p53 Gene mutations in esophageal squamous cell carcinoma and their relevance to etiology and pathogenesis: Results in Japan and comparisons with other countries

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Esophageal squamous cell carcinoma is a form of cancer that has varying incidence rates among different countries, distinct geographic areas and different ethnic groups. According to previous reports, *p53* gene mutations have been identified in 20–80% of these tumors, and these mutations have occurred at an early stage. These findings suggest that such mutations play an important role in esophageal carcinogenesis, and highlight the importance of mutagens, which cause sequence alterations in the *p53* gene. In order to clarify the environmental factors and the molecular mechanisms that may be responsible for the occurrence and prevention of a specific mutation in the process of esophageal carcinogenesis, we analyzed *p53* gene mutations in 95 samples of esophageal squamous cell carcinoma. We further reviewed published reports investigating the frequency of *p53* gene mutations in esophageal cancer from high-risk areas to normal-risk areas and compared these findings to our results in Japan. The frequency of *p53* gene mutations in Japanese esophageal cancer is 47.4% and there are three prominent features: (1) a predominance of transversions, in particular the G:C to T:A transversion; (2) a relatively low frequency of transitions; and (3) a relatively high percentage of frameshift mutations. These results indicate the possible importance of the benzo[a]pyrene metabolite and oxidative DNA damage in esophageal carcinogenesis and scarcely correlate with DNA replication errors or alkylation in comparison to other gastrointestinal cancers. In addition, we observed a peculiar sequence of frameshift mutations. Taken together, these data suggest that this tumor suppressor gene plays a critical role in the multistep carcinogenesis process for esophageal squamous cell cancer. (*Cancer Sci* 2007; 98: 1152–1156)

Esophageal cancer is the sixth most common cause of cancer death in the world. There are two major histological types of esophageal cancer: squamous cell carcinoma and adenocarcinoma. Although the incidence of the latter type has been increasing, especially in the USA and Europe, the former histological type is predominant worldwide, including in Japan. There is a marked geographic and ethnic variation in the incidence of this cancer. The high-risk regions are North Central China,[1–3] Northern Iran,[4,5] Normandy in France[6,7] and some areas in South Africa.[8] The different etiological factors, such as thermal irritation, dietary factors, and nutritional deficiencies in antioxidants, could therefore play a role in carcinogenesis. Alcohol consumption and tobacco smoking have been shown to be major risk factors for esophageal cancer in most Western countries.[9]

The development and progression of esophageal cancer are multistep processes that require the accumulation of specific alterations in the genes regulating cell growth, differentiation, apoptosis, and so on. Among them, the wild-type p53 protein plays a crucial role in cell proliferation by arresting the cell cycle in G_1 phase, regulating apoptosis and suppressing angiogenesis. Mutations in the *p53* gene have been reported in over half of all human cancers and they appear to occur at an early stage of esophageal cancer, thus indicating the important role of such mutations in esophageal carcinogenesis. Alcohol and tobacco are well-known factors contributing to the induction of *p53* gene mutation in ESCC. Moreover, dietary carcinogens or habits have also been reported to be causal factors inducing *p53* mutations in ESCC in some high-risk areas such as China, Southern Brazil and Taiwan.[3,10–12]

Ionizing irradiation, ultraviolet exposure and chemical mutagens are all known to cause various kinds of DNA damage. In addition to those exogenous factors, endogenous factors such as oxygen radicals, deamination and the loss of bases can also cause DNA damage. Under normal conditions, such DNA damage can usually be efficiently repaired or removed by DNA repair enzymes. However, in cases of excess mutagen exposure or a deficiency in the DNA repair enzyme activity, such damage remains and thereafter causes mutations after DNA replication. Such DNA damage can induce particular mutations. For example, 8-oxoguanine, which occurs in oxygen radicals, can be incorporated opposite adenine as well as cytosine. As a result of such misincorporation, G:C to T:A transversion can occur. Furthermore, the misincorporation of non-damaged nucleotides by DNA polymerase can cause mutation. DNA mismatch repair systems are responsible for repairing these misincorporated and misaligned bases in order to prevent base substitutions as well as frameshift mutations.[13]

A mutational analysis of tumors could provide clues to exogenous as well as endogenous mutagenesis mechanisms because the observed mutation reflects the specific DNA damage. In particular, the mutation spectrum of the *p53* gene has been used as a tool in predicting the role of carcinogenic factors in specific types of cancer.[12]

In order to clarify the environmental factors and the molecular mechanisms that may be responsible for the occurrence and prevention of specific mutations in the process of esophageal carcinogenesis, we analyzed the *p53* gene mutations in 95 samples of ESCC using the PCR direct sequencing technique. In addition, we reviewed reports investigating the frequency of *p53* gene mutations in esophageal cancer from high-risk areas to normal-risk areas and then compared these findings with our results in Japan.

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Abbreviations: EDTA, ethylene diamine tetra-acetic acid; ESCC, esophageal squamous cell carcinoma; HEX, 6-carboxy-2′,4′,7′,4,7-hexachloro-fluorescein; MSI, microsatellite instability; PCR, polymerase chain reaction; ROX, 6-carboxy-x-rhodamine, SSCP, single strand conformation polymorphism.

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Materials and Methods

Tissue collection. Surgically resected esophageal carcinomas with no preoperative therapy were collected from 95 patients who underwent an esophagectomy between 1995 and 2005 at the Second Department of Surgery, Kyushu University, Japan. All of the tissue specimens were obtained after receiving the patients' written informed consent. All of the samples were diagnosed histologically to be squamous cell carcinoma by means of hematoxylin and eosin staining.

DNA preparation. DNA was extracted as described previously.[14] 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% sodium dodecyl sulfate) containing proteinase K (100 μg/mL) at 37°C for 2 h. After doing phenol extraction two times, phenol–chloroform extraction and chloroform extraction were done, then ethanol precipitation was carried out following dilution 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 using primers (Nippon Gene). The PCR primers for amplification of the 406-bp fragment containing exon 5 of the *p53* gene were designed as follows: exon 5 forward, TGC AGG AGG TGC TTA CACATG; exon 5 reverse, TCC ACT CGG ATA AGA TGC TG.

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

High-resolution fluorescent microsatellite analysis. Five human dinucleotide microsatellites, D2S123, D5S107, D10S197, D11S904 and D13S175, were used as markers for MSI. Using the genomic DNA derived from tumor specimens and matched normal tissue, five microsatellite loci were analyzed as described previously.[15] Briefly, the oligonucleotide primers corresponding to the microsatellite sequences were synthesized and purified by high-performance liquid chromatography and 5′ primers were labeled with the fluorescent compounds ROX or HEX. The PCR were carried out using Taq Reagent Kits (Takara). The thermal conditions of the system were as follows: one cycle at 95°C for 4 min; 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s; one cycle at 72°C for 10 min. Then 0.5 μL of T4 DNA polymerase was added to the mixture, followed by incubation at 37°C for 10 min. A 1.5-μL aliquot of the product was mixed with 0.5 μL loading buffer (blue dextran, 25 mM EDTA), 2.5 μL formamide and 0.5 μL dH_2O. To compare electrophoretic profiles between two samples, 1.2 μL of the ROX-labeled product and 0.3 μL of the HEX-labeled product were mixed, denatured and loaded onto the ABI 310 autosequencer. All data were processed using the ABI program GeneScan.

Table 1. Summary of the *p53* gene mutations in esophageal squamous cell carcinoma in Japan

Case	Spectrum	Codon	Base change	Amino acid change
1	G:C→A:T, TS	167	cag→tag	Gln→Stop
2	G:C→T:A, TV	157	gtc→ttc	Val→Phe
3	A:T→T:A, TV	132	aag→atg	Lys→Met
	G:C→A:T, TS	192	cag→tag	Gln→Stop
4	Deletion	152	Frameshift	
5	G:C→T:A, TV	176	tgc→tga	Cys→Stop
6	G:C→T:A, TV	215	agt→att	Ser→Ile
7	G:C→A:T, TS	282	cgg→tgg	Arg→Trp
8	G:C→T:A, TV	203	gtg→ttg	Val→Leu
9	A:T→T:A, TV	193	cat→ctt	His→Leu
10	Insertion	230	Frameshift	
11	G:C→A:T, TS	193	cat→tat	His→Leu
12	G:C→T:A, TV	282	cgg→agg	Silent
13	G:C→A:T, TS	248	cgg→tgg	Arg→Trp
14	Insertion	294	Frameshift	
15	G:C→T:A, TV	294	gag→tag	Glu→Stop
16	Insertion	151	Frameshift	
17	A:T→C:G, TV	275	tgt→tgg	Cys→Trp
18	G:C→A:T, TS	179	cat→tat	His→Tyr
19	G:C→A:T, TS	213	cga→caa	Arg→Gln
20	A:T→G:C, TS	134	ttt→ctt	Phe→Leu
21	G:C→A:T, TS	282	cgg→tgg	Arg→Trp
22	G:C→T:A, TV	242	tgc→ttc	Cys→Phe
23	G:C→T:A, TV	242	tgc→ttc	Cys→Phe
24	A:T→T:A, TV	206	ttg→tag	Leu→Stop
	Deletion	209	Frameshift	
25	A:T→C:G, TV	251	atc→agc	Ile→Ser
26	G:C→T:A, TV	151	ccc→cac	Pro→His
27	A:T→T:A, TV	195	atc→ttc	Ile→Phe
28	G:C→A:T, TS	176	tgc→tac	Cys→Tyr
29	G:C→C:G, TV	278	cct→cgt	Pro→Arg
30	A:T→C:G, TV	254	atc→agc	Ile→Ser
31	G:C→A:T, TS	275	tgt→tat	Cys→Tyr
32	G:C→T:A, TV	285	gag→tag	Glu→Stop
33	Insertion	246	Frameshift	
34	G:C→C:G, TV	224	gag→gat	Glu→Asp
35	Deletion	258	Frameshift	
36	Deletion	238	Frameshift	
37	G:C→T:A, TV	245	ggc→tgc	Gly→Cys
38	A:T→C:G, TV	193	cat→cct	His→Pro
39	A:T→G:C, TS	179	cat→cgt	His→Arg
40	A:T→G:C, TS	220	tat→tgt	Tyr→Cys
	Deletion	254	Frameshift	
41	G:C→A:T, TS	245	ggc→gac	Gly→Asp
42	Deletion	133	Frameshift	
43	G:C→C:G, TV	282	cgg→ccg	Arg→Pro
44	A:T→T:A, TV	255	atc→ttc	Ile→Phe
45	G:C→T:A, TV	157	gtc→ttc	Val→Phe

TS, transition; TV, transversion.

Results

Frequency of *p53* gene mutations in esophageal squamous cell carcinoma. Of the 95 patients with ESCC investigated in this study in Japan, *p53* gene mutations in exons 5–9 were found in 45 patients, and three patients had double mutations. The frequency of *p53* gene mutations in esophageal cancer was therefore 47.4% in our study.

Distribution of *p53* gene mutations in ESCC. The mutations were almost equally distributed over exons 5–8 of the *p53* gene. Thirteen of the 49 mutations (26.5%) were located in exon 5, 12 (24.4%) in exons 6 and 7, 10 (20.4%) in exon 8, and no mutations were detected in exon 9 (Table 1).

There were nine codons in which mutations were identified from two or more patients in this study. However, among these mutations only two were the same (codon 242, tgc to ttc; codon 282, cgg to tgg).

Of the three known major mutation hot spots in the *p53* gene, codon 248 was mutated in two cases and no mutation was observed at codons 175 and 273 in this study. Among the three codons 157, 248 and 273, there are known to be sites of adduct formation for the metabolites of benzo(a)pyrene; a G:C to T:A transversion at codon 157 was observed in two cases.

Features of the *p53* gene mutation spectrum in ESCC. Among the 48 mutations identified, transversions were predominant (24 mutations out of the total 48 mutations, or 50.0%), followed by transitions (14/48, 29.2%) and frameshifts (10/48, 20.8%) (Table 2).

Table 2. Mutation spectrum of *p53* gene in esophageal squamous cell carcinoma in Japan

Type of mutation	Occurrence	
	n	%
Transition	14	29.2
G:C→A:T	11	23.0
A:T→G:C	3	6.3
Transversion	24	50.0
G:C→T:A	12	25.0
G:C→C:G	3	6.3
A:T→T:A	5	10.4
A:T→C:G	4	8.3
Frameshift	10	20.8
Deletion	7	14.6
Insertion	3	6.3
Total	48	100.0

Of the 24 transversions, 12 mutations were G:C to T:A and they counted for 25.0% of all of the mutations identified. Of the 10 frameshifts, seven of the mutations had deletions of 1–18 bases and three had insertions of 1 or 2 bases.

MSI in ESCC. As we and others have previously reported, the frequency of MSI is low in Japanese ESCC.[16,17] Out of the 80 cases examined in the present study, we observed alterations at the microsatellite loci in nine cases. The frequency of MSI in ESCC was thus 11.3%.

Sequence context of frameshift mutations. Table 3 shows the sequence contexts of the *p53* gene around the deletion mutation sites. The deleted bases are represented in bold, and the repeated sequences around the deleted bases are in italics. All of the deletion sites had one to five repeated bases that were adjacent to the deleted sequences.

These repeated sequences could play a role in the occurrence of deletion mutations. Misalignments between the template and the newly synthesized strands could be caused by the slippage of replicative DNA polymerase, and this misalignment could be fixed as a deletion if the DNA mismatch repair is not carried out efficiently. However, it is interesting to note that no MSI, which reflects the ability of the DNA mismatch repair system, was found in the five tumors harboring deletion mutations in the present study (Table 3).

Discussion

We herein investigated the characteristics of *p53* gene mutation in Japanese patients with esophageal cancer. Among the 95 patients, 45 cases (47.4%) had a *p53* gene mutation. The mutation frequency observed in the present study was moderate among the previously reported results and had a prevalence that was compatible with patients from other middle-risk areas and in contrast to high-risk areas such as Northern Iran and Normandy (about two-thirds of patients with esophageal tumors harbor *p53* mutation) (Table 4).

As shown in Table 4, the reported frequencies of *p53* gene mutation in esophageal cancer varied widely from 17 to 84%.[1–8,10,11,18–21] There are several reasons for this variation. First, the variation may depend on the sensitivity of the mutation detection system. For example, PCR SSCP analysis and PCR direct sequencing may differ in sensitivity. Moreover, the interpretation of the results may differ among the reports. We used

Table 3. Characteristics of the *p53* mutation sequence context at the deleted site in the current study

Case	Sequence around the mutation	Nucleotides	Exon	Microsatellite sequence
1	ctgccctcaacaagat**gt**ttttgccaactggccaagacc	401–402	5	Stable
2	gattcca*caccc***ccgcccggcaccc***gcgtccgcgccatg	456–468	5	Stable
3	ggagtatttggatgac*a***ga***aacactttttcgacatagtgt	628–629	6	Stable
4	tacaacta*catg***tgtaacagttcctgcatg***ggcggcatgaa	714–731	7	Stable
5	atcatcacactgga*g***agactcc***aggtcaggagccacttgc	776–783	7	Stable

The bases in bold are deleted bases and the underlined bases are repeated sequences.

Table 4. Reported frequencies of *p53* mutations in esophageal cancer

Pt (*n*)	Pt with mutation (%)	TS (%)	G:A at CpG (%)	TV (%)	TV (G:T) (%)	FS (%)	A:T (%)	Exons examined	Histology	Country (region)	Reference
76	17	–	–	15	–	85	15	5–8	SCC	South Africa	(8)
76	18	57	14	21	7	21	21	5–8	SCC	Japan	(18)
135	35	53	18	35	18	12	18	5–9	SCC	Southern Brazil	(10)
55	36	55	10	30	15	15	25	5–8	SCC	India (Kashmir)	(19)
42	40	59	35	29	18	12	18	5–8	SCC 11 Adeno 31	USA	(20)
56	41	36	8	44	24	20	20	5–8	SCC	Thailand	(21)
75	46	43	8	51	24	–	38	4–8	SCC	Taiwan	(11)
95	48	29	22	49	24	22	24	5–9	SCC	Japan	The present study
98	50	59	29	28	12	14	26	5–8	SCC	Northern Iran	(4)
29	55	41	10	18	12	12	25	5–8	SCC	China (Linxian)	(1)
74	65	62	34	20	13	18	11	5–8	SCC	Northern Iran	(5)
43	70	38	26	44	23	18	14	4–9	SCC	China (Linzhou)	(2)
56	77	38	18	18	13	38	9	4–9	SCC	China (Shanxi)	(3)
56	84	46	10	42	17	12	41	4–10	SCC 50 Adeno 6	France (Normandy)	(7)
32	84	45	10	34	3	21	48	2–8	SCC	France (Brittany)	(6)

Adeno, adenocarcinoma; FS, frameshift; Pt, patients; SCC, squamous cell carcinoma; TS, transition; TV, transversion.

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Table 5. Characteristics of sequence context at the deletion site in the *p53* gene in esophageal squamous cell carcinoma from the literature

Case	Sequence around the mutation	Nucleotides	Exon	Reference
1	atggccatcta**ca*agca***gtcacagcacatgac	491–494	5	(3)
2	tctacaagca**gtca**cagcacatgacggaggt	495–498	5	(6)
3	tgtgag*gcgctg***ccccccaccatga*gcgctgc***tcagatagcgat	529–546	6	(2)
4	gagcgctgctcaga**tagcga**tggtgagcagctggggc	552–557	5	(3)
5	tagtgtggtggtgcc**ctatgagcc**gcctgaggtctggttt	657–668	6	(19)
6	ccatccactacaa**ctac**atgtgtaacagttcctgcatgg	706–708	7	(18)

The bases in bold are deleted bases and the italic bases are repeated sequences.

the PCR direct sequencing technique and a precise interpretation. If an extra peak was detected by the first sequencing reaction with the forward primer, we did the second sequencing reaction using the same PCR product with the reverse primer. In addition, we confirmed the same extra peak with the forward primer using an independent PCR product.

Another factor is the length of the examined region. Except for hot spot regions (exons 5–9), there are several mutations in the coding region of the *p53* gene (exons 2–4, and exons 10 and 11). As shown in Table 4, a broader area of the coding region, exon 4 in particular, was investigated in the reports, which showed a high frequency (>70%) of *p53* gene mutation. In fact, there are several mutations in exon 4 of the *p53* gene in Japanese esophageal cancer (A. Egashira, Y. Maehara, unpublished data 2007). However, the mutation spectrum observed in that extended analysis, which contained exon 4, was similar to that seen in the present study. We thus considered it to be sufficient to analyze exons 5–9 in order to investigate the features of *p53* mutation.

However, the most significant factor that could be responsible for this variation may be the mutagens, which could play an important role in esophageal carcinogenesis. In the high-risk areas, such as Normandy and Brittany, a well-known risk factor for esophageal cancer is alcohol consumption.[6,7] In both cases, many mutations were found at the A:T base pair (41 and 48%, respectively). The transition or transversion at this site has been suggested to result from exposure to DNA-reactive agents such as acetaldehyde, a metabolite of ethanol.[22] In contrast, in North Central China, which is another high-risk area for esophageal cancer, the mutation at the A:T base pair is not predominant.[2,3] In this area, the etiology of esophageal cancer remains unclear. We do no know why there was a significant difference in the mutation frequency and spectrum among our findings in the present report and the previous one.[17]

In the middle-risk areas, such as Japan, Taiwan and Thailand, the most frequent mutation is a G:C to T:A transversion and the known risk factors are cigarette smoking and alcohol consumption.[11,21] A G:C to T:A transversion occurred preferentially at the defined codons known to be sites of adduct formation for the metabolites of benzo[a]pyrene, a major tobacco carcinogen (codons 157, 248 and 273). We summarized the distribution of *p53* gene mutation in ESCC (Fig. 1). Five, one and three G:C to T:A transversions were reported at codons 157, 248 and 273, respectively, in previous reports (Fig. 1).

In addition to the well-known hot spot codons of the *p53* gene (codons 175, 248 and 273), many mutations occurred at codons 179 and 282 (Fig. 1). Most of the mutations in these five codons were G:C to A:T transitions. However, semi-hot spot codons were also found, such as codons 135, 151, 158, 159, 173, 176, 193, 213, 220, 242, 245 and 248. Among these semi-hot spot codons, four, five, four, four, three, three and two G:C to T:A transversions were identified in codons 135, 151, 158, 173, 242, 245 and 248, respectively. A carcinogen may thus be involved in the occurrence of the G:C to T:A transversion at these codons, similar to the relationship between G:C to T:A transversion at codon 249 in hepatocellular carcinomas with aflatoxin B1 contamination.[23]

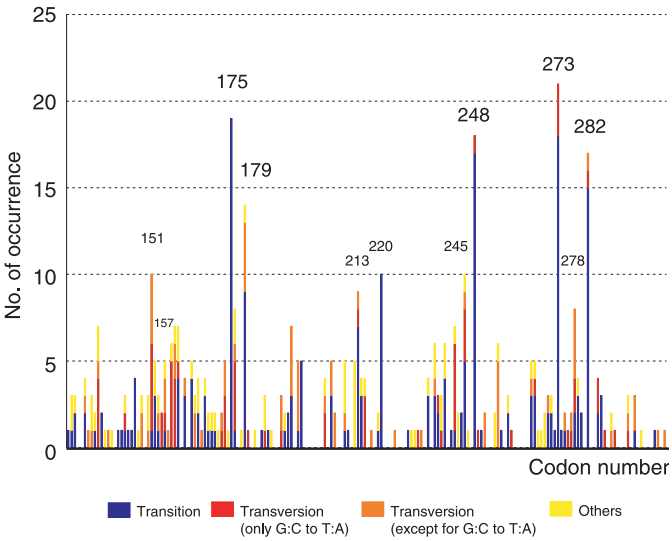

Fig. 1. Site distribution of *p53* gene mutations in esophageal cancer from the literature. The vertical axis represents a number of mutations at each codon (horizontal axis). Five hot spot codons (175, 179, 248, 273 and 282) were identified. Among the 12 semi-hot spot codons, several G:C to T:A transversions are found in codons 135, 151, 158, 173, 242, 245 and 248.

The number of patients investigated in the present study was large enough to evaluate the characteristics of the mutation frequency of the *p53* gene in Japanese patients. When we compared the spectrum of the *p53* gene mutations in esophageal cancer investigated in our study with the other reports, three features of esophageal cancer were identified: (1) the predominance of transversions, in particular the G:C to T:A transversion, which accounts for about one-fourth of all mutations; (2) a low frequency of transitions, the percentage of which is only 29.2%; and (3) the relatively high percentage of frameshift mutations, which account for over one-fifth of all mutations.

Among the many sources of transversion, oxidative DNA damage and benzo[a]pyrene metabolite are most likely associated with esophageal carcinogenesis because in Japan smoking is the major risk factor for developing esophageal cancer, and benzo[a]pyrene is an important component of cigarette smoke. There have been many reports regarding the association between oxidative DNA damage and cancer, including esophageal cancer. We are now investigating the role of these two factors in esophageal cancer.

However, most of the transitions could be caused by the deamination of 5-methylcytosine at the CpG site, misincorporation by DNA polymerase (replication error) during replication or

the mispairing of the O6-methyguanine adduct with thymine.[12] We previously reported the low frequency of MSI, which reflects the ability of DNA mismatch repair in Japanese esophageal cancer.[16] In contrast to colon cancer, the involvement of DNA mismatch repair may be low in esophageal cancer.[24] Interestingly, we observed that some tumors harboring the G:C to A:T transition in the *p53* gene also demonstrated MSI in this panel (data not shown).

In the present study, as shown in Table 3, the sequence contexts of the *p53* gene around the deletion mutation sites contained one to five repeated bases, which were adjacent to the deleted sequences. Similar to our study, shown in Table 5, some of the repeated sequences were observed near the deletion sites in previous reports. Greenblatt *et al*. indicated that most of the deletions in the *p53* gene may be explained by the features of the monotonic base runs, the adjacent or nonadjacent repeats of the short tandem sequences, palindromes and runs of purines or pyrimidines,[25] and the features of the deletion site have also been observed in other cancers.

Regarding the cause of frameshift mutations in which repeats were completely deleted in a run or in which one of the flanking repeats was removed, Tang *et al*. explained these mutations using the slippage model.[26] However, none of the five esophageal tumors listed in Table 3 showed MSI, which reflects the ability of DNA mismatch repair responsible for the collection of slippage error. The impairment of other repair systems, such as single-strand annealing, may be involved in the occurrence of these mutations.

We have investigated the relationship between the clinicopathological data and the mutation spectrum of the *p53* gene. However, we could hardly detect any correlations between such factors as prognosis, histology, tumor depth, nodal involvement, and even smoking and drinking habit. We hypothesized that not only the exposure of mutagens but the dysfunction of the repair system against mutagens, such as benzo[a]pyrene or alcohol metabolism, could thus possibly influence the occurrence of mutation. We are presently investigating the capacity of the repair system against benzo[a]pyrene.

In summary, we analyzed *p53* gene mutations in 95 samples of ESCC by PCR direct sequencing in order to clarify the environmental factors and molecular mechanisms that may be responsible for the occurrence and prevention of specific mutations in the process of esophageal carcinogenesis. The frequency of *p53* gene mutations in Japanese esophageal cancer patients has been reported to be 47.4% and has three prominent features:(1) predominance of transversion, in particular, G:C to T:A transversion:(2) relatively low frequency of transition: and(3) relatively high percentage of frameshift mutation. Taken together, these data suggest a critical role for this tumor suppressor gene to play a critical role in the multistep carcinogenesis process of esophageal squamous cell cancer.

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