

Double Cancer in a 74-Year-Old Woman: A Case Report with Genetic Findings

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KATADA, F., MURAKAMI, K., UZUKI, M. and HORII, A. *Double Cancer in a 74-Year-Old Woman: A Case Report with Genetic Findings.* Tohoku J. Exp. Med., 1996, 178 (4), 437-445 — An autopsy case of double cancer is reported. The patient had undergone right hemicolectomy for colon carcinoma in 1982, and the second cancer was detected in the pancreas in April 1989, which was diagnosed as intraductal papillary adenocarcinoma that is known for its favorable prognosis after surgical resection. However, as the patient did not consent to the operation, she died in August 1994, five years after the diagnosis of the second cancer. Histopathological study revealed neither recurrence nor metastasis of colon carcinoma. The pancreatic carcinoma metastasized to the lung, liver, and peritoneum. DNAs were extracted from paraffin-embedded tissue for molecular pathological examinations. Different mutations were found at codon 12 of the K-ras gene by nucleotide sequencing analysis: one in the colon and the other in the pancreas and lung. Over-expression of p53 protein was also detected in the colon by immunostaining. Replication error was not observed in these three tumors suggesting that a factor(s) other than genetic instability was playing a role in the development of double cancer in this patient. ————— multiple primary cancers; mucus hypersecreting tumor of the pancreas

Recent advances in medicine enabled molecular analyses of cancers, and various kinds of oncogenes and tumor suppresser genes have been found to play important roles in human carcinogenesis. Increasing evidences of genetic alterations in human cancers have indicated that carcinogenesis proceeds in multisteps with the accumulation of genetic changes. Among other cancers, those of the colon and rectum are studied most extensively, giving a molecular basis for the concept of the adenoma-carcinoma sequence (Hill et al. 1978; Fearon and Vogelstein 1990). Moreover, some genetic backgrounds of multiple cancers have been studied, and the presence of replication error (RER) which is caused by a mutation of one of the mismatch repair genes is recognized as one of the important

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risk factors for developing multiple primary cancers (Horii et al. 1994). In this study, we analyzed a patient with a double cancer: a colonic carcinoma and a pancreatic carcinoma. The routine histopathological studies were done macroscopically and microscopically. To compare the colonic carcinoma and the pancreatic carcinoma genetically, tumor tissues from both of the carcinomas and metastatic lesions were compared in mutations of the *K-ras* and *p53* genes by nucleotide sequencing and immunohistochemically. To ascertain the genetic background of the occurrence of a double cancer, RER test was performed and found that no microsatellite instabilities were detectable in these tumors.

CASE REPORT

The patient was a 74-year-old Japanese female. There was no cancer patient in her family within the second degree of relatives. She had no history of alcohol consumption or smoking, and had been healthy until 61 years of age when a cancer was found in the transverse colon. Right hemicolectomy was performed at Tohoku University Hospital. Her post-operative course was well and she was followed up as an outpatient. Since the age of 66, she was treated with insulin for diabetes mellitus (DM). In 1989, at the age of 69, she was admitted to Tohoku University Hospital for further evaluation of DM. CT scan and endoscopic retrograde cholangio-pancreato-graphy (ERCP) examination at the admission revealed a cystic lesion of the pancreatic head, and the clinical diagnosis was mucin-producing tumor of the pancreas. As the patient did not consent to a surgical operation, she was treated with a conservative therapy as an outpatient. In May 1994, at the age of 74, she was admitted again to Tohoku University Hospital for progressive jaundice and enlargement of multiple metastatic tumors in both lungs. After admission, her general condition rapidly deteriorated and she died on August 21, 1994.

Histopathological findings

The tumor of the transverse colon resected 12 years prior to death was an advanced carcinoma of Borrmann III type. This carcinoma formed irregular tubules or small nests of atypical cells, and were diagnosed as moderately differentiated adenocarcinoma (see Fig. 1a). Cancer cells infiltrated into proper muscle layer and invaded some lymph and blood vessels. Metastases to the paracolic lymph nodes were also observed. TNM classification of the colon cancer was pT3N1M0.

Fig. 1. Representative histopathology of the colonic carcinoma, pancreatic carcinoma and metastatic carcinoma of the lung. a, In the colonic carcinoma, cancerous cells formed irregular tubules. b, In the pancreas, cancerous cells proliferated papillary with extension of fibrovascular stalk. c, In the metastatic carcinoma of the lung, cancerous cells, closely resembling to the carcinoma cells of the pancreas in morphologic feature, proliferated along the alveolar walls and showing papillary configuration.

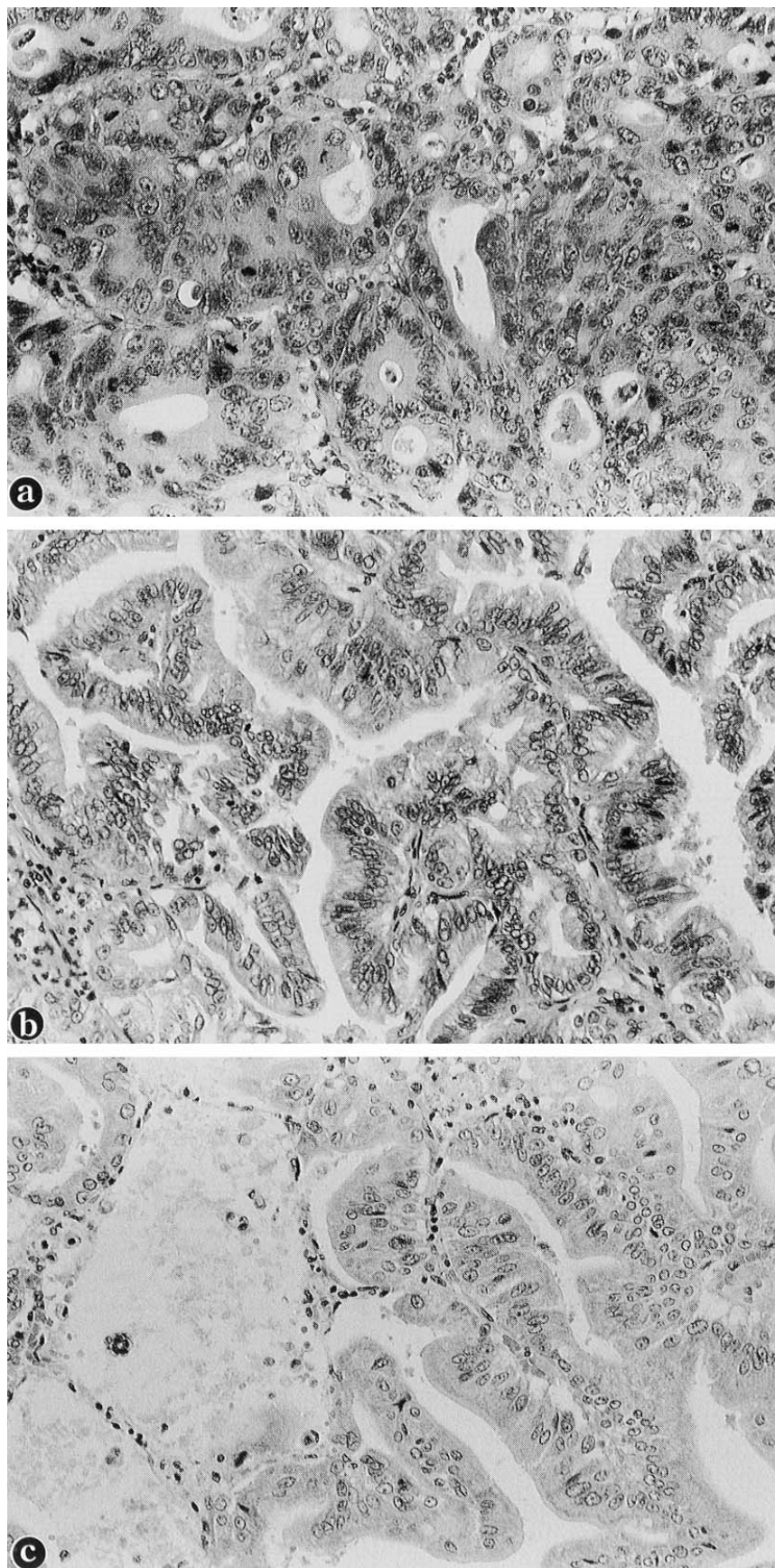


Fig. 1.

An autopsy was performed three hours after death. A hen's egg-sized mucinous tumor was found in the pancreatic head. The tumor was partially cystic, and papillary proliferation of high columnar carcinoma cells and collection of massive mucinous contents were observed in the cystic lesion (Fig. 1b). Infiltration of carcinoma cells forming irregular tubules was also observed in the pancreatic head around the cystic lesion. From the above pathological findings, we diagnosed the tumor as invasive ductal carcinoma originating from intraductal papillary adenocarcinoma. In the abdominal cavity, massive collection of ascites (7,500 ml) and peritoneal dissemination of carcinoma cells were prominent. Metastases of carcinoma in the form of many mucinous nodules were found in both lungs. Microscopic metastasis of carcinoma was also found in the liver. In these metastatic and disseminated lesions, papillary proliferation of carcinoma cells with high columnar-shape was observed (Fig. 1c). Carcinoma cells in the pancreatic head and the metastatic lesions were positively stained with a monoclonal antibody to CA19-9 (data not shown). Thus, metastases to the lung, liver, and peritoneum were all considered from the pancreatic cancer histologically and immunohistochemically; no sign of recurrence and metastasis of the colonic carcinoma was observed. TNM classification of the pancreatic cancer was pT2N0M1.

Analysis of genetic alterations

Mutations of the *K-ras* gene at codons 12 and 13 of the exon 1 were analyzed by the nucleotide sequencing method. As all the samples were fixed by formalin and embedded in paraffin, we collected samples carefully, under a microscope, from lesions that harbor more than 50% of cancerous cells to avoid contamination of normal cells. DNAs were prepared from tumors of colonic and pancreatic cancers, as well as of metastatic lesions of the lung, according to the methods described previously (Goelz et al. 1985; Yanagisawa et al. 1991). Myocardium of the heart was also analyzed as the control for a noncancerous tissue. Exon 1 of the *K-ras* gene was then amplified by polymerase chain reaction (PCR) with the primer set of 5'-TTGTTGGATCATATTCGTCC-3' and 5'-GGCCTGCTGAAAATGACTGA-3', which yielded an 118-base pair DNA fragment including codons 12 and 13 (Yanagisawa et al. 1991). PCR was performed as described (Miyoshi et al. 1992) for 40 cycles with the following regime: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec. After the PCR, products were purified and cloned in *EcoRV* site of the pBluescript II SK(-) (Stratagene, La Jolla, CA, USA). Plasmid DNAs were purified and sequenced by the method described previously (Hattori and Sakaki 1986). As shown in Fig. 2, transversions from GGT to TGT and GTT in colonic and pancreatic cancers, respectively, were observed at the 12th codon which would cause amino acid change from glycine to cysteine and valine. DNA from metastatic lesion of the lung contained the same mutation as detected in the

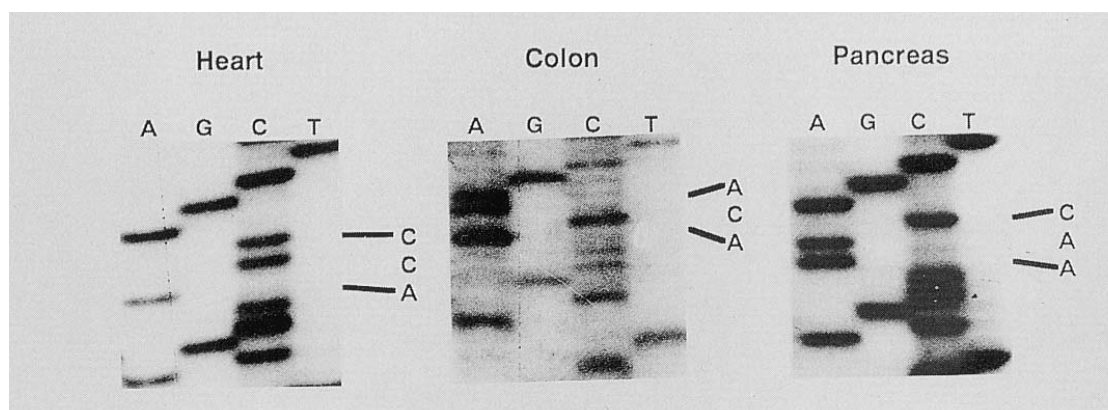


Fig. 2. Nucleotide sequencing analysis around codon 12 of the *K-ras* gene. Result of the heart represents the normal nucleotide sequence. Transversions from G to T (C to A in the antisense strand) at the first and the second letters of the 12th codon were detected in DNA samples purified from colon and pancreas. These alterations would result in amino acid changes from glycine to cysteine or valine, respectively. Nucleotide sequences of the antisense strands are shown.

pancreatic cancer (data not shown).

Mutations of the *p53* gene were examined immunohistochemically using a monoclonal antibody to human p53 protein which reacts both wild and mutant p53 in the N-terminus at the amino acid residues of 35th to 45th (Vojtesek et al. 1992). Briefly, 3 μ m sections were cut from the paraffin blocks and were incubated overnight with a mouse monoclonal antibody to human p53 product (DO-7) (Dakopatts, Copenhagen, Denmark) at 4°C. After washing with PBS, each section was further incubated with horseradish peroxidase-conjugated second antibody for 1 hr at room temperature, and immunoreactive products were visualized with diaminobenzidine. Cancer cells of the transverse colon were positively stained with the antibody (Fig. 3a and 3b). However, carcinoma cells in the pancreatic head and the metastatic lesions of the lung were not stained (Fig. 3c and 3d). These findings, along with the results of mutation analyses in the *K-ras* gene, were in good agreement with those obtained from the histopathological examinations. Tissues of the heart and kidney were used as the control; no staining was observed with the antibody (data not shown).

To determine whether genetic instability was playing an important role in the development of multiple primary cancers in this patient, RERs were also analyzed at five selected microsatellite loci. Primer sets examined in the present study were as follows; D1S226, D4S404, D5S474, D7S490, D11S900. Nucleotide sequences of each primer set were described previously (Gyapay et al. 1994). PCR and electrophoresis were performed as described (Horii et al. 1994). As the results, no RERs were observed in any of the tumors tested (data not shown).

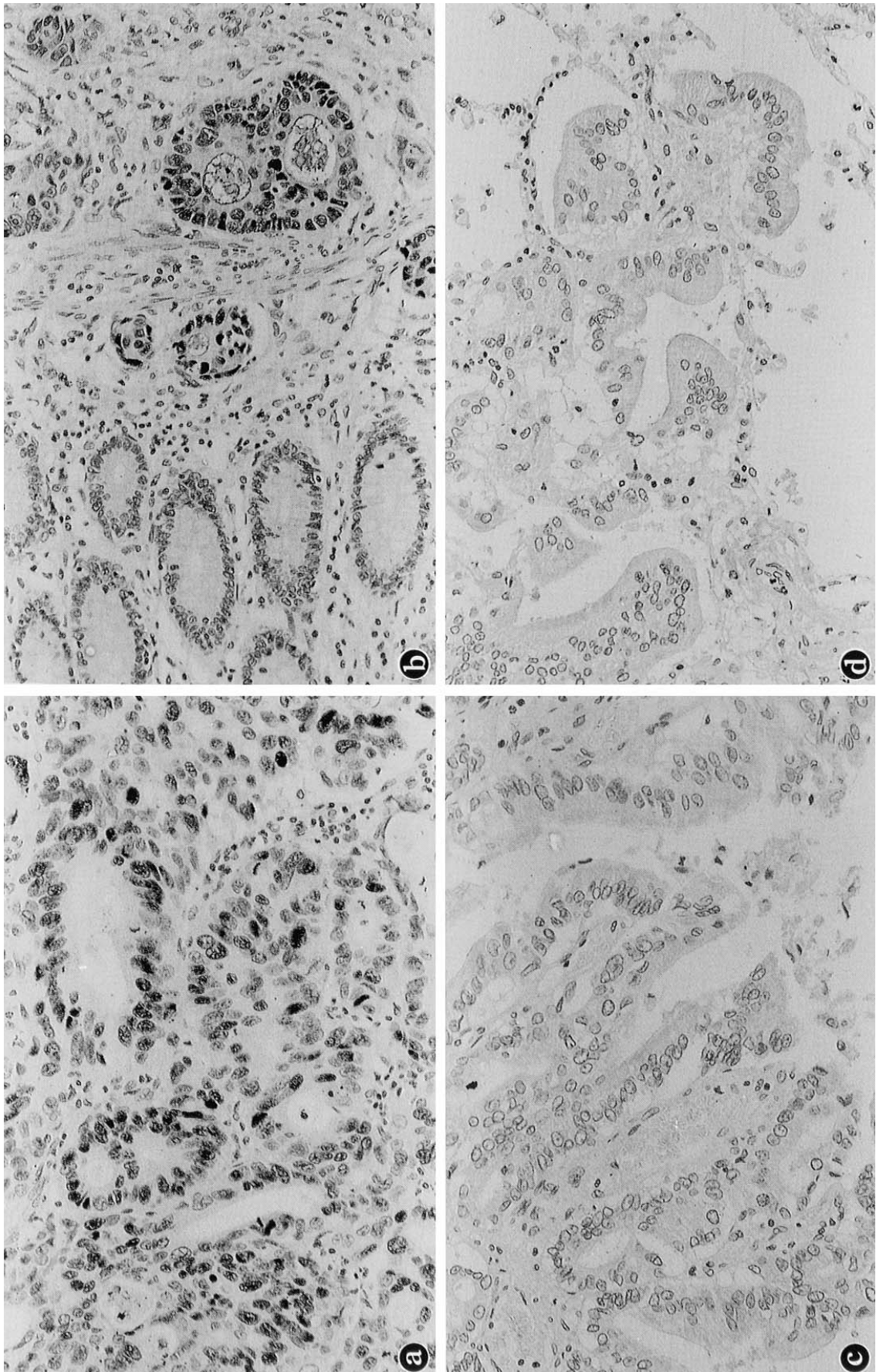


Fig. 3.

DISCUSSION

Recent advances in molecular biology revealed that an accumulation of multiple genetic changes involving oncogenes and tumor suppressor genes is playing an important role during the development of cancers. It is well characterized in the colonic carcinogenesis, which is known to advance in the form of "adenoma-carcinoma sequence" that involves sequential genetic alterations of adenomatous polyposis coli (*APC*), *K-ras* and *p53* (reviewed by Fearon and Vogelstein 1990). In our study, cancer cells of the colon proved to harbor a missense mutation of glycine to cysteine at 12th codon of *K-ras* as well as an overexpression of the p53 protein, which is in good agreement with the model of the adenoma-carcinoma sequence. In the present study, the carcinoma of the colon was operated 12 years prior to death. Histological diagnosis at the time of operation was moderately differentiated adenocarcinoma. As the cancer cells invaded in vessels and metastasized to lymph nodes, prognosis of this patient was not expected to be fair. After 12 years, however, neither metastasis nor recurrence was observed, and thus, treatment of colon cancer was perfect.

The carcinoma of the pancreas was clinically diagnosed as mucin-producing tumor, and histologically as invasive ductal carcinoma derived from intraductal papillary adenocarcinoma. These entities are established recently by the advances in the diagnostic methods. Intraductal papillary adenocarcinoma is known for its slow progression and better prognosis if it is surgically resected (Morohoshi et al. 1989). In this case, the patient survived five years after clinical detection without surgical operation. Although there was a possibility that the pancreatic tumor was intraductal papillary adenoma at the clinical detection, still it is notable that the patient survived relatively long period without any surgical treatment.

According to our genetic and immunohistochemical analyses, several different mutations were found in the two cancers that developed in this patient: (i) mutation in *K-ras* was different in colonic and pancreatic cancers; (ii) only colonic cancer showed overexpression of p53 protein. Since metastatic tumors in the lung showed the same characteristics as observed in the pancreatic cancer, it is evident that (i) the colonic and pancreatic cancers were developed independently in this patient, and (ii) the metastasis to the lung was originated from the

Fig. 3. Immunohistochemical staining for detection of p53 protein in sections of the colonic carcinoma, pancreatic carcinoma, and metastatic carcinoma of the lung. a and b, In a section of the colonic carcinoma, nuclei of almost all of the carcinoma cells were positively stained with a monoclonal antibody to human p53 protein. At the border of the carcinoma, nuclei of infiltrating carcinoma cells (right) were positively stained with the antibody but those of normal glandular epithelial cells were not stained (left). c and d, In sections of the pancreatic carcinoma (c) and metastatic carcinoma of the lung (d), p53 protein was not detected in nuclei of the carcinoma cells.

pancreatic cancer. Moreover, the pancreatic carcinoma and metastasis to the lung have the common histological features, e.g., mucin-producing character and papillary growth; colonic carcinoma was different.

Recently, diagnosis and treatment for cancer patients have progressed so as to ensure that about 40% of cancer patients may survive for five years after the diagnosis of the cancer (Hanai et al. 1994). However, about 10% of cancer patients develop second primary cancer within ten years after the diagnosis (Tsukuma et al. 1994). Thus, it is important for clinical management to find out patients that are at high risk of developing multiple primary cancers. A mechanism of RER, due to DNA mismatch repair error, was found as one of the major causes for developing cancers. Germline mutations of a subset of those genes, called mismatch repair (MMR) genes such as *hMSH2*, *hMLH1*, *hPMS1* and *hPMS2* that are playing essential roles in DNA mismatch repair system, are reported in hereditary nonpolyposis colorectal cancer (HNPCC), one of the susceptibility syndromes for multiple primary cancers (Fishel et al. 1993; Leach et al. 1993; Bronner et al. 1994; Papadopoulos et al. 1994; Nicolaides et al. 1994). Moreover, most of the patients with multiple primary cancers other than HNPCC showed RERs in their tumors (Horii et al. 1994). In the present study, we also analyzed RERs in tumors of the patient at five distinct microsatellite loci, but no microsatellite instability was observed. This result implied that (i) an intrinsic genetic alteration(s) other than MMR genes lies in the development of the two primary cancers, or (ii) factors other than intrinsic genetic alterations, such as mutagens from the environment or food, were playing crucial role in the development of these cancers in this patient. Although the great majority of patients with multiple primary cancers exhibit RER(+) phenotype, there are still considerable number of patient with multiple primary cancers who are not RER(+). Further studies are necessary to clarify factors for cancer susceptibilities, and to develop effective way to prevent from development of cancers for those who are at high risk.

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