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Expression of Transforming Growth Factor-β1 mRNA in Human Hepatocellular Carcinoma

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We investigated the expression of transforming growth factor β1 (TGF-β1) mRNA in tumor tissues surgically removed from ten patients with hepatocellular carcinoma (HCC). All HCC tissues expressed TGF-β1 mRNA at different levels, indicating the presence of activated transcription of TGF-β1 gene in human HCC tissues *in vivo*. The level of TGF-β1 mRNA expression showed no relationship to main tumor size or plasma α-fetoprotein level. Some HCC tissues presenting a relatively low grade of histological differentiation showed the highest levels of TGF-β1 mRNA expression.

Key words: Transforming growth factor-β1 — Hepatocellular carcinoma

Transforming growth factor-β (TGF-β) is a potent cell growth regulator having a disulfide-linked structure with a molecular weight of 25 kD.[1-3] Five types of TGF-β have been identified,[1,4-7] and TGF-β1,[1] β2[4] and β3[5] have been cloned from human materials. TGF-β1 and β2, whose amino acid sequences show about 70% homology,[4] have different affinities for TGF-β receptors, but display equivalent biological functions,[8,9] promoting collagenesis[10] and chondrogenesis,[11] but inhibiting epithelial cell growth,[12] hematopoiesis,[13-15] adipogenesis[16] and myogenesis[17] *in vitro*.

TGF-β1, the predominant form in humans, is widely distributed in different organs,[18,19] but recent studies indicate that TGF-β1 mRNA is undetectable in normal human liver.[19] On the other hand, it has been reported that transformed and malignant cells in culture, including the human hepatoblastoma-derived cell line Hep G2, secrete abundant TGF-β into conditioned media and overexpress TGF-β1 mRNA,[18,19] suggesting a profound relationship between the production of TGF-β and carcinogenesis. However, the production of TGF-β1 by human hepatocellular carcinoma (HCC) has not yet been investigated. The aim of this study was to clarify whether human HCC tissues express TGF-β1 mRNA *in vivo*, like malignant liver epithelial cells in culture. We also examined the relation of the levels of TGF-β1 mRNA expression to the size of the main tumors, production of α-fetoprotein (AFP) and histological differentiation of the tumors.

Ten patients (6 males and 4 females; mean age, 65.0± 6.9 yr) with histologically proven HCC were included in this study. We obtained informed consent from all patients before operation. All subjects were negative for HBsAg and had no histological evidence of alcoholic liver diseases. Plasma AFP was present in all cases and the mean value was 4640 ng/ml (range, 10–39800 ng/ml). The tumors were solitary in six patients, but daughter nodules were found in the remaining four patients by angiographic examinations. The mean size of the main tumors was 51.1 mm in diameter, ranging from 27 to 185 mm. However in nine of the ten patients, the tumor sizes were smaller than 50 mm. In the case with the largest HCC (185 mm in diameter), the tumor was solitary, without daughter nodules. The underlying liver diseases were chronic hepatitis in three cases and liver cirrhosis in seven. Histological examination showed well differentiated carcinomas in four cases and moderately differentiated ones in six.

The tumor tissues were obtained at operations and were frozen in liquid nitrogen immediately after removal and preserved at −70°C until RNA extraction. Twenty micrograms of total RNA isolated by the guanidine/cesium chloride procedure from each main tumor was electrophoresed on 1% agarose gel, with PLC/PRF/5, a human hepatoma-derived cell line,[20] serving as a positive control. After blotting, the nylon filters were prehybridized in a solution of 50% formamide, 5×SSPE, 0.5% (w/v) SDS, and 5×Denhardt's solution at 42°C. Next, they were hybridized overnight with ^{32}P-labeled human TGF-β1 c-DNA (1.05 kb corresponding to λβc1, a generous gift from Dr. R. Derynck, Department of Molecular Biology, Genentech Inc.)[19] in the same solution at 42°C. Filters were then exposed to Kodak XAR film at −70°C. The signal intensity of the mRNA was

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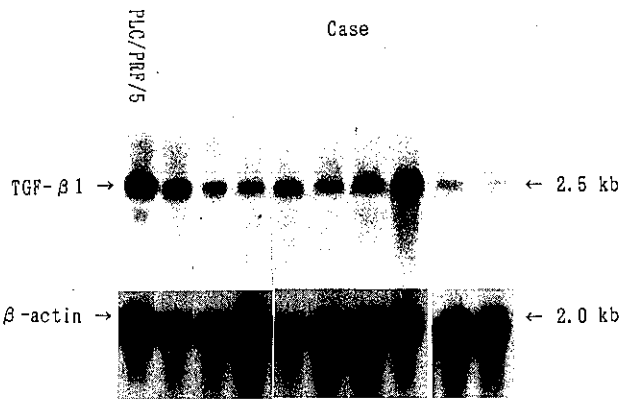

Fig. 1. TGF-β1 mRNA expression in PLC/PRF/5 cells and HCC tissues. TGF-β1 mRNA was demonstrated at the position of 2.5 kb. Hybridization with β-actin c-DNA served as an internal standard.

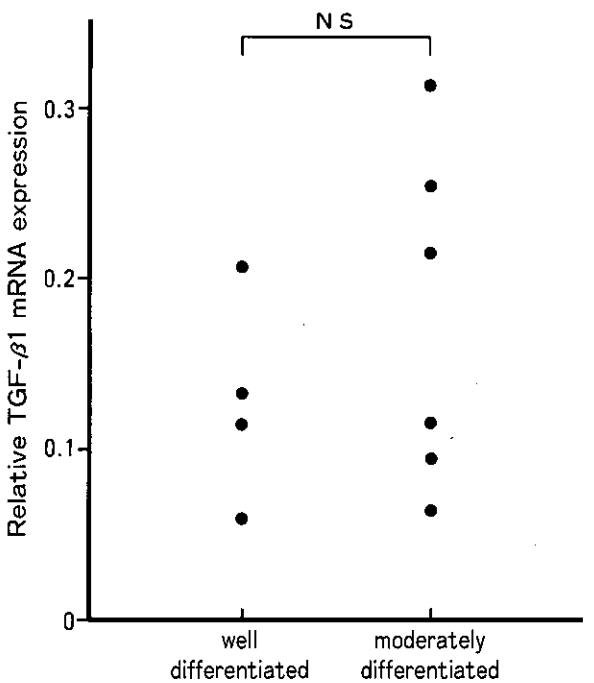

Fig. 2. Comparison of the levels of TGF-β1 mRNA expression between well and moderately differentiated HCC. NS: not significant.

analyzed by scanning the autoradiographs with a densitometer. After washing off the hybridized TGF-β1 c-DNA, the filters were rehybridized with ^{32}P-labeled β-actin c-DNA.[21] The levels of TGF-β1 mRNA expression were expressed as the ratio of the signal intensity of TGF-β1 mRNA normalized by that of β-actin mRNA (TGF-β1 mRNA/β-actin mRNA). The Mann-Whitney method was used for statistical analysis.

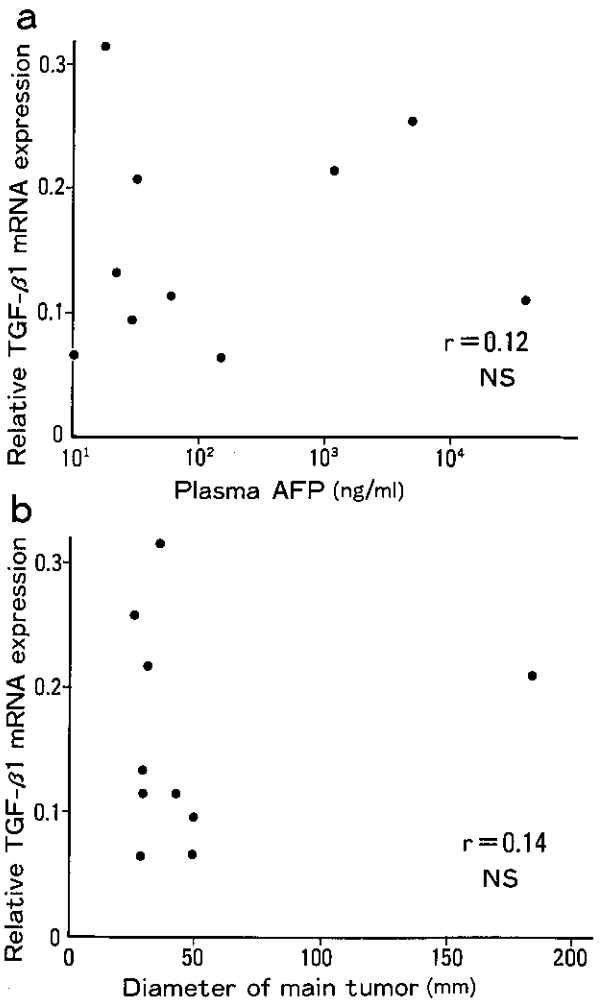

Fig. 3. Relationship between the levels of TGF-β1 mRNA expression and plasma AFP levels (a) and the size of the main tumors (b). NS: not significant.

Northern hybridization showed a fair amount of TGF-β1 mRNA expression in the PLC/PRF/5 cells (Fig. 1). The overexpression of TGF-β1 mRNA in human hepatoblastoma cell line Hep G2 has been described by Derynck *et al.*[19] In the present study, the expression of TGF-β1 mRNA was demonstrated in all tumors removed from the ten patients with HCC, indicating that malignant liver epithelial cells express TGF-β1 mRNA *in vivo*, as well as *in vitro*. This is the first demonstration of TGF-β1 mRNA expression in excised HCC tissues.

The levels of TGF-β1 mRNA expression (TGF-β1 mRNA/β-actin mRNA) varied among the cases (range,

0.06–0.31; that in PLC/PRF/5 cells was 0.24), and four cases showed high levels of TGF-β1 mRNA expression (>0.2) (Fig. 2). This variety of TGF-β1 mRNA expression implies that transcriptional regulation of TGF-β1 gene may be different in each tumor. However, little is known about the regulation of TGF-β1 gene transcription in a malignant tumor *in vivo*. Recent studies have demonstrated that TGF-β1 plays an important role in tissue differentiation and development.[22–24] Differentiation of tumor tissue may be dependent on the level of TGF-β1 gene expression. An indicator of the differentiation of a tumor is the histological grade of the cancer tissue. Histological examination showed well differentiated carcinomas in four patients and moderately differentiated ones in six patients. Although no statistically significant difference was observed in the levels of TGF-β1 mRNA expression between well and moderately differentiated HCCs (Fig. 2), it is noteworthy that three tumors of the four showing high levels of TGF-β1 mRNA expression (>0.2), excluding the one whose expression was lowest, were moderately differentiated carcinomas. This suggests that some tumors presenting a low grade of cell differentiation may express a high level of TGF-β1 mRNA.

It is well known that TGF-β1 is a strong promoter of collagenesis.[10] High expression of TGF-β1 mRNA in scirrhous-type gastric cancer has been reported,[25] suggesting a close relation of TGF-β1 to tissue fibrosis. No cases with scirrhous-type HCC, a rare histological type, were included in this study, and the role of TGF-β1 in the formation of marked fibrosis in this type of HCC remains to be clarified.

AFP has been used as a marker protein for detection of HCC. Production of AFP may indicate overall hepatic differentiation.[26, 27] However, we found that the levels of plasma AFP showed no correlation with the levels of TGF-β1 mRNA expression (Fig. 3a), even at high levels of plasma AFP (more than 100 ng/ml). The size of the main tumors also showed no relationship with the levels of TGF-β1 mRNA expression (Fig. 3b).

This study revealed activated transcription of TGF-β1 gene in all HCC tissues studied. The levels of TGF-β1 mRNA expression were not related to plasma AFP levels or the size of the main tumors. However, some HCC tissues presenting a relatively low grade of histological differentiation showed the highest levels of TGF-β1 mRNA expression.

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