Microsatellite Instability and Loss of Heterozygosity in Breast Cancer1

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Abstract

Microsatellite instability (MSI) has been described in colorectal and other cancers. The purpose of this study was to determine the presence of MSI in breast cancer and to correlate its occurrence with clinicopathological parameters. For microsatellite markers we examined mono-, di-, tri-, and tetranucleotide repeats that, due to their polymorphic nature, may also be used to investigate loss of heterozygosity. In 20 paired breast cancer-peripheral blood DNA samples we identified four tumors (20%) with somatic MSI. All four tumors were stage I or II, grade 1 or 2, and estrogen receptor positive. To study MSI in relation to tumor progression we also examined paired DNA samples from two ipsilateral and three contralateral breast cancers, as well as two matched tumor-metastatic lymph node specimens. None of these seven cases showed MSI, but two of the contralateral tumors revealed allelic loss of polymorphic repeats. These data suggest that MSI is an early event in mammary tumorigenesis while loss of heterozygosity may occur at a later stage.

Introduction

Extensive studies of colorectal cancer suggest that tumorigenesis proceeds through a series of genetic alterations involving both protooncogenes and tumor suppressor genes (1). Most of these genetic alterations are in the form of point mutations providing evidence for substitution mutagenesis as the major mechanism of damage to these genes. Recently, a second, distinct type of genetic alteration based on substitution mutagenesis as the major mechanism of damage to these genes. The MSI1 was correlated significantly with tumor location in the ascending colon and with increased patient survival. A second study also reported instability of dinucleotide repeats in 13% of sporadic and 79% of familial colorectal cancers (2). Finally, a third study observed contractions and expansions of mononucleotide repeats in 12% of sporadic colorectal cancers (4). The combined evidence of these studies suggests a generalized defect in DNA replication or repair in both sporadic and familial colorectal cancer. Recently, a defect in the DNA mismatch repair gene MSH2 on chromosome 2p22-21 has been implicated as the cause of the most common form of inherited colorectal cancer, hereditary nonpolyposis colorectal cancer (5, 6).

In this study, we investigated the frequency and types of MSI in 20 primary breast cancers and correlated the findings with clinicopathological parameters. To determine the stage during tumor development when MSI may occur, we also analyzed two ipsilateral and three contralateral breast cancers as well as two matched primary tumor-metastatic lymph node specimens. Finally, we made use of the highly polymorphic nature of microsatellites to assess the allelic loss of heterozygosity in all tumor samples.

Materials and Methods

Patients. The study is based on 26 women with primary invasive breast cancer who were treated at Vanderbilt University Medical Center between 1983 and 1991. All patients had tumors of sufficient size (≥ 1 cm) to allow multipoint hormone-binding analyses of steroid hormone receptors and extraction of DNA in addition to routine histopathological studies. Demographic and clinical data were obtained from patients’ records with follow-up information provided by the Vanderbilt Tumor Registry. Disease stage was determined using the tumor-node-metastasis classification and tumor types were evaluated according to WHO criteria (7). All patients were treated by modified radical mastectomy.

Tissue and DNA Samples. All breast cancer biopsies were examined in the Surgical Pathology Laboratory of Vanderbilt University Medical Center. After establishing the diagnosis of infiltrating breast cancer on frozen tissue sections, a portion of tumor tissue was fixed in formalin and embedded in paraffin for routine histopathological examination. The remainder of the tumor tissue was stripped of adherent fat and frozen at -70°C for steroid receptor studies. The frozen tumor specimens were pulverized in liquid nitrogen; the resulting fine powder was suspended in low salt buffer (50 mM Tris, pH 7.4–1 mm EDTA-10% glycerol-1 mM monothioglycerol) and centrifuged for 30 min at 100,000 × g and 4°C. The supernatant cytosol was used for estrogen and progesterone receptor assays while the pellet was used to extract DNA by standard methods (8). In total, the genomic tumor DNA was compared with matched DNA from peripheral blood leukocytes (20 patients), axillary lymph node metastases (2 patients), ipsilateral tumors (2 patients), and contralateral tumors (3 patients). In one of the latter patients, we compared DNA from both tumors and blood.

DNA Analysis. The DNA samples were examined for genetic alterations at seven different microsatellites by PCR amplification. The microsatellites were selected to include mono-, di-, tri-, and tetranucleotides and to represent different chromosomes, some of which have been involved in LOH or linked to familial breast cancer (9). In brief, a GAAA repeat, at Ip32 (10)(5'-ATCGTGCACTGAAACGAGCT; 5'-CTCCGACCTCAAGGCATCA- CTT), a GT repeat, at D9S63 (11)(5'-TTATATGCGTACACTGCT; 5'-CCGGAATTACTCTAGTCTA), a TG repeat, on chromosome 15q11 in intron 4 of the cardiac muscle actin gene (12)(5'-TTGACTCTGAACGACT CTTA; 5'-TCCATACCTGGAACGAGT), a CA repeat, at D17S807 (13)(5'- GCTTTAGAAGGGAAAATCTAAG; 5'-AGATATGTACACTCTCATCA), a CA repeat, at D18S34 (14)(5'-CAGAAATCTCTCTGAGCA; 5'-CTCATGTTCCTGGCAAGATA), a CAG repeat, in the coding region of the androgen receptor gene at Xq11-12 (15)(5'-GTCCCGGCAATGATCGA GAA; 5'-TCTGGGAGCCACCTTCTCCT), and a TG repeat within Alu sequences (4)(5'-GAGGCCCAAACTCTGAC; 5'-AAAGATCAGTATAA GGAGA) were amplified using the listed primers.

Approximately 0.25–0.6 μg of DNA was amplified in each 100-μl PCR reaction consisting of 10 mm Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2 (4 mM MgCl2 for Alu), 200 mM of each deoxynucleotide triphosphate, 1 μM

Received 1/5/94; accepted 2/21/94.

The cost of publication of this article was defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by American Cancer Society Grant EDT-26A to F. F. P.

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3 The abbreviations used are: MSI, microsatellite instability; LOH, loss of heterozygosity; PCR, polymerase chain reaction.
MKROSSATellite INSTABILITY IN BREAST CANCER

Table 1 Microsatellite instability and loss of heterozygosity in breast cancer

<table>
<thead>
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<th>Patient</th>
<th>Age (yr)</th>
<th>Stage</th>
<th>Type</th>
<th>Grade</th>
<th>ER*</th>
<th>PR</th>
<th>DNA samples</th>
<th>MSI</th>
<th>LOH</th>
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<td>51</td>
<td>135</td>
<td>Blood-tumor</td>
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* ER, estrogen receptor (fmol/mg); PR, progesterone receptor (fmol/mg); NA, not applicable; ND, not done.

Concentrations of each primer, and 2.5 units of Taq DNA polymerase (Promega, Madison, WI). Prior to the PCR reaction, each sense primer was end-labeled with [γ-32P]ATP (10 mCi/ml; DuPont New England Nuclear, Boston, MA) using 1 unit T4 polynucleotide kinase/reaction. The PCR conditions consisted of an initial denaturation step followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. A 4-μl aliquot of each PCR reaction was diluted 1:1 with loading buffer [95% (v/v) formamide, 10 mM EDTA (pH 8.0), 0.1% (w/v) bromophenol blue, and 0.1% (w/v) xylene cyanol FF], heat denatured, and electrophoresed on 6% polyacrylamide-8 M urea gels at 60 W for 1.5–3 h depending on the fragment size. The gels were dried and exposed to Kodak X-omat-AR film (Eastman Kodak Co., Rochester, NY) at −80°C with intensifying screens. As defined in Refs. 2–4, MSI is characterized by a shift and/or gain of electrophoretic bands. In contrast, LOH is characterized by a loss of bands.

Results

Four of the 20 breast cancers (20%) showed evidence of MSI (Table 1). A comparison of the electrophoretic mobility of nucleotide repeats from paired normal and tumor tissue DNA samples showed a shift in the latter indicating an alteration in microsatellite size (Fig. 1). The shift in electrophoretic mobility was reproducible in replicate experiments. In three of the four tumors two or more loci were affected with contraction or expansion of dinucleotide (CA)n or trinucleotide (CAG)n repeats (Fig. 1). The fourth tumor showed an instability of mononucleotide repeats (T)n within Alu sequences. All four patients were 50 years or older and had no family history of breast cancer. Three of the patients had stage I tumors and were alive and well 3–5 years after mastectomy. The fourth patient had a stage II tumor and died of breast cancer 18 months after mastectomy. Two tumors were moderately well differentiated ductal carcinomas; the others were classified as lobular and tubular cancers. All four tumors were positive for estrogen and progesterone receptors. Using the same set of microsatellite markers, we identified two tumors with LOH affecting one allele of the androgen receptor gene at the Xq11 locus. Both patients were less than 50 years old (Table 1).
MICROSATELLITE INSTABILITY IN BREAST CANCER

23  
TI  LN  
24  
TI  TO  
25  
TR  TL  

Fig. 2. Example of LOH in breast cancer. Genomic DNA samples from three patients were subjected to PCR amplification of (TG)n repeat at 15q11 followed by denaturing gel electrophoresis and autoradiography. Patient 23, tumor (T) versus lymph node (LN) metastasis; patient 24, ipsilateral tumor (TI, inner quadrant; TO, outer quadrant); patient 25, contralateral tumor (TR, right breast; TL, left breast). Arrow, absent polymorphic repeat indicating LOH in the contralateral tumor of patient 25. The numbering of the patients is the same as that used in Table 1.

To study MSI in relation to tumor progression we examined paired DNA samples from two ipsilateral and three contralateral cancers as well as two matched primary tumor - metastatic lymph node specimens. None of these seven cases showed MSI, but two of the contralateral tumors revealed allelic loss of polymorphic repeats at the 15q11 and Xq11 loci (Fig. 2: Table 1).

Discussion

Colorectal cancer was the first type of human malignancy in which MSI was described (2–4). The reported genomic instabilities involved mono-, di-, and trinucleotide repeats and occurred in hereditary nonpolyposis as well as sporadic colorectal cancer. Subsequently, MSI was described in endometrial, pancreatic, gastric, and urinary bladder cancers (11, 16, 17). In this study, we identified MSI in four primary invasive breast cancers. The types of instability were similar to those described in the other malignancies; i.e., they affected mono-, di-, and trinucleotide repeats and, in three of the four tumors, involved two or more loci suggesting a more widespread genomic instability in this subset of breast cancers.

The hereditary nonpolyposis colorectal cancer syndrome is characterized by a familial predisposition to colorectal carcinoma and exacral cancers of the gastrointestinal, urological, and female reproductive tract (18). Recently, a germline defect in the DNA mismatch repair gene MSH2 was identified (2–4). The reported genomic instabilities involved mono-, di-, and trinucleotide repeats and occurred in hereditary nonpolyposis as well as sporadic colorectal cancer. We extended our exploration to a group of seven more advanced tumors including ipsilateral and contralateral cancers as well as lymph node metastases. While we found two additional cases with LOH among the seven tumors we did not observe any MSI. Overall, these data suggest that MSI may be an early event in mammary tumorigenesis whereas LOH occurs at a later stage. The latter is consistent with a recent study of 86 breast cancers in which LOH was shown to represent a relatively late event in tumor progression (22).

With regard to the role of MSI in tumorigenesis, it is interesting to note that MSI in urinary bladder cancer occurs in low stage tumors (11) and in colorectal cancer is associated with a good prognosis (3). Further studies on a larger population of breast cancer patients will be important to verify these initial observations and to determine if these DNA changes are independent prognostic indicators.

References


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