

Expression of insulin like growth factor II and its receptor in hepatocellular carcinogenesis

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INTRODUCTION

Insulin-like growth factor II (IGF-II) is a mitogenic peptide of 74 kD and is mostly synthesized in fetal liver tissue. IGF-II is believed to play an important role in fetal growth and development and is involved in cellular proliferation and differentiation^[1-5]. Recently, several researchers have reported increased expression of the IGF-II gene in human hepatocellular carcinoma (HCC) and adjacent non-cancerous liver tissues^[6-10]. It is suggested that the aberrant overexpression of insulin-like growth factor II might be related to the deregulation of growth control in hepatocytes during malignant transformation via an autocrine or paracrine mechanism^[11-14]. We have designed a study using an *in situ* hybridization technique to assess the effect of IGF-II and its receptor mRNAs on the carcinogenesis and development of HCC.

MATERIALS AND METHODS

Tissue samples

Thirty surgically resected HCC specimens were collected in the Tumor Hospital affiliated to Sun-Yet Medical University during the period of 1996-1997. All the HCC specimens were classified according to Edmondson-Steiner criteria^[15]. Eighteen chronic hepatitis and 25 liver cirrhosis specimens were taken by liver biopsy. The tissues were fixed in 10% paraformaldehyde and embedded in paraffin. Continuous sections were cut for *in situ*

hybridization staining of IGF-II and IGF-IIR, respectively.

Hybridization probes

We designed the oligonucleotide probes of IGF-II and IGF-IIR. They were synthesized at the Shanghai Biochemistry Research Institute. The sequence of the oligonucleotide probes is: IGF-II, 5'-GTG-CTT-CTC-ACC-TTC-GCC-TTC-GCC-TCG-TGC-TGC-ATT-G-3'^[16] IGF-IIR, 5'-ACA-ATG-CCT-GTC-TGT-GGG-ACC-ATC-CTG-GGA-AAA-CCT-GTC-T-3'^[17].

In situ hybridization

The *in situ* hybridization protocols used are described by Tomita *et al*^[18]. Briefly, the hybridization solution contained 50% formamide, 5 × SSC, 0.15 mol/L NaCl, 0.1% degraded herring sperm DNA, 0.1% bovine serum albumin, 0.1% SDS, 0.1% polyvinylpyrrolidone (PVP), and digoxin labeled the oligonucleotide probes of IGF-II or IGF-IIR and were kept at 40°C overnight. The slides were digested prior to proteinase K (30 mg/L) treatment at 37°C for 30 minutes. After the procedures, the slides were counter stained with nuclear fast red.

Controls

The fetal liver tissues with a high level of expression of insulin-like growth factor II served as positive controls. Negative control slides were treated with PBS and were subjected to all other steps of the staining procedure.

DNA extraction and Southern-blot hybridization

Genomic DNA was prepared using the proteinase K and Phenol-Chloroform extraction method. Southern-blot hybridization was used to detect HBV-DNA integration in various kinds of liver tissues^[19].

Statistical analysis

Chi-square test was used for significance analysis. The difference was regarded as significant if *P* value was less than 0.05.

RESULTS

Expression of IGF-II and IGF-IIR mRNAs in chronic hepatitis, liver cirrhosis and HCC tissue

In normal adult human liver sections and negative control slides, IGF-II and IGF-IIR mRNAs were

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not detected exclusively in the hepatocytes. However, in normal fetal liver tissue, very strong signals of IGF-II and IGF-IIR mRNAs were detected. The positive or ratios of IGF-II and IGF-IIR mRNAs increased stepwise starting with chronic hepatitis (33.3%), then HCC (66.7%) and finally liver cirrhosis (72.0%). There was a statistically significant difference in IGF-II and IGF-IIR mRNAs expression between chronic hepatitis and liver cirrhosis ($\chi^2 = 6.34$, $P < 0.05$) or HCC ($\chi^2 = 5.04$, $P < 0.05$). No significant difference of IGF-II and IGF-IIR mRNAs expression was observed between liver cirrhosis and HCC ($\chi^2 = 0.29$, $P > 0.05$). The positive expression of IGF-II and IGF-IIR mRNAs was anchored locally in hepatocellular cytoplasm in a diffuse pattern. The signal intensity of IGF-II and IGF-IIR mRNAs was found to vary with cell type. The lower signal intensity was detected in chronic hepatitis sections, whereas the stronger signal intensity was detected in liver cirrhosis and HCC tissue. An intensive signal intensity was detected in liver regenerative nodules, liver cell dysplasia (LCD) and poorly differentiated HCC cells.

Expression of IGF-II and IGF-IIR mRNAs in differently differentiated HCC tissue

Expression of IGF-II and IGF-IIR mRNAs in HCC tissue of Edmondson grades I-IV were 0, 25.0%, 76.2% and 100.0% respectively. The poorly differentiated tissues (Edmondson grades III and IV) showed higher positive ratios than the well-differentiated tissues (Edmondson grades I and II). (comparison among all groups: $\chi^2 = 9.48$, $P < 0.05$).

Relationship between expression of IGF-II/IGF-IIR mRNAs and HBV-DNA integration ratios

HBV-DNA integration ratios in chronic hepatitis, liver cirrhosis and HCC tissues were 61.1% (11/18), 84.0% (21/25) and 83.3% (25/30), respectively. The positive ratios of HBV-DNA integration in chronic hepatitis, liver cirrhosis and HCC tissues were 54.5% (6/11), 81.0% (17/21) and 76.0 (19/25), respectively. The negative ratios of HBV-DNA integration in chronic hepatitis, liver cirrhosis and HCC tissues were 0% (0/7), 25.0% (1/4) and 20.0 (1/5), respectively. These data showed that IGF-II and IGF-IIR mRNAs expression was closely related to HBV-DNA integration.

DISCUSSION

The occurrence of HCC is a process which involves multiple-steps^[20,21]. Chronic hepatitis, liver cirrhosis and liver cell carcinogenesis are three important different steps in the occurrence of HCC.

Our study demonstrated that IGF-II and IGF-IIR mRNAs were both abnormally expressed in chronic hepatitis, liver cirrhosis and HCC tissue. The expression ratio of IGF-II and IGF-IIR mRNAs in liver cirrhosis was the highest, while that in chronic hepatitis was the lowest. The expression of IGF-II and IGF-IIR mRNAs in liver cirrhosis and HCC was remarkably higher than that in chronic hepatitis. The results suggest that IGF-II and IGF-IIR participate in the process of HCC development at different stages^[22,23].

IGF-II and IGF-IIR were expressed at low levels in chronic hepatitis. With the progression of chronic hepatitis to liver cirrhosis, the expression of IGF-II and IGF-IIR increased significantly (especially in regenerative nodules and of liver cell dysplasia). Not only the number of positive cells increased, but also the intensity of the positive signal, which suggests that their over-expression was related to the formation of liver cell dysplasia and liver cell regenerative nodules^[24,25]. It is generally accepted that regenerative nodules and liver cell dysplasia are pre-cancerous pathological changes of HCC^[26]. The over-expression of IGF-II and IGF-IIR in the pre-cancerous pathological changes of HCC suggests an early event in hepatocellular carcinogenesis. IGF-II and IGF-IIR possibly may play important roles at the early stage of hepatocellular carcinogenesis^[27].

In the developmental process of chronic liver disease, repeated inflammation and necrosis of liver cells occurs, which leads to over-expression of IGF-II and IGF-IIR. All these stimulate the abnormal proliferation of liver cells, which creates conditions for the formation of regenerative nodules and liver cell dysplasia. The hepatocytes of liver cell dysplasia and regenerative nodules proliferate actively. IGF-II and IGF-IIR were highly expressed in the two kinds of pre-cancerous pathological changes of HCC, which further stimulates the abnormal proliferation of pre-cancerous liver cells and increases the mutation rate of pre-cancerous liver cells^[28]. Their high expression could also induce the malignant transformation of pre-cancerous hepatocytes under certain conditions and eventually lead to hepatocellular carcinogenesis^[29-31]. If the over-expressions of IGF-II and IGF-IIR are related to the abnormal proliferation of pre-cancerous hepatocytes, they could be regarded as markers for abnormal proliferation of pre-cancerous hepatocytes^[32-34]. The pre-cancerous lesions with high expression of IGF-II and IGF-IIR may be high risk factors of carcinogenesis^[35,36].

The expressions of IGF-II and IGF-IIR were related to the differentiation of HCC. Stronger positive signal were detected in poorly differentiated HCC cells. The expression of IGF-II and IGF-IIR in poorly differentiated HCC cells were significantly

higher than that in well differentiated HCC cells. All these magnify the signal transduction, stimulating cells to grow persistently what accelerates the growth of HCC cells and maintained the malignant phenotype of HCC cells^[37-41]. If the expressions of IGF-II and IGF-IIR are related to the differentiation of HCC cells, it IGF-II and IGF-IIR may be regarded as a marker for HCC differentiation, which could contribute to better determination of prognosis for patients with HCC^[42].

We observed that the expressions of IGF-II and IGF-IIR mRNA in liver cirrhosis were higher than that in HCC. This observation is similar to results reported by Yang Dong-Hua^[43]. The over-expressions of IGF-II and IGF-IIR mRNAs were mainly detected in regenerative nodules and liver cell dysplasia. Their over-expressions provided necessary conditions for the malignant evolvement of pre-cancerous liver cells. Once the pre-cancerous liver cells became malignant, they got auto-growth ability and did not simply depend on the high expression of IGF-II and IGF-IIR to maintain their malignant proliferation. Although the expressions of IGF-II and IGF-IIR in HCC tissue were less than that in pre-cancerous liver cirrhosis tissue, when compared with normal more redundancy hepatocytes and non-pre-cancerous liver tissue, the expressions of IGF-II and IGF-IIR in HCC tissue were higher. So the expressions of IGF-II and IGF-IIR in liver cirrhosis tissue were higher than that in HCC tissue.

Human HCC frequently occurs in patients with chronic hepatitis B virus (HBV) infection^[44-46]. However, the exact molecular biological mechanism by which HBV leads to HCC is still unknown. HBV-DNA integration was reported in 80%-90% of HCC tissue derived from HBV infected patients, which would lead to the deletion, rearrangement, translocation or replication of cellular DNA at the integration site^[47,48]. Therefore, the integration of HBV-DNA might play an important role in pathogenesis of some HCC cases. The 11th chromosome is the main site for HBV-DNA integration. The IGF-II gene is located on the short arm of 11th chromosome^[49]. Hence, the integration of HBV-DNA on ¹¹P chromosome was probably related to the aberrance of IGF-II gene and therefore lead to abnormal over-expression of IGF-II^[50]. Another possibility is that the instability of genome DNA caused by chronic liver disease interrupts the normal regulating sequence of IGF-II gene and therefore leads to the replacement, deletion and transition of IGF-II gene, leading to the persistent over-expression of IGF-II and eventually resulting in hepatocellular carcinogenesis. Our data indicates that the over-expressions of IGF-II and IGF-IIR were significantly higher with than without HBV-DNA

integration in liver disease tissue. However, the accurate regulating mechanism and possible motivating or initiating relationship between over-expression of IGF-II/IGF-IIR and HBV-DNA integration remained obscure.

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