 Concurrent and Independent Genetic Alterations in the Stromal and Epithelial Cells of Mammary Carcinoma: Implications for Tumorigenesis

Farid Moinfar, Yan Gao Man, Laurent Arnould, Gary L. Bratthauer, Manfred Ratschek, and Fattaneh A. Tavassoli

ABSTRACT

The high frequency of loss of heterozygosity (LOH) in epithelial cells of mammary ductal carcinoma in situ (DCIS) and IDC is a well known phenomenon, whereas the genetic abnormalities in the mammary stroma and its influence on the epithelial component have not been sufficiently studied. Using the PCR, we examined DNA extracts from microdissected stromal and epithelial tissues of 11 breast samples containing DCIS, including five cases associated with IDC. In each case, the mesenchymal tissue consisting of normal-appearing stroma at a distance from DCIS and IDC or stroma close to either DCIS or IDC was manually microdissected. Epithelial cells from morphologically clear-cut normal ducts and lobules, DCIS, and IDC were also microdissected. Twelve polymorphic DNA markers were tested to identify possible genetic alterations in the mesenchymal and epithelial cells on chromosomes 2p, 3p, 11q, 16q, and 17q. Samples from bilateral reduction mammoplasty from 10 women without any clinical, radiological, or pathological abnormalities were also selected as a control (reduction mammoplasty group). Whereas most cases (8/11, 73%) displayed at least one identical LOH in both epithelial and mesenchymal components, LOH at several loci was noted exclusively in stromal cells. The most frequent genetic alterations in the mesenchymal cells were at chromosomes 17q24, 16q23.1–24.2, 3p14.2, and 11q21–23.2, in 87.5, 62, 60, and 45% of informative cases, respectively. The LOH frequency in the stroma close to cancer ranged from 10 to 66.5% for DCIS and from 20 to 75% of informative cases for IDC. Furthermore, 10 of the 12 polymorphic markers revealed LOH in the stroma at a distance, ranging from 11 to 57% of informative cases. None of the control cases (women without any breast disease) revealed LOH either in the epithelial or in the stromal components. Our findings strongly support the concept of stromal-epithelial interaction in the development and progression of mammary neoplasia. Furthermore, this study suggests that genetic alterations in the stromal cells may precede genotypic changes in the epithelial cells. At least in some cases, the mammary stroma in DCIS or IDC apparently represents a neoplastic interactive component rather than a reactive response to the carcinoma. The frequent allelic loss (LOH) in the mammary stroma, identified in our study, may explain some of the fibroblastic abnormalities previously observed in patients with breast carcinoma or a variety of cancer-associated hereditary diseases. We conclude that the mammary stroma may play a key role in inducing neoplastic transformation of epithelial cells, recapitulating its role in normal mammary duct development.

INTRODUCTION

The interaction between epithelial and mesenchymal cells in different organs plays a critical role in development (1), differentiation (1, 2), and proliferation (2, 3) of epithelial cells. Although during embryogenesis the mesodermal (mesenchymal) cells have a key role in inducing differentiation and proliferation in the ectodermal cells (4), the role of stromal cells in the development and progression of epithelial neoplasia has not been thoroughly investigated. Despite frequent genetic alterations in the form of microsatellite instability (5), LOH (5, 6, 7), and gene amplification (6, 8) observed in many benign and malignant epithelial neoplasms, the issue of possible genetic abnormalities in the background supportive stroma of these neoplasms has not been addressed properly.

In a previous study (9), we detected frequent occurrence of genetic alterations (LOH) in an early “nonhyperplastic” intraductal neoplasia of the breast [ductal intraepithelial neoplasia, (DIN)-flat type, also known as “clinging ductal carcinoma in situ”]. In addition to the epithelial cells, the stroma in each case was manually microdissected at a distance (at least 15 mm) from the intraductal neoplasia and invasive ductal carcinoma (IDC) to serve as a normal control. Although the stroma in the vast majority of cases (22/25 cases) did not show any genetic abnormality, three cases had definite LOH in the stroma; therefore, these cases were excluded from our previous study for further investigation. This rather surprising finding prompted initiation of a separate study to examine the possibility and frequency of LOH in the mammary stroma from women with and without breast cancer.

MATERIALS AND METHODS

Samples from 11 female patients with DCIS, including five cases with IDC, were selected from the files of the Armed Forces Institute of Pathology. In each case, the mesenchymal tissue consisting of normal-appearing SAD (at least 15-mm distance) from DCIS and IDC and stroma close to either DCIS or IDC was manually microdissected. Epithelial cells from morphologically clear-cut normal ducts and ductules (acinis), DCIS, and IDC were also microdissected. Samples from 10 women with bilateral RM were also included in the study and served as a control for normal epithelium and stroma unassociated with any neoplastic process; these women did not show any clinical, radiological, or histomorphological abnormalities in their breasts. In each RM case, the morphologically normal epithelium and the intervening normal stroma were microdissected and analyzed for possible LOH. Accurate microdissection and DNA extraction were carried out as previously described (Ref. 10 and Fig. 1).

Using the PCR, we examined DNA extracts from the microdissected tissues with 12 polymorphic DNA markers on chromosomes 2p, 3p, 11q, 16q, and 17q, which are mostly known for a high frequency of LOH in DCIS and/or IDC of the breast (11, 12). Gene Amp PCR kits, Taq gold DNA polymerase, and DNA size markers were obtained from Perkin-Elmer (Foster City, CA). Fluorescent-labeled polymorphic DNA markers, including TPO (2pter), D3S106 (3p14.1–14.3), D3S1300 (3p14.2), D3S1581 (3p14.2–21.2), DSS2432 (3p22–24.2), DSS766 (3q26.2–27), D11S1311 (11q21–23.2), D15S802 (15q24.1), D16S518 (16q23.1–24.2), D17S579 (17q21), and D17S791 (17q21), were purchased from Research Genetics (Huntsville, AL). PCR amplification was carried out in a programmed thermal cycler (Perkin-Elmer) at the following settings: after a denaturation at 94°C for 14 min, samples were amplified for 35–40 cycles at 94°C, 55–60°C, and 72°C, each for 1 min, with a final extension at 72°C for 10 min. Amplified PCR products were subjected to electrophoresis in 5–6% polyacrylamide gels.

Received 11/23/99; accepted 3/1/00.

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.

1The abbreviations used are: LOH, loss of heterozygosity; IDC, infiltrating ductal carcinoma; DCIS, ductal carcinoma in situ; SAD, stroma at a distance; SC-DCIS, stroma close to DCIS; SC-IDC, stroma close to IDC; RM, reduction mammoplasty; NE, normal epithelium; TSG, tumor suppressor gene; ECM, extracellular matrix; FHIT, fragile histidine triad.

The opinion and assertions contained herein are the private views of the authors and are not to be construed as official or as representing the views of the Department of the Army or the Department of Defense.

2To whom requests for reprints should be addressed, at Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology, Washington DC 20306. Phone: (202) 782-1612; Fax: (202) 782-3939; E-mail: man@afip.osd.mil.
LOH IN MAMMARY CARCINOMA-ASSOCIATED STROMAL CELLS

RESULTS

LOH was a frequent finding in the mammary stroma in patients with DCIS and IDC (Table 1). In the presence of either DCIS or IDC, all but one (D3S2432) of the polymorphic DNA markers showed LOH in the stroma. Among 11 DNA markers that revealed LOH in the stroma, the LOH frequency ranged from 10% (1/10) to 66.55% (4/6) of informative cases in the SC-DCIS. The frequency of LOH in the SC-IDC was even higher, ranging from 20% (1/5) to 80% (4/5) of informative cases.

A surprising finding in our study was the common occurrence of LOH in the morphologically normal-appearing SAD from DCIS and IDC. The morphologically normal epithelium in samples containing either DCIS or invasive carcinoma occasionally displayed LOH. Although 8 of the 12 DNA markers did not show any LOH in NE, four markers revealed LOH in the NE in three cases. The LOH frequency in IDC ranged from 25% (1/4) to 100% (3/3) of informative cases.

The morphologically normal epithelium in samples containing either in situ or invasive carcinoma occasionally displayed LOH. Although 8 of the 12 DNA markers did not show any LOH in NE, four markers revealed LOH in the NE in three cases. The LOH frequency in IDC ranged from 25% (1/4) to 100% (3/3) of informative cases.

A comparison of LOH frequency in the epithelial and stromal cells revealed that although most cases (8/11, 73%) were associated with at least one identical LOH in both the epithelial and stromal components, several microsatellite loci (D11S1311, D3S106, D17S785, and TPO) were lost only in the stromal cells in five cases (Table 3). The most common genetic alterations in the stromal cells were at chromosomes 17q24, 16q23.1–24, 3p14.2–21.2, and 11q21–23.2 in 87.5, 62, 60, and 45.5% of informative cases, respectively. Selected case examples are shown in Figs. 2, 3, and 4. Interestingly, two cases showed LOH (D3S1581, D11S1311, D16S402, D16S518, D17S579, and D17S791) in their malignant epithelial cells (DCIS) but not in either the stroma adjacent or distant from the DCIS (Table 3). In contrast to the cases with DCIS and IDC, not a single case in the control RM group (10 bilateral RM specimens) revealed LOH in either its epithelial or stromal components.

DISCUSSION

This study is the first to show that LOH in the mammary stroma of patients with breast cancer is a common event. Although most cases (8/11, 73%) revealed at least one identical LOH in both epithelial and

Table 1. LOH frequency in the stroma in patients with DCIS (11 cases) and IDC (five cases)

<table>
<thead>
<tr>
<th>Marker</th>
<th>SAD (%)</th>
<th>SC-DCIS (%)</th>
<th>SC-IDC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPO</td>
<td>1/9 (11%)</td>
<td>1/9 (11%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>D3S1067</td>
<td>2/11 (18%)</td>
<td>6/11 (54.5%)</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>D3S1300</td>
<td>2/8 (25%)</td>
<td>3/9 (33%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>D3S1581</td>
<td>3/10 (30%)</td>
<td>6/10 (60%)</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td>D3S2432</td>
<td>0/11</td>
<td>0/11</td>
<td>0/5</td>
</tr>
<tr>
<td>D15S666</td>
<td>2/9 (22%)</td>
<td>2/9 (22%)</td>
<td>0/4</td>
</tr>
<tr>
<td>D15S1311</td>
<td>2/10 (20%)</td>
<td>2/11 (18%)</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>D16S402</td>
<td>4/7 (57%)</td>
<td>4/7 (57%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>D16S518</td>
<td>4/11 (36%)</td>
<td>5/11 (45.5%)</td>
<td>4/5 (80%)</td>
</tr>
<tr>
<td>D17S579</td>
<td>3/7 (43%)</td>
<td>1/10 (10%)</td>
<td>2/4 (50%)</td>
</tr>
<tr>
<td>D17S785</td>
<td>3/8 (37.5%)</td>
<td>4/6 (66.5%)</td>
<td>3/4 (75%)</td>
</tr>
<tr>
<td>D17S791</td>
<td>0/8</td>
<td>2/7 (28.5%)</td>
<td>1/4 (25%)</td>
</tr>
</tbody>
</table>

Table 2. LOH frequency in the epithelial cells

<table>
<thead>
<tr>
<th>Marker</th>
<th>Normal epithelium</th>
<th>DCIS</th>
<th>IDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPO</td>
<td>1/6 (16.5%)</td>
<td>1/9 (11%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>D3S1067</td>
<td>0/6</td>
<td>5/11 (45.5%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>D3S1300</td>
<td>0/5</td>
<td>3/8 (37.5%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>D3S1581</td>
<td>0/7</td>
<td>5/10 (50%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>D3S2432</td>
<td>0/6</td>
<td>0/11</td>
<td>0/5</td>
</tr>
<tr>
<td>D3S766</td>
<td>0/6</td>
<td>1/10 (10%)</td>
<td>0/3</td>
</tr>
<tr>
<td>D15S1311</td>
<td>0/8</td>
<td>1/11 (9%)</td>
<td>0/5</td>
</tr>
<tr>
<td>D16S402</td>
<td>0/6</td>
<td>7/8 (87%)</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>D16S518</td>
<td>2/8 (25%)</td>
<td>6/10 (60%)</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>D17S579</td>
<td>0/6</td>
<td>1/10 (10%)</td>
<td>0/3</td>
</tr>
<tr>
<td>D17S785</td>
<td>2/5 (40%)</td>
<td>5/8 (62.5%)</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td>D17S791</td>
<td>1/4 (25%)</td>
<td>2/8 (25%)</td>
<td>2/4 (50%)</td>
</tr>
</tbody>
</table>
carcinoma of skin (21) or even highly aggressive acute leukemia (22),
malignant tumors, including prostatic adenocarcinoma (20), basal cell
in circumstances, normal mesenchymal cells (fibroblasts) can convert
of epithelial neoplasms (18, 19). On the other hand, under certain
investigated. Earlier recognized phenomenon (13, 14), a possible role for mesenchymal
stroma may even precede genotypic changes in the epithelial cells. Recently, hepatocyte growth factor, which is mainly produced by fibroblasts, has been identified as one of the most potent mitogenic factors for proliferation of epithelial cells in a variety of organs (27). Genetic alterations with loss of TSGs in the mesenchymal cells may lead to subsequent abnormal production of growth factors with constant signal transduction in the adjacent epithelial cells (28). Using a line of transgenic mice expressing the Aequorea victoria green fluorescent protein, a recent study (29) has demonstrated that stromal cells (fibroblasts) with abnormal vascular endothelial growth factor promoter activity not only influence tumor angiogenesis but also may induce spontaneous mammary tumors. Furthermore, it is well-documented that stromal cells play a key role in the production and possible dissolution of the ECM (30, 31). Therefore, genetic abnormalities in the stroma may change the physiological composition of the ECM with subsequent alteration in the epithelial-ECM interaction (30, 31). Moreover, the observed concurrent genetic alterations with at least one identical LOH in the stromal and epithelial cells of mammary carcinoma raises the provocative possibility that the elusive “pluripotent stem cells” (32) in the breast give rise to the malignant epithelial cells and the associated altered supportive stroma. During malignant transformation, the genetically altered, neoplastic “pluripotent (primitive) stem cells” could differentiate into morphologically recognizable, epithelial (carcinoma), mesenchymal (sarcoma), or even mixed epithelial-mesenchymal (carcinosarcoma) cancers (32).

Interestingly, the most common loci with LOH in the stromal cells, identified at chromosomes 17q24 (87%), 16q23.1–24.2 (62%), 3p14.2–21.2 (60%), and 11q21–23.2 (45.5%), contain several putative TSGs. The region 17q24-q25 harbors several potential TSGs (33) that are frequently lost in alveolar soft-part sarcoma (34), dermatofibrosarcoma protuberance (35), and fibrosarcoma in patients with von Recklinghausen’s neurofibromatosis (36) as well as breast carcinoma (37). The cytogenic locus 17q21 (D17S579, D17S791) contains the

Table 3 Distribution of LOH among the epithelial and mesenchymal components

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cases with LOH in either Ep or St/informative cases</th>
<th>Cases with LOH in both Ep and St</th>
<th>Cases with LOH only in Ep</th>
<th>Cases with LOH only in St</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>3/9 (33%)</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>DSS1067</td>
<td>7/11 (64%)</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>DSS1300</td>
<td>3/3 (33%)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DSS1581</td>
<td>7/10 (70%)</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DSS2452</td>
<td>0/11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DSS1766</td>
<td>3/10 (30%)</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>D11S1311</td>
<td>5/11 (45.5%)</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>D16S402</td>
<td>6/8 (75%)</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D16S518</td>
<td>7/11 (64%)</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>D17S579</td>
<td>5/10 (50%)</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D17S785</td>
<td>7/8 (87.5%)</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>D17S791</td>
<td>3/8 (37.5%)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Ep, epithelium; St, stroma.

Fig. 2. A, LOH on chromosome 17q (D17S785; expected size, 181–207 bp) in SC-IDC (Lane 2), SC-DCIS (Lane 3), clear-cut normal epithelial cells (Lane 4), DCIS (Lane 5), and IDC (Lane 6). Lane 1, normal SAD from DCIS or IDC. B, graphical (densitometric) representation of lanes seen in A.

Fig. 3. LOH on chromosome 3p (D3S1300; expected size, 217–241 bp) in SAD (Lane 1), SC-IDC (Lane 2), SC-DCIS (Lane 3), epithelium with mild cytological atypia (Lane 4), DCIS (Lane 5), and IDC (Lane 6). Lane 7, clear-cut normal epithelium (normal ducts and ductules).

Fig. 4. LOH on chromosome 11q (D11S1311; expected size, 127–147 bp) in SC-DCIS (Lane 3), using the polymorphic marker D11S1311, normal SAD from DCIS (Lane 1), fibrotic SAD from DCIS (Lane 2), morphologically normal epithelial cells (Lane 4), and DCIS (Lane 5) are not associated with LOH.

Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 2000 American Association for Cancer Research.
D3S1300 and D3S1581, which suppresses invasion which is frequently altered in prostate (39) and breast carcinomas (40). The CDH1 gene encodes the adhesion molecule E-cadherin, which suppresses invasion in vitro (41). The polymorphic loci D3S1300 and D3S1581 (3p14.2, 3p14.2–21.2) contain the FHIT gene, a recently recognized TSG (42). The loss of FHIT gene has been reported as one of the earliest genetic abnormalities in several malignant neoplasms including breast carcinoma (43), osteosarcoma (44), and Ewing sarcoma (45). The cytogenic locus 11q21–23.2 (D11S1311) is distal and close to the ataxia telangiectasia gene (45), another potential TSG that is commonly lost in breast carcinoma (45), particularly in tubular carcinoma (43).

The presence of LOH in the morphologically normal-appearing fibroconnective tissue SAD from DCIS and IDC and the lack of any LOH in 10 RM specimens from women without breast disease are in concordance with a few reports that have shown abnormal fibroblastic functions in morphologically normal-appearing fibroblasts in the skin of patients with breast carcinoma (46–48). Indeed, abnormal skin fibroblasts displaying various oncotical characteristics were demonstrated in 90% of patients with familial breast cancer and in 50% of the clinically unaffected first-degree relatives of patients suffering from familial breast cancer (46, 47, 49). Moreover, abnormal skin fibroblasts with a high level of enhanced activation of herpes simplex virus have been found (50) in a variety of hereditary cancer-prone syndromes such as retinoblastoma, polyposis coli, neurofibromatosis type 1 and 2, dysplastic nevus syndrome, von Hippel-Lindau syndrome, and multiple endocrine neoplasia type 2, suggesting that loss of one allele of putative TSGs may activate cellular processes that result in the induction of the enhanced reactive response and that functionally abnormal fibroblasts may be related to the process of carcinogenesis (50). The frequent allelic loss (LOH) in the mammary stroma, particularly LOH near some of the putative TSGs such as CDH1, FHIT, and ataxia telangiectasia genes, as identified in our study, may partly explain some of the abnormal fibroblastic functions that have been observed in patients with breast cancer (47, 48) or some of the cancer-associated hereditary diseases (50). Furthermore, at least in some cases, the genetic alterations in the mammary stroma can occur without, and perhaps before, genotypic abnormalities in the epithelial cells, possibly to facilitate invasion.

The results of our study strongly favor the concept of reciprocal stromal-epithelial interaction in mammary tumorigenesis (3, 13, 14, 17, 21, 51, 52). We conclude that the mammary stroma, at least in some patients with DCIS or IDC, most likely represents part of a neoplastic process or interaction rather than a reactive response to breast carcinoma. Furthermore, stromal cells in the breast may play a key role in inducing neoplastic transformation of epithelial cells, a situation that recapitulates their role in embryological development of mammmary ducts. Conversely, epithelial cells may influence various important aspects of fibroblast function such as matrix production, deposition, and secretion of collagenases and other matrix metalloproteinases, as suggested in prior publications (30, 52). Understanding the role of epithelial-stromal interaction in mammary carcinogenesis could ultimately provide alternate therapeutic approaches to the regulation of cancer growth. The involvement of genetically altered stromal cells in mammary carcinogenesis raises the intriguing possibility that novel therapeutic modalities could be developed to specifically target the stromal cells rather than the epithelial component of mammary carcinoma. Ultimately, transformation of the malignant epithelial cells to a benign or less aggressive form could be induced by manipulation of the stromal environment.

REFERENCES


Concurrent and Independent Genetic Alterations in the Stromal and Epithelial Cells of Mammary Carcinoma: Implications for Tumorigenesis

Farid Moinfar, Yan Gao Man, Laurent Arnould, et al.

*Cancer Res* 2000;60:2562-2566.

---

**Updated version**

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/9/2562

**Cited articles**

This article cites 51 articles, 21 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/9/2562.full.html#ref-list-1

**Citing articles**

This article has been cited by 41 HighWire-hosted articles. Access the articles at:
/content/60/9/2562.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.