INTRODUCTION
Cancer progression has long been considered to be a multistage process. Accumulation of genetic abnormalities such as activation of oncogenes and inactivation of tumor suppressor genes are recently revealed to be involved in this process. The purpose of present study is to clarify the significance of the p53 tumor-suppressor gene in the genesis and development of Japanese hepatocellular carcinoma (HCC).

MATERIALS AND METHODS
Patients and pathologic samples: One hundred and sixty-nine frozen samples of hepatocellular carcinoma (HCC) were obtained from 140 patients who underwent surgical treatment at the National Cancer Center Hospital, Tokyo. SSCP method: DNA was extracted and analyzed for presence of p53 gene mutations. Each exon 5-8, mutation hotspots, of the p53 gene was amplified by Polymerase chain reaction (PCR) followed by single-strand conformation polymorphism (SSCP) analysis, simple method for detecting mutation. Then, abnormally shifted bands detected upon SSCP analysis were selectively further analyzed by the direct DNA sequencing to identify the mutated sequence.

RESULT
Forty-nine tumors (29%) showed a p53 mutation (39 point mutations and 10 frameshifts). The point mutations comprised 18 transitions, only 4 of which occurred at CpG sites, and 21 transversions. Two evolutionarily conserved domains, IV and V, contained 65% of all mutations and codon 249 was the most frequent mutation site (7/49). The spectrum of p53 mutation did not differ among HCCs in relation to the type of hepatitis virus infection, sex, age and background liver disease of patients, tumor size or presence of metastasis, but incidence and site were significantly associated with the degree of differentiation of cancer cells. In poorly differentiated HCC, p53 mutation was frequent (54%)
and clustered on domains IV and V, whereas in well or moderately differentiated HCC, the mutation was less frequent (21%) and equally distributed on domains II to V.

DISCUSSION

Carcinogens can induce mutations by direct adduct-driven mutagenesis or by triggering increased cellular turnover (=mitogenesis), which increases the mutation rate indirectly. In the present study, no specific type of mutation indicative of an association with particular carcinogens was found in Japanese HCCs. These data suggest that various carcinogens are involved in the genesis of p53 gene mutation, or if one or more specific carcinogens are associated, their effect is indirect, most probably due to DNA polymerase infidelity linked to mitogenesis.

It has been reported that G to T transversion at the third base of codon 249 is a specific feature of aflatoxin-related endemic HCCs. In our series, we found 7 mutations at codon 249, at different sites (two in the first, three in the second and two in the third base), of different types (five G to T and two A to T) and in different types of hepatitis virus infection (three HBV, three HCV and the last one not tested). Our data suggest that codon 249 is a common hotspot for p53 mutations in HCC, occurring not only in aflatoxin high-exposure areas but also in low-exposure areas.

We hypothesize that damage to domains IV and V surrounding codon 249 largely destroys the tumor-suppressing activity of p53, providing a considerable growth advantage, whereas mutation outside these regions results in only weakly altered function. As domains IV and V constitute one of two binding sites for SV40 T antigen in transformed cell lines, these regions might play an important role as a binding site for an unknown protein in vivo.

Our comprehensive analysis of this large series of HCCs suggests a role for p53 gene mutation in the late stage of the multistage process of hepatocarcinogenesis. However, the earliest and the latest genetic events in hepatocarcinogenesis remain undetermined, and identification of such genetic alterations seems to be important for further understanding the molecular basis of multistage hepatocarcinogenesis.

REFERENCES


Key Words; Hepatocellular carcinoma, p53 gene mutation, Multistage cancer progression