

EXPRESSION OF INSULIN-LIKE GROWTH FACTOR II (IGF-II) IN HUMAN HEPATOCELLULAR CARCINOMA AND LIVER CIRRHOSIS: ITS RELATIONSHIP WITH HEPATITIS B VIRUS X PROTEIN EXPRESSION*

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Sixty cases of hepatocellular carcinoma (HCC) and 47 cases of liver cirrhosis (LC) were examined with immunocytochemistry method using antibodies against IGF-II and HBxAg on formalin-fixed, paraffin-embedded tissue sections. 32 HCC and 37 LC were found to be positive to HBxAg, in which the positive rates of IGF-II were 100% (32/32) and 94.6% (35/37) respectively. 28 HCC and 10 LC were found to be HBxAg negative, IGF-II was positive in 23 HCC (83.1%) and 6 LC (60%). The positive expression rates of IGF-II in HBxAg positive tissues were significantly higher than those in HBxAg negative tissues ($P<0.05$). There were three types of distribution of IGF-II expression in HCC and LC: (1) perinucleus; (2) diffuse in cytoplasm; (3) inside nucleus. IGF-II was highly expressed in most of hyperplastic and neoplastic nodules hepatocytes and some of regeneration nodules. Small polygonal liver cells (SPLCs) were found in the liver tissues surrounding the tumor and cirrhosis and they were positive to both IGF-II and HBxAg. The positive rates of IGF-II in SPLC were 86.4% (38/44) in the HBxAg-positive tissues and 40.5% (15/37) in the HBxAg-negative tissues. The above findings suggest that IGF-II plays an important role in abnormal proliferation of HCC and SPLC. The relation between IGF-II and

HBxAg and the nature of SPLCs are also discussed.

Key words: Liver neoplasms, Liver cirrhosis, Insulin-like growth factor II, Hepatitis B virus antigens, Immunocytochemistry.

Insulin-like growth factor II (IGF-II) is mitogenic peptide of 47 kDa^{1,2} and it is expressed in fetal liver and believed to play an important role in the proliferation and differentiation of embryonic hepatocytes.³⁻⁵ Recent studies demonstrated that IGF-II and some oncogenes were overexpressed in hepatomas and pericancerous liver tissues.⁶⁻⁹ Hepatocellular carcinoma (HCC), one of the most common human cancer, frequently occurs in patients who have pre-existing chronic hepatitis B virus (HBV) infection.^{10,11} Individuals with chronic HBV infection have been shown to have a 200-fold greater risk of developing HCC than age-matched noninfected controls.¹¹ Essentially high percentage of HCC occurring in HBV infected patients contain integrated HBV DNA. But up to date no direct relationship have been found between the activation of oncogenes and HBV integration.^{12,13} We investigated the expression and distribution of IGF-II in human HCC and liver cirrhosis (LC) by means of immunocytochemical techniques and preliminary study has also been finished to disclose the relationship between

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IGF-II expression and the expression of HBV viral X protein.

MATERIALS AND METHODS

Samples

sixty cases samples of HCC (34 cases with pericancerous liver tissues) and 47 cases of LC were collected from Department of Pathology of Xijing Hospital, The Fourth Military Medical University. All the hepatomas were classified according to Edmondson-Steiner criteria¹⁴ and cirrhotic liver samples were typed in accordance with the criteria of WHO.

Reagents

Rabbit antibody to IGF-II was kindly provided by Dr. Charles E. Rogler (Marion Bessin Liver Research Center, USA); rabbit antibody against HBV X protein (HBxAg) was kindly donated by Dr. Mark Feitelson (Fox Chase Cancer Center, USA). ABC kit was purchased from Victor Laboratories, Inc. (USA). Peroxidase anti-peroxidase (PAP) complex reagents were from Immunocytochemical Laboratory of Fourth Military Medical University.

Immunocytochemistry

All the tissue sections in this research were stained using combination methods of PAP and ABC techniques reported by Davidoff M. et al.¹⁵

Control Examinations

Normal control: 5 cases of liver tissues of autopsy with normal structure and negative reaction for immunological tests of HBV antigens and antibodies in the clinical were employed in this research. Negative control: the primary antibodies were replaced by PBS and EHFV antibody respectively in the reactions.

Statistics Analyses

The data of positive rates of IGF-II and HBxAg were compared by χ^2 test and the data of the intensities of positive staining were treated with Rigit analysis.

RESULTS

Distributions of IGF-II Expression in HCC and LC

The positive cells of IGF-II immunostaining could be found in the tissues of HCC and LC. In neoplastic nodules the numbers of positive cells of IGF-II expression was great higher than that in the pericancerous liver tissues and LC.

There were three patterns of distribution of IGF-II expression in HCC and LC tissues; (1) inclusionlike pattern: the positive materials were concentrated nearby the nucleus (Figure 1); (2) diffuse pattern: positive signals were diffused in the cytoplasm (Figure 2); (3) inside-nuclear pattern: a few samples (2 cases HCC and 3 cases LC) were showed to be positive staining in the nucleus (Figure 3).

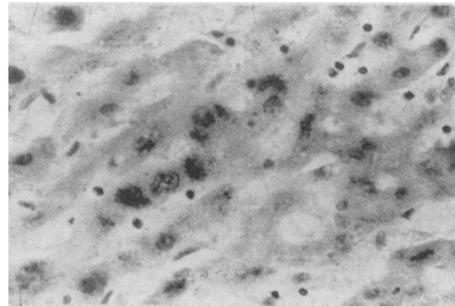


Fig. 1. IGF-II is localized as inclusionlike pattern in paranuclear area in dysplastic hepatocytes in pericancerous liver tissue. PAP-ABC staining $\times 600$

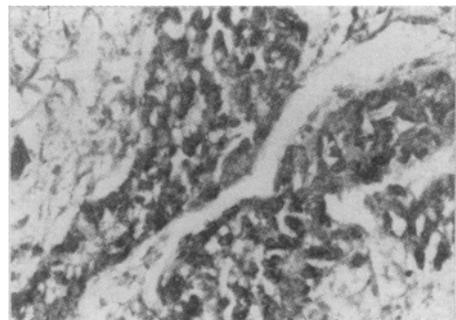


Fig. 2. IGF-II is showed to be positivity expression in neoplastic nodules of HCC as diffuse pattern. PAP-ABC staining $\times 600$

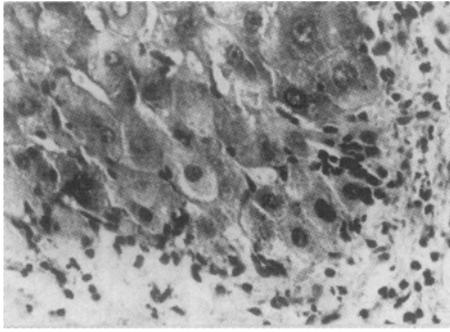


Fig. 3. IGF-II is positive expression in the tissues of cirrhotic liver and its positive matters are positively stained in nucleus. PAP-ABC staining $\times 600$

In the pericancerous liver tissues and LC, some polyploid and multinuclear giant hepatocytes and some proliferation ductules were found to be IGF-II positive staining and most of hyperplastic nodules were also stained strongly positive for IGF-II, in which many dysplastic hepatocytes could be found (Figure 1, 3).

Distributions of HBxAg Expression and its Relationship to the Positive Tissues of IGF-II Expression in HCC and LC

Immunostaining results showed that HBxAg could be detected in the tissues of HCC and LC. The positive intensity of HBxAg staining in the pericancerous liver tissues was stronger than did in the tumor cells of HCC (Figure 4). The relation between HBxAg and IGF-II expressions was displayed at Table

Of 47 cases LC, 37 cases showed HBxAg positive staining, in which 35 cases were also positive for IGF-II (94.6%). 10 cases LC being HBxAg negative detection, 6 cases were found to be IGF-II positive (60%). It was significant different for IGF-II expression between the positive and negative tissues of HBxAg expression ($P < 0.05$, χ^2 test).

A kind of small liver parenchymal cell, which we named small polygonal liver cells (SPLCs),¹⁶ was positively stained for both HBxAg and IGF-II in LC and pericancerous liver tissues (Figure 5, 6). In SPLCs being HBxAg positive expression, the positive rate of IGF-II expression was 86.36% (38/44), but

40.54% (15/38) of SPLCs detected negatively for HBxAg were found to express IGF-II. The positive expression rate of IGF-II in HBxAg-positive SPLCs was much higher than it in HBxAg-negative SPLCs ($P < 0.01$, χ^2 test).

Table 1. The positive rates of IGF-II expression in HBxAg-positive and HBxAg-negative tissues of HCC.

Edmondson-Steiner grade	Cases	HBxAg (+)	HBxAg (-)
		IGF-II (+)	IGF-II (+)
I	7	3	4
II	36	20	11
III	10	5	5
IV	7	4	3
Total (%)	60	32 (100) [*]	23 (82.1)

$P < 0.05$ HBxAg (+) group versus HBxAg (-) groups (χ^2 test).

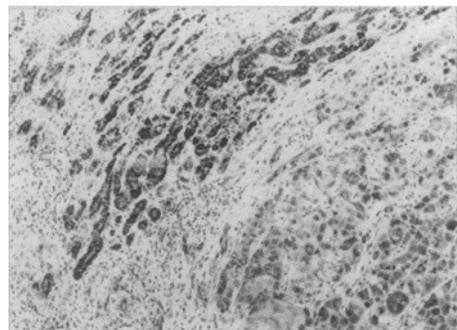


Fig. 4. HBxAg are positively expressed in the tissue of pericancerous liver and HCC. The pericancerous liver tissue is much more strongly labeled by HBxAg antibody than cancer tissue. PAP-ABC staining $\times 200$

The Positive Intensities of IGF-II and HBxAg Staining in HCC

The positive intensities of IGF-II and HBxAg staining with immunocytochemistry were classified in four classes in 60 cases HCC: (-) negative: no definite staining; (+) weakly positive: stained yellow; (++) medium positive: yellow-brown or brown; (+++) strong positive: dark brown. The comparison of

staining intensities of IGF-II and HBxAg in HCC was showed in Table 2.

Table 2. The comparison of IGF-II and HBxAg positive intensities stained with immunocytochemistry in 60 HCC

Edmondson-Steiner grade	IGF-II				HBxAg			
	-	+	++	+++	-	+	++	+++
I	0	4	3	0	5	1	1	0
II	6	11	14	5	12	19	2	0
III	0	2	5	3	3	4	2	0
IV	0	3	3	1	4	2	1	0

Right analysis: In Edmondson-Steiner grade II and III, IGF-II staining intensity was significantly higher than HBxAg ($P<0.05$); no definite statistic different between I and IV grades ($P>0.05$).

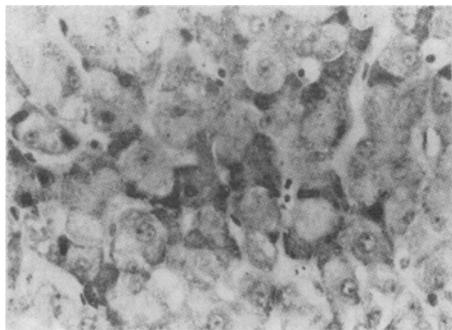


Fig. 5. SPLCs are showed to be IGF-II positivity expression in hyperplastic nodules of cirrhosis liver tissue. PAP-ABC staining $\times 600$

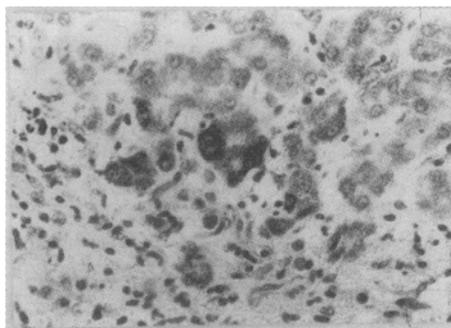


Fig. 6. SPLCs in hyperplastic nodules of pericancerous liver tissue are also found to be HBxAg positive. PAP-ABC staining $\times 600$

All of control sections were shown to be negative reactions to IGF-II and HBxAg immunostaining.

DISCUSSION

The genesis of human HCC has been found to be closely related to persistent HBV infection; However, the mechanism of the HBV induced hepatocyte transformation is yet unknown. Some investigator have thought that HBV DNA integration into human hepatocytes genome to be essential step, but no common integration site of HBV within the HCC genome has been found among groups of HCC patients^{10 13} and the integration of HBV was later shown to be a random event.⁷ The HBV X protein (HBxAg) was considered to be a important regulator with transactivation effect during the hepatocyte transformation.¹⁷⁻¹⁹ The numbers of oncogenes including IGF-II were overexpressed in HCC and pericancerous tissues, in which HBxAg was also expressed.^{6-9,20} The results in this research showed, the positivity rate of IGF-II expression in HBxAg-positive-expression tissues was significantly higher than that in HBxAg-negative-expression tissues in HCC and LC. In poor-differented HCC tissues (Edmondson-Steiner II, III grades) 60 cases of hepatocellular carcinoma (HCC) and 47 cases the expression abundant of HBxAg was slight but IGF-II was stronger. Therefor, in the benign liver disorders (LC and pericancerous liver tissues) IGF-II expression was weak but HBxAg was higher. Rogler et al.²¹ had investigated the woodchuck hepatitis virus (WHV), which is similar with HBV in structure and biologic functions, they thought, WHV might be a promoter to transforming hepatocytes through *hit and run* mechanism. In another hand, WHV would escaped from the target cells after hepatocytes transforming have been induced. In this research, it was revealed that IGF-II expression was closely related to HBV X protein expression both in benign and malignant liver tissues. We suggest that HBV X protein can active IGF-II gene by way of its transactivation effects^{17,18} and take part in human hepatocarcinogenesis.

Distribution types of IGF-II expression in the positive cells were mainly inclusionlike and diffuse patterns in cytoplasm. In some HCC tumor cells and some hepatocytes in LC, IGF-II was observed to accumulate in a paranuclear area, corresponding to the

Golgi region.²² Whether IGF-II molecules are synthesized and modulated here or bound to IGF-II/mannose-6-phosphate receptors that have been demonstrated to be concentrated in this area.²³ The diffuse distribution of IGF-II polypeptide was showed in the dysplastic hepatocytes of the regenerative nodules in LC and pericancerous hepatocytes and tumor cells in HCC, which seemed to be morphological sign of IGF-II overexpression. It was interesting that IGF-II was expressed inside nucleus of hepatocytes in a few cases of LC and HCC. The functions and mechanisms of IGF-II expression inside nuclear were unclear and the further study will be needed.

The morphological characteristics of small polygonal liver cells (SPLCs) demonstrated that they were one kind of immature hepatocyte and localized in LC and chronic activity hepatitis described by Liu et al.¹⁶ SPLCs might be a sort of epithelial stemlike cells differentiating toward typical hepatocytes.⁹ In this research, most of SPLCs were not only found to express both IGF-II and HBxAg but the expressions of these polypeptides were also showed to be closely related. SPLCs, in our opinion, may be an new type of juvenile and active proliferating hepatocytes in the chronic liver disorder with HBV infection and they were attacked by HBV more easily. The role of SPLCs in LC and BCC and it relationship with human hepatocarcinogenesis will be continued to study.

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