World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v3.i3.160

World J Gastroenterol 1997 September 15; 3(3): 160-162
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL RESEARCH

Preliminary study on the loss of heterozygosity at 17p13 in gastric and colorectal cancers

Guo-Jun Wu, Xiang-Nian Shan, Ming-Fa Li, Shao-Lin Shi, Qi-Ping Zheng, Long Yu, Shou-Yuan Zhao

Guo-Jun Wu, Shao-Lin Shi, Qi-Ping Zheng, Long Yu, Shou-Yuan Zhao, Institute of Genetics, Fudan University, Shanghai 200433, China

Xiang-Nian Shan, Ming-Fa Li, Biological Department of Nanjing Railway Medical College, Nanjing 210009, China

Guo Jun Wu, male, was born on Nov. 2, 1967 in Yuyao County, Zhejiang Province. He graduated from Nanjing Railway Medical College, and is a PhD candidate in genetics at the Institute of Genetics at Fudan University. He has published seven papers.

Author contributions: All authors contributed equally to the work.

Presented at the Fifth Congress of Chinese Genetics Society.

Supported by the National Natural Science Foundation of China (No. 39480018), Natural Science Foundation of Jiangsu Province, No. BK93154315, and Outstanding Youth Teachers Foundation of Jiangsu Province, No. QZ91014.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Guo Jun Wu, Institute of Genetics, Fudan University, Shanghai 200433, China
Telephone: +86-21-65492222-4107

Received: November 2, 1996
Revised: January 25, 1997
Accepted: February 22, 1997
Published online: September 15, 1997

Abstract

AIM: To evaluate the role of *p*53 in the development and progression of colorectal cancer and gastric carcinoma by analyzing the loss of heterozygosity (LOH) at 17p13.1 and 17p13.3.

METHODS: LOH at the *p*53 gene locus and 17p13.3 were examined in 22 cases of gastric carcinoma and 14 cases of colorectal cancer by Southern blot analysis.

RESULTS: Of the 22 gastrocarcinoma cases, 12 (54%) were heterozygous and LOH was detected in 6 (50%) of the 12 informative cases. In the 14 colorectal cancer cases, 10 (71%) were heterozygous, and LOH was detected in 6 (60%) of the 10 informative cases.

CONCLUSION: LOH at the *p*53 gene locus is a frequent event in multiple step carcinogenesis progression. The high frequency of LOH at 17p13.3 suggests that there may be another tumor suppresser gene in that chromosome region.

Key words: Stomach neoplasms; Colorectal neoplasms; *p*53 gene; Heterozygosity loss; Genes, suppressor, tumor

Wu GJ, Shan XN, Li MF, Shi SL, Zheng QP, Yu L, Zhao SY. Preliminary study on the loss of heterozygosity at 17p13 in gastric and colorectal cancers. *World J Gastroenterol* 1997; 3(3): 160-162 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/160.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.160

INTRODUCTION

Recent molecular biology studies have revealed that carcinogenesis is a multiple-step process involving many factors. The activation of some oncogenes and inactivation of some tumor suppresser genes play key roles in this process[1]. *p53* is a widely related tumor suppresser gene, but its function has not been clearly defined[2,3]. In this research, LOH of the *p*53 gene in two common cancers, gastric cancer and colorectal cancer, was examined by Southern blot hybridization and RFLP analysis.

MATERIALS AND METHODS

Samples

Twenty-two cases of gastric cancer and 14 cases of colorectal cancer were acquired from the affiliated hospital of Nanjing Railway Medical College. Tumor tissues and their corresponding normal tissues were obtained during surgery. The classification of tumor and normal tissues was performed by the Department of Pathology of Nanjing Railway Medical College.

Probes

php53B is a *p*53 cDNA probe located at 17p13.1. pYNZ22 is a VNTR (variable number of tandem repeats) probe located at 17p13.3. Both probes were obtained from ATCC (American Type Culture Collection).

Southern blot

Genomic DNA was isolated from tumor and normal tissues according to standard methods[4]. It was then completely digested with specific restriction endonucleases, electrophoresed on agarose gels, denatured, and transferred to nylon filters. The filters were prehybridized for 8-12 h, hybridized for 24-36 h in 50% formamide at 42°C, washed and autoradiographed for 1-4 d at -70°C. Probes were radiolabeled using the random primer method.

LOH analysis

The hybridization results of each tumor tissue were compared to that of its normal tissue. If the normal tissue had two heterozygosity bands and its corresponding tumor tissue had only one

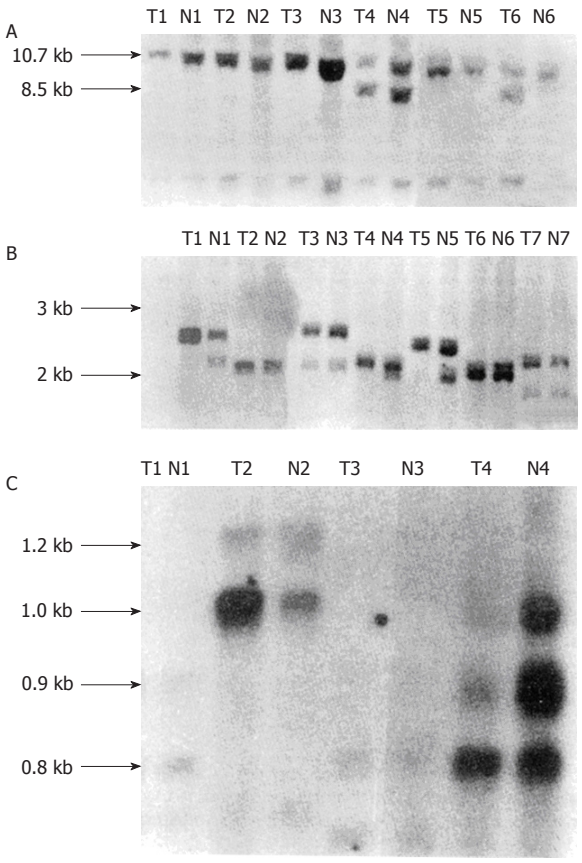

Figure 1 Loss of heterozygosity (LOH) at 17p13 in gastric carcinoma. N: Normal DNA; T: Tumor DNA. (A) LOH at the *p*53 locus. Genomic DNA was digested with ScaI. Sample 4 showed heterozygosity, and Sample 6 showed LOH. (B) LOH at YNZ22. Genomic DNA was digested with TaqI. Samples 3 and 7 showed heterozygosity. Samples 1 and 5 showed LOH. (C) LOH at YNZ22. Genomic DNA was digested with MsqI, LOH was detected in Samples 2 and 4.

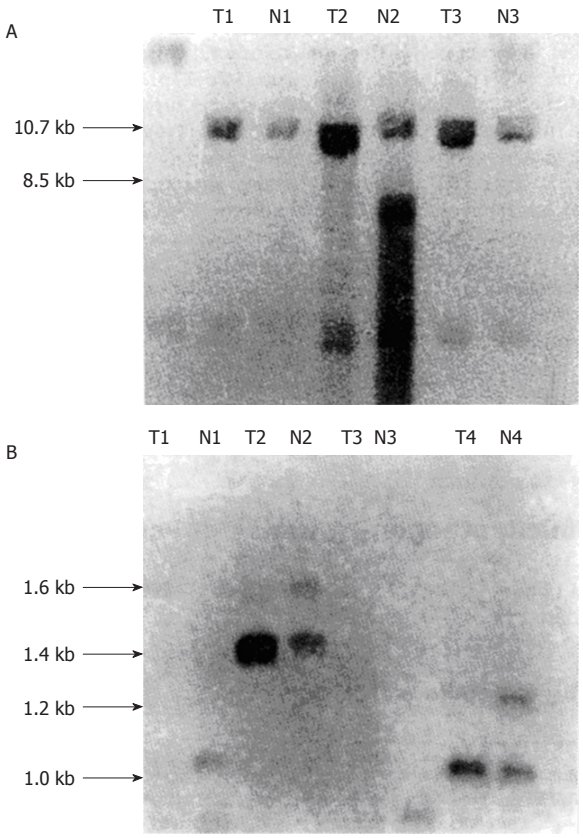

Figure 2 Loss of heterozygosity (LOH) at 17p13 in colorectal cancer. N: Normal DNA; T: Tumor DNA. (A) LOH at *p*53. Genomic DNA was digested with ScaI. LOH was detected in Sample 2. (B) LOH at YNZ22. Genomic DNA was digested with MspI. LOH was detected in Samples 2 and 4.

homozygosity band, the patient was classified as having *p*53 LOH.

RESULTS

php53B and pYNZ22 RFLP fragments

Using the ScaI restriction enzyme, we identified 8.5 kb and 10.7 kb allelic fragments with the php53B probe. After use of the MsqI restriction enzyme, we identified a group of alleles 0.5 kb to 1.3 kb with the pYNZ22 probe. While using TaqI, we identified allele fragments ranging from 2 kb to 3 kb with the pYNZ22 probe (Figures 1 and 2).

Table 1 Loss of heterozygosity (LOH) detected by the php53B and pYNZ22 probes in gastric cancer

Locus	Restriction enzyme	Number of sample	Heterozygosity and rate (%)	LOH and rate (%)
*p*53	*Sca* I	22	3 (14)*	2 (66)*
YNZ22	*Taq* I	8	4 (50)	2 (50)
	Msp I	14	6 (43)*	3 (50)*
17p13		22	12 (54)	6 (50)

*One sample showed heterozygosity and LOH detected with both probes.

Table 2 Loss of heterozygosity (LOH) detected by the php53B and pYNZ22 probes in colorectal cancer

Locus	Restriction enzyme	Number of sample	Heterozygosity and rate (%)	LOH and rate (%)
*p*53	*Sca* I	7	2 (20)	1 (50)
YNZ22	*Msp* I	14	8 (57)	5 (62)
17p13		14	10 (71)	6 (60)

The number in bracket shows the rate of heterozygosity or LOH.

LOH of 17p13 in gastric and colorectal cancer

We identified heterozygosity in all of the normal tissues from the 12 gastric cancer cases and ten colorectal cancer cases. They all showed hybridization bands of different lengths. We determined that six gastric cancer cases and six colorectal cancer cases exhibited LOH. The rate of detection was 50% and 60%, respectively. These details are shown in Tables 1 and 2.

DISCUSSION

In his "two hit" theory, Knudson showed that the loss of function of tumor suppressor genes generally involves at least two genetic mutation events[5]. These mutations can be detected by Southern blot hybridization and analysis of the LOH occurrence rate. The closely linked polymorphic gene probes located nearby or inside the possible tumor suppressor gene are used to examine the tumor tissue and its normal adjacent tissue. Detection of LOH suggests that there is a tumor suppressor gene located in the region covered by the gene probe, and two mutation events may have occurred in the tumor suppressor gene.

In this study, we detected LOH in two gastric cancer cases and one colorectal cancer case using the php53B probe. As php53B is a *p*53 cDNA probe, our data suggest that two mutation events occurred in the *p*53 gene of some gastric cancer and colorectal cancer patients, and that the normal function of the wild type *p*53 gene was lost. We also detected LOH in five gastric and colorectal cancer cases using the pYNZ22 probe, which is located at 17p13.3 and is tightly linked with p53. These results further suggest that the inactivation of *p*53 at 17p13.1 is involved in gastric and colorectal carcinogenesis.

To date, *p*53 is the only tumor suppresser gene that has been assigned to chromosome 17p13. There are conflicting reports on whether the LOH detected by the pYNZ22 probe only reflects *p*53 inactivation. Studies in breast cancer have shown that LOH detected by pYNZ22 primarily represents *p*53 inactivation[6]. However, Coles *et al*[7,8]suggested that there may be certain regulatory genes located between YNZ22 and *p*53 that control *p*53 gene expression. Damage to this gene, together with *p*53 inactivation supposedly contribute to breast cancer carcinogenesis. In our research, only one case of gastric cancer showed LOH in both *p*53 and the YNZ22 region, while the other cases did not demonstrate LOH in both *p*53 and the YNZ22 locus. Therefore, further studies are necessary, including collecting more cases and using more restriction endonucleases, to determine whether LOH detected within pYNZ22 in gastric and colorectal cancers represents *p*53 inactivation or whether there is an additional regulatory gene near the YNZ22 locus that is mutated in

gastric and colorectal cancers.

REFERENCES

1 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-I]

2 **Lane DP**. Cancer. p53, guardian of the genome. *Nature* 1992; **358**: 15-16 [PMID: 1614522 DOI: 10.1038/358015a0]

3 **Hollstein M**, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991; **253**: 49-53 [PMID: 1905840 DOI: 10.1126/science.1905840]

4 **Sambrook J**, Fritsch EF, Maniatis T. Molecular cloning Ed 2. New York: Cold Spring Harbor Laboratory Press, 1989: 914-923

5 **Knudson AG**. Hereditary cancer, oncogene and antioncogene. *Cancer Res* 1985; **24**(6): 1437-1443

6 **Singh S**, Simon M, Meybohm I, Jantke I, Jonat W, Maass H, Goedde HW. Human breast cancer: frequent *p*53 allele loss and protein overexpression. *Hum Genet* 1993; **90**: 635-640 [PMID: 8444469]

7 **Coles C**, Thompson AM, Elder PA, Cohen BB, Mackenzie IM, Cranston G, Chetty U, Mackay J, Macdonald M, Nakamura Y. Evidence implicating at least two genes on chromosome 17p in breast carcinogenesis. *Lancet* 1990; **336**: 761-763 [PMID: 1976143 DOI: 10.1016/0140-6736(90)93236-I]

8 **Kim CJ**, Kim WH, Kim CW, Lee JB, Lee CK, Kim YL. Detection of 17p loss in gastric carcinoma using polymerase chain reaction. *Lab Invest* 1995; **72**: 232-236 [PMID: 7853854]

S- Editor: Filipodia **L- Editor**: Jennifer **E- Editor**: Hu S

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327
0 3>