The p53 gene in patients under the age of 40 with gastric cancer: mutation rates are low but are associated with a cardiac location

M Rugge, Y-H Shiao, G Busatto, M Cassaro, C Strobbe, V M Russo, G Leo, A R Parenti, A Scapinello, P Arslan, E Egarter-Vigl

Abstract

Background—Determining both the frequency and the spectrum of p53 gene mutation in young patients with gastric cancer might provide clues to the host related genetic mechanism(s) in gastric carcinogenesis.

Patients and methods—p53 mutations were assessed (by means of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), followed by DNA sequencing) in a cohort of 105 consecutive Italian patients in whom gastric cancer was ascertained before the age of 41.

Results—A low prevalence of p53 mutations (eight of 105) was observed, with no significant difference between intestinal (three of 31; 10%) and diffuse (five of 74; 7%) phenotypes. A significantly higher prevalence of p53 mutations was associated with the cardiac location (odds ratio, 7.09; confidence interval, 1.56 to 32.11). In all but one case, p53 mutations were associated with a stage higher than I. All eight mutations were located at CpG sites, where G : C to A : T transitions have been associated with frequent methylation at the C5 position of cytosine.

Conclusions—These findings show that, unlike what has been consistently demonstrated in the general population, p53 mutations are uncommon in gastric cancer occurring in young patients, and in such patients, p53 alterations are significantly associated with the cardiac location.

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Keywords: stomach neoplasm; genes; p53

The incidence of gastric cancer reaches a peak in the 7th decade and gastric epithelial malignancies are uncommon before the age of 40 years. In the developing world, environmental agents have been found to play a major aetiological role in gastric oncogenesis. In such an epidemiological context, the late onset of the disease (after years of exposure to several potentially oncogenic environmental agents) makes it difficult, not to say impossible, to evaluate any coexisting, host related factors. In countries with a low prevalence of gastric malignancies, it has been suggested that host related mechanisms might be relevant in gastric cancer development; the increased risk of gastric cancer seen in twins and in patients with other family member(s) affected is thought to reflect a host related genetic susceptibility to gastric adenocarcinoma. Patients under 40 years of age with gastric cancer are a unique population, in which the early onset (that is, short latency period) of the disease suggests a minimal aetiological role of environmental agents.

Mutations of the p53 gene are detected frequently in gastric cancer, mainly in the distal stomach and in those cancers featuring a glandular phenotype (that is, intestinal-type gastric cancer, so called epidemic gastric adenocarcinoma). In the mutational spectrum of the p53 gene, G : C to A : T transitions and C : G to T : A transitions at CpG sites have been considered as reliable markers of environmental genotoxic damage. On this basis, p53 mutations might also contribute towards clarifying the relative importance of either host or environment related mechanism(s) in the gastric oncogenetic process. Examining the p53 gene in gastric cancer of young patients might help to characterise the genetic abnormalities associated with its development.

In our study, both the frequency and spectrum of p53 gene mutation were determined in patients under 41 years of age with gastric cancer. Associations of these p53 mutations with other demographic and pathological data were also examined.

Patients and methods

PATIENTS

One hundred and five consecutive patients with gastric cancer under 41 years of age (16–40 years old, mean age of 35 years) were selected from the electronic archives of several Italian pathology departments from January 1990 to December 1996. All centres involved in our study have comparable gastric cancer incidences. Demographic and pathological data (age, sex, tumour site, and tumour stage according to the TNM system) were obtained by consulting the original pathology reports. Multiple postsurgical specimens were available in all cases, including neoplastic and non-neoplastic gastric samples. By re-examining all of the histological samples, gastric cancers were classified as being of the intestinal or diffuse type according to Lauren’s system. When both phenotypes coexisted, tumour categorisation was based on the most representative histology. Gastritis (non-atrophic versus atrophic/metaplastic) was classified using the Houston...
updated Sydney system. All cases were jointly assessed by two authors (VR and MC).

DNA EXTRACTION AND POLYMERASE CHAIN REACTION—SINGLE STRAND CONFORMATION POLYMORPHISM (PCR—SSCP) ANALYSIS

In each case, neoplastic and adjacent non-neoplastic, non-metaplastic areas were micro-dissected separately from unstained sections obtained from formalin fixed, paraffin wax embedded samples. Dewaxing, proteinase K digestion, DNA purification, and amplification of p53 exons 5–8 were performed as described elsewhere. Briefly, a total of 25 µl PCR mixture (75 mM Tris base (pH 9.0), 20 mM ammonium sulphate, 0.01% Tween, 1.5 mM MgCl2, 200 µM deoxyribonucleoside triphosphates, 0.5 µM primers, and 0.5 U thermostable DNA polymerase (Advanced Biotechnologies, Surrey, UK)) containing 20 ng of DNA template was run in parallel for each amplification. SSCP mobility shifts of PCR fragments of p53 exons 5–8 resulting from mutations were determined under SSCP conditions described elsewhere. In summary, a mixture of 1 µl of PCR product and 5 µl of denaturing buffer (95% formamide, 20 mM disodium EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue) was heated to 95°C for five to 10 minutes and then immediately placed on ice to prevent renaturation. Samples (5 µl) of each denatured PCR product were loaded on to 0.75 mm, 12% slabs (Bio-Rad, Richmond, California, USA). Electrophoresis was carried out at ambient temperature at a constant 100 V for four to six hours. Single stranded DNAs were visualised by means of silver staining. Samples with mobility shifts were verified by a second independent PCR-SSCP.

DNA SEQUENCING

PCR products (20 µl) were gel purified on a 6% polyacrylamide gel (Novex, San Diego, California, USA). A one third volume of purified product was used for DNA sequencing. The same primer sets used for the PCR were also used for DNA sequencing, which was performed by the dideoxynucleotide method with a Sequenase cycle sequencing kit (Amersham Life Science, Arlington Heights, Illinois, USA) and labeled ddATP, ddCTP, ddGTP, and ddTTP terminators. The sequencing ladder was resolved on a 7.7 M urea/6% polyacrylamide gel. After electrophoresis, the gel was dried in a Bio-Rad gel dryer before exposure to a x-ray film for one to three days at room temperature. Mutations were confirmed by sequencing both sense and antisense DNA strands.

STATISTICAL ANALYSIS

The odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to compare the frequency of p53 mutations among different pathological variables. The bio-medical data processing software (BMDP; University of California) was used for these calculations.

Results

Mutations of the p53 gene were detected in eight of 105 (8%) patients with gastric cancer. Three of these mutations occurred in 31 intestinal-type gastric cancers and five were seen in 74 diffuse tumours. Base changes were never observed in non-neoplastic, non-metaplastic gastric mucosa. Table 1 lists the distributions of p53 mutations and demographic and pathological variables in these young patients. Mutations of the p53 gene did not correlate with with age, sex, histological type according to Lauren’s classification, or type of gastritis (atrophic/metaplastic versus non-atrophic) coexisting with adenocarcinoma. A significant association with p53 mutations was observed with the tumour site concerned. A more than sevenfold greater risk of mutation was seen for tumours located in the cardia as opposed to the antrum or corpus. In all but one case, p53 alterations were associated with a cancer stage higher than I.

When the mutation spectrum was considered, base changes at CpG sites were uniformly observed for all eight mutations (table 2). Figure 1 illustrates DNA sequencing results from representative cases. Of these mutations, seven were G : C to A : T transitions and one was a C : G to G : C transversion. These mutations appeared to be distributed randomly in the coding region of the p53 gene and not at a specific codon.

Discussion

In the general population, environmental rather than host related factors are thought to be the main aetiological determinants for...
intestinal-type gastric cancer. The late onset of this adenocarcinoma is consistent with the long latency period required for the progressive genotypic and phenotypic transformations described in the multistep gastric oncogenetic model.\textsuperscript{14} In our study, p53 mutations were observed in only three of 31 (10%) intestinal-type gastric cancers in patients \( \leq 40 \) years of age, a much lower frequency than the 40–60% of p53 mutations reported in intestinal-type tumours in elderly populations.\textsuperscript{13–18} This suggests that environmental and/or dietary genotoxic agents might contribute less to intestinal-type tumours of the young than of aged patients. Conversely, the frequency of mutation in diffuse-type tumours is similar between our study (five of 74; 7%) and reports in the literature (10–25%).\textsuperscript{16–18} In our cohort of young patients, the comparable (low) rate of p53 mutations associated with both intestinal-type and diffuse-type adenocarcinomas prompts us to hypothesise an equivalent (marginal) role of environmental agents in both histological variants.\textsuperscript{19–21} In addition, our data support the hypothesis that, among young patients, genetic susceptibility is important in the development of both intestinal-type and diffuse-type gastric cancers.\textsuperscript{19–21}

Despite the similar frequency of mutation detected in intestinal-type and diffuse-type gastric cancers in patients \( \leq 40 \) years of age, a significantly different risk of mutation was observed at tumour sites. The association of p53 mutations with tumours involving the cardia suggests biological similarities between gastric cancers arising in the proximal stomach and adenocarcinomas detected at the gastrooesophageal junction, in which p53 alterations are common.\textsuperscript{22–26} The consistency of our results compared with those obtained in cohorts of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{Mutations detected by DNA sequencing analysis in exons 5–8 of the p53 gene.}
\end{figure}
older patients (characterised by high p53 mutation rates) suggests an important oncogenic role for genotoxic (environmental?) agents, which could have the gastrointestinal and urogenital junction as their elective target.21–26

In contrast to the most common model of gastric adenocarcinomas developing in older patients,11 in our cohort, p53 mutations were detected in only one of 28 patients having an early (stage I) adenocarcinoma. This observation suggests two different (although not conflicting) possibilities, namely:

(1) In gastric cancer arising in young patients, p53 abnormalities may represent a late event. This hypothesis would be consistent with a minor action role for environmental agents in gastric malignancies arising in such patients. Among these patients, however, p53 abnormalities might contribute to both tumour promotion and progression.

(2) Loss of p53 function is most frequently associated with a more aggressive cancer phenotype. This would result in an advanced tumour at the stage of clinical presentation.

In the elderly gastric cancer population, G : to A : T transversions at CpG sites have been detected in 20–70% of all base substitutions, depending on the geographical location.37 The mutation pattern (that is, CpG sites) found in our study is consistent with the spectrum of mutations most frequently detected in older age groups. Frequent G : C to A : T transversions at CpG sites have been associated with methylation at the C5 position of the cytosine of CpG dinucleotides.3 DNA methylation is catalysed by endogenous C5 cytosine methyltransferase.38–40 Because methylation at the C5 position of cytosine is a key event in the inactivation of G : C to A : T transversions at CpG sites (through either deamination or alkylation), DNA methylation status in these young patients appears to be an important determinant for p53 mutations. A further comparison of DNA methylation status between patients with gastric cancer and a control population is needed to clarify the role of DNA methylation in gastric carcinogenesis.

In conclusion, unlike the situation in older patients with gastric cancer, a low frequency of p53 mutations was demonstrated in our cohort of young patients with gastric cancer and no difference in the mutation rate was shown between intestinal-type and diffuse-type adenocarcinoma. Furthermore, p53 alterations were associated with significant differences in the most proximal tumour location, which suggests biological similarities among the adenocarcinomas involving the gastrointestinal and upper digestive tract. The uniform base changes at CpG sites prompts the hypothesis that a similar (perhaps host related) molecular mechanism might be involved.

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