MYC Is Amplified in BRCA1-Associated Breast Cancers

Tatyana A. Grushko,1 James J. Dignam,2 Soma Das,3 Anne M. Blackwood,4,5 Charles M. Perou,7 Karin K. Ridderstråle,1 Kristin N. Anderson,1 Min-Jie Wei,1 April J. Adams,1 Fitsum G. Hagos,1 Lise Sveen,1 Henry T. Lynch,8 Barbara L. Weber,4,6 and Olufunmilayo I. Olopade1

1Section of Hematology/Oncology, Department of Medicine, Committees on Genetics and Cancer Biology, and 2Departments of Health Studies and 3Human Genetics, University of Chicago, Chicago, Illinois; 4Department of Medicine, Division of Hematology/Oncology, 5Center for Clinical Epidemiology and Biostatistics, and 6Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; 7Departments of Genetics and Pathology, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; and 8Department of Preventive Medicine, Creighton University, Omaha, Nebraska

ABSTRACT

Purpose: Germ-line mutations in the BRCA1 tumor suppressor gene predispose to early onset breast cancers with a distinct phenotype characterized by high tumor grade, aneuploidy, high proliferation rate, and estrogen receptor-negativity. The molecular mechanisms and cooperative oncogenes contributing to multistep tumor progression in cells lacking BRCA1 are not well defined. To examine whether C-MYC (MYC), a transforming oncogene associated with genetic instability, contributes to multistep tumor progression in BRCA1-associated breast cancer, we have analyzed tumors from women with hereditary BRCA1-mutated and sporadic breast cancers.

Experimental Design: We performed fluorescence in situ hybridization using a MYC:CEP8 assay on formalin-fixed paraffin-embedded tumor tissues from 40 women with known deleterious germ-line BRCA1 mutations and 62 sporadic cases, including 20 cases with hypermethylation of the BRCA1 gene promoter.

Results: We observed a MYC:CEP8 amplification ratio ≥2 in 21 of 40 (53%) BRCA1-mutated tumors compared with 14 of 62 (23%) sporadic tumors (P = 0.003). Of the 14 sporadic cases with MYC amplification, 8 (57%) were BRCA1-methylated. In total, MYC amplification was found in a significantly higher proportion of tumors with BRCA1 dysfunction (29 of 60, 48% versus 6 of 42, 14%; P = 0.0003). In a multivariable regression model controlling for age, tumor size, and estrogen receptor status, BRCA1-mutated tumors demonstrated significantly greater mean MYC:CEP8 ratio than sporadic tumors (P = 0.02).

Conclusions: Our data indicate that MYC oncogene amplification contributes to tumor progression in BRCA1-associated breast cancers. Thus, we conclude that the aggressive histopathological features of BRCA1-associated tumors are in part due to dysregulated MYC activity.

INTRODUCTION

BRCA1 (MIM 113705) is a classical tumor suppressor gene, the loss of the wild-type allele of which is required for breast and ovarian tumorigenesis in germ-line mutation carriers (1, 2). BRCA1 encodes a multifunctional protein, which together with other proteins contributes to homologous recombination, DNA damage response, and transcriptional regulation, and serves to maintain genomic stability (3). Breast cancers arising in BRCA1 mutation carriers are usually high grade, aneuploid, highly proliferative, and estrogen receptor (ER)-negative (ER−; Ref. 2). Moreover, the BRCA1-mutated tumors have unique gene expression profiles (4, 5). Interestingly, hypermethylation of the BRCA1 promoter may be an important mechanism for functionally inactivating BRCA1 in nonhereditary forms of breast cancer (6), as 7–31% of sporadic breast tumors are reported to be BRCA1-methylated. It appears that BRCA1-methylated sporadic tumors may display pathological features and gene-expression profiles similar to BRCA1-mutated hereditary breast cancers (4, 6, 7). However, the cooperating oncogenes and tumor suppressor genes contributing to multistep carcinogenesis in BRCA1-deficient cells remain largely unknown. In a previous study, we showed that HER-2/neu oncogene amplification is not a feature of BRCA1-associated breast cancers despite the clinicopathological similarities among HER-2/neu-amplification-positive tumors and BRCA1-associated tumors (8).

MYC oncogene encodes a proliferative nuclear DNA-binding protein, the deregulated expression of which has been shown to play an important role in the induction and progression of lymphomas, lung cancer, and breast cancer (9, 10). MYC amplification has been reported as a poor prognostic biomarker.

Received 6/26/03; revised 10/17/03; accepted 10/28/03.

Grant support: O. I. O. is supported by the United States Army Department of Defense Grant DAMD17-99-1-9123, the Falk Medical Research Trust, the Breast Cancer Research Foundation, and CA 14955 to the University of Chicago Cancer Research Center. O. I. O. is a Doris Duke Distinguished Clinical Scientist. B. L. W. is supported by grants from the Breast Cancer Research Foundation, the NIH (CA57601), and is an Investigator of the Abramson Family Cancer Research Institute at the University of Pennsylvania Cancer Center. The costs of publication of this article were 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.

Requests for reprints: Olufunmilayo I. Olopade, Section of Hematology/Oncology, Department of Medicine, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637-1463. Phone: (773)702-1632; Fax: (773) 702-0963; E-mail: folopade@medicine.bsd.uchicago.edu.

MYC is the officially accepted name for C-MYC (Human Genome Organization Gene Nomenclature Committee http://www.gene.ucl.ac.uk/nomenclature/).
in ~25% (1–48%) of breast tumors and is associated with tumor aggressiveness, including genetic instability, high tumor grade, and ER-negativity (10, 11).

In the present study, using breast tumors from BRCA1 mutation carriers and sporadic tumors with known BRCA1 promoter methylation status, we tested the hypothesis that MYC oncogene is preferentially amplified in BRCA1-associated cancers. To our knowledge, this is the first study to evaluate MYC amplification in BRCA1-deficient breast cancer.

MATERIALS AND METHODS

Patient Materials. The study was conducted under research protocols approved by the University of Chicago, University of Pennsylvania, and Creighton University Institutional Review Boards. BRCA1 mutation carriers were identified through the high-risk clinics at the corresponding institutions where genetic testing is provided as a clinical service. The details of patient enrollment have been reported previously (8). All of the BRCA1 mutation carriers for whom tumor blocks were available were included. Sporadic breast tumors were identified from cases operated on at the University of Chicago Hospitals as described previously (8). The 102 breast tumors analyzed in the present study were composed of 40 specimens from BRCA1 germ-line mutation carriers and 62 sporadic cases, used as control for our hybridization assays. Twenty-two of the 40 (55%) specimens from BRCA1-mutation carriers were included in our previous study and were negative for HER-2/neu amplification/overexpression (8, 12). Specimens from BRCA1-mutation carriers were predominantly from young patients (mean age 43), with infiltrating ductal carcinomas (89%) of high grade (70%) that were ER – (71%). The sporadic cancers were skewed toward cases with tumors large enough to contribute to a tissue bank. Pathological features such as tumor grade and ER status were generally distributed in a manner consistent with that in the general population of women with breast cancer. Sporadic tumors were from older individuals and were more frequently ER-positive relative to the BRCA1-mutated cases. All 62 of the sporadic cases were evaluated for methylation of the BRCA1 promoter region. Twenty of the 62 sporadic tumors were BRCA1-methylated and displayed tumor characteristics that were intermediate between hereditary and sporadic unmethylated tumors (Table 1).

Fluorescence in Situ Hybridization (FISH). Probes and corresponding hybridization mixtures (Vysis/Abbott, Inc., Downers Grove, IL) included MYC, labeled with Spectrum-Orange, and chromosome enumeration probe CEP8, labeled with SpectrumGreen. The MYC probe contains DNA sequences specific for the MYC human gene locus and hybridizes to 8q24.12-q24.13 of human chromosome 8. The CEP8 probe contains α-satellite DNA that hybridizes to the centromeric region of chromosome 8 (8p11.1-q11.1). The CEP8 probe was used in dual hybridizations with the MYC probe as an internal control for chromosome 8 aneusomy. FISH for detection of MYC gene amplification in 4–6 μm thick formalin-fixed, paraffin-embedded breast tumor tissue specimens was performed according to protocols described previously (8). In each tumor sample an average of 86 (30–200) well-defined malignant nuclei and in each normal sample an average of 40 (20–70)
nonmalignant nuclei were scored (8). Both the absolute number of MYC signals and the ratio of MYC signals to chromosome 8 centromere signals were recorded. Tumors with a ratio of MYC:CEP8 signals of ≥2.0 were considered MYC-amplified. The chromosome 8 copy number alteration was estimated by scoring the reduction of CEP8 signals to one copy (monosomy) and gain of CEP8 signals to three or more copies (polysomy; Ref. 8). The CEP9 probe, labeled with SpectrumOrange, and CEP17 probe, labeled with SpectrumAqua, contains a-satellite DNA that hybridizes to the centromeric regions of chromosome 9 (9p11-q13) and chromosome 17 (17p11.1-q11.1), respectively. Tumor ploidy was identified by scoring and comparing the mean numbers of CEP8, CEP9, and CEP17 signals per cell in a triple-color FISH experiment conducted on a representative group of 12 tumors (7 BRCA1-mutated and 5 sporadic).

**BRCA1 Methylation Analysis.** Methylation analysis was performed on DNA isolated from patient tumor slides using a methylation-specific PCR-based approach (13). Methylation-specific primers were designed to the promoter region in exon 1A in the 5′ untranslated region of the BRCA1 gene (14) taking care to design primers to regions that differ between the BRCA1 gene and its pseudogene (15). A nested PCR protocol was used. Primers that do not discriminate between methylated and unmethylated BRCA1 sequence (5′-GTATTTGAGGTTTGGTTTA-3′ and 5′-CTAAAAACCCACACCTATC-3′, annealed at 62°C for 15 cycles) were first used followed by primers specific to the methylated and unmethylated sequence (methylated sequence-specific primers: 5′-TCTTGTTTGAACG-GAAAGCGGC-3′ and 5′-AACGAACTCAGCCGCGC-3′, annealed at 66°C for 35 cycles; and unmethylated sequence-specific primers: 5′-TTAGAGGTGTGTTTATGG-3′ and 5′-AACAAACTCACCACACAA-3′, annealed at 54°C for 40 cycles). The methylated primers resulted in a 68-bp product, whereas the unmethylated primers resulted in a 100-bp product. As a control for the methylated-specific primers, Ss1 methylase-treated DNA was used that generates DNA completely methylated at all of the CpG sites.

**Statistical Analysis.** Demographic and disease characteristics expressed on a continuous scale were summarized and compared between BRCA1-mutated and sporadic case groups using the F test, followed by pair-wise comparisons where indicated. To obtain more symmetric distributions suitable for these tests, logarithms of values were used. For these tests and those described above, results of the parametric procedures and their nonparametric counterparts (e.g., Kruskal-Wallis, Wilcoxon rank-sum, and Wilcoxon signed-rank tests) were similar. MYC:CEP8 ratios were also classified into amplification status categories (e.g., no amplification, amplification) and proportions with amplification compared between groups using Fisher’s exact test. Analogous tests were computed for amplification status cross-classified with tumor aneugenic status (monosomic, disomic, or polysomic).

Linear model methods were used to evaluate the relationship between tumor group and MYC amplification taking into account differences in patient/tumor characteristics between BRCA1-mutated and sporadic tumors. Specifically, a linear regression model was fit, with the logarithm of the MYC:CEP8 ratio as the response variable and covariates for tumor group and other patient/tumor characteristics that differed between tumor groups as the predictors.

**RESULTS**

Control values for gene copy numbers for tumor cells were established from evaluation of the adjacent nontumor breast epithelium from 18 BRCA1-mutation carriers and from 10 patients with sporadic tumors. Because mean signal copy numbers per cell and copy number ratios were similar between the two groups (data not shown), the control values were evaluated from a pool of all 28 of the nontumoral specimens. The mean number of MYC copies per nontumoral cell was 1.87 ± 0.08 (mean ± SD) with no case from either group having more than four MYC signals per nucleus. Similarly, the mean number of CEP8 copies per cell was 1.88 ± 0.06, resulting in a mean ratio MYC:CEP8 = 1.00 ± 0.05. The mean proportion of cells having only one copy of CEP8 was 12.5% (± 5.1%). The mean proportion of cells with two CEP8 signals per nucleus was 87.2% (± 5.4%), and the mean proportion of cells with more than two CEP8 signals per nucleus was 0.4% (± 0.11%).

Among both BRCA1-mutated and sporadic cases, tumor cells had on average significantly greater number of MYC copies per cell than the adjacent normal cells (P < 0.01 in both cases). In BRCA1-mutated tumors, the mean number of absolute MYC signals per cell (5.57 ± 2.62) was greater than in the sporadic group (4.10 ± 3.58; P = 0.02). CEP8 copies per cell did not differ between groups, suggesting that MYC was targeted for amplification in the BRCA1-mutated tumors (Table 2). The mean MYC:CEP8 ratio in BRCA1-mutated tumors (2.37 ± 1.17) was significantly greater than the ratio among sporadic tumors (1.80 ± 1.29; P = 0.002). Fig. 1 shows the mean number of MYC copies per cell plotted against the mean number of CEP8 copies per cell, depicted separately for tumors from BRCA1-mutated (Fig. 1A) and sporadic (Fig. 1B) groups. Fourteen (23%) of the sporadic tumors had a MYC:CEP8 ratio of two or higher, a proportion of tumors comparable with the ~25% of breast tumors that have been reported with MYC amplification in the literature (10). However, the proportion of MYC-amplified tumors was significantly higher in the BRCA1-mutated group (21 of 40, 53%; P = 0.003). (A representative photomicrograph of a MYC-amplified BRCA1-mutated tumor is shown on Fig. 2A).

Next we asked whether MYC amplification in the sporadic cases was associated with BRCA1-methylation. Twenty of the 62 sporadic tumors were BRCA1-methylated, and 8 of these showed MYC amplification (Fig. 1B). We observed significant heterogeneity among these tumors because BRCA1 methylation was incomplete in the majority of cases (methylated and un-
methylated DNA species were observed; data not shown). The mean absolute MYC copy number per cell was 5.32 ± 5.09 in BRCA1-methylated tumors, compared with 3.53 ± 2.45 in unmethylated tumors (P = 0.07); mean MYC:CEP8 signal ratios were 2.36 ± 1.91 in BRCA1-methylated tumors and 1.53 ± 0.74 in unmethylated tumors (P = 0.06; Table 2). Pairwise comparisons between the BRCA1-mutated and sporadic groups (i.e., BRCA1-methylated, unmethylated), however, revealed that BRCA1-mutated tumors had significantly higher MYC copy numbers per cell (P < 0.0001) and MYC:CEP8 signal ratios (P < 0.0001) than sporadic unmethylated cancers (Table 2).

Because the three groups differed with respect to age, tumor size, and ER status (Table 1), we additionally examined whether the MYC:CEP8 signal ratio remained greater in BRCA1-mutated tumors after these differences were taken into account. A multiple regression model was fit, with log (MYC:CEP8 signal ratio) as the response variable, and the tumor group (BRCA1-mutated versus all sporadic), age, tumor size, and ER status as the predictors. Results indicated a significant difference (univariate comparison of the mean number of MYC:CEP8 signals per cell revealed that the majority of tumors from both the BRCA1-deficient and sporadic unmethylated groups had definite gains of chromosome 8 as has been reported in breast cancer literature (Ref. 17; Table 5; mean MYC:CEP8 copies per cell ≥2; Fig. 1, A and B). However, as described above, the mean absolute MYC signals per cell was greater in the BRCA1-deficient cases (5.49 ± 3.59) than in the sporadic unmethylated group (3.53 ± 2.45; P = 0.0002), as was the mean MYC:CEP8 ratio (2.37 versus 1.53; P < 0.0001). In all, 35 of 102 (34%) tumors analyzed in our study were MYC-amplified (Table 5), and the majority of tumors with MYC amplification displayed chromosome 8 polysomy (Fig. 1, A and B). To demonstrate that the gain of chromosome 8 was not random or due to tissue preparation artifact, we evaluated aneusomy for chromosome 8 relative to chromosome 9 and chromosome 17 in triple color CEPS/CEP9/CEP17 FISH on a representative group of 12 tumors (7 BRCA1-mutated and 5 sporadic). The comparisons among CEP17, CEP8, and CEP9 in BRCA1-mutated tumors indicate significant reduction of CEP17 to one copy as compared with either CEP8 (mean paired difference = −0.77) or CEP9 copy number (mean difference is −1.01). There was a higher mean copy number of CEP8 per cell and a larger proportion of cells with ≥3 copies/cell than was observed with CEP9 (data not shown). It does appear that CEP8 tended to differ by MYC amplification status, whereas the CEP9 and CEP17 did not (data not shown). Thus, consistent with our previous publication (8) we observed increased copy number of chromosome 8 (correlated with MYC amplification), reduction of chromosome 17 to one copy, and more or less stable copy number of chromosome 9.

### Table 2: Mean numbers of MYC and CEP8 copies per cell and mean ratio in tumor tissues from BRCA1-mutated and sporadic cases

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BRCA1-mutated tumors (n = 40)</th>
<th>Sporadic tumors (n = 62)</th>
<th>P (pair-wise test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRCA1-methylated (n = 20)</td>
<td>BRCA1-unmethylated (n = 42)</td>
<td></td>
</tr>
<tr>
<td>MYC</td>
<td>5.57</td>
<td>3.53</td>
<td>0.0005</td>
</tr>
<tr>
<td>CEP8</td>
<td>2.46</td>
<td>2.23</td>
<td>0.43</td>
</tr>
<tr>
<td>MYC:CEP8</td>
<td>2.37</td>
<td>1.53</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the present study, we showed that MYC amplification is a frequent event in breast tumors from BRCA1 germ-line mutation carriers and in sporadic tumors with BRCA1 promoter hypermethylation. This observation is in contrast to our previous work showing that MYC amplification is more frequent in sporadic tumors than in BRCA1-deficient tumors. We also found that MYC amplification is associated with the presence of chromosome 8 polysomy, which is consistent with previous reports in breast cancer. However, our study also highlights the importance of considering other factors, such as age and tumor size, when analyzing MYC amplification in BRCA1-deficient tumors.
finding that no BRCA1-associated tumors had high levels of HER-2/neu amplification (8). Thus, our data support an association of MYC amplification with breast tumors from BRCA1 mutation carriers. In addition, the similarity observed between tumors from germ-line BRCA1-mutation carriers and some methylated sporadic tumors suggest that MYC is a cooperative oncogene in tumor cells lacking BRCA1. To our knowledge, this is the first study to address the contribution of MYC to hereditary BRCA1-mutated and BRCA1-methylated sporadic breast cancers.

MYC amplification in sporadic breast cancers has been intensely studied using different methods. However, the results
have been controversial, partly due to low sensitivity of some of the analytic methods used (10, 18). Studies, in which MYC amplification was detected by FISH method, listed in Table 5, show that 12–45% of breast cancer cases have MYC amplification. The proportion of MYC-amplified tumors found in our study is comparable with the majority of these reports. Similar to previous studies, we did not find any correlation between MYC amplification and age or tumor size. We also found no association between MYC amplification and ER-negativity, which is in agreement with some reports (17, 19, 20) but in contradiction to others (21). For the first time, however, we found, that MYC oncogene amplification can be associated with BRCA1 inactivation status.

An association among BRCA1 methylation, loss of BRCA1 transcripts, and reduced or undetectable BRCA1 protein expression has been described in about 20–30% of sporadic tumors (6, 22). We observed MYC amplification in 40% of BRCA1-methylated sporadic tumors. Interestingly, despite the methylation heterogeneity, the mean MYC and CEP8 copy numbers per cell, copy number ratios, the proportion of MYC-amplified tumors, and the pathological features of BRCA1-methylated tumors were comparable with the parameters of BRCA1-mutated hereditary tumors and appear to be intermediate between BRCA1-mutated hereditary and sporadic unmethylated tumors. Thus, these data suggest that loss of BRCA1 in some sporadic breast cancers through epigenetic mechanism(s) such as promoter methylation contribute to the development of those tumors (6), and appears to precede and, hence, promote MYC amplification, as we observed in the hereditary BRCA1-mutated tumors.

Our data are consistent with previous studies in familial breast cancers. DNA microarray-based analyses have suggested

Table 3  Multivariable regression model for MYC:CEP8 ratio

The analysis indicates that BRCA1-mutated tumors have significantly greater MYC:CEP8 ratio than sporadic tumors after adjustment for age, tumor size, and estrogen receptor (ER). None of these factors were significant predictors of MYC:CEP8 ratio.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>β</th>
<th>SE (β)</th>
<th>P for test: β = 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1-mutated (vs. all sporadic)</td>
<td>0.333</td>
<td>0.142</td>
<td>0.02</td>
</tr>
<tr>
<td>Age at diagnosis (vs. ≤2.0 cm)</td>
<td>−0.0006</td>
<td>0.004</td>
<td>0.88</td>
</tr>
<tr>
<td>Tumor size ≥2.0 cm (vs. ≤2.0 cm)</td>
<td>0.114</td>
<td>0.126</td>
<td>0.37</td>
</tr>
<tr>
<td>Tumor size unknown (vs. ≤2.0 cm)</td>
<td>0.056</td>
<td>0.171</td>
<td>0.74</td>
</tr>
<tr>
<td>ER negative (vs. ER positive)</td>
<td>0.140</td>
<td>0.114</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* Tumor group, tumor size, and ER are represented as (0,1) indicator variables in the model, and coefficients represent change in log MYC:CEP8 ratio relative to reference category shown in parentheses. Analysis is based on 88 of 102 cases with complete data for age and ER. Because tumor size was unavailable for 15 cases, a category “unknown” was created and used as a covariate in order to retain these cases in the model. Results of the same analysis omitting these 15 cases were similar. The estimated intercept for the model is 0.353.

Fig. 2  Representative photomicrographs of breast tumor tissue sections from BRCA1-deficient cancers after fluorescence in situ hybridization. The MYC gene is identified by red fluorescent signals, and the chromosome 8 centromere (CEP8) is identified by green fluorescent signals. The nuclei were counterstained with 4',6-diamidino-2-phenylindole (blue). Original magnification, ×1250. A, highly MYC-amplified cancer from BRCA1 germ-line mutation carrier (ratio = 6.0). A mean of 12 (3–40) MYC signals per nuclei were observed. The tumor was heterogeneous, monosomic/polysomic for chromosome 8. The patient was 32 years old. Inset, nucleus from the neighboring field of the same tissue section. B, MYC-amplified BRCA1-methylated sporadic tumor (ratio = 3.9). The mean copy number of MYC per cell was 8 (4–14); 75% of nuclei revealed two to three CEP8 signals. The patient was 40 years old.
that breast cancers arising in the setting of germ-line BRCA1 mutations have unique gene expression profiles, and sporadic tumors with methylated BRCA1 may be misclassified with the BRCA1-mutation-positive group (4, 5). We reviewed the set of genes published by Hedenfalk et al. (Ref. 4; 7 BRCA1 tumors) and by van ’t Veer et al. (Ref. 5; 18 BRCA1 tumors), and found that MYC on 8q was overexpressed in BRCA1 mutation carriers (data not shown). By conventional comparative genomic hybridization, 8q23–24 amplicon has been described in hereditary as well as in sporadic breast cancers, and MYC has been suggested

Table 5  Frequency of MYC amplification in fluorescence in situ hybridization studies of breast cancer reported in the literature and in current study

<table>
<thead>
<tr>
<th>Cases analyzed</th>
<th>MYC amplified</th>
<th>Correlations and comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>261</td>
<td>38 (14.6%)</td>
<td>DNA aneuploidy, PR(−), high tumor grade, node positive, high S-phase, tumor aggressiveness. No association with ER(−), age, tumor size, metastases.</td>
<td>Runnukainen et al., 2001b (20)</td>
</tr>
<tr>
<td>177</td>
<td>25 (14%)</td>
<td>DNA aneuploidy, PR(−), high tumor grade, node positive, high S-phase. No association with ER(−), age, tumor size, metastases.</td>
<td>Runnukainen et al., 2001a (19)</td>
</tr>
<tr>
<td>100</td>
<td>21 (21%)</td>
<td>S phase, DNA index and ER(−). No correlation with tumor size. Only node negative IDC analyzed.</td>
<td>Persons et al., 1997 (21)</td>
</tr>
<tr>
<td>74</td>
<td>9 (12%)</td>
<td>No clinicopathologic parameters mentioned. Amplification detected in tissue microarray.</td>
<td>Schraml et al., 1999 (31)</td>
</tr>
<tr>
<td>53</td>
<td>24 (45%)</td>
<td>Hypertetraploidy/hypertetrasomy. All cells aneuploid for chromosome 8 exhibited extensive MYC amplification.</td>
<td>Janocko et al., 2001 (17)</td>
</tr>
<tr>
<td>26</td>
<td>22 (86%)</td>
<td>DNA aneuploidy, chromosome 8 polysomy, high tumor grade, high tumor stage.</td>
<td>Visscher et al., 1997 (32)</td>
</tr>
<tr>
<td>23</td>
<td>7 (30.4%)</td>
<td>High tumor grade. No correlation with chromosome 8 polysomy. Only DCIS analyzed.</td>
<td>Fiche et al., 2000 (33)</td>
</tr>
<tr>
<td>20</td>
<td>5 (25%)</td>
<td>DNA aneuploidy. No other clinicopathologic parameters mentioned. Amplification detected in fine needle aspirates.</td>
<td>Heselmeyer-Haddad et al., 2002 (34)</td>
</tr>
<tr>
<td>102</td>
<td>35 (34%)</td>
<td>BRCA1 status (mutation and methylation). No correlation with age, tumor size, ER(−). 60 BRCA1-deficient (40 hereditary BRCA1-mutated and 20 sporadic BRCA1 methylated) and 42 sporadic tumors with presumably normal BRCA1 were analyzed.</td>
<td>Grushko et al. (current study)</td>
</tr>
</tbody>
</table>

PR, progesterone receptor; ER, estrogen receptor.
as a target of this amplification (11, 23, 24). Moreover, mice carrying conditional BRCA1 mutation display gain of chromosome 15 (orthologous to human chromosome 8q24) by CGH and overexpression of MYC protein by Western blot analysis (25, 26). However, the observation of preferential MYC amplification in our study does not itself rule out the possible importance of other genes in the 8q24 region, which may be amplified with MYC (27).

The observed similarities between BRCA1-mutated and BRCA1-methylated sporadic tumors support a tumor progression model in which early loss of BRCA1 function causes defects in chromosome structure, cell division, and viability, so that a BRCA1-deficient cell must acquire additional alterations that overcome these problems and presumably force tumor evolution down a limited set of pathways (3). Our data suggest that MYC function might be critical or important in these pathways. BRCA1 protein contains several functional domains that interact directly or indirectly with a variety of molecules, and it likely serves as an important central component in multiple biological pathways (22). BRCA1 contains at least two nuclear localization sequences, which are required for translocation into the nucleus. The presence of a transactivation domain and the association of BRCA1 with the RNA polymerase II holoenzyme suggest that BRCA1 might be involved in gene transcription. Consistent with this notion, Wang et al. (16) demonstrated that BRCA1 physically binds to MYC and represses its transcriptional and transforming activity. Furthermore, they showed that BRCA1 reverses the phenotype of rat embryonic fibroblasts transformed by myc-ras activation. Another group found that in addition to direct binding to C-MYC, BRCA1 specifically binds to Nmi (N-MYC-interacting protein; Ref. 28) and that later is functioning as an adaptor molecule to recruit MYC to a complex with BRCA1. The authors showed that through disruption of Nmi-BRCA1-MYC tri-complex constructs with BRCA1 mutations within Nmi binding sites are unable to indirectly suppress the oncogenic potential of MYC. These data indicate that BRCA1 is a component of a transcription factor complex and may in part function as a tumor suppressor by regulating MYC activity (28). Thus, our observation that MYC activation through gene amplification occurs in a high proportion of human BRCA1-mutated hereditary and BRCA1-methylated sporadic cancers provides additional support for a role for MYC in BRCA1-associated tumor progression. Future work will evaluate the mechanisms of MYC amplification in BRCA1-deficient cells.

In our study not all of the BRCA1-mutated tumors displayed MYC amplification, suggesting a possible association between the type of BRCA1 mutation and MYC amplification. However, no such association was found. It was shown previously that BRCA1 contains two regions that independently interact with MYC and require amino acid residues 175–303 and 343–433. The two regions span exons 8, 9, and 10 and the NH2-terminal portion of exon 11 (16). MYC amplification was observed not only in tumors with BRCA1 mutations located upstream or within MYC binding sites, but also in cases with mutations located downstream of binding sites. Apparently, the regions downstream of MYC binding sites may indirectly affect BRCA1-MYC interaction, or the BRCA1 truncated protein formed might be unstable and incapable of strong interaction with MYC. For example, mutations of BRCA1 within Nmi binding sites (298–683 and 1301–1863 amino acids) may disrupt Nmi-BRCA1-MYC tricomplex thereby suppressing the oncogenic potential of MYC (Ref. 28; Table 4). In addition, because DNA methylation is a quantitative process, the heterogeneity observed in the BRCA1-methylated cases is possibly related to the degree of methylation of the promoter region (the quantity and density of CpG islands involved), causing different levels of concentration or complete absence of the protein product.

The BRCA1 mutant tumors appear to have a profile that is most consistent with the basal-like subtype suggested by Perou et al. (29) and Sorlie et al. (30) based on the following observations. First, both (meaning sporadic basal-like tumors and BRCA1 mutant tumors) tend to be high grade, ER/progesterone-receptor negative and HER-2/neu-negative, and both show MYC amplification. In fact, MYC emerged as one of the most relevant genes that defined the basal-like group and was expressed 2–4 fold above background in the majority of cases.10 Moreover, we have shown previously that BRCA1-mutated tumors express specific basal cytokeratins in a manner suggestive of an ER−, basal-like epithelial cell of origin (12) and are never associated with high levels of HER-2/neu amplification (8). Therefore, it is reasonable to suggest that BRCA1-mutated tumors are mostly basal-like (ER−, HER2−), and that MYC amplification additionally defines a subset of these tumors. Additional studies of a larger cohort of BRCA1-associated tumors are ongoing to dissect the role of cooperative oncogenes and tumor suppressor genes in the progression of these breast cancers.

ACKNOWLEDGMENTS

We thank Dr. Katrin Carlson for critical reading of the manuscript.

REFERENCES


10 C. M. Perou, unpublished observations.
MYC Is Amplified in BRCA1-Associated Breast Cancers

Tatyana A. Grushko, James J. Dignam, Soma Das, et al.


Updated version  Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/10/2/499

Cited articles  This article cites 33 articles, 13 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/10/2/499.full.html#ref-list-1

Citing articles  This article has been cited by 17 HighWire-hosted articles. Access the articles at:
/content/10/2/499.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.