Selective Loss of Heterozygosity in Multiple Breast Cancers from a Carrier of Mutations in Both BRCA1 and BRCA2

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Abstract

Carriers of one mutant allele of either BRCA1 or BRCA2 are at risk for somatic loss of the second wild-type allele, leading to the initiation of breast tumorigenesis. We identified a patient of Ashkenazi Jewish heritage with germ-line heterozygous mutations in both BRCA1 (5382insC) and BRCA2 (6174delT), who had developed three independent breast cancers by age 47. Two breast cancers demonstrated inactivation of both BRCA2 alleles but retention of the wild-type BRCA1 allele, and the third showed loss of heterozygosity for BRCA1 but not BRCA2. The observation that breast tumors arising in a double heterozygote show biallelic inactivation of either BRCA1 or BRCA2, but not both, suggests that these genetic events are functionally equivalent in initiating tumorigenesis. The distinct histopathological features of these tumors may reflect the acquisition of subsequent genetic events.

Introduction

The occurrence of founder mutations in the BRCA1 and BRCA2 genes within the Ashkenazi Jewish population is responsible for carrier frequencies of 1.09% and 0.13% for the 185delAG and 5382insC alleles of BRCA1, respectively, and 1.52% for the 6174delT mutation in BRCA2 (1). Within this population, carriers of mutant alleles in both genes would be predicted to comprise 1.85/10,000 individuals, and 4 such cases have been identified in an analysis of 1,500 patients of Ashkenazi descent diagnosed with either breast or ovarian cancer (2). Initiation of breast cancer in carriers of one mutant allele of either BRCA1 or BRCA2 follows somatic loss of the second allele (LOH) and appears to result from chromosomal instability associated with homozygous inactivation of either gene (reviewed in Ref. 3). However, it is unknown whether loss of either BRCA1 or BRCA2 is more likely to lead to tumor formation, and whether inactivation of both genes confers an additional proliferative advantage over that of either gene alone. Histological analyses of breast tumors arising in patients with a positive family history have suggested that BRCA1-associated tumors may have a more aggressive phenotype than sporadic cancers, whereas BRCA2-linked tumors do not appear to have a consistent histological appearance (4). Here, we describe a molecular and histological analysis of three primary breast tumors arising in a single carrier of mutations in both BRCA1 and BRCA2, to determine the relative contribution of each mutation to the development of breast cancer.

Materials and Methods

Nucleotide Sequence Analysis. The patient was identified as a heterozygous carrier of the 5382insC mutation in BRCA1 and of the 6174delT mutation in BRCA2 by screening a blood specimen for these known founder mutations in the Ashkenazi Jewish population. DNA was extracted from paraffin-embedded tumor sections using a standard proteinase K-phenol-chloroform procedure. A subset of tumors was estimated to be composed of at least 80% tumor cells. Nested PCR was used to amplify sequences flanking the BRCA1 mutation (external primers: 5'-ATATAGCAGTCTGCCTCCTC-3' and 5'-CACAGGTCAGACGCTTCG-3'; internal primers: 5'-CCACTTCCATGGAAAGAAGCTTCTC-3' and 5'-TGGGTTGAGAATTCTGTCACACCTTG-3'), and the BRCA2 mutation (external primers: 5'-GTTAAATTGTATGAGGAAGCTCCT-3' and 5'-ATGTTCTGGAGTACGATGACCA-3'). Internal primers included a 5' M13 tail in both the forward and reverse directions to facilitate dimer primer sequencing of uncloned PCR products, using an ABI 3100 sequencer (BigDye Primer kit; Applied Biosystems, Foster City, CA), with heterozygous nucleotide positions marked using Factura and displayed using Sequence Navigator software. For TP53 sequencing, the four conserved exons were also PCR-amplified and sequenced (exon 5 external primers: 5'-CTGTTACCTTGTGCTCCGTACCTTCTCTCTTACGTTCCAC-3' and 5'-GGACCCTTGGCACCACCCCGCTTG-3'; internal primers: 5'-GACCTTTCAACTCTGTCCTTCCTCTCTCTC-3' and 5'-CACGGCTCTGTCCTTCCGACC-3'); exon 6 external primers: 5'-CAGGGCTGTGTTGCCAGGACCTCCTTAACC-3' and 5'-GCAGGACCTTCCAGGAGCTCTCC-3'; internal primers: 5'-GTCCCTCCAGGTCCCTCCTGCTTGAACTC-3' and 5'-CTAATCCACCTGCTCTGCTGCTG-3'); exon 7 external primers: 5'-CAGGACCTTCCAGGAGCTCTCC-3' and 5'-CGACCCCGTGCCTCCTTGTTGTCCTG-3'; internal primers: 5'-CTAATCCACCTGCTCTGCTGCTG-3' and 5'-CGAGCCGGAAGTGTCAGGGTG-3'); exon 8 external primers: 5'-CTGGTTCTTCCTACTGCAGCTTCGTTGGGTCCTG-3' and 5'-CATCTGCTCGCCTGCTGCTCCTG-3'; internal primers: 5'-CTGGTTCTTCCTACTGCAGCTTCGTTGGGTCCTG-3' and 5'-CTTGTGCTGCTGCTCCTCCACCGCGTCTCAG-3').

Histological Analysis. Slides for each specimen were reviewed, blind to the BRCA1 and BRCA2 sequencing results, by a breast cancer pathologist (D. C. S.), and the tumors were classified using the modified version of the Bloom-Scarff-Richardson grading system. Formalin-fixed, paraffin-embedded tissue blocks, along with original slides (5-µm sections) were available for review for all three of the breast tumors. For immunohistochemical staining, sections from the original paraffin blocks were cut and stained using avidin-biotin-peroxidase technique using primary antibodies to ER (clone ID5; DAKO), PR (A 0098; DAKO), and HER2/neu (polyclonal; Neomarkers).

Results and Discussion

A patient was diagnosed with three independent primary breast cancers at ages 33, 44, and 47. She was of Ashkenazi Jewish ancestry; she had a paternal aunt with breast cancer around age 70, and her paternal grandmother died at a young age of unknown cause. There...
was no other known family history of breast or ovarian cancer. The patient had no history of reproductive dysfunction. Nucleotide sequencing of the blood cells of the patient demonstrated the presence of two heterozygous mutations: the 5385insC mutation in \textit{BRCA1} and the 6147delT mutation in \textit{BRCA2}. Family members were not available to determine the parental origin of each mutation. The patient ultimately died of metastatic breast cancer; there was no evidence of malignancy in either ovary.

We obtained paraffin-embedded tumor blocks for each of the three independent breast cancers and analyzed microdissected specimens for presence of wild-type and mutant alleles of \textit{BRCA1} and \textit{BRCA2}. Analysis of the first (83T) and second (94T) primary breast tumors demonstrated reduction to homozygosity (LOH) for the 6174delT \textit{BRCA2} mutation but retention of heterozygosity for the \textit{BRCA1} mutation (Fig. 1). In contrast, the third tumor (98T) showed reduction to homozygosity for the 5382insC \textit{BRCA1} mutation but retained heterozygosity for the \textit{BRCA2} mutation.

The selective somatic inactivation of either \textit{BRCA1} or \textit{BRCA2} suggests that loss of both gene products within a breast tumor cell does not confer a proliferative advantage beyond that associated with loss of either gene alone. In the absence of such selection pressure, tumor cells that have sustained LOH at both \textit{BRCA1} and \textit{BRCA2} loci would not become predominant in the population. Similar observations have been made for genes of which the products function within a common cellular pathway, such as the retinoblastoma tumor suppressor gene \textit{RB1} and the cyclin-dependent kinase inhibitor \textit{p16 INK4a}, both of which regulate the same critical step in cell cycle progression (5). Although \textit{BRCA1} and \textit{BRCA2} proteins have no amino acid similarity, recent studies have suggested that a fraction of both gene products may be components of a large multiprotein complex, which is required for genomic stability (6). Therefore, homozygous inactivation of both genes within a single tumor cell may be functionally redundant. Our observations are consistent with a previous case report of a patient

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<th>Tumor</th>
<th>\textit{BRCA1}</th>
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<tr>
<td>83T</td>
<td>5382insC</td>
<td>6174delT</td>
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<td>94T</td>
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<td>98T</td>
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Fig. 1. Mutually exclusive biallelic inactivation of \textit{BRCA1} or \textit{BRCA2} in breast tumors from a carrier of heterozygous mutations in both genes. Biallelic inactivation of either \textit{BRCA1} or \textit{BRCA2} in three primary breast tumors that developed in this double heterozygote. Representative nucleotide sequence analysis of \textit{BRCA2} demonstrates presence of the heterozygous mutation in the germ-line (top panel) and reduction to homozygosity in tumor 83T (bottom panel).

Fig. 2. Histological appearance of tumors associated with biallelic inactivation of \textit{BRCA1} or \textit{BRCA2}. Histological appearance of tumors associated with biallelic inactivation of \textit{BRCA1} or \textit{BRCA2}. Tumors 83T and 94T demonstrate small clusters and nests of malignant cells infiltrating in an irregular pattern into surrounding fibroadipose tissue, whereas tumor 98T invades into the surrounding fibroadipose tissue with a predominantly continuous broad-faced pushing margin (arrows). Numerous mitoses (arrowheads) are present in tumor 98T, consistent with a poorly differentiated (grade III) invasive carcinoma. Tumors 83T and 94T are moderately differentiated (grade II) with rare mitoses and focal tubule (gland) formation.
that follow the loss of either tumors may reflect the accumulation of additional genetic alterations

tological characteristics of BRCA1-cancers (11). The examination of molecular expression profiles in hereditary breast and no TP53 BRCA1 characteristics that are seen frequently in BRCA2 -linked carcinomas, a unique histopathological phenotype has not emerged for BRCA2 contrast to the exception of the observed ER and PR positivity, and HER2/neu negativity, all of the histological features of the BRCA1-mutant tumors studied here lacked the proliferative characteristics that have been linked recently with inactivation of these two genes. BRCA1-related breast cancers differ from sporadic cases in having a higher frequency of high grade histology, high mitotic counts, a prominent pushing margin, and a lymphocytic infiltrate (8, 9). These tumors are also more likely to be negative for expression of the ER and PR, overexpression of HER2-neu, and to have somatic mutations of TP53. With the exception of the observed ER and PR positivity, and HER2/neu negativity, all of the histological features of the BRCA1-mutant tumor 98T were consistent with these characteristics (Fig. 2). The tumor was also found to harbor a somatic TP53 mutation (Arg179His), documented previously in cancers of the lung, pancreas, and brain (10). In contrast to BRCA1-linked breast cancers, a unique histopathological phenotype has not emerged for BRCA2-associated breast cancers (4). The two BRCA2-mutant tumors studied here lacked the proliferative characteristics that are seen frequently in BRCA1-linked carcinomas, and no TP53 mutations were detected. Presumably, the distinct histological characteristics of BRCA1- and BRCA2-dependent breast tumors may reflect the accumulation of additional genetic alterations that follow the loss of either BRCA1 or BRCA2, as suggested by examination of molecular expression profiles in hereditary breast cancers (11).

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References

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