of *HEPT3* is represented in the left inset (mean ± SD, n=23). *P<0.0001 as revealed by paired t-test.

ended with 10 min at 72˚C. ß-actin served as an internal control. RT-PCR products were analysed by gel electrophoresis. The real-time RT-PCR conditions were: (1) 55˚C for 10 min; (2) 95˚C for 10 sec; and (3) for PCR amplification, 45 cycles of 5 sec at 95˚C, 10 sec at 55˚C, and 12 sec at 72˚C. The relative expression level of *HEPT3* in each sample was presented in fold computed against the expression level of the internal control GAPDH.

Northern blot analysis. Total RNA (40 μg) was separated on a 1% formaldehyde/MOPS [3-(N-morpholino) propanesulfonic acid] gel and transferred to Hybond-N$^+$ (Amersham, Germany) membrane by capillary blotting. Labeling of *HEPT3* cDNA probe (nucleotides 645-1199) and hybridisation were carried out by using the DIG High Prime DNA labeling and detection starter kit I (Roche) according to the manufacturer's procedures.

Antisense plasmid construct and transfection studies. The antisense construct of *HEPT3* was generated by PCR with primers AS-forward and AS-reverse (Table I), and cloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). Transfections were carried out with the Lipofectamine Plus Reagents (Invitrogen). To establish stable transfection, HepG2 cells transfected with either the antisense HEPT3 plasmid or pcDNA3.1 vector were selected in the medium containing 800 μg/ml of G418 (Sigma, St. Louis, MO, USA) for 1 month, and then cloned and propagated. Real-time RT-PCR was performed to examine the mRNA levels of *HEPT3* in a number of cell clones. Three cell clones, namely HEPT3-AS1, HEPT3-AS2 and HEPT3-AS3, with a significant reduction of *HEPT3*, were selected for further study. Two cell clones with pcDNA3.1 vector and the parental cells served as controls. To assess the rate of cell proliferation, ~2x10^4 cells were seeded and cultured in 6-well plates. At every 24 h for 5 days, both microscopic analysis and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay were performed to evaluate the cell density and viability, respectively. To establish the base line of cell proliferation, MTT assay was carried out once the cells had attached to the culture dishes on the day of plating. The growth rate of each cell line was presented as a fold increase in cell viability against the respective base line.

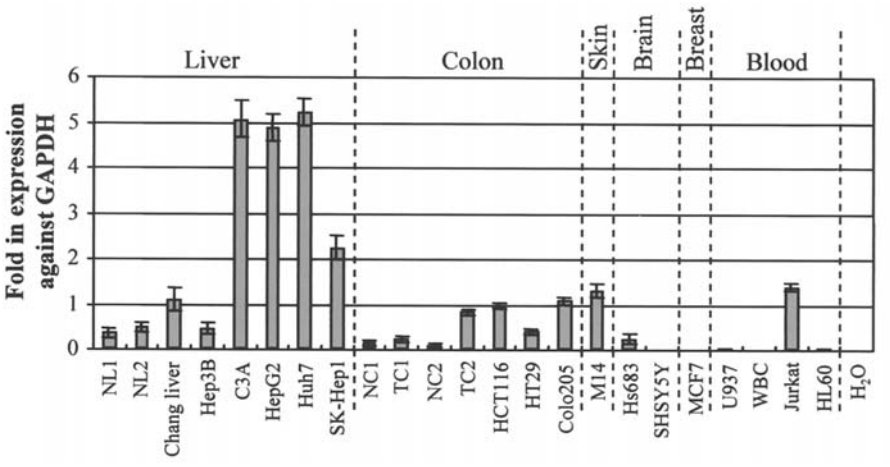

Figure 3. Expression level of *HEPT3* in a variety of human tumours. Real-time RT-PCR was performed to determine the mRNA levels of *HEPT3* in tissues and cell lines derived from different tumour types. The relative expression level of *HEPT3* is presented in fold computed against the expression level of GAPDH in each sample (means ± SD, n=3 repeats). The name and tissue type of each cell line are indicated at the bottom and the top of the graph, respectively. NL, normal liver tissue; NC, normal colon tissue; TC, colon cancer tissue; WBC, normal white blood cells; H_2O, negative control.

Table II. Correlation between *HEPT3* overexpression and the clinicopathological parameters in 23 HCC patients.[a]

Parameters	HEPT3 overexpression	HEPT3 unchanged	Overexpression rate (%)	P
Total number	20	3	87	
Sex				
Male	16	2	89	NS
Female	4	1	80	NS
Grade				
Well	3	0	100	NS
Moderate	11	3	79	NS
Poor	6	0	100	NS
Cirrhosis	16	3	84	NS
Hepatitis virus				
HBV	15	3	83	NS
HCV	3	0	100	NS
HBV+HCV	2	0	100	NS

[a]The identities and personal data of all the patients are strictly kept confidential. Grade, histological differentiation of HCC. NS, not significant; P was revealed by the Fisher's exact test.

For colony formation, HepG2 cells transfected with antisense HEPT3 construct or the vector alone were cultured in the medium containing 800 μg/ml of G418 for 3 weeks without trypsinisation. The cell colonies formed at the end of experiment were visible and the number of colonies in each plate was quantitated.

Bioinformatics and statistical analysis. Bioinformatics was carried out through database searches facilitated by the National Center for Biotechnology Information (NCBI), and motif searches with the RepeatMasker program (9). Software InStat 3.0 (GraphPad, San Diego, CA, USA) was use for statistical analysis. For a small sample size (n=6), the Mann-Whitney test was performed to compare two means while the nonparametric ANOVA for more than two means. Paired t-test was used to assess data with sample size >6. The Fisher's exact test was used to reveal the correlation between *HEPT3* overexpression and the clinicopathological parameters in 23 HCC patients. P<0.05 was considered significant.

Results

Identification of the novel gene HEPT3 in hepatocellular carcinoma. Using the technique of suppression subtractive hybridisation, we compared mRNA expression profiles of HCC and its adjacent non-tumourous liver tissue obtained from a randomly chosen HCC patient (No. 8 in Fig. 2A), and cloned 134 transcripts that were upregulated in HCC (data not shown). Five novel genes were identified as they did not have significant homology to known genes when subjected to database search. The differential expression of the five genes was confirmed by RT-PCR in the same HCC patient (Fig. 1). However, when screened against a panel of patients, only *HEPT3* appeared to be constantly upregulated in HCC and was therefore chosen for further investigations.

Frequent upregulation of HEPT3 in HCC. Semi-quantitative RT-PCR was used to examine the expression of *HEPT3* in 23 HCC specimens. The results showed that the expression of *HEPT3* was elevated in 87% (20/23) of the HCC samples (Fig. 2A). Real-time RT-PCR was performed to determine the mRNA levels of *HEPT3* in the liver specimens. In addition to the reproducible frequency of *HEPT3* overexpression in HCC, real-time RT-PCR revealed an average of 2.5-fold upregulation of *HEPT3* in HCCs compared to their adjacent non-tumourous liver tissues (P<0.0001; Fig. 2B). Moreover, real-time RT-PCR analysis was performed in tissues and cell lines of a variety of human malignancies, including cancers of the liver, colon, skin, brain, breast and blood (Fig. 3). The results showed an overexpression of *HEPT3* in 4 of the 5

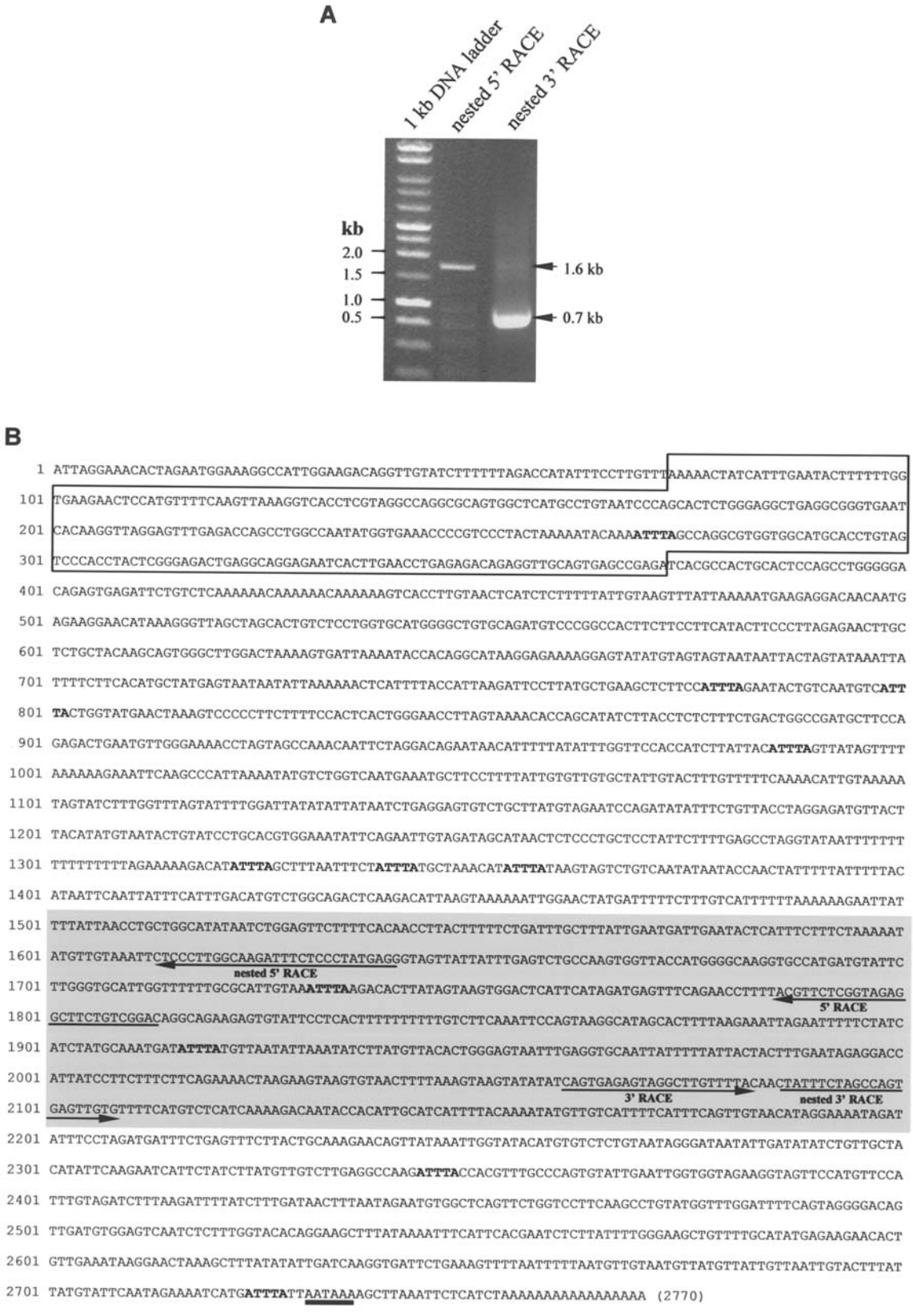

Figure 4. Isolation of full-length cDNA of *HEPT3*. (A) Nested 5' and 3' RACE products were analysed by gel electrophoresis. (B) Full-length cDNA sequence of *HEPT3*. The full-length cDNA of *HEPT3* is ~2.7 kb. The Alu sequence is boxed, the mRNA destabilisation motif, ATTTA, is in bold, and the polyadenylation signal, AATAAA, is underlined. The primers used in RACE are indicated by arrows. The fragment of 700 bp generated by SSH is shaded.

HCC cell lines, but the upregulation of *HEPT3* appeared significantly less in tissues and cell lines derived from other tumour types. The data indicate that *HEPT3* overexpression is common in HCC, suggesting that the gene may be associated with the tumorigenesis of hepatocytes. However, no significant correlations were observed between *HEPT3* overexpression and the clinicopathologic parameters in the 23 HCC patients (Table II).

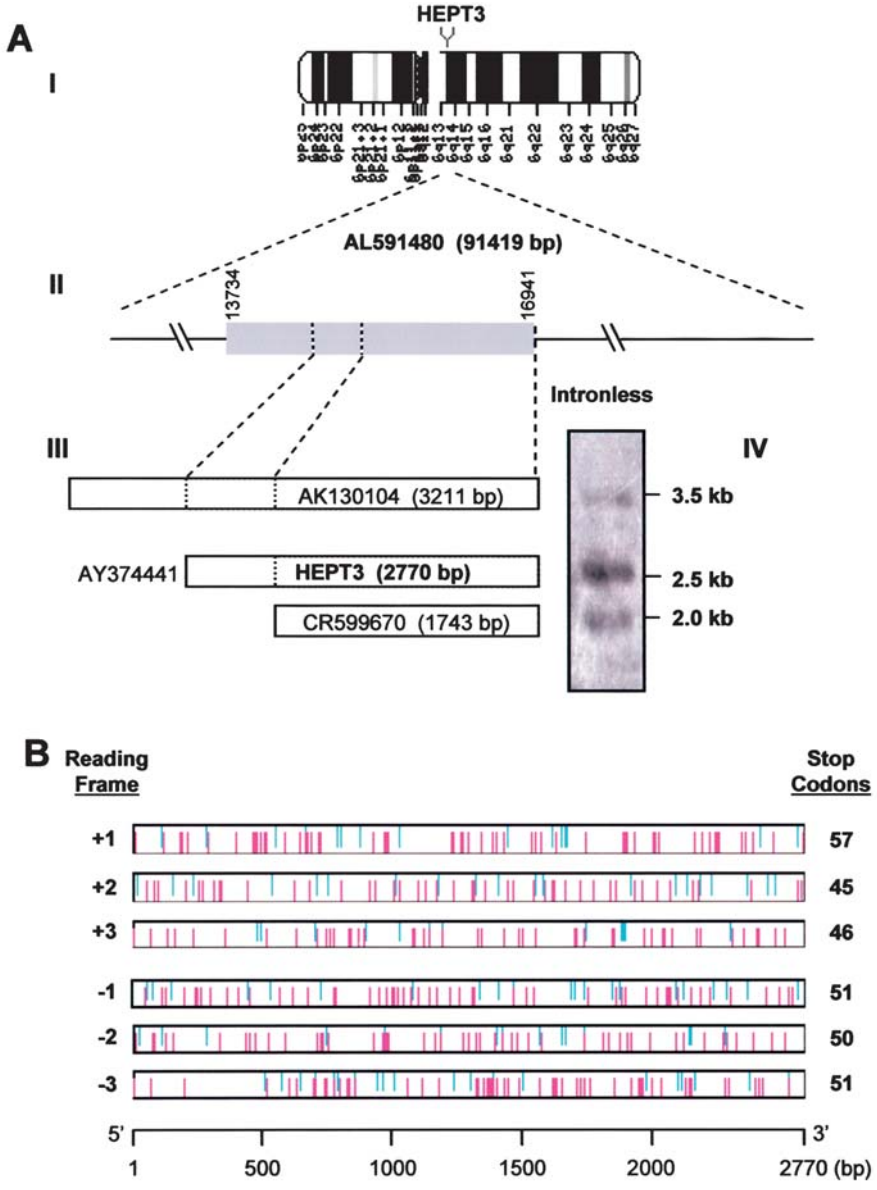

Figure 5. Characteristics of *HEPT3*. (A) Structure and isoforms of *HEPT3*. (I) *HEPT3* is located on human chromosome 6q13-14. (II) The genomic DNA of *HEPT3* (GenBank AL591480) lacks introns. (III) Two isoforms of *HEPT3*, AK130104 and CR599670, were retrieved from the database. (IV) Northern blot analysis of *HEPT3* and the 2 isoforms. (B) Noncoding potential of *HEPT3*. Open reading frame was predicted and analysed in six possible frames. The high frequency of the stop codons on each frame is indicated by the small bars on the lower line and reflected in the right panel.

Sequence analysis of HEPT3 full-length cDNA. Four *HEPT3* gene-specific primers designed on the fragment of 700 bp obtained by SSH, as indicated in Fig. 4B, were used in the two consecutive rounds of 5' and 3' RACE reactions. The respective RACE products, ~1.6 kb and ~0.7 kb fragments (Fig. 4A), were cloned and sequenced. The overlapping sequences of the SSH and the 5' and 3' RACE products were aligned to obtain the full-length cDNA of *HEPT3* of ~2.7 kb (GenBank AY374441). As shown in Fig. 4B, the sequence contained a consensus polyadenylation signal, AATAAA, 23 bp upstream of the poly(A) tail at the 3' terminus. Eleven copies of the mRNA destabilisation motif, AUUUA (ATTTA in the cDNA sequence) were identified throughout the sequence. Furthermore, through the RepeatMasker program (9), a 300-bp long Alu repetitive element was identified between the nucleotides 73 and 372 on the *HEPT3* cDNA sequence.

HEPT3, an intronless gene mapped to chromosome 6q13-14. To determine the localisation of *HEPT3* on human chromosomes, the *HEPT3* full-length cDNA sequence was subjected to searches against the human genome database of the NCBI algorithm. The results showed that *HEPT3* was specifically mapped to the human chromosome 6q13-14 (Fig. 5AI). Comparative analysis showed that *HEPT3* cDNA was collinear to the genomic sequence (GenBank AL591480), indicating that the gene *HEPT3* is intronless (Fig. 5AII).

Northern blot analysis was performed using a cDNA probe without the *HEPT3* Alu element to avoid non-specific hybridisation. The results revealed three differently sized signals of ~3.3, 2.7 and 2.0 kb (Fig. 5AIV), suggesting that *HEPT3* may have isoforms. Indeed, database analysis revealed two uncharacterised full-length cDNAs that matched both the cDNA sequence and the chromosomal location of *HEPT3*

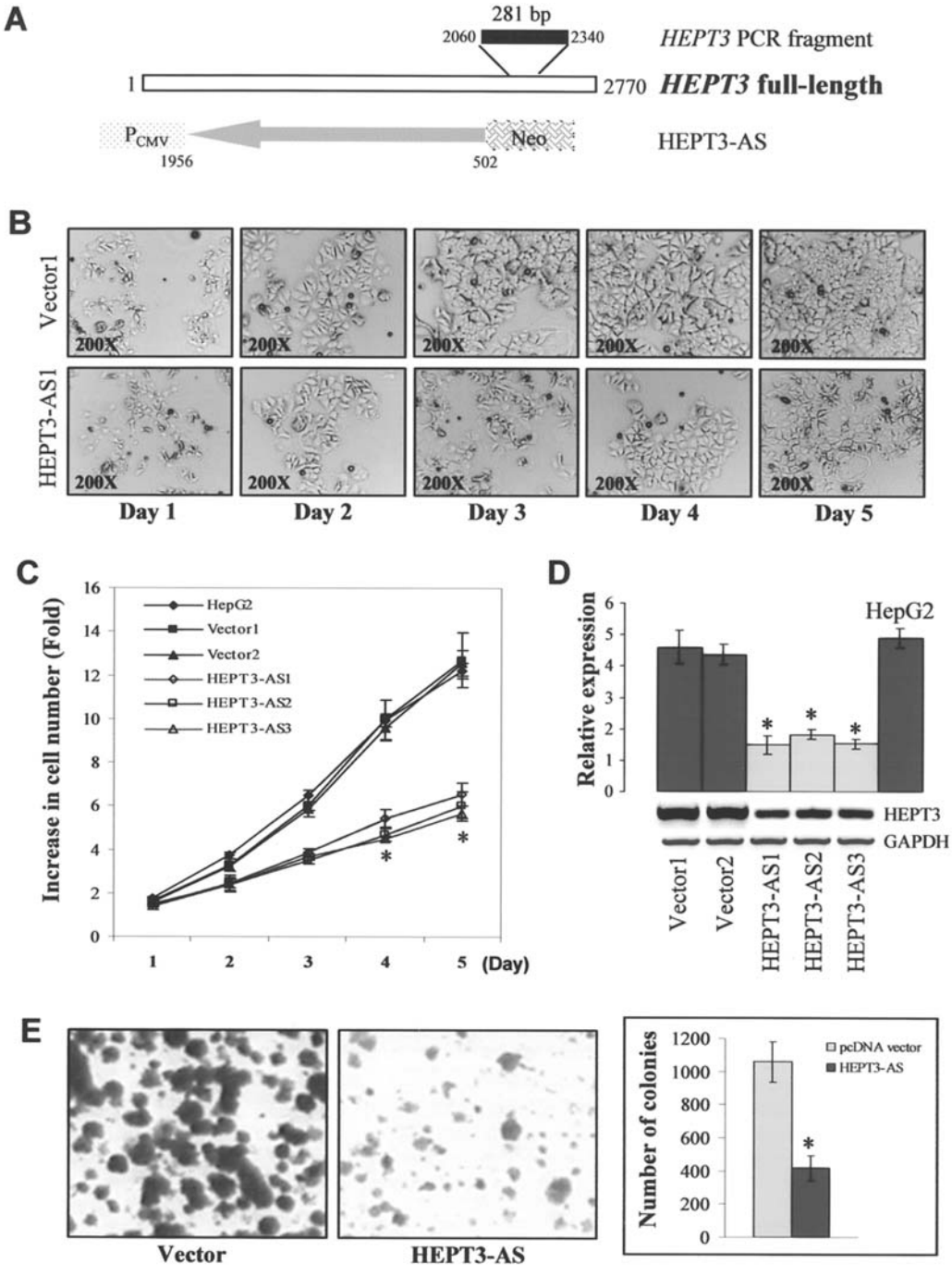

Figure 6. Antisense studies. (A) Schematic representation of HEPT3 antisense construct, namely HEPT3-AS. The construct was generated by cloning a fragment of *HEPT3* cDNA (from 502 to 1956) in the reverse order into the pcDNA3.1 vector. The forward and reverse *HEPT3* gene-specific primers located at 2060 and 2340, respectively, were used to generate the PCR product of 281 bp. (B and C) Analysis of cell growth rate. Three cell clones stably transfected with HEPT3 antisense construct were compared to two cell clones with pcDNA3.1 vector and the parental cells for their growth rate. At every 24 h for 5 days, both microscopy (B) and MTT assay (C) were performed to evaluate cell density and viability, respectively. The growth rate is represented as a fold increase in cell viability (means ± SD, n=6); *P<0.001 as revealed by nonparametric ANOVA. (D) Suppression of endogenous *HEPT3* evaluated by real-time RT-PCR. The expression level of *HEPT3* is presented in fold against the expression level of GAPDH in each sample (means ± SD, n=3 repeats); *P<0.001 as revealed by nonparametric ANOVA. The PCR product was examined by gel electrophoresis for its specificity (under each bar). (E) Colony formation. The size and density of cell colonies are shown in the photographs. The number of colonies on each plate is represented in the bar chart (means ± SD, n=6). *P<0.001 as revealed by the Mann-Whitney test. Vector, HepG2 cells transfected with pcDNA3.1 vector and cell clones Vector 1 and 2; HEPT3-AS, HepG2 cells transfected with HEPT3 antisense construct and cell clones HEPT3-AS1, -AS2, and -AS3.

(Fig. 5AIII). One was a 3211-bp human cDNA (GenBank AK130104), which contained the entire *HEPT3* cDNA in its 3' region. The other was a 1743-bp full-length cDNA of human fetal brain (GenBank CR599670), which was 99% homologous to the 3' portion of *HEPT3* cDNA. On the Northern blot, the ~2.7 kb band with the strongest signal appeared to correspond to the full-length *HEPT3* cDNA while the ~3.3 and ~2 kb bands corresponded to the two isoforms identified in the database. Similar to *HEPT3*, both transcripts were encoded by intronless genomic DNA. These findings

indicate that *HEPT3* and its variants may be transcribed from the same genomic DNA.

HEPT3, a potential noncoding RNA. The NCBI ORFinder revealed a high density of stop codons in all the 6 theoretical reading frames (Fig. 5B). Although numerous start codons were scattered throughout the *HEPT3* gene, the frequent stop codons resulted in a lack of an extensive open reading frame. Similar observations were obtained when the other two *HEPT3* isoforms, the 3.2-kb and 1.7-kb transcripts, were analysed. These data suggest that *HEPT3* may not possess coding capacity. However, searches with both the full-length and fragmented *HEPT3* sequences failed to reveal any significant homology to the known microRNAs in the available databases.

Reduction of HepG2 cell growth rate by antisense HEPT3. As *HEPT3* was markedly upregulated in HCC cell lines, we employed the antisense approach to interfere with the expression of *HEPT3* in HepG2 cells and then to evaluate the involvement of *HEPT3* in regulating cell growth. The antisense plasmid was constructed with a partial cDNA of *HEPT3* in the antisense orientation (Fig. 6A). The Alu element was excluded to avoid binding to non-specific sequences. The endogenous *HEPT3* mRNA levels were clearly decreased by antisense HEPT3 in three clones (Fig. 6D). Light microscopy (Fig. 6B) and MTT assay (Fig. 6C) revealed that the cell proliferation rate of these three cell clones was reduced ~5-fold on days 4 and 5 ($P<0.001$) when compared to the controls, two cell clones transfected with the empty vector and the parental HepG2 cells. In addition, disruption of *HEPT3* reduced the colony formation of HepG2 cells. Not only did the antisense HEPT3 decrease the number of colonies by >50% ($P<0.001$) but also the size and cell density of the colonies (Fig. 6E). These data implicate that overexpressed *HEPT3* exerts proliferative effect on the HCC cells.

Discussion

Using suppression subtractive hybridisation to compare the mRNA expression patterns of HCC and its adjacent non-tumourous liver tissue, we identified a novel transcript, *HEPT3*, which is highly upregulated in 87% of 23 HCC tissues examined. However, no significant correlations could be observed between *HEPT3* overexpression and the clinico-pathological parameters in the 23 HCC patients. This observation could be due to the high frequency (20/23) of *HEPT3* overexpression in the patients and/or the relatively small sample size examined in this study. An elevated expression of *HEPT3* was also detected in most (4/5) of the HCC cell lines tested, but no apparent overexpression of *HEPT3* was observed in tissues and cell lines of other tumour types. The data indicate that *HEPT3* overexpression is specific to HCC, suggesting that the gene may be associated with the tumorigenesis of hepatocytes. Liver-specific genes that are differentially expressed in HCC have been identified. For example, the human LFIRE-1/HFREP-1 is frequently down-regulated or lost in HCC (10). Hopefully, the emerging of *HEPT3* will shed new light on liver carcinogenesis.

The mechanism responsible for regulating *HEPT3* expression is still elusive. The polyadenylated transcript of *HEPT3* contains eleven copies of the mRNA destabilisation motif, AUUUA. Such motifs have been reported in lymphokines, cytokines and proto-oncogene mRNAs; and have been proposed to interact with the AU-binding factor that degrades mRNA (11). This finding offers a clue that the RNA levels of *HEPT3* may be regulated by mechanisms involving RNA stability.

The gene *HEPT3* encodes a transcript of ~2.7 kb and displays several intriguing characteristics. The full-length cDNA of *HEPT3* is collinear to its genomic DNA, indicating that *HEPT3* represents an intronless gene. Naturally intronless genes account for <6% of the genes in human (12). Since intronless genes do not require post-transcriptional splicing, they can be transcribed more efficiently leading to a potentially faster rate of expression, greater abundance and higher fidelity. Classic examples of intronless proto-oncogenes are the *myc* (13) and *c-jun* (14) genes. The lack of intron/exon boundaries in the genomic sequence implies that alternative splicing of *HEPT3* is unlikely to occur. However, Northern blot analysis detected three differently sized transcripts, suggesting that *HEPT3* has isoforms. Database analyses revealed two uncharacterised full-length cDNAs matching with *HEPT3* both in sequence and in chromosomal location. These findings indicate that *HEPT3* and its variants are transcribed from the same genomic DNA. However, the mechanism of generating these transcripts by the DNA machinery remains unclear.

Another striking feature of *HEPT3* is the high density of stop codons in all the reading frames, resulting in a lack of extensive open reading frame. In addition, an Alu repetitive element was identified in the *HEPT3* transcript. The presence of short interspersed repetitive sequences (SINEs) and other repeats is a frequent event in non-translatable transcripts (15), but rare in translated transcripts (16). Collectively, the atypical structural features of the cDNA suggest that *HEPT3* may function as a noncoding RNA. Two examples of relatively well-studied mammalian genes that act at the RNA level are *XIST* (X (inactive)-specific transcript) and *H19* (17,18). *XIST* RNA coats one of the two X chromosomes in females, resulting in transcriptional silencing of the X chromosome. *H19* has been implicated in imprinting of the insulin-like growth factor 2 gene important for tumour suppression (19). Moreover, many miRNAs (microRNAs), another group of noncoding RNAs, have been implicated in carcinogenesis including HCC (20-22). However, database searches failed to reveal significant homology or complementarities between *HEPT3* sequences and the known miRNAs.

The gene *HEPT3* is mapped to human chromosome 6q13-14. Chromosome 6 is best recognised for the major histocompatibility complex (MHC) that is critical to the human immune response. Additionally, it was documented to carry genes related to genetic diseases such as arthritis (23), diabetes (24), schizophrenia (25) and cancers. Gains in chromosome 6 have also been reported in HCC (26); however, it is unclear if *HEPT3* is involved in such gains.

To determine if *HEPT3* mRNA contributes to hepatocyte survival, we used an antisense inhibition approach to interfere with the biological activity of *HEPT3*. Suppression of the endogenous *HEPT3* mRNA by antisense HEPT3 significantly

inhibited both cell proliferation and colony formation of HepG2 cells, demonstrating that antisense HEPT3 exhibits antiproliferative effect on the HCC cells that express the endogenous *HEPT3* gene. These results implicate that transcriptional activation of the *HEPT3* gene may stimulate the oncogenic pathway that induces cell proliferation.

In conclusion, the gene *HEPT3* is more commonly upregulated in HCC than in other tumour types. *HEPT3* is an intronless gene and maps to chromosome 6q13-14. Its transcript displays the typical characteristics of noncoding RNA, including the lack of extensive ORF and the presence of an Alu sequence. Antisense studies revealed that the growth of HCC cells was significantly suppressed when *HEPT3* expression level was reduced. The data indicate that *HEPT3* may function through its noncoding RNA and play a role in the malignant transformation of hepatocytes. Further studies will be carried out to elucidate how *HEPT3* transcription is activated in hepatocarcinogenesis and then promotes the progression of HCC.

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