BRCA2 and p53 Mutations in Primary Breast Cancer in Relation to Genetic Instability

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Abstract

The products of the BRCA breast cancer susceptibility genes have been implicated in cell cycle control and DNA repair. It has been suggested that mutations in the p53 gene are a necessary step in tumorigenesis in BRCA tumors. We tested samples from 402 breast cancer patients for germ-line BRCA2 and p53 mutations in tumors. p53 mutations are more frequent in BRCA2 mutation carriers than in controls. Tumors with mutations in either gene had multiple chromosomal abnormalities, as shown by cytogenetic analysis.

Introduction

Germ-line mutations in the BRCA1 and BRCA2 genes have been shown to predispose to breast cancer (1–3), and the most common somatic changes found in breast tumors are mutations in the p53 gene (4). Both the BRCA and p53 genes have been implicated in cell cycle control (5–7). A number of studies have shown that p53 mutations are associated with genomic instability in tumors (8, 9), and recent studies on the functions of the BRCA proteins suggest that they are also involved in control of genomic integrity (10, 11). Both BRCA proteins and p53 have been shown to interact with hRad51, a human homologue of the yeast Rad51 protein that is involved in recombination and DNA double-strand repair (10–12). BRCA1 and BRCA2 deficiencies cause cell lethality in early mouse embryos (13, 14), and hypersensitivity to γ-irradiation is seen in BRCA2 —/- cells (11). Recent studies show that BRCA1- and BRCA2-deficient embryos can be partially rescued by p53 or p21 null mutations (13, 14). This suggests that cells with nonfunctional BRCA can only survive if they also have defective checkpoint control. Germ-line mutations in BRCA1 or BRCA2 increase the risk of breast cancer, but even in these hereditary cases, breast cancer is a multistep process, and somatic mutations in other genes play a part in the tumor formation. Recent publications suggest that p53 abnormalities are a necessary step in tumorigenesis in BRCA1 carriers (15, 16). We wanted to examine this in an unselected group of breast cancer patients and test whether BRCA2 abnormalities were associated with genomic instability in tumors.

Family and population studies indicate that there is only one mutation in each BRCA gene in the Icelandic population, one rare BRCA1 mutation (D1692N) and a common BRCA2 founder mutation (999del5), found in 8% of all breast cancer cases in Iceland (17, 18). There are no indications of other BRCA1 or BRCA2 mutations in the population. This enabled us to analyze over 400 consecutive breast cancer cases for somatic p53 mutations, as well as for the germ-line BRCA2 founder mutation. A subset of samples were studied by conventional cytogenetic methods, FISH, and flow cytometry, thus allowing analysis of genetic instability in relation to these two genes.

Materials and Methods

The study material consisted of 402 breast tumor samples from the Icelandic Cancer Society Tumor Specimen Bank.

Samples were screened for mutations in exons 5–8 of the p53 gene with the constant denaturant gel electrophoresis method (19). p53 mutation status in relation to prognosis has been previously published for the majority of these samples (20–22). BRCA2 exon 9 fragments were PCR-amplified and run on 6% denaturing polyacrylamide gels for mutation detection (17). Mutants were identified by the presence of an extra allele.

Tumors were harvested directly for cytogenetic analyses or cultured on average for 6–7 days before harvesting (23). Analysis of karyotypes was done according to an International System for Human Cytogenetic Nomenclature (24). Karyotypic clones with one to three numerical changes (i.e., additional or lacking chromosome) or a single structural change (i.e., translocation or deletion) were listed as having simple clonal changes. Clones with more than three numerical changes and/or more than two structural changes were called complex.

FISH was performed using PCR-amplified whole chromosome probes for chromosomes 1, 3, 16, and 17, as described by Anamthawat-Jonsson et al. (25), in which chromosomes 1 and 3 were labeled with red rhodamine and chromosomes 16 and 17 were labeled with green fluorescein.

Flow cytometry was used to analyze the DNA content of the tumor cells. DNA ploidy index and S-phase assessment were performed as described previously (26). Histograms were classified as diploid (DNA index = 1), if there was a single G0/G1 peak, or aneuploid (DNA index > 1), if there were at least two clearly distinct peaks, including multiploid, if more than two peaks were seen. The median S-phase value of <7% was defined as low S-phase fraction, and ≥7% was defined as high S-phase fraction.

For statistical analysis, the χ² test and Fisher’s exact test were used as appropriate.

The germline mutation screening was approved by the Icelandic Cancer Society Institutional Review Board. All DNA analyses were performed on samples without personal identification.

Results

p53 and BRCA2 Mutation Analysis. Samples from unselected breast cancer patients (n = 402) were screened for BRCA2 and p53 mutations. The BRCA2 999del5 germ-line mutation was detected in samples from 34 patients (8.4%), and somatic mutations in the p53 gene were detected in 72 (17.9%) tumors. The p53 mutations were distributed as follows: 25 mutations in exon 5 (35%), 9 in exon 6 (12%), 17 in exon 7 (24%), and 21 in exon 8 (29%).

We compared the frequency and pattern of p53 mutations in tumors
from BRCA2 carriers and noncarriers. We found that 10 BRCA2 mutation carriers (29%) had p53 mutations in their tumors or nearly twice as many as in the BRCA2 wild-type group (17%; Table 1). This difference was, however, not significant. The p53 mutation distribution in the BRCA2-positive group was similar to that found in the whole group (Table 2).

Flow Cytometry, Cytogenetic, and FISH Analyses. Flow cytometric analysis of DNA content was performed on 266 of the samples (Table 3). Aneuploidy was detected in 65% of all samples, and the prevalence was similar to that in BRCA2 mutants and wild-type samples. The same was true for the p53 mutated samples. Cytogenetic analysis was performed on fresh tumor samples from 60 patients (Table 3). There was no significant difference between samples from tumors with BRCA2 mutation compared to wild type, based on the occurrence of simple and/or complex clonal changes. The cytogenetic and flow cytometry data combined showed that 73% of the samples from BRCA2 carriers were aneuploid, compared to 63% of the BRCA2/p53 normal samples. The instability trend was further strengthened if BRCA2 and p53 mutated samples were pooled (76.5%) and compared to the controls. There was, however, a clear difference observed in the complexity of the clonal changes. All of the clonal changes detected in the BRCA2 and p53 mutated tumors were complex as compared to about half of the clones in the mutant-negative tumors (Table 3). The chromosomal aberrations included both structural and numerical changes, they were not restricted to any particular chromosome and polyploidy was highly prevalent. However, among the primary breast tumor samples, chromosomes 1, 3, 16, and 17 were most frequently abnormal and were, therefore, selected for FISH analysis with whole chromosome paint. The FISH analysis confirmed the structural instability detected by G-banding and, furthermore, showed that chromosomal rearrangements, gains, and losses were common. Multiple rearrangements of the painted chromosomes were detected in samples with BRCA2 germ-line mutations, p53 somatic mutations, and both BRCA2 and p53 mutations (Fig. 1, B, D, and F). Tumors with both BRCA2 and p53 mutations were not more complex than tumors with either mutation alone.

S Phase. The proliferation rate of the tumors was examined. Both BRCA2 and p53 mutations were associated with a very high proliferative rate, with approximately 70% of tumors showing high mitotic activity, indicated by a high S-phase fraction (≥7.00). This association was significant in all groups (Table 3).

Discussion

Here, we screened samples from 402 unselected breast cancer patients for BRCA2 germ-line mutation and p53 mutations in primary tumor samples. The frequency of BRCA2 mutation carriers (8.4%) was in agreement with our previous studies on Icelandic breast cancer patients (18). We detected mutations in exons 5–8 of the p53 gene in 17.9% of the tumors. This was also in agreement with our previous findings (20–22) and those of others (4, 27).

p53 mutations were found to be more common in tumors from BRCA2 mutation carriers than they were in controls. However, the frequency was not as high as those recently reported in breast tumors from BRCA1 mutation carriers (15). In that study, p53 mutations were found in eight of 17 tumors with known BRCA1 mutations, and the majority of p53 mutations were located in exon 5. The distribution of p53 mutations found in our study was the same in BRCA2 mutation carriers as in the whole group. None of the mutations reported in the BRCA1 carriers by Crook et al. (15) were found. p53 protein overexpression in BRCA1 tumors from high-risk families also indicate a fairly high frequency of

Table 1 Frequency of p53 mutations in tumors from BRCA2 mutation carriers and noncarriers

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<th>Sample</th>
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<th>Codon</th>
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<td>5</td>
<td>182</td>
<td>1-base deletion</td>
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<td>2</td>
<td>5</td>
<td>173</td>
<td>GTG→ATG</td>
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<td>3</td>
<td>5</td>
<td>179</td>
<td>CAT→CTT</td>
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<tr>
<td>4</td>
<td>5</td>
<td>141</td>
<td>TGC→ACC</td>
</tr>
<tr>
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<td>6</td>
<td>ND</td>
<td>Large deletion</td>
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<td>ND</td>
<td>Large deletion</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>298-302</td>
<td>14-bp deletion</td>
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6 ND, not determined.

Table 2 p53 mutations found in tumors from BRCA2 carriers

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<th>Exon</th>
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BRCA2 AND p53 MUTATIONS IN PRIMARY BREAST CARCINOMAS

Fig. 1. FISH with whole chromosome painting showing structural abnormalities in metaphases (B, D, and F). The cells were counterstained with 4',6-diamidino-2-phenylindole (A, C, and E). Scale bar, 10 μm. The chromosome abnormalities involved both structural and numerical changes, and they are of very complex type, based on G-banding and FISH analysis. A and B, near-triploid cell from a tumor from a BRCA2 mutant carrier after painting of chromosomes 1 and 16 (red and green fluorescence, respectively). Chromosome 1 is involved in eight translocations and shows overall gain of material, but chromosome 16 is seen in one translocation and shows loss of material. C and D, near-triploid cell from p53 mutated tumor after painting of chromosomes 1 and 16 (red and green fluorescence, respectively). Here, chromosome 1 takes part in two translocations, and chromosome 16 shows gain. E and F, near-diploid cell from a BRCA2 and p53 mutated tumor after painting of chromosomes 3 and 17 (red and green fluorescence, respectively). In this cell, four rearrangements can be seen, one of which is a translocation between the two painted chromosomes.
p53 abnormalities, 40 and 70%, respectively (16, 27). p53 overexpression was not associated with BRCA2 mutation in our study (data not shown).

The samples analyzed in this study were from breast cancer patients who had not been previously tested or found to belong to known high-risk families (17). Of those, only three had p53 mutations, and there was one additional case of p53 overexpression. Sobol et al. (16) suggest that specific mutations in the BRCA1 gene are more often associated with p53 abnormalities as measured by abnormal staining. Because all of the BRCA2 mutation carriers in our study have the same 999del5 mutation, no conclusion can be drawn about association between p53 abnormalities and BRCA2 mutations in general. As mentioned previously, only one rare BRCA1 mutation has been found in this population. A single case of this mutation was found in this cohort. Neither p53 mutation nor abnormal p53 staining was detected in a tumor sample from this patient.

We previously described a significant association between p53 abnormalities and genomic instability in primary breast tumors (9, 22). This study supports this and, furthermore, shows that BRCA2 mutated tumors have complex chromosomal changes as well. Culturing primary breast tumor cells is difficult, and in particular, it is hard to get cells with complex karyotypes to divide. The most abnormal cancer cells may, therefore, be lost in the process of cell culture and chromosome harvesting. The BRCA2 samples were, indeed, highly complex showing multiple chromosomal rearrangements (e.g., Fig. 1B), which seems to fit the notion of BRCA2 involvement in double-strand DNA repair.

It is well known that p53 participates in cell cycle control by activating p21 in response to DNA damage (29). It has been shown that the expression of the BRCA1 gene is cell cycle dependent (5, 30), and in a recent study, BRCA1 was found to activate p21 in a p53-dependent manner (31). It is as yet unknown how BRCA2 participates in cell cycle control. We found that tumors from BRCA2 mutation carriers were highly proliferative, as judged by high S phase. This was also true for tumors without additional p53 abnormalities, suggesting a possible role for BRCA2 in cell cycle control.

In conclusion, our results support findings that implicates BRCA2 in DNA repair and cell cycle control. Inactivation of p53 may be important in initiation of tumorigenesis in BRCA2 carriers. However, our results show that two of three of the BRCA2 mutated tumors have normal p53 and, therefore, inactivation of other integrity control genes must be involved in these tumors, allowing cells with highly abnormal chromosomes to go through the cell cycle.

Acknowledgments

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References

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