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Infrequent Replication Errors at Microsatellite Loci in Tumors of Patients with Multiple Primary Cancers of the Esophagus and Various Other Tissues

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Patients with esophageal cancer are at high risk of developing other primary tumors, especially squamous cell carcinoma in the head and neck. Heavy smoking and excessive consumption of alcohol are considered to be crucial environmental risk-factors for development of these multiple primary cancers. To investigate whether any genetic background, such as defects in the DNA-mismatch repair system, may influence the development of these multiple primary tumors, we examined replication errors (RER) at six microsatellite loci in DNAs of 46 tumors from 33 patients who had developed primary cancers in various tissues in addition to the esophagus. RER(+) (RER-positive) phenotype was observed in three tumors in two patients of the 33 patients examined. Our results suggested that development of multiple primary tumors in these patients would not be affected by an abnormality in the DNA repair system(s) detected as the RER phenotype. However, it is noteworthy that a single patient who developed multiple cancers revealed RER(+) phenotypes at multiple microsatellite loci in both tumors, indicating that a defect in the DNA repair gene(s) may have played an important role in the development of the tumors in this patient.

Key words: Genetic instability — RER — Multiple cancers — Esophageal cancer — Head-and-neck cancer

In recent years, research in molecular biology has revealed that an accumulation of genetic alterations in oncogenes and tumor suppressor genes transforms cells of the normal lineage to cancer cells. In esophageal carcinomas, for example, inactivation of *p53*, *Rb*,[1,2] and activation of the c-*myc*, *cyclinD*, and *EGFR* genes[3,4] often play important roles during development and progression. Observations that mutations of genes involved in the DNA mismatch repair system in yeast (e.g. *MSH2*, *MLH1*, *PMS1*) decreased the stability of poly(GT) tracts[5] suggested that mutations in the mammalian homologue of these yeast genes might accelerate accumulation of the genetic alterations that transform normal cells. Subsequently, genetic defects in the DNA mismatch repair system were proven to increase the risk for mutations in oncogenes and tumor suppressor genes; germline mutations of hMSH2, hMLH1, hPMS1, or hPMS2, which are human homologues of these yeast genes, were found in patients with hereditary non-polyposis colorectal cancer (HNPCC).[6–8] This observation suggested that germ line mutations in the human homologues of these yeast genes would have an increased accumulation of the genetic alterations that transform normal cells to cancer cells in any organ, and primary cancers might develop in multiple organs.

Genetic defects in the mismatch repair system can be monitored by examining replication errors (RER) at microsatellite loci in cancer cells. The RER(+) phenotype has been reported in tumors associated with HNPCC and Muir-Torre syndrome, which develop multiple primary cancers.[9–11] Moreover, it has been reported that genetic instability may play an important role in development of multiple primary cancers not associated with HNPCC and Muir-Torre syndrome.[12] However, RER phenotype has not been investigated in detail in patients with squamous cell carcinoma combined with esophagus and head-and-neck tumors. Although esophageal cancer is infrequent in patients with HNPCC and Muir-Torre syndrome, it is well known that patients with squamous cell carcinoma of the esophagus have a high risk of developing a second primary cancer in the head or neck.[13] The increased risk has been attributed to

Table I. Summary of Microsatellite Alterations among 46 Tumors from 33 Patients

Patient	Tumors	Microsatellite loci					
		D1S226	D2S136	D2S393	D3S1067	D5S644	TP53
1	Esophagus	−	−	−	−	−	−
	Hypopharynx			NA			
2	Esophagus	−	−	+	−	+	−
	Hypopharynx	+	−	+		−	+
3	Esophagus	−	−	−	−	−	−
	Hypopharynx	−	−	−	−	−	−
4	Esophagus	−	−	−	−	−	−
	Hypopharynx	−	−	−	−	−	−
5	Esophagus	−	−	−	−	−	−
	Tongue	−	−	−	−	−	−
6	Esophagus	−	−	−	−	−	−
	Hypopharynx	−	−	−	−	−	−
7	Esophagus		−	−	−	−	−
	Hypopharynx			NA			
8	Esophagus	−	−	−	−	−	−
	Hypopharynx			NA			
9	Esophagus	−	−	−	−	−	−
	Hypopharynx			NA			
10	Esophagus	−	−	−	−	−	−
	Floor of Mouth			NA			
11	Esophagus	−	−	−	−	−	−
	Hypopharynx			NA			
12	Esophagus	−	−		−	−	−
	Hypopharynx			NA			
13	Esophagus	−	−	−	−	−	−
	Oropharynx			NA			
14	Esophagus		−	−		−	−
	Tongue			NA			
15	Esophagus	−	−	−	−	−	−
	Hypopharynx			NA			
	Stomach			NA			
16	Esophagus	−	−	−	−	−	−
	Hypopharynx			NA			
17	Esophagus	−	−	−	−	−	−
	Larynx			NA			
	Skin			NA			
18	Esophagus	−	−	−	−	−	−
	Oropharynx			NA			
19	Esophagus	−	−	−	−	−	−
	Tongue			NA			
20	Esophagus	−	−	−	−	−	−
	Rectum			NA			
	Stomach			NA			
21	Esophagus	−	−	−	−	−	−
	Kidney			NA			
22	Esophagus	−	−	−	−	−	−
	Stomach	−	−	−	−	−	−
23	Esophagus	−	−	−	−	−	−
	Stomach	−	−	−	−	−	−
24	Esophagus	−	−	−	−	−	−
	Liver	−	−	−	−	−	−
25	Esophagus	−	−	−	−	−	−
	Stomach	−	−	−	−	−	−
26	Esophagus	−	−	−	−	−	−
	Rectum			NA			
27	Esophagus	−	−	−	−	−	−
	Stomach			NA			

Table I. Continued

Patient	Tumors	Microsatellite loci					
		D1S226	D2S136	D2S393	D3S1067	D5S644	TP53
28	Esophagus	−	−	−	−	−	−
	Stomach	−	+	−	+	−	−
29	Esophagus	−	−	−	−	−	−
	Skin			NA			
30	Esophagus	−	−	−		−	−
	Stomach			NA			
31	Esophagus	−	−	−	−	−	−
	Liver	−	−	−	−	−	−
32	Esophagus	−	−	−	−	−	−
	Stomach	−	−	−	−	−	−
33	Esophagus	−	−	−	−	−	−
	Rectum	−	−	−	−	−	−

Abbreviations used are: +=RER(+), −=RER(−), NA=not available.

life style factors, such as heavy smoking and excessive consumption of alcohol.[14] However, it is also possible that genetic background, including an abnormality in the mismatch repair pathway, may influence the risk for developing multiple primary tumors in various tissues including esophagus.

To investigate this possibility, we analyzed genetic instability at six microsatellite loci in 46 tumors from 33 patients who had multiple primary tumors in various tissues in addition to the esophagus. The results presented here support the idea that development of tumors in these patients is influenced mainly by environmental risk factors, but not by dysfunction of the mismatch repair system.

Forty-six tumors and corresponding normal tissues were obtained from the Cancer Institute Hospital (Tokyo), the Osaka University Hospital (Osaka), the Center for Adult Diseases (Osaka), the Kinki Central Hospital (Hyogo), and the Kansai Rosai Hospital (Hyogo). Thirty-three esophageal cancers (squamous cell carcinoma), 5 head-and-neck cancers (squamous cell carcinoma), 5 gastric cancers (adenocarcinoma), 2 liver cancers (hepatocellular carcinoma and carcinoid), and 1 rectal cancer (adenocarcinoma) were obtained from 33 patients with multiple primary carcinomas of the esophagus and other organs. Genomic DNAs from paraffin-embedded or frozen tissues were prepared as described previously.[15]

Primer sets for the six loci examined in the present study were described previously: D1S226,[16] D2S136,[16] D2S393,[16] D3S1067,[17] D5S644,[16] TP53.[18] The polymerase chain reaction (PCR) was performed in a 15 μl reaction mixture containing 10 ng of DNA, 6.7 m*M* Tris-HCl (pH 8.8), 16.6 m*M* $(NH_4)_2SO_4$, 10 m*M* beta-mercaptoethanol, 6.7 m*M* EDTA, 6.7 m*M* $MgCl_2$, 0.33 m*M* of labeled (with [γ-^{32}P]ATP) and unlabeled primer,

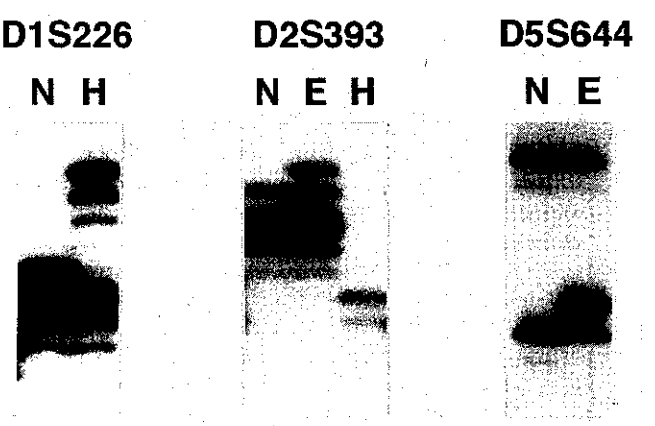

Fig. 1. Representative results of replication error (RER) phenotype at three of the six tested microsatellite loci (D1S226, D2S393, and D5S644) in tumors from one patient with multiple primary cancers (Patient #2). N, normal DNA from the patient; E and H, tumor DNA from corresponding carcinomas of the esophagus (E) and hypopharynx (H).

1.5 m*M* of each deoxynucleotide, 10% (v/v) DMSO and 0.75 unit of Taq DNA polymerase. Amplification was carried out with 40 cycles of the following regime: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. PCR products were electrophoresed in 6% polyacrylamide/8 *M* urea/32% formamide gels and autoradiographed overnight at room temperature on Fuji RX film.

Forty-six tumors in 33 patients who had developed multiple primary tumors in various organs in addition to the esophagus were analyzed for RER at six microsatellite loci. RER(+) phenotype was observed in three tumors of two patients of the 33 patients examined. The

results of RER analyses at the six microsatellite loci in 33 patients are summarized in Table I.

Fig. 1 shows the RER(+) phenotype detected in tumors of patient #2. Each of the two tumors in patient #2 exhibited bands of different sizes at D2S393 in comparison to their normal controls, indicating that the esophageal and hypopharyngeal tumors were of different clonal origin. In this case, RERs were observed at multiple microsatellite loci in each of the two tumors, suggesting the possible involvement of some defect in the mismatch repair system in the development of these tumors.

Our study revealed that the frequency of the RER(+) phenotype in tumors from patients with multiple primary tumors of the esophagus and various other tissues was very low. The results indicated that genetic defects in the DNA mismatch repair pathway do not play important roles in the development of these multiple tumors.

However, it is notable that replication errors at multiple microsatellite loci were found in one patient, who developed hypopharyngeal cancer at the age of 45 and esophageal cancer at age 49. As patients with HNPCC or Muir-Torre syndrome frequently develop tumors under the age of 50, we suspected that germline mutations in one of the genes involved in the mismatch repair pathway may have played an important role in the multiple-tumor phenotype of this patient.

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