Chinese Journal of Cancer Research 9(1):24-27,1997.

LOSS OF HETEROZYGOSITY AT 17p13 IN GASTRIC CANCER AND COLORECTAL CANCER*

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Southern hybridization was done on DNA samples of 22 gastric tumors and corresponding normal tissues, 14 colorectal tumors and corresponding normal tissues by probe php53B mapping at 17p13.1 and probe pYNZ22 mapping at 17p13.3 which were purchased from the American Type Culture Collection. RFLP heterozygosity was observed in 12 normal tissues of gastric cancers and 10 normal tissues of colorectal cancers. Among these informative tumors, 6(50%) cases of gastric cancers and 6(60%) cases of colorectal cancers showed the loss of heterozygosity at 17p13. Our results demonstrated that the inactivation of wild type p53 might be involved in the carcinogenesis of gastric cancers and colorectal cancers. Furthermore, the mode of inactivation of p53 was in accord with the "two hits" hypothesis by knudson. The significance was discussed regarding the presence of LOH detected by probe pYNZ22 mapping at 17p13.3.

Key words: Gastric cancer, Colorectal cancer, Loss of heterozygosity, p53.

Both gastric cancer and colorectal cancer are common human tumors. Although little is known regarding the molecular pathogenesis of the neoplasms, it is now generally accepted that the activation of oncogenes and inactivation of tumor suppressor genes might be critical for the tumor carcinogenesis and progression. In the present study, we employed the Southern hybridization by probes php53B and pYNZ22, which were obtained from the American Type Culture Collection, to detect the loss of heterozygosity(LOH) at 17p13 in gastric cancer and colorectal cancer. We further analysed the involvement of p53 inactivation in the pathogenesis of these two tumors and discussed the significance of presence of 17p13 LOH.

MATERIALS AND METHODS

Patients

Tumors and corresponding normal tissues were collected from 22 patients with gastric cancers and 14 patients with colorectal cancers at the affiliated hospital of Nanjing Railway Medical College. All tissue specimens were obtained from surgery. The definite pathological diagnosis and discrimination between tumors and normal tissues for all patients were done in department of pathology at Nanjing Railway Medical College.

Probes

The probes php53B mapping at 17p13.1 and pYNZ22 mapping at 17p13.3 were used.

Accepted January 10,1997

* This work was supported by The Natural Science Fund of Jiangsu Province and The Outstanding Youth Teacher Fund of Jiangsu Province, China (No. BK 93154315 and No. QZ 91014).

Southern Hybridization

DNA preparation from tumor specimens and normal tissues was according to standard phenol-chloroform procedures. Different length DNA fragments after digestion with specific restriction enzymes were separated by agarose electrophoresis, then transferred to the nylon membrane by Southern blotting. Probes were labelled with α-^{32}p-dCTP by hexanucleotide-primed labelling after they were transformed, amplified, extracted and purified. Prehybridization was performed at 42 ℃ for 8–12 h in 50%(v/v) formamide hybridization solution. For hybridization (at least 36–48 h at 42 ℃), labelled DNA probe was added to a fresh sample of prehybridization solution. Autoradiography was done at -70 ℃ for 1–4 days after the DNA membrane was washed to eliminate the unspecific hybridization bands.

LOH Measurement

Measurement of LOH was based on Southern hybridization results of pairs of tumors and normal samples. LOH was scored when the tumor became homozygous, while the corresponding normal tissue showed the heterozygous for RFLP bands.

RESULTS

Detection of RFLPs by Probes php53B and pYNZ22

8.5Kb and 10.7kb bands were detected in ScaI restriction digest probed with php53B. MspI restriction digest probed with pYNZ22 showed allelic RFLPs which ranged from 0.5kb to 1.3kb while presence of different sizes of alleles ranging from 2kb to 3kb was observed in MspI restriction digest probed with pYNZ22.

LOH at 17p13 in Gastric Cancer and Colorectal Cancer

Typical examples of restriction patterns after hybridization with probes php53B and pYNZ22 are presented in Figure 1. The results of the investigation on LOH are summarized in Table 1, 2. LOH was examined in 22 patients with gastric cancers and 14 patients with colorectal cancers with probes php53B and pYNZ22 combined with restriction enzymes, ScaI, MspI and TaqI. Heterozygosity was observed in 12 cases of gastric cancers and 10 cases of colorectal cancers which were informative for LOH analysis. Of the informative tumors, 6(50%) cases of gastric cancers and 6(60%) cases of colorectal cancers showed the loss of heterozygosity respectively. In the total tumors with LOH at 17p13, only one case of gastric cancer was found to be LOH detected by probes php53B and pYNZ22 simultaneously.

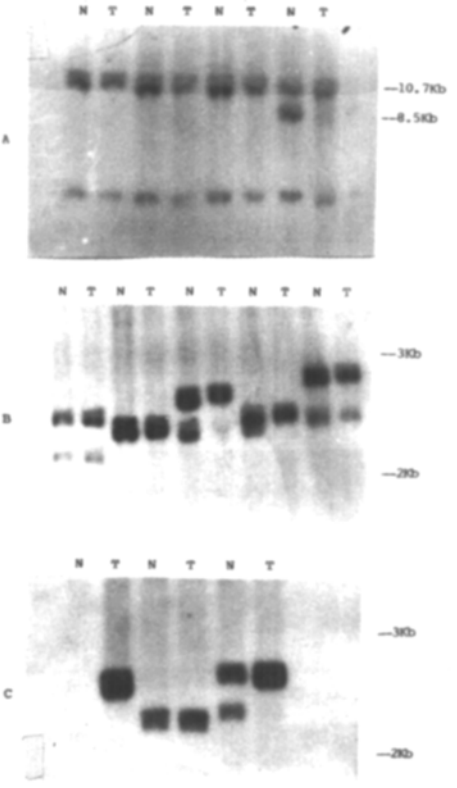

Fig. 1. Typical examples of Southern hybridization of pairs of tumor and corresponding normal tissue' with probes php53B and pYNZ22. A: ScaI restriction digest probed with php53B. B,C: TaqI restriction digest probed with pYNZ22. Band loss of tumors was evaluated as LOH.

DISCUSSION

It had been hypothesized by knudson that a classic tumor suppressor gene inactivation often occurs by at least two mutation vents which include

mutation of one allele and a second somatic event, i.e, nondisjunction, leading to loss of the other allele.[1] This recessive gene inactivation could uaually be detected as LOH when Southern molecular hybridization was done on DNA samples from the tumors and corresponding normal tissues by polymorphic probes which come from the tumor suppressor gene locus or are closely linked to it. In other word, detection of LOH always suggests that there exists a tumor suppressor gene which is located nearby the polymorphic probe used in LOH analysis and the tumor suppressor gene has been inactivated by two mutation events.[2] In this study, LOH in two cases with gastric cancers and one case of colorectal cancer was detected with probe php53B, indicating that the normal function of p53 gene has been lost by two recessive mutation events happening in the carcinogenesis of some cases of gastric and colorectal cancer, because probe php53B itself is the full length cDNA of p53 gene. In addition, we found LOH in another five patients with gastric cancer and five patients with colorectal cancer by probe pYNZ22 mapping at 17p13.3 close to the p53 locus at 17p13.1. These data would argue for an involvement of inactivation of p53 gene mapping at 17p13 in the carcinogenesis of human gastric cancer and colorectal cancer.

Table 1. Results of LOH in patients with gastric cancers detected by probes php53B and pYNZ22

Locus	Enzyme	Number tested	Heterozygosity & Frequency (%)	LOH &Frequency (%)
php53B	ScaI	22	3(14)*	2(66)*
pYNZ22	TaqI	8	4(50)	2(50)
	MspI	14	6(43)*	3(50)*
17p13	total	22	12(54)	6(50)

*Includes one tumor with LOH detected by probes php53B and pYNZ22 simultaneously.

Table 2. Results of LOH in patients with colorectal cancers detected by probes php53B and pYNZ22

Locus	Enzyme	Number tested	Heterozygosity & Frequency (%)	LOH & Frequency (%)
php53B	ScaI	7	2(29)	1(50)
pYNZ22	MspI	14	8(57)	5(62)
17p13	total	14	10(71)	6(60)

So far, only p53 gene is the tumor suppressor gene which has been definitely mapped at 17p13. Conflicting reports exist regarding the significance of detection of LOH by probe pYNZ22 at 17p13.3. Singh et al. found a considerably higher rate of LOH (64%) by probe php53B than by probe pYNZ22 (43%) in human breast cancer. Furthermore, no tumoramong the subset of 19 tumors informative for both probes showed LOH only at pYNZ22, whereas 4 tumors had lost one php53B allele and retained both pYNZ22 alleles. Thus their data support the view that LOH detected by probe pYNZ22 mainly reflects the inactivation of p53 gene itself.[3] Coles et al. also reported LOH at p53 locus decreased to 27% in a total of 81 informative tumors by using a p53 probe php53B and the LOH at the pYNZ22 locus was independent of LOH at php53B locus. A correlation between pYNZ22 LOH and p53 mRNA overexpression led them to propose a separate gene 20 megabases telomeric of p53, colse to pYNZ22 locus, the function of which is to regulate p53 expression. Both lesions of this regulatory gene and inactivation of p53 seem to be involved in the breast cancer carcinogenesis.[4] More recently, phillips et al. examined 57 sporadic ovarian epithelial tumors for LOH at multiple chromosome 17 loci, including pYNZ22 mapping at 17p13.3 and php53B mapping at 17p13.1. Their results showed that eighty percent (37/46) of informative tumors had LOH in 17p13.3 at pYNZ22 locus, whereas forty-five percent (19/42) of LOH was

detected by php53B. Furthermore, LOH at pYNZ22 was independent of that at php53B. 12 tumors had LOH at pYNZ22 but not at php53B whereas no tumors had loss of one allele at php53B but retained heterozygosity at pYNZ22. They concluded that the 17p13.3 region defined by pYNZ22 may contain a tumor suppressor gene that is involved early in ovarian carcinogenesis, whereas the p53 gene may play a role in tumor progression.[5] Recent single-stranded conformation polymorphism(SSCP) analysis of p53 in ovarian tumors support phillips' conclusion that p53 alterations occur late in tumor development. No missense or nonsense p53 mutations have been seen in tumors of low malignant potential, whereas mutations have been seen in up to 40% of stage I carcinomas and up to 84% of higher stage carcinomas.[6,7] In present study, the polymorphic information and LOH at pYNZ22 and php53B couldn't be available for all cases but one case with gastric cancer simultaneously. We hope that further investigations will clarify the significance of LOH detected by probe pYNZ22 at 17p13.3 based on the establishment of the parallel relationship between LOH at pYNZ22 and php53B respectively by broadening the cases and using more restriction enzymes. It is to be determined that LOH at pYNZ22 at 17p13.3 will only reflect the inactivation of p53 or involvement of an unidentified regulatory gene or a separate tumor suppressor gene, close to pYNZ22 locus in the carcinogenesis of human gastric cancer and colorectal cancer.

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