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Aflatoxin sufferer and p53 gene mutation in hepatocellular carcinoma *

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Subject headings Aflatoxin B1; genes, p53; mutation; carcinoma, hepatocellular; liver neoplasms

Abstract

AIM To study the p53 gene mutation and its relationship to aflatoxin B1 exposure in hepatocellular carcinoma (HCC).

METHODS Restriction fragment length polymorphism analysis method was used in 62 HCC samples, and DNA direct sequencing in another 45 HCC samples.

RESULTS In HCC and AFB1 high and low-risk areas, 36/52 (69%) and 2/10 (20%) cases were found losing the HaeIII allele respectively, suggesting one of the base G mutation at the p53 gene codon 249. Similar results appeared in DNA direct sequencing, 20/35 (57%) and 1/10 (10%) respectively mutated at the codon 249 third base G to C transversion.

CONCLUSION In HCC after AFB1 exposure, mutation of p53 gene is fixed at codon 249 third base and take the form of G to T transversion. This is a definite marker of mutation which is induced by AFB1 mutagen. It is applicable for molecular epidemiologic survey of the sufferers of AFB1 among HCC cases and for discovering more unknown natural AFB1 contaminated areas.

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INTRODUCTION

Previous reports from Qidong and southern Africa discovered that the p53 gene of hepatocellular carcinoma (HCC) had a mutational hot-spot at codon 249[1-3]. Later there were repeated reports from Qidong. However, reports from worldwide other than those two areas showed no such special mutational hot-spot in HCC[4-6]. It was supposed that codon 249 mutation might be caused by exposure to aflatoxin B1 (AFB1) which was a common enviromental factor prevalent in the two mentioned areas. Aspergillus flavus is widely distributed throughout the world. However, well-known natural high AFB1 contaminated areas are uncommon. It is of significance to study one more known natural high AFB1 risk area Fusui and its neighboring counties where HCC mortality rate was high up to 40/100 000 annually. AFB1 exposure and hepatitis B virus (HBV) were both suspected to be the principal aetiological factors.

MATERIALS AND METHODS

Surgical specimens of HCC were fixed in formalin and embedded in paraffin. The pathological diagnosis was made by standard histologic criteria. The DNA of 62 HCC samples were amplified focus on the p53 gene exon 7 by polymerase chain reaction (PCR). Primers were introduced by Murakami *et al*. PCR products containing exon 7 were digested with the restriction enzyme Hae III, then eletrophoresed for restriction fragment length polymorphism (RFLP) analysis. Another 45 HCC samples were sent for DNA direct sequencing. In the meantime, all samples were stained for HBsAg by ABC method of immunohistochemistry.

RESULTS

A lot of HCC samples came from high HCC prevalent and high AFB1 exposed area in Guangxi lost the Hae III restriction site GG/CC by method of RFLP analysis. It suggested a mutation occuring at the sequence AGG, CCC. Undoubtedly, the mutational point occurred at the hot-spot codon 249 nucleotides G rather than non-hot-spot C of codon 250. Surprisingly, this mutation was found in 36/52 samples (69%). Compared to the samples from a low prevalent area in Guangxi, the frequency was 2/10 (20%). The difference was significant (P<0.01). The results by direct DNA sequencing were similar. The samples from high prevalent area were 20/35 (57%) mutation at codon 249 third

nucleotide G to T transversion, while low prevalent area showed this transversion only in 1/10(10%). The difference was also significant ($P<0.01$) . The total positive rate of HBsAg was 94.4%, with no difference between high and low prevalent areas.

DISCUSSION

The p53 tumor suppressor gene is one of the genes with great interest, because it is commonly mutated in human cancer, and the spectra of p53 mutations in these cancers provide clues to the etiology and molecular pathogenesis of tumors. In HCC, p53 gene mutation is related to AFB1 and HBx protein of HBV. The present data is a research on the spot on great number of cases. The conclusions are as follows: the principal etiology and molecular pathogenesis of HCC at the part of high prevalent area in Guangxi are caused by AFB1 without doubt, and HBV chronic infection is also high. AFB1 induces p53 gene mutational hot-spot in the high prevalent local area up to 57% - 69%. This hot-spot is located at exon 7 codon 249 third nucleotide G to T transversion. It is a special and stable mutational point. It is useful for HCC molecular epidemiologic study for general survey whether the residents are at the risk of aflatoxin exposure, and distinguish the AFB1 sufferers of HCC cases individually. Until now, there has been no report from high AFB1 contaminated area where HCC samples escape this p53 gene mutational hot-spot. More and more human materials from high AFB1 areas show the mutation point clustering at the codon 249 third nucleotide rather than the first or second nucleotide, and only G to T single form but not G to A or C multiple forms mutation. Therefore it would be confirmed as a mutational marker of a AFB1 sufferer in human HCC.

REFERENCES

1 Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hot-spot in the p53 gene in human hepatocellular carcinomas. *Nature*,1991;350 (6317):427-428
2 Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from Southern Africa. *Nature*, 1991;350(6317): 429-431
3 Ozturk M and Collaborators. p53 mutation in hepatocellular carcinoma after aflatoxin exposure. *Lancet*, 1991;338(8779):1356-1359
4 Li D, Cao Y, He L, Wang NJ, Gu JR. Aberrations of p53 gene in human hepatocellular carcinoma from China. *Carcinogenesis*,1993;14(1):169-173
5 Fujimoto Y, Hampoton LL, Wirth PJ, Wang JP, Xie JP, Thorgeirsson SS. Alterations of tumor suppressor genes and allele losses in human hepatocellular carcinomas in China. *Cancer Res*,1994;54(2):281 - 285
6 Scorsone KA, Zhou YZ, Butel JS, Slagle BL. p53 mutations cluster at codon 249 in hepatocellular carcinomas from China. Cancer Res, 1992;52(7):1635-1638
7 Yet FS, Yu MC, Mo CC. Hepatitis B virus, aflatoxin, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Res*, 1989;49(10):2506 - 2509
8 Murakami Y, Hayashi K, Hirohashi S, Sckiya T. Aberrations of the tumor suppressor p53 and retinoblastoma genes in human hepatocellular carcinomas. *Cancer Res*,1991;51(10):5520 - 5525

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Effect of garlic and garlic-green tea mixture on serum lipids in MNNG-induced experimental gastric carcinoma and precancerous lesion*

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INTRODUCTION

To study effect of garlic and garlic-green tea mixture on serum contents of Tch, LDL and HDL in MNNG-induced gastric carcinoma (GC) and precancerous lesion (PL) in Wistar rats.

METHODS

Serum contents of Tch, LDL and HDL in normal control group (n = 10, NG), MNNG group (n=30, MG), prevention group (n = 30, PG), treatment group I (n = 20, TG I) and treatment group II (n = 20, TGII) were detected by PGE 6000/COD.

RESULTS

Serum Tch and LDL of rats of MG (6.86 ± 1.39, 3.72 ± 1.10) and its GC(6.95 ± 1.37, 3.77 ± 1.08) and PL(6.42 ± 1.04, 3.56 ± 0.74) were lower than that of NG (8.74 ± 1.89, 5.89 ± 1.61),PG(7.73 ± 3.18,4.96 ± 2.89) and its GC(8.36 ± 3.41, 5.93 ± 3.31) and PL (7.45 ± 3.16,4.55 ± 2.71), TGI (8.86 ± 1.75, 5.38 ± 1.76) and its GC (9.10 ± 2.27, 5.55±2.51) and PL (8.61 ± 1.17, 5.22 ± 0.55) and TGII (8.16 ± 0.76, 5.32± 0.72) aod its GC(8.52 ± 0.67,5.96 ± 0.48) and PL (8.02 ± 0.79,5.09 ± 0.65), respectively ($P<0.01$-0.05). Serum HDL of MG rats (2.76 ± 0.48) and its GC(2.79 ± 0.48) were remarkably higher than that of MG (2.20 ± 0.85) and GC of PG (2.24 ± 0.38) ($P<0.05$).

CONCLUSION

Experimental gastric carcinoma and precancerous lesion were associated with hypocholesterolaemia, LDL and HDL. Garlic and garlic-green tea mixture can inhibit and reverse MNNG-induced gastric carcinoma and precancerous lesion in Wistar rats.