The distribution and expression profiles of human Aspartyl/Asparaginyl beta-hydroxylase in tumor cell lines and human tissues

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Abstract. Human aspartyl beta-hydroxylase (HAAH) is a highly conserved enzyme that hydroxylates epidermal growth factor-like domains in transformation-associated proteins. Previous studies showed that the HAAH gene was over-expressed in many human malignancies. The present study investigates the distribution and expression profiles of HAAH in a variety of tumor cell lines and human tissues. One hundred and four cases of carcinomas were retrieved from the pathology archives of Department of Pathology, First Affiliated Hospital of Medical College of Xi'an Jiaotong University. Paraffin-embedded tissue specimens were immunostained with the HAAH monoclonal antibodies (MAb) generated to naked plasmid DNA containing N-terminal domain of the encoding HAAH gene and recombinant HAAH. Immunoreactivity was detected in 90.4% (94 positive cases) of carcinomas, including 88.5% of strong positive cases. In addition, HAAH mRNA levels were measured in seven tumor cell lines and in normal tissue. The results demonstrated that HAAH was highly expressed in the tumor cell lines in contrast to its low level of expression in normal tissue. The protein expression level of HAAH in the tumor cell lines by Western bolt analysis was comparable to the mRNA expression level. HAAH distribution in seven tumor cell lines was analyzed by immunofluorescence cell staining. The antigen was primarily localized in cytoplasm and plasmalemma. HAAH mAb exhibited high level binding to the four tumor cell lines (breast carcinoma MCF-7, hepatic carcinoma SMMC-7721, cervix cancer HeLa and ovary cancer SKOV) and lower degrees of binding were observed with the other three (renal adenocarcinoma ACHN, bladder cancer BIU-87 and laryngeal cancer Hep-2). In contrast, normal mouse embryonic osteoblasts MC3T3 exhibited no staining. In conclusion, HAAH is overexpressed frequently a variety of carcinomas, indicating that overexpression of the enzyme correlates with the development and progression of carcinomas and it could serve as a prognostic biomarker to predict the clinical course of some carcinomas.

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Introduction

Cancer is one of the most serious diseases in the world today, also occurring with high frequency in China. Early detection, diagnosis and treatment are important for improving the treatment of malignant tumors and prolonging the lives of patients. Although many tumors are detectable by ultrasonography, helical computed tomography, or magnetic resonance imaging, these approaches seldom reveal tumors that are <1 cm in diameter (1,2). Therefore, these methods are limited for detecting tumors at an early stage. Some molecular factors such as expression of genes, tumor proliferative indices, nuclear DNA ploidy, growth factors, and hormone receptors have been used to predict clinical outcome in patients with tumors (3,4). Specific and sensitive biomarkers for tumors would be used to detect preclinical disease, and to target therapy if the associated antigens are expressed on the cell surface.

The human aspartyl beta-hydroxylase (HAAH) is a protein belonging to the α-ketoglutarate-dependent dioxygenase family of prolyl and lysyl hydroxylases, which play a key role in collagen biosynthesis. This molecule hydroxylates aspartic acid or asparagine residues in certain EGF-like domains of several proteins in the presence of ferrous iron (5-10). These EGF-like domains contain conserved motifs that form repetitive sequences in diverse proteins, such as clotting factors, extracellular matrix proteins, low-density lipoprotein receptor, Notch homologues or Notch ligand homologues, which have demonstrated roles in cell motility or adhesion (5,6,8,11). Aspartyl beta-hydroxylase studies have shown that the gene is upregulated in a broad range of human malignancies such as breast, hepatic, biliary, colon, pulmonary, pancreatic and neural origin (12-16). Overexpression of the protein is closely related with tumor cell formation, proliferation, invasion, metastasis, differentiation, oncogenesis and cellular functions that propagate the malignant phenotype.

It is considered as a potential broad-spectrum tumor marker. However, the roles of the enzyme in these functions are still not well understood.

In this study, the expression and distribution of HAAH was examined in different tumor cell lines and tissues of Chinese patients using reverse transcriptase-polymerase chain reaction, Western blot and immunofluorescence cell staining. We also determined whether the enzyme could serve as a prognostic biomarker to predict behavior of the clinical course of some carcinomas.

Materials and methods

Cell lines and human tissues. The tumor cell lines of human renal adenocarcinoma ACHN, bladder cancer BIU-87, breast carcinoma MCF-7, hepatic carcinoma SMMC-7721, laryngeal cancer Hep-2, cervix cancer HeLa, ovary cancer SKOV and mouse embryonic osteoblasts MC3T3 were purchased from Wuhan Cell Institute of Chinese Academy of Sciences. They were maintained in MEM or RPMI-1640 cell culture mediums (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal calf serum heat-inactivated at 56˚C, 10 mM nonessential amino acids, 1,000 IU/ml of penicillin, and 100 mg/ml of streptomycin in a humidified 5% CO_2 atmosphere at 37˚C.

Samples of normal human tissues and tumor tissues paired with adjacent uninvolved tissues of cancer patients were obtained by surgical excision. One part of the tissues was snap-frozen in liquid nitrogen immediately and stored at -80˚C. The other part was fixed in 10% buffered formalin and embedded in paraffin. This study was approved by the institutional review board at the First Affiliated Hospital of Medical College of Xi'an Jiaotong University.

Extraction of mRNA and preparation of cDNA. Total RNA was respectively extracted from ~4x10^6 cells of the tumor and normal cell lines and then was used to establish and validate the RT-PCR assays. Serial dilutions of cDNA from target PCR products served as positive controls and for quantification in each PCR run. Total RNA was extracted using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA) in accordance with the manufacturer's standard protocol. cDNA was reverse-transcribed from 2 μg of the extracted RNA in a 20-μl reaction system containing the following: 2 μl of 5X reaction buffer, 1 μl of M-MLV reverse transcriptase (Promega), 1 μl of RNAsin (Toyobo), 2 μl of 0.1 M DTT, 1 μl of oligo-dT primers (50 μg/ml) (Promega), 6 μl of dNTP mix (10 mM) (Promega) and DEPC-treated water. The samples were heated to 70˚C for 5 min, followed by incubation at 42˚C for 1 h. Reactions were terminated by heating at 95˚C for 5 min. ß-actin RNA levels measured in parallel reactions were used to calculate relative abundance of each mRNA transcript.

RT-PCR. RT-PCR was performed in a 25-μl reaction mixture containing 2 μl of cDNA template, 20 μM of each primer, and 1U of Taq DNA Polymerase (AmpliTaq Gold; Roche Molecular Systems, Pleasanton, CA, USA), as follows: after one cycle at 95˚C for 5 min, 30 cycles at 95˚C for 30 sec, 56˚C for 30 sec, and 72˚C for 30 sec, followed by 72˚C for 10 min. The nucleotide sequences for the primer pairs are shown in Table I. Experiments were performed in triplicate and repeated twice. The PCR products were separated by electrophoresis on 1% agarose gels.

Table I. Specific primers for RT-PCR studies.

Primer	Sequence (5'→3')	Amplicon size (bp)
HAAH-F	GTTACCACGTGGAAGAGAC	160
HAAH-R	GCTTGTTCCTCATAGACTTG	
ß-actin-F	ATTGAACACGGTATTGTCACC	385
ß-actin-R	GTCAGGATCTTCATGAGGTAGTC	

Immunofluorescence cell staining. Cultured cells were grown on sterile glass slides overnight at 37˚C. They were washed briefly with PBS and fixed by the following procedures: 15 min in 4% paraformaldehyde in PBS, and washed 3 times with PBS. The fixed cells were then permeabilized for 30 min with 2% Triton X-100, blocked at 37˚C for 1 h with 1% BSA, and incubated for 2 h with anti-HAAH monoclonal antibody (generated by immunizing mice with naked plasmid DNA containing N-terminal domain of encoding HAAH gene and recombinant HAAH polypeptide) (17) in 0.2 ml of PBS-BSA. After washing thrice, fluorescein isothiocyanate-conjugated goat anti-mouse IgG (1:200) (Wuhan BoShiDe Biotechnology, Wuhan, China) and Hoechst 33258 were added to the cells and incubated at 37˚C for 1 h in dark. The cells were washed thrice again, and mounted with 50% glycerol/PBS mountant. The slides were then viewed under a fluorescent microscopy.

Western blotting. Western blotting was performed as previously described (18). In brief, 1x10^7 cells were washed once with ice-cold PBS and then detached by the addition of trypsin-EDTA solution. The cell suspensions were transferred into 1.5 ml tubes. After centrifugation (10 min at 1,500 x g), the supernatant was discarded, and the pellets were resuspended in 1 ml of sample loading buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% ß-mercaptoethanol, 0.1% bromophenol blue). Samples were electrophoresed on a 12.5% SDS-polyacrylamide gel (19) and then transferred onto a nitrocellulose membrane (Millipore Co., Billerica, MA, USA). The membranes were blocked and then incubated for 2 h with mouse anti-HAAH antibody or rabbit anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) antibody (Guangzhou LanBo Biotechnology, Guangzhou, China). After washing, the membrane was incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (1:2,000) or goat anti-rabbit IgG (1:2,000) (Wuhan BoShiDe Biotechnology). Development was performed with H_2O_2 and dianilinobenzene as the substrates. The signal was detected under a fluorescent microscopy.

Immunohistochemistry. MAb HAAH was preliminarily applied to evaluate and localize the expression of HAAH in 19 kinds of tumor tissues of 104 specimens and non-tumor tissues by immunohistochemical stain. Paraformaldehyde fixed (2%), paraffin-embedded tumor tissues, together with adjacent

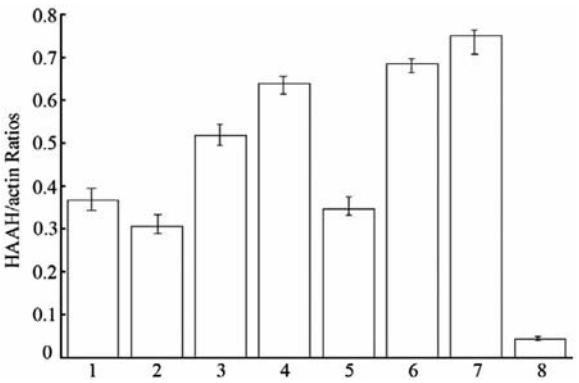

Figure 1. Mean ± SEM levels of HAAH mRNA in tumor cell lines and normal tissue are depicted. In addition, the mean levels of ß-actin measured in the paired samples are illustrated. 1, ACHN; 2, BIU-87; 3, MCF-7; 4, SMMC-7721; 5, Hep-2; 6, HeLa; 7, SKOV; 8, Normal tissue.

uninvolved tissues were studied. In addition, some normal human tissues were also fixed and paraffin embedded. The sections (8-mm) were deparaffinized in xylenes, rehydrated in graded alcohol solution, and equilibrated in PBS. After an overnight incubation at 4˚C with HAAH mAb (5 or 10 μg/ml), the sections were immunostained by the avidin-biotin horseradish peroxidase complex method, according to the manufacturer's protocol (Vector Laboratories, Inc., Burlingame, CA), and with 3-3'-diaminobenzidine (0.5 mg/ml in 0.03% hydrogen peroxide) as the chromagen. The sections were lightly counterstained with hematoxylin, dehydrated, cleared, and mounted with cover glass. We regarded cells as immunoreactive for HAAH when the signal was clearly observed in the cell surface membranes and/or cytoplasm. Equivocal stains were considered negative.

The intensity of HAAH staining was scored as negative (0), weak (+1), positive (+2), or strong positive (+3). The scores corresponding to overall distribution of HAAH immunoreactivity were averaged across the different tumor plugs per case. All sections were scored independently by 2 pathologists (K. Song and T. Huyan) in a blinded fashion, that is, without knowledge of the clinicopathologic features or clinical course.

Statistical analysis. All statistical analyses were performed using the SPSS software (SPSS, Chicago, IL). The differences between the HAAH mRNA or protein overpression in tumor and non-tumor cells or tissues were statistically analyzed using paired t-test or unpaired Student's t-test for single comparisons, and one-way analysis of variance (ANOVA) for multiple comparisons. P-values were set at the 0.05 level of significance.

Results

RT-PCR results of overexpression of Aspartyl beta-hydroxylase in tumor cell lines. HAAH mRNA levels were measured by RT-PCR, with values normalized to ß-actin quantified in the same samples. The relative HAAH and ß-actin gene expression levels in tumor cell lines and normal tissues were depicted in Fig. 1. The results demonstrated higher levels of HAAH mRNA in all 7 (100%) tumor cell lines relative to the normal tissue (Fig. 2). There are statistically significant differences between them ($P<0.01$). In contrast, the mean levels of ß-actin were similar in the tumor cell lines and the normal tissue. The mean level of HAAH mRNA in normal tissue was respectively 16.7-fold (SKOV), 15.3-fold (HeLa), 14.3-fold (SMMC-7721), 11.5-fold (MCF-7), 8.2-fold (ACHN), 7.74-fold (Hep-2) and 6.8-fold (BIU-87) lower compared with those in the tumor cell lines. These RT-PCR results correlated well with the findings by Western blot analysis using the HAAH monoclonal antibody.

Immunofluorescence cell stain analysis of localization of HAAH in tumorous cells. The HAAH mAb was initially

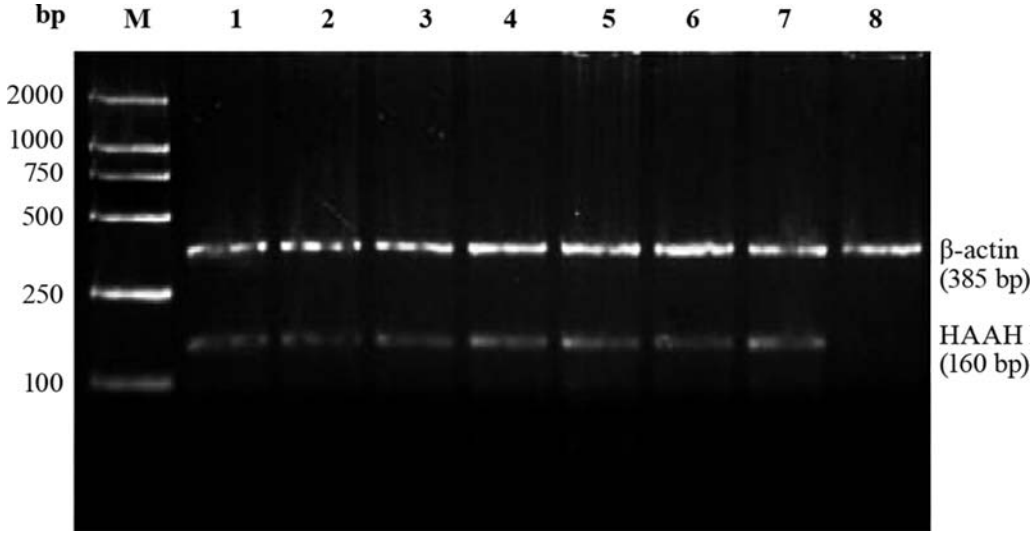

Figure 2. RT-PCR amplification of mRNA showed aspartyl beta-hydroxylase mRNA expression in seven tumor cell lines, normal tissue and mouse embryonic osteoblasts MC3T3 cell line. Marker, DNA ladder marker DL2000; 1, ACHN; 2, BIU-87; 3, MCF-7; 4, SMMC-7721; 5, Hep-2; 6, HeLa; 7, SKOV; 8, Normal tissue.

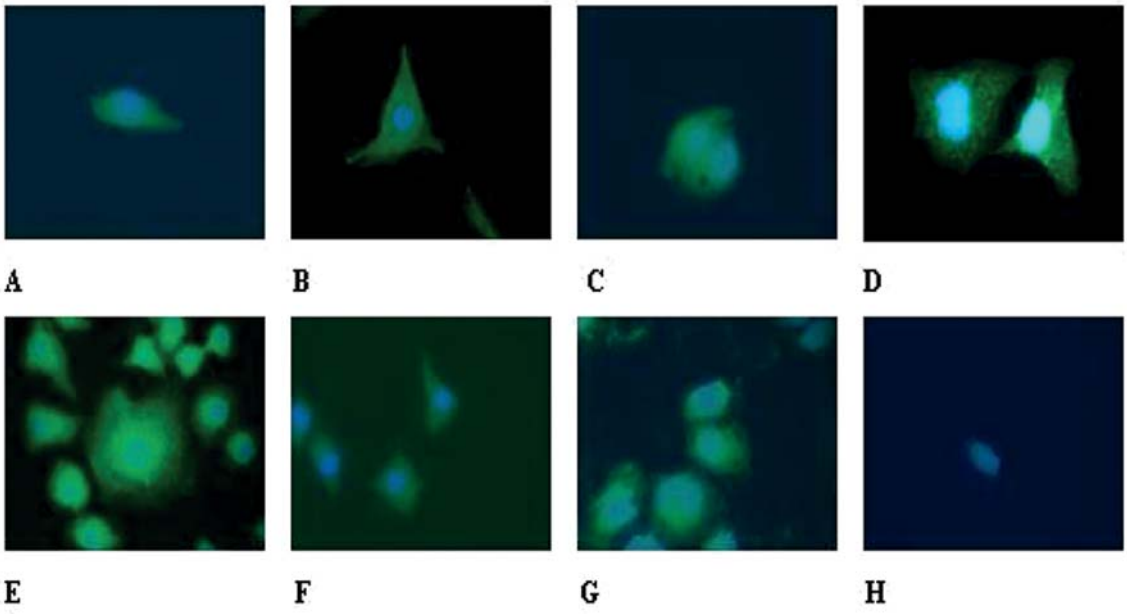

Figure 3. HAAH distribution in seven tumor cell lines detected by cell immunofluorescence staning. (A) ACHN; (B) BIU-87; (C) MCF-7; (D) SMMC-7721; (E) Hep-2; (F) HeLa; (G) SKOV; (H) MC3T3; (x100).

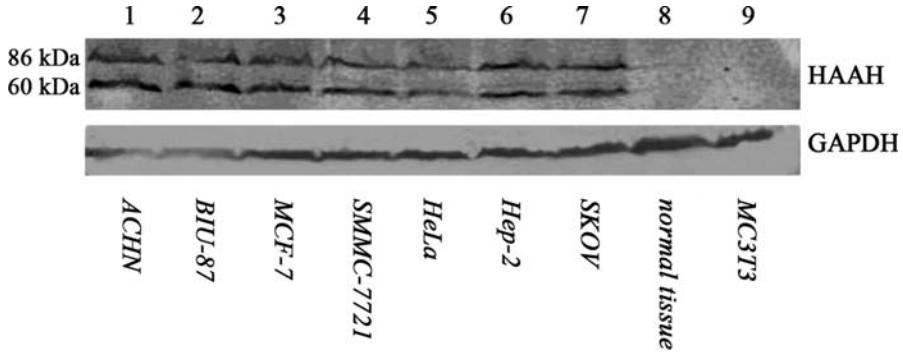

Figure 4. Western immunoblot analysis of HAAH extracted from tumor cell lines and the normal tissue specimens. Total proteins (~20 mg/ml) from extracts of each tumor cell line and the normal tissue were fractionated. The approximate increases of enzyme protein in the tumor cell lines compared to the normal tissue were: 5.6-fold (ACHN), 6.2-fold (BIU-87), 4.3-fold (MCF-7), 6-fold (SMMC-7721), 4.3-fold (Hep-2), 7.9-fold (HeLa), and 10.1-fold (SKOV).

generated by co-immunizing mice with naked plasmid DNA containing N-terminal domain of encoding HAAH gene and recombinant HAAH polypeptide, which was highly specific and sensitive used as the immunogen (17). Antigen distribution was assessed by determining the binding of HAAH mAb to a variety of tumor cell lines. As shown in Fig. 3, HAAH mAb exhibited high level binding to the four human tumor cell lines (MCF-7, SMMC-7721, HeLa and SKOV). Lower degrees of binding were observed with ACHN, BIU-87 and Hep-2 cell lines. In contrast, normal mouse embryonic osteoblasts MC3T3 were unreactive.

The staining pattern for HAAH in the tumor cells was primarily cytoplasmic with a distinct perinuclear accentuation (Fig. 3) and plasmalemmal, while the normal mouse embryonic osteoblasts exhibited no staining.

Elevated levels of hydroxylase protein in tumor cell lines. When Western blot was carried out using the HAAH mAb, the levels of HAAH in 7 (100%) tumor cells were much greater than in the normal tissue ($P<0.01$) (Fig. 4). Equivalent amounts of total protein from the normal tissue or tumor cell lines were prepared for the assay. The molecular weights of the major two species in the tumor cells were respectively ~86 kD and ~60 kD, and only very weak bands of the proteins were observed in the normal tissue. The levels of HAAH in the tumor cells were respectively 10.1-fold (SKOV), 7.9-fold (HeLa), 6.2-fold (BIU-87), 6-fold (SMMC-7721), 5.6-fold (ACHN), 4.3-fold (MCF-7) and 4.3-fold (Hep-2) greater than its levels in the normal tissue and mouse embryonic osteoblasts (Fig. 5). Estimates were made by scanning densitometry after normalizing for the internal controls. These results were quite comparable to those previously detected by RT-PCR; thus, the marked increases seen with the tumor cell lines represented true increments over what was observed in the normal tissue.

Immunohistochemistry validation studies. The cellular localization of HAAH in human tumor tissues was confirmed

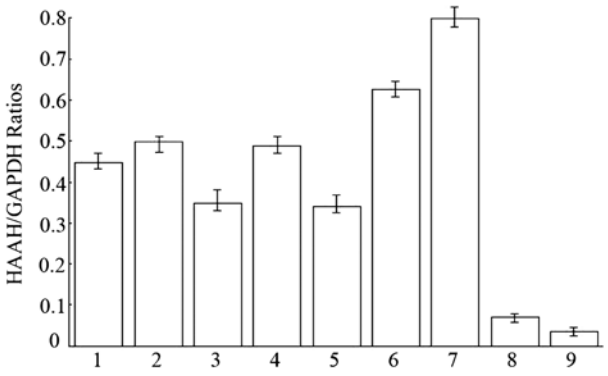

Figure 5. Mean ± SEM levels of the expressed HAAH in tumors and tumor-free adjacent tissues are depicted graphically. In addition, the mean levels of GAPDH measured in the paired samples are illustrated. 1, ACHN; 2, BIU-87; 3, MCF-7; 4, SMMC-7721; 5, Hep-2; 6, HeLa; 7, SKOV; 8, Normal tissue; 9, MC3T3; (x100).

Table II. Immunohistochemistry results.

	No.	Expression of HAAH			
		Negative (0)	Weak Positive (1+)	Strong Positive (2+/3+)	Positive ratio (%)
Normal tissue	8	8	0	0	0.0
Tumor tissue	104	10	2	92	90.4

Table III. HAAH expressed in different sources of tumor tissues.

Tumor tissue	No.	Negative	Positive	Positive ratio (%)
Liver cancer	16	0	16	100
Cholangiocarcinoma	11	0	11	100
Kidney cancer	14	1	13	92.8
Breast cancer	16	1	15	93.7
Cervical cancer	3	0	3	100
Ovarian cancer	3	1	2	66.7
Fallopian tube carcinoma	2	0	2	100
Laryngo carcinoma	4	1	3	75
Lung cancer	3	1	2	66.7
Thyroid cancer	4	1	3	75
Pancreatic	3	0	3	100
Thymic carcinoma	1	0	1	100
Prostate cancer	5	1	4	80
Bladder	4	1	3	75
Esophagus cancer	4	1	3	75
Gastric carcinoma	3	1	2	66.7
Gall bladder	2	0	2	100
colon cancer	3	0	3	100
Rectum carcinoma	1	0	1	100
Total	104	10	94	88.5

by immunohistochemical staining studies (Tables II and III). Immunoreactivity was detected on the cell surface membranes, in the cytoplasm, and in perinuclear zones (Fig. 6). Adjacent benign tissues were negative or weakly focal expressed. Of the 104 tumor samples, 94 (90.4%) exhibited aspartyl beta-hydroxylase immunoreactivity using the monoclonal antibody to HAAH.

Fifteen breast carcinomas exhibited high levels (2+ or 3+) of HAAH immunoreactivity, and only the remaining one had the staining pattern seen in the normal tissue. Distribution of antigen was heterogeneous among the cells in the tumors with high level immunoreactivity. All 16 hepatocellular carcinomas, 11 cholangiocarcinomas and 3 pancreatic cancers examined exhibited intense and uniform immunoreactivity (2+ or 3+) (Table III; Fig. 6). In the samples of lung carcinomas and colon carcinomas, HAAH immunoreactivity was localized throughout the cytoplasm, but in a distinct and prominent perinuclear pattern (figure not shown). Mucin-producing and well differentiated neoplastic foci within the specimens exhibited the most striking HAAH immunoreactivity. In contrast, nests, cords, and diffusely infiltrative neoplastic cells exhibited low level or absent HAAH immunoreactivity. The high level HAAH expression was a feature of both primary and metastatic lesions. All specimens exhibited immunoreactivity for glyceraldehyde-3-phosphaste dehydrogenase (positive control) and absent immunolabeling with the primary antibody omitted.

Discussion

Aspartyl beta-hydroxylase (AAH) is a member of the α-ketoglutarate-dependent dioxygenase family of molecules and catalyzes the hydroxylation of specific aspartyl and asparaginyl residues in epidermal growth factor (EGF)-like domains of certain proteins (5-10). The consensus sequence for AAH hydroxylation is present in proteins such as notch, the notch ligand, jagged, and extracellular matrix molecules such as laminin and tenascin, which have demonstrated roles in cell motility or adhesion (5,6,8,11). The DNA sequence encoding the full-length AAH contains 27 exons and it has a predicted molecular mass of ~86 kD. The AAH gene encodes 4 proteins: AAH, humbug, junctate, and junctin, which are generated by alternative splicing of an ~6-kb messenger RNA (mRNA) transcript, with exon sharing and exon swapping mainly within the first 13 exons (6,20). The AAH (~86 kD) and humbug (~60 kD) proteins are the main expressed products of the gene (21). In our study, the two kinds of proteins were detected in all 7 tumor cell lines.

The AAH genes have been documented to be over-expressed in a variety of malignant neoplasms and transformed cell lines, including those of colonic, breast, pancreatic, biliary, or hepatic origin (12-16). In contrast, most normal mature tissues have relatively low levels of AAH expression with the exception in proliferating trophoblastic cells of the placenta and in adrenal glands (12). AAH was initially concerned due to the research report of the Panacea Pharmaceutical Company

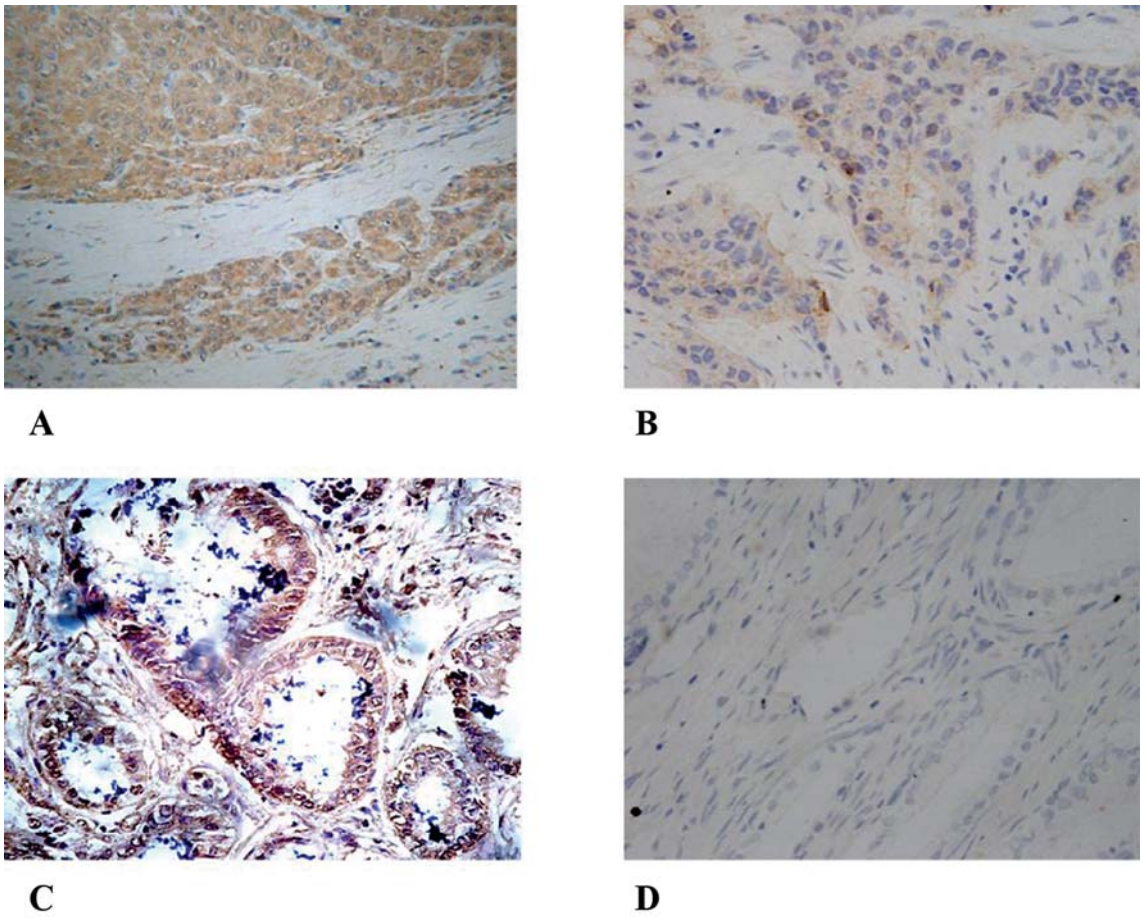

Figure 6. Representative examples of immunoreactivity. HAAH immunoreactivity in liver (A), breast (B), pancreatic (C) carcinomas, and in the normal tissue (D). Normal tissue was negative or weakly focal positive; high immunoreactivity was detected in the cytoplasm, and in membranes and perinuclear zones in carcinomas. No immunoreactivity was observed with the primary antibody omitted.

in the United States (22). The company detected 598 cases of 12 kinds of frequent tumors, and AAH was found to be overexpressed in 594 cases (99%). Lavaissiere *et al* (12) reported that AAH was expressed at high levels in primary hepatocellular carcinomas, cholangiocarcinomas, breast carcinomas, colon carcinomas and in a variety of transformed cell lines. Studies on AAH expression in Chinese patients with hepatocellular carcinoma (23) showed that AAH was overexpressed frequently in hepatocellular carcinoma, including early-stage small hepatocellular carcinoma, indicating that overexpression of AAH played a role in the development and progression of hepatocellular carcinoma. They suggested that it could possibly be used as a prognostic marker and an immunotarget for anti-tumor agents for hepatocellular carcinoma and other carcinomas (23). Other *in vitro* assays demonstrated the correlation between increased AAH expression and directional cell motility of neuroblastoma (16) and cholangiocarcinoma (24) cell lines. In addition, overexpressed AAH could be released from the tumorous cells and existed in soluble or free states in human serum and body fluids. The malignant tumors could be screened by titers of AAH in serum (25).

In our previous study, HAAH encoding gene has already been cloned, expressed and purified (17). HAAH-specific monoclonal antibody (MAb) was generated by co-immunizing mice with naked plasmid DNA containing N-terminal domain of encoding HAAH gene and recombinant HAAH polypeptide. The specificity and sensitivity of the obtained MAb was assessed, and it was preliminarily applied to detect the expression of HAAH in seven tumor tissues, including hepatocellular carcinoma, lung cancer, kidney cancer, cholangiocarcinoma, prostate cancer, breast cancer, and glioblastoma by immunohistochemical stain. The results showed that the MAb was highly specific and effective as the immunogen (17). Our present study indicates that HAAH mRNA was overexpressed in all seven (100%) tumor cell lines examined, although the levels of the gene expression varied from each other. These RT-PCR results correlated well with the findings by Western blotting using the HAAH monoclonal antibody. The molecular weights of the expressed major two species in the tumor cells were respectively ~86 kD and ~60 kD. It could be the result of alternative splicing of the mRNA (26,27). The level of HAAH in SKOV cell line was highest, ~10.1-fold over than in the normal tissue. Also in other tumor cell lines, the levels of the protein were respectively 7.9-fold (HeLa), 6.2-fold (BIU-87), 6-fold (SMMC-7721), 5.6-fold (ACHN), 4.3-fold (MCF-7) and 4.3-fold (Hep-2) greater than in normal tissue and mouse embryonic osteoblasts. The protein expression levels of HAAH in the tumor cell lines were

parallel with the mRNA expression level, indicating that the expression is regulated at the transcription level, and mRNA level can represent the protein expression level. Further investigation is required to determine whether the substantially increased expression of HAAH in tumors is merely associative or contributes to the development and/or maintenance of the malignant phenotype.

Antigen distribution was assessed by determining the binding of HAAH mAb to a variety of tumor cell lines. The staining of HAAH was found to mainly exit in the cytoplasm focused around the nucleus and the plasmalemma in the tumor cell lines, while the normal mouse embryonic osteoblasts exhibited no staining. It was suggested that all AAH-related proteins share common N-terminal exons (exons 2 and 3) that encode the transmembrane domain and a portion of the cytoplasmic domain (5,6,20). The distribution of HAAH in the tumor cell lines may be associated with the sequences of its N-terminus. The immunohistochemical staining studies demonstrated that HAAH was overexpressed in a significant proportion (90.4%) of 104 different sources of tumor tissue samples, while adjacent benign tissues were negative or weakly focal expressed. Among the 19 kinds of tumor specimens detected, 9 kinds showed 100% positive rates of immunoreactivity to HAAH MAb, and other 3 kinds presented the lowest positive rate of 66.7% (Table III). The results suggest highly correlation between the overexpression of HAAH and many human malignancies. However, the present studies were not sufficient to be concluded that HAAH was detected to be overexpressed at high positive rates in some tumor tissues or at low positive rates in others due to the limited quantities of tumor tissue samples detected. Further investigation is required to include many more tumor specimens and their stage, location, patient age, gender, and vital statistics.

In conclusion, HAAH gene is frequently overexpressed in varieties of carcinomas of Chinese patients, indicating that overexpression of the enzyme plays a role in the development and progression of these carcinomas. It could possibly be used as a prognostic marker and an immunotarget for anti-tumor agents for these carcinomas. This would be especially beneficial for patients with early stage of cancer for which the addition of adjuvant chemotherapy is debatable. Further investigation is required to define the roles of HAAH in the development and/or maintenance of tumors and the exact molecular pathways leading to carcinogenesis.

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