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Comparison of p53 Gene Mutations in Paired Primary and Metastatic Gastric Tumor Tissues

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Our previous study revealed that mutations of the p53 gene were detected by cDNA sequencing in one of four (25%) primary gastric tumors and in five of six (83%) gastric cancer cell lines. It was of interest that all five cell lines established from metastatic lesions had p53 gene mutations, while the single cell line established from a primary tumor lacked an abnormality. Thus, the current study was initiated to determine the frequency of p53 mutations in 10 pairs of samples from primary gastric carcinomas and their lymph node metastases, in addition to morphologically normal gastric mucosa. In addition, we correlated the findings with other relevant molecular markers including the metastasis associated nm23-H1 gene and loss of heterozygosity (LOH) using multiple polymorphic markers for chromosome 17p and sequencing the entire open reading frame (ORF) of the p53 gene.

Five of ten (50%) patients were constitutionally heterozygous for one or more 17p and/or p53 probes (pYNZ 22, BamHI RFLP; pMct35.1, MspI RFLP; php53cl, Bg/II RFLP), while none had LOH at the 17p and/or p53. A Bg/II RFLP for analysis of possible nm23-H1 somatic allelic deletion revealed no LOH out of four informative cases.

One paired sample demonstrated the substitution of valine for isoleucine at codon 41 (GTT to ATT) in both primary gastric tumor and metastasis. Another metastatic sample demonstrated the substitution of proline for threonine at codon 278 (CCT to C/ACT) in addition to a non-mutated codon, while only the wild-type p53 sequence was present in the paired primary gastric tumor tissue. We conclude: 1) p53 gene mutation is relatively rare in primary gastric cancers and their lymph node metastases; 2) mutations are preserved in lymph node metastases; 3) p53 gene mutations may be involved in gastric cancer progression.

Key Words: *p53 gene, Gastric cancer, Metastasis, Mutation, Cancer progression*

INTRODUCTION

Recently, several line of evidence have indicated that the wild-type p53 gene, located at chromosome 17p13.1, may function as a tumor suppressor gene and that a mutant p53 gene could promote transformation by inactivating normal p53 function in a dominant

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negative fashion (Baker et al., 1989; Eliyahu et al., 1989; Finlay et al., 1989). Many different point mutations in the highly conserved regions of the open reading frame (ORF) have been observed in a variety of common cancers, such as lung, colon, and breast cancers (Baker et al., 1990 Nigro et al., 1980; Osborne et al., 1991; Takahashi et al., 1989).

Our previous study revealed that abnormalities of the p53 gene were detected by complementary DNA (cDNA) sequencing in one of four (25%) primary gastric tumors and in five of six (83%) gastric cancer cell lines (Kim et al., 1991). It was of interest that all five cell lines established from distant metastatic lesions had p53 mutations, while the single cell line established from a primary tumor lacked an abnormality. This mutations of the p53 gene were present in gastric cancer cell lines established from distant metastatic lesions, but were less common in primary gastric cancers. Yamada et al. (1991) reported similar results, finding that p53 gene mutations were present both in cell lines established from gastric cancer metastases and tissue specimens from gastric cancer metastases but not in primary lesions. These differences have two possible explanations: a) p53 gene mutations confer an *in vitro* growth advantage; or b) occasional p53 gene mutations which arise in primary gastric tumors are more likely to result in metastatic spread.

The current study was initated to determine the frequency of p53 gene mutations in samples from paired primary and metastatic gastric carcinomas for evaluating the role of p53 mutation in gastric cancer progression, and to correlate these mutations with other relevant molecular markers.

MATERIALS AND METHODS

Sample acquisition and preparation

Paired primary and metastatic gastric carcinoma tissues, and non-malignant gastric mucosal tissues were obtained during routine surgical diagnostic or curative procedures. All the metastatic samples were obtained from regional lymph nodes. All samples were obtained at Yonsei Medical Center, Seoul, Korea, and were stored at-70°C until processed. Histologic diagnosis was confirmed with H-E sections and areas of the specimen with a predominant neoplastic component were collected using the stereoscope in the primary and metastatic tumor tissues before processing.

DNA and RNA Preparation

Frozen tissues were pulverized on a metal surface placed on a bed of dry ice prior to DNA and RNA extraction. Methods for preparing DNA and RNA were performed as previously described (Davis et al., 1968).

DNA probes and Analysis of Genomic DNA

The following probes were used to measure allelic loss; pYNZ 22 (Nakamura et al., 1988) after *Bam*HI digestion of gemominc DNA and another 17p probe pMCT35.1 (Carson et al., 1988) after *Msp*I digestion; php53cl (Zahut-Houri et al., 1985) after *Bg*/II digestion; and pnm23, a cDNA clone derived by reverse transcriptase PCR amplification of the normal ORF of nm23 gene and inserted into the *Bam*HI and *Eco*RI site of pGem7 (Promega, U.S.A.), after *Bg*/II digestion. Genomic DNAs from corresponding normal mucosa, primary gastric tumor, and lymph node metastasis (10μg each) were digested to completion with the restriction enzyme of choice (Gibco-BRL, U.S.A.), subjected to electrophoresis and hybridized as described (Kim et al., 1991).

Amplification and Sequencing of cDNA

First-strand cDNA synthesis using 5μg of total cellular RNA and subsequent PCR amplification were performed using p53-specific oligonucleotide primers located just outside the ORF, as previously described (Takahashi et al., 1989; Zakut-Houri et al., 1985). The primers used were; sense, 5'-ATGCGAATTCCAGCCAGACTGCCTTCCG GGTCACT-3', and antisense, 5'-ATGCGAATTCAGGCTGTCAGTGGGGAACAAG AAG-3'.

The PCR products were cloned into the *Eco*RI site of the plasmid pGem 4 (Promega, U.S.A.). Plasmid DNAs were prepared from pooled clones and sequenced using p53 ORF-specific primers and a Sequenase II kit (USB, U.S.A.) with [a-^{35}S] dATP (Amersham, U.S.A.). All nucleotide sequencing abnormalities were confirmed again with the independent PCR amplification product.

RESULTS

RFLP analysis of pYNZ 22, pMCT35.1, p53, and pnm23

To determine whether loss of heterozygosity (LOH) on chromosome 17p correlates with mutations in the p53 gene, genomic DNA smaples from the paired primary and metastatic gastric tumor tissues were compared to the constitutional (normal gastric mucosa) genomic DNAs from the same patients for LOH at the pYNZ22, pMCT35.1, p53, and pnm23-H1 loci.

Table 1. Summary of RFLP analysis on chromosome 17p and sequencing data of p53 gene in gastric cancer

Tumor No.	RFLP probe				p53 mutation	
	p53	YNZ22	MCT35.1	nm23	Codon	Nucleotide change
1	NI*	hete†	NI	NI		ND‡
2	NI	hete	hete	NI		ND
3	NI	NI	NI	hete		ND
4	hete	NI	hete	hete		ND
5	NI	NI	NI	NI		ND
8	NI	NI	NI	hete	278 in 8M§	CCT to ACT
9	NI	NI	NI	NI		ND
12	hete	NI	NI	NI		ND
13	NI	NI	NI	NI		ND
16	NI	hete	NI	hete	31 in P* & M	GTT to ATT

* not informative
† heterozygous
‡ not detected
§ lymph node metastasis of gastric cancer
* primary gastric tumor tissue

Five of ten (50%) patients were constitutionally heterozygous for 17p and/or p53 (analyzed by pYNZ 22, *Bam*HI RFLP; pMCT35.1, *Msp*I RFLP ;php53cl, *Bg*/II RFLP), while none had LOH at the 17p and/or p53 (Table 1). A *Bg*/II RFLP for analysis of possible nm23-H1 somatic allelic delection revealed no LOH out of 4 informative cases (Table 1).

Sequence Analysis

We performed nucleotide sequencing of p53 cDNA containing the entire ORF on all 10 paired primary and metastatic gastric tumor tissues. Sequencing demonstrated missense mutations in a paired primary gastric tumor and lymph node metastasis, while another missense mutation was encountered in the metastasis only but not in the corresponding primary tumor (Fig. 1). One paired sample demonstrated the substitution of valine for isoleucine at codon 31 (transition from GTT to ATT) in both primary gastric tumor and metastasis. Another metastatic sample demonstrated the substitution of proline for threonine at codon 278 (transversion from CCT to ACT) in addition to a non-mutated codon, but only a wild-type p53 sequence was found in paired primary gastric tumor tissue.

DISCUSSION

Gastric carcinoma is the most common form of gastrointestinal malignancy in certain parts of the world, including most of the Far East, yet relatively little is known about the molecular events leading to its development and progression.

Recent data have shown that mutations of the p53 gene are common in various human tumors (Baker et al., 1990; Osborne et al., 1991 Takahashi et al., 1989). But the role of p53 gene mutation on tumor and/or progression is obscure. Baker et al. (1990) suggested that p53 gene mutations occur near the transition from benign to malignant growth, and may play a causal role in this progression in colorectal tumorigenesis. On the other hand, Sidransky et al. (1992) suggested that mutations of p53 gene lead to selective growth advantage in vivo and seem to be involved in the histological progression of brain tumors. In the gastric canacer, previous data suggested that mutations in the p53 gene may be involved in gastric cancer progression (Kim et al., 1991; Yamada et al., 1991).

In the present study, ten paired primary and metastatic gastric tumor tissues (lymph nodes) were examined to determine at what stage mutational inactivation occurs and whether it is mainained during tumor progression. Sequencing of p53 mRNA from these paired tumors revealed an identical nucleotide substitution (codon 31, GTT to ATT) in one paired sample of the primary and metastatic tumor. This observation confirms the derivation of the metastatic tumor from a clonal population in the primary tumor and is evidence for selection of a mutant p53 gene prior to the metastatic event. The other metastasis sequenced appeared to retain both wild-type and mutant p53 (codon 278, CCT to C/ACT), but only the wild-type was

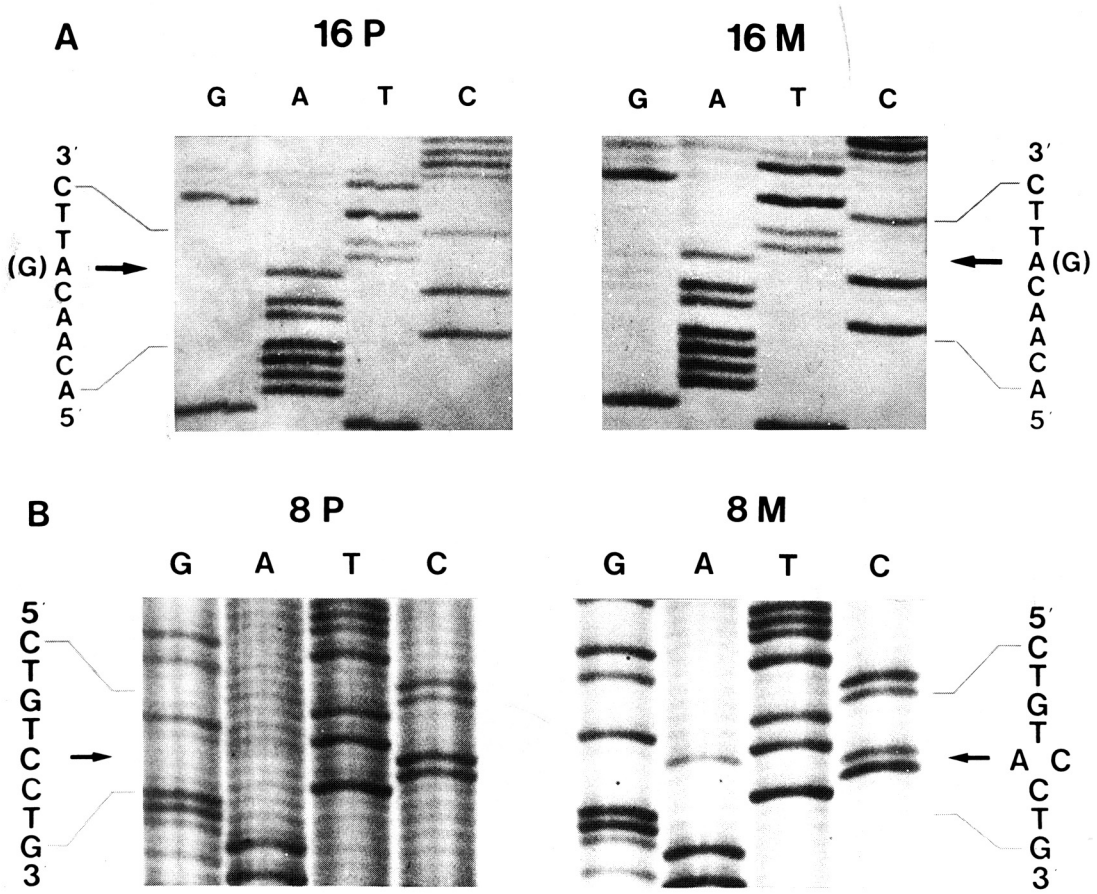

Fig. 1. Abnormalities of the p53 gene demonstrated by sequencing of cDNA/PCR products. Panel A: Primary gastric carcinoma tissue (P) and corresponding lymph node metastasis (M) contain a point mutation at codon 31 (transition from GTT to ATT). Panel B: Lymph node metastasis shows point mutation at codon 278 (transversion from CCT to ACT) in addition to a non-mutated codon, but wild type codon only in the paired primary gastric carcinoma tissue.

present in the primary tumor. It is possible that the wild-type allele present in the metastasis was derived from normal stromal cells in the tumor. Another possible explanation is that the metastatic tumor was heterogenous, and contained mixtures of tumor cells with and without the p53 mutation. The finding of lack of mutation in the primary tumor and the presence of a mutated p53 gene in the lymph node metastasis suggests that mutation of the p53 gene may be involved in tumor progression rather than in the origin of gastric cancer. Retention of the wild-type p53 allele in the metastasis suggests the complete elimination of the wild-type p53 may not be necessary for the tumor progression. Yamada et al. (1991) reported a p53 gene mutation rate in two of four (50%) of distant gastric cancer metastases compared to our findings (2/10, 20%) in lymph node metastases. While the number of cases in these series are too small to determine whether they are significantly different, the findings may reflect differences in the mutation frequencies between regional and distant metastases.

Our findings and the published literature (Kim et al., 1991; Tamura et al., 1991; Yamada et al., 1991), indicate that G:C to A:T transitions constitute the majority of the p53 gene mutations in gastric cancer (5/12, 41.6%) which is similiar to that of colon cancer but different to that of hepatocellular carcinoma in terms of mutation pattern (Hollstein et al., 1991). This finding may reflect differences in the carcinogens that induce mutagenic events in gastrointestinal and hepatic carcinomas.

Our previous work demonstrated a LOH of chromo-

some 17p markers in 29% of gastric cancers (Kim et al., 1991). However, in the current study, five of ten cases were informative with pYNZ 22, pMCT 35.1 and/Or php53cl probes, but none had LOH. In two cases which has mutated p53 genes, one sample was heterozygous even in the metastasis and another was not informative. Approximately 10% of p53 gene alleles were found to contain an additional *Bg*/II site in a region of introl I (Buchman et al., 1988). In the current study we observed two cases (20%) of p53 *Bg*/II polymorphism without LOH. The metastasis associated nm23-H1 gene localized near the centromeric region of chromosome 17 may contribute to some aspects of the tumorigenesis process as well as metastasis (Leone et al., 1991). Leone et al. reported an incidence of nm23-H1 allelic deletion in informative cases of breast and renal carcinomas varying from 20 to 67%. In the current study no LOH at nm23-H1 locus was observed in four informative cases, even in metastatases. Thus, detection of point mutations of the p53 gene is either a more sensitive method, or may precede the onset of LOH in this region of chromosome 17. However, further studies are required to dissect the genetic lesions on chromosome 17p and document the stage of tumor progession at which p53 point mutations and LOH begin to appear.

In summary, we found mutations of the p53 gene in the lymph node metastases of gastric cancers but not in corresponding primary lesions. These findings may be important in understanding the molecular events associated with progression and metastasis in gastric carcinoma.

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