The Difference in p53 Mutations between Cancers of the Upper and Lower Gastrointestinal Tract

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Key Words

p53 • Spectrum • Gastric cancer • Esophageal cancer • Colon cancer

Abstract

Background: *p53* gene mutations have been reported in over half of all human cancers and they appear to occur in the early stage of cancer, thus indicating the important role that such mutations may play in the carcinogenesis of the digestive tract. This study investigated the differences in *p53* abnormalities between cancers of the upper and lower gastrointestinal tract. **Materials and Methods:** The DNA of 354 specimens of gastrointestinal cancer (esophagus 85, stomach 112, colon 157) was extracted and then *p53* gene mutations were investigated by direct sequencing; the loss of heterozygosity was also synchronously analyzed in all cases. **Results:** (1) *p53* gene mutation: *p53* gene mutations were found in 41 samples (48.2%) in the esophagus, 18 samples (16.0%) in the stomach and 36 samples (22.9%) in the colon. *p53* mutations were more frequently identified in well-differentiated cancers and a close correlation was recognized between *p53* mutations and loss of heterozygosity. (2) Mutation spectrum: the ratio of transversion was 43.9% in esoph-

agus, 33.3% in stomach and 25.0% in the colon tumors. Reciprocally, the ratio of transition was 31.7, 66.7, and 72.2%, respectively. **Discussion:** The frequency of *p53* transversion mutations was extremely high in the upper digestive tract, whereas transition mutations were more frequently observed in the lower digestive tract. The investigation of the spectrum of mutations in *p53* is therefore expected to lead to a better understanding of the agents responsible for inducing cancer.

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Introduction

p53 gene mutations have been reported in over half of all human cancers and they appear to occur at an early stage of cancer, thus indicating the important role of such mutations in carcinogenesis of the digestive tract [1]. Dietary carcinogens, ultraviolet exposure and chemical mutagens are all known to cause various kinds of DNA damage and to induce *p53* mutations. A mutation of *p53* is thought to increase the protein half-life and it is also often associated with an overexpression of *p53* protein in the nucleus [2, 3]. Therefore, the overexpres-

sion of p53 protein has often been used as a surrogate marker for the presence of abnormalities [4, 5]. The large majority of studies has used an immunohistochemical analysis to detect abnormalities of p53, showing different results, since the antibodies, tissue preparation and immunohistochemical detection techniques varied [6, 7]. Many investigators have tried to determine the importance of p53 immunoreactivity or mutation as prognostic factor. However, the role of p53 abnormalities in prognosis is not known. The importance of p53 as prognostic factor varies in different organs and the mutation rate and spectrum are quite different in individual gastrointestinal tracts. There are many factors that cause gene mutations and a different mutation spectrum is produced by each factor. Different types of mutation might show different immunoreactivities. Dietary carcinogens, ionizing irradiation, ultraviolet exposure and chemical mutagens are all known to cause various kinds of DNA damage and thereby induce p53 mutations. Endogenous factors such as oxygen radicals, deamination and loss of bases can also cause DNA damage. This study investigated the difference in p53 mutations between cancers of the upper and lower gastrointestinal tract to determine what contributes to carcinogenesis for each organ.

Materials and Methods

Tissue Collection

The surgically resected esophageal, gastric and colorectal carcinomas with no preoperative therapy were collected from patients who underwent surgery between 1995 and 2005 at the Second Department of Surgery, Kyushu University, Japan. All tissue specimens were obtained after receiving the patients' written informed consent. All samples were diagnosed to be carcinomas histologically by means of hematoxylin and eosin staining by pathologists.

DNA Preparation

DNA was extracted as previously described. Briefly, the frozen samples were incubated in a lysis buffer (0.01 M Tris-HCl, pH 8.0, 0.1 M EDTA, pH 8.0, 0.5% SDS) containing proteinase K (100 µg/ml) at 37°C for 2 h. The samples were extracted twice in phenol, then in phenol/chloroform and chloroform. Following ethanol precipitation, the samples were diluted in TE (0.01 M Tris-HCl, pH 8.0, 0.01 M EDTA, pH 8.0) buffer.

PCR Direct Sequencing of the p53 Gene

The 275-bp fragment containing exon 6, the 439-bp fragment containing exon 7 and the 445-bp fragment containing exons 8 and 9 of the p53 gene were amplified by PCR (Nippon Gene). The PCR primers for the amplification of the 406-bp fragment containing exon 5 of the p53 gene were: exon 5 forward: TGC AGG

Table 1. p53 mutations in digestive tract cancers

TP53 status	Esophageal cancer (n = 85)	Gastric cancer (n = 118)	Colon cancer (n = 157)
Wild type	44 (51.8%)	100 (84.0%)	121 (77.1%)
Mutant	41 (48.2%)	18 (16.0%)	36 (22.9%)

AGG TGC TTA CAC ATG; exon 5 reverse: TCC ACT CGG ATA AGA TGC TG.

Mutations of the p53 gene were detected by PCR direct sequencing of all PCR products by using each forward and reverse primer with the dideoxynucleotide chain-termination method (Bigdye sequencing kit; Applied Biosystems, Norwalk, Conn., USA) and then were sequenced using the ABI Prism 310 Genetic Analyzer (Applied Biosystems).

Loss of Heterozygosity Analysis

Loss of heterozygosity (LOH) was analyzed using a DNA sequencer with microsatellite markers. The PCR reactions and running conditions with Perkin-Elmer Genetic Analyzer 310 were previously described [8]; 2 MSI markers, D17S796 and D17S1353 which are close to the 5' and 3' end of the TP53 gene, respectively, were used. The relatively highest peak in the curve cluster in the PCR product's electrophoresis profile from the cancerous tissues and corresponding noncancer tissue was compared. However, when the 2 alleles overlapped either partially or totally, the case was not informative for LOH estimation. When the peak of cancer tissue decreased by more than 30% in comparison to its normal counterpart, it was defined as LOH.

Results

Frequency of p53 Gene Mutations in Upper and Lower Gastrointestinal Cancer

Of the 85 patients with esophageal squamous cell carcinoma investigated in this study, p53 gene mutations in exons 5–9 were found in 41 patients (48.2%). The mutations were found in 18 (16.0%) of 112 gastric cancer patients and in 36 (22.9%) of 157 colon cancer patients (table 1). p53 mutations were more frequently identified in well-differentiated gastric and colon cancers (tables 2 and 3).

Frequency of p53 LOH in Upper and Lower Gastrointestinal Cancer

A close correlation was recognized between p53 mutations and LOH (fig. 1). The cases which are not informative for the LOH analysis were excluded in the analysis. In esophageal cancers, both the rate of LOH and muta-

Fig. 1. The relationship between p53 mutations and LOH. The horizontal axis shows the rate of LOH. The longitudinal axis shows the rate of mutation frequency. The mutation frequency and LOH were observed to correlate with each organ. ROH = Retention of heterozygosity.

Table 2. p53 mutations and clinicopathological factors in gastric cancer

Factors	p53		p value
	wild type	mutant	
Gender			
Male	64	14	NS
Female	30	4	
Average age	62.2	67.8	NS
Nodal metastasis			
Negative	34	5	NS
Positive	59	13	
Lymph vessel involvement			
Negative	37	8	NS
Positive	56	10	
Vascular involvement			
Negative	46	9	NS
Positive	47	9	
Histology			
Intestinal type	30	13	<0.01
Diffuse type	64	5	

NS = Not significant.

tions were high. In gastric cancer, the rates of LOH and mutations were not high, however, the correlation between LOH and the mutation rate was more significant. In colon cancer, the correlation between LOH and mutations was also significant.

Features of p53 Gene Mutation Spectrum in the Upper and Lower Gastrointestinal Tract

Among the 41 mutations identified in esophageal cancer, transversions were predominant (18/41, 43.9%), followed by transitions (12/41, 31.7%) and frameshifts (9/41, 22.0%; table 2). Of the 18 transversions, 10 mutations were G:C to T:A and they accounted for 25% of all mutations identified. Of the 9 frameshifts, 6 of the mutations were deletions from 1 to 18 bases and 3 were insertions of 1 or 2 bases. In gastric and colon cancer, transitions were predominant, followed by transversion. The ratio of transversion was 43.0% in the esophagus, 33.3% in the stomach and 25.0% in the colon. Reciprocally, the ratio of transition was 31.7, 66.7 and 72.2%, respectively.

Discussion

p53 Gene Mutation of Esophageal Cancer

The characteristics of *p53* gene mutations were investigated in Japanese patients with esophageal cancer [9]. Among 85 patients, 41 (48.2%) had a *p53* gene mutation. The reported frequencies of *p53* gene mutation in esophageal cancer vary widely from 17 to 84% [9–13]. The mutations are almost equally distributed over exons 5–8 of the *p53* gene [9]. Among the 41 mutations identified, transversions were predominant (18/41 mutations, 43.9%), followed by transitions (12/48, 29.2%) and frameshifts (9/41, 22.0%). Of the 18 transversions, 10 mutations were G:C to T:A and they accounted for 24.4% of all mutations identified. Of the 9 frameshifts, 6 of the mutations were deletions from 1 to 18 bases and 3 were insertions of 1 or 2 bases. Transversion mutants are the major feature of esophageal cancer. The esophagus is exposed to external carcinogens more frequently than the stomach or colon. Dietary carcinogens or habits have been reported to be causal factors inducing *p53* mutations in esophageal squamous cell carcinomas in some high-risk areas, such as China, Southern Brazil and Taiwan. Among the many sources of transversion, oxidative DNA damage and benzo[a]pyrene metabolites are most likely associated with esophageal carcinogenesis, since smoking is the major risk factor for developing esophageal cancer and benzo[a]pyrene is an important component of cigarette smoke. A G:C to T:A transversion occurred preferentially at the defined codons which are known to be the sites of adduct formation for the metabolites of benzo[a]pyrene, a major tobacco carcinogen (codons 157, 248 and 273).

p53 Gene Mutation of Gastric Cancer

The reported incidence of *p53* mutations in gastric cancer ranges from 3.2 to 65%. The characteristics of *p53* gene mutation were investigated in Japanese patients with gastric cancer. Among 112 patients, 18 (16.0%) had a *p53* gene mutation (table 3); 12 (66.7%) were transition mutants and 6 (33.3%) were transversion mutants. The mutational site of *p53* in gastric cancer is wide. However, there are several sites where mutations are more common than others. These include, in a decreasing order of frequency, codons 175, 248, 273, 282, 245 and 213, all of which are CpG sites. G:C to A:T transitions at CpG sites are the most common type of mutation, regardless of the histological type of the tumor. Interestingly, there appears to be a difference in the frequency of G:C to A:T and A:T to G:C transitions in European compared to

Table 3. *p53* mutations and clinicopathological factors in colon cancer

Factors	<i>p53</i>		p value
	wild type	mutant	
Gender			
Male	66	21	NS
Female	51	15	
Average age	63.5	65.8	NS
Nodal metastasis			
Negative	60	17	NS
Positive	45	18	
Lymph vessel involvement			
Negative	58	21	NS
Positive	48	14	
Vascular involvement			
Negative	62	28	NS
Positive	43	8	
Histology			
Well, moderately ¹	86	35	<0.05
Poorly	20	1	

NS = Not significant.

¹ Well and moderately differentiated adenocarcinoma.

Asian populations [6]. The spectrum of observed mutations was consistent with the predicted spectrum for dietary mutagens associated with the metabolism of nitrogenous compounds, thus resulting in the deamination of nucleic acids. C to T mutations are induced by nitric oxide [14, 15], a substance known to be produced during *Helicobacter pylori* infections. G:C to A:T transitions are also specifically induced by N-methyl-N'-nitro-N-nitrosoquandine and N-nitroso compounds found in foods, substances considered to be carcinogens involved in gastric carcinogenesis [16]. *H. pylori* induces an aberrant expression of activation-induced cytidine deaminase (AID), a member of the cytidine deaminase family that acts as a DNA- and RNA-editing enzyme, via the IκB kinase-dependent nuclear factor-κB activation pathway. *H. pylori*-mediated upregulation of AID results in the accumulation of nucleotide alterations in the TP53 tumor suppressor gene in gastric cells in vitro [17]. Indeed, the constitutive expression of AID in transgenic mice induces tumor development in various organs in association with high mutation frequencies. The constitutive active expression of AID generates mainly C to T mutations [18, 19].

Table 4. *p53* mutation spectrum in digestive tract cancers

Spectrum	Esophagus		Stomach		Colon	
	n	incidence, %	n	incidence, %	n	incidence, %
Transition	12	29.2	12	66.7	26	72.2
G:C→A:T	9	22.0	10	55.6	20	55.5
A:T→G:C	3	7.3	2	11.1	6	16.7
Transversion	22	43.9	6	33.3	9	25
G:C→T:A	10	24.4	5	27.8	3	8.3
G:C→C:G	2	4.9	0	0	1	2.8
A:T→T:A	3	7.3	1	5.6	2	5.6
A:T→C:G	3	7.3	0	0	3	8.3
1-bp frameshift	9	22.0	0	0	0	0
1-bp delation	6	14.6	0	0	0	0
1-bp insertion	3	7.3	0	0	0	0
Large deletion	2	4.9	0	0	0	0
Others	0	0	0	0	1	2.8
Total	41	100	18	100	36	100

p53 Gene Mutations of Colon Cancer

Information on 1,517 *p53* mutations held in the UMD-*p53* database indicates that 80% are G:C to A:T transitions occurring predominantly at CpG dinucleotides [20, 21]. The characteristics of *p53* gene mutations were investigated in Japanese patients with colon cancer and a similar result was observed (table 4). Among 157 patients, 36 (22.9%) had a *p53* gene mutation [unpubl. data]. Twenty-six cases (72.2%) were transition mutants and 20 of those were G:C to A:T transitions. These mutations are thought to arise by endogenous processes related to the deamination of 5-methylcytosine. Mutations in 5 hot-spot codons (175, 245, 248, 273 and 282) account for approximately 43% of all *p53* mutations in colon cancer [20, 22]. Three of these (codons 175, 248 and 273) contain a CpG dinucleotide. Interestingly, mutations occurring in the conserved regions of *p53* are more frequent in tumors from the distal than from the proximal colon and this has been suggested to reflect a different etiology [23]. Transversion rather than transition mutations have also been reported to occur more frequently in distal tumors [24].

The Difference in p53 Abnormalities between Cancers of the Upper and Lower Gastrointestinal Tract

The nuclear overexpression of *p53* is found in approximately 20–70% of the upper and lower gastrointestinal tract [25–28]. The positive rate is higher in esophageal cancer than in colon and gastric cancers [29–32]. The relationship between *p53* nuclear expression and patient

survival is controversial. In esophageal cancer, there is no association between patient survival and *p53* overexpression [25, 26, 33, 34]. However, most articles demonstrate that the *p53*-positive group shows worse survival in gastric cancer [27, 28, 35, 36]. We need a careful evaluation of *p53* overexpression in colon cancer, since completely opposite results are often reported.

It is apparent that a nuclear *p53* overexpression could sometimes occur in the absence of a mutation. On the other hand, no nuclear *p53* overexpression is observed in *p53*-mutated cancer. There are various mutation spectra for *p53*. Mutations of *p53* are found in approximately half of all gastrointestinal tracts, with a higher frequency observed in esophageal cancer and distal colon and rectal tumors, and a lower frequency in proximal, mucinous and gastric cancer. Transversion mutants are the major feature of esophageal cancer. The esophagus is exposed to external carcinogens more frequently than the stomach or colon. On the other hand, the frequency of transition mutants is greater in the lower than the upper gastrointestinal tract. According to the current investigation, the incidence of transition mutants of all *p53* mutations in the esophagus, stomach and colon was 31.7, 66.7 and 72.2%, respectively (table 4). In the stomach and colon, *p53* mutation is influenced more often by endogenous factors than external carcinogens such as benzo[a]pyrene metabolite in the esophagus.

Therefore, the *p53* mutation spectrum varies in each organ, revealing a difference in the carcinogenesis in the

upper and lower gastrointestinal tract. Both the LOH ratio and the frequency of *p53* transversion mutations were extremely high in the esophagus, and oxidative DNA damage was thus considered to be associated with carcinogenesis. In the lower digestive tract, transition mutations were more frequently observed than transversion mutations, thus indicating that DNA alkylation and methylation are associated with these mutations. The investigation of the spectrum of mutations in *p53* in cancer tissue is therefore expected to lead to a better understanding of the agents ultimately responsible for inducing cancer.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

References

- Hollstein M, Hergenhahn M, Yang Q, Bartsch H, Wang ZQ, Hainaut P: New approaches to understanding *p53* gene tumor mutation spectra. *Mutat Res* 1999;431:199–209.
- Giaccia AJ, Kastan MB: The complexity of *p53* modulation: emerging patterns from divergent signals. *Genes Dev* 1998;12:2973–2983.
- Bodner SM, Minna JD, Jensen SM, et al: Expression of mutant *p53* proteins in lung cancer correlates with the class of *p53* gene mutation. *Oncogene* 1992;7:743–749.
- Sun XF, Carstensen JM, Zhang H, et al: Prognostic significance of cytoplasmic *p53* oncoprotein in colorectal adenocarcinoma. *Lancet* 1992;340:1369–1373.
- Bosari S, Viale G, Bossi P, et al: Cytoplasmic accumulation of *p53* protein: an independent prognostic indicator in colorectal adenocarcinomas. *J Natl Cancer Inst* 1994;86:681–687.
- Fenoglio-Preiser CM, Wang J, Stemmermann GN, Noffsinger A: TP53 and gastric carcinoma: a review. *Hum Mutat* 2003;21:258–270.
- Iacopetta B: TP53 mutation in colorectal cancer. *Hum Mutat* 2003;21:271–276.
- Oki E, Tokunaga E, Nakamura T, et al: Genetic mutual relationship between PTEN and *p53* in gastric cancer. *Cancer Lett* 2005;227:33–38.
- Egashira A, Morita M, Kakeji Y, et al: *p53* Gene mutations in esophageal squamous cell carcinoma and their relevance to etiology and pathogenesis: results in Japan and comparisons with other countries. *Cancer Sci* 2007;98:1152–1156.
- Gao H, Wang LD, Zhou Q, Hong JY, Huang TY, Yang CS: *p53* tumor suppressor gene mutation in early esophageal precancerous lesions and carcinoma among high-risk populations in Henan, China. *Cancer Res* 1994;54:4342–4346.
- Shi ST, Yang GY, Wang LD, et al: Role of *p53* gene mutations in human esophageal carcinogenesis: results from immunohistochemical and mutation analyses of carcinomas and nearby non-cancerous lesions. *Carcinogenesis* 1999;20:591–597.
- Sepehr A, Tanieri P, Martel-Planche G, et al: Distinct pattern of TP53 mutations in squamous cell carcinoma of the esophagus in Iran. *Oncogene* 2001;20:7368–7374.
- Hu N, Huang J, Emmert-Buck MR, et al: Frequent inactivation of the TP53 gene in esophageal squamous cell carcinoma from a high-risk population in China. *Clin Cancer Res* 2001;7:883–891.
- Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnok JS, Tannenbaum SR: DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci USA* 1992;89:3030–3034.
- Wink DA, Kasprzak KS, Maragos CM, et al: DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991;254:1001–1003.
- Sugimura T, Tanaka N, Kawachi T, Kogure K, Fujimura S: Production of stomach cancer in dogs by N-methyl-N'-nitro-N-nitrosoguanidine. *Gann* 1971;62:67.
- Matsumoto Y, Marusawa H, Kinoshita K, et al: *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nat Med* 2007;13:470–476.
- Ramiro AR, Stavropoulos P, Jankovic M, Nussenzeig MC: Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the non-template strand. *Nat Immunol* 2003;4:452–456.
- Yoshikawa K, Okazaki IM, Eto T, et al: AID enzyme-induced hypermutation in an actively transcribed gene in fibroblasts. *Science* 2002;296:2033–2036.
- Soussi T, Dehouche K, Beroud C: *p53* website and analysis of *p53* gene mutations in human cancer: forging a link between epidemiology and carcinogenesis. *Hum Mutat* 2000;15:105–113.
- Beroud C, Soussi T: The UMD-*p53* database: new mutations and analysis tools. *Hum Mutat* 2003;21:176–181.
- Soong R, Powell B, Elsaleh H, et al: Prognostic significance of TP53 gene mutation in 995 cases of colorectal carcinoma: influence of tumour site, stage, adjuvant chemotherapy and type of mutation. *Eur J Cancer* 2000;36:2053–2060.
- Jernvall P, Makinen M, Karttunen T, Makela J, Vihko P: Conserved region mutations of the *p53* gene are concentrated in distal colorectal cancers. *Int J Cancer* 1997;74:97–101.
- Borresen-Dale AL, Lothe RA, Meling GI, Hainaut P, Rognum TO, Skovlund E: TP53 and long-term prognosis in colorectal cancer: mutations in the L3 zinc-binding domain predict poor survival. *Clin Cancer Res* 1998;4:203–210.
- Sarbia M, Porschen R, Borchard F, Horstmann O, Willers R, Gabbert HE: *p53* protein expression and prognosis in squamous cell carcinoma of the esophagus. *Cancer* 1994;74:2218–2223.
- Kanamoto A, Kato H, Tachimori Y, et al: No prognostic significance of *p53* expression in esophageal squamous cell carcinoma. *J Surg Oncol* 1999;72:94–98.
- Lee HS, Lee HK, Kim HS, Yang HK, Kim WH: Tumour suppressor gene expression correlates with gastric cancer prognosis. *J Pathol* 2003;200:39–46.
- Victorzon M, Nordling S, Haglund C, Lundin J, Roberts PJ: Expression of *p53* protein as a prognostic factor in patients with gastric cancer. *Eur J Cancer* 1996;32A:215–220.
- Kim YW, Lee S, Park JH, Yoon TY, Park YK, Yang MH: An immunohistochemical study of the expression of *p53* protein in colon cancer. *J Korean Med Sci* 1995;10:176–182.

- 30 Soong R, Grieu F, Robbins P, et al: p53 alterations are associated with improved prognosis in distal colonic carcinomas. *Clin Cancer Res* 1997;3:1405–1411.
- 31 Manne U, Weiss HL, Myers RB, et al: Nuclear accumulation of p53 in colorectal adenocarcinoma: prognostic importance differs with race and location of the tumor. *Cancer* 1998;83:2456–2467.
- 32 Allegra CJ, Paik S, Colangelo LH, et al: Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with Dukes' B and C colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project collaborative study. *J Clin Oncol* 2003;21:241–250.
- 33 Hardwick RH, Barham CP, Ozua P, et al: Immunohistochemical detection of p53 and c-erbB-2 in oesophageal carcinoma; no correlation with prognosis. *Eur J Surg Oncol* 1997;23:30–35.
- 34 Lam KY, Law S, Tung PH, Wong J: Esophageal small cell carcinomas: clinicopathologic parameters, p53 overexpression, proliferation marker, and their impact on pathogenesis. *Arch Pathol Lab Med* 2000;124:228–233.
- 35 Okuyama T, Maehara Y, Kabashima A, Takahashi I, Kakeji Y, Sugimachi K: Combined evaluation of expressions of p53 and p21 proteins as prognostic factors for patients with gastric carcinoma. *Oncology* 2002;63:353–361.
- 36 Maehara Y, Okuyama T, Kakeji Y, Endo K, Yamamoto M, Sugimachi K: A tumour-associated cell-surface glycoprotein accompanying p53 overexpression and higher growth potential for gastric cancer. *Br J Cancer* 1995;71:999–1002.