Allelic Imbalance on Chromosome 13q: Evidence for the Involvement of BRCA2 and RB1 in Sporadic Breast Cancer

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Abstract

Recently, the breast cancer susceptibility gene BRCA2 has been identified in chromosome 13q, a region that also contains the retinoblastoma gene RB1. To elucidate a possible role of BRCA2 and RB1 in sporadic breast tumorigenesis, allelic imbalance (AI) at 13q loci was examined in 78 primary sporadic breast tumors. AI was found in 52–63% of tumors. Nine tumors showed AI only in the BRCA2 region but not at RB1. Six tumors showed AI at RB1 but not in the BRCA2 region. AI in the BRCA2 region correlated significantly with aneuploidy (P = 0.032) and AI at RB1 with small tumor size (P = 0.025). Our data suggest that BRCA2 and RB1 may be both distinct target loci for AI on chromosome 13 in sporadic breast cancer.

Introduction

There is evidence that several tumor suppressor genes contribute to breast tumorigenesis. On chromosome 17p these are TP53 and a gene located distally to TP53 (1). On 17q these are the breast cancer susceptibility gene BRCA1, prohibitin, and nm23 (2–4). A second breast cancer susceptibility gene, BRCA2, was mapped in 13q12-q13 and recently identified (5, 6). Frequent LOH in this region was detected in sporadic breast tumors (7, 8) and in breast tumors from a family linked to BRCA2 (9). The RB1 gene is also located in this region. Structural changes in the RB1 gene have been reported in a small proportion of primary breast tumors and breast tumor cell lines (10). No correlations of LOH2 and loss of expression of the RB1 protein have been observed (11). This study was directed to evaluate the possible involvement of both BRCA2 and RB1 in sporadic breast cancer. AI was analyzed at multiple microsatellite loci on chromosome 13q in 78 primary breast tumors. Correlations of AI with ploidy at D13S260 and tumor size at RB1 indicate a biological role in breast cancer development.

Materials and Methods

Tissues. Breast tumors and matching peripheral blood lymphocytes were collected at the Universitätsklinikum Heidelberg (Heidelberg, Germany) and the Städtisches Klinikum Karlsruhe (Karlsruhe, Germany). Tumor tissue was snap frozen in liquid nitrogen and stored at -80°C. The percentage of tumor cells was estimated by visual inspection of H&E-stained sections. Seventy-eight breast tumors with a tumor cell content of >50% were analyzed. Tumor size and ploidy are given in Table 1.

DNA Analysis. Genomic DNA was isolated according to standard procedures. The loci in the 13q12-q14 region were D13S229, D13S260, D13S219/D13S220, D13S218, D13S263, D13S291, D13S155, and D13S153 (within RB1; Refs. 12 and 13). DNA was amplified using PCR and visualized with autoradiography. AI was loss or gain of alleles and ascertained by comparison of allelic patterns to corresponding normal tissue in heterozygotes in two independent readings. Any change in signal intensity was determined using densitometry in cases with weak intensity differences on autoradiographs and evaluated with respect to the tumor cell content.

Statistical Analysis. Analysis of statistical significance between AI at chromosome 13q loci and clinicopathological parameters was tested with Fisher’s exact test. Parameters were age at first diagnosis, tumor size, lymph node status, histopathological type, S-phase, ploidy, and hormone receptor status. Statistical significance was taken at a 5% confidence interval.

Results

AI on Chromosome 13q. Normal and tumor DNA of 78 breast cancer patients were tested for AI at four microsatellite loci on chromosome 13q. AI was observed in 31 (53%) of 58 tumors at D13S289, 29 (52%) of 56 tumors at D13S260, 38 (63%) of 60 tumors at D13S267, and 39 (61%) of 64 tumors at RB1. Tumors were distinguished into those with AI (n = 62) and those without AI (n = 16; Table 2). Among those affected we observed different patterns of AI. Whereas 32 tumors showed AI in the BRCA2 region and at RB1, 9 tumors showed AI in the BRCA2 region only and 6 tumors at RB1 only. All tumors with preferential AI in the BRCA2 region were informative at RB1, and tumors with preferential AI at RB1 were informative at loci in the BRCA2 region. Fig. 1 shows microsatellite analyses of representative tumors.

A schematic representation of the pattern of AI in tumors with breakpoints between BRCA2 and RB1 is shown in Fig. 2. Tumors R53, R73, P196, P369, P331, and R58 showed AI at D13S153 only. Of these tumors, R53, R73, and P196 are informative but unaffected at D13S289, D13S260, and D13S267; tumors P369, P331, and R58 are informative but unaffected at two of these loci and not informative at one.

Tumors with AI in the BRCA2 region only were analyzed at additional 13q loci. Five tumors (P63, P252, P309, P360, and R67) showed AI at all informative loci, whereas four tumors showed interstitial AI. Of these tumors, P135 showed interstitial AI at D13S260, D13S267, D13S219/D13S220, D13S263, D13S291, and D13S155; the flanking loci D13S289 and D13S153 are informative but unaffected. Tumor P211 showed two interstitial AIs at D13S260, D13S267, and at D13S263 but no AI at the flanking loci D13S289, D13S218, and D13S155. Tumors P236 and R48 showed interstitial AI at D13S267 but no AI at D13S260 and D13S219/D13S220. In addition to AI, tumor R48 showed a microsatellite instability at D13S267.
Correlation of AI with Clinicopathological Parameters. We compared the clinicopathological parameters age at first diagnosis, tumor size, lymph node status, histopathological type, histological tumor grading, ploidy, S-phase, and hormone receptor status of the patients with AI only in the BRCA2 region or with AI only at RB1 with those who were informative but unaffected at either locus. Correlations of AI with clinicopathological parameters were obtained with Fisher's exact test for small tumor size and aneuploidy. A small tumor size was more frequent in tumors with AI at RB1 only (4/6, 66%) compared with tumors with no AI (2/16, 12%; P = 0.025). In addition, aneuploidy was more frequent in tumors with AI at D13S260 (14/21, 66%) compared with tumors with no AI (3/12, 25%; P = 0.032).

Discussion

In this study, we show high frequencies of AI on chromosome 13q in the BRCA2 region (52–63%) and at RB1 (61%) in 78 primary sporadic breast tumors. Fifteen tumors with breakpoints between BRCA2 and RB1 were identified. Nine tumors showed AI only at loci in the BRCA2 region but not at RB1, and six tumors showed AI only at RB1. These results suggest that BRCA2 and RB1 are distinct target genes inactivated by AI in sporadic breast cancer.

Previous studies showed frequent LOH on chromosome 13q over a range of loci containing BRCA2 and RB1 (14). Only six primary breast tumors and one breast cancer cell line have been reported with breakpoints between BRCA2 and RB1. In these tumors and cell line, LOH was detected only in the BRCA2 region and not at RB1 (15, 16). However, we found six tumors that showed AI only at RB1 and not in the BRCA2 region. The relationship between RB1 and breast cancer is not clear. Several reports suggest the involvement of RB1 in a subset of breast tumors (10, 17, 18). However, no correlation of LOH and loss of RB1 protein expression was observed (11). Our findings of AI at RB1 only support the evidence that RB1 is inactivated during sporadic breast tumorigenesis, at least in a subset of tumors.

We identified four breast tumors with interstitial deletions in the BRCA2 region. The deleted region in these tumors is consistent with the location of the familial BRCA2 gene (6), suggesting that BRCA2 may be involved in both sporadic and familial breast tumors.

Another tumor suppressor gene BRUSH1 was recently isolated from the 13q12-q13 region (19). The relationship between BRUSH1 and BRCA2 needs to be elucidated. Since up to now no mutations in the BRCA2 gene have been identified in sporadic breast tumors, it may be possible that the target gene inactivated by AI may be BRUSH1 and not BRCA2.

Our results of a significant correlation of AI at RB1 only with small tumor size in a small number of patients suggests that this molecular marker may be suitable as a prognosis factor. This possibility should be explored in a large number of patients. Since small tumor size is indicative for early tumor stage, AI at RB1 may be an early event in the progression of sporadic breast cancer. In addition, we found a correlation of AI in the BRCA2 region with aneuploidy. These data are in agreement with previous reports (15). Thus, the data presented support evidence that both BRCA2 and RB1 may be involved in sporadic breast tumorigenesis.

Acknowledgments

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Table 1 Clinicopathological data of the 78 analyzed breast tumors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size (cm)</td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>20</td>
</tr>
<tr>
<td>≥2</td>
<td>58</td>
</tr>
<tr>
<td>Ploidy</td>
<td></td>
</tr>
<tr>
<td>Diploid</td>
<td>20</td>
</tr>
<tr>
<td>Aneuploid</td>
<td>30</td>
</tr>
<tr>
<td>Unknown</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 2 Summary of AI results in 78 sporadic breast tumors

<table>
<thead>
<tr>
<th>AI</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2 region and RB1</td>
<td>32 (51%)</td>
</tr>
<tr>
<td>None</td>
<td>16 (25%)</td>
</tr>
<tr>
<td>BRCA2 region</td>
<td>9 (14%)</td>
</tr>
<tr>
<td>RB1</td>
<td>6 (10%)</td>
</tr>
</tbody>
</table>

* Fifteen tumors were not informative at RB1.
Fig. 2. Patterns of AI on chromosome 13q in 15 breast tumors. The ideogram of chromosome 13q shows the location of microsatellite loci. Numbers on the top, breast tumors. O, loci, informative with AI; O, loci, informative and unchanged; O, loci, not informative.


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