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Concurrent and Independent Genetic Alterations in the Stromal and Epithelial Cells of Mammary Carcinoma: Implications for Tumorigenesis[1]

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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[3] (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 (SC-DCIS) and/or IDC (SC-IDC) were manually microdissected. Epithelial cells from morphologically clear-cut normal ducts and ductules (acini), 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* (*3p4.1–14.3*), *D3S1300* (*3p14.2*), *D3S1581* (*3p14.2–21.2*), *D3S2432* (*3p22–24.2*), *D3S1766* (*3q26.2–27*), *D11S1311* (*11q21–23.2*), *D16S402* (*16q24.2*), *D16S518* (*16q23.1–24.2*), *D17S579* (*17q21*), *D17S785* (*17q24*), *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

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[3] The 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.

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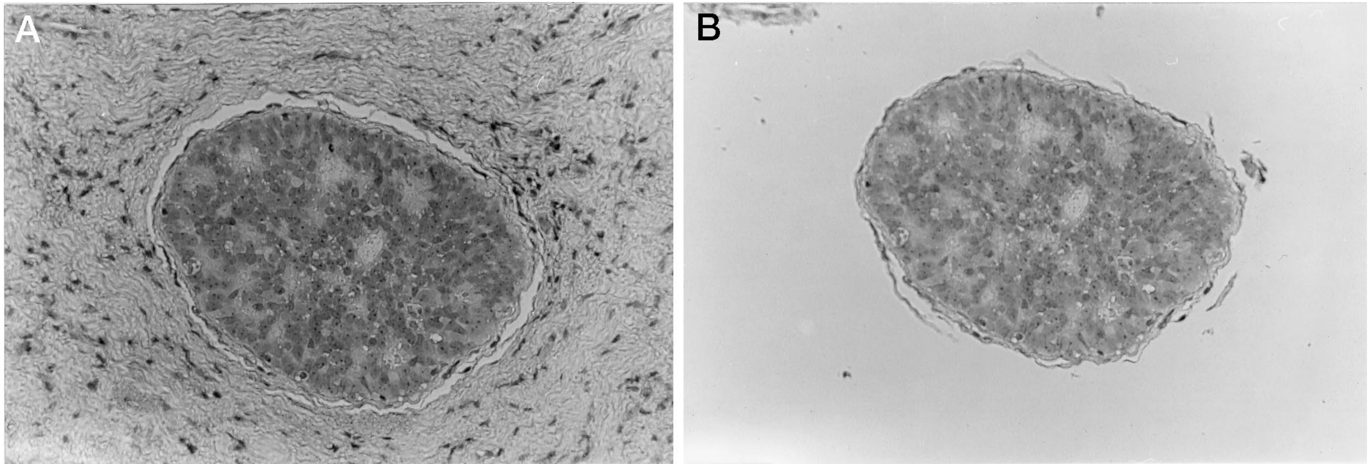

Fig. 1. Microdissection of stromal cells close to the epithelial component. Magnification, ×200. *A*, before microdissection. *B*, after microdissection.

(Bio-Rad, Foster City, CA), and the signal was detected with a model 377 DNA sequencer (Perkin-Elmer) according to the manufacturer's instruction. The amplified products were located and examined by comparing the mobility of the predominant DNA bands in each case with that of the size markers and by comparing the mobility and intensity of the amplified specific DNA bands from the tumor cells with those of normal controls. LOH was defined as complete absence or at least 75% reduction of one allele as assessed by direct visualization. To evaluate the reproducibility of the results, the assay was repeated two to three times on each sample under the same conditions. In all cases, results identical to the original reactions were observed, demonstrating the fidelity of the reactions.

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. Ten of the 12 tested DNA loci had LOH in the SAD; the LOH frequency in SAD ranged from 11% (1/9) to 57% (4/7) of informative cases. Although SC-DCIS and SC-IDC usually appeared altered with either a hypercellular, fibrous ("desmoplastic") or hypocellular, edematous appearance, the fibroconnective tissue in SAD did not reveal any morphologically recognizable alterations. As expected, LOH occurred frequently in the epithelial components in DCIS and IDC (Table 2). All but one (*D3S2432*) of the polymorphic DNA markers showed LOH in the DCIS, ranging from 10% (1/10) to 87% (7/8) of informative cases. Furthermore, 8 of the 12 DNA markers revealed LOH in the IDC. The LOH frequency in IDC ranged from 25% (1/4) to 100% (3/3) of informative cases.

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

Marker	SAD	SC-DCIS	SC-IDC
TPO	1/9 (11%)	1/9 (11%)	1/4 (25%)
D3S1067	2/11 (18%)	6/11 (54.5%)	1/5 (20%)
D3S1300	2/8 (25%)	3/9 (33%)	1/3 (33%)
D3S1581	3/10 (30%)	6/10 (60%)	3/5 (60%)
D3S2432	0/11	0/11	0/5
D3S1766	1/9 (11%)	2/9 (22%)	0/4
D11S1311	2/10 (20%)	2/11 (18%)	1/5 (20%)
D16S402	4/7 (57%)	4/7 (57%)	2/2 (100%)
D16S518	4/11 (36%)	5/11 (45.5%)	4/5 (80%)
D17S579	3/7 (43%)	1/10 (10%)	2/4 (50%)
D17S785	3/8 (37.5%)	4/6 (66.5%)	3/4 (75%)
D17S791	0/8	2/7 (28.5%)	1/4 (25%)

Table 2 *LOH frequency in the epithelial cells*

Marker	Normal epithelium	DCIS	IDC
TPO	1/6 (16.5%)	1/9 (11%)	1/3 (33%)
D3S1067	0/6	5/11 (45.5%)	1/4 (25%)
D3S1300	0/5	3/8 (37.5)	1/4 (25%)
D3S1581	0/7	5/10 (50%)	1/4 (25%)
D3S2432	0/6	0/11	0/5
D3S1766	0/6	1/10 (10%)	0/3
D11S1311	0/8	1/11 (9%)	0/5
D16S402	0/6	7/8 (87%)	3/3 (100%)
D16S518	2/8 (25%)	6/10 (60%)	3/3 (100%)
D17S579	0/6	1/10 (10%)	0/3
D17S785	2/5 (40%)	5/8 (62.5%)	3/5 (60%)
D17S791	1/4 (25%)	2/8 (25%)	2/4 (50%)

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 in NE ranged from 16.5% (1/6) to 40% (2/5) of informative cases (Table 2).

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

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Table 3 *Distribution of LOH among the epithelial and mesenchymal components*

Marker	Cases with LOH in either Ep or St/informative cases[a]	Cases with LOH in both Ep and St	Cases with LOH only in Ep	Cases with LOH only in St
TPO	3/9 (33%)	1	0	2
D3S1067	7/11 (64%)	4	0	3
D3S1300	3/9 (33%)	3	0	0
D3S1581	7/10 (70%)	4	1	2
D3S2432	0/11	0	0	0
D3S1766	3/10 (30%)	1	0	2
D11S1311	5/11 (45.5%)	0	1	4
D16S402	6/8 (75%)	5	1	0
D16S518	7/11 (64%)	5	2	0
D17S579	5/10 (50%)	3	1	1
D17S785	7/8 (87.5%)	5	0	2
D17S791	3/8 (37.5%)	0	1	2

[a] Ep, epithelium; St, stroma.

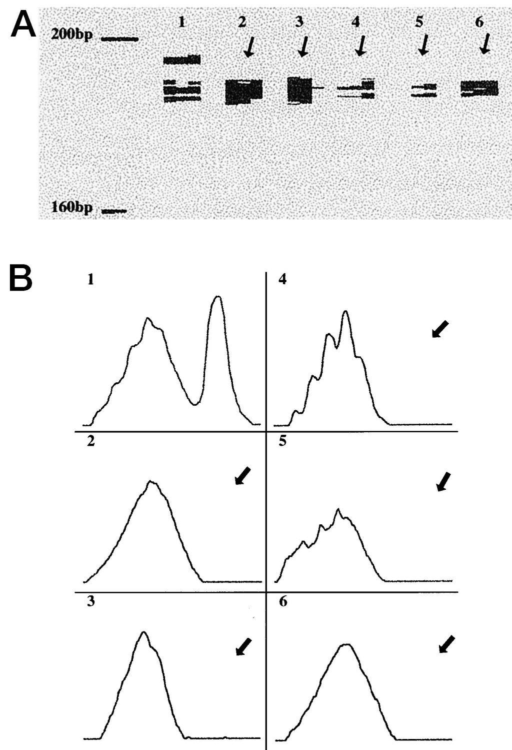

Fig. 2. *A*, LOH on chromosome 17q (*D17S785*; expected size, 181–207 bp) in SC-IDC (*Lane 2*), SC-DCIS (*Lane 3*), clear-cut normal epithelial cells *(Lane 4)*, DCIS *(Lane 5)*, and IDC *(Lane 6)*. *Lane 1*, normal SAD from DCIS or IDC. *B*, graphical (densitometric) representation of lanes seen in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

stromal cells, several cases were associated with loss of certain microsatellite loci exclusively in the stroma (Table 3). Our findings suggest that occasionally certain genetic alterations in the mammary stroma may even precede genotypic changes in the epithelial cells.

Although a dynamic, reciprocal interaction between ectodermal and mesodermal (mesenchymal) cells during embryogenesis is a well-recognized phenomenon (13, 14), a possible role for mesenchymal cells in the evolution of epithelial neoplasms has not been thoroughly investigated. Earlier *in vitro* and *in vivo* studies have demonstrated that the stroma (mesenchyme) not only exerts significant influences on epithelial differentiation (15), cell death (16), proliferation (16, 17), and motility (18), but that it also impacts the metastatic behavior of epithelial neoplasms (18, 19). On the other hand, under certain circumstances, normal mesenchymal cells (fibroblasts) can convert malignant tumors, including prostatic adenocarcinoma (20), basal cell carcinoma of skin (21) or even highly aggressive acute leukemia (22), into morphologically and functionally normal (22), benign (20, 21), or at least biologically less aggressive cell populations (20).

Although the underlying molecular-biological mechanisms for these stromal functions remain speculative, a number of growth factors such as fibroblastic growth factor(s) (23), transforming growth factor (β family; Ref. 24), stem cell factor (25), and hepatocyte growth factor (26) are known to contribute to the morphogenic and mitogenic functions of stromal 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

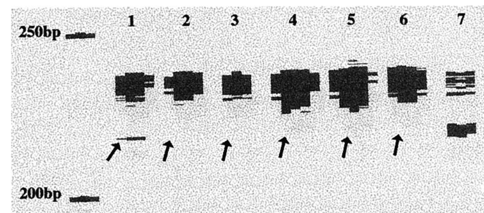

Fig. 3. LOH on chromosome 3p (*D3S1300*; expected size, 217–241 bp) in SAD *(Lane 1)*, SC-IDC *(Lane 2)*, SC-DCIS *(Lane 3)*, epithelium with mild cytological atypia *(Lane 4)*, DCIS *(Lane 5)*, and IDC *(Lane 6)*. *Lane 7*, clear-cut normal epithelium (normal ducts and ductules).

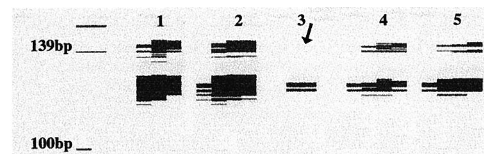

Fig. 4. LOH on chromosome 11q (*D11S1311*; expected size, 127–147 bp) in SC-DCIS *(Lane 3)*. using the polymorphic marker *D11S1311*, normal SAD from DCIS (*Lane 1*), fibrotic SAD from DCIS *(Lane 2)*, morphologically normal epithelial cells *(Lane 4)*, and DCIS *(Lane 5)* are not associated with LOH.

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BRCA1 gene, a TSG that is commonly lost in the familial breast and ovarian carcinomas (38). It is of note that the polymorphic locus *D17S579* showed LOH in the stroma (SAD, SC-DCIS, and SC-IDC), ranging from 10% (1/10) to 50% (2/4) of informative cases (Table 1). The cytogenic locus 16q23.1–24.2 is close to a putative TSG, *CDH1*, 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 teleangiectasia* 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 oncofetal 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 reactivation 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 Hipple-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 reactivation 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 teleangiectasia* 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 mammary 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

1. Hom, Y. K., Young, P., Wiesen, J. F., Miettinen, P. J., Derynck, R., Werb, Z., and Cunha, G. R. Uterine and vaginal organ growth requires epidermal growth factor receptor signaling from stroma. Endocrinology, *139:* 913–921, 1998.
2. Donjacour, A. A., and Cunha, G. R. Stromal regulation of epithelial function. Cancer Treat. Res., *53:* 335–364, 1991.
3. Cunha, G. R., Bigsby, R. M., Cooke, P. S., and Sugimura, Y. Stromal-epithelial interactions in adult organs. Cell Differ., *17:* 137–148, 1985.
4. Ohuchi, H., Nakagawa, T., Yamamoto, A., Araga, A., Ohata, T., Ishimaru, Y., Yoshioka, Y., Kuwana, T., Nohno, T., Yamasaki, M., Itoh, N., and Noji, S. The mesenchymal factor, FGF10, initiates and maintains the outgrowth of the chick limb bud through interaction with FGF8, an apical ectodermal factor. Development, *124:* 2235–2244, 1997.
5. Rohrbach, H., Haas, C. J., Baretton, G. B., Hirschmann, A., Diebold, J., Behrendt, R. P., and Lohrs, U. Microsatellite instability and loss of heterozygosity in prostatic carcinomas: comparison of primary tumors, and of corresponding recurrences after androgen-deprivation therapy and lymph-node metastases. Prostate, *40:* 20–27, 1999.
6. Agapova, L. S., Ivanov, A. V., Sablina, A. A., Kopnin, P. B., Sokova, O. I., Chumakov, P. M., and Kopnin, B. P. P53-dependent effects of RAS oncogene on chromosome stability and cell cycle checkpoints. Oncogene, *18:* 3135–3142, 1999.
7. Smith, H. S., Lu, Y., Deng, G., Martinez, O., Krams, S., Ljung, B. M., Thor, A., and Lagios, M. Molecular aspects of early stages of breast cancer progression. J. Cell. Biochem. Suppl., *17G:* 144–152, 1993.
8. Grooteclaes, M., Vernimmen, D., Plaza, S., Pasleau, F., Hodzic, D., and Winkler-Gol, R. A new *cis* element is involved in the HER2 gene overexpression in human breast cancer cells. Cancer Res., *59:* 2527–2531, 1999.
9. Moinfar, F., Man, Y. G., Bratthauer, G. L., and Tavassoli, F. A. Genetic abnormality in ductal intraepithelial neoplasia of the flat type ("clinging carcinoma *in situ*") of the breast. Cancer, *88:* 2072–2081, 2000.
10. Mooney, E. E., Man, Y. G., Bratthauer, G. L., and Tavassoli, F. A. Evidence that Leydig cells in Sertoli-Leydig cell tumors have a reactive rather than a neoplastic profile. Cancer, *86:* 2312–2319, 1999.
11. O'Connell, P., Pekkel, V., Fuqua, S. A., Osborne, C. K., Clark, G. M., and Allred, D. C. Analysis of loss of heterozygosity in 399 premalignant breast lesions at 15 genetic loci. J. Natl. Cancer Inst., *90:* 697–703, 1998.
12. Kerangueven, F., Noguchi, T., Coulier, F., Allione, F., Wargniez, V., Simony-Lafontaine, J., Longy, M., Jacquemier, J., Sobol, H., Eisinger, F., and Birnbaum, D. Genome-wide search for loss of heterozygosity shows extensive genetic diversity of human breast carcinomas. Cancer Res., *57:* 5469–5474, 1997.
13. Ronnov-Jesson, L., Petersen, O. W., and Bissell, M. J. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. Physiol. Rev., *76:* 69–125, 1996.
14. Kedinger, M., Duluc, I., Fritsch, C., Lorentz, O., Plateroti, M., and Freund, J. N. Intestinal epithelial-mesenchymal cell interactions. Ann. NY Acad. Sci., *859:* 1–17, 1998.
15. Kleinman, H. K., Graf, J., and Iwamoto, I. Role of basement membranes in cell differentiation. Ann. NY Acad. Sci., *513:* 134–145, 1987.
16. Kratochwil, K. The stroma and the control of cell growth. J. Pathol., *149:* 23–24, 1986.
17. Sialg, P., Coulomb, B., Lebreton, C., Bell, E., and Dubertret, L. Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model *in vitro*. Science (Washington DC), *230:* 669–672, 1985.
18. Nakamura, T., Matsumoto, K., Kiritoshi, A., Tano, Y., and Nakamura, T. Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: *in vitro* analysis of tumor-stromal interactions. Cancer Res., *57:* 3305–3313, 1997.
19. Beviglia, L., Matsumoto, K., Lin, C. S., Ziober, B. L., and Kramer, R. H. Expression of the c-met/HGF receptor in human breast carcinoma: correlation with tumor progression. Int. J. Cancer, *74:* 301–309, 1997.
20. Hayashi, N., and Cunha, G. R. Mesenchyme-induced changes in the neoplastic characteristics of the Dunning prostatic adenocarcinoma. Cancer Res., *51:* 4924–4930, 1991.
21. Cooper, M., and Pinkus, H. Intrauterine transplantation of rat basal cell carcinoma as a model for reconversion of malignant to benign growth. Cancer Res., *37:* 2544–2552, 1977.
22. Fibach, E., Landau, T., and Sachs, L. Normal differentiation of myeloid leukaemic cells induced by a differentiation-inducing protein. Nat. New Biol., *237:* 276–278, 1972.
23. Giri, D., Ropiquet, F., and Ittmann, M. Alterations in expression of basic fibroblast growth factor (FGF) 2 and its receptor FGFR-1 in human prostate cancer. Clin. Cancer Res., *5:* 1063–1071, 1999.
24. Akhurst, R. J., and Balmain, A. Genetic events and the role of TGF-β in epithelial tumor progression. J. Pathol., *187:* 82–90, 1999.
25. Nishida, K., Tsukamoto, T., Uchida, K., Takahashi, T., and Ueda, R. Introduction of the c-*Kit* gene leads to growth suppression of a breast cancer cell line, MCF-7. Anticancer Res., *16:* 3397–3402, 1996.
26. Kasai, S., Sugimura, K., Matsumoto, K., Nishi, N., Kishimoto, T., and Nakamura, T. Hepatocyte growth factor is a paracrine regulator of rat prostate epithelial growth. Biochem. Biophys. Res. Commun., *228:* 646–652, 1996.
27. Takahashi, M., Ota, S., Shimada, T., Hamada, E., Kawabe, T., Okudaira, T., Matsumura, M., Kaneko, N., Terano, A., Nakamura, T., and Omata, M. Hepatocyte

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growth factor is the most potent endogenous stimulant of rabbit gastric epithelial cell proliferation and migration in primary culture. J. Clin. Investig., *95:* 1994–2003, 1995.

28. Klint, P., and Claesson-Welsh, L. Signal transduction by fibroblast growth factor receptors. Front. Biosci., *4:* D165–D177, 1999.
29. Fukumura, D., Xavier, R., Sugiura, T., Chen, Y., Park, E. C., Lu, N., Selig, M., Nielsen, G., Taksir, T., Jain, R. K., and Seed, B. Tumor induction of VEGF promoter activity in stromal cells. Cell, *94:* 715–725, 1998.
30. Noel, A., Hajitou, A., L'Hoir, C., Maquoi, E., Baramova, E., Lewalle, J. M., Remacle, A., Kebers, F., Brown, P., Calberg-Bacq, C. M., and Foidart, J. F. Inhibition of stromal matrix metalloproteinases: effects on breast tumor promotion by fibroblasts. Int. J. Cancer, *76:* 267–273, 1998.
31. Wolf, C., Rouyer, N., Lutz, Y., Adida, C., Loriot, M., Bellocq, J. P., Chambon, P., and Basset, P. Stromelysin 3 belongs to a subgroup of proteinases expressed in breast carcinoma fibroblastic cells and possibly implicated in tumor progression. Proc. Natl. Acad. Sci. USA, *90:* 1843–1847, 1993.
32. Pierce, G. B., and Speers, W. C. Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. Cancer Res., *48:* 1996–2004, 1988.
33. Kalikin, L. M., George, R. A., Keller, M. P., Bort, S. , Bowler, N. S., Law, D. J., Chance, P. F., and Petty, E. M. An integral physical and gene map of human distal chromosome 17q24-proximal 17q25 encompassing multiple disease loci. Genomics, *57:* 36–42, 1999.
34. Heimann, P., Devalck, C., Debusscher, C., Sariban, E., and Vamos, E. Alveolar soft-part sarcoma: further evidence by FISH for the involvement of chromosome band 17q25. Genes Chromosomes Cancer, *23:* 194–197, 1998.
35. Pedeutour, F., Simon, M. P., Minoletti, F., Barcelo, G., Terrier-Lacombe, M. J., Combemale, P., Sozzi, G., Ayraud, N., and Turc-Carel, C. Translocation, t(17;22; q22;q13), in dermatofibrosarcoma protuberans: a new tumor-associated chromosome rearrangement. Cytogenet. Cell Genet., *72:* 171–174, 1996.
36. Lothe, R. A., Karhu, R., Mandahl, N., Mertens, F., Saeter, G., Heim, S., Borresendale, A. L., and Kallioniemi, O. P. Gain of 17q24-qter detected by comparative genomic hybridization in malignant tumors from patients with von Recklinghausen's neurofibromatosis. Cancer Res., *56:* 4778–4781, 1996.
37. Steeg, P. S., Clare, S. E., Lawrence, J. A., and Zhou, Q. Molecular analysis of premalignant and carcinoma *in situ* lesions of the human breast. Am. J. Pathol., *149:* 733–738, 1996.
38. Narod, S., Feunteun, J., and Lynch, H. T. Familial breast-ovarian cancer locus on chromosome 17q12–q23. Lancet, *338:* 82–83, 1991.
39. Latil, A., Cussenot, O., Fournier, G., Driouch, K., and Lidereau, R. Loss of heterozygosity at chromosome 16q in prostate adenocarcinoma: identification of three independent regions. Cancer Res., *57:* 1058–1062, 1997.
40. Chen, T., Sahin, A., and Aldaz, M. Deletion map of chromosome 16q in ductal carcinoma *in situ* of the breast: refining a putative tumor suppressor gene region. Cancer Res., *56:* 5605–5609, 1996.
41. Hiraguri, S., Godfrey, T., Nakumara, H., Graff, J., Collins, C., Shayesteh, L., Doggett, N., Johnson, K., Wheelock, M., Herman, J., Baylin, S., Pinkel, D., and Gray, J. Mechanisms of inactivation of E-cadherin in breast cancer cell lines. Cancer Res., *58:* 1972–1977, 1998.
42. Siprashvili, Z., Sozzi, G., Barnes, L. D., McCue, P., Robinson, A. K., Eryomin, V., Sard, L., Tagliabue, E., Greco, A., Fusetti, L., Schwartz, G., Pierotti, M. A., Croce, C. M., and Huebner, K. Replacement of Fhit in cancer cells suppresses tumorigenicity. Proc. Natl. Acad. Sci. USA, *94:* 13771–13776, 1997.
43. Man, S., Ellis, I. O., Sibbering, M., Blamey, R. W., and Brook, J. D. High levels of allele loss at the FHIT and ATM genes in non-comedo ductal carcinoma *in situ* and grade I tubular invasive breast cancers. Cancer Res., *56:* 5484–5489, 1996.
44. Hinorhara, S., Satake, N., Sekine, K., and Kaneko, Y. Abnormalities of the FHIT transcripts in osteosarcoma and Ewing sarcoma. Jpn. J. Cancer Res., *89:* 887–894, 1998.
45. Laake, K., Odegard, A., Anderson, T. I., Bukholm, J. K., Karesen, R., Nesland, J. M., Ottestad, L., Shiloh, Y., and Borresen-Dale, A. L. Loss of heterozygosity at 11q23.1 in breast carcinomas: indication for involvement of a gene distal and close to ATM. Genes Chromosomes Cancer, *18:* 175–180, 1997.
46. Antecol, M. H., Darveau, A., Sonenberg, N., and Mukherjee, B. Altered biochemical properties of actin in normal skin fibroblasts from individuals predisposed to dominantly inherited cancers. Cancer Res., *46:* 1867–1873, 1986.
47. Durning, P., Schor, S. L., and Sellwood, R. A. S. Fibroblasts from patients with breast cancer show abnormal migratory behaviour *in vitro*. Lancet, *2:* 890–892, 1984.
48. Azzarone, B., Pedulla, D., and Romanzi, C. A. Spontaneous transformation of human skin fibroblasts derived from neoplastic patients. Nature (Lond.), *262:* 74–75, 1976.
49. Azzarone, B., Mareel, M., Billard, C., Scemama, P., Chaponnier, C., and Macieira-Coelho, A. Abnormal properties of skin fibroblasts from patients with breast cancer. Int. J. Cancer, *33:* 759–764, 1984.
50. Abrahams, P. J., Houweling, A., and van der Eb, A. J. High levels of enhanced reactivation of herpes simplex virus in skin fibroblasts from various hereditary cancer-prone syndromes. Cancer Res., *52:* 53–57, 1992.
51. Schor, S. L., and Schor, A. M. Clonal heterogeneity in fibroblast phenotype: implications for the control of epithelial-mesenchymal interactions. BioEssays, *7:* 200–204, 1987.
52. Uria, J. A., Adida, C., Backdahl, M. S., Seiki, M., Fueyo, A., and Lopez-Otin, C. Regulation of collagenase-3 expression in human breast carcinomas is mediated by stromal-epithelial cell interactions. Cancer Res., *57:* 4882–4888, 1997.

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