PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 June 21; 12(23): 3770-3775
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Purification and characterization of α-L-fucosidase from human primary hepatocarcinoma tissue

Chao Li, Jie Qian, Ju-Sheng Lin

Chao Li, Jie Qian, Ju-Sheng Lin, Institute of Liver Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
Supported by the National High Technology Research and Development Program of China (863 Program), No. 2002AA2Z2011
Correspondence to: Dr. Ju-Sheng Lin, Institute of Liver Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China. linjusheng2001@163.com
Telephone: +86-27-83662578 Fax: +86-27-83662578
Received: 2006-01-13 Accepted: 2006-02-20

Abstract

AIM: To purify and characterize α-L-fucosidase from human liver cancer tissue and to detect the localization of α-L-fucosidase in tumor tissue.

METHODS: Cation exchange chromatography on CM-52 and ultrafiltration were used to separate α-L-fucosidase (AFU) from crude extract of liver cancer tissue. 4-methylumbelliferyl-α-L-fucopyranoside was used as a fluorescent substrate to quantify the purified AFU activity in each step. A polyclonal antibody (pAb) against the purified AFU was obtained by anion exchange chromatography on DEAE-52 after ammonium sulfate fractionation and ultrafiltration. Immuohistochemical staining was used to observe the expression of AFU in malignant and adjacent liver tissues.

RESULTS: Human α-L-fucosidase was purified 74–fold to apparent homogeneity with 15% yield. SDS-PAGE indicated the presence of one subunit of molecular weight of 55 Ku. The specific activity of AFU in pooled fraction by chromatography was 10085 IU/mg. Western blot analysis indicated that the pAb could recognize one protein band of molecular weight of 55 Ku. The expression of AFU was observed in cytoplasm membrane of liver cancer tissue but not in that of adjacent tissue.

CONCLUSION: The purified α-L-fucosidase from primary hepatocarcinoma (PHC) is different in its properties from α-L-fucosidase in human other organs. The polyclonal antibody prepared in this experiment can be applied to the diagnosis of PHC.

Key words: α-L-fucosidase; Primary hepatocarcinoma; Polyclonal antibody

Li C, Qian J, Lin JS. Purification and characterization of α-L-fucosidase from human primary hepatocarcinoma tissue. *World J Gastroenterol* 2006; 12(23): 3770-3775

http://www.wjgnet.com/1007-9327/12/3770.asp

INTRODUCTION

α-L-fucosidase (AFU) is a glycosidase primarily found in lysosome and involved in the degradation of a variety of fucose-containing fucoglycoconjugates[1,2]. The necessity and importance of AFU (α-L-fucoside fucohydrolase, EC 3.2.1.51) are demonstrated by deficiency of its enzymatic activity leading to fatal neurovisceral storage disorder designated fuocosidosis characterized by mental and motor retardation [3]. The alterations of AFU catalytic activity in human cells, tissues and body fluids have a diagnostic value for human tumors such as primary hepatocarcinoma (PHC)[4-8], colorectal cancer[9-11], ovarian cancer[12].The deficiency of AFU activity in female sera is probably a hereditary condition related to higher risk of ovarian cancer[2,12]. The persistently elevated AFU level in sera of patients with liver cirrhosis contributes to early detection of PHC[5]. In addition, information of the structure and biochemical characteristics of AFU helps to understand the pathogenesis of fuocosidosis on molecular level and to discover therapeutic approaches for the disease[1,13]. In the present investigation, we described an improved purification procedure for AFU by ion exchange chromatography and immunochemical studies using polyclonal antibody against the homogeneous enzyme from human PHC tissue.

MATERIALS AND METHODS

Materials

Liver specimens were obtained from patients with PHC who underwent partial hepatectomy at Tongji Hospital. The procedure conformed to the local ethical guidelines. Informed consents were obtained from the patients or their relatives. The study protocol followed the ethical guidelines of the Helsinki Declaration of 1975. Rabbits used for antiserum production procedure received human care. All procedures were performed at 4℃ unless otherwise specified. Substrate 4–methylumbelliferyl-α–L–fucopyranoside (4-MU-Fuc) and standard product 4–methylumbelliferone (4-MU) were purchased from

Sigma. CM-52 and DEAE-52 cellulose resin were purchased from Whatman, USA.

Enzyme assay

AFU activity was assayed using 4-MU-Fuc as previously described with a minor modification[14-15]. Thirty μL aliquots of enzyme preparation was incubated at 37℃ for 30 min with 150 μL of 0.2 mol/L citrate buffer (pH 5.0) containing 60 nmol/L 4-MU-Fuc. The reaction was terminated by addition of 4 mL of 0.2 mol/L K_2CO_3 (pH 10.0). The fluorescent absorbance was read on a HITACHI F-4500 spectrophotometer with excitation at 365 nm and emission peak at 460 nm. Readings were corrected by subtracting tissue and substrate blanks. One unit of enzyme activity was defined as the amount generating 1 nmol of product 4-MU per min at 37℃. Protein concentration was determined by the traditional method of Bradford[16] using Coomassie brilliant blue G-250, following the standard assay and microassay with a protein concentration assay kit (Nanjing, China) using bovine serum albumin as standard protein. To quantify AFU activity in each purification step, we used a modified procedure to determine enzyme activity which assays the activity of β-galactose and generates a standard curve of known product concentration[17].

Extraction and concentration of enzymes

Crude extract of PHC tissue was prepared as previously described[18] with certain modifications. Portions of human liver from patients with PHC diagnosed by pathological examination were washed in distilled water and cut into small sections, stored in 30% glycerol at -70℃ until use. About 25 g of liver tissue (wet weight) was cut into small blocks within 0.05 mol/L cold sodium acetate pH 5.0 (10 mL per gram of tissue, wet weight). The blocks were then homogenized in a glass homogenizer, heated to 37℃ for 1 h to release the enzyme. The pH was then immediately adjusted to 5.0 with glacial acetic acid. The mixture was heated to 60℃ for 10 min before it was cooled to room temperature on ice and then centrifuged at 0℃ for 30 min at 35 000 r/min. The precipitate was discarded and supernatant pH was adjusted to 5.0 with 15 N NH_4OH. Solid ammonium sulfate was added to the supernatant to 35% saturation. The solution was allowed to stand at 4℃ for 4 h, and then centrifuged at 0℃ for 30 min at 29000 r/min. The precipitate was discarded, and the supernatant was saturated to 50% ammonium sulfate by addition of solid ammonium sulfate and allowed to stand at 4℃ for 20 h, then centrifuged at 0℃ for 30 min at 29000 r/min. The precipitate was dissolved in 4.0 mL of 0.1mol/L sodium citrate (pH 5.0), 0.02% in NaN_3 to yield 35%-50% ammonium sulfate fractions. The supernatant was saturated to 60% of ammonium sulfate by addition of solid ammonium sulfate and allowed to stand at 4℃ for 20 h, then centrifuged at 0℃ for 30 min at 29 000 r/min. The precipitate was dissolved in 2.0 mL of 0.1mol/L sodium citrate (pH 5.0), 0.02% in NaN_3 to yield 50%-60 % ammonium sulfate fractions. The 35%-50% and 50%-60% ammonium sulfate fractions were stored at -20℃ for chromatography.

Chromatographic methods

Further fractionations of the 35%-50% and 50%-60% ammonium sulfate fractions were obtained by chromatography on CM-cellulose (Whatman CM-52). To keep the stability of enzyme activity as possible the whole procedure was performed at 4℃. The chromatography column (2.5 cm × 35 cm) was prepared in 0.011 mol/L citric acid-NaOH (pH 4.5), 0.02 % in NaN_3 as previously described[18]. Details are given in Figure1.

Molecular weight determination of purified AFU

The presence of subunits was determined in 12 % polyacrylamide gels (8 cm × 7.3 cm, 0.75 mm) containing 0.1% SDS as previously described[19]. Briefly, 5% stacking and 12 % running gels were run at room temperature using 25 mmol/L Tris-HCl, 0.2 mol/L glycine buffer (pH 8.3) containing 0.1% SDS for 30-40 min at 80 V and 1-1.5 h at 100 V respectively. AFU samples (20-25 μg/lane) were subjected to electrophoresis after treated with 2% SDS, 5% 2-mercaptoethanol, 25% glycerol, 0.1% bromophenol blue in 60 mmol/L Tris-HCl (pH 6.8) in boiling water bath for 3 min. Prestained molecular mass standards (Fermentas, SM0671) were used and stained at the same condition. The gels were stained as previously described[20] with 0.25% Coomassie brilliant blue R-250 in glacial acetic acid: methanol: distilled water (10:45:45, v/v/v) for 3 h, then distained with the mixture of glacial acetic acid : methanol: distilled water (10:45:45, v/v/v) without Coomassie brilliant blue R-250 for 1 h, 20 min per time.

Immunization of animals

All procedures were performed as previously described[21] unless otherwise specified. Antiserum against AFU was produced by immunizing rabbits with 0.5 mg of purified AFU in equal volume of Freund's complete adjuvant, and 5 mL serum was collected before immunization as a normal control. The emulsion was injected subcutaneously at multiple sites on the rabbit metapodium palm. After 2 wk, the rabbits were injected with 0.5 mg purified AFU in Freund's incomplete adjuvant intramuscularly in each caudal thigh muscle. After 3 wk, the rabbits were given 0.2, 0.2, 0.4 mg of purified AFU respectively in a week at different sites on ear central artery for booster injection. A blood sample from ear central artery was collected for determination of the tilter after the second immunization. One week after the last booster injection, the rabbits were exsanguinated *via* cardiac puncture under general anaesthesia using diethyl ether.

Purification of α-L-fucosidase IgG

Blood was stood at room temperature for 1 h at 4℃ overnight, then centrifuged at 13000 r/min for 30 min at 4℃.The resulting pellet was discarded with the supernatant collected. The whole serum was precipitated with saturated ammonium sulfate to a final saturation of 33%, then desalted with Amicon Ultra-15 PLGC centrifugal filter unit (Millipore, NMWL, 10 KDa) (http://www.millipore.com/catalogue.nsf/docs/C7715).

Anion exchange chromatography

The desalted antiserum was added to anion exchange

Table 1 Purification of AFU from human PHC tissue

Fraction	Protein (mg)	AFU (units)	Specific activity (units/mg protein)	Purification fold	Yield (%)
Homogenate and incubate at 37℃	878.4	120024	137		
35%-50% $(NH_4)_2SO_4$ precipitation	126.55	95352	753	5	79
50%-60% $(NH_4)_2SO_4$ precipitation	46.55	16420	353	3	14
Ultrafiltration fraction					
35%-50% $(NH_4)_2SO_4$ precipitation	22	46676	2122	16	39
50%-60% $(NH_4)_2SO_4$ precipitation	5.6	6835	1221	9	6
CM-cellulose fraction	1.82	18354	10085	74	15

column (DEAE-52, Whatman) pre-balanced with 0.005mol/L balancing buffer, pH 8.6, Tris-PO_4 and stood at 4℃ for 30 min. Fractionations were eluted using 0.055 mol/L (pH 6.0) and 0.5 mol/L (pH5.1) Tris-PO_4 by a stepwise developing method, and pooled according to their protein content determined by absorbance of optical density at 280 nm. Because of instability of the purified antibody, chromatography should be carried out at 4℃. Pooled fractionations from DEAE-52 were adjusted to pH 6.4 with 10mol/L NaOH and stored at 4℃ to preserve their activity.

Western blot analysis

The proteins from slab gels were electrotransferred to 0.2 μm-pore-size nitrocellulose membrane (Schleicher and Schuell, Keene, NH) in 48 mmol/L Tirs/HCl transferring buffer containing 39 mmol/L glycine, 0.037% SDS, 20% methanol, at 4℃ and 350 mA for 70 min. A portion of nitrocellulose was stained as previously described[22] for 5 min with a working solution of 10-fold dilution of 2% ponceau S, 30% trichloracetic acid (TCA), 30% salicylsulfonic acid, solved in distilled water to 100 mL total volume, then destained vigorously in TBST with shaking until the ponceau S was washed off. The remainder of nitrocellulose was blocked for 2 h under constant shaking at room temperature in 5% non-fat dry milk dissolved in Tris-buffered saline-Tween-20 (TBST) containing 10 mmol/L Tirs/HCl (pH 7.5), 0.15 mol/L NaCl and 0.05% Tween-20. The membrane was incubated overnight at 4℃ in 100-fold dilution of immunoglobulin G (IgG) fraction of anti-AFU polyclonal antibody, and washed three times with TBST under constant shaking for 1 h, 20 min per time. The membrane was incubated with the secondary antibody at room temperature for 2 h under constant shaking, 5000-fold dilution of horseradish peroxide-conjugated immunoPure goat anti-rabbit IgG antibody [IgG (H+L),blotting grade; Pierce]. After three more 20 min washes with shaking (10 mmol/L-Tirs/HCl buffer, pH 7.4), development was accomplished by enhanced chemiluminescence (ECL) for 1 min following the manufacturer's instructions (Pierce), and the membrane was exposed to Kodak X-ray film. Exposure time was determined on the basis of signals generated by the reaction between membrane and mixture solution from ECL kit. The results were obtained through Kodak medical X-ray processor 102 (Eastman Kodak, Rochester, USA).

Streptavidin-peroxidase-biotin (SP) immunohistochemistry

The samples were incubated with the primary antibody against AFU (1:50, purified polyclonal, diluted in PBS) at 4℃ overnight. SP-immunohistochemistry (SP-IHC) was performed according to the manufacture's instructions (Zhong Shan Ltd Co, China) for SP kit. Sections were stained with 3, 3'-diaminobenzidine (DAB) and counterstained with haematoxylin for visualization of nuclei. In negative controls, phosphate-buffered saline (PBS) was chosen as the primary antibody instead of anti-human AFU polyclonal antibody.

RESULTS

Purification of AFU

An effective procedure was developed for the purification of AFU from human primary hepatocarcinoma tissue. The process included homogenization, high speed centrifugation, ammonium sulfate precipitation, ultrafiltration and cation exchange chromatography. The results are summarized in Table1. This procedure typically resulted in a purification of 74-fold with a very high specific activity of 10085 (nmol.min/mg) protein in 15% yield.

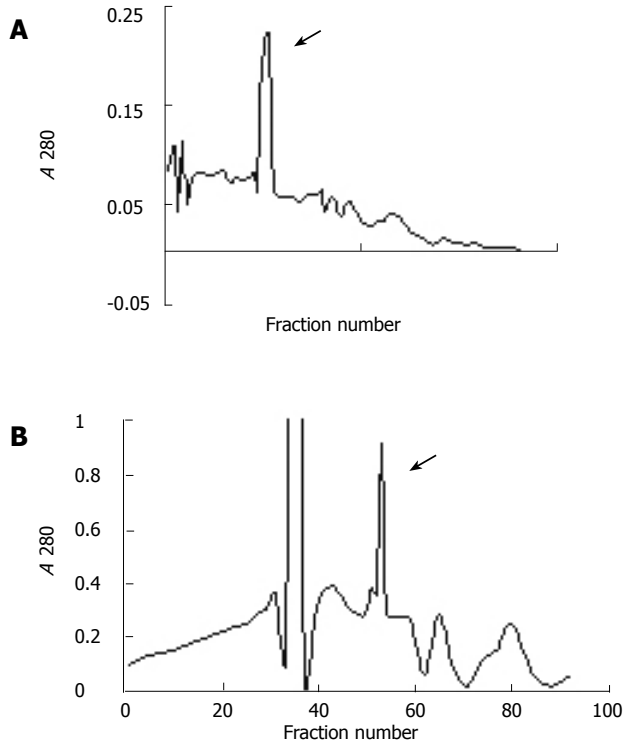
The stability of purified AFU was evaluated following storage at -20℃, 4℃ and 20℃ (pH 5.0) in the presence of sample buffer respectively. Samples in the frozen condition retained 100% of enzyme activity for at least two months. While samples in refrigerated condition showed slow but progressive decrease of enzyme activity with about 50%-80% of initial activity after two-month storage. Observations also exhibited that 100% enzyme activity remained in aseptic condition at room temperature within two days.

The elution schemes of two fractionations by chromatography are shown in Figure 1.

Standard curve of AFU

To quantify AFU activity in each purification step, we modified a procedure to assay the activity of β-galactose[17]. Using this method, enzyme activity in unknown samples could be compared according to a standard curve.

The absorbance of known concentrations of commercially available products generated at excitation wavelength 365 nm and emission wavelength 460 nm was



Figurge 1 Elution of fractionations on cation exchange. **A:** Chromatography of the ultrafiltration fraction of 35% to 50% $(NH_4)_2SO_4$ ammonium sulfate precipitation (22 mg of protein) on CM-cellulose. The procedure was performed with a column (2.6 cm × 30 cm) at a flow rate of 65 to 75 mL per hour. About 5 mL fractions was pooled. The column was developed initially with 750mL of 0.011 M citric acid-NaOH (pH 4.5), 0.02% NaN_3, and then a stepwise dilution over 400 mL from 0.05, 0.10, 0.15 to 0.25 mol/L NaCl was used to accomplish the development; **B:** Chromatography of the ultrafiltration fraction of 50% to 60% $(NH_4)_2SO_4$ ammonium sulfate precipitation (5.6 mg of protein) on CM-cellulose. The arrows indiente the elution peak of the interest protein.

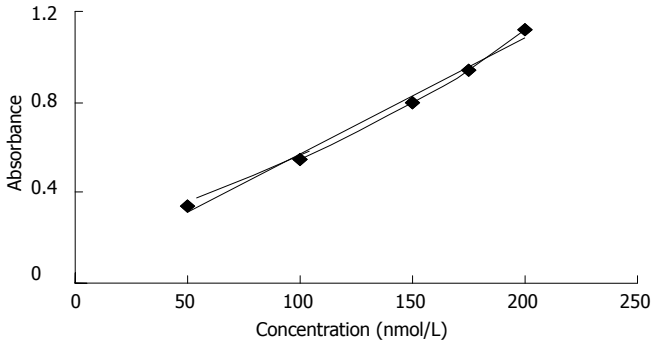


Figure 2 Stand curve of AFU. Solid line represents an artifact line generated by the Microsoft excel™ and dot line represents the practical values in linear range.

shown using HITACHI fluorescence spectrophotometer (Figure 2).

Purity of AFU in human liver cancer tissue

The migration pattern of crude extracts and purified AFU in denaturing polyacrylamide gel electrophoresis is shown in Figure 3. The SDS-PAGE indicated the presence of one subunit of purified AFU at 55 Ku.

Purification and purity of AFU antiserum

Antiserum was obtained via cardiac puncture by high

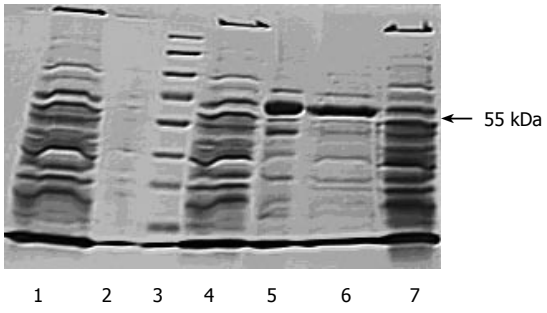


Figure 3 Electrophoretic analysis of AFU in crude and purified human liver. Lane 1: 50% to 60% $(NH_4)_2SO_4$ precipitation; lane 2: supernatant after high speed centrifugation; lane3: protein standards(Fermentas , prestained); lane 4: 35% to 50% $(NH_4)_2SO_4$ precipitation; lane 5: ultrafiltration fraction after 35% to 50% $(NH_4)_2SO_4$ precipitation; lane6: CM-cellulose fraction; lane 7: Homogenate and incubate at 37℃.

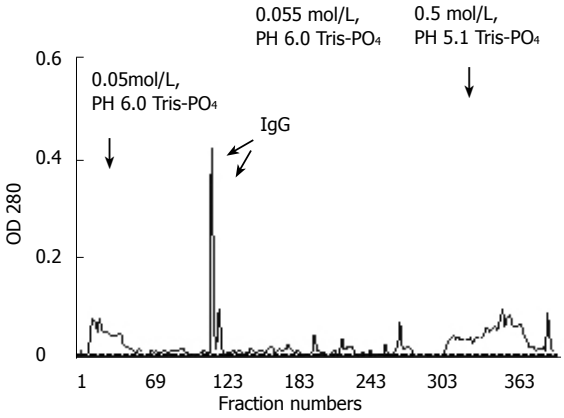


Figure 4 Anion exchange chromatography of crude antiserum against human PHCα-L-fucosidase using a column (2.6 cm × 30 cm) of DEAE-cellulose. The column was eluted as described in Materials and methods. The arrows indicate the applied buffers and the emerged elution peak of IgG.

speed centrifugation, ammonium sulfate precipitation, ultrafiltration and anion exchange chromatography on DEAE-cellulose. The elution profile of anti-serum from anion exchange column is depicted in Figure 4. The curve showed an elution peak of IgG fraction of antiserum against AFU around the 115th collecting tube. SDS-PAGE analysis illustrated the single subunit of 55 kDa of purified IgG and appeared to be highly purified.

Western-blot analysis of human liver AFU

Western blot analysis indicated that purified AFU antiserum recognized the 55 kDa band of the enzyme compared with immunized rabbit serum used as a control. The density of bands seemed to become thicker with prolonged exposure time (Figure 5).

Location and distribution of AFU expression

Immunohistochemistry staining indicated that predominant expression of AFU could be seen primarily in plasma membrane and cytoplasm of primary hepatocarcinoma cells compared with adjacent tissue and negative control. This staining was not found in the negative control treated identically except for primary antibody against human liver AFU.

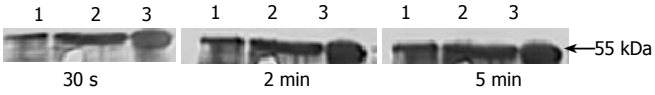


Figure 5 Western blot analysis showing that purified AFU could recognize a single subunit of 5 Ku (transverse arrow of the enzyme at 30s, 2 min, 5 min, respectively. Lane 1: purified AFU (5 μg) detected with a 10^3 dilution of antiserum; lane 2: purified AFU (10 μg) detected with a 5×10^2 dilution of antiserum; lane 3: purified AFU (5 μg) detected with a 5×10^2 dilution of antiserum.

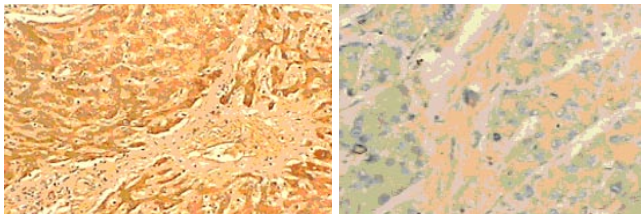


Figure 6 AFU expression in cytoplasm and/or cell membrane of PHC.

DISCUSSION

A number of investigations on α-L-fucosidase (EC 3.2.1.51) in different species have provided evidence for its importance in organism metabolism and diseases[1-3]. Although AFU is of considerable interest in human disease research because of fucosidosis, neurovisceral storage disease lacking of its enzymatic activity, many studies on its role in cancer research have been reported. To our knowledge, this is the first report of purification and characterization of human AFU from primary hepatocarcinoma tissue.

In this study, several criteria were chosen to purify and characterize AFU including ammonium sulfate precipitation, ultrafiltration, ion exchange chromatography, SDS-PAGE, Western blot, immunohistochemsitry staining and production of monospecific antibody against purified AFU. The results showed that this procedure was straightforward and successful for AFU purification. Contaminating glycosidases were removed during the course of each purification step and purified enzyme appeared to be homogeneity.

The molecular weight of purified enzyme determined by SDS-PAGE was a single subunit of 55 Ku, which is different from those reported previously[23-27]. These results may prove that purified AFU from PHC tissue is tetrameric, consisting of four identical subunits.

Western blot analysis showed enzyme of the 55 Ku subunit recognized by anti-AFU polyclonal antibody. None of the bands reacted with a preimmune serum (data not shown). This immunochemical recognition is consistent with previous studies on human colon AFU[11], one band of about 55 Ku was detected when incubated with an immunized serum, which is similar to the report of Johnson *et al*[28].

Immunohistochemistry staining showed predominant expression of AFU in plasma membrane and/or cytoplasm of primary hepatocarcinoma tissue, which is similar to previous results[29](Figure 6). The precise localization of AFU may suggest that expression level and specific site of AFU expression in primary hepatocarcinoma tissue can be a useful marker for early detection of PHC and a prognosis indicator of PHC development. It was reported that serum AFU activity is a useful marker for early detection of PHC particularly in α-fetoprotein -low or negative PHC patients[4-8,30]. However, the mechanism of elevated AFU in serum or tissues of patients with PHC is still obscure. One possible explanation is the increased synthesis and secretion of AFU by tumor cells[31]. Genetic studies suggest that three different phenotypes of AFU are dominated by two alleles exhibiting autosomal inheritance with a gene dosage effect. Individuals with high activity of AFU in serum are considered homozygous. In addition, sialic acid of glycoproteins determines the clearance rate in circulation and its transformation may cause retarded clearance of glycoproteins[32].

In conclusion, purified AFU and corresponding polyclonal antibody can be used as antigen-antibody candidates to detect PHC at early stage.

ACKNOWLEDGEMENTS

The authors thank Hui-Fen Zhu and Xiao-Dan Jiang for their excellent technical assistance, He-Jun Zhou and Wei Yan for preparation of the antiserum.

REFERENCES

1 **Johnson SW,** Alhadeff JA. Mammalian alpha-L-fucosidases. *Comp Biochem Physiol B* 1991; **99**: 479-488
2 **DiCioccio RA**, Barlow JJ, Matta KL. Substrate specificity and other properties of alpha-L-fucosidase from human serum. *J Biol Chem* 1982; **257**: 714-718
3 **Seo HC**, Willems PJ, Kretz KA, Martin BM, O'Brien JS. Fucosidosis: four new mutations and a new polymorphism. *Hum Mol Genet* 1993; **2**: 423-429
4 **Giardina MG**, Matarazzo M, Varriale A, Morante R, Napoli A, Martino R. Serum alpha-L-fucosidase. A useful marker in the diagnosis of hepatocellular carcinoma. *Cancer* 1992; **70**: 1044-1048
5 **Giardina MG**, Matarazzo M, Morante R, Lucariello A, Varriale A, Guardasole V, De Marco G. Serum alpha-L-fucosidase activity and early detection of hepatocellular carcinoma: a prospective study of patients with cirrhosis. *Cancer* 1998; **83**: 2468-2474
6 **Deugnier Y**, David V, Brissot P, Mabo P, Delamaire D, Messner M, Bourel M, Legall JY. Serum alpha-L-fucosidase: a new marker for the diagnosis of primary hepatic carcinoma? *Hepatology* 1984; **4**: 889-892
7 **Bukofzer S**, Stass PM, Kew MC, de Beer M, Groeneveld HT. Alpha-L-fucosidase as a serum marker of hepatocellular carcinoma in southern African blacks. *Br J Cancer* 1989; **59**: 417-420
8 **Leray G**, Deugnier Y, Jouanolle AM, Lehry D, Bretagne JF, Campion JP, Brissot P, Le Treut A. Biochemical aspects of alpha -L-fucosidase in hepatocellular carcinoma. *Hepatology* 1989; **9**: 249-252
9 **Ayude D**, Fernández-Rodríguez J, Rodríguez-Berrocal FJ, Martínez-Zorzano VS, de Carlos A, Gil E, Páez de La Cadena M. Value of the serum alpha-L-fucosidase activity in the diagnosis of colorectal cancer. *Oncology* 2000; **59**: 310-316
10 **Ayude D**, Páez De La Cadena M, Martínez-Zorzano VS, Fernández-Briera A, Rodríguez-Berrocal FJ. Preoperative serum alpha-L-fucosidase activity as a prognostic marker in colorectal cancer. *Oncology* 2003; **64**: 36-45
11 **Fernández J,** Rodríguez-Berrocal FJ, de Carlos A, de Castro G, de la Cadena MP. Nonradioactive immunoquantification of alpha-L-fucosidase protein in human colon tissues. *J Biochem*

Biophys Methods 1996; **31**: 39-47
12 **Abdel-Aleem H**, Ahmed A, Sabra AM, Zakhari M, Soliman M, Hamed H. Serum alpha L-fucosidase enzyme activity in ovarian and other female genital tract tumors. *Int J Gynaecol Obstet* 1996; **55**: 273-279
13 **Cragg H**, Winchester B, Seo HC, O'Brien J, Swallow D. Molecular basis of the common electrophoretic polymorphism (Fu1/Fu2) in human alpha-L-fucosidase. *J Med Genet* 1994; **31**: 659-660
14 **Di Matteo G**, Orfeo MA, Romeo G. Human alpha-fucosidase. Purification and properties. *Biochim Biophys Acta* 1976; **429**: 527-537
15 **Alhadeff JA**, Miller AL, Wenger DA, O'Brien JS. Electrophoretic forms of human liver alpha-L-fucosidase and their relationship to fucosidosis (mucopolysaccharidosis F). *Clin Chim Acta* 1974; **57**: 307-313
16 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254
17 A TD-700 Laboratory Fluorometer Method for b-Galactosidase. Turner Biosystems, Inc. Available from: URL: http://www.turnerdesigns.com/
18 **Carlsen RB**, Pierce JG. Purification and properties of an alpha-L-fucosidase from rat epididymis. J Biol Chem 1972; **247**: 23-32
19 **Wang JZ**, Fan M. Protein technology Manual. 1st ed. Science Publish Company, 2002: 77-110
20 **Sambrook J**, Fritsch EF, Maniatis T. Molecular Cloning. 2nd ed. Cold Spring Harbor Laboratory Press, 1989. Translated into Chinese by Jin DY, Li MF. Science Publish Company, 1996: 885
21 **Shen GX**, Zhou RL. Contemporary immunology experiment technology. 2nd ed. Wuhan: Hubei Science and Technology Publish Company, 2002: 23-25, 47-50
22 **Ed Harlow**, David Lane. Using Antibodies: A Laboratory Manual; Translated into Chinese by Shen GX, Gong FL. 2nd ed. Beijing: Science Publish Company, 2002: 159-186
23 **Alhadeff JA**, Khunsook S, Choowongkomon K, Baney T, Heredia V, Tweedie A, Bean B. Characterization of human semen alpha-L-fucosidases. *Mol Hum Reprod* 1999; **5**: 809-815
24 **Alhadeff JA**, Janowsky AJ. Human serum alpha-L-fucosidase. *Clin Chim Acta* 1978; **82**: 133-140
25 **Khunsook S**, Alhadeff JA, Bean BS. Purification and characterization of human seminal plasma alpha-L-fucosidase. *Mol Hum Reprod* 2002; **8**: 221-227
26 **Alhadeff JA**, Janowsky AJ. Purification and properties of human brain alpha-L-fucosidase. *J Neurochem* 1977; **28**: 423-427
27 **Alhadeff JA**, Miller AL, Wenaas H, Vedvick T, O'Brien JS. Human liver alpha-L-fucosidase. Purification, characterization, and immunochemical studies. *J Biol Chem* 1975; **250**: 7106-7113
28 **Johnson SW**, Piesecki S, Wang RF, Damjanov I, Alhadeff JA. Analysis of purified human liver alpha-L-fucosidase by western-blotting with lectins and polyclonal and monoclonal antibodies. *Biochem J* 1992; **282** (Pt 3): 829-834
29 **Khunsook S**, Bean BS, McGowan SR, Alhadeff JA. Purification and characterization of plasma membrane-associated human sperm alpha-L-fucosidase. *Biol Reprod* 2003; **68**: 709-716
30 **Wang JJ**, Cao EH. Rapid kinetic rate assay of the serum alpha-L-fucosidase in patients with hepatocellular carcinoma by using a novel substrate. *Clin Chim Acta* 2004; **347**: 103-109
31 **Takahashi H**, Saibara T, Iwamura S, Tomita A, Maeda T, Onishi S, Yamamoto Y, Enzan H. Serum alpha-L-fucosidase activity and tumor size in hepatocellular carcinoma. *Hepatology* 1994; **19**: 1414-1417
32 **Hutchinson WL**, Johnson PJ, Du MQ, Williams R. Serum and tissue alpha-L-fucosidase activity in the pre-clinical and clinical stages of hepatocellular carcinoma. *Clin Sci (Lond)* 1991; **81**: 177-182

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Liu Y