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Identification of a Novel Transcriptional Repressor (HEPIS) That Interacts with nsp-10 of SARS Coronavirus

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ABSTRACT

A novel gene was previously isolated from a cDNA library of human embryo lung tissue by its encoded protein, which interacts with non-structural protein 10 (nsp-10) of the severe acute respiratory syndrome coronavirus (SARS-CoV). The protein was named human embryo lung cellular protein interacting with SARS-CoV nsp-10 (HEPIS), and it is composed of 147 amino acids with several CK II phosphorylation sites. In the present study, we demonstrated that HEPIS was capable of suppressing chloramphenicol acetyltransferase (CAT) gene expression controlled by different enhancer elements in a transcription assay. HEPIS interacted specifically with the HSP70 TATA sequence, and not with various other enhancer elements in a binding test. Furthermore, we co-immunoprecipitated HEPIS with BTF3, a component of the RNA pol II initiation complex, and observed reduced proliferation of HeLa cells transfected with the HEPIS gene. Taken together, our results suggest that HEPIS may function as a potential transcriptional repressor.

INTRODUCTION

RESEARCH ON THE REGULATION OF EUKARYOTIC GENE EXPRESSION during the previous decade focused on the identification and characterization of the various factors that are involved in the transcriptional process (5,16). Despite the lack of fine details, a model was established to demonstrate the process of transcription in eukaryotic cells (1). In this model, at least two kinds of factors are involved in transcriptional initiation and regulation (15,22). In the initiation of transcription by RNA polymerase II (RNA pol II), the multi-protein complex, which includes some basic transcriptional factors such as BTF1, BTF2, and BTF3 among other proteins (10), functions through binding to the TATA box cap-site element. While transcription is initiated by these protein factors and RNA pol II, it is controlled by other regulatory factors, including Sp1, NF-κB, and YY1. These regulatory factors bind to specific proximal sequence elements located upstream of the TATA box to enhance transcriptional activity (18). Such a model also suggests that various transcriptional repressors function by binding to different initiators, regulatory factors, or specific DNA sequences, to slow the rate of transcription (21). Identification of various repressors, such as p53, support this model and indicate that the repressor, as a transcriptional regulator, is very important for eukaryotic cell proliferation and metabolism (14). During viral replication in host cells, some proteins encoded by viral genes also function in viral gene transcription through interaction with different cellular transcriptional factors (17). Such interactions between viral proteins and cellular transcriptional factors determine, in some cases, the progress of viral replication and host cell survival (20). Studies on the interaction of virus with host cells led to the identification of some proteins involved in both viral and cellular transcriptional regulation (7).

The non-structural protein 10 (nsp-10) of the severe acute respiratory syndrome coronavirus (SARS-CoV) is

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produced by $3CL^{pro}$ cleaving pp1a-pp1ab during infection, and is thought to function as a viral transcriptase (19). In our previous work, we isolated a gene from a cDNA library of human embryo lung tissue, which encoded a novel protein that specifically interacted with nsp-10 of SARS-CoV in a yeast trap experiment. This interaction was confirmed in a series of experiments (13). This newly identified protein, which is composed of 147 amino acids with several CK II phosphorylation sites, was named human embryo lung cellular protein interacting with SARS-CoV nsp-10 (HEPIS) (Fig. 1). Since nsp-10 of SARS-CoV is involved in viral genomic replication and was observed to interact with ATF5, the cellular initiation factor of the RNA pol II complex (13), we inferred that HEPIS may also be involved in cellular gene transcription. Therefore, the significance of HEPIS expression in cells needed to be further investigated. The work we describe here suggests that HEPIS represses cellular transcription initiation through interaction with a component of the RNA pol II complex, and may be a potential member of the anti-oncoprotein family.

MATERIALS AND METHODS

Cells

Human embryo fibroblasts (KMB-17 strain, maintained in our lab) (12) and HeLa cells (also maintained in our lab) were grown in DMEM-5% (v/v) fetal bovine serum (FBS) to form monolayers in culture flasks. CHK cells (also maintained in our lab) were grown in Ham's F12 medium with 5% FCS at 37°C and 5% CO_2 to 80% confluency in 50-mm plates.

Plasmid construction

The HEPIS eukaryotic expression vector, pcDNA-hepis, was constructed using pcDNA3 (Invitrogen, Carlsbad, CA) and the hepis gene-encoding sequence. pGBK-hepis, the screen vector for yeast trap analysis, was constructed using pGBK-T7 (Invitrogen) and the hepis gene encoding sequence. Three mutants of the hepis gene were produced as described in Fig. 2 and were cloned into pGBK-T7 to construct pGBK-hepis1, pGBK-hepis2, and pGBK-hepis3. pcDNA-BTF3 was constructed using pcDNA3 and the BTF3 encoding sequence obtained by reverse transcription from mRNA extracted from KMB-17 cells. The reporter plasmid pCAT-SV, containing the TATA-box from the SV40 promoter sequence, was constructed using pCAT-basic. pCAT-SV-CA, pCAT-SV-SP1, pCAT-SV-AP1, pCAT-SV-OCTA, pCAT-SV-ATF, pCAT-SV-Ad, and pCAT-SV-HSV-α were constructed using pCAT-SV and different regulatory element sequences, namely CAT: GATTGGCT; SP1: GGGGCGGGGC; AP1: TGAGTCAG; OCTA: ATTTGCAT; ATF: TGACGTCA; AdV promoter, and HSV-1 α-gene promoter, respectively.

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  1    GGGTGTAGCTTTGGA AATCTGTCTTTGTTG CTCTGGGAGAGGGGA CTCCTGGAATGTGTC TGTGAATAAAGACTA
                                        M  S  A  H  M   S  G  L  E  I   M  D  E  D  Q

 76    GCCGAAGATACTGTG GCCTCATGAATAGGA ATGTCTGCCCATATG TCAGGATTGGAAATA ATGGATGAAGATCAA
        L  I  K  D  V   L  D  K  F  L   N  C  H  E  Q   T  Y  D  E  E   F  L  N  T  F

151    TTAATCAAAGACGTC TTGGATAAATTCCTT AATTGTCATGAGCAA ACATATGATGAAGAA TTTCTGAACACTTTT
        T  H  L  S  Q   D  L  L  L  L   P  G  E  V  E   Q  D  V  S  T   S  I  P  S  C

226    ACTCATCTTTCACAA GATTTGCTGCTGCTT CCAGGAGAAGTGGAG CAGGATGTAAGCACC AGCATTCCTTCCTGT
        I  P  F  V  A   Q  P  P  T  C   E  V  K  P  K   P  S  V  K  R   M  D  K  Q  T

301    ATCCCTTTTGTGGCC CAGCCTCCTACCTGT GAAGTGAAGCCAAAG CCCAGTGTTAAAAGA ATGGACAAACAGACG
        E  E  I  L  G   D  E  V  Q  L   F  S  L  D  E   E  F  D  Y  D   N  V  M  L  T

376    GAAGAGATACTTGGA GATGAAGTTCAACTT TTTTCACTTGATGAA GAATTTGATTATGAC AATGTGATGCTAACC
        S  K  F  S  P   A  E  I  E  N   I  K  E  L  C   K  Q  Q  K  R   K  D  T  S  P

451    TCCAAGTTTAGTCCT GCAGAGATAGAGAAC ATCAAAGAGCTATGC AAGCAGCAGAAGAGA AAGGACACCAGCCCA
        D  L  E  K  S   C  D

526    GACTTAGAGAAATCC TGTGACTGATTCACA GAGGCATTTTGTGTG TGTGTGCTTATTTTA ATTTTGTTCTTATTC
601    TAGCAACATTAGAAT AAAAGATAAACCTAC TATAAAAAAAAAAAA AAAAAAAAAAAAAAA AAAAAA
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FIG. 1. Nucleotide sequence and deduced amino acid sequence of HEPIS. The HEPIS protein is composed of 147 amino acids with five CK II phosphorylation sites at residues 6, 31, 102, 119, and 138 (underlined). The pattern of CK II phosphorylation motif is [ST]-X(2)-[DE], as predicted by Omiga 2.0 software. The GenBank accession number of HEPIS is DQ121386.

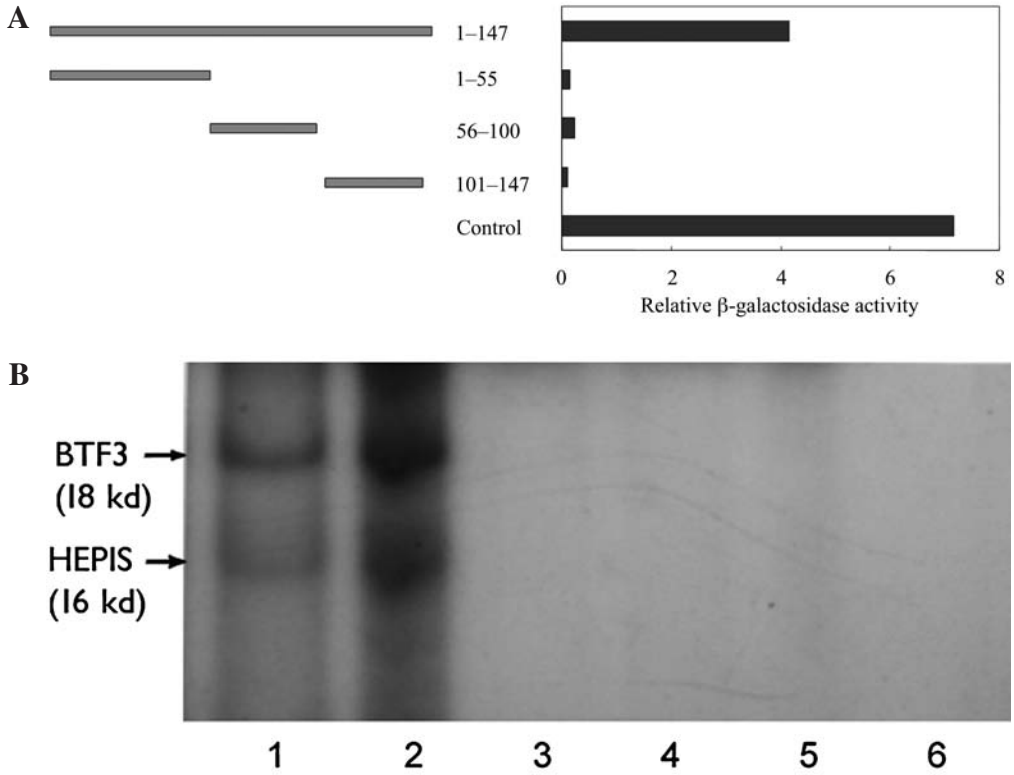

FIG. 2. (**A**) Mapping the region of HEPIS interacting with BTF3. Three plasmids encoding amino acid residues 1–55, 56–100, and 101–147 of HEPIS were constructed as pGBK-hepis1, pGBK-hepis2, and pGBK-hepis3, respectively, and transfected into yeast AH109. These transfected clones were fused with Y187 transfected with pACT-btf3, and then the β-galactosidase assay was used to evaluate the interaction between different HEPIS domains and BTF3. (**B**) Co-immunoprecipitation of the HEPIS and BTF3 interaction complex. Co-immunoprecipitation with anti-HEPIS and anti-BTF3 antibodies was performed separately. The ^{35}S-methionine-labeled interaction complex from CHO cells co-transfected with pcDNA-HEPIS and pcDNA-BTF3 as described in the text was incubated with anti-HEPIS or anti-BTF3 antibodies. Cells transfected with the empty vector pcDNA3 were used as controls. The conjugated complex was then absorbed to A protein-Sepharose 4B beads. After washing with RIPA buffer, the samples were eluted in sample buffer and run on an SDS-PAGE gel and auto-exposed to x-ray film. Lane 1: the specific interaction between complexes immunoprecipitated by anti-HEPIS antibody; lane 2: the specific interaction between complexes immunoprecipitated by anti-BTF3 antibody; lane 3: pcDNA3 control transfection immunoprecipitated by anti-HEPIS antibody; lane 4: pcDNA3 control transfection immunoprecipitated by anti-BTF3 antibody; lane 5: control cells; lane 6: negative control with normal mouse IgG.

Yeast two-hybrid screen

The pGBK-hepis plasmid was used to screen a cDNA library of human embryo lung. The procedure was conducted according to the manufacturer's protocol. After screening twice using the QDO plate and the β-galactosidase assay, cDNA gene fragments from the library that encoded proteins capable of interacting with the HEPIS protein were isolated and identified by sequencing. The β-galactosidase assay to evaluate the interaction between different HEPIS mutants and BTF3 was performed according to the manufacturer's protocol.

Transfection of cells

Recombinant plasmids, including pcDNA-hepis, were linearized by digestion with suitable restriction enzymes. CHO cells and HeLa cells were transfected by electroporation as described elsewhere (12). The control transfections were performed with linearized pcDNA3 plasmid. The transfected cells were maintained in DMEM-5% fetal bovine serum for 24–48 h prior to analysis.

Co-immunoprecipitation

Cells transfected by pcDNA-hepis and pcDNA-BTF3 or by pcDNA3 were grown in methionine-free MEM or phosphate-free MEM for 1 h. Then, the same media supplemented with ^{35}S-methionine (100 moi/mL) or ^{32}P-phosphate (100 moi/mL) was added to the cells, which were then incubated at 37°C for 1.5–2 h. The harvested cells were lysed in buffer containing detergent and centrifuged at 12,000 rpm for 5 min, followed by discarding

of the nuclei. The supernatant was incubated with anti-HEPIS or anti-BTF3 antibodies and the immune complex was precipitated by protein A-Sepharose followed by washing (4). Finally, the labeled protein was separated from the complex by boiling in the presence of detergent, and was submitted to 12% SDS-PAGE and auto-exposed to x-ray film.

Northern blot

A pre-made Northern blot of normal human tissue containing 10 μg of total RNA per lane from eight different human normal tissues including lung, heart, muscle, kidney, colon, bone, ovary, and liver, was hybridized with a hepis sequence probe containing 60 nt between positions 241 $\rightarrow$ 300 in its cDNA according to standard protocol (13). In addition, a pre-made Northern blot of human tumor tissue containing 10 μg of total RNA per lane from eight different human tumor tissues, including breast tumor, ovary tumor, uterus tumor, lung tumor, kidney tumor, stomach tumor, colon tumor, and rectum tumor, was treated as described above.

Binding test

The different regulatory element sequences of CAAT, Sp1, Ap1, OCTA, ATF (see above), and TATA box (9), were labeled with r-^{32}p-ATP and T4 kinase according to the standard protocol (4). Each labeled sequence (1–2 μci) was added to 1–2 μg of purified HEPIS protein, which was expressed in bacteria in 20 μL of binding buffer (8). After incubation of this HEPIS protein mixture and labeled sequence at 37°C for 1 h, the whole reaction volume was loaded onto a 5% non-denaturing PAGE gel and electrophoresed at 60 V. The result was analyzed with auto-exposed x-ray film after drying the gel.

Transcriptional activity assay

The transfected CHO cells were maintained in Ham's F12 media with 5% fetal calf serum for 36 h, collected with a rubber policeman, and washed three times with PBS. The washed cells from each well were lysed with 200 μL 1$\times$ reporter lysis buffer for the CAT enzyme assay system (Promega Corp. Madison, WI) and incubated at 60°C for 10 min. The supernatant was collected by centrifugation at 11,000 $\times$ g at 4°C for 5 min. The CAT assay system was composed of extract from transfected cells (10–100 μL), [3,5-^{3}H] chloramphenicol (0.25 μCi; New England Lab, Woburn, MA), n-butyryl-CoA (5 μL, 5 mg/mL), and Tris-HCl (pH 8.5, 0.25 M), for a total reaction volume of 125 μL. This reaction system was incubated at 37°C for 1–5 h, shaken strongly for 30 sec in a Vortex with 300 μL of mixed xylenes, and spun at 11,000 $\times$ g to separate the two phases. The upper-phase xylenes were collected and added to 100 μL of 0.25 M Tris-HCl for one additional extraction. After a second collection, the upper xylenes phase was put into a scintillation counter with 800 μL of scintillation fluid, and the standard curve of CAT activity was established using serial CAT enzyme (Promega) dilutions from 0.1 to 0.003125 units. One unit (U) was defined as the amount of enzyme required to transfer 1 nmol of acetate to chloramphenicol in 1 min at 37°C.

Stable expression of HEPIS in HeLa cells

HeLa cells transfected with pcDNA-hepis were grown in DMEM-5% FBS containing 350 μg/mL of G418 to form resistant clones. The cloned cells were grown and passaged in the presence of G418 and were detected with Western blotting using antibody against the HEPIS protein. Once stable expression of HEPIS was observed in the cloned cells, they could be used in the following experiments.

Cellular proliferation assay

Transfected and control HeLa cells grown in DMEM media containing 350 μg/mL of G418 were monitored for proliferation potential at 12, 24, 36, 48, 60, and 72 h, using the 3-[4,5-dimethythialzol-2-yi]-2,5-diphenyl tetrazolium bromide (MTT) assay as described elsewhere. These tests were performed in triplicate for statistical analysis.

Soft agar assay

Cells grown in DMEM growth medium were diluted to 10^3 cells/mL and mixed with 1.2% agar (Voigt Global Distribution, Lawrence, KS) in an equal volume for growth in six-well plates. Clones grown in soft agar were examined 2 weeks later. The data were expressed as the percentage of colonies containing $>$200 cells.

Statistical analysis

All data were statistically analyzed according to standard protocol. Results were expressed as means $\pm$ standard deviation (SD). Statistical significance was determined using two-way analysis of variance (ANOVA) and the Student's *t*-test by SPSS 11.0 software (SPSS, Inc., Chicago, IL), and p values <0.05 were considered statistically significant.

RESULTS

Identification of the HEPIS transcript in human cells

Since our previous work suggested that HEPIS was a novel cellular protein by virtue of its interaction with nsp-

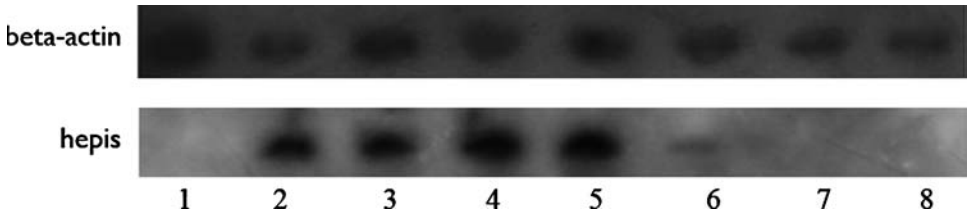

FIG. 3. HEPIS gene expression in different normal human tissues. A pre-made membrane (Multiple Tissue Northern Blot; BD Biosciences, Palo Alto, CA) containing 10 μg of total RNA from normal human tissues per lane was used in a Northern blot to detect the HEPIS transcript with a specific probe (a fragment of 60 nt between positions 241 → 300 in its cDNA). β-Actin mRNA was detected as a normalizing control with a 491-nt probe. Lanes 1–8: heart, lung, liver, kidney, ovary, colon, muscle, and bone.

10 of SARS-CoV in a yeast trap assay (13), we performed further studies to confirm the transcription of the HEPIS gene in normal tissues. A Northern blot of normal human tissue RNA was probed with an oligonucleotide from the HEPIS gene. The results showed that the HEPIS transcript was produced mainly in the lung, liver, ovary, and kidney, and at a lower level in muscle and bone (Fig. 3). This result supports the prediction that HEPIS is a cellular protein in normal tissues. Structural analysis also indicated that the HEPIS gene located on chromosome 11 is composed of four exons (data not shown).

Identification of a protein that interacts with HEPIS in vivo

Given that HEPIS interacted with nsp-10, a putative viral transcriptase, we hypothesized that HEPIS may function through interaction with proteins involved in cellular transcription. To identify these potential interacting proteins in cells, HEPIS was used as a bait protein to screen a cDNA library of human embryo lung tissue using a yeast trap assay. After a standard screening procedure, several proteins were identified that included a component of the RNA pol II initiation complex, BTF3, and other proteins of unknown function (Table 1). We confirmed that HEPIS interacted functionally with BTF3 in a β-galactosidase activity assay. However, no specific domain responsible for this interaction was found (Fig. 2A). Our efforts to immunoprecipitate endogenous HEPIS from normal human embryo fibroblast cells failed, perhaps due to low expression. Therefore, we investigated the *in vivo* interaction of HEPIS and BTF3 by co-transfection of CHO cells with pcDNA-hepis and pcDNA-BTF3, followed by co-immunoprecipitation of these proteins using antibodies raised in mice immunized with recombinant HEPIS or BTF3. Both ^{35}S-methionine-labeled HEPIS and BTF3 were precipitated together by antibodies against HEPIS or BTF3 (Fig. 2B). Given that BTF3 is one component of the RNA pol II initiation complex, it was expected that other weaker protein bands were observed in this co-immunoprecipitate. Certainly, such an experiment depends on the transfection system and may not reflect completely the behavior of the endogenous proteins in the cell. However, our results confirmed that HEPIS and BTF3 were able to interact when expressed *in vivo.*

HEPIS binds to the TATA sequence

In the general model of transcriptional regulation in eukaryocytes, transcriptional repressors are thought to function through interaction with the RNA pol II complex and/or specific element(s) of the promoter region (6). However, some previous reports also suggested that a transcriptional repressor is able to inhibit transcription of a specific gene through binding to a certain upstream proximal sequence element of the promoter (3,6). Since HEPIS is capable of interacting with BTF3 physically, it was important to investigate the possible interaction of this protein with elements in the promoter region. Six sequences of specific DNA elements that are general enhancers or initiators distributed in eukaryotic promoters, including CAAT, Sp1, Ap1, OCTA, TATA, and ATF, were selected for a binding test. Interestingly, HEPIS protein showed weak but specific binding to the TATA sequence, and no interaction with other enhancer elements (Fig. 4). This result combined with the finding that HEPIS co-immunoprecipitated with BTF3 suggests that HEPIS may be involved in the transcriptional regulation of the RNA pol II complex.

Table 1. The Isolated Genes Coding Proteins Interacting with HEPIS from Yeast Trap

No.	*Gene*	*GenBank accession no.*
45	RNA pol II initiation complex	NM 000937
118	BTF3	NM 001207
134	Unknown protein 1	AC113385
199	Unknown protein 2	BX247988

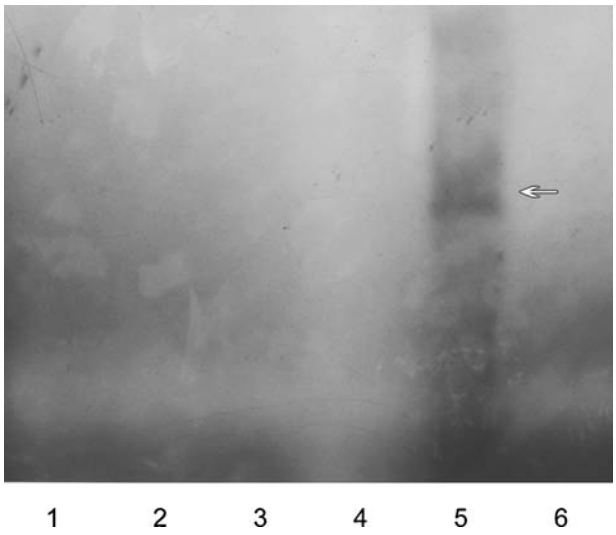

FIG. 4. Assay of HEPIS binding to different promoter/enhancer sequences. Purified HEPIS was examined for the ability to bind to DNA of different regulatory elements by EMSA. The oligonucleotides were labeled with ^{32}P at their 5′ ends and gel purified prior to EMSA. Binding reactions were performed as described in the materials and methods section. To separate the protein-DNA complexes, the reaction mixtures were loaded onto a running non-denaturating 5% polyacrylamide gel and electrophoresis was carried out. Gels were dried and band patterns were analyzed. The arrow indicates weak binding to the TATA sequence. Lanes 1–6 of each gel contain radiolabeled oligonucleotides of CAAT, Sp1, Ap1, OCTA, TATA, and ATF box, respectively.

HEPIS repressed transcription induced by various elements

To investigate further the possible activity of HEPIS in transcriptional regulation, a chloramphenicol acetyltransferase (CAT) reporter system was used for an *in vitro* transcription assay. Transcription induced by different upstream proximal sequence elements, including a unique TATA element, was inhibited to different extents by HEPIS (Fig. 5). Combined with the previous results of the binding assay and immunoprecipitation, this finding suggested that HEPIS impacted the rate of transcription by interacting with a component of the transcription initiation complex and TATA element.

The biological role of HEPIS as a transcriptional repressor

To investigate the biological role of HEPIS *in vivo*, we generated a cell line to stably express the protein. HeLa cells transfected with pcDNA-hepis were grown in selection media containing G418, and clones stably expressing the protein were selected by Western blotting with the anti-HEPIS antibody (Fig. 6A). Growth of these cells was compared with control cells using the proliferation assay and soft agar assay. The results showed that the growth rate and colony-forming ability of the HEPIS-expressing HeLa cells were lower than those of control cells (Fig. 6B and 6C). A Northern blot of RNA from human tumor tissues further indicated that HEPIS was expressed at a higher level in ovary, lung, and stomach, and especially in uterus tumor tissues, and at a lower level in kidney and rectum tumor tissues (Fig. 6D). Compared with normal tissues, this result suggests that expression of HEPIS in some tumor tissues is strongly enhanced and is perhaps related to the development of these tumors. On the other hand, as a transcriptional repressor potentially capable of decreasing the proliferation of tumor cells, HEPIS could impact the growth regulation of normal cells if its expression is inhibited in some way. Therefore, we reduced the expression of HEPIS in human fibroblast cells by RNAi to investigate whether the lack of this protein could upregulate the proliferation of cells. However, proliferation of the cells in which the expression of HEPIS was inhibited by a specific RNA molecule was not significantly different from the control. This result suggests that HEPIS does not function as a primary regulator of cellular proliferation in human fibroblasts.

DISCUSSION

HEPIS was originally observed and studied by virtue of its interaction with nsp-10 of SARS-CoV, a putative

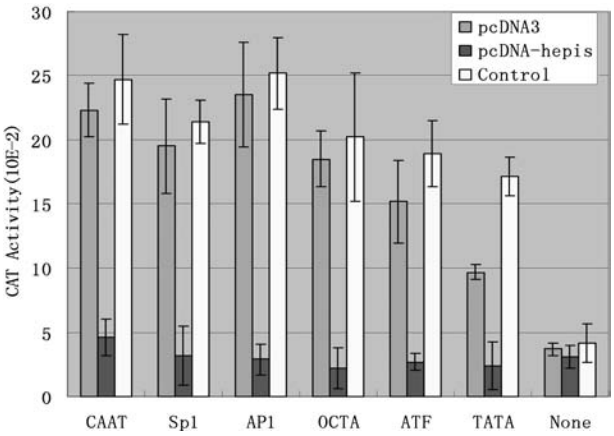

FIG. 5. Transcriptional repression by HEPIS of different cellular proximal sequence elements in CHO cells. The pCAT-CAAT, pCAT-Sp1, pCAT-Ap1, pCAT-OCTA, pCAT-ATF, pCAT-TATA, and pCAT-none reporter plasmids were constructed with pCAT-Basic and gene specific sequences. The plasmid pcDNA-hepis was co-transfected with the above reporter plasmids into CHO cells, while cells co-transfected with the empty vector pcDNA3 and untransfected cells were used as controls.

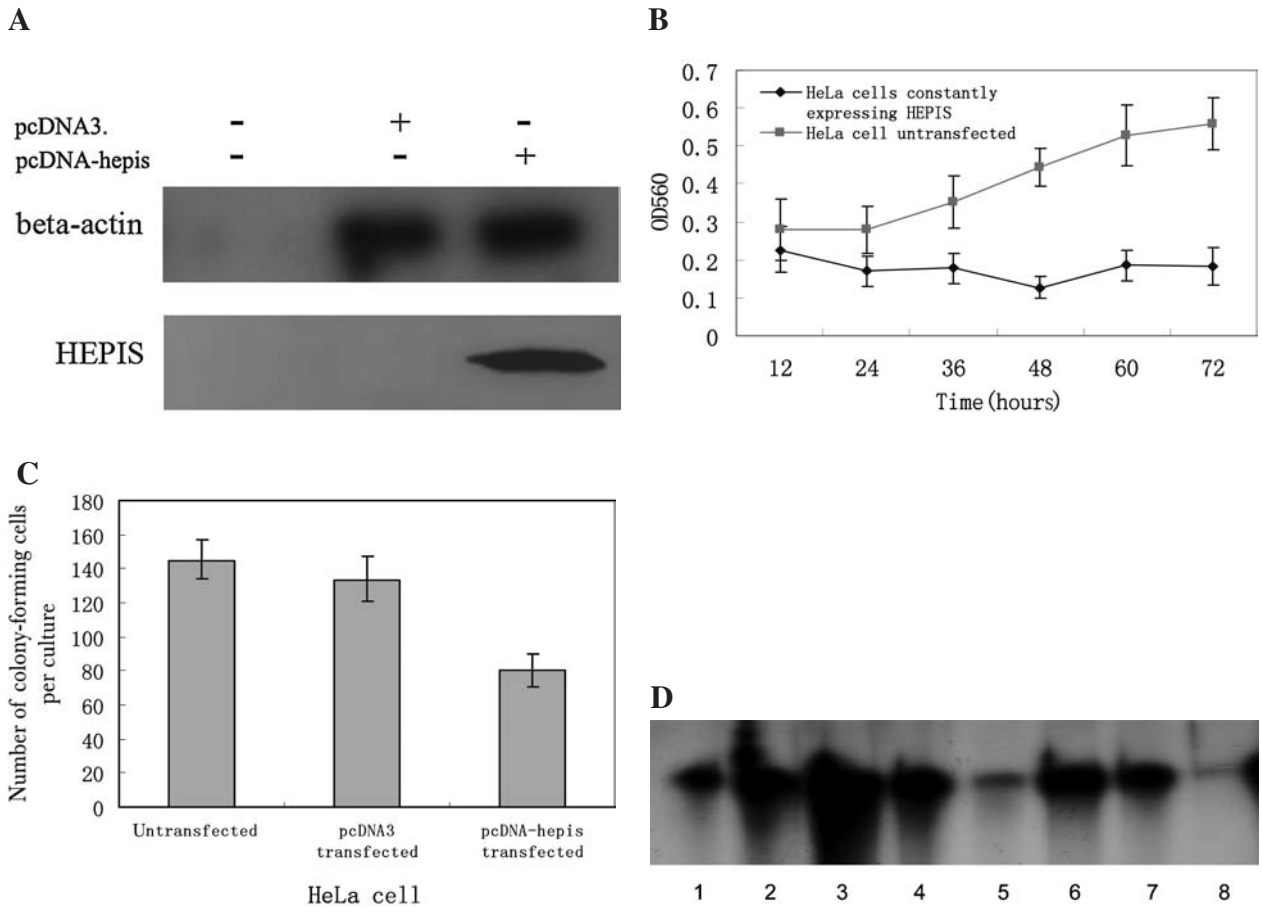

FIG. 6. HEPIS acts as a transcriptional repressor in cells. (**A**) Western blot assay of HeLa cells stably expressing HEPIS. The cellular extract of HeLa cells stably expressing HEPIS was transferred to nitrocellulose membranes and the protein was detected with an anti-HEPIS antibody in a Western blot. Lane 1: negative control with normal mouse IgG; lane 2: cellular extract of HeLa cells transfected with pcDNA3 and detected by anti-HEPIS antibody; lane 3: cellular extract of HeLa cells transfected with pcDNA-hepis and detected by anti-HEPIS antibody. β-Actin was detected as a control with anti-actin antibody, and is shown in the upper panel. (**B**) Cell proliferation analysis in HeLa cells stably expressing HEPIS. Cell growth rate of HEPIS-stable cells was measured with the MTT assay and compared to untransfected HeLa cells as a negative control. Monitoring for proliferative potential at 12, 24, 36, 48, 60, and 72 h was performed in triplicate and analyzed statistically. (**C**) Colony-forming assay of HeLa cells stably expressing HEPIS. HEPIS-stable cells and relative controls were seeded in soft agar medium and representative colonies were observed by microscopy (100× magnification). Colonies were counted after 2 weeks and the number of colony-forming cells per culture was calculated. Means and standard errors from two independent experiments each containing three replicates are shown. (**D**) HEPIS gene expression in different tumor tissues. Pre-made membranes (Human Tumor MTN Blot; BD Biosciences) containing 10 μg of total RNA from tumor tissues per lane were detected by Northern blotting. The HEPIS transcript was detected using a specific probe (a fragment of 60 nt between positions 241 $\rightarrow$ 300 in its cDNA). β-Actin mRNA was detected as a normalizing control with a 491-nt probe. Lanes 1–8: breast tumor, ovary tumor, uterus tumor, lung tumor, kidney tumor, stomach tumor, colon tumor, and rectum tumor.

viral transcriptase, and with proteins found by yeast trap analysis. In particular BTF3, a component of the transcription initiation complex, was identified as the target molecule of HEPIS by a β-galactosidase assay and co-immunoprecipitation experiment *in vivo,* suggesting that HEPIS may be one of the molecules involved in the transcriptional regulation of specific genes required for certain cellular functions. The fact that the HEPIS transcript was produced in certain normal tissues, but induced at a higher level in some tumor tissues, as shown by Northern blot assays supports this notion. Our attempts to confirm HEPIS protein expression in normal and tumor tissues by immunoprecipitation or Western blot failed, probably due to low protein expression levels. However, based on the observations in our study of HEPIS gene expression, it is possible that HEPIS is specifically reg-

ulated and may yet play a significant role in cell proliferation.

Previously reported data showed that the expression of transcriptional regulators such is p53 and pRb are usually controlled by changes in the cellular physiological environment (2). These transcriptional regulators are capable of acting positively or negatively on the transcriptional regulation of cellular genes by interacting with other regulators or DNA elements (11). The interaction of HEPIS with BTF3 by yeast trap analysis and the HEPIS expression in tumor tissues further confirms that HEPIS might be involved in cellular transcriptional regulation. Our transcription assay indicated that HEPIS possesses the ability to suppress the rate of CAT gene expression, which was controlled by different enhancers with either an HSP70 TATA initiator or only a TATA element. Because HEPIS showed no interaction with various enhancer elements except the HSP70 TATA sequence in the binding assay and co-immunoprecipitated with BTF3, it is reasonable to conclude that HEPIS functions in transcriptional repression by interacting with both BTF3 and the TATA element. However, this activity of HEPIS may be regulated by protein phosphorylation. An *in vivo* phosphorylation test in cells transfected with the HEPIS expression vector indicated that HEPIS was phosphorylated, and a structural analysis suggested that it is a potential substrate of CK II (data not shown). However, the detailed mechanism of this phosphorylation in HEPIS and the nature of its interaction with BTF3 are still unclear.

The investigation of the biological role of HEPIS *in vivo* provided strong evidence to support our hypothesis that HEPIS may be a new transcriptional repressor. As a tumor cell line, HeLa cells show a higher proliferation rate than normal cells in media, and a higher rate of colony formation in soft agar. The proliferation and colony-formation rates of transfected HeLa cells expressing HEPIS decreased in comparison with control cells. These observations indicated that HEPIS expressed *in vivo* was capable of suppressing the proliferation of tumor cells.

CONCLUSION

In summary, the work described in this paper demonstrates that HEPIS is a potentially new transcriptional repressor. Although characterization of its biochemical interaction with other molecules in cells is still somewhat unclear, its biological role in transcriptional repression implies that it may be a significant factor in anti-oncogenesis and other cellular biological processes.

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