Loss of Heterozygosity in Familial Tumors from Three BRCA1-linked Kindreds

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Abstract

BRCA1, a breast-ovarian cancer susceptibility gene which has been localized to 17q21, appears to be a tumor suppressor gene based on evidence from loss of heterozygosity (LOH) studies. We analyzed 14 ovarian and breast tumors from BRCA1 carriers and 1 sporadic breast tumor from 3 kindreds for 17q21 LOH. Thirteen of the 14 tumors from gene carriers exhibited LOH of the wild-type allele. Tumors from one gene carrier and the sporadic breast case did not exhibit any LOH in the region. There was loss of the wild-type allele from both maternally and paternally derived chromosomes, therefore excluding the possibility of genomic imprinting and providing further evidence that BRCA1 is a tumor suppressor. Three tumors showed interstitial LOH in the region, and thus established the utility of familial tumors in refining a region surrounding a tumor suppressor gene in a manner analogous to using genetic recombinants.

Introduction

BRCA1, a breast-ovarian cancer susceptibility gene, was originally mapped to chromosome 17q by linkage analysis (1, 2). Based on multipoint linkage analysis in early-onset familial breast cancer kindreds, it was localized initially to an interval bounded proximally by D17S250 and distally by D17S800 (3) and, more recently, to an interval bounded proximally by D17S776 (4) and distally by D17S78 (5). To date, most of the cloned genes involved in the pathogenesis of human familial cancers have been tumor suppressor genes (6). Knudson (7) originally proposed a two hit model for explaining retinoblastoma. In hereditary cancer, the first hit would be a germine mutation in a specific cancer gene and the second hit would be a mutation in, or loss of, the second copy of that gene in the somatic cell (8, 9). BRCA1 fits the model of a tumor suppressor gene (10, 11) based on LOH studies on familial tumors in which the wild-type allele is always lost. However, in the previously reported cases (10, 11), the BRCA1 haplotype was inherited from the mother so that LOH of the wild-type allele could be due to genomic imprinting. In this study, we examined LOH in 14 tumors from BRCA1-linked individuals from three families in order to: (a) confirm that BRCA1 is a tumor suppressor gene; (b) disprove the possibility that LOH of the wild-type chromosome is due to imprinting; and (c) define the minimal region of loss which must contain the gene and thereby refine the BRCA1 region.

Materials and Methods

Families. Kindred 2035 is a single extended family of 42 individuals with 11 cases of breast cancer (age of onset, 27 to 52 years) and one case of both breast and ovarian cancer (diagnosed at age 59 years). It has been linked previously to BRCA1 (12) and currently has a LOD score of 2.6 with D17S1327. Kindred 2082 contains 31 cases of breast cancer (age of onset, 28 to 68 years) and 22 cases of ovarian cancer (age of onset, 46 to 73 years) (4). It has a LOD score of 9.5 with D17S1327. Kindred 2099 is of African-American descent and contains 22 cases of breast cancer (age of onset, 28 to 63 years), one case of ovarian cancer (diagnosed at age 69 years), and one case with both breast and ovarian cancer. It is linked to BRCA1 and has a LOD score of 2.4 with a multipoint analysis of D17S800 and D17S855.

DNA Extraction. Samples of tumor and normal tissue were obtained from surgical material previously embedded in paraffin blocks. Five-μm sections from one or more blocks from each case were placed on glass slides and stained with either hematoxylin and eosin or toluidine blue, without coverslipping, for histological examination. Small fragments of tissue determined to contain either tumor cells or normal cells were then removed from the slide and placed into 0.2 ml buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCI, 2.5 mM MgCl2, 0.1% Tween 20, 0.5 mg/ml proteinase K, and 0.0025% 100 X BSA. Samples were incubated for 8 h at 55°C and then boiled for 10 min. Four μl were used in subsequent PCR reactions.

Microsatellite Marker Analysis. The 17q12–21 polymorphic STRs used were D17S250 (13), D17S800 (14), D17S776 (15), D17S1328 (16), D17S855 (17), D17S1322 (16), D17S1325 (16), D17S1184 (16), and D17S579 (18). The order of markers on 17q from D17S776 to D17S1184 were ascertained from physical maps of the BRCA1 region (16), and the order of the other markers relative to those is according to published linkage study (17).

For genotyping, PCR reactions were performed in a 10-μl volume with 35 ng of each oligonucleotide primer, 0.25 unit Taq polymerase (Perkin-Elmer), 200 μM concentrations each of dGTP, dATP, and dTTP and 5 μM dCTP, with 0.5 μCi 32P, in a standard PCR buffer. Samples were amplified for 35 cycles of 5 s at 94°C, 5 s at 55°C, and 10 s at 72°C in a PE9600 (Perkin-Elmer). Products were electrophoresed in 6% denaturing polyacrylamide gels. Alleles were detected after autoradiography by exposure to Kodak X-Omat film for 1 to 24 h.

LOH. Allelic losses were scored as decreases in intensity of one allele relative to the other as determined from comparison of tumor and normal DNAs. A matched normal DNA control either from archival sections or blood was analyzed. At least two assays were performed for each marker.

Results

With the exception of one tumor from a sporadic breast cancer case in K2082, all tumors described in Table 1 were from cases with linked BRCA1 haplotypes. Five ovarian and five breast tumors from K2082, three breast tumors from K2035, and two breast tumors from K2099 were tested for LOH using nine polymorphic i7q12—21 STRs. Based on inheritance of haplotypes through these families, the allele lost could be distinguished as either from the noncarrier parent or from the carrier parent.

In Fig. 1a, LOH at D17S1325 is shown for tumors from K2082. In all cases, the wild-type allele was lost and the linked-BRCA1 allele was retained. Tumor 17594, which exhibited no LOH, was from a sporadic breast cancer case and did not share the linked BRCA1 allele (Fig. 1a). Variation in extent of allele loss as measured by intensity of one allele in relation to the other was likely due to the proportion of normal tissue contamination within the tumor tissue. For example, 17558, 17560, and 17561 exhibited almost total loss of the wild-type allele, whereas 17759 exhibited only a slight decrease in intensity of

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The abbreviations used are: LOH, loss of heterozygosity; STRs, short tandem repeats; LOD, logarithm of the odds ratio.

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The minimum region of loss defined by these tumors is bounded proximally by D17S800 on 17p (data not presented). Of the 14 gene carriers, only 17563 did not show some degree of loss on 17q (Table 1; Fig. 2). The minimum region of loss defined by these tumors is bounded proximally by D17S800 and distally by D17S1184 (an STR distal to D17S789). The minimum region of loss defined by these tumors is bounded proximally by D17S800 and distally by D17S1184.

Discussion

By analyzing a genetic recombinant from K2082, BRCA1 had been previously localized to a region bounded proximally by D17S776 (4). However, there was not a distal breakpoint in this kindred which would refine the region beyond D17S779. Using tumors from BRCA1 carriers in this kindred, we were able to confirm the D17S776 boundary and identify a distal boundary at D17S1184. In conjunction, these two methods may allow for greater refinement of regions containing tumor suppressor genes.

In this report, we add an additional 14 tumors from BRCA1 carriers to support evidence that BRCA1 is a tumor suppressor gene. Thirteen of these tumors showed LOH within the BRCA1 region and the loss was always of the wild-type allele with retention of the BRCA1 haplotype allele (Table 1). Kelsell et al. (11) examined 8 familial tumors and observed loss of the wild-type allele in all of them for the entire 17q region examined. Smith et al. (10) reported that 9 of the 13 tumors showed allele losses for the 4 markers analyzed on chromosome 17q. Although LOH was not detected in the remaining four cases, these may have had areas of LOH which were too small for detection with the STRs examined. Alternatively, these tumors may contain mutations in each of the BRCA1 alleles, with no LOH.

In the previous studies of Smith et al. (10) and Kelsell et al. (11), the most logical interpretation of the data is that BRCA1 is a tumor suppressor gene. However, because each of the individuals who showed LOH had inherited the BRCA1 haplotype from the mother, the possibility of genomic imprinting, as suggested by Smith et al. (10), could not be excluded. In this study, eight individuals inherited the BRCA1 haplotype from their mothers and six inherited it from their fathers (Table 1). Our results therefore provide evidence against genomic imprinting and strengthen the supposition that BRCA1 is a tumor suppressor gene.

Familial tumors rather than sporadic or nonfamilial ones were analyzed because these tumors likely contain BRCA1 mutations, and LOH in the region therefore would be centered around the BRCA1 gene. In this report, the sporadic breast tumor examined did not show LOH on 17q. With the recent isolation of the BRCA1 gene (19), Futreal et al. (20) analyzed 32 breast and 12 ovarian tumors exhibiting LOH at the BRCA1 locus for mutations within the gene. Only four mutations were identified and they were germline, suggesting that BRCA1 may not be critical in development of breast and ovarian cancers that do not arise from an initial germline mutation in BRCA1.
There have been many other studies examining LOH in sporadic breast and/or ovarian tumors, and when sufficient markers are analyzed to delineate the BRCA1 region, the minimal region of LOH does not include BRCA1 as defined by genetic breakpoints (21–23). Jacobs et al. (21) examined LOH in 120 ovarian cancers and found a common region of deletion distal to the BRCA1 region. Saito et al. (22) found a similar result when examining 94 ovarian and 246 breast tumors. Cropp et al. (23) examined 130 sporadic breast cancers and found a minimal region of deletion between D17S846 and D17S746, a region centromeric to BRCA1 (4). One explanation for these disparate results is that there are other genes on 17q involved in breast or ovarian cancer and LOH results from studies using sporadic tumors could be defining regions around these other genes rather than surrounding BRCA1. Obviously, this would not allow one to define the minimum region of loss for BRCA1. By using tumors from individuals carrying the BRCA1 haplotype, we were able to localize BRCA1 on 17q21 to an interval bounded proximally by D17S776 and distally by D17S1184.

For tumor suppressor genes, localization can be accomplished by defining genetic breakpoints and/or by defining a minimal area of loss from LOH studies. There appear to be two limitations to the use of tumors to define a minimal area of loss: (a) the DNA contained in paraffin blocks is degraded to the degree that amplification of products greater than 180 base pairs is limited, restricting the number of STRs which can be utilized to define a common area of loss; (b) the availability of familial tumors can limit the ability to define a minimal region of loss. In the study by Kelsell et al. (11), the entire region exhibited LOH in all tumors allowing no resolution of the region. In this report, only 3 of the 14 tumors from individuals carrying the linked-BRCA1 haplotype had small interstitial deletions that were useful in the delineation of the region. We were interested in defining the area of loss between D17S776 and D17S855 but were unable to do so because the STRs available to us in this region were uninformative or could not be amplified on some of the tumor DNAs. Therefore, it is important to have many tumors from linked haplotypes available to analyze. In summary, tumors from BRCA1 carriers, with inheritance from both maternal and paternal sources, were used to disprove the possibility that LOH of the wild-type allele was due to genomic imprinting and to corroborate that BRCA1 is a tumor suppressor gene. In addition, a minimum area of loss was defined as bounded proximally by D17S776 and distally by D17S1184. These results illustrate that familial tumors may be a useful adjunct to genetic recombinants in localization of tumor suppressor genes.

References


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