Frequent Replication Errors at Microsatellite Loci in Tumors of Patients with Multiple Primary Cancers

Akira Horii, Hye-Jung Han, Mamoru Shimada, Akio Yanagisawa, Yo Kato, Hirotoshi Ohta, Wataru Yasui, Eiichi Tahara, and Yusuke Nakamura

Abstract

Nearly 10% of cancer patients develop a second primary cancer within 10 years after surgical removal of the first tumor. Hence, detection of a genetic risk for developing multiple primary tumors would be of clinical importance. To investigate whether a genetic defect(s) involving the mismatch repair system constitutes an important risk factor in patients with multiple primary cancers, we examined replication errors (RER) at microsatellite loci in 79 primary cancers which had developed among 38 patients with multiple primary cancers. The RER(+) phenotype was observed at five microsatellite loci on chromosomes 2, 3, 11, or 17 in tumors from 34 (89%) of 38 patients with multiple primary cancers but only in 19 tumors from 174 patients (11%) with a single primary cancer. Our results suggested that: (a) genetic instability may play an important role in development of multiple primary cancers, and (b) testing for RER in a primary cancer may be an appropriate approach to detection of patients at high risk for developing multiple primary cancers.

Introduction

Recent developments in medicine have improved treatment of cancer patients to the point that nearly 40% of patients are cured now by surgery, chemotherapy, and/or radiotherapy (1). However, the incidence of a second primary cancer is increasing significantly; nearly 10% of cancer patients develop another primary cancer within 10 years after their first operation (2). Hence, an ability to identify patients at high risk for multiple primary cancers would contribute significantly to decisions concerning clinical management.

Studies based on molecular biology have revealed that an accumulation of genetic alterations transforms normal cells to cancer cells. For example, inactivation of APC, p53, and DCC and activation of the K-ras gene play important roles during development and progression of colorectal tumors. In addition to mutations in oncogenes and tumor suppressor genes, defects in genes associated with the mismatch repair system increase the risk of the development of cancers; for example, mutations of MSH2 or MLH1, genes playing important roles in the mismatch repair system, have been found in germline DNA of patients with hereditary nonpolyposis colorectal cancer but also in sporadic forms of tumors that developed in colon, rectum, stomach, pancreas, and endometrium (7–13). In yeast, the mutation rate is significantly increased in cells defective in MSH2, PMS1, and/or MLH1 (14). Hence, individuals who carry germline mutations in the human homologues of yeast MSH2 or MLH1 probably also have a higher risk of accumulating mutations in cancer-associated genes and of developing multiple primary cancers. Although it would be laborious to examine cancer patients for germline mutations in various mismatch repair genes, the presence or absence of RER in tumors may constitute a useful marker for screening patients to identify a group at high risk for additional primary cancers.

To investigate this possibility, we analyzed genetic instability at five microsatellite loci in 79 tumors from 38 patients who had developed two to four primary cancers. Our results imply that abnormality in the mismatch repair system increases the risk for development of multiple primary malignant tumors.

Materials and Methods

Materials and DNA Preparation. A total of 79 tumors and corresponding normal tissues, fixed with formalin and embedded in paraffin, were obtained from 38 Japanese patients with multiple primary cancers at the Cancer Institute Hospital (Tokyo), the Hiroshima University Hospital (Hiroshima), and Kure Kyosai Hospital (Hiroshima). Genomic DNA was purified as described previously (15, 16).

RER Test at Microsatellite Loci. Primer sets for the five loci examined in the present study were described previously: D2S123 (17), D2S136 (17), D3S1067 (18), D11S922 (17), and TP53 (19). Polymerase chain reaction was performed as described previously (20) with some modification. In brief, each 15-μl reaction mixture, containing 10 ng of DNA, 6.7 mM Tris-HCl (pH 8.8), 16.6 mM (NH4)2SO4, 10 mM β-mercaptoethanol, 6.7 mM EDTA, 6.7 mM MgCl2, 0.33 mM of labeled (with [γ-32P]ATP) and unlabeled primer, 1.5 mM of each deoxynucleotide, 10% (v/v) dimethylsulfoxide, and 0.75 units of Taq DNA polymerase was amplified for 40 cycles with 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. Polymerase chain reaction products were electrophoresed in 6% polyacrylamide-8 M urea-32% formamide gels and autoradiographed overnight at room temperature on Fuji RX film.

Results

A total of 79 tumors that developed in 38 Japanese patients with 2–4 primary cancers were analyzed for RER at five selected microsatellite loci. Fig. 1A shows a typical result; size alterations were observed in two of the three cancers that developed in this patient. Differences in the respective sizes of the altered microsatellites seemed to indicate that the gastric and colonic tumors had different clonal origins in this patient. Fig. 1B and C, show some other results obtained from tumors of multiple primary cancer patients along with those of single cancer patients. We did not find any correlation between the type of RER (gain or loss of number of repeats, or both of these) and the number of tumors. Results of RER testing in all 38 patients are summarized in Table 1. In most cases, DNA from tumors

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2To whom requests for reprints should be addressed.

3The abbreviations used are: RER, replication error; RER(+), replication error positive.
Fig. 1. A, a typical result of an RER test at D2S123 in one multiple primary cancer patient (case C5). DNA from normal tissue (N), two independent cancers of the stomach (S1 and S2), and a colon cancer (C) were analyzed. Genetic instability was observed in stomach tumors S1 and C. B, results of an RER test at three loci (D2S123, D11S922, and TP53) in four multiple primary cancer patients (cases C4, C7, H2, and H9). N, DNA from normal tissue; others are DNA from cancers of the colon (C), stomach (S), gallbladder (G), lung (L), and bladder (B). C, typical results of RER(+) cases at TP53 in three gastric cancer patients (cases S3, S23, and S106). N and T, DNA from normal and tumor tissue, respectively.

obtained from the same individual carried the same RER phenotypes, suggesting that such patients may harbor an underlying defect in the mismatch repair system.
cancers, screening of the RER phenotype can be applied as a rapid, simple, and efficient method for selecting a high-risk group. Since we observed RERs at multiple loci in multiple tumors in cancer patients, we suggest that germline mutations of genes associated with DNA mismatch repair systems are the most likely cause of the genetic instability, although exposure to environmental mutagens or carcinogens cannot be totally excluded. To identify patients at high risk for multiple cancers, detection of mutations in genes associated with mismatch repair systems in patients' DNA or measurement of environmental exposure would constitute useful approaches. However, it would be time consuming and laborious to examine all of these factors. Although we did not observe the RER(+) phenotype in every tumor from patients with multiple cancers, the incidence was high; nine-tenths of the patients showed the RER(+) phenotype in at least one, and in many cases all, of their tumors. Hence, we believe that screening of RER is a potentially useful way to identify patients at high risk of having additional primary cancers and for directing clinical management of these patients.

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Table 2 Frequency of RER(+) cases

<table>
<thead>
<tr>
<th>Patient#</th>
<th>RER</th>
<th>Tumor D2S123 D2S136 D3S1067 D11S922 TP53</th>
</tr>
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<tbody>
<tr>
<td>H7</td>
<td>+</td>
<td>Bile Duct + + + + + +</td>
</tr>
<tr>
<td>H8</td>
<td>+</td>
<td>Pancreas - + + + + + +</td>
</tr>
<tr>
<td>H9</td>
<td>+</td>
<td>Lung - - - - + + + +</td>
</tr>
<tr>
<td>H10</td>
<td>+</td>
<td>Liver + + + + + + + + +</td>
</tr>
<tr>
<td>H11</td>
<td>+</td>
<td>Pancreas - + + + + + + + + +</td>
</tr>
<tr>
<td>H12</td>
<td>+</td>
<td>Esophagus - - + + + + + + +</td>
</tr>
<tr>
<td>H13</td>
<td>-</td>
<td>Bladder - - - - - + + + + +</td>
</tr>
<tr>
<td>H14</td>
<td>-</td>
<td>Liver Paranasal Sinus - - - + + + + + + +</td>
</tr>
<tr>
<td>H15</td>
<td>+</td>
<td>Esophagus + + + + + + + + + + + +</td>
</tr>
<tr>
<td>H16</td>
<td>+</td>
<td>Bladder - - - + + + + + + + + + +</td>
</tr>
<tr>
<td>H17</td>
<td>+</td>
<td>Myeloma + + + + + + + + + + + + + + + + + +</td>
</tr>
</tbody>
</table>

Total RER(+) 34/38 (89%)

As a control, we analyzed RER in 174 tumors from patients with single cancers to compare the frequency of RER(+) cases with multiple primary cancers. The results summarized in Table 2 shows that: (a) a significantly higher incidence of RER(+) phenotype was observed in tumors from patients with multiple cancers ($P < 0.0001, \chi^2 = 98.50$), and (b) in about nine-tenths of the patients multiple primary cancer patients, tumors showed an RER(+) phenotype.

Discussion

It was recently reported that disruption of the genes involved in mismatch repair systems in yeast, such as *MSH2*, *MLH1*, and *PMS1*, significantly increased the incidence of RERs at microsatellite loci (14). This observation suggested to us that individuals that harbor mismatch repair systems in patients' DNA or measurement of environmental exposure would constitute useful approaches. However, it would be time consuming and laborious to examine all of these factors. Although we did not observe the RER(+) phenotype in every tumor from patients with multiple cancers, the incidence was high; nine-tenths of the patients showed the RER(+) phenotype in at least one, and in many cases all, of their tumors. Hence, we believe that screening of RER is a potentially useful way to identify patients at high risk of having additional primary cancers and for directing clinical management of these patients.

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