Clinical Observations

EXPRESSION OF BLOOD AFPmRNA IN THE PATIENTS WITH DISTANT METASTASIS OF HUMAN HEPATOCELLULAR CARCINOMA (HCC)

LIU Yang 刘扬, QIAN Guangxiang 钱光相, ZHANG Baihe 张柏和,
CHEN Han 陈汉, WU Mengchao 吴孟超

Institute of East Hepatobiliary Surgery, Second Military Medical University, Shanghai 200438, China

ABSTRACT

Objective: To study blood AFPmRNA expression in patients with distant metastasis of human hepatocellular carcinoma (HCC). Methods: Using nested reverse transcriptase polymerase chain reaction (nested RT-PCR) to detect 93 blood samples from human HCC cases. Results: AFPmRNA was detected in 21 blood samples from 72 human HCC (40.28%) without distant metastasis, all HCC patients with distant metastasis were detected with AFPmRNA (100%). Conclusions: AFPmRNA can be used as a distant metastasis marker of HCC.

Key words: Hepatocellular carcinoma, RT-PCR, AFPmRNA

Hepatocellular carcinoma (HCC) is common in China; about 20,000 patients died of HCC every year. One of the difficulties in management of HCC is its involvemtnt characteristic, including intrahepatic metastasis, portalvenous invasion and spreading into peripheral blood, which is the main reason for HCC recurrence and distant metastasis after HCC resected. In this study, we attempt to demonstrate in HCC patients with distant metastasis whose peripheral bloods expressed AFPmRNA, the effects and significance in the recurrence or distant metastasis of HCC after their resection.

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Correspondence to: LIU Yang, The Hospital of East Hepatobiliary Surgery, Second Military Medical University, No. 225, Chan-hai Road, Shanghai 200438, China; Phone: (0086-021)-25070837; Fax: (0086-021)-65562400; E-mail: Lsyshcn@online.sh.cn

PATIENTS AND METHODS

Patients

Patients were diagnosed by B ultrasonography, CT or MRI and AFP levels in serum (range from 600 μg/L to 300,000 μg/L). Group A: 21 HCC patients with distant metastasis (17 with lung metastasis. 4 with bone metastasis). Group B: 72 HCC patients without distant metastasis.

Methods

Five ml of whole blood was withdrawn from a peripheral vein of each subject into a heparinized tube when HCC produced a distant metastasis. We detected AFPmRNA in peripheral blood with nested RT-PCR. The detailed procedures are as follows:

Detection of AFPmRNA

Heparinized whole blood was centrifuged and the plasma fraction was removed. The cellular fraction of it was enriched for mononuclear cells or possible tumor cells according to the method described by Komeda.[1] Total cellular RNA was extracted with single-step method of RNA isolation.[2] The reverse transcription reaction was carried out in 20 μl reaction mixture using a first-strand cDNA synthesis kit (Promega USA) following the manufacture's instructions. Nested PCR was conducted by adding solution 5 μl of cDNA to 100 μl of reaction mixture containing 10 mM Tris-HC1 (*p*H 9.0), 50 mM postassium chloride, 4.5 mM magnesium chloride, 250 nM dNTP 15 pmol of each outer primer (EX-sense and EX-antisense) and 2.5 units of Taq DNA polymerase (Promega, USA). The reaction mixtures were subjected to 35 cycles of amplification in a programmable thermal cycler (Perkin-Elmer Cetus, USA) by using the following sequence: 94°C for 1.5

min, 57°C for 1.5 min and 72°C for 2.5 min, plus a final extension step at 72°C for 10 min. A sample of 10 μl of the first amplification product was further amplified using an inner pair of primers (IN-sense and IN-antisense). To verify the amplified AFPcDNA fragment, samples were digested with the restriction enzyme Pst I and analyzed by electrophoresis on a 2% agarose gel and stained with ethidium bromide for the specific bands of 174 base pairs (first amplification product) and 101 base pairs (second amplification product). Nested PCR was conducted two or three times for samples with conflicting results. The designing of external and inner pair of primers are as follows:

EX-sense 5'ACTGAATCCACAACACTGCATAG-3'
Ex-antisense 5'TGCAGTCAATGCATCTTCACCA-3'
IN-sense 5'-TGGAATAGCTTCCATATTGGATTC-3'
IN-antisense 5'-AAGTGGCTTCTTGAACAAACTGG-3'

According to the designing of Primer Pairs, the PCR products of 176 and 101 base pairs were amplified from AFPcDNA by external (EX-sense and EX-antisense) and internal (IN-sense and IN-antisense) primer pairs, respectively. The primer pairs were located as follow: EX-sense in exon 1 (AFPmRNA nucleotides 90–112), EX-antisense in exon 2 (AFPmRNA nucleotides 263~241), IN-sense over exon 1 and exon 2 (AFPmRNA nucleotides 122~145), IN-antisense in exon 3 (AFPmRNA nucleotides 222~200). cDNA sequences were based upon those previously reported.

Statistical Analysis

The relationship between the presence of AFPmRNA in peripheral blood and various clinical parameters was examined by chi-square test.

RESULTS

Expression of AFPmRNA

In all of the 21 HCC patients with distant metastasis AFPmRNA was detected (21/21, 100%). In 29 within 72 HCC patients without distant metastasis AFPmRNA was detected (29/72, 40.28%), shown as in Table 1.

Table 1. The detectable rates of AFPmRNA between HCC patients with or without distant metastasis

Style	Number	Number of AFPmRNA(+)	Positive rate
Group A	21	21	100
Group B	72	29	40.28*

Compared with group *$P<0.01$ (Group A vs Group B $P<0.01$)

Differentiation of PCR Products

The first cycle products of PCR are 174 base pairs, which can be cut into two pieces of 102 and 72 base pairs by restriction enzyme Pst I. The second cycle products of PCR are 101 base pairs, which can be cut into two pieces of 60 and 41 base pairs by restriction enzyme Pst I, shown as in Figure 1 and Figure 2.

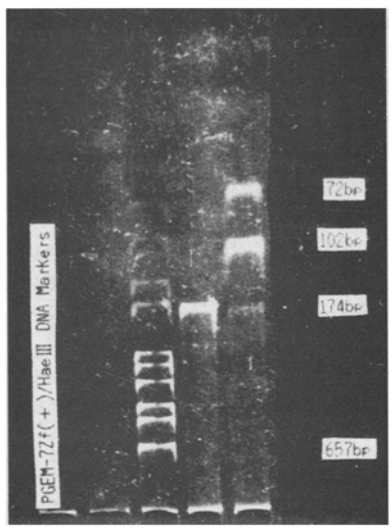

Fig. 1. The first products of PCR were cut into two pieces of 102 bp and 72 bp by nuclease Pst I.

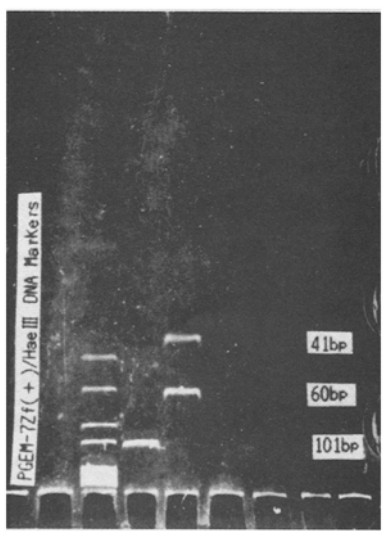

Fig. 2. The final products of PCR were cut into two pieces of 60 bp and 41 bp by nuclease Pst I.

DISCUSSION

Recently, there have been some reports of the

employment of the reverse transcriptase-polymerse chain reaction (RT-PCR) technique to detect tumor cells spreading into the peripheral blood, bone marrow, and lymph nodes.[3-5] These trials have aimed to amplify tumor-specific gene transcripts that are not detected in these tissues under normal conditions. Free mRNA is so fragile under conditions of abundant RNAse activity that the specific mRNA in blood will indicate the presence of intact cells producing such proteins just before the extraction of RNA, not circulating free mRNA.

In this study, 29 of 72 HCC without distant metastasis expressed AFPmRNA (29/72, 40.28%); the blood of 21 HCC with distant metastasis all expressed AFPmRNA (21/21, 100%), which showed the blood of HCC patients with distant metastasis all exist circulating tumor cells or micrometastasis. As Paget's theory "seeds and soils", the circulating tumor cells or micrometastasis served as "seeds", which might be an important factor in metastasis or recurrence after HCC resection.

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