GENE EXPRESSION OF TRANSFORMING GROWTH FACTOR β_1 TYPE II RECEPTOR IN HCC AND ITS CLINICAL SIGNIFICANCE

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ABSTRACT

Objective: Transforming Growth Factor-β_1 (TGF-β_1) plays a central role in the process of growth suppression of the hepatocytes, and its type II receptor (TGF-β_1R II) transfers the signal of growth suppression. In this study, the gene expression of TGF-β_1R II in HCC and its clinical significance was investigated. Methods: The expression of TGF-β_1R II mRNA in 30 cases of HCC tissue and the surrounding liver tissue was separately detected using reverse transcription-PCR. Results: The positive expression rate of TGF-β_1R II mRNA was significantly lower in HCC tissue (11/30) than that in the surrounding liver tissue (23/30) (P<0.01). Further, the less the cancer tissue expressed TGF-β_1R II mRNA, the more poorly the tumoral hepatocyte differentiated (P<0.01) and the more portal vein cancer embolus existed (p=0.0465). Conclusion: The decrease expression of TGF-β_1R II mRNA by tumoral hepatocyte results in the defect of its negative growth regulation, and this may be one of the most important reasons for its carcinogenesis and uncontrolled growth.

Key words: Hepatocellular carcinoma, Transforming growth factor-β_1 receptor II, Gene expression

Transforming growth Factor-Beta 1 (TGF-β_1) is an important negative mediator of liver cell proliferation and replication.[1] Three cell surface proteins have been identified according to their ability to bind with high affinity to TGF-β_1: type I (55 kd), type II (80 kd), type III (280 kd) receptors. Type II, possibly in conjunction with type I, is thought to be the receptor mediating the signal transduction of growth inhibition.[2] Because some tumor cell lines and certain human carcinomas have few or no TGF-β_1 receptors,[3-6] we explored and compared TGF-β_1 type II receptors (TGF-β_1 R II) in HCC tissue with the surrounding liver tissue using reverse transcription polymerase chain reaction (RT-PCR).

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PATIENTS AND METHODS

Tissue Preparation

Surgical specimens of 30 patients (24 men, 6 women; median age, 47 years) with liver cirrhosis secondary to viral hepatitis B and hepatocellular carcinoma were obtained. Immediately after removal, small pieces of the liver were taken from cirrhotic parenchyma (the surrounding liver tissue) and neoplastic nodules (HCC tissue), They were frozen and preserved in liquid nitrogen until taken out for RNA extraction. The diagnosis of HCC was confirmed by pathology.

RT-PCR

Total cellular RNA were prepared by the single-step method of acid guanidium thiocyanatephenol-chloroform extraction (AGPC).[7] RNA was washed in ethanol and dissolved in diethyl pyrocarbonate (DEPC)-treated water. Recovered RNA and its purity were assessed by optical densimeter at 260 nm and 280 nm.

Oligonucleotide primers were designed according to the sequence of TGF-β_1 R II cDNA[8] and were synthesized by Bio-synthesis Co. (American). The primer I (Forward) sequence is: 5'-TCA CTG ACA ACA ACG GTG CAG T-3' (113-134), and the primer II (Reverse) sequence is: 5'-ATA GAC CTC AGC AAA GCG ACC T-3' (756-777).

2 μg RNA of each sample was heated to 65°C for 3 minutes before cooling and adding to the next RT reaction mixture. The mixture consisted of 4 μl dNTP

(2.5 μM), 4 μl AMV-RT 5×Buffer, 2 μl DTT (0.1 M), 0.5 μl Rnasin (10 unit), 40 pmol primer II and 1 μl AMV-Rtase (5 unit), (Promega company) to a total volume of 20 μl. The reaction was allowed to proceed at 42°C for 1 hour.

The PCR mixture consisted of 2 μl of the RT reaction solution, 20 pmol of each primer, 2 μl dNTP (2mM), 2 μl 10 × Buffer and 1 unit Taq polymerase (Promega company) to a total volume of 20 μl. This reaction mixture was overlaid with 40 μl of mineral oil. PCR was carried out in a DNA thermal cycler (Perkin-Elmer Cetus) for 30 cycles; after initial denaturation by heating to 93°C for 7 minutes, each cycle consisted of denaturation at 93°C for 45 seconds, annealing at 55°C for 90 seconds and extension at 72°C for 90 seconds. An extra extension at 72°C for 5 minutes was performed after the final cycle.

10 μl of each amplification product was electrophoresed through 2% agarose gel, stained with ethidium bromide, and photographed. The specimen with a special band of 665 bp in between 515 bp and 695 bp on the electophoretogram was considered positive for TGF-β_1 R II mRNA.

RESULTS

Altogether 30 cases of HCC were examined. HCC tissues were graded according to the suggestions of Edmondson. In 30 cases of the surrrounding liver tissue, 23 cases were positive for TGF-β_1 R II mRNA. But in HCC tissue, only 11 out of 30 cases were positive. That is to say, the expression of TGF-β_1 R II mRNA in HCC tissue decreases compared with the surrounding liver tissue. (χ^2=9.77, P<0.01, Table 1). Figure 1 shows the amplified fragment of TGF-β_1 R II mRNA.

Table 1. The expression of TGF-β_1 R II mRNA in HCC tissue and the surrounding liver tissue

	TGF-β_1R II mRNA (+)	TGF-β_1 R II mRNA (−)
HCC tissue	11	19
The surrounding liver tissue	23	7

χ^2=9.77, P<0.01

In 11 positive cases of HCC tissue, 8 belonged to Edmondson I and II, and 3 belonged to Edmondson III and IV. However, in 19 negative cases of HCC tissue, only 2 belonged to Edmondson I and II, and as many as 17 belonged to Edmondson III and IV. There was statistical significance among the cancer tissue of different grades in expression of TGF-β_1 R II mRNA. (P<0.01, Table 2)

It was also found that there was a strong correlation between the expression of TGF-β_1 R II mRNA and the portal vein metastasis of tumoral hepatocyte. Portal vein cancer embolus were found in 4 out of 11 positive cases of HCC tissue, and in 15 out of 19 negative cases. (P=0.0465, Table 3)

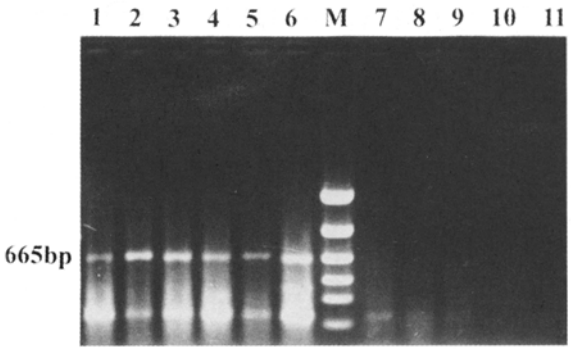

Fig. 1. The expression of TGF-β_1 R II mRNA in HCC
Lane 1-6: HCC tussue; lane 7-11: the surrounding liver tissue
M: standard DNA fragment, molecular weight is 237, 377, 515, 695, 994, 1543 bp from the bottom to the top.

Table 2. The cancer tissue of different grades in expression of TGF-β_1 RII mRNA

	TGF-β_1R II mRNA (+)	TGF-β_1R II mRNA (−)
I~II	8	2
III~IV	3	17

P<0.01

Table 3. The portal vein cancer embolus and the expresion of TGF-β_1R II mRNA in HCC tissue

	TGF-β_1R II mRNA (+)	TGF-β_1 R II mRNA (−)
embolus(+)	4	15
embolus(−)	7	4

P=0.0465

DISCUSSION

Hepatocyte growth is thought to be controlled by positive and negative growth regulation. Hepatocyte growth factor (HGF), Epidermal growth factor(EGF) and transforming growth factor-α (TGF-α) are representative hepatotrophic factors. TGF-β_1, interleukin-1 and interleukin-6 serve as growth-inhibitory factors.[9-13] TGF-β_1 seems to play a central role in the process of growth Suppression of the hepatocytes.[1,9-13] In the process of liver regeneration after hepatoectomy in rat, TGF-β_1 mRNA increases in the regenerating liver and reaches a peak (about 8 times higher than basal levels) after the major wave of hepatocyte cell division and mitosis have taken place, and this may prevent umcontrolled hepatocyte proliferation.[1]

Several types of TGF-β_1 binding proteins have

been detected at the cell surface. The cloned type II receptor is predicted to function as a transmembrane serine-threonine kinase and is required for the antiproliferative activity of TGF-β_1.[2]

It has been postulated that during the process of carcinogenesis, epithelial cells, whose proliferation normally is inhibited by TGF-β_1, may escape from an autocrine or paracrine growth control by TGF-β_1 and become autonomous. The loss of responsiveness by tumor cells to negative growth control can be achieved by a variety of mechanisms including inability to activate the laten form of the peptide, loss of cellular receptors for TGF-β_1, and loss of functional intracellular signal transduction pathways.[14]

In this study, a significant reduction in the mRNA expression of TGF-β_1 R II was demonstrated in human HCC tissue as compared with that in the surrounding liver tissue. Several tumor cell lines, such as retinoblastoma, breast tumor cell lines, gastric cancer cells and colon carcinoma cell lines, lack detectable TGF-β_1 R II or TGF-β_1 R I and show no growth inhibition of TGF-β_1.[3-6] Bedossa found a significant decrease in the expression of TGF-β_1 R II in human HCC at the protein level.[15] The downregulation of the receptor in cancer tissue compared with the surrounding liver tissue suggest that human HCC also may obtain a growth advantage by escaping the mito-inhibitory effects of TGF-β_1. TGF-β_1 R II has been considered a new tumor suppressor gene, and its mutation or loss in epithelial cells may lead to carcinogenesis.[16]

In this study, it was also found that the less the cancer tissue expressed TGF-β_1 R II mRNA, the more poorly the tumoral hepatocyte differentiated, and the more portal vein metastasis existed. Therefore, whether tumoral hepatocyte expresses TGF-β_1 R II is another biological feature of HCC. It is well known that the prognosis of HCC patients is largely dependent upon the degree of differentiation of cancer tissue or/and whether portal vein metastasis existed. So, to detect the expression of TGF-β_1 R II mRNA in cancer tissue is of referential value for the judgment of the prognosis of HCC patients, and the transfer of TGF-β_1 R II cDNA to the defective tumoral hepatocyte might become another way for gene therapy of HCC.

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