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ORIGINAL RESEARCH

Expression of insulin-like growth factor Ⅱ and its receptor in liver cells from patients with chronic liver diseases

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Abstract

AIM: To study the relationship between insulin-like growth factor Ⅱ (IGF-Ⅱ), IGF-Ⅱ receptor, and chronic liver diseases and to investigate the clinical mechanisms of human hepatocellular carcinoma (HCC) development.

METHODS: We analyzed IGF-Ⅱ and IGF-Ⅱ receptor poly (A)+ mRNA in dysplasia liver cell (DLC; n = 10), liver cirrhosis (LC; n = 9), and chronic active hepatitis (CAH; n = 9) specimens by Northern blot using human IGF-Ⅱ and IGF-Ⅱ receptor DNA probes labeled with ^{32}P through nick translation.

RESULTS: Expression of IGF-Ⅱ in DLC samples (10/10, 100%) was higher than in CAH (3/9, 33%) and LC samples (3/9, 33%) ($P < 0.01$). Expression of IGF-Ⅱ receptor in DLC samples (7/10, 70%) was significantly higher than in CAH (2/9, 22%) and LC samples (3/9, 33%). Data on hepatitis B virus (HBV) infection status from different chronic liver disease samples were also analyzed.

CONCLUSION: Overexpression of IGF-Ⅱ and IGF-Ⅱ receptor in DLC samples was associated with a preceding step to malignant phenotype hepatocyte transformation and may be of diagnostic value for early detection of hepatocellular carcinoma (HCC). Persistent HBV infection was strongly associated with abnormal IGF-Ⅱ and IGF-Ⅱ receptor mRNA expression, suggesting that an autocrine or paracrine mechanism is involved in the regulation of growth in liver cell carcinogenesis.

Key words: Insulin like growth factor Ⅱ; Receptors; Carcinoma; Hepatocellular; Hepatitis; Liver neoplasms; Liver cirrhosis; Liver diseases

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INTRODUCTION

The role of insulin-like growth factor Ⅱ (IGF-Ⅱ) and IGF-Ⅱ receptor in liver cell carcinoma progression was investigated by examining the transcriptional expression of IGF-Ⅱ and IGF-Ⅱ receptor in chronic active hepatitis (CAH), liver cirrhosis (LC), and dysplasia liver cell (DLC) samples by Northern blot. In addition, data on hepatitis B virus (HBV) infection status from the different groups of chronic liver disease samples were analyzed. Our results suggest that detection of IGF-Ⅱ and IGF-Ⅱ receptor mRNA expression may be useful for early diagnosis and in gene therapy studies of hepatocellular carcinoma (HCC).

MATERIALS AND METHODS

Tissue specimens

Specimens from 10 patients with DLC (7 men and 3 women; mean age: 45 years) were surgically obtained. Specimens from 9 patients with CAH (5 men and 4 women; mean age: 38 years) and from 9 cases with LC (8 men and 1 woman; mean age: 42 years) were obtained by liver tissue biopsy. Normal human liver specimens were obtained from two accidental death cases. Specimens were immediately frozen and stored at -80 ℃ until analysis.

mRNA extraction and DNA probe preparation

Recombinant IGF-Ⅱ and recombinant IGF-Ⅱ receptor plasmid DNA were generated as previously described and digested with restriction endonucleases *Pst* I and *Eco*R I to isolate IGF-Ⅱ (0.7 kb) and IGF-Ⅱ receptor (5.1 kb) DNA fragments[1]. These fragments were

Table 1 Hepatitis B virus markers in serum from different liver disease cases

Groups	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc
Dysplasia liver cell	10	0	0	2	8
Chronic active hepatitis	9	1	1	1	6
Liver cirrhosis	9	0	1	1	7
Total	28	1	2	4	21

labeled with α-^{32}P-dATP by nick translation. The specific activities of the labeled IGF-Ⅱ and IGF-Ⅱ receptor DNA probes were 4.2 × 10^7 cpm/μg and 5.6 × 10^7 cpm/μg, respectively. mRNA extracted from the different liver disease specimens was loaded (10 μg) into 1% agarose gels, electrophoresed, and blotted into membranes. Membranes were hybridized with radiolabeled probes and autoradiographed after 48 h at -70 ℃.

Detection of HBV infection markers

HBV infection markers in serum from the three groups of chronic liver disease cases were examined by ELISA.

RESULTS

IGF-Ⅱ expression

IGF-Ⅱ was differentially expressed at the mRNA level in specimens from the three different groups. In CAH, 2 cases showed moderate and 1 case showed mild IGF-Ⅱ mRNA expression (3/9, 33%); in LC cases, 3/9 (33%) showed moderate IGF-Ⅱ mRNA expression; in DLC, 6 cases presented high and 4 cases showed moderate IGF-Ⅱ mRNA expression (10/10, 100%). In 1 of the samples from normal liver tissue a mild signal of IGF-Ⅱ mRNA hybridization could be detected.

IGF-Ⅱ receptor expression

IGF-Ⅱ receptor mRNA level was moderate in 2/9 (22%) CAH cases. In LC, 2 cases showed moderate and 1 case showed mild IGF-Ⅱ receptor mRNA expression (3/9, 33%) and in DLC cases, high expression was observed in 5 cases and mild expression in 2 cases (7/10, 70%). No distinct signal of IGF-Ⅱ receptor mRNA hybridization was detected in normal liver tissue samples.

HBV infection status

We analyzed HBV infection status of the different chronic liver disease cases by detection of HBV markers in serum (Table 1).

DISCUSSION

Overexpression of IGF-Ⅱ and IGF-Ⅱ receptor mRNA was observed in human HCC cancer cells and surrounding liver tissue, suggesting that liver cancer cells might produce IGF-Ⅱ factors in large amounts and, through an autocrine or paracrine mechanism, stimulate IGF-Ⅱ receptor expression in cancer cells or neighboring liver cells, therefore accelerating or amplifying the growth of liver cancer cells[2-4]. Expression of a fetal isoform of IGF-Ⅱ mRNA was remarkably higher in pericancerous liver tissues than in liver cancer tissues. This observation reflects the abnormal activation of a second promoter controlling *IGF-Ⅱ* gene expression in precancerous hepatocytes and could be responsible for the persistent proliferation of hepatocytes, leading to the development of liver cancer.

We showed moderate and high expression of the IGF-Ⅱ mRNA in DLC samples, suggesting that liver cell dysplasia may characterize cells with potential cancerous activity and ability to transform into a malignant phenotype. Persistent overexpression of the *IGF-Ⅱ* gene could indicate the preceding steps to the malignant transformation of liver cells. Therefore, the study of IGF-Ⅱ expression may be important for early stage diagnosis of HCC. IGF-Ⅱ receptor is a transmembranous glycoprotein present in the cytoplasm and Golgi apparatus of hepatocytes. It binds to IGF-Ⅱ with high affinity, which might promote the proliferation of liver cancer cells. IGF-Ⅱ binds not only to the IGF-Ⅱ receptor but also to the IGF-Ⅰ receptor, which belongs to the tyrosine kinase family of receptors and plays a role in promoting the division and growth of cells. The DNA sequence of the IGF-Ⅱ receptor gene is 99.4% homogenous with that of the cation-independent mannose 6 phosphate receptor gene, which is associated with autocrine growth of cancer cells. The binding of IGF-Ⅰ to IGF-Ⅰ and -Ⅱ receptors suggests a multi-pathway mechanism for the transmission of signals stimulating cell growth, which would accelerate the transformation of precancerous liver cells into their malignant counterparts. Our observation that 7/10 DLC cases express mild to high IGF-Ⅱ receptor mRNA levels suggests that the IGF-Ⅱ receptor is involved in the dysplasia of liver cells.

HBV infection is closely associated with the development of human HCC. Serum HBsAg was positive in samples from the three different chronic liver disease groups, which overexpressed IGF-Ⅱ and its receptor to different extents. These results suggest that HBV infection is involved in the degeneration, necrosis, or cirrhosis of liver cells, which leads to the abnormal activation of the *IGF-Ⅱ* gene and its receptor, stimulating aberrant division and unlimited growth of liver cells via the autocrine or paracrine system.

We suggest testing the use of IGF-Ⅱ RNA in gene therapy studies for the treatment of human HCC. Introduced sense or antisense IGF-Ⅱ mRNA may potentially reduce abnormal activation of HBV, suppress the biological activation of IGF-Ⅱ and its receptor, and retard the progress of malignant transformation in liver cells.

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