

Activation of c-Yes in hepatocellular carcinoma: A preliminary study

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Supported by Grants-in Aid for Scientific Research (C-17590649) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Alumni Association of Faculty of Medicine, Kagawa University

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Received: 2006-03-31 Accepted: 2006-04-24

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Key words: Hepatocellular carcinoma; Tyrosine kinase; c-Yes; Oncogene

Feng H, Masaki T, Nonomura T, Morishita A, Jian G, Nakai S, Deguchi A, Uchida N, Himoto T, Iwama H, Usuki H, Wakabayashi H, Izuishi K, Yoshiji H, Kurokohchi K, Kuriyama S. Activation of c-Yes in hepatocellular carcinoma: A preliminary study. *World J Gastroenterol* 2006; 12(35): 5743-5745

<http://www.wjgnet.com/1007-9327/12/5743.asp>

TO THE EDITOR

Hepatocellular carcinoma (HCC) is thought to develop through a multistep process^[1]. A long history of viral

hepatitis or prolonged exposure to environmental toxins predisposes liver cells to mutations of the genes critical in the control of hepatocyte growth. In fact, both activation of cellular oncogenes and inactivation of tumor-suppressor genes are involved in the development of HCC. Activation of oncogenes by hepatitis virus integration has been shown in the woodchuck animal model^[2], although the significance of this finding in human hepatocarcinogenesis is still under investigation. Tyrosine kinases, though a minor class of cellular protein, represent a major class of oncogenes. These tyrosine kinases are classified into two major groups^[3,4]. The first is receptor-type protein tyrosine kinases, the second is non-receptor type tyrosine kinases. The main representatives of the latter group are non-receptor-linked and membrane-associated Src family tyrosine kinases. At least nine Src-related tyrosine kinases have been identified thus far, including c-Yes, c-Src, c-Lck, c-Fyn, c-Hck, c-Lyn, c-Blk, c-Fgr and c-Yrk protooncogene products. The cellular oncogene c-Yes, a member of the Src family, encodes a 62-kilodalton, cytoplasmic and membrane-associated protein-tyrosine kinase^[5]. c-Yes expression and its kinase activity have been shown to be increased in colorectal cancer^[6,7], melanoma^[8] and metastatic liver cancer^[9]. However, the activation of c-Yes in human HCC has not yet been investigated at all. In the present study, we determined the activity of c-Yes both in the normal liver (NL) tissues and in chronic hepatitis (CH), tumorous (T) and adjacent nontumorous (N) cirrhotic liver tissues. This is the first report on the activity of c-Yes in various liver diseases including HCC.

Tissue samples, including the tumorous and surrounding nontumorous cirrhotic tissues, were obtained during surgery from 9 patients with HCC (7 males and 2 females; mean age, 68.2 ± 4.4 years; range, 59-73 years). All the patients were positive for hepatitis C virus (HCV), as determined by the reverse-transcriptase polymerase chain reaction method (Amplicor, Roche Diagnostics Ltd). The number of patients with well-, moderately- and poorly-differentiated HCC was 2, 5 and 2, respectively. The fibrosis stage of the surrounding liver tissues was assessed as cirrhosis in all the HCC cases. Liver tissue samples from three patients with HCV-induced CH were obtained by liver biopsy. During surgery, two normal liver tissue samples were also obtained from patients with liver metastases of colon cancer. The serum samples of these patients with NL were negative for HCV and hepatitis B virus (HBV).

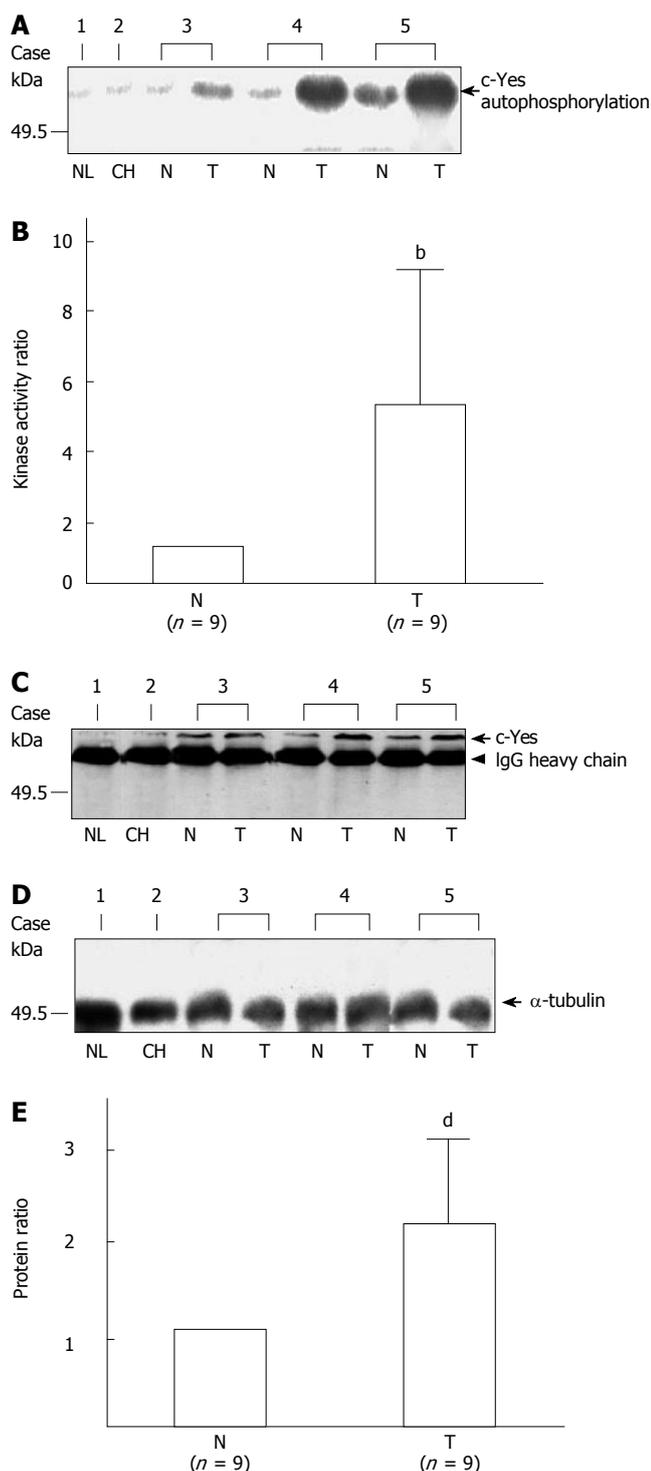


Figure 1 Activities and levels of c-Yes kinase in different liver tissues. **A:** Activity of c-Yes in normal liver (NL), chronic hepatitis (CH), nontumorous cirrhotic (N) and tumorous (T) tissues. The arrow indicates the band corresponding to c-Yes autophosphorylation; **B:** Relative levels of total c-Yes activity (mean \pm SE, Student's *t*-test, $^bP < 0.001$); **C:** Levels of c-Yes in the NL, CH, N, and T portions of HCC. The arrow and arrowhead indicate the bands corresponding to c-Yes protein and the heavy-chain of the c-Yes antibody, respectively; **D:** Level of α -tubulin in lysate containing 100 μ g of cellular protein used in the immunoprecipitation (arrow); **E:** Relative levels of c-Yes protein in T and N tissues (mean \pm SE, Student's *t*-test, $^dP < 0.01$).

To determine the protein kinase activity of c-Yes in the NL, CH, N and T portions of HCC, we prepared lysates containing 100 μ g of cellular protein of tissue samples, precipitated the protein with a monoclonal antibody specif-

ic for c-Yes [MAb (3H9), Wako Pure Chemical Co, Tokyo], and measured the autophosphorylation of c-Yes using an *in vitro* protein kinase assay described previously^[6,8]. Activities of c-Yes in the representative results of NL (patient 1), CH (patient 2), N and T tissues (patients 3-5), respectively, are shown in Figure 1A. When the kinase level of N tissues was used as a reference level ($n = 1$), c-Yes activity ratio in the T tissues ($n = 9$) was 5.0 ± 3.7 times higher than that in the N tissues, as measured by autophosphorylation (Student's *t*-test, $P < 0.001$) (Figure 1B). Patients 3-5 were classified as well-differentiated, moderately-differentiated and poorly-differentiated HCC, respectively. In addition, the c-Yes activity in all NL and CH tissues used in this study was very low as measured by autophosphorylation of c-Yes. Thus, c-Yes activity was notably high in human liver tissues with malignancy. In addition, our results showed that although the kinase activity of c-Yes was very low in NL and CH, it was already activated in liver cirrhosis (nontumorous cirrhotic tissues). Because the kinase activity of c-Yes was already activated at the preneoplastic stage (cirrhosis), *i.e.*, before the development of HCC, these data suggest that HCV-induced liver cirrhosis is a precancerous condition.

Protein levels of c-Yes were determined by Western blot analysis. Immunoprecipitates used for the protein kinase assays were also applied to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) before transfer to nitrocellulose membranes. As shown in Figure 1C, two proteins were detected in each lane. The upper protein was c-Yes, and the lower one was the heavy chain of the c-Yes monoclonal antibody. Although the level of c-Yes was very low in NL and CH, it was elevated in N and T tissues of HCC (Figure 1C). As an internal control, the amount of α -tubulin in lysate containing 100 μ g of cellular protein used in the immunoprecipitation was almost the same in each lane (Figure 1D). When the protein level of N tissues was used as a reference level ($n = 1$), the level of c-Yes protein in T tissues ($n = 9$) was 2.1 ± 0.84 times higher than that in the surrounding N tissues from the same patients (Student's *t*-test, $^dP < 0.01$) (Figure 1E). The ratio (T tissue *vs* surrounding N tissue) of the protein level of c-Yes was smaller than that of the kinase activity. These data suggest that the high c-Yes kinase activity in the HCC is probably caused not only by an increase of c-Yes protein but also by an increase of the enzyme activity.

In conclusion, activation of the protooncogene product c-Yes may play a significant role in the malignant transformation of hepatocytes. The suppression of c-Yes kinase activity may offer a novel strategy for overcoming the development and invasion of HCC. Further studies are necessary to investigate such processes.

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S- Editor Wang GP **L- Editor** Wang XL **E- Editor** Ma WH