Expression profile and promoter analysis of HEPIS

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Abstract. Human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10 (HEPIS) is a novel transcriptional repressor, the expression profile and promoter activity of which have not been well studied. In the present study, *in situ* hybridization of RNA was used to study differential *HEPIS* expression levels in different types of cancer and normal tissues. A total of six truncated lengths of the HEPIS promoter regulatory sequences were cloned into the pGL3-basic vector, and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and dual luciferase reporter assays were performed. The results of RT-qPCR demonstrated that HEPIS expression levels differed across four breast cancer cell lines. The results of the dual luciferase reporter assays revealed that the activities of the reporter gene fragments spanning -1334/+373, -1203/+373, -1060/+373 and -899/+373 bp were higher compared with the reporter gene fragments spanning -759/+373 and -279/+373 bp. A search of the transcription factor database TRANSFAC identified numerous octamer transcription factor-1 (OCT-1), nuclear factor (NF)-κB and C-JUN transcription factor binding sites located on the HEPIS promoter (pHEPIS). Furthermore, the results revealed that mutations of the OCT-1 (-1236/-1223 bp), NF-κB (-1186/-1176 bp) and C-JUN (-856/-846 bp) sites on the human pHEPIS resulted in a decrease in luciferase activity. A chromatin immunoprecipitation assay revealed that OCT-1, NF-κB and C-JUN bound to pHEPIS in a site-dependent manner at the basal state. The TRANSFAC database was used to analyze the pHEPIS of multiple species and several activator protein-1, NF-κB and OCT-1 transcription factor binding sites were predicted. In conclusion, the results of the present study suggest that *HEPIS* is expressed at different levels in multiple organs and breast cancer cell lines. Furthermore, these findings indicate that OCT-1, NF-κB and C-JUN transcription factors are associated with transcriptional regulation of the *HEPIS* gene.

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Introduction

The human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10 (SARS-CoV nsp-10; HEPIS) gene is a novel gene that was initially discovered by Hong *et al* (1) in 2008 from a cDNA library of human embryo lung tissues. The HEPIS protein is able to interact with SARS-CoV nsp-10 (1). SARS-CoV nsp-10 is produced by the coronavirus main protease, which cleaves polyproteins pp1a-pp1ab during infection; this protein is able to function as a viral transcriptase (2). The HEPIS protein consists of 147 amino acids and has several casein kinase II phosphorylation sites (1). In a previous study, HEPIS was demonstrated to interact specifically with the TATA sequence of the heat shock protein 70 promoter, suggesting that HEPIS may be associated with gene transcriptional regulation (1). However, the expression profile and promoter activity of HEPIS are yet to be elucidated.

Changes in the expression of specific gene products are regulated by a wide range of mechanisms, including transcriptional and translational regulation (3). Octamer transcription factor-1 (OCT-1), nuclear factor κB (NF-κB) and activator protein 1 (AP-1) are important transcription factors that serve roles in cancer cell proliferation, survival, transformation, invasion, metastasis, angiogenesis and chemotherapy/radiotherapy resistance (4). OCTs are a class of transcription factor that bind to the 'ATTTGCAT' sequence of the gene promoter (5). OCT-1 (also termed POU2F1) is a ubiquitously expressed transcription factor containing a POU domain with a homeobox subdomain (6). OCT-1 serves an important regulatory role in cellular transcription via binding to a specific promoter octamer sequence on the target genes (7). Furthermore, OCT-1 binds to cofactors that interact with the POU DNA-binding domain to either positively or negatively regulate a variety of genes (8). Previous studies have reported that OCT-1 affects the occurrence and development of several cancers, including breast cancer (9), LNCaP prostate cancer (10), esophageal squamous cell carcinoma (11) and colorectal cancer (12). NF-κB is a dimeric transcription factor that belongs to the Rel/NF-κB family and is formed by hetero- or homodimerization (13). NF-κB is known to serve a vital role in the regulation of inflammation, immunity, cell

proliferation and apoptosis (13-16). AP-1, which is a dimeric transcriptional activator composed of Jun, Fos, activating transcription factor and musculoaponeurotic fibrosarcoma protein subunits (17,18), serves important roles in the regulation of cellular proliferation, transformation, differentiation and apoptosis via binding to a common AP-1-binding site in the target gene promoter (19,20).

In the present study, *in situ* RNA hybridization and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were used to detect the *HEPIS* gene expression profile in several organ tissues and breast cancer cell lines. The promoter activity of the *HEPIS* gene was also investigated. The first step was to identify the core *HEPIS* promoter to enable subsequent determination of the important transcription factors. The promoter region and transcription factor binding sites of the *HEPIS* gene were predicted by bioinformatics analysis. The AP-1, NF-κB and OCT-1 binding sites of the *HEPIS* promoter region were identified using site-directed mutagenesis, dual luciferase reporter assays and chromatin immunoprecipitation (ChIP) assays, respectively.

Materials and methods

RNA in situ hybridization. A DNA microarray containing samples from 72 cases of tumor and normal tissue was obtained from Shaanxi Chaoying Biotechnology Co., Ltd. (cat. no. BCN721; Xian, China). The samples were from the following 12 organs: Esophagus, stomach, colon, rectum, liver, lung, kidney, breast, uterine cervix, ovary, prostate and pancreas; with 3 cores positive for cancer and 3 cores of adjacent normal tissue from each organ and one cancer tissue core and one adjacent normal tissue core per case. The following sense and antisense probes matching the *HEPIS* core responding sequence were used: Antisense, digoxigenin (DIG)-TCTGCCCATATGTCAGGATTGGAAATAATGGAT-3' and sense, DIG-ATCCATTATTTCCAATCCTGACATATGGGCAGA-3'. All probes were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Hybridization procedures were performed as previously described (21). Staining was scored using a 0-3+ scale. 0, no staining; 1+, 2+ and 3+ indicate increased intensity of the staining. Sub-regions excluding necrosis, macrophages and infiltrated neutrophils and lymphocytes were selected and scored. The intensity score for an array spot is the mean of all its sub-regions.

Cell culture. MDA-MB-231, MCF-7, T-47D, ZR-75-30 and 293T cells (China Center for Type Culture Collection, Wuhan, China) were maintained in high-glucose Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) and incubated at 37˚C with 5% CO_2. 293T cells were seeded at a density of $15x10^4$ cells/well in 6-well plates for quantitative ChIP assays. 293T cells were seeded at a density of $5x10^4$ cells/well in 24-well plates for luciferase assays.

RT-qPCR. Total RNA was extracted from MDA-MB-231, MCF-7, T-47D and ZR-75-30 breast cancer cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The extracted total RNA (0.5 μg per sample) was then used to synthesize first-strand cDNA using a GoScript™ Reverse Transcription System kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. The primers used for PCR were as follows: HEPIS, forward, 5'-ATGTGG CTCAGTTTGTCCTC-3' and reverse, 5'-AGCAAGATT TCCTCCAGGTC-3'; GAPDH, forward, 5'-TGACTTCAA CAGCGACACCCA-3' and reverse, 5'-CACCCTGTTGCT GTAGCCAAA-3'. GAPDH was used as an internal control. qPCR was performed using a SYBR Master Mixture (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol using the following cycling conditions: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. The expression of HEPIS was analyzed as previously described (22).

Plasmid construction. The promoter sequence of the *HEPIS* gene (pHEPIS) was obtained by PCR from MCF-7 cell genomic DNA using the following primers: pHEPIS-F1.7k (-1334), forward 5'-ATCCTCGAGCATCACAAGTAGGGC AGCAT-3'; pHEPIS-F1.6k (-1060), forward 5'-ATCCTCGAG GAGTCTTCAAAGGGAGTG-3'; pHEPIS-F1.4k (-1203), forward 5'-ATCCTCGAGTCCTGGTATGCCAAGAAA-3'; pHEPIS-F1.3k (-899), forward 5'-ATCCTCGAGCAAGCT GATAGCCACCAA-3'; pHEPIS-F1.1k (-759), forward 5'-ATC CTCGAGAGGTTGGCAGGCCGGATAT-3'; pHEPIS-F0.6k (-279), forward 5'-ATCCTCGAGCGAAGAGGAGGGAGG TAG-3'; pHEPIS-R (+373), reverse 5'-AGTAAGCTTACTTCG CACCTTCGGCTA-3'. PCR was performed using pyrobest DNA polymerase (Takara Biotechnology Co., Ltd.). The PCR amplification reaction system conditions and PCR products were purified as previously described (23). Purified products were cloned into the *Xho*I (CTCGAG) and *Hind*III (AAGCTT) restriction enzyme sites of the pGL3-basic vector (Promega Corporation) using T4 DNA ligase (Sangon Biotech Co., Ltd.) according to the manufacturer's protocol.

In the present study, the transcription factor database TRANSFAC (www.cbrc.jp/research/db/TFSEARCH.html) was used for the search, and several AP-1, NF-κB and OCT-1 transcription factor-binding sites were predicted within the *HEPIS* promoter region. Site-directed mutageneses of the OCT-1 (-1236/-1223, negative numbers indicate that it is upstream of the transcription initiation site), NF-κB (-1186/-1176) and C-JUN (-856/-846) binding sites in the HEPIS promoter were performed using a Quick Change Site-Directed Mutagenesis kit (Stratagene; Agilent Technologies, Inc., Santa Clara, CA, USA) according to manufacturer's protocol, using the following primers: pHEPIS-OCT-1-M, forward 5'-TTATAGGTGT CAAATTCATCATCACCATCAAAACTGCGTGCTTCTG CACTGAAACA-3' and reverse 5'-TGTTTCAGTGCAGAA GCACGCAGTTTTGATGGTGATGATGAATTTGACACC TATAA-3'; pHEPIS-NF-κB-M, forward 5'-GAGTCTTCA AAGGGAGTGGAATTACCTGGATCTTCTGTTG-3' and reverse 5'-CAACAGAAGATCCAGGTAATTCCAACTCCC TTTGAAGACTC-3'; pHEPIS-C-JUN-M, forward 5'-AAT AACAAATTCATCATTGTTAGTTTGTAGCAGGATTGC ACTGGAGACAGAGATTCC-3' and reverse 5'-GGAATC TCTGTCTCCAGTGCAATCCTGCTACAAACTAACAAT GATGAATTTGTTATT-3'. Underlined base pairs indicate mutation sites.

Transfection and dual luciferase reporter assay. 293T cells were cotransfected with 1 μg pGL3-basic vector, pHEPIS-1.7K, pHEPIS-1.6K, pHEPIS-1.4K, pHEPIS-1.3K, pHEPIS-1.1K, pHEPIS-0.6K, pHEPIS-1.7K-M-OCT-1, pHEPIS-1.6K-M-NF-κB, pHEPIS-1.3k-M-C-JUN or pHEPIS-1.7K-3M and 0.2 μg pRL-TK (Promega Corporation) plasmid DNA/well in 24-well plates using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The luciferase activity of the extracts was assessed 24 h following transfection using a Betascope analyzer Infinite M200, (Tecan Group Ltd., Männedorf, Switzerland) and analyzed as previously described (23). The pRL-TK plasmid containing the *Renilla* luciferase gene was used as an internal control.

ChIP assays. ChIP assays were performed according to the manufacturer's protocol using a Millipore ChIP assay kit (EMD Millipore, Billerica, MA, USA). The following primary antibodies were used: Rabbit polyclonal antibodies against NF-κB p65 (cat. no. ab7970, 1:200), OCT-1 (cat. no. ab66132, 1:200; both Abcam, Cambridge, UK) and C-JUN (cat. no. sc-1694, 1:100), and anti-rabbit normal immunoglobulin G (cat. no. sc-2345, 1:100; both Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was used as an negative control. The above antibodies were used per chromatin sample and rotated overnight at 4˚C. Protein A/G Agarose/Salmon Sperm DNA Secondary antibody (1:400; cat. nos. 16-157 and 16-201; EMD Millipore) was added per sample for 1 h at 4˚C with rotation. The amount of each specific DNA fragment in the immunoprecipitates was determined using PCR reactions with the following primers: OCT-1, forward 5'-ATGTAATCCAGTAGCCTGTC-3' and reverse 5'-CTCCCTTTGAAGACTCTGA-3'; NF-κB, forward 5'-TTCAGAGTCTTCAAAGGGAG-3' and reverse 5'-GCA TACCAGGAGACAATAAAC-3'; C-JUN, forward 5'-GCC ACCAACAATAACAAA-3' and reverse 5'-AGGAGGACA TTCACTTGC-3'. The PCR was performed using a PCR Master Mix (Sangon Biotech, Shanghai, China) according to the manufacturer's protocol using the following cycling conditions: 95˚C for 30 sec, followed by 30 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 10 sec; 72˚C for 5 min.

Statistical analysis. Statistical analysis was performed using SPSS 9.0 software (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation. Student's t-test and one-way analysis of variance followed by a Dunnett's test were used to analyze data. P<0.05 was considered to indicate a statistically significant difference.

Results

HEPIS expression profile in tissues and breast cancer cells. The *HEPIS* expression profile was detected by RNA *in situ* hybridization in a tissue microarray. *HEPIS* expression in esophageal squamous cell carcinoma and rectal adenocarcinoma tissues was the opposite of that in normal esophageal and rectal tissues (Table I; Fig. 1); *HEPIS* expression was positive in esophageal squamous cell carcinoma and negative in normal esophageal tissue, whereas it was positive in normal rectal tissue and negative in rectal adenocarcinoma. *HEPIS* was positively expressed in tumor and normal tissues

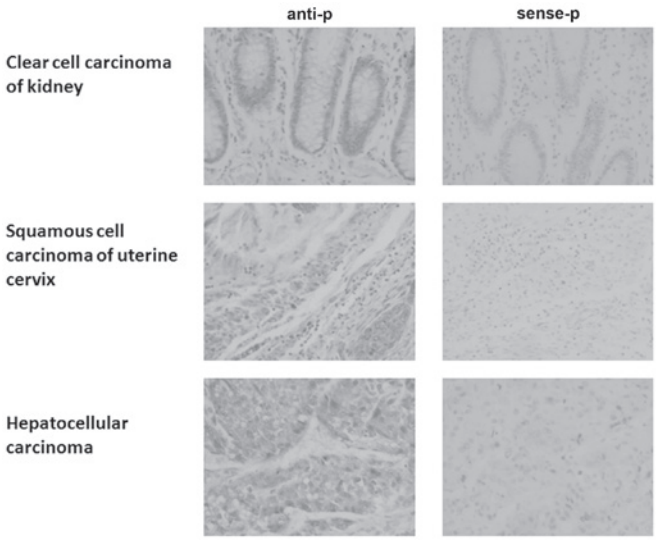

Figure 1. RNA *in situ* hybridization demonstrating HEPIS expression in clear cell carcinoma of the kidney, squamous cell carcinoma of the uterine cervix, and hepatocellular carcinoma. Magnification, x200. HEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10; anti-p, antisense probe used to detect HEPIS mRNA expression; sense-p, sense probe used as a negative control.

from the stomach, liver, colon, prostate, lung, uterine cervix and pancreas (Table I). The expression of *HEPIS* was positive in some tumor and normal tissues of the kidneys and ovaries and negative in others. *HEPIS* was positively expressed in nonspecific infiltrating duct carcinoma of the breast and partial positive expression was observed in normal breast tissue. *HEPIS* expression levels in four human breast cancer cell lines was examined using RT-qPCR (Fig. 2). The expression of *HEPIS* was significantly increased in the osteolytic breast cancer T-47D cell line compared with ZR-75-30, MDA-MB-231 and MCF-7 cells. *HEPIS* mRNA levels in T-47D cells were ~8-fold higher compared with MCF-7 cells (P<0.01), and in ZR-75-30, MDA-MB-231 cells were ~1.8-fold higher compared with MCF-7 cells (P<0.05). These results suggest that HEPIS is expressed at different levels in various organs and breast cancer cell lines.

Cloning and activity of the human HEPIS promoter. To understand the mechanism by which *HEPIS* gene transcripts are expressed, dual luciferase reporter assays were used to detect *HEPIS* promoter activity. A total of six different truncated lengths of the *HEPIS* promoter regulatory sequences were amplified and the PCR products were cloned into the pGL3-basic vector (Fig. 3A). Dual luciferase reporter assay analysis of the six recombined plasmids revealed that the -1334/+373, -1203/+373, -1060/+373, and -899/+373 bp reporter gene fragments exhibited higher activity levels compared with pGL3-basic (P<0.01); and the -759/+373 bp and -279/+373 bp reporter gene fragments exhibited higher activity levels compared with pGL3-basic (P<0.05; Fig. 3B).

Mutations at transcription factor binding sites and luciferase activity analysis. To investigate whether these putative response elements regulate the transcription of *HEPIS*,

Table I. HEPIS expression in multiple organ cancer and normal tissue.

Organ	Pathology diagnosis	Tissues/samples (n)	HEPIS mRNA-positive tumors, n (+/++/+++)	HEPIS mRNA-negative tumors, n
Esophagus	Squamous cell carcinoma	3	3 (0/3/0)	0
	Normal tissue	3	0	3
Stomach	Adenocarcinoma	3	3 (1/2/0)	0
	Normal tissue	3	3 (0/1/2)	0
Colon	Adenocarcinoma	3	3 (0/2/1)	0
	Normal tissue	3	3 (0/0/3)	0
Rectum	Adenocarcinoma	3	0	3
	Normal tissue	3	3 (0/3/0)	0
Liver	Hepatocellular carcinoma	3	3 (0/2/1)	0
	Normal tissue	3	3 (0/0/3)	0
Lung	Squamous cell carcinoma	3	3 (0/3/0)	0
	Normal tissue	3	3 (3/0/0)	0
Kidney	Clear cell carcinoma	3	2 (2/0/0)	1
	Normal tissue	3	1 (1/0/0)	2
Breast	Non-specific infiltrating duct carcinoma	3	3 (2/1/0)	0
	Normal tissue	3	2 (1/1/0)	1
Uterine cervix	Squamous cell carcinoma	3	3 (3/0/0)	0
	Normal tissue	3	3 (3/0/0)	0
Ovary	Serous cystadenocarcinoma	3	1 (1/0/0)	2
	Normal tissue	3	1 (1/0/0)	2
Prostate	Adenocarcinoma	3	3 (1/1/1)	0
	Normal tissue	3	3 (2/1/0)	0
Pancreas	Duct adenocarcinoma	3	3 (3/0/0)	0
	Normal tissue	3	3 (0/3/0)	0

HEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10.

the OCT-1 (5'-CTATTTGCTTCTG-3', -1236/-1223 bp), NF-κB (5'-GGAATCCCCT-3', -1186/-1176bp), and C-JUN (5'-TTGAGTCAGG-3', -856/-846bp) response elements on the human HEPIS promoter were mutated to generate pHEPIS-1.7K-M-OCT-1, pHEPIS-1.6K-M-NF-κB and pHEPIS-1.3K-M-C-JUN, which were constructed individually (Fig. 4A). The dual luciferase assay results demonstrated that the luciferase activities of pHEPIS-1.7K-M-OCT-1, pHEPIS-1.6K-M-NF-κB and pHEPIS-1.3k-M-C-JUN were significantly decreased compared with the activities of pHEPIS-1.7K, pHEPIS-1.6K and pHEPIS-1.3K, respectively (P<0.05; Fig. 4B), suggesting that C-JUN, OCT-1 and NF-κB activate the reporter. Furthermore, the OCT-1, NF-κB and C-JUN binding elements of the *HEPIS* promoter were simultaneously mutated to generate pHEPIS-1.7K-3M. When all three sites were mutated, the pHEPIS-1.7K-3M promoter activity was significantly decreased compared with the pHEPIS-1.7K (P<0.05; Fig. 4B); however, the level of suppression with the three mutations did not exceed the combined level of suppression by the individual point mutations, which suggests that the three mutations act jointly. Taken together, these results suggest that the OCT-1, NF-κB and C-JUN sites serve an important role in inhibiting the transcriptional activity of HEPIS.

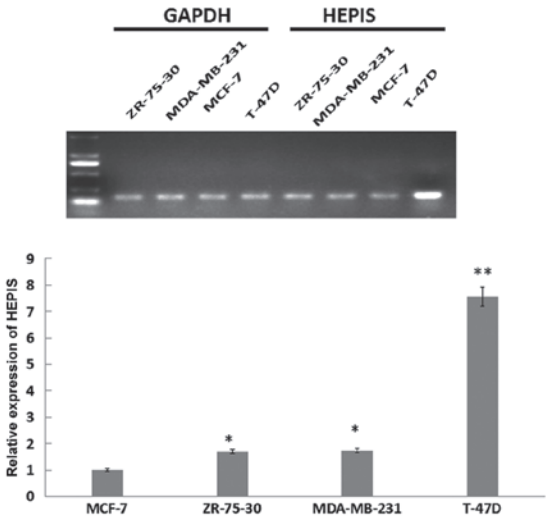

Figure 2. *HEPIS* mRNA levels in MDA-MB-231, MCF-7, T-47D and ZR-75-30 breast cancer cells. *P<0.05 and **P<0.01 vs. MCF-7. HEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10.

Identification of transcription factors in the HEPIS promoter. Identifying the transcription factor binding sites within

Table II. Analysis of *HEPIS* promoters in multiple species.

Species	Context of *HEPIS* promoter (-2.0k)	Transcriptional factor binding sites, n				
		OCT-1	NF-κB	AP-1	C-JUN	C-Fos
Rattus norvegicus (Norway rat)	Chr3: 91195981-91197981 [-]	11	0	6	2	0
Mus musculus (house mouse)	Chr2: 101629105-101631105 [-]	13	11	6	2	1
Homo Sapiens (human)	Chr11: 36592229-36594229 [+]	14	5	5	2	0
Bos Taurus (cattle)	Chr15: 67842229-67844229 [+]	17	3	9	4	1
Pan troglodytes (chimpanzee)	Chr11: 36583771-36585771 [+]	14	6	6	2	0
Canis lupus familiaris (dog)	Chr18: 31618122-31620122 [-]	15	3	7	4	3
Macaca mulatta (rhesus monkey)	Chr14: 29326253-29328253 [-]	14	4	5	2	0

HEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10; OCT-1, octamer-binding transcription factor 1; NF-κB, nuclear factor-κB; AP-1, activator protein 1.

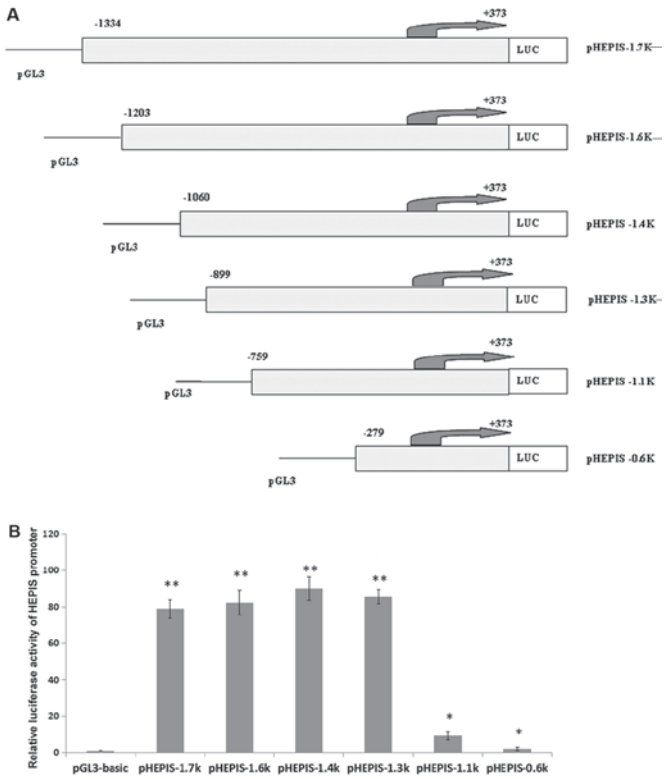

Figure 3. Cloning and activity of pHEPIS. (A) Six different truncated pHEPISs were cloned into a pGL3-basic LUC expression vector. These plasmids were designated as pHEPIS-1.7K, pHEPIS-1.6K, pHEPIS-1.4K, pHEPIS-1.3K, pHEPIS-1.1K and pHEPIS-0.6K. (B) Dual LUC activity assays of six pHEPIS constructs. Six recombinant vectors containing pHEPISs of different lengths and pRL-TK were cotransfected into 293T cells. *P<0.05, **P<0.01 vs. pGL3-basic. HEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10; pHEPIS, HEPIS promoter; LUC, luciferase.

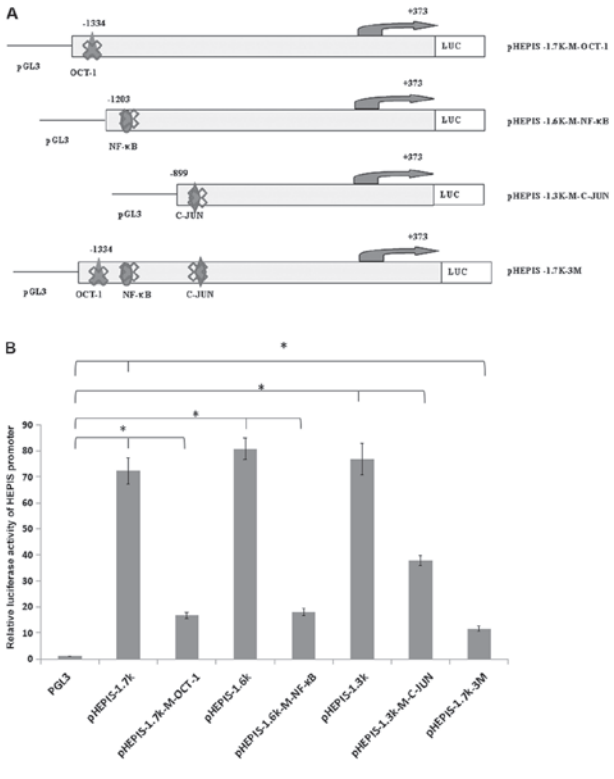

Figure 4. Mutation of transcription factor binding sites and LUC assay analysis of human pHEPIS. (A) The OCT-1, NF-κB and C-JUN elements were mutated individually on pHEPISs and designated as pHEPIS-1.7K-M-OCT-1, pHEPIS-1.6K-M-NF-κB and pHEPIS-1.3k-M-C-JUN. All three binding elements were mutated on the pHEPIS to generate pHEPIS-1.7K-3M. (B) Dual LUC activity assays of mutated pHEPIS constructs. A total of six recombinant vectors containing mutated pHEPIS fragments and pRL-TK were cotransfected into 293T cells. *P<0.05. LUC, luciferase; pHEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10 promoter; OCT-1, octamer-binding transcription factor 1; NF-κB, nuclear factor-κB.

the *HEPIS* promoter region is important for determining the mechanism of *HEPIS* gene transcription. To determine whether OCT-1, NF-κB and C-JUN were able to bind to the endogenous HEPIS promoter, a ChIP assay was performed to investigate transcription factor binding. The results indicated that OCT-1, NF-κB and C-JUN bind to the endogenous *HEPIS* promoter in 293T cells, which suggests that they may serve an important role in regulating *HEPIS* expression (Fig. 5).

Analysis of HEPIS promoters in multiple species. Table II lists the putative *HEPIS* promoter among various species with

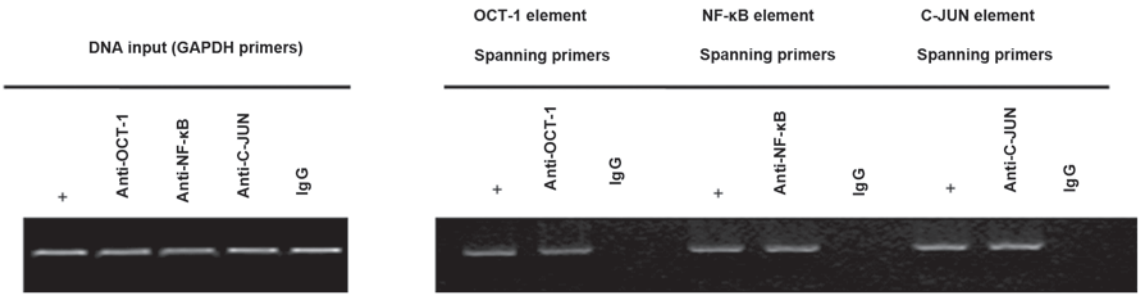

Figure 5. Binding of OCT-1, NF-κB and C-JUN to the endogenous pHEPIS promoter was analyzed using a chromatin immunoprecipitation assay in 293T cells. An amplified pHEPIS fragment with the OCT-1, NF-κB and C-JUN elements is presented. The amount of DNA in the input confirms equal loading of chromatin. '+' indicates the positive control in which the template of genomic DNA fragments from 293T cells was used. OCT-1, octamer-binding transcription factor 1; NF-κB, nuclear factor-κB; pHEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10 promoter.

the same sequence length. The *HEPIS* promoter is conserved among vertebrates (Fig. 6). The sequence of the *Homo sapiens* (human) *HEPIS* promoter shares the highest homology (100%) with that of *Pan troglodytes* (chimpanzees). The *HEPIS* promoters of *Rattus norvegicus* (Norway rats), *Mus musculus* (house mice), *Bos taurus* (cattle), chimpanzees, humans, *Canis lupus familiaris* (dogs) and *Macaca mulatta* (Rhesus monkeys) were analyzed using TRANSFAC and several AP-1, C-JUN, C-Fos, NF-κB and OCT-1 transcription factor binding sites were predicted within the promoter region (Table II).

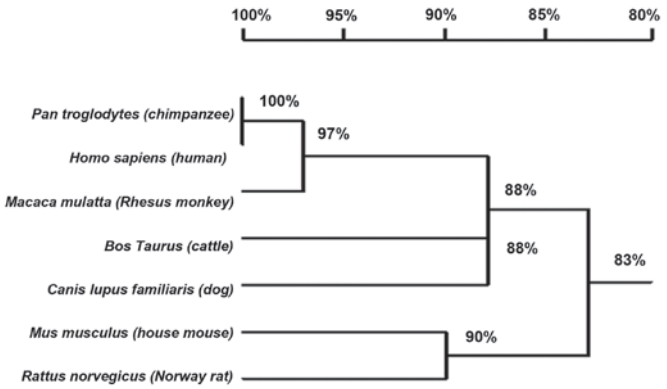

Figure 6. Homology tree of the HEPIS promoter (2.0kb upstream from the 5'-end of the HEPIS gene). HEPIS, human embryo lung cellular protein interacting with severe acute respiratory syndrome-coronavirus nonstructural protein-10.

Discussion

It has previously been reported that *HEPIS* is able to inhibit the proliferation of HeLa cells and may serve as an anti-oncoprotein (1). *HEPIS* is also able to inhibit the expression of the chloramphenicol acetyltransferase gene and may function as a factor of transcriptional repression (1). The aim of the present study was to determine the expression profile of the *HEPIS* gene and further elucidate the mechanism by which *HEPIS* transcriptional levels differ. RNA *in situ* hybridization (RISH) is a method of identifying the mRNA transcriptional expression pattern within the cytoplasm by hybridizing the sequence of interest to a labeled probe (24). Probes include radioactive probes and non-radioactive probes and RISH experiments performed with non-radioactive probes have several advantages over the radioactive procedures, including signal resolution, safety, shelf-life and cost (25). Due to the limited availability of the HEPIS antibody, the expression of the *HEPIS* gene in cancer and adjacent normal tissues in 12 organs was assessed using RNA *in situ* hybridization with a specific digoxigenin-labelled probe. However, due to the limited number of specimens available, it was necessary to further increase the number of specimens analyzed in order to obtain accurate results. HEPIS expression levels in four human breast cancer cell lines was examined using RT-qPCR, however, HEPIS expression in other types of cell lines remains unknown. Determining the differential expression of HEPIS allows analysis of its function in a variety of diseases.

Promoters control gene transcription. They may be located upstream of the gene transcription start site and can be very long (26). The binding of transcription factors to a promoter is an important mechanism by which gene expression is controlled (26). Investigating *HEPIS* promoter activity revealed that the luciferase activity varied between pHEPIS-1.3k and pHEPIS-1.1k, suggesting that transcriptional regulation occurs at the-899/-759 bp region of the promoter. Furthermore, the results suggest that mutations of C-JUN (TTGAGTCAGG, -856/-846 bp), OCT-1 (CTATTTGCTTCTG, -1236/-1223 bp) and NF-κB (GGAATCCCCT, -1186/-1176 bp) result in a marked reduction in luciferase activity, which indicated that C-JUN, OCT-1 and NF-κB are activators. However, no significant changes in luciferase activity were observed following truncation of the -1334/1203 bp and -1203/-1060 bp regions. These results suggest that the -1334/1203 bp and -1203/-1060 bp regions also contain repressor-binding sites. The findings of the present study indicate that the apparent changes in transcriptional activity of the *HEPIS* gene may result from complex interactions of different transcription factors with the promoter. The association of *HEPIS* gene and the above transcription factors maybe widespread and therefore, further study is required.

In the present study, sequence analysis identified numerous transcription factor-binding sites within the HEPIS promoter sequence. Of these, OCT-1 NF-κB and C-JUN were ubiquitously expressed; these have previously been reported to serve a variety of roles in the progression of numerous cancers (5,9,16,17,19,20). The results of the ChIP assay indicated that OCT-1, NF-κB and C-JUN are able to bind to the endogenous HEPIS promoter in 293T cells. Several AP-1,

C-JUN, C-Fos, NF-κB and OCT-1 transcription factor-binding sites were predicted within the putative *HEPIS* promoter in various species.

In conclusion, the results of this present research revealed that HEPIS has different expression levels in multiple types of cancer and normal tissues, and four breast cancer cell lines; and the OCT-1, NF-κB and C-JUN transcription factors are associated with transcriptional regulation of the *HEPIS* gene. These findings provide further insight into the expression profile and the mechanism of *HEPIS* gene transcriptional regulation.

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