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TAKAHASHI, Kazuhiro
First Department of Internal Medicine, Faculty of Medicine, Kyushu University

KUDO, Jiro
First Department of Internal Medicine, Faculty of Medicine, Kyushu University

ISHIBASHI, Hiromi
First Department of Internal Medicine, Faculty of Medicine, Kyushu University

MASUMOTO, Naoko
First Department of Internal Medicine, Faculty of Medicine, Kyushu University

他

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原　　著

Serial Chromosomal Change during the Progression of Hepatocellular Carcinoma

Kazuhiro TAKAHASHI, Jiro KUDO, Hiromi ISHIBASHI, Naoko MASUMOTO, Yasuhiko HIRATA and Yoshiyuki NIHO

First Department of Internal Medicine, Faculty of Medicine, Kyushu University 60, Fukuoka 812-82

Abstract We investigated the chromosomal loss pattern of a primary large nodule and one of the disseminated intrahepatic small nodules of hepatocellular carcinoma in one patient using RFLP analysis. Using D17S30 probe mapping to 17p, loss of heterozygosity was observed both the main nodule and the disseminated nodule, however, using IGLV probe mapping to 22q, loss of heterozygosity was observed only in the disseminated nodule. This is the case in which serial loss of chromosome in the progression on hepatocellular carcinoma was highly suggested.

Introduction

Recent progress in molecular analysis identified genetic changes that contribute to the development of malignancies mainly through the study of familial cancers[4)8)18)]. Vogelstein and collaborators[27)] demonstrated that colorectal cancers from patients of familial or non-familial forms accumulate alteration of the *ras* gene and several tumor suppressor genes in a fashion that paralleled the clinical progression of the tumors. From these studies tumor progression can be considered as a serial process of genetic changes involving activation of oncogenes or recessive loss of functional mutation of tumor suppressor genes[12)].

We have investigated 24 cases of hepatocellular carcinomas (HCCs) from Japanese patients to find alternation of genome and found high frequency of chromosomal loss on 10q, 17p, and 22q[24)]. During this detailed investigation of the chromosomal loss as well as histological changes of the HCCs, we found a difference in the RFLP pattern of DNAs which were derived from two different HCC nodules in the liver of one patient. We present here data showing a serial chromosomal change in the main and the metastatic nodules of HCC, that paralleled clinical progression.

Patient and Method

Case. The patient is a 55-year-old man who died of hepatic failure in 1990. Since 1985, he had been diagnosed as having liver cirrhosis. In 1989, a large mass of 6 cm in diameter was detected in the right lobe of the liver which was diagnosed as HCC. Serum hepatitis B surface antigen was positive and antibody to hepatitis C virus was negative. The autopsied liver revealed dissminated intrahepatic small nodules of HCC in addition to the large main nodule with a fibrous capsule (Fig. 1). Tumor thrombi were observed in the portal veins.

DNA Isolation. High molecular weight DNA was extracted from each of the main nodule, one of the disseminated small nodules and spleen tissue, by digestion with proteinase K containing sodium dodecyl sulfate(SDS) and phenol/chloroform extraction[14)24)].

Southern Blot Analysis. Southern blot

Fig. 1 The autopsied liver revealed the liver had a main large nodule in the right lobe (A) and many desseminated intra-hepatic small nodules of HCC.

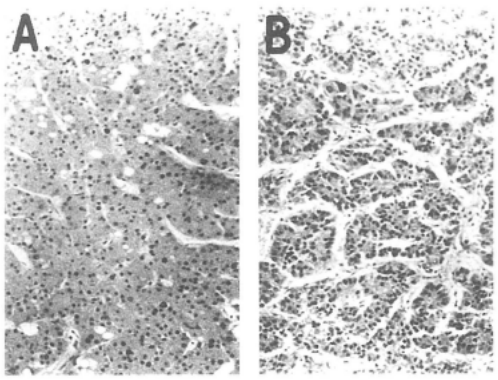

Fig. 2 A histopathological view of the main large nodule (A) and the small disseminated nodule (B).

analysis was performed as described earlier[14)24)]. Briefly, 10 μg of DNA digested with appropriate restriction enzymes, was separated by electrophoresis in 1.0% agarose gels, and transferred to nylon membranes(Hybond N ; Amersham). The membranes were prehybridized for 6 h at 42°C in 50% formamide, 5× SSPE, 5× Denhardt's solution, 0.1% SDS, and 200 μg denatured herring sperm DNA, and hybridized with ^{32}P-labeled probes in the same solution as that for prehybridization for 48 h at 42°C. After hybridization the filters were washed twice at 42°C in 1× SCC / 0.1% SDS for 20 min and then once at 42°C for 20 min in 0.1% SCC / 0.1× SDS. Filters were exposed to X-ray films (Fuji RX, Tokyo) with an intensifying screen at −80°C for autoradiography.

Determination of Loss of Heterozygosity. After developing the films, the autoradiographic signals were measured by scanning with a densitometer (Shimadzu CS-930 ; Shimadzu Ltd, Tokyo) to quantitate the signal intensity of polymorphic alleles was calculated to correct for differences in DNA loading[24)].

DNA Probes. DNA probes used in this study were D17S30 (pYNZ22) ; an anonymous DNA segment mapping to 17p13.3[16)], IGLV (pV3.3) ; the λ - chain immunoglobulin variable-region locus mapping to 22q11[1)] and hepatitis B virus (HBV) genome (pBRHBadr72) ; the HBV DNA probe of the adr subtype with a size of 3.2 kb[10)].

Result

Autopsy revealed the liver had a main large nodule and many disseminated small nodules. A histopathological examination revealed that both part of the tumor consisted of moderately differentiated HCC though the disseminated nodule presented less mature and predominance with a pseudoglandular pattern (Fig. 2).

We examined DNAs from both part of the main and the disseminated tumors to search if there might exist some genetical differences with probes which detect restriction fragment length polymorphism (RFLP). The D17S30 probe, which locate 17p13.3 detected RFLP with DNA of 1.8 kilobase (kb) and 1.3 kb by *MspI* digestion in non-tumorous tissue (spleen tissue) in this patient. Both the main nodule and the disseminated nodule lost the corresponding 1.3 kb allelic band (Fig. 3). The IGLV probe, which locate 22q11, detected allelic fragments of 5.4 and 3.6 kb by *BamHI* digestion in the spleen tissue. At this locus, howere, the disseminated nodule lost the 3.6 kb allelic band, whereas the main nodule retained it (Fig. 3). To examine HBV DNA integration to celllular DNAs of both part of the tumors, Southern blot analysis

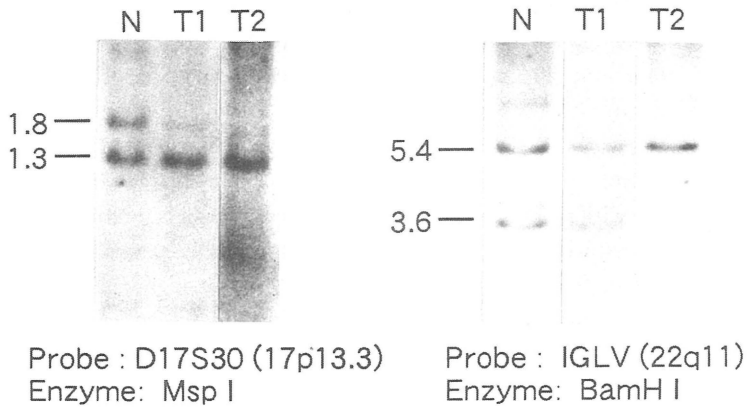

Fig. 3 Autoradiograms from Southern blot analysis of DNAs from the spleen tissue (N), the main large nodule (T1) and the desseminated small nodule (T2). The observed polymorphic allele size (kilobase) is shown at the left. The D17S30 probe detected a RFLP with fragments of 1.8 kb and 1.3 kb in *MspI* degestion of high molecular weight DNA from the spleen tissue. Both main large nodule and the disseminated small nodule lost the 1.3 kb allelic band. The IGLV probe detected RFLP fragments of 5.4 kb and 3.6 kb in *BamHI* degestion. Only the disseminated nodule lost the 3.6 kb allelic band.

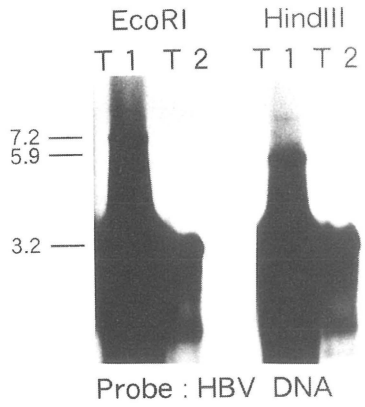

Fig. 4 An integration pattern of HBV DNA in DNAs from the main large nodule (T1) and the disseminated small nodule (T2). In *EcoRI* digestion a 7.5 kb band was observed only in the main large nodule. In *HindIII* digestion a 5.9 kb band was observed only in the main large nodule. The 3.2 kb band was considered to represent free HBV DNA as also detected in undigested DNA samples.

was performed with a HBV DNA probe (Fig. 4). The main nodule possessed a 7.2 kb band by *EcoRI* digestion and a 5.9 kb band by *HindIII* digestion indicating HBV DNA integration, while the disseminated nodule consisted of only a 3.2 kb band by either *EcoRI* or *HindIII* digestion indicating infected free HBV DNA.

Discussion

Recent studies have demonstrated that multiple genetic alteration, such as activation of oncogenes and inactivation of tumor suppressor genes play important roles in the development or progression of wide variety of human tumors[6)7)20)]. For HCC, loss of heterozygosity on chromosome 1p[22)], 4q[3)17)30)], 5q[5)9)], 10q[9)], 11p[9)29)], 13q[9)17)28)29)], 16q[9)17)21)25)30)] and 17p[9)23)26)] have been reported. Mutation of the p53 gene, a putative tumor suppressor gene, was also reported[2)11)15)]. Recently we investigated 24 cases of hepatocellular carcinoma, from Japanese patients to find alteration of genome and found high frequency chromosomal loss on chromosome 10q, 17p and 22q[24)].

In colorectal carcinoma, the serial chromosomal changes were closely related to the development of colorectal adenoma and subsequent progression to advanced carcinoma[27]. In HCC, loss of heterozygosity on chromosome 16 and mutation of p53 gene have been reported to associate with increasing sizes and grades of the tumor, hence these genetic changes are thought to correlate with the tumor progression[15)25)].

In our case, a large main nodule and many disseminated small nodules were observed. Though HCC sometimes occur multicentric, we considered these disseminated small nodules as intrahepatic metastasis by following reasons. First, many portal tumor thrombi were observed around the main tumor. Second, the histology of the disseminated nodules was less mature than that of the main tumor in spite of a much smaller size[13)19)]. Therefore, we examined DNAs from both part of the tumor to search for some possible genetical differences. Using the D17S30 probe, loss of heterozygosity was observed in both the main and the disseminated nodules. However, using the IGLV probe, loss of heterozygosity was observed only in the disseminated nodule. The schema presenting these serial changes is shown in Fig. 5.

From the findings presented here, we assume that p53 or an unknown tumor suppressor gene on chromosome 17p had been inactivated in the tumor cell in the large primary tumor. Subsequently, a tumor cell which had an inactivated unknown tumor suppressor gene on chromosome 22q newly appeared in the main nodule, and then disseminated to other parts of the liver. It is unclear whether this additional loss of heterozygosity at chromosome 22q related to the intrahepatic metastasis, rapid growth of the tumor, or a slight histopathological change because the nature of the tumor suppressor gene on 22q11 is presently unknown. However, this change can be related to the progression of HCC.

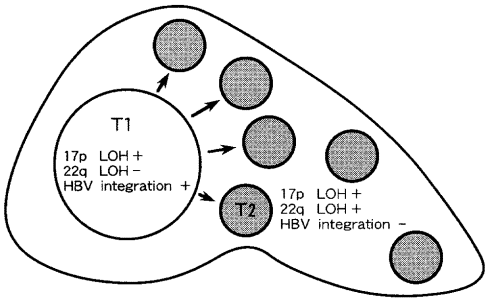

Fig. 5 The chromosomal pattern of the case. T1; Main large nodule, T2; disseminated small nodule, LOH; loss of heterozygosity.

The reason why the intrahepatic metastatic lesion lacked the HBV-integration is unclear. However, it would be possible that HBV-integration might have occurred on chromosome 22q or on some other genomic sequence which were lost during the metastasis.

The finding presented in this report is a direct evidence of an additional event of chromosomal changes during the progression of HCC. These data should help to elucidate the molecular mechanism underlying the progression of HCC.

Acknowledgments

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(和文抄録)

肝細胞癌における段階的遺伝子変異

九州大学医学部内科学教室第一講座
高橋和弘・工藤二郎・石橋大海・桝本直子
平田泰彦・仁保喜之

肝内にびまん性に播種転移巣を伴った肝細胞癌の1症例において，制限酵素断片長多型を利用して，原発巣と播種転移巣の遺伝子欠失の有無を検討した．17番染色体短腕のRFLPプローブD17S30を使用すると，原発巣と播種転移巣共にヘテロ接合性の消失を認めた．一方，22番染色体長腕のRFLPプローブIGLVを使用すると，播種転移巣のみでヘテロ接合性の消失を認めた．このことより，肝細胞癌の進展に段階的な遺伝子欠失が関与することが示唆された．