Loss of Heterozygosity on the Short Arm of Chromosome 8 in Male Breast Carcinomas

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ABSTRACT

Identification of loss of heterozygosity (LOH) at specific genetic loci in neoplastic cells suggests the presence of a tumor suppressor gene within the deleted region. LOH on chromosome 8p has been identified in colorectal, bladder, hepatocellular, and prostatic carcinomas. Little is currently known about the molecular events occurring during the development of male breast cancer. We studied LOH on chromosome 8p in 23 male breast carcinomas. Five polymorphic DNA markers were used: D8S136 and D8S137 on 8p12-21.3; and D8S254, D8S258, and D8S349 on 8p22. DNA was extracted from microdissected normal and tumor cells obtained from formalin-fixed, paraffin-embedded tissue sections and amplified by the PCR. LOH was identified in 19 of 23 cases (83%) with at least one marker. Seven cases showed LOH only at 8p22, six cases showed LOH only at 8p12-21.3, and six cases showed LOH at both 8p22 and 8p12-21.3. In five of these last six cases, at least one locus was retained between the two deleted regions; thus, the whole short arm of chromosome 8 was not lost in these tumors. Our results show that there are two discrete areas of deletion on chromosome 8p in male breast cancer, suggesting the presence of one or more tumor suppressor genes that may play a role in the development or progression of the disease.

INTRODUCTION

The process of carcinogenesis is thought to occur as a stepwise accumulation of genetic alterations, including activation of oncogenes and inactivation of tumor suppressor genes (1). Frequent allelic loss at specific loci in tumor cells implies functional inactivation of a putative tumor suppressor gene.

Very little is currently known of the molecular events leading to breast cancer in males. Male breast carcinoma is an uncommon disease with a prevalence of 1% that of female breast carcinomas in the United States (2). The histology of the normal male breast is similar to that of the prostate, and carcinomas arising in each tissue are histologically quite similar (3, 4). In prostatic carcinomas, a high frequency of LOH has been detected on chromosome 8p, ranging from 29 to 90% of informative cases (5–11). To investigate similarities with the somatic genetic progression of prostatic cancer, we analyzed LOH in microdissected sporadic male breast carcinomas with five polymorphic DNA markers on the short arm of chromosome 8.

MATERIALS AND METHODS

Tumor Samples. Cases were obtained from the Pathology files of the National Cancer Institute and the Hospital Universitario “San Carlos.” In each case, paraffin-embedded, hematoxylin and eosin-stained sections were reviewed. Twenty-three cases in which slides contained both invasive carcinoma and normal breast tissue were selected for the study. None of the patients included in our study were determined to have a strong family history of breast cancer; additionally, none of the patients had a history of prostatic cancer.

Microdissection Technique. Microdissection of selected areas from formalin-fixed, paraffin-embedded tissue was performed under direct light microscopic visualization as has been described previously (Ref. 12; Fig. 1). Briefly, pure tumor cells were selected from the eosin-stained slides and microdissected using a disposable, sterile, 30-gauge needle. Adjacent normal stromal or inflammatory cells were also dissected from each lesion.

DNA Extraction. Procured cells were immediately resuspended in a 20-μl solution containing 0.05 M Tris-HCl, 0.001 M EDTA, 1% Tween 20, and 0.1 mg/ml proteinase K (pH 8.0) and incubated overnight at 37°C. The mixture was boiled for 10 min to inactivate proteinase K, and 1.5 nl of this solution was used for PCR.

Primers and PCR Conditions. A total of five polymorphic DNA markers were used. Markers D8S136 and D8S137 are located on 8p12-21.3 (13–15). Markers D8S254, D8S258, and D8S349 are located on 8p22 (13, 16). Reactions were cycled in a Perkin-Elmer Cetus thermal cycler as follows: D8S136, 94°C for 45 s, 55°C for 45 s, 72°C for 1 min, total 35 cycles; D8S137, 94°C for 45 s, 62°C for 45 s, 72°C for 1 min, total 35 cycles; D8S254 and D8S258, 94°C for 45 s, 58°C for 45 s, 72°C for 1 min, total 35 cycles; and D8S349, 94°C for 45 s, 60°C for 45 s, 72°C for 1 min, total 35 cycles. PCR was performed in 10 μl volumes and contained 1 μl 10X PCR buffer [100 mM Tris-HCl (pH 8.3); 500 mM KCl; 15 mM MgCl2; 0.1% w/v gelatin; 1.5 μl DNA extraction buffer; 50 μl of each primer; 20 mM each of dCTP, dGTP, dTTP, and dATP; 0.2 μCi [32P] dCTP (6000 Ci/mM); and 0.1 unit Taq DNA polymerase]. Labeled amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol). The samples were denatured for 5 min at 94°C and loaded onto a gel consisting of 6% acrylamide (49:1 acrylamide:bis). Samples were electrophoresed at 1800 V for 1 to 2 h. Gels were transferred to 3 mm Whatman paper and dried, and autoradiography was performed with Kodak X-OMAT film. LOH was considered with complete or near complete absence of one allele in the tumor DNA as defined by direct visualization (Figs. 2–4).

RESULTS

Chromosome 8p LOH was identified with at least one marker in 19 of 23 cases (83%; Fig. 5). Thirteen cases showed LOH with one or more markers on chromosome 8p22. One case showed LOH with three 8p22 markers, 2 cases with two 8p22 markers, and 10 cases showed LOH with one 8p22 marker. The D8S349 locus had the highest rate of loss on 8p22 (10 of 18 informative cases), and the D8S258 had the lowest rate of loss on 8p22 (3 of 16 informative cases). Seven of the 13 cases showing LOH on 8p22 did not show LOH with any of the markers on 8p12–21.3, whereas 6 of the 13 cases also showed LOH, at least with one marker on 8p12–21.3.

Twelve cases showed LOH with at least one marker on chromosome 8p12–21.3. Four cases showed LOH with both markers on 8p12–21.3, and eight cases showed LOH with one of the markers. Multiple bands for each allele were observed in some cases with marker D8S136. This is not uncommon with markers which yield a small product, like marker D8S136 (71- to 89-bp product). The same pattern was reproduced dissecting different tissue areas from these cases. LOH on D8S137 was found in 10 of 16 informative cases, whereas LOH was found on D8S136 in 7 of 20 informative cases. Six of the 12 cases showing LOH at 8p12–21.3 did not show LOH with any marker at 8p22.
In the four cases in which a sample from lymph node metastasis was available for microdissection, samples from the metastasis and from the primary tumor in each case showed identical results with all of the markers. One case (case 15) showed LOH, both at 8p12–21.3 (D8S137) and 8p22 (D8S254), and one case (case 13) showed LOH at 8p22 (D8S349). Both cases showed loss of the same allele in the primary tumor and metastasis (Figs. 2 and 3). Two cases (cases 11 and 14) did not show LOH at either loci.

DISCUSSION

Little is known about the genetic events that lead to the development and progression of breast cancer in males. Mutations in p53 (17) and K-ras genes (18) have been reported, but they seem to occur infrequently. Familial breast cancer predisposition in males does not appear to be linked to the breast cancer susceptibility gene 1 (BRCA1) locus (19), and previously we did not identify a significant rate of LOH at this locus (17q21.3–22) in male breast cancer (20). Mutations in the androgen receptor gene on Xp11.2–q12 have been reported in a few families with male breast cancer history (21, 22). Wooster et al. (23) have located the breast cancer susceptibility gene BRCA2 on chromosome 13q12–13 and have identified several cases of mutations at BRCA2 in male breast cancer (23). We identified a high frequency of LOH in male breast cancers on 11q13, suggesting the presence of a tumor suppressor gene in male breast carcinomas in this region (20). No other LOH studies identifying loci with putative tumor suppressor genes have been reported in male breast cancer.

The presence of one or more tumor suppressor genes located on the short arm of chromosome 8 has been suggested by the detection of LOH in several tumors including prostate (5–11), colorectal (7, 24–27), hepatocellular (24), bladder (28), and lung carcinomas (24). In prostatic carcinomas, several of the studies have detected the highest frequency of loss in a distal region of 8p, at 8p21–23.3 (5–7, 9). However, others have identified 8p12–21 as the most commonly

Fig. 1. Eosin-stained sections before dissection (A) and after dissection of three (B) and seven (C) nests of pure invasive carcinoma (case 21).

Fig. 2. Sequencing gel showing three cases with D8S136 primer. N, normal sample; T, tumor sample; M, sample from the metastasis. Normal samples show two alleles (brackets); tumor samples show LOH (arrowheads). Sample from the metastasis also shows loss of the same allele as the primary tumor (cases 15, 4, and 20).

Fig. 3. Sequencing gel showing four cases with D8S349 primer. N, normal sample; T, tumor sample; M, sample from the metastasis. Normal samples show two alleles (brackets); tumor samples show LOH (arrowheads). Sample from the metastasis also shows loss of the same allele as the primary tumor (cases 13, 5, 20, and 21).
deleted region in prostatic carcinomas (10, 11). Some LOH studies of chromosome 8p in other tumors also suggest that there are two separate regions on the short arm of chromosome 8 that contain different tumor suppressor genes. In colorectal carcinomas, LOH on 8p has been detected in 36.8–51% of informative cases (24–26), with the highest frequency of loss on 8p22 (25). However, two areas of deletions on 8p separated by several retained loci have been reported in colorectal carcinomas (25, 26). One area is more centromeric, at 8p12–21.3, and the other is more telomeric, at 8p22–23.2 (25, 26). We used two polymorphic DNA markers located on 8p12–8p21.3 (D8S136 and D8S137) and three located on 8p22 (D8S254, D8S258, and D8S349; Refs. 13–15). At 8p12–21.3, D8S137 is located more centromerically than D8S136 (Fig. 6), the distance between both markers being 7.4 to 18 cM (8, 29, 30). At 8p22, D8S258 is located more centromerically, close to the lipoprotein lipase gene (13), whereas D8S349 is more distal, close to the macrophage scavenger receptor gene (Ref. 13; Fig. 6).

In the present study, LOH on 8p was identified with at least one marker in 19 of 23 cases (83%). The majority of the cases showed LOH only on the telomeric (8p22) region (seven cases) or only on the centromeric (8p12–21.3) region (six cases) of chromosome 8p. This suggests that these are two different regions of deletions on 8p. Furthermore, five of the six cases showing LOH at both the proximal and distal areas on 8p showed at least one retained locus between the two deleted regions, further suggesting that there are two discrete regions of deletion on chromosome 8p, each potentially containing a different suppressor gene. Only one case (case 5) showed LOH with all of the informative loci, suggesting a large 8p deletion.

In four cases, tissue from lymph node metastasis was available for microdissection. In the primary tumor, one of these cases showed LOH selectively on the distal region of chromosome 8, and the other case showed LOH on both the distal and proximal regions of 8p. The other two cases did not show LOH in the primary tumor. In all four cases, the same results were detected in both the primary tumor and metastasis. Thus, chromosome 8p LOH does not appear to be a late event in male breast cancer progression. Interestingly, LOH on 8p has been identified in preinvasive lesions such as prostatic intraepithelial neoplasia (9, 11) and
dysplasia in ulcerative colitis (7), suggesting that inactivation of a tumor suppressor gene on 8p is an early event in tumor development. Whether similar genetic changes exist in male and female breast cancers is not known. Male breast carcinomas are histologically similar to female ductal breast carcinomas. High frequency of LOH at chromosome 11q13 was identified in sporadic female breast cancers (31), as well as in sporadic male breast cancers (20), suggesting that a tumor suppressor gene on 11q13 plays a role in both carcinomas. In female breast carcinomas, Emi et al. (24) reported LOH on 8p in only 5 of 56 informative patients. However, with the microdissection technique, pure tumor cell populations for DNA extraction can be obtained, avoiding contamination with normal or inflammatory cell DNA. Higher rates of LOH than reported previously were identified using the microdissection technique at 11q13 in female breast carcinomas (31), on chromosome 8p in prostate intraepithelial neoplasia (11), and in the von Hippel-Lindau disease gene on chromosome 3p in colorectal carcinomas. Very little is known about the possible presence of tumor suppressor genes on chromosome 8p involved in female breast carcinomas. Interestingly, Pykett et al. (32) recently identified LOH in 6 of 8 female breast carcinoma cell lines on chromosome 8p. One tissue sample was included in the study, also showing LOH. A probe (mac 30) located between 8p11 and 8p21 was used. No marker on 8p22 was analyzed. In a recent study, Keranguen et al. (33) looked for deletions on chromosome 8p in female breast carcinomas, using eight microsatellite markers on region 8p12–22. LOH was detected in almost one-half of the tumors. High frequency of LOH was detected with marker D8S258 and low frequency with marker D8S137. Thus, it seems that the pattern of LOH is not the same as reported in the present study of male breast carcinoma, although only two of the eight markers correspond to markers used in the present study. In conclusion, our results suggest the presence of a tumor suppressor gene(s) on the short arm of chromosome 8p that may play a role in the development of male breast cancer. Thus, it seems that similar histological and genetic alterations may commonly occur in male breast and prostatic carcinomas.

REFERENCES

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