• LIVER CANCER •

Alpha-fetoprotein stimulated the expression of some oncogenes in human hepatocellular carcinoma Bel 7402 cells

Meng-Sen Li, Ping-Feng Li, Qian Chen, Guo-Guang Du, Gang Li

Meng-Sen Li, Qian Chen, Department of Biochemistry, Hainan Medical College, Haikou 571101, China
Ping-Feng Li, Guo-Guang Du, Gang Li, Department of Biochemistry and Molecular Biology, Health Science Center, Peking University, Beijing 100083, China
Supported by the National Natural Science Foundation of China, No. 30260117, Natural Science Foundation of Hainan Province, No. 30315 and the Nursery Foundation of Hainan Medical College, No. 200202
Correspondence to: Dr. Meng-Sen Li, Department of Biochemistry, Hainan Medical College, Haikou 571101, China. mengsenli@163.com
Telephone: +86-898-66893779
Received: 2003-09-18   **Accepted:** 2003-11-15

Abstract

AIM: To investigate the molecular mechanism of alpha-fetoprotein (AFP) on regulating the proliferation of human hepatocellular carcinoma cells.

METHODS: Alpha-fetoprotein purified from human umbilical blood was added to cultured human hepatocellular carcinoma Bel 7402 cells *in vitro* for various treatment periods. The expression of *c-fos*, *c-jun*, and *N-ras* mRNA involved in proliferation and differentiation of cells was analyzed by Northern blot, and the expression of mutative p53 and p21^{ras} proteins was determined by Western blot.

RESULTS: The results showed that AFP (20 mg/L) stimulated mRNA expression of these oncogenes in Bel 7402 cells. The expression of *c-fos* mRNA increased by 51.1%, 60.9%, 96.0%, and 25.5% at 2, 6, 12, and 24 h, respectively. The expression of *c-jun* and *N-ras* mRNA reached to the maximum which increased by 81.3% and 59.9% as compared with the control after 6 h and 24 h incubation with AFP, respectively. Western blot assay also demonstrated that AFP promoted the expression of mutative p53 and p21^{ras} proteins, and the increased rate of those proteins was 13.0%, 39.9%, and 70.9%, as well as 35.2%, 102.6%, and 46.8% at 6, 12, and 24 h, respectively, as compared with the control. Both human serum albumin (the same dosage as AFP) and monoclonal anti-AFP antibody failed to stimulate the expression of these oncogenes, but anti-AFP antibody could block the functions of AFP.

CONCLUSION: The data indicate that AFP can stimulate the expression of some oncogenes to enhance the proliferation of human hepatocellular carcinoma Bel 7402 cells.

INTRODUCTION

Alpha-fetoprotein is the major serum protein in human embryos, and is synthesized by embryonic liver and yolk sac. During the embryonic growth course, AFP expresses much more (3 g/L), and falls to the adult level (0.02×10^{-3} g/L) after one-year birth. Although many investigations for the function of AFP had been carried out, the biological role of AFP is still a riddle so far. Because the composition and the sequence of the amino acid resides of AFP were very similar to those of human serum albumin, thus, people always think that the function of AFP just like human serum albumin, which functions to transport materials and stabilize blood colloid osmotic pressure in the life course of the fetus. However, the concentration of serum AFP increases apparently with liver cancer or liver optimum regeneration in humans. AFP always accompanies with the growth of liver cells, and it is possible that AFP may be related to the proliferation of tumor or fetal cells. Some investigations had showed that AFP could be individually synergy with other growth factors to promote the growth of many tumor or normal cells[1-6]. Alpha-fetoprotein (MW 69 ku) is a kind of biomacromolecules, and it is impossible to directly permeate the cells to regulate cell proliferation. We previously found that AFP could enhance growth of human hepatocellular carcinoma Bel 7402 and NIH3T3 cells, and there were two typical receptors of AFP existed on the membranes of these cells[7,8]. However, it has not been reported in former investigations how AFP influences the expression of oncogenes which are mediated by AFP receptors to regulate growth of human hepatocellular carcinoma cells. This study used human hepatocellular carcinoma Bel 7402 cell line, which is closely related to AFP, to observe mRNA expression of the oncogene *c-fos*, *c-jun*, and *N-ras*, and protein expression of mutative p53 and p21^{ras}, which are correlated with cell proliferation, after treated with AFP. Additionally, the study explored some molecular mechanisms for AFP-mediated growth of human hepatocellular carcinoma cells.

MATERIALS AND METHODS

Materials

Human hepatocellular carcinoma Bel 7402 cells, crude AFP, and monoclonal anti-AFP antibody were provided by Endocrine Research Group of the Department of Biochemistry and Molecular Biology, Health Science Center, Peking University; RPMI 1640 medium was purchased from GIBCO; Fetal calf serum (FCS) was from the Blood Research Institute of Chinese Medicine Science Academy (Tianjin, China); Human serum albumin (HSA) and MOPS were purchased from Sigma Company; Diethyl pyrocarbonate (DEPC), sodium dodecyl sulfate (SDS), agarose, and Tris were obtained from Bio-Rad Company; Total RNA extraction kit was purchased from Promega Company; α-^{32}P-dCTP was bought from YaHui Biology Engineer Company (Beijing, China); *N-ras*, *c-fos*, *c-jun,* and β-actin cDNA probes were from the Department of Endocrinology, Northwestern University (Chicago); Random primer labeling kit was the product of Takara company (Japan); Salmon sperm DNA, fraction V of bovine serum albumin (BSA), and Ficoll-400 were bought from the Jingke Chemical

Reagents Company (Beijing, China); Monoclonal antibodies for mutative p53 and p21[ras] were from NEOMARKERS Company.

Methods

Purification of human AFP Human AFP was prepared by the method as described elsewhere[9]. Briefly, human cord blood AFP was precipitated by ammonium sulfate and passed through an anti-AFP antibody affinity chromatography column. The AFP-positive fractions were collected and concentrated. The purity of prepared AFP was 92.7% determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The protein was stored at -80 ℃ until analysis.

Cells culture Human hepatocellular carcinoma Bel 7402 cells ($1.5×10^4$/mL) were cultured in RPMI 1640 medium supplemented with 100 mL/L FCS at 37 ℃ in a humidified atmosphere of 50 mL/L CO_2. The cultured medium was changed after 24 h.

RNA isolation and Northern blot analysis The cells were treated with AFP (20 mg/L), HSA (20 mg/L), anti-AFP antibody (40 mg/L), or AFP (20 mg/L) + anti-AFP antibody (40 mg/L) for 2, 6, 12, and 24 h, respectively. Total cellular RNA was isolated from Bel 7402 cells using a TRIzol reagent kit (Promega, Madison, WI) according to the manufacturer's protocol. Then, RNA was quantitated by the absorbance at 260 nm, and RNA (10-20 μg/lane) was fractionated by electrophoresis through 10 g/L formaldehyde agarose gel. The fractionated RNA was transferred in 20×SSC buffer to the nitrocellulose membrane (Millipore Corporation, Bedford, MA) using the standard procedure[10]. These membranes were hybridized with α-^{32}P labeled probe, and washed using the standard protocol. The membranes were then exposed to the X-ray film at -80 ℃.

Purification of protein and Western blot analysis The cells were treated with AFP (20 mg/L), HSA (20 mg/L), anti-AFP antibody (40 mg/L), or AFP (20 mg/L) + anti-AFP antibody (40 mg/L) for 6, 12, and 24 h, respectively. After washed three times with PBS (pH 7.4, 0.15 mol/L), the cells were lyzed in 10 μL of lysis buffer containing 2 mL/L Triton X-100, 500 mmol/L NaCl, 500 mmol/L sucrose, 1 mmol/L EDTA, 0.15 mmol/L spermine, 0.5 mmol/L spermidine, 10 mmol/L HEPES (pH 8.0), 200 μmol/L phenylmethylsulfonyl fluoride, 2 mg /L leupeptin, 2 mg /L pepstatin, 24 000 IU /L aprotinin, and 7 mmol/L β-mercaptoethanol. Proteins (40 μg) were subjected to SDS-PAGE, and transferred to the PVDF membrane for immunodetection. The SDS-PAGE molecular weight markers (Bio-Rad) verified the correct location of the visualized bands. The membranes were blocked with 50 mL/L nonfat milk in PBS-Tween, probed with anti-p53 or anti-p21 antibody, and followed by secondary antibody (goat anti-mouse IgG-alkaline phosphatase). Immunoreactive proteins were detected using a color develop system (NBT/BCIP) by the standard procedure[10].

RESULTS

Expression of c-fos mRNA

Northern blot analysis demonstrated the overexpression of *c-fos* mRNA in Bel 7402 cells after treated with AFP for 2, 6, and 12 h. The data showed that AFP (20 mg/L) treated for 2 h significantly increased the expression of *c-fos* mRNA in Bel 7402 cells by 51.1% compared with the control group. The expression of *c-fos* mRNA continuously increased by 60.9% and 96.0% when treated with AFP for 6 h and 12 h, respectively, and declined thereafter to 25.5% increase at 24 h, compared with control group (Figure 1). However, HSA at the same dosage (20 mg/L) as AFP and anti-AFP antibody (40 mg/L)

had no significant influence on the expression of *c-fos* mRNA in Bel 7402 cells. Anti-AFP antibody partially blocked an increase in the expression of *c-fos* mRNA by AFP (Figure 2).
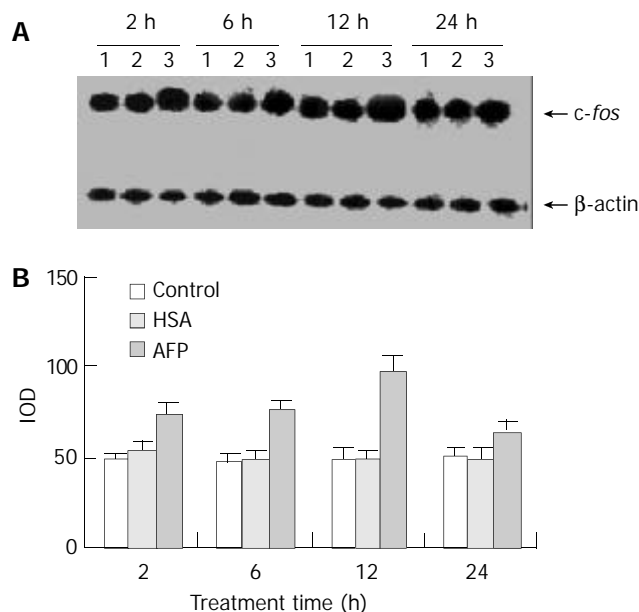

Figure 1 Effects of AFP (20 mg/L) or HSA (20 mg/L) on the expression of *c-fos* mRNA in human hepatocellular carcinoma Bel 7402 cells analyzed by Northern blot. The cells were incubated with AFP or HSA for 2, 6, 12, and 24 h, respectively. A: Autoradiogram of Northern blot. Lane 1: control group; Lane 2: HSA treated group; Lane 3: AFP treated group. B: Densitometric intensity of absorbance (IOD) of *c-fos* mRNA expression in Bel 7402 cells. The data were selected from 3 independent experiments.
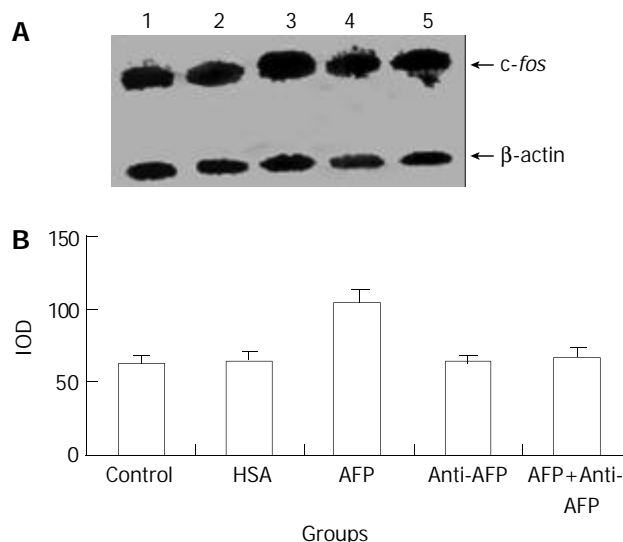

Figure 2 Effects of AFP (20 mg/L), HSA (20 mg/L), anti-AFP antibody (40 mg/L), and AFP (20 mg/L) + anti-AFP antibody (40 mg/L) on *c-fos* mRNA expression in human hepatocellular carcinoma Bel 7402 cells analyzed by Northern blot after 2 h treatment. A: Autoradiogram of Northern blot. Lane 1: control group; Lane 2: HSA treated group; Lane 3: AFP treated group; Lane 4: anti-AFP antibody treated group; Lane 5: AFP + anti-AFP antibody treated group. B: Densitometric intensity of absorbance (IOD) of *c-fos* mRNA expression in Bel 7402 cells. The data were selected from 3 independent experiments.

Expression of c-jun mRNA

AFP (20 mg/L) had no significant influence on the expression

of *c-jun* mRNA in human hepatocellular carcinoma cells when treated for 2 h, but when treated for 6 h the expression of *c-jun* mRNA increased obviously, the increase rate was 81.3%, but when treated for 24 h, increased rate fell to 14.6% as compared with the control group (Figure 3). However, HSA (20 mg/L) at the same dosage as AFP and anti-AFP antibody (40 mg/L) had no significant influence on the expression of *c-jun* mRNA in Bel 7402 cells. Anti-AFP antibody partially inhibited an increase in the expression of *c-jun* mRNA by AFP (Figure 4).
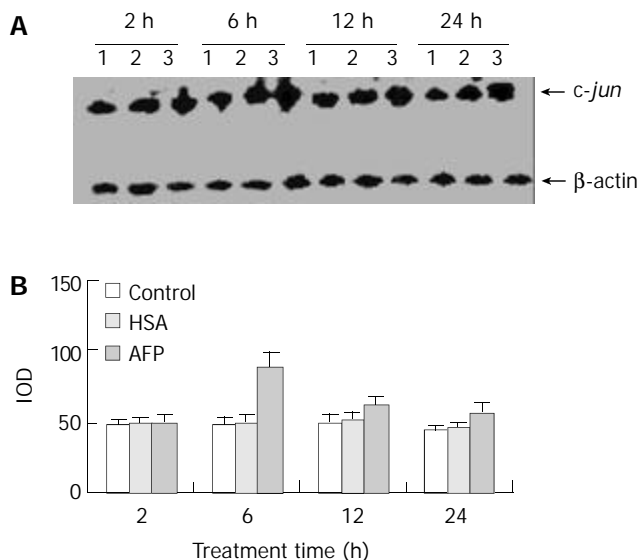

Figure 3 Effects of AFP (20 mg/L) or HSA (20 mg/L) on the expression of *c-jun* mRNA in human hepatocellular carcinoma Bel 7402 cells analyzed by Northern blot. The cells were incubated with AFP or HSA for 2, 6, 12, and 24 h, respectively. A: Autoradiogram of Northern blot. Lane 1: control group; Lane 2: HSA treated group; Lane 3: AFP treated group. B: Densitometric intensity of absorbance (IOD) of *c-jun* mRNA expression in Bel 7402 cells. The data were selected from 3 similar experiments.
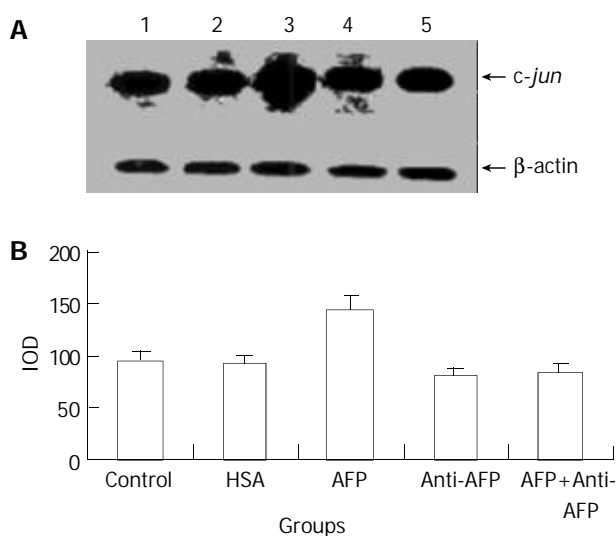

Figure 4 Effects of AFP (20 mg/L), HSA (20 mg/L), anti-AFP antibody (40 mg/L), and AFP (20 mg/L) + anti-AFP antibody (40 mg/L) on the expression of *c-jun* mRNA in human hepatocellular carcinoma Bel 7402 cells analyzed by Northern blot after 6 h treatment. A: Autoradiogram of Northern blot. Lane 1: control group; Lane 2: HSA treated group; Lane 3: AFP treated group; Lane 4: anti-AFP antibody treated group; Lane 5: AFP + anti-AFP antibody treated group. B: Densitometric

intensity of absorbance (IOD) of *c-jun* mRNA expression in Bel 7402 cells. The data were selected from 3 independent experiments.
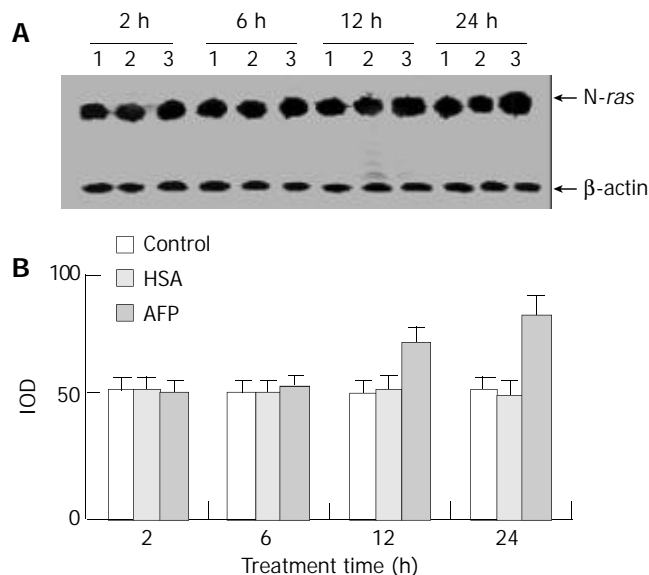

Figure 5 The effects of AFP (20 mg/L) or HSA (20 mg/L) on the expression *N-ras* mRNA in human hepatocellular carcinoma Bel 7402 cells analyzed by Northern blot. The cells were incubated with AFP or HSA for 2, 6, 12, and 24 h, respectively. A: Autoradiogram of Northern blot. Lane 1: control group; Lane 2: HSA treated group; Lane 3: AFP treated group. B: Densitometric intensity of absorbance (IOD) of *N-ras* mRNA expression in Bel 7402 cells. The data were selected from 3 similar experiments.
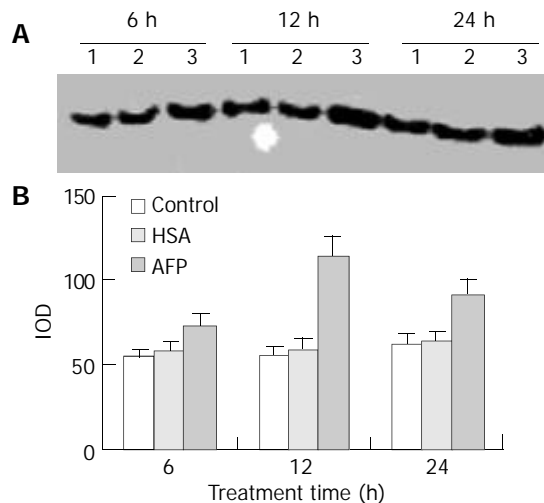

Figure 6 Effects of AFP (20 mg/L) or HSA (20 mg/L) on the expression of p21^{ras} in Bel 7402 cells analyzed by Western blot. The cells were incubated with AFP or HSA for 6, 12, and 24 h, respectively. A: Western blot analysis. Lane 1: control group; Lane 2: HSA treated group; Lane 3: AFP treated group. B: Densitometric intensity of absorbance (IOD) of p21^{ras} protein. The data were selected from 3 similar experiments.

Expression of N-ras mRNA

Northern blot analysis showed that AFP (20 mg/L) had no significant influence on the expression of *N-ras* mRNA in Bel 7402 cells when treated for 2 h and 6 h, but *N-ras* mRNA was overexpressed when treated with AFP (20 mg/L) for 12 h and 24 h. The increased ratios were 22.6% (12 h) and 59.9% (24 h), respectively, compared with the control group, and HSA (20 mg/L) did not significantly affect the expression of *N-ras* mRNA (Figure 5).

Expression of p21ras protein

Western blot analysis demonstrated that AFP significantly enhanced the expression of p21ras protein in Bel 7402 cells by 35.2%, 102.6%, and 46.8% at 6, 12, and 24 h, respectively, compared with the control group. However, HSA (20 mg/L) did not influence the expression of p21ras protein (Figure 6).

Expression of mutative p53 protein

The data showed that AFP significantly influenced the expression of mutative p53 protein in Bel 7402 cells by 13.4%, 39.9%, and 70.6% at 6, 12, and 24 h, respectively, compared with the control group. However, HSA (20 mg/L) did not affect the expression of mutative p53 protein (Figure 7).
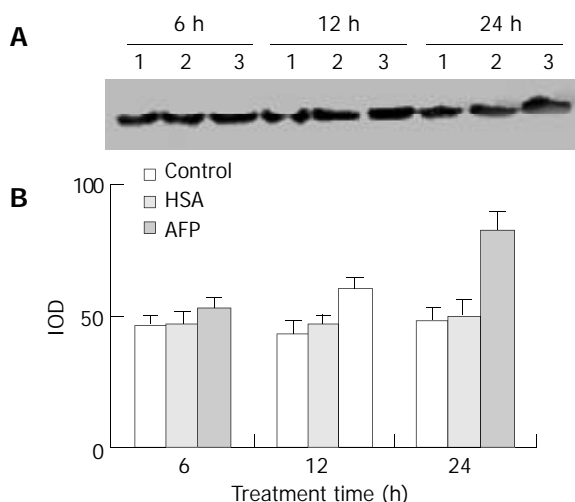

Figure 7 Effects of AFP (20 mg/L) or HSA (20 mg/L) on the expression of mutative p53 protein in Bel 7402 cells analyzed by Western blot. The cells were treated with AFP or HSA for 6, 12, and 24 h, respectively. A: Western blot analysis. Lane 1: control group; Lane 2: HSA treated group; Lane 3: AFP treated group. B: Densitometric intensity of absorbance (IOD) of p53 protein. The data were selected from 3 similar experiments.

DISCUSSION

Many investigations have shown that AFP receptors exist on the membrane of various tumor cells[5,7,8,11-13], and play an important role in regulating growth of the cells[7,8,14]. The receptor may mediate intercellular signal transduction which influences the expression of genes related to proliferation, and the expression of these genes is the most direct factor that controls cell cycle. Our results showed that AFP stimulated the expression of *c-fos*, *c-jun*, and *N-ras* mRNA in hepatocellular carcinoma Bel 7402 cells. Both *c-fos* and *c-jun* have the characteristics of early response genes. When treated with AFP, the two oncogenes in Bel 7402 cells express promptly, thereafter the expression of these oncogenes dramatically declines. Previous researches had showed that AFP promoted the proliferation[1-6] and some oncogene expression of many tumor cells[15,16]. Cell growth was regulated by various factors, in which, some oncogene coding proteins have an important function in modulating growth and differentiation of the cells. *c-fos* and *c-jun* are the immediate early genes (IEG) with the characteristics of early response gene. When Bel 7402 cells were treated with AFP, the expression of these oncogenes had the characteristics of dynamics, which was independent on the translation of RNA. Additionally, IEG coding regulated-proteins can control the expression of some later response genes through the activation of nuclear transcription factors. Furthermore, c-*Fos* and c-*Jun* proteins can interact with each other to form a heterodimer (*Fos-Ju*n) called AP-1 (activator

protein-1). The dimer has leucine zipper structure, which has stronger binding affinity to DNA, and is the third messenger of signal transduction. The AP-1 protein is a nuclear transcription factor, which can regulate gene transcription by binding to a gene transcription-regulated element, and can increase transcriptional activation of downstream target genes. A previous study showed that the transcriptional properties of AP-1 depended on the site of cAMP-response elements to influence the expression of growth-related genes in hepatocellular carcinoma[17]. Therefore, we thought that AFP regulated cell cycle of Bel 7402 cells, which was related to the expression of early-response genes. Our result also showed that AFP promoted the expression of *N-ras* in Bel 7402 cells. The *N-ras* gene is closely related to cell proliferation, and p21ras, the *N-ras* coding protein, is an important intermediate of tyrosine-protein kinase (TPK) signal pathway in the cells to regulate growth-related gene expression. Therefore, we suggested that AFP could affect signal transduction to regulate growth of Bel 7402 cells probably through stimulating the expression of *N-ras*.

The wild type p53 is a very important tumor suppressor gene to inhibit proliferation of tumor cells. Some studies had showed that hepatocellular carcinoma was closely related to abnormal expression of p53 gene[18,19]. Additionally, p53 and beta-catenin mutation rates were inversely correlated in hepatocellular carcinoma[20]. The inactivation of p53 was an important cause of aberrant accumulation of beta-catenin, which was involved in both cell-cell interactions and wnt pathway-dependent cell fate determination in many cancer cells[20]. A previous study found that p53 mutation was correlated significantly with invasiveness including vascular permeation of hepatocellular carcinoma cells, and p53 mutation in the primary lesion was useful as an indicator of the biological behavior of recurrent hepatocellular carcinoma[21]. It showed that mutative p53 protein played a critical role in cell proliferation of hepatocellular carcinoma. The p53 gene could influence AFP expression to regulate the biological behavior of tumor cells[22,23]. Our data had showed that AFP could enhance the expression of mutative p53 protein. When p53 gene is mutated, it can not suppress the growth of tumor cells, on the contrary, it possesses the functions of oncogenes. There are two types of mutative p53 genes in tumor cells, one of the mutative p53 genes can restrain the growth-suppressing activity of wild type p53 gene, then display apparently its negative regulation; the other type of mutative p53 genes can cooperate with *ras* gene to actuate cell transformation, and then become a dominant oncogene, which promotes the growth of tumor cells. Because *Ras* protein is an important intermediate in TPK signal transduction pathway, we analyzed the expression of *N-ras* mRNA, and then further detected its protein in this study. The results showed that AFP promoted the expression of p21ras protein in Bel 7402 cells. The functions of p21ras protein are similar to those of guanylate binding protein, and it can phosphorylate the intermediate in the downstream of TPK signal pathway to activate mitogen-activated protein kinase (MAPK) and to participate in intercellular signal transduction. The p21ras protein can transduce the signal from TPK to threoine/serine protein kinase chain through Ras-Raf-MAPK signal pathway. These responses further activate some transcription factors which promote gene expression to enhance the growth of various cells. Our results demonstrated that AFP increased the expression of p21ras. Therefore, we speculated that AFP affected the expression of p53 and p21ras proteins through AFP receptor-mediated signal transduction pathway to accelerate the proliferation of Bel 7402 cells. Similar to the findings in this study, our previous study found that AFP enhanced the growth of HeLa and NIH3T3 cells through stimulating the expression of some oncogenes[8,15,16], suggesting

that AFP might unselectively regulate proliferation of tumor cells in different tissues and species.

The mechanism for growth-promoting activity of AFP is still unclear. It has been known for a long time that AFP has the ability to transport the substances essential for cell proliferation to enter the cells. Studies have indicated that AFP is an important protein in the embryos to regulate growth of the fetus[24], and required for female fertility, but not essential for embryonic development[25]. Up to date, it is not clear whether the biological function of AFP is involved in regulating cell proliferation. Some studies indicated that AFP inhibited immune response to promote the growth of tumor cells *in vivo*[26,27]. Escaping from the surveillance of immune system was the primary cause for malignant growth of tumor cells. Hepatocellular carcinoma cells can escape from the surveillance of immune system through altering the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors[28], which can sustain the growth of tumor cells *in vivo*. However, our *in vitro* study could not establish the immune response without immune factors to affect the expression of the oncogenes in Bel 7402 cells. Therefore, it could not explain that AFP inhibited immune response to affect cell growth. Because AFP is a macromolecule, it is impossible to enter the cells through the cell membrane directly. Although AFP can transport some substances required for cell proliferation, it is not enough to completely support cell proliferation. Many studies found that AFP receptors existed on the membrane surface of various tumor cells, and mediated signal transduction to regulate the expression of the genes. The expression of these genes was the ultimate determination factor in regulating cell growth. Our results showed that AFP had the ability to stimulate the expression of the oncogenes *c-fos*, *c-jun*, and *N-ras* in Bel 7402 cells, and the response of these genes to AFP was various. Both the expression of *c-fos* and *c-jun* responded early, whereas the expression of *N-ras* and mutative p53 gene responded thereafter. Some oncogenes such as p53 and ras are important prognostic molecular markers in human hepatocellular carcinoma[29]. According to previous studies[5,7,8] and our results, we considered that the action of AFP was via TPK signal transduction pathway to stimulate the expression of some oncogenes which could regulate the growth of tumor cells. Many investigations had showed that the expression of some oncogenes was up-regulated in hepatocellular carcinoma, and some factors such as intergrin gene, p28/gankyrin, and HLA class I could influence cell proliferation of hepatocellular carcinoma[30-32]. However, some studies had shown that treatment of tumor cells *in vitro* with high dosage of AFP (1-10 μmol/L) significantly suppressed the growth of tumor cells, because AFP positively regulated cytochrome c-mediated caspase activation, apoptosome complex formation, and low-dose cytochrome c-mediated signals[33,34]. The mechanisms for how AFP regulates the expression of these genes and signal transduction, and why it is not essential for embryonic development[24], although it promotes the growth of some tumor cells[1-8] remain to be studied.

A previous study showed that AFP could coordinate other growth factors existed in HSA and serum to promote the growth of porcine granulosa cells, and AFP could function to modulate growth factor-mediated cell proliferation during development and neoplasia[4]. To further verify the up-regulation of these oncogenes was mediated by AFP, this study used HSA as a negative control due to similar amino acid sequences of AFP as HSA. The results showed that HSA or anti-AFP antibody did not stimulate the expression of these genes in Bel 7402 cells, but anti-AFP antibody efficiently blocked the function of AFP, which can be explained that AFP specifically stimulated the expression of these oncogenes in human hepatocellular carcinoma Bel 7402 cells.

REFERENCES

1   **Dudich E**, Semonkova L, Gorbatova E, Dudich I, Khromyh L, Tatulov E, Grechko G, Sukhikh G. Growth- regulative activity of human alpha-fetoprotein for different types of tumor and normal cells. *Tumour Biol* 1998; **19**: 30-40

2   **Wang XW**, Xie H. Alpha-fetoprotein enhances the proliferation of human hepatoma cells *in vitro*. *Life Sci* 1999; **64**: 17-23

3   **Wang XW,** Xu B. Stimulation of tumor-cell growth by alpha-fetoprotein. *Int J Cancer* 1998; **75**: 596-599

4   **Keel BA,** Eddy KB, Cho S, May JV. Synergistic action of purified alpha-fetoprotein and growth factors on the proliferation of porcine granulosa cells in monolayer culture. *Endocrinology* 1991; **129**: 217-225

5   **Li MS**, Li PF, Zhou AR, Li G, Du GG. Growth factor like activity of alpha-fetoprotein in human hepatoma cell line, Bel7402 and HeLa cell (abstract). *Endo 2000 the Endocrine Society 82nd Annual Meeting, Toronto, Canada* 2000: 143

6   **Nunez EA**. Biological role of alpha-fetoprotein in the endocrinological field: data and hypothesis. *Tumor Biol* 1994; **15**: 63-72

7   **Li MS**, Li PF, He SP, Du GG, Li G. The promoting molecular mechanism of alpha-fetoprotein on the growth of human hepatoma Bel7402 cell line. *World J Gastroenterol* 2002; **8**: 469-475

8   **Li MS**, Li PF, Yang FY, He SF, Du GG, Li G. The intracellular mechanism of alpha-fetoprotein promoting the proliferation of NIH 3T3 cells. *Cell Res* 2002; **12**: 151-156

9   **Yamada T**, Kakinoki M, Totsuka K, Ashida Y, Nishizono K, Tsuchiya R, Kobayashi K. Purification of canine alpha-fetoprotein and alpha-fetoprotein values in dogs. *Vet Immunol Immunopathol* 1995; **47**: 25-33

10   **Sambrook J**, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual, 2nd ed. *New York*: *Cold Spring Harbor Laboratory Press* 1989: 888-897

11   **Esteban C**, Geuskens M, Uriel J. Activation of an alpha-fetoprotein (AFP)/receptor autocrine loop in HT-29 human colon carcinoma cells. *Int J Cancer* 1991; **49**: 425-430

12   **Villacampa MJ**, Moro R, Naval J, Failly-Crepin C, Lempreave F, Uriel J. Alpha-fetoprotein receptors in a human breast cancer cell line. *Biochem Biophys Res Commun* 1984; **122**: 1322-1327

13   **Naval J**, Villacampa M J, Goguel AF, Uriel J. Cell-type-specific receptor for alpha-fetoprotein in mouse T-lymphoma cell line. *Proc Natl Acad Sci U S A* 1985; **82**: 3301-3305

14   **Laderoute MP**, Pilarski LM. The inhibition of apoptosis by alpha-fetoprotein (AFP) and the role of AFP receptors in anti-cellular senescence. *Anticancer Res* 1994; **14**(6B): 2429-2438

15   **Li MS**, Li PF, Du GG, Li G. The enhancement effects of alpha-fetoprotein on the expression on *N-ras* and p53 and p21ras in HeLa cells. *Chin J Biochem Mol Biol* 2002; **18**: 750-754

16   **Li MS**, Li PF, Li G, Du GG. Enhancement of proliferation of HeLa cells by the α-fetoprotein. *Shengwu Huaxue Yu Shengwu Wuli Xuebao* 2002; **34**: 769-774

17   **Guberman AS**, Scassa ME, Giono LE, Varone CL, Canepa ET. Inhibitory effect of AP-1 complex on 5-aminolevulinate synthase gene expression through sequestration of cAMP-response element protein (CRE)-binding protein (CBP) coactivator. *J Biol Chem* 2003; **278**: 2317-2326

18   **Caruso ML**, Valentini AM. Overexpression of p53 in a large series of patients with hepatocellular carcinoma: a clinicopathological correlation. *Anticancer Res* 1999; **19**(5B): 3853-3856

19   **Zhang XW,** Xu B. Differential regulation of P53, c-*Myc*, Bcl-2, Bax and AFP protein expression, and caspase activity during 10-hydroxycamptothecin-induced apoptosis in Hep G2 cells. *Anticancer Drugs* 2000; **11**: 747-756

20   **Cagatay T**, Ozturk M. P53 mutation as a source of aberrant beta-catenin accumulation in cancer cells. *Oncogene* 2002; **21**: 7971-7980

21   **Sheen IS**, Jeng KS, Wu JY. Is p53 gene mutation an indicatior of the biological behaviors of recurrence of hepatocellular carcinoma? *World J Gastroenterol* 2003; **9**: 1202-1207

22   **Ogden SK**, Lee KC, Wernke-Dollries K, Stratton SA, Aronow B, Barton MC. p53 targets chromatin structure alteration to repress alpha-fetoprotein gene expression. *J Biol Chem* 2001; **276**: 42057-42062

23   **Lee KC**, Crowe AJ, Barton MC. p53-mediated repression of alpha-fetoprotein gene expression by specific DNA binding. *Mol*

Cell Biol 1999; **19**: 1279-1288

24 **Mazure NM**, Chauvet C, Bois-Joyeux B, Bernard MA, Nacer-Cherif H, Danan JL. Repression of alpha-fetoprotein gene expression under hypoxic conditions in human hepatoma cells: characterization of a negative hypoxia response element that mediates opposite effects of hypoxia inducible factor-1 and c-*Myc*. *Cancer Res* 2002; **62**: 1158-1165

25 **Gabant P**, Forrester L, Nichols J, Van Reeth T, De Mees C, Pajack B, Watt A, Smitz A, Alexandre H, Szpirer C, Szpirer J. Alpha-fetoprotein, the major fetal serum protein, is not essential for embryonic development but is required for female fertility. *Proc Natl Acad Sci U S A* 2002; **99**: 12865-12870

26 **Semeniuk DJ**, Boismenu R, Tam J, Weissenhofer W, Murgita RA. Evidence that immunosuppression is an intrinsic property of the alpha-fetoprotein molecule. *Adv Exp Med Biol* 1995; **383**: 255-269

27 **Gotsman I**, Israeli D, Alper R, Rabbani E, Engelhardt D, Ilan Y. Induction of immune tolerance toward tumor-associated enables growth of human hepatoma in mice. *Int J Cancer* 2002; **97**: 52-57

28 **Chen XP**, He SQ, Wang HP, Zhao YZ, Zhang WG. Expression of TNF-related apoptosis-inducing Ligand receptors and anti-tumor tumor effects of TNF-related apoptosis-inducing Ligand in human hepatocellular carcinoma. *World J Gastroenterol* 2003;

9: 2433-2440

29 **Qin LX**, Tang ZY. The prognostic molecular markers in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 385-392

30 **Liu LX** , Jiang HC, Liu ZH, Zhou J, Zhang WH, Zhu AL, Wang XQ, Wu M. Intergrin gene expression profiles of human hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 631-637

31 **Fu XY**, Wang HY, Tan L, Liu SQ, Cao HF, Wu MC. Overexpression of p28/gankyrin in human hepatocellular carcinoma and its clinical significance. *World J Gastroenterol* 2002; **8**: 638-643

32 **Huang J**, Cai MY, Wei DP. HLA class I expression in primary hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 654-657

33 **Dudich E,** Semenkova L, Dudich I, Gorbatova E, Tochtamisheva N, Tatulov E, Nikolaeva M, Sukhikh G. Alpha-fetoprotein causes apoptosis in tumor cells via a pathway independent of CD95, TNFR1 and TNFR2 through activation of caspase-3-like proteases. *Eur J Biochem* 1999; **266**: 750-761

34 **Semenkova L,** Dudich E, Dudich I, Tokhtamisheva N, Tatulov E, Okruzhnov Y, Garcia-Foncillas J, Palop-Cubillo JA, Korpela T. Alpha-fetoprotein positively regulates cytochrome c-mediated caspase activation and apoptosome complex formation. *Eur J Biochem* 2003; **270**: 4388-4399

Edited by Chao JCJ **Proofread by** Xu FM