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Multiple Primary Cancers with Microsatellite Instability: Report of a Case

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We report here a patient who developed a variety of tumors both synchronously and metachronously over a 2-year period. The involved organs were the uterus, ureter, and small and large intestines. The patient underwent open surgery 3 times and polypectomies 6 times. Postoperative histopathologic analysis showed 2 adenomas and 8 carcinomas. Genetic analysis revealed microsatellite instabilities at the tested loci in all 10 tumors, indicating that replication errors played an essential role in the tumorigenesis. Early identification of microsatellite instability could be useful for predicting development of additional primary cancers.

Key words: Multiple cancer — Microsatellite instability — Colorectal cancer

The role played by hereditary factors in the etiology of common human cancers is of great scientific and clinical importance. For colorectal cancer, in particular, there has been much debate about the relative contribution of hereditary and environmental factors. Predisposition to colorectal cancer takes two major genetic forms: a) familial adenomatous polyposis, and b) hereditary nonpolyposis colorectal cancer (HNPCC). In the latter group, an epoch-making discovery in unveiling a so-called black box in oncogenesis has recently been reported[1]: four human genes, *MSH2*, *MLH1*, *PMS1*, and *PMS2*, have been cloned and characterized. These genes have been shown to play an important role in the DNA mismatch repair system.[2–5]

It is possible that abnormalities in the mismatch repair system may also be related to oncogenesis in patients who do not meet the criteria of HNPCC.[6] We have recently experienced an interesting case in a patient who had no family history of colorectal cancer but who developed a variety of tumors both synchronously and metachronously. Genetic analysis of this patient should provide information useful for explaining the basis of the oncogenetic mechanism. We present here the clinical details and genetic features of this patient.

MATERIALS AND METHODS

Case report A 55-year-old woman visited our hospital because of anal bleeding and hematuria in December, 1992. A villous polyp, 20 mm in diameter, was found in the rectum; histologically, it was cancer in adenoma. Dripped intravenous pyelography and urethral endoscopy revealed a papillary tumor in the right ureter; histologically, this tumor was transitional cell carcinoma. Total colonoscopy revealed a polyp in the transverse colon; histologically it was also cancer in adenoma. Transanal polypectomy, endoscopic polypectomy, and right ureterectomy with nephrectomy were performed to extirpate the rectal polyp, the polyp in the transverse colon, and ureteral cancer, respectively. All of the surgical margins were free of malignancy.

Seven months later, the patient again visited our hospital, complaining of lower abdominal pain. A palpable tumor was found in the right upper quadrant of the abdomen. Emergent X-ray investigation revealed the presence of intussusception; this was readily reduced by barium enema. Total colonoscopy revealed 2 polyps, in the sigmoid colon and rectum, which had not been found on the previous endoscopic examination. However, we could not identify the cause of the intussusception. Endoscopic polypectomy was performed and, histologically, the resected specimens showed adenoma with moderate dysplasia. Subsequently an open laparotomy was performed to determine the cause of the intussusception. The open laparotomy revealed an ileoileal intussusception and a tumor 30 mm in diameter located at 40 cm proximal from the terminal ileum. Postoperative histopathological examination showed well-differentiated adenocarcinoma and no malignancy of the surgical margin.

After the second episode, the patient was periodically followed up with colonoscopy and other screening. In January 1994, a third rectal polyp, a second ileal tumor, and endometrial cancer were found. Transanal polypectomy, partial resection of the ileum and an extended hysterectomy were performed. Histologically, the ileal tumor was well-differentiated adenocarcinoma and the rectal tumor was cancer in adenoma.

Ten months later, a fourth rectal polyp, 20 mm in diameter, was detected and transanal resection of the

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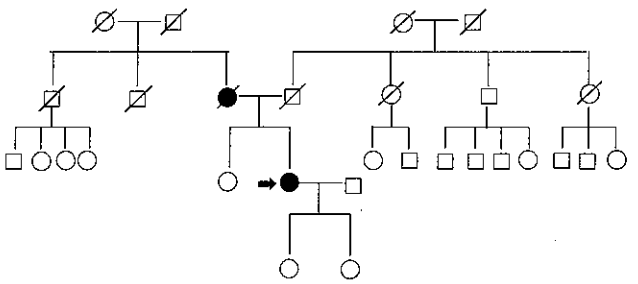

Fig. 1. Pedigree of the patient. The arrow indicates this proband. Except for her mother, who had endometrial cancer and died at the age of 56 years, no first-degree relative or second-degree relative had either colorectal cancer or extracolonic cancer. □, ○, males and females unaffected by cancer; ■, ●, males and females affected by cancer.

tumor was performed. Histopathologic study showed well-differentiated submucosal adenocarcinoma; the surgical margin was free of malignancy.

Since this operation, the patient has had periodic colonoscopies at 3-month intervals and was well, with no evidence of tumor, in May 1995. The pedigree of this patient is shown in Fig. 1.

Genetic analysis Formalin-fixed paraffin-embedded specimens of all the tumors, and two specimens of normal tissues (normal ileal and colon mucosas), were subjected to DNA extraction, performed as described previously.[7,8] Peripheral blood lymphocytes and fresh frozen specimens of the fourth rectal tumor were also subjected to DNA extraction.

Microsatellite instability was examined using five different (CA)n repeats, described previously: D1S495,[9] D2S123,[9] D3S1067,[10] TP53[11] and D19S412.[9] Polymerase chain reaction (PCR) was carried out in 25-μl volumes of a mixture containing 1×PCR buffer [6.7 mM Tris (pH 8.8); 16.6 mM NH_4SO_4; 6.7 μM EDTA; 10 mM β-mercaptoethanol]; and 10 pmol each of unlabeled primer and labeled primer with [γ-^{32}P]ATP, 20 ng of DNA, 0.5 unit of Taq DNA polymerase, 250 μM of each dideoxynucleotide, and 5 mM $MgCl_2$. PCR conditions consisted of 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. The PCR products were electrophoresed in 6% polyacrylamide gel containing 8 M urea and 32% formamide and the gel was exposed to X-ray film for 2–24 h.

RESULTS

On comparing samples amplified from these tumor DNAs with constitutional DNA, we detected genetic instability at more than four loci tested in all the malignancies and one adenoma (Table I and Fig. 2). The other small adenoma, however, showed this phenotype at only one locus (Fig. 2, lane 7 at D1S495). Bands seen in samples amplified from the same tissues, fresh-frozen and paraffin-embedded tissues, showed the same instability patterns. Results at five loci tested are summarized in Table I.

DISCUSSION

Recent advances in the diagnosis and treatment of malignant tumors have resulted in an increase in the incidence of second malignancies after treatment of the initial malignancy.[12] Multiple primary malignancy occurs in approximately 5–10% of all patients with colorectal cancers.[12] However, reports of more than quadruple primary cancers are rare. In this paper, we present an extremely rare case, of a patient in whom 10 tumors occurred metachronously and synchronously over a period of only two years. During each hospital stay, the patient was subjected to extensive screening tests for possible tumor development as well as surgical treatment

Table I. Results of RER Test in the Patient

Sample	Microsatellite loci				
	D1S495	D2S123	D3S1067	TP53	D19S412
Transverse colon cancer	+	+	+	+	−
Rectal cancer (1)	+	+	+	+	−
Normal colon mucosa	−	−	−	−	−
Ureteral cancer	+	+	+	+	+
Rectal adenoma	+	+	+	+	+
Sigmoidal colon adenoma	+	−	−	−	−
Ileal cancer (1)	+	+	+	+	+
Rectal cancer (2)	+	+	+	−	+
Ileal cancer (2)	+	+	+	+	+
Normal ileal mucosa	−	−	−	−	−
Endometrial cancer	+	+	+	−	+
Rectal cancer (3)	+	+	+	+	+

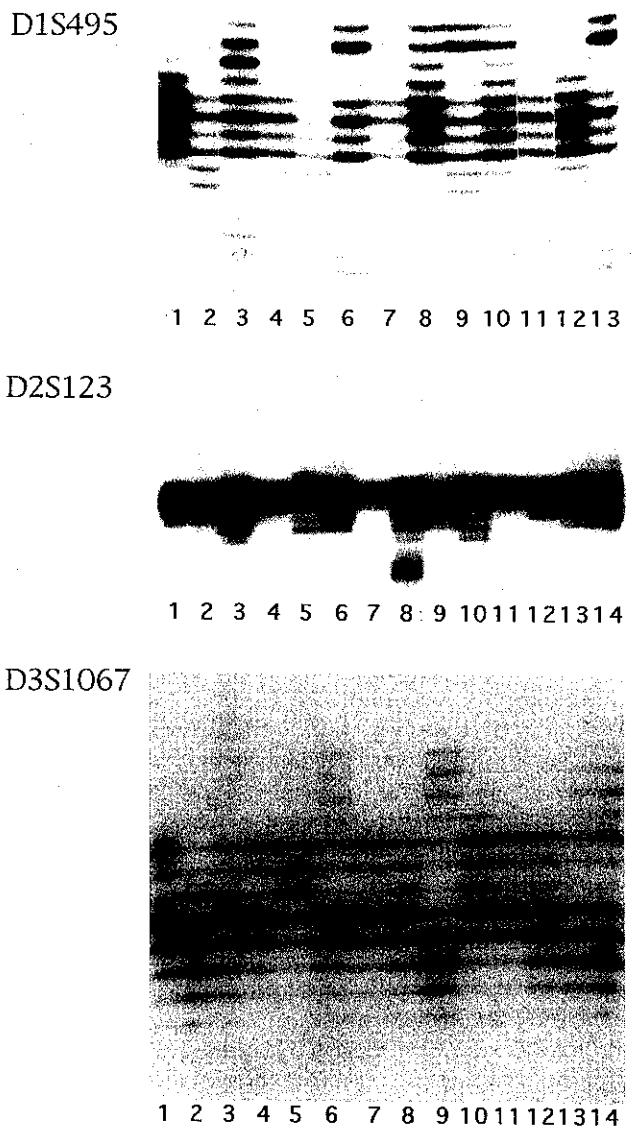

Fig. 2. Replication error test at D1S495, D2S123 and D3S1067. Microsatellite instability was observed in all 8 malignancies and 2 adenomas at these loci. DNA derived from patient's lymphocytes (lane 1), transverse colon cancer (lane 2), first rectal cancer (lane 3), normal colonic mucosa (lane 4), ureteral cancer (lane 5), rectal adenoma (lane 6), sigmoid colon adenoma (lane 7), first ileal cancer (lane 8), second rectal cancer (lane 9), second ileal cancer (lane 10), normal ileal mucosa (lane 11), endometrial cancer (lane 12), third rectal cancer (lane 13: paraffin-embedded tissue and lane 14: fresh-frozen specimen). Except for lanes 1 and 14, DNA was extracted from paraffin-embedded tissues. Microsatellite instabilities were clearly observed in lanes 2, 3, 5, 6, 9, 12, 13 and 14 at three loci, in lanes 8 and 10 at D1S495 and D2S123, and in lane 7 at D1S495. This phenotype was also detectable in lanes 8 and 10 at D3S1067, because the density of the third band (from the top in each lane) was stronger than that of the third band of constitutional DNA due to allelic shift.

for the tumors that had been detected. Each of the tumors detected on every admission had developed independently and was not a metastasis or a dissemination from the tumors treated previously.

To explain this repeated oncogenesis in the patient, we must consider possible genetic or environmental factors. Instability of microsatellite repeat sequences has been reported to occur frequently in tumors from HNPCC patients who often develop multiple cancers.[1] This genetic phenotype is considered to be a direct outcome of alterations in the DNA mismatch repair system. These patients are characterized by a familial predisposition to colorectal carcinoma and to extracolonic cancers of the gastrointestinal, urinary and female reproductive tracts.[13, 14] Our patient can not, strictly speaking, be classified as HNPCC, but she appears to exhibit a clinical phenotype similar to that of HNPCC.[15] Moreover, her mother was affected by endometrial cancer, which is the most frequently observed extracolonic tumor in HNPCC families,[16] at the age of 54 years. We, therefore, considered that the repeated tumorigenesis in this patient could be explained by the participation of a genetic aberration such as replication error.

Microsatellite instabilities were found in all of the tumors in our patient, but not in 2 specimens of normal bowel mucosa. Since microsatellite instability is observed as a consequence of replication error, we believe that impairment of the DNA repair system is closely connected with the repeated carcinogenesis in this patient.

Horii *et al.*[17] have recently reported that positive replication errors were found in 34 of 38 patients (89%) with multiple cancers, whereas this finding was observed in only 19 of 174 patients (11%) with a single primary cancer. These observations indicate that multiple primary cancerous mutations are probably caused by replication errors. Identification of a specific mismatch repair gene for each patient is time-consuming and laborious. Therefore, for the management of patients likely to develop multiple primary cancers, screening of microsatellite instability would be a sufficient and practical procedure.

In conclusion, microsatellite instabilities were found in a patient who was not classified as HNPCC, but who developed multiple cancers. This observation indicates that replication error plays an important role in repeated oncogenesis and that the detection of microsatellite instability would be useful for predicting secondary oncogenesis.

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