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A MOLECULAR EPIDEMIOLOGIC MARKER OF HEPATOCELLULAR CARCINOMA FROM AFLATOXIN B1 CONTAMINATED AREA IN THE SOUTHWEST OF GUANGXI

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HCC specimens from high and low AFB1 risk areas in Guangxi showed different frequency of p53 mutational hot-spot, which were 20/35 (57%) and 1/10 by DNA sequencing and 36/52 (69%) and 2/10 by RFLP analysis respectively. Their differences were significant ($P<0.01$). Mutational points of p53 gene induced by AFB1 mutagen almost exclusively clustered at codon 249 third nucleotide and by the form of G to T transversion only. We call it "AFB1 mutational hot-spot". It turns out to be a significant marker for molecular epidemio-logic survey to decide how many HCC and which individuals are induced by AFB1 mutagen, and if emergence of this marker in HCC is frequent in certain region it indicated that there is heavy contamination by AFB1.

Key words: Hepatocellular carcinoma, Aflatoxin B1, p53 gene, Mutational hot-spot, Molecular epidemiologic marker.

Southern Africa, Qidong of Jiangsu, and Fusui of Guangxi are well known for their high prevalence of hepatocellular carcinoma (HCC), and have been identified as areas of high hepatitis B virus (HBV) infection and aflatoxin B1 (AFB1) contamination. Recently, several reports about p53 mutational hot-spot in HCC samples of the former two areas have been published.[1-3] We present here the informations about p53 gene mutation in the third high HCC prevalent area (Fusui) by methods of DNA direct sequencing and restriction fragment length polymorphism (RFLP) analysis targeted for the exon 7 of p53 gene.

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MATERIALS AND METHODS

Specimens were from surgical resection of hepatocellular carcinomas collected during the past 8 years at our Department of Pathology. Samples were formalin fixed and paraffin embedded tissue blocks. The histological diagnosis was made on standard pathological criteria.

PCR-RFLP Analysis and DNA Sequencing

The focus of our attention was on the exon 7 codon 249 third nucleotide of p53 gene. Primers used for amplification of exon 7 were introduced by Murakami et al.[4] PCR products containing exon 7 were digested with the restriction enzyme HaeIII at 37 ℃ overnight. The digested DNA was then eletrophorized on a 15% polyacrylamide gel. The DNA sequence contained a HaeIII enzyme restriction site between codons 249 and 250 at nucleotides GG/CC. Any point mutations at these mucleotides would result

in abolition of the restriction site and yielding a 110 bp fragment instead of two (75 bp and 35 bp) fragments, as seen in the wild-type p53 gene. DNA direct sequencing was done by Dr. Pan and Dr. Peng at Molecular Biology and Molecular Diagnostics Laboratory, University College London Medical School.

RESULTS

Cases were divided into two groups according to the results of traditional epidemiologic survey.

Group 1

Cases were from high prevalent regions of HCC including Fusui and its neighbouring counties. The average mortality rate of HCC was higher than 20 per hundred thousand population per year, and up to 78 in some villages. It was higher than the average level of this country.[5] It is well known that HBV infection and AFB1 exposure are principal risk factors.[6] In this group, 20/35 samples (57%) have a point mutation of G to T clustered at codon 249 third position by the method of DNA direct sequencing. In the meantime, in 36/52 samples (69%) the HaeIII restriction site is abolished and only one fragment of 110 bp is yielded.

Group 2

Cases were from two low prevalent regions of HCC in Guangxi, where only HBV was principal risk factor while AFB1 contamination was low. The average mortality rate of HCC was lower than 20 per hundred thousand population per year. It is equal to or a little lower than the average level of nationwide figure. In this group, 10 samples were analysed by the method DNA direct sequencing, only one G to T transversion occurred at codon 249 third base. Another 10 samples were treated with RFLP analysis, two of them lost the HaeIII restriction site. Comparing to group 1, the difference of the third base G to T transversion is significant ($P<0.01$).

There were other mutational points of exon 7 in the remainder samples as well. However, they did not cluster at any codon to form another hot-spot, but widely distributed at different codons (Table 1).

Table 1. Mutations in exon 7 of p53 gene in HCC

Codon	Nucleotide alterations	Amino-acide alterations	Number		Total
			Group 1	Group 2	
236	TAC->TAA	Tyr->end		1	1
238	TGT->AGT	Cys->Ser	1		1
239	AAC->AAG	Asn->Lys	1		1
242	TGC->TGA	Cys->end	1		1
	TGC->CGC	Cys->Arg		1	1
243	ATG->ATA	Ser->Ile	1		1
	ATG->AGG	Ser->Arg	1		1
243–244	ATGGGC->AAGTGC	SerGly->LysCys	1		1
244	GGC->GGA	Gly->Gly	1	1	2
247	AAC->AGC	Asn->Ser	1		1
249	AGG->AGT	Arg->Ser	20	1	21
	AGG->TGT	Arg->Cys		1	1
251	ATC->ATA	Ile->Ile	1		1
258	GAA->GGA	Glu->Gly	1	1	2
No alteration			5	4	9
In total			35	10	45

DISCUSSION

The two far separated areas — Qidong and Southern Africa in the world have been discovered that there were high frequency in p53 gene mutation in prevalent HCC and surprisingly found that more than half of the mutational points clustered at codon 249 to form a special mutational hot-spot. Their common risk factors were high HBV infection and especially high AFB1 contamination. However, low AFB1 contaminated areas throughout the world could hardly find this mutational hot-spot in HCC, regardless of high or low HBV infection. One more far separated area with similar mortality rate of HCC and risk factors of HBV and AFB1 is presented here. Though there are differences of natural environment and nationalities from the former two, p53 gene mutation is high up to 80% in total number, from Table 1 we can see that this is because the mutational hot-spot at codon 249 third base G to T transversion accounts for 20/35 (57%), similar to the former two areas as well. This surprising result indicates that AFB1 is principal agent responsible for the special point mutation. Therefore to call G to T transversion in codon 249 third base as "AFB1 mutational hot-spot" is perfectly justifiable. We do not think the other two bases of codon 249 and other form of mutation at the third base possess the same significance in human case. This hot-spot is a useful marker of AFB1 inducing the development of HCC. It can be used in molecular epidemiologic survey to decide how many HCC and which individuals are induced by AFB1 mutagen. If emergence of this marker in HCC is frequent in certain region, then heavy contamination by AFB1 can not be excluded. In this article, we have just used this marker as a checking point for molecular epidemiologic survey and have compared to traditional epidemiologic survey, we have obtained very identical and convincing result. Though we do not know the significance of chronic HBV infection yet, however, so far the AFB1 hot-spot cluster present surely cooperates with high HBV infection, so do in this series too.

Using HaeIII-RFLP analysis to recognize the AFB1 mutational hot-spot is reliable in this study. Because any alteration among GGCC 4 bases in its restriction site is negligible except the third base G to T transversion (Table 1). With DNA direct sequencing results control, we believe that RFLP instead of DNA sequencing is suitable for searching AFB1 mutational hot-spot in human HCC. It is a more popular and economic method and good enough for large samples survey.

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