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 esophagus, 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.

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 a prognostic factor. However, the role of p53 abnormalities in prognosis is not known. The importance of p53 as a 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 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.

Table 1. p53 mutations in digestive tract cancers

<table>
<thead>
<tr>
<th>TP53 status</th>
<th>Esophageal cancer (n = 85)</th>
<th>Gastric cancer (n = 118)</th>
<th>Colon cancer (n = 157)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>44 (51.8%)</td>
<td>100 (84.0%)</td>
<td>121 (77.1%)</td>
</tr>
<tr>
<td>Mutant</td>
<td>41 (48.2%)</td>
<td>18 (16.0%)</td>
<td>36 (22.9%)</td>
</tr>
</tbody>
</table>

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-
Mutations in Cancers of the Upper and Lower Gastrointestinal Tract

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.

Table 2. p53 mutations and clinicopathological factors in gastric cancer

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

NS = Not significant.
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 frame-shifts (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 frame-shifts, 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 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 IkB 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 3. p53 mutations and clinicopathological factors in colon cancer

<table>
<thead>
<tr>
<th>Factors</th>
<th>p53</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wild type</td>
<td>mutant</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
</tr>
<tr>
<td>Average age</td>
<td>63.5</td>
<td>65.8</td>
</tr>
<tr>
<td>Nodal metastasis</td>
<td>Negative</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>45</td>
</tr>
<tr>
<td>Lymph vessel involvement</td>
<td>Negative</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>48</td>
</tr>
<tr>
<td>Vascular involvement</td>
<td>Negative</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>43</td>
</tr>
<tr>
<td>Histology</td>
<td>Well, moderately¹</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Poorly</td>
<td>20</td>
</tr>
</tbody>
</table>

NS = Not significant.

¹ Well and moderately differentiated adenocarcinoma.
**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

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

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Esophagus</th>
<th>Stomach</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>incidence, %</td>
<td>n</td>
</tr>
<tr>
<td>Transition</td>
<td>12</td>
<td>29.2</td>
<td>12</td>
</tr>
<tr>
<td>G:C→A:T</td>
<td>9</td>
<td>22.0</td>
<td>10</td>
</tr>
<tr>
<td>A:T→G:C</td>
<td>3</td>
<td>7.3</td>
<td>2</td>
</tr>
<tr>
<td>Transversion</td>
<td>22</td>
<td>43.9</td>
<td>6</td>
</tr>
<tr>
<td>G:C→T:A</td>
<td>10</td>
<td>24.4</td>
<td>5</td>
</tr>
<tr>
<td>G:C→C:G</td>
<td>2</td>
<td>4.9</td>
<td>0</td>
</tr>
<tr>
<td>A:T→T:A</td>
<td>3</td>
<td>7.3</td>
<td>1</td>
</tr>
<tr>
<td>A:T→C:G</td>
<td>3</td>
<td>7.3</td>
<td>0</td>
</tr>
<tr>
<td>1-bp frameshift</td>
<td>9</td>
<td>22.0</td>
<td>0</td>
</tr>
<tr>
<td>1-bp deletion</td>
<td>6</td>
<td>14.6</td>
<td>0</td>
</tr>
<tr>
<td>1-bp insertion</td>
<td>3</td>
<td>7.3</td>
<td>0</td>
</tr>
<tr>
<td>Large deletion</td>
<td>2</td>
<td>4.9</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>100</td>
<td>18</td>
</tr>
</tbody>
</table>
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 alkylolation 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

7 Iacopetta B: TP53 mutation in colorectal tissue is therefore expected to lead to a better understanding of the agents ultimately responsible for inducing cancer.

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References

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