Base Transitions Are the Most Frequent Genetic Changes at P53 in Gastric Cancer

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ABSTRACT

We searched for P53 mutations in gastric carcinoma by analyzing tumor DNAs from 29 patients. We detected 13 different somatic mutations in 15 patients (52%) and a biallelic polymorphism in exon 6 (5 heterozygous subjects). The somatic mutations were mainly localized in the sequences corresponding to the highly conserved domains of the protein. Twelve samples showed a single base change: 11 missense and 1 nonsense mutations. Three samples showed deletions leading to a frame shift, to the in-frame loss of 2 amino acids, and to the deletion of a splicing site. All point mutations, except one, were transitions, and 91% of them were G: C→A:T changes.

We previously analyzed this panel of tumors for allelic loss at the 17p13 chromosomal region, where the P53 gene had previously been located: the results showed an increasing incidence of allelic loss in late-stage tumors. On the contrary, in the present study no trend between P53 mutations and tumor stages was found. This observation indicates that mutation events precede allelic loss in gastric cancer. Half (54%) of the mutations occurred in samples without allelic loss, suggesting that specific mutated alleles, acting in a dominant negative fashion, can alter in vivo the P53 protein function.

INTRODUCTION

Although gastric cancer is a frequent human neoplasia throughout the world, with about 92,000 new cases per year in Europe alone, the genetic changes contributing to its genesis are poorly understood. Previous studies failed to correlate a precise genetic pattern to the initiation and/or to the progression of the disease (reviewed in Ref. 1). This is in contrast with the molecular genetics of colorectal cancer for which evidence supports a precise model of tumor progression which includes activation of the Ki-ras oncogene and inactivation of several tumor suppressor genes by both mutations and loss of the corresponding chromosomal sequences (2-4). Restriction fragment length polymorphism analysis on DNA from gastric tumors has revealed LOH3 at different loci (5-7) including the 17p13.1 chromosomal region where the P53 tumor suppressor gene has previously been localized. This gene encodes for a 53-kDa phosphoprotein which is involved in the control of cell proliferation (reviewed in Ref. 8). P53 gene mutations are the most common genetic changes known to occur in human cancer, and both point mutations and allelic losses have been detected in a wide variety of primary tumors (9). Most of the mutations found within the gene are largely confined to exons 5-8, which correspond to the highly conserved domains of the protein. On the other hand, the incidence, distribution, and nature of P53 mutations show cancer type and tissue specificity (reviewed in Ref. 10).

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3 The abbreviations used are: LOH, loss of heterozygosity; PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; Arg, arginine; Met, methionine; Ile, isoleucine; Gly, glycine; Ser, serine; Tyr, tyrosine; Cys, cysteine; Trp, tryptophan; Pro, proline; His, histidine; Ala, alanine.


To investigate the role of the P53 gene in stomach cancer, we searched for mutations in a panel of tumors that had been characterized for LOH at the 17p13 chromosomal region.4 The analysis of the P53 gene, which represents the target of numerous mutational events, can provide some clues on both endogenous cellular mechanisms and exogenous agents connected with mutagenic processes.

We screened patients from a defined geographic area (Forli, North-Central Italy) characterized by one of the world's highest incidences of stomach cancer (11). To date, molecular and epidemiological studies have been unable to identify specific genetic and/or environmental risk factors responsible for such a high incidence (12, 13).

DNA samples from primary stomach carcinomas were analyzed by PCR-based DGGE and sequencing methods.

The high mutation frequency we found (52%) indicates that P53 alterations represent one of the most important genetic events known to occur in primary gastric carcinoma.

MATERIALS AND METHODS

Tissue Samples. Tumor samples were obtained from surgically resected material from 29 untreated patients affected by primary gastric carcinoma. Necrotic and nonneoplastic tissue was removed as completely as possible from tumor tissue fragments. These were stored in liquid nitrogen until utilized for DNA extraction. Microscopic slides from tissue areas adjacent to the fragments utilized were available to evaluate the amount of neoplastic cells (at least 35-40%).

Tumors were staged according to the American Joint Committee on Cancer and histologically classified according to the procedure of Laurén (14). The samples included 22 tumors of intestinal type, 3 of mixed type, and 4 of diffuse type, stage classified as follows: 1 tumor of stage I, 3 of stage II, 9 of stage III, and 16 of stage IV.

Peripheral blood was available from all patients, and samples were stored at ~20°C until used. Biological material was collected at the Morgagni-Pierantoni Hospital of Forli (North-Central Italy).

DNA Preparation. High molecular weight DNA was prepared from tumor samples and peripheral blood leukocytes as previously described (15).

PCR. The sequences of the utilized primers are reported in Table 1. Exon 5 was amplified in two fragments, 5a and 5b, as described by Borresen et al. (16). One member of each primer pair contained a 5′ 40-base pair GC-rich sequence (GC-clamp).

PCR reactions were performed in an automatic device developed at the Department of Human Genetics in Leiden (17).

Genomic DNA (0.3 μg) was amplified in 50 μl of PCR reaction buffer [50 mM KCl-10 mM Tris-HCl (pH 8.4)-1.5 mM MgCl2 (1.25 mM for exon 7)-0.2 mM dNTPs-0.2 mg/ml of BSA-25 pmol of each primer]. Formamide was added at a specific percentage for each exon: 2.5% for exons 6 and 7 and 5% for exon 5; 0.25 unit of Supertaq polymerase (HT Biotechnology, Cambridge, England) was added to each assay. The reaction mixtures were subjected to 35 PCR cycles, each consisting of 1 min at 95°C; 1.5 min at 58°C for exons 6, 7, and 8 (55°C for exon 5); and 2 min at 70°C.

DGGE. The PCR products obtained with the GC-clamped amplimers were analyzed by DGGE. The introduction of a GC-clamp into the amplified fragment brings the probability to detect any base change within the fragment close to 100% (18).
an A to G nucleotide substitution at codon 236 (Tyr—»Cys) in patient
or DGGE homoduplex bands. The PCR product was cloned into the plasmid
(Met-Hle) in patient 108 and at codon 245 (G!y->Ser) in patient 35,
4 different missense mutations: a G to A replacement at codon 237
of patients 33, 35, 37, and 108. By nucleotide sequencing, we found
codon 213 in patient 29 and a 4-base pair deletion at the exon/intron
dure according to conventional protocols (21).

Alternatively, PCR was performed by using biotinylated primers; the product
vector PCR 2(KH) (Inv itrogen) as instructed by the supplier and then sequenced.

Four different exon 7 variants were observed in the tumor samples
33, and a C to T substitution at codon 248 (Arg—>Trp) in patient 37.
DGGE patterns and DNA sequences of samples 33 and 35 are shown in Fig. 2.

The greatest number of mutations was found in exon 8. Six different
DGGE variants were observed in 8 different tumors (23, 24, 30, 41, 50, 54, 116, 118); identical patterns were shared by samples 41 and
118 and by samples 23 and 54. No corresponding control DNAs showed any variant. Sequence analysis confirmed the presence of the same
mutation in cases with identical patterns, i.e., two different missense substitutions at the same codon 273, Arg to Cys at codon 282 in patient 50 and a C to G substitution (Pro—»Arg) at codon 278 in patient 116 and 2 deletions (a 6-base pair deletion in patient 24 and a 2-base pair deletion in patient 30).

The mutations found during the present study are summarized in
Table 3.

DISCUSSION

We found P53 mutations in 15 of 29 (52%) gastric cancer patients. This high incidence is comparable with those found in few other human neoplasias (10). Previous studies of gastric cancer showed
either no P53 mutations (22) or a lower incidence (1 of 5; 9 of 24) (23, 24). This discrepancy may be due to the different technical approaches utilized for the mutation analysis. Alternatively, it may result from

RESULTS

We performed PCR-based DGGE analysis of exons 5, 6, 7, and 8 of
the P53 gene in 29 gastric tumors. The chosen exons encompass four
highly conserved domains of the P53 protein (codons 124–308) and
include several mutational hot spots (10). The screening allowed us to
identify specific DGGE variants in tumor samples. We defined the
effect nature of the mutations by subsequent sequencing of the variants.
In order to distinguish between somatic and germinal mutations,
leukocyte and tumor DNAs from the same patients were analyzed
simultaneously. Leukocyte DNA is referred to here as control DNA.

Mutational analysis of exon 3 was performed with two sets of
primers, 5a and 5b, spanning the 5' and 3' halves of the exon, re-
spectively. No abnormal electrophoretic pattern was observed in the 5'
half of the exon. Analysis of the 3' half showed a variant in one tumor
(sample 111) but not in its control DNA. Sequence analysis of the
variant revealed a C to T change at codon 159 resulting in an alanine
to isoleucine substitution.

DGGE analysis of exon 6 revealed the presence of three different
electrophoretic variants. While two of these were present in only two
samples (29 and 46) of tumor DNA, the third variant was found in five
unrelated samples both in tumor and control DNAs (Fig. 1). Analysis
of the somatically acquired variants revealed a nonsense mutation at
codon 213 in patient 29 and a 4-base pair deletion at the exon/intron
boundary (GAGG) in patient 46. Sequence analysis of the third var-
ant showed a silent nucleotide substitution (CGA—>CGG) at codon 213 with no change in the corresponding amino acid (Arg).

Four different exon 7 variants were observed in the tumor samples
of patients 33, 35, 37, and 108. By nucleotide sequencing, we found
4 different missense mutations: a G to A replacement at codon 237
(Met—>Ile) in patient 108 and at codon 245 (Gly—>Ser) in patient 35,
an A to G nucleotide substitution at codon 236 (Tyr—>Cys) in patient

![Fig. 1. DGGE analysis of amplified fragments of exon 6 from 7 different patients. Leukocyte (L) and tumor (T) DNAs were loaded in parallel, h. exon 7 sequence analysis
Het., heteroduplexes; Hom., homoduplexes.](image)

![Fig. 2. a. DGGE analysis of amplified fragments of exon 7 from samples 33 and 35; leukocyte (L) and tumor (T) DNAs were loaded in parallel. b. exon 7 sequence analysis
antisense strand) of tumor DNAs from patients 33 and 35. Tumor 33 shows a T—>C change at codon 236 leading to a Tyr—>Cys substitution.; tumor 35 shows a C—>T change at
codon 245 leading to a Gly—>Ser substitution.](image)
than defined factors, may account for the high incidence of gastric
cancer in the area (11, 12). Therefore, it is difficult to directly correlate
the P53 mutation pattern with any cancerogenic agent to which the
population of Forli may be exposed.

In the present group of patients we observed the biallelic polymor-
phism due to a silent substitution in exon 6 at codon 213 already
reported in other populations (32).

The tumor samples investigated for P53 mutations also have been
previously analyzed for LOH in the 17p13 chromosomal region
(37.5% incidence). LOH turned out to be a predominant feature of
tumors at late stages. On the contrary, in this study we found that P53
mutations were present in tumors at both early and late stages without
any trend (2 of 3 samples of stage II, 3 of 9 samples of stage III, and
10 of 16 of stage IV). This observation indicates that mutation events
precede P53 allelic loss in the progression of malignant tumors from
early to late stages. On the contrary, the role of P53 mutation events
in the genesis of gastric cancer may only be assessed by further studies
of precancerous lesions, such as metaplasia or displasia (mild to
severe).

In our study 6 of the 8 samples with LOH (75%) showed a muta-
tion. Mutations located outside the P53 gene region analyzed here
may account for the 2 samples with allelic loss only. Seven of 13
tumors at late stages. On the contrary, in this study we found that P53
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any trend (2 of 3 samples of stage II, 3 of 9 samples of stage III, and
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allele at the P53 locus. Recently, it was demonstrated by transfection experiments that the wild-type P53 allele suppresses growth of human gastric cancer cells carrying given P53 mutations (25). However, these changes are at codons different from those found mutated in this survey.

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REFERENCES

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