DETECTION OF t(14; 18) CHROMOSOMAL TRANSLOCATION IN PARAFFIN-EMBEDDED HEPATOCELLULAR CARCINOMA TISSUE BY *IN SITU* PCR

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Abstract

Objective: To understand the relationship between (14; 18) chromosomal translocation and hepatocellular carcinoma. Methods: Semi-nested *in situ* PCR (SNISPCR) technique was used to detected bcl-2/JH fusion gene in 40 cases of hepatocellular carcinoma (HCC). Results: Bcl-2/JH fusion gene was detected in 10 of 40 HCC. There were no significant differences in bcl-2/JH fusion formation between histopathological grades and metastases ($P>0.05$). Conclusion: By detecting bcl-2 fusion gene in HCC, we think that t(14; 18) chromosomal translocation is not a specific change in lymphoma. t(14; 18) chromosomal translocation may not an important cause in pathologensis of HCC.

Key words: t(14; 18), Hepatocellular carcinoma, Bcl-2/JH fusion gene.

Bcl-2 gene on chromosome 18 translocates to the chromosome 14 and juxtaposes with the immunoglobulin heavy-chain joining region (JH) on chromosome 14. Because of high incidence in follicular lymphoma (85%) and some cases of diffuse non-Hodgkin's (NHL) (30%), t(14; 18) chromosomal translocation is regarded as an important cause in the pathogenesis of lymphomas.[1-4] With the purpose of understanding the relationship between t(14; 18) chromosomal translocation and HCC, bcl-2/JH fusion gene was examined with semi-nested *in situ* PCR (SNISPCR) technique in 40 cases of HCC.

MATERIALS AND METHODS

Accepted for publication: February 29, 1998
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Tissue Preparation

Fifty-one samples were obtained by surgical resection in our department. Of them, 13 were hepatic cirrhosis. The cases of HCC were classified according to the criteria described by Edmondso-Steiner as follows: grade I, 7 cases; grade II, 18 cases and grade III–IV, 15 cases. Among 33 cases of grades 2–4, intrahepatic and extrahepatic metastases were found in 10 and 9 cases respectively. The diagnosis of intrahepatic metastasis was made on the criteria described by Oda. Seven normal liver tissues were uses as control. All tissues were fixed in 10% formalin and embedded in paraffin wax and then 4 μl serial sections were cut.

Primer and Probe

Two and half sets of primers and one set of probes specific for the two hot breakpoint regions on of bcl-2 gene were synthesized according to the DNA sequences elsewhere published by Gribben.[5] The oligonucleotide probe was labeled with biotin. Primers: A, 5'-CAGCCTTGAAACATTGATGG-3' for the mbr (external primer in major breakpoint region). B, 5'-CGTGCTGGTACCACTCCTG-3' for the mcr (external primer in minor breakpoint region). C, 5'-TATGGTGGTTTGACCTTTAG-3' for the mbr (internal primer in major breakpoint region). D, 5'-GGACCTTCCTTGGTGTGTTG-3' for the mcr (external primer in minor breakpoint region). Immunoglobulin heavy chain (JH), 5'-CCTGAGGAGACGG-TGACC-3'. Probes: mbr, 5'-CCCTCCTGCCCTCC-TTCCG-3', mcr, 5'-GGACCTTCCTTGGTGTGTTG-3'.

SNISPCR

Pretreatment: Sections were deparaffinized and dehydrated routinely, then washed in 0.1N HCl and PBS and digested with proteinase K (10 mg/L).

Placed on platform in DNA thermal cycler for 98°C 2 min.

Amplification *in situ*: The final volume of 20 μl (200 μmol/L each buffer of dATP, dCTP, dGTP and dTTP, 1.5U Taq polymerase 2 μl, 10X buffer). At first, mbr-JH fusion gene was amplified by primer A and JH, mcr-JH fusion gene was amplified by primer B and JH. Covered the slides adding reaction mixture. The samples were subjected to 25 amplification cycles. Each cycle was performed with 1 min of denaturation at 94°C, 1 min of annealing at 55°C and 1 min of extension at 72°C. The final extension period was extended to 10 min. Reamplification was performed for 30 cycles using primer C, JH for mbr-JH fusion gene and D, JH for mcr-JH fusion gene. The reaction condition is the same as stated above.

Hybridization *in Situ*

Removed the covers and dehydrated routinely. The slides were incubated in 4% poly formeldehyde for 10 min. 40 μl hybridization solution (probe concentration 50 pmol/L) was added on slides overnight at 37°C in wet box.

Non-specific antigen was blocked with 2% bovine serum and 0.3% triton X-100, followed by incubated with to secondary antibody for 1 h. Slides were then visualized with alkaline phosphate BCIP/NBT kit. Purple-blue granules in nuclear were regarded as the positive (Figure 1-4).

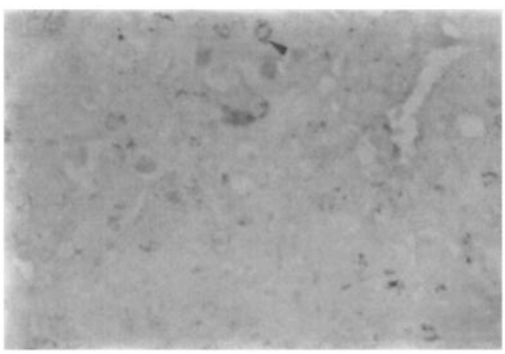

Fig. 1. Purple-blue deposits in granules in nuclear of hepatocarcinoma cells, contributing on nuclear membrane predominently
(positive for mbr, see arrow) *in situ* PCR × 400

Controls

Follicular lymphoma was used as positive control.

Negative controls: (1) Empty control. (2) Missing dNTP or Taq polymerase in PCR reaction solution. (3) Missing probe in hybridization solution. To exclude the false positive or negative results, each sample was analyzed at least twice occasions. Non-corresponding tissue such as normal skin tissue was also treated to exclude the false positive,

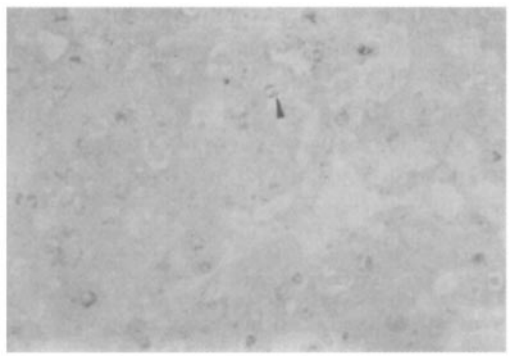

Fig. 2. Purple-blue deposits in granules in nuclear of hepatocarcinoma cells, contributing on nuclear membrane predominently
(positive for mcr, see arrow) *in situ* PCR × 400

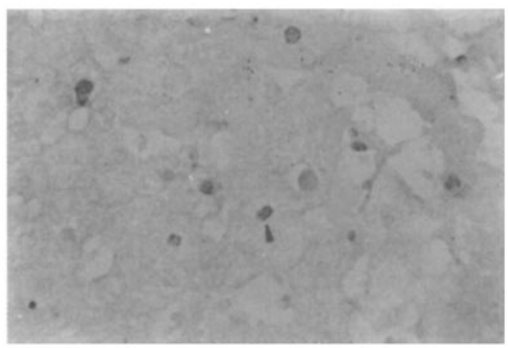

Fig. 3. Purple-blue deposits in granules in nuclear of adjacent hepatocytes
(positive for mbr, see arrow) *in situ* PCR × 400

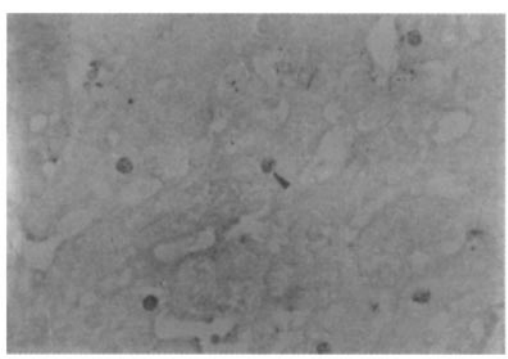

Fig. 4. Purple-blue deposits in granules in nuclear of adjacent hepatocytes
(positive for mcr, see arrow) *in situ* PCR × 400

RESULTS

Detection of bcl-2/JH fusion gene in HCC. Bcl-2/JH fusion gene was detected in 10 of 40 HCC, in which 1 of 7 in grade I, 5 of 18 in grade II and 4 of 15 in grade III-IV were positive. There were no

statistically significant differences between histopathological grades ($P>0.05$). 5 cases were positive for mbr or mcr region in bcl-2 breakpoint. Intrahepatic or extrahepatic metastasis rates were found in 40% (4/10) bcl-2/JH positive group and 30% (9/30) bcl-2/JH negative group. No significant difference was demonstrated between them ($P>0.05$).

Bcl-2 fusion gene was not detected in cirrhosis and normal liver tissues.

DISCUSSION

In this study, bcl-2/JH positive rate was 25% (10/40) in HCC, in which 5 cases for mbr were positive and 5 cases for mcr positive. Bcl-2 fusion gene has been regarded as an important molecular change in follicular lymphoma genesis. By detecting bcl-2 fusion gene in HCC, we think that t(14; 18) chromosomal translocation is not a specific change in lymphoma.

Bcl-2 gene has two hot breakpoint regions, mbr and mcr, in which mbr is the major breakpoint region and mcr is the minor breakpoint region. Approximately 50% to 70% of the bcl-2 translocation occurred in mbr breakpoint region in follicular lymphoma reported by Bskhshi.[3] In Wu's study, 91.7% breakpoint region present in mbr in follicular lymphoma.[4] In our study, 2 cases for mbr positive and 2 cases for mcr positive in HCC. It suggested that mbr and mcr region are the two important breakpoint regions in HCC. Although t(14; 18) chromosomal translocation was detected in HCC, it may not play an important role in pathogenesis of HCC for little positive rate.

Bcl-2 fusion gene may be related to the clinical prognosis of patients with lymphoma. Patients with lymphoma who had a PCR-amplifiable breakpoint were insensitive to conventional chemotherapy and easy to recur than that who had not PCR-amplifiable breakpoint.[5] In the present study, bcl-2/JH fusion formation did not correlate significantly with histological grade and metastasis in HCC. The results indicated that bcl-2 gene aberrant may be not related to cell differentiation and malignant behavior of HCC. The clinical significance of detecting bcl-2/JH fusion gene needs to be investigated further.

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