**Breast Stroma Plays a Dominant Regulatory Role in Breast Epithelial Growth and Differentiation: Implications for Tumor Development and Progression**

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**Abstract**

Although growth factors and extracellular matrix (ECM) are recognized as important contributors to breast epithelial growth, morphogenesis, hormone responsiveness, and neoplastic progression, the influence of functional interactions between breast stromal and epithelial cells on these processes has not been defined. Using a novel three-dimensional cell-cell interaction model, we have compared the abilities of different mesenchymal cell types, including breast fibroblasts derived from reduction mammoplasty and tumor tissues, and human umbilical endothelial cells (HUVECs) to induce three-dimensional morphogenesis and growth of normal MCF10A and preneoplastic MCF10AT1-EIII8 (referred as EIII8) human breast epithelial cells. Our data demonstrate a requirement for organ-specific fibroblasts in the induction of epithelial morphogenesis. Whereas inclusion of normal reduction mammoplasty fibroblasts inhibit or retard morphological conversion and growth of MCF10A and EIII8 cells, respectively, tumor-derived breast fibroblasts evoke ductal-alveolar morphogenesis of both MCF10A and EIII8 cells. The growth and morphogenesis inhibitory effects of normal fibroblasts remain even in the presence of estrogen because they are able to suppress the estrogen-induced growth of EIII8 cells, whereas tumor fibroblasts support and maintain estrogen responsiveness of EIII8 cells. The inductive morphogenic effects of tumor fibroblasts on EIII8 cells is further augmented by the inclusion of HUVECs because these cocultures undergo a dramatic increase in proliferation and branching ductal-alveolar morphogenesis that is accompanied by an increase in invasion, degradation of coincident ECM, and expression of MMP-9. Therefore, tumor fibroblasts confer morphogenic and mitogenic induction of epithelial cells, and further enhancement of growth and progression requires active angiogenesis. These data illustrate the importance of structural and functional interactions between breast stromal and epithelial cells in the regulation of breast epithelial growth and progression.

**Introduction**

Reciprocal cellular interactions between epithelial and stromal cells have been demonstrated as a key determinant in the morphogenesis, proliferation, and cytodifferentiation of both endocrine and nonendocrine target organs (1–3). Carcinomas of the breast are composed of not only tumor epithelial cells but also of infiltrating endothelial cells, fibroblasts, macrophages, and lymphocytes (4). The stroma provides vascular supply and specific soluble and ECM molecules that are required for tumor growth and progression (5). Several lines of evidence indicate that stromal cells play a central role via ECM remodeling in tumor invasion and dissemination (6–8). However, a recent report has shown that stromal alteration(s) precede the malignant conversion of tumor cells (9).

Because commitment to the morphogenetic and differentiation programs requires the establishment of intercellular communication between breast stromal and epithelial cells, we have established a novel three-dimensional cell-cell interaction model to study the molecular and cellular basis of epithelial-fibroblast-endothelial cell interactions. In this experimental system, we have compared the abilities of specific mesenchymal cell types and HUVECs to induce three-dimensional morphogenesis and growth of normal-behaving MCF10A and preneoplastic MCF10AT1-EIII8 human breast epithelial cells. Our results not only demonstrate a requirement for breast-specific fibroblasts but also show the dominant manner by which normal (reduction mammoplasty) and tumor-derived breast fibroblasts suppress or induce, respectively, growth and ductal-alveolar morphogenesis of MCF10A and MCF10AT1-EIII8 breast epithelial cells.

**Materials and Methods**

**Cell Lines and Primary Cultures.** The MCF10AT system is a xenograft model of progressive human proliferative breast disease in which the progression of a T24-Ha-ras transformed derivative of MCF10A (10), i.e., MCF10AneoT (11) can be followed in immunodeficient mice from a histologically precancerous stage to development of frank invasive carcinoma (12). MCF10AneoT and lines derived by alternating in vivo transplantation and in vitro culture (MCF10ATn) are collectively known as the MCF10AT system (13). The lesions formed by lines of the MCF10AT system are composed of a heterogeneous spectrum of ductular tissues with a range of morphology as seen in human breast tissues that include mild hyperplasia, moderate hyperplasia, atypical ductal hyperplasia, carcinoma in situ, and moderately differentiated and undifferentiated carcinoma, as well as histologically normal ducts. The present studies used parental MCF10A cells and MCF10AT1-EIII8 cells (14). The latter, referred to as EIII8 cells, are breast preneoplastic epithelial cells that were derived from MCF10AT1 xenografts arising in E2-supplemented animals (15) and respond to E2 with increased growth (14). MCF10A and EIII8 cells were maintained in phenol red-free DMEM-F12 medium supplemented with 0.1 μg/ml cholera toxin, 10 μg/ml insulin, 0.5 μg/ml hydrocortisone, 0.02 μg/ml EGF, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2.5% horse serum. Charcoal-stripped serum was not used because it reduced both the viability and proliferative capacity of MCF10A cells. Thus, the only sera used routinely were those that were unable to support the growth of the estrogen-dependent cell line MCF-7, indicating the absence of biologically significant levels of E2 or other estrogenic compounds.

HUVECs purchased from ATCC at passage 13 were maintained in SFM (Life Technologies, Inc., Grand Island, NY) supplemented with 10 ng/ml EGF, 20 ng/ml bFGF, and 10 μg/ml fibronectin. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2 in air and cultured up to 15 passages.

**Primary cultures of human breast fibroblasts were established from reduction mammoplasty tissues, tumors, and benign tissues ipsilateral but distal to the tumors acquired after protocol review and approval by the Wayne State University human investigation committee (16). Tumors, confirmed by histol-
ogy, were finely minced and digested overnight with collagenase (150 IU/ml) in DMEM/Ham’s F-12 medium (1:1) containing 20% calf serum. Larger pieces were allowed to settle, and cells in the supernatant, predominantly fibroblasts, were recovered by centrifugation and plated in Waymouth’s MB752 supplemented with 10 mmol/liter HEPES and 15% FCS (FIB; Ref. 16). Reduction mammoplasty and benign tissues were digested as above with the addition of hyaluronidase (100 IU/ml) and were further processed as described for tumors. Portions of reduction mammoplasty and benign tissues used for culture were examined histologically to confirm the absence of neoplasia. Fibroblasts were routinely cultured up to 15 passages and were used at passages 4 to 6. Fibroblasts were characterized immunocytochemically with monoclonal antibodies to pan cytokeratin (Dako Corp., Carpintaria, CA), cytokeratin 14 (Novacastra Laboratories, Ltd., Newcastle upon Tyne, United Kingdom), and vimentin (V9; Dako Corp.) as described in Ref. 16. Normal human lung fibroblasts (IMR-90) and the mesenchymally derived malignant human fibrosarcoma cell line HT1080 were obtained from ATCC and maintained in Eagle’s MEM supplemented with 2 mm l-glutamine, 0.1 mm non-essential amino acids, 1.0 mm sodium pyruvate, and 10% fetal bovine serum. The NIH 3T3 mouse embryo fibroblast line was purchased from ATCC and maintained in DMEM supplemented with 4 mm glutamine, 4.5 g/liter glucose, 1.0 mm sodium pyruvate, and 10% FCS.

Homotypic and Heterotypic Three-Dimensional Basement Membrane Culture of Epithelial, Fibroblast, and Endothelial Cells. Homotypic three-dimensional cultures were set up as described in Ref. 14. Briefly, 10 × 10⁴ cells were seeded as a single-cell suspension in eight-well chamber slides coated with Matrigel (Collaborative Biomedical Products, Bedford, MA) in DMEM-F12-supplemented medium for MCF10A/EIII8 cells, in SFM-supplemented medium for HUVECs, in FIB medium for human breast fibroblasts, or in the appropriate medium for IMR-90, HT1080, or NIH 3T3 cells as described above. For heterotypic cocultures, 50 × 10⁴ MCF10A or EIII8 cells were mixed with an equal number of human breast fibroblasts (reduction mammoplasty, benign or tumor-derived), IMR-90, HT1080, or NIH 3T3 cells. For heterotypic tricultures, MCF10A or EIII8 cells were mixed with equivalent numbers of human breast fibroblasts (reduction mammoplasty, benign or tumor-derived) and HUVECs and seeded onto chamber slides coated with Matrigel as described for homotypic cultures. Typically, heterotypic cultures were performed in SFM supplemented with EGF and bFGF because it allows optimal viability, growth, and three-dimensional organization of HUVECs, fibroblasts, and MCF10A cells. Heterotypic cocultures were routinely maintained for up to 3 weeks, and morphological development was analyzed by phase-contrast microscopy.

Quantitation of Effects of Interaction of Preneoplastic Breast Epithelial Cells with Reduction Mammoplasty/Tumor-derived Breast Fibroblasts and Endothelial Cells on Three-Dimensional Growth. Three-dimensional cultures were set up by seeding 50 × 10⁴ EIII8, HUVECs, 31R or 14R (reduction mammoplasty fibroblasts), and 38T or 12T (tumor-derived breast fibroblasts) either alone or by mixing 50 × 10⁴ EIII8 cells with an equivalent number of 31R (or 14R) or 38T (or 12T) in the absence or presence of an molar excess of ICI 182,780.

Results

Heterotypic Coculture of Reduction Mammoplasty or Tumor-derived Fibroblasts with MCF10A or EIII8 Cells Cause Dominant Growth Inhibitory and Growth Stimulatory Effects, Respectively, on Three-Dimensional Growth of Human Breast Epithelial Cells. We compared the effects of normal fibroblasts and tumor-derived human breast fibroblasts to initiate, support, and maintain three-dimensional organization and growth of MCF10A and EIII8 cells. Homotypic culture of MCF10A or EIII8 cells produce tubular networks (Fig. 1A, panels A and E; Ref. 14). In contrast, MCF10A or EIII8 cells, seeded on Matrigel in contact-dependent cocultures with fibroblasts derived from reduction mammoplasty or breast tumor tissues, produce sealed structures consisting of centrally located fibroblasts with epithelial buds emerging from the central fibroblast core (Fig. 1A and B). When cocultured with MCF10A or EIII8 cells, reduction mammoplasty fibroblasts 31R or 14R retain their capacity for chemotaxis but are unable, or only weakly able, to support growth and differentiation of MCF10A and EIII8 cells, respectively (Fig. 1B, panels B and F). In contrast, cocultures of MCF10A or EIII8 cells with 12T tumor-derived (or 12B benign), or 38T tumor-derived (or 38B benign) breast fibroblasts, besides concentrating cells by chemotaxis, induce growth and ductal-alveolar morphogenesis of both MCF10A and EIII8 cells (Fig. 1B, panels A, C, and E). However, a principal difference between the MCF10A and EIII8 cultures is the formation of a larger number of highly proliferative, ductal-alveolar units with EIII8 cells as compared with those formed by MCF10A cells (Fig. 1B, compare panel A with C and E). These results indicate major differences in the ability of fibroblasts derived from reduction mammoplasty versus benign (data not shown) or tumor breast tissues to support the growth and morphogenesis of normal and preneoplastic breast epithelial cells.

Inclusion of HUVECs into MCF10A cocultures with fibroblasts from either reduction mammoplasty or benign/tumor tissues (Fig. 1A, panel C, and Fig. 1B, panel B), or EIII8 cocultures with reduction mammoplasty fibroblasts (Fig. 1A, panel G) failed to alter the growth characteristics induced by fibroblasts. In contrast, the inclusion of HUVECs into EIII8 cocultures with fibroblasts from benign/tumor tissues (12B, 12T, 38B, or 38T) resulted in a dramatic (Fig. 1B, panels D and F) induction of branching ductal-alveolar morphogenesis. These results are consistent with our previous data that showed that,
unlike normal MCF10A cells, preneoplastic EIII8 cells have the capacity to support endothelial survival and growth (14). Similar cocultures set up with HT1080 fibrosarcoma cells, normal IMR-90 lung cells, or embryonal NIH 3T3 fibroblasts failed to elicit similar effects on breast epithelial growth and morphogenesis as those induced by human breast fibroblasts (data not shown).

Quantitative Evaluation of the Effects of Fibroblasts and Endothelial Cells on Three-Dimensional Growth of EIII8 Cells, and EIII8 Responsiveness to Estrogen. Because the results from Fig. 1, A and B, revealed remarkable differences in the abilities of fibroblasts from reduction mammoplasty and breast tumor tissues to mediate three-dimensional organization and morphogenesis of EIII8 cells and further induction by endothelial cells, we measured cell growth in homotypic (EIII8, 31R, 38T, HUVEC) and heterotypic cultures containing fibroblasts derived from reduction mammoplasty. ×100, panels A, E, H); bar, 40 μm (panels B, C, D, F, G). b, phase-contrast morphology of heterotypic three-dimensional Matrigel cultures containing breast tumor-derived fibroblasts in the presence or absence of HUVECs. Panels A and B, MCF10A+38T and MCF10A+38T+HUVEC, respectively; panels C and D, EIII8+38T and EIII8+38T+HUVEC, respectively; panels E and F, EIII8+12T and EIII8+12T+HUVEC, respectively. There is a difference in the morphologies of the three-dimensional structures formed between MCF10A and EIII8 cells with tumor-derived fibroblasts, and, in addition, there is a dramatic increase in proliferation and branching ductular-alveolar morphogenesis induced in EIII8–38T and EIII8–12T cultures upon inclusion of HUVECs. Bar, 40 μm.

Unlike normal MCF10A cells, preneoplastic EIII8 cells have the capacity to support endothelial survival and growth (14). Similar cocultures set up with HT1080 fibrosarcoma cells, normal IMR-90 lung cells, or embryonal NIH 3T3 fibroblasts failed to elicit similar effects on breast epithelial growth and morphogenesis as those induced by human breast fibroblasts (data not shown).

Because EIII8 cells respond to E2 treatment with pronounced growth enhancement (14), we evaluated the effects of 17 β-E2 on growth of EIII8-fibroblast-endothelial triculures to determine whether maintenance of close contact with breast fibroblasts will influence responsiveness of EIII8 cells to E2. Treatment of EIII8 triculures containing reduction mammoplasty fibroblasts with either 1 or 10 nM E2 failed to stimulate growth (Fig. 2A). Similar treatment of EIII8 triculures containing 38T tumor-derived breast fibroblasts invoked only a marginal increase in growth (P < 0.03) that was apparent only with 10 nM E2 (Fig. 2B). However, the inductive effects
of exogenous E2 became more obvious on addition of the pure estrogen antagonist ICI 182,780 because it significantly inhibited ($P < 0.001$) growth in both vehicle- and E2-treated cultures (Fig. 2B). Because no E2-mediated stimulatory effects were observed in EIII8 cocultures with reduction mammaplasty fibroblasts, these results suggest that the E2-induced growth effects observed in vehicle-treated EIII8–38T-HUVEC tricultures were not caused by contaminating estrogen or estrogenic compounds in the phenol red-free culture media but probably originated from the tumor-derived fibroblasts. From these data, it can be inferred that breast fibroblasts play a dominant role in the maintenance and induction of epithelial growth and morphogenesis: on the one hand, fibroblasts from normal breast tissues exert growth inhibitory effects that can override genetic constraints imposed by preneoplastic breast epithelial cells whereas, on the other hand, tumor-derived breast fibroblasts exercise growth stimulatory effects that can overcome or augment the genetic constraints imposed by normal or preneoplastic breast epithelial cells, respectively.

Contact-dependent Existence With Tumor-derived Breast Fibroblasts Facilitate Structural and Functional Organization That Is Reminiscent of the in Vivo Situation. The results seen in Figs. 1 and 2 demonstrate that benign- or tumor-derived breast fibroblasts exert inductive branching morphogenic effects on preneoplastic EIII8 cells that is further enhanced by the presence of endothelial cells. Histological analysis of H&E-stained paraffin-embedded EIII8 cocultures revealed the presence of a central core of fibroblasts from which, and around which, branching epithelial buds emerge (Figs. 3 and 4) confirming the data from whole-mount (Fig. 3A) and phase-contrast microscopy (Fig. 1, A and B). Further confirmatory biochemical evidence for the localization and functionality of epithelial, fibroblast, and endothelial components was obtained by immunohistochemical analysis of expression of relevant markers: cytokeratins and muc-1 (epithelial), vimentin (fibroblast), cd31, Factor VIII, VEGF receptor-2, and $\alpha$, $\beta_3$ integrin (endothelial cells). Results in Fig. 3 show that the majority of the cytokeratin-positive muc-1-expressing epithelial cells (Fig. 3, F and G) are organized as buds around the central vimentin-positive fibroblast core (Fig. 3H), whereas a small fraction of epithelial cells are retained in the stromal core (Fig. 3, F and G). This residual epithelial cell population may represent “fragments of ducts emerging from the core” or cells that did not participate in the organization process. The epithelial structures exhibit pronounced branching and increased potential to invade and degrade the coincident ECM in EIII8 cocultures containing endothelial cells (Fig. 4A). This ability to degrade the ECM is consistent with the strong reactivity to MMP-9 antibody in the immediate vicinity of epithelial buds in EIII8–12T-HUVEC tricultures (Fig. 4, E and F). The ability of epithelium to invade and proteolytically degrade the surrounding ECM requires the presence of endothelial cells because EIII8 cocultures, containing only benign- or tumor-derived fibroblasts, lack this property (data not shown). It is interesting to note that the central fibroblast core in EIII8 cocultures is nonproliferating in contrast to the high Ki-67 labeling of epithelial buds (Fig. 3E), which suggests that the metabolically active yet nonproliferating fibroblasts are sufficient to serve as a structural platform for maintenance and support of epithelial survival, growth, and morphogenesis (Fig. 3). An interesting feature observed in the EIII8 tricultures is the establishment of contact between epithelial, fibroblast, and endothelial cells that is most suitable for paracrine interaction. In EIII8–12T-HUVEC tricultures, the cd31-positive, factor-VIII-expressing endothelial cells are situated in the central stromal core but predominantly close to the base of the branching epithelial buds (Fig. 4, A–D). These vascular units are functional because they not only express factor VIII but are also positive for the endothelial-specific VEGF receptor-2 (Flk-1) and $\alpha$, $\beta_3$ integrin, markers that are indicative of active angiogenesis (Fig. 4, H and I). The maintenance of endothelial cell survival and growth appears to be ensured by large amounts of VEGF expressed and secreted by EIII8 epithelium because intense immunoreactivity to VEGF antibody is observed in the epithelial compartment, whereas only focal immunostaining to VEGF antibody (which is similar to staining patterns of Flk-1 and $\alpha$, $\beta_3$ integrin) is observed in the central stromal core (Fig. 4G).

Discussion

Several studies have shown that stroma is a key determinant of epithelial proliferation (18), cell death (19), motility (20), and differentiation (21). In our present study, we have established a novel three-dimensional culture system in which human breast epithelial cells, breast fibroblasts and endothelial cells, seeded as a mixed single cell-suspension on a reconstituted basement membrane matrix, retain their inherent ability to segregate (or compartmentalize) and organize in a three-dimensional manner with formation of a central stromal core composed of fibroblasts and functional endothelial cells from and...
around which epithelial buds emerge. This bears resemblance to the in vivo situation because, in the breast, terminal ductules or acini are set within a rich and specialized stroma that define the lobular unit. This lobular connective tissue is usually loose, possesses many capillaries, and is sharply demarcated from the surrounding fat and more dense fibrous tissue of the structural rather than the functional portion of the breast (22). Our coculture assay system bears physiological relevance because not only does it permit the mammary epithelial cells to exhibit a homotypic affinity for itself and form an interface with adjacent stromal core or compartment as observed in vivo but it also facilitates an arrangement, observed in vivo, that is most suitable for functional paracrine interactions between epithelial-fibroblast and epithelial-endothelial cells. It must be noted that, although the structural and functional organization observed in this assay system mimics several characteristics of the mammary gland, it is representative not of normal mammary gland morphology but rather of alterations occurring during early breast cancer.

Our data also show that only breast fibroblasts, but not soft tissue sarcoma-derived malignant mesenchymal cells, normal lung, or embryonal 3T3 fibroblasts, confer morphogenesis-inducing effects. Thus, although the cultures are performed in Matrigel, a reconstituted basement membrane matrix containing a variety of ECM molecules (23), induction of breast epithelial growth and morphogenesis appears to be mediated by the ECM that is laid down by organ-specific mesenchyme. The ability of normal fibroblasts to convert or revert malignant tumor lines such as basal cell carcinoma of skin (24) and prostatic adenocarcinoma (25) into morphologically benign (24, 25) or biologically less aggressive cell populations has been demonstrated (25). Our data show that, although normal reduction mammoplasty fibroblasts can inhibit or retard morphological transformation of both normal MCF10A and preneoplastic EIII8 breast epithelial cells, respectively, tumor-derived breast fibroblasts can override or augment genetic constraints imposed by MCF10A or EIII8 cells, respectively, causing them to undergo ductal-alveolar morphogenesis. This difference in the activities between normal and tumor-derived fibroblasts cannot be accounted for by variations in the ages of cells in culture, because both sets of fibroblasts grew equally well at the passages four to six used for coculture experiments. Although the normal and tumor-derived fibroblasts were derived from donors with mean ages of 30 and 48 years, respectively, deviations in the activities of the fibroblasts cannot be ascribed to differences in age because both normal and tumor-derived fibroblasts exhibited sample-to-sample variations in growth rates that overwhelmed any age-related differences in growth.

Alterations in fibroblasts in the stroma immediately adjacent to transformed epithelial cells have been documented in several tumor systems (26, 27). Desmoplasia, a profound stromal response consisting of changes in cellular composition and ECM, is observed in infiltrating ductal carcinoma (28). Differences in the activity of normal versus tumor-derived fibroblasts may result from variations in the establishment of reciprocal communication between epithelial and fibroblast compartments, which, in turn, could result from differences in molecular and/or cellular mechanisms that are responsible for the production and release of a number of soluble paracrine factors such as FGF(s) (29), transforming growth factor β (30), IGFs (27), and hepatocyte growth factor (31), and/or the ability to respond to epithelial-derived signals. Although, potentially, any of the growth factors derived from breast fibroblasts may function as an important regulator of mammary tumor growth, IGFs, and IGF-II in particular, may play a key role in mediating breast tumor growth because IGF-II expression has been reported in the stroma of invasive breast cancers, but not in normal breast (27), and in the stroma of N-nitrosomethylurea-induced rat mammary tumors (32). Whereas a principal role for the stroma has often been proposed in mediating steroid action during growth and differentiation in many adult tissues (3), our study shows that normal fibroblasts have the ability to suppress E2-induced growth of estrogen receptor-positive EIII8 cells (14), whereas tumor-derived fibroblasts support E2-induced growth of EIII8 cells. Although the addition of 10 nm E2 was required to see a marginal increase in growth over that of control cultures, the E2-induced effect is estrogen receptor-mediated because the addition of the pure estrogen antagonist ICI 182,780 effectively blocked growth in both vehicle-treated and E2-treated cultures. These data suggest that the marginal induction of growth by exogenous estrogen may be attributable to the presence of...
significant levels of E2 in the control cultures that are probably derived from tumor-derived fibroblasts via aromatase-mediated synthesis of estrogen (16, 33, 34).

An alternate possibility for differences between reduction mammoplasty and tumor-derived fibroblasts may be the presence of genetic abnormalities in the background supportive stroma, i.e., the benign tumor fibroblasts. Moinfar et al. (9) have recently reported frequent allelic loss in the stroma close to breast cancer, ranging from 10 to 66.5% for ductal carcinoma in situ and from 20 to 75% for invasive ductal cancers, and the absence of loss of heterozygosity either in epithelial or in stromal components in women without any breast disease (9). Thus, the presence of genetic alterations present in pre-neoplastic EIII8 cells (which is akin to many benign or early epithelial neoplasms), combined with the presence of a genetically altered stroma (such as that which may be present in benign or malignant tumors of the breast), may underlie the acute inductive responses observed in EIII8–12T or EIII8–38T cocultures. These data are further supported by the absence of similar effects in EIII8-reduction mammoplasty fibroblast cocultures and the occurrence of inductive response in MCF10A-tumor fibroblast cocultures. Stromal-epithelial interaction has a fundamental role in determining normal duct development. Schor et al. (35) have suggested that perturbations in epithelial-mesenchymal interactions caused by the presence of fibroblast subpopulations with “fetal-like” phenotypic properties in breast carcinomas and in histologically normal breast tissue adjacent to a carcinoma may significantly enhance the susceptibility of epithelium to develop cancer. Aberrations in stromal-epithelial interactions resulting from differences in the production and release of growth factors, motility factors, and/or ECM molecules that may potentially ensue from genetic alterations in the stromal compartment may play a vital role in the development and progression of human breast cancer.

It is interesting to note that survival and functionality of endothelial cells are observed only in EIII8 cocultures, because the addition of HUVECs to EIII8–12T or EIII8–38T cocultures resulted in dramatic augmentation of proliferation and branching ductal-alveolar morphogenesis that was accompanied by an increase in invasion and degradation of the coincident ECM and up-regulation of MMP-9. These data are consistent with our previous report (14) that the maintenance of endothelial cell survival and function is promoted by preneoplastic, but not by normal, epithelial cells because the former responds to the presence of endothelial cells with the synthesis and release of the endothelial cell survival factor VEGF (14, 36, 37). These data imply that tumor fibroblasts play an active role as a morphogenic and mitogenic inducer of epithelial cells; however, further enhancement of tumor growth and progression requires the establishment of active angiogenesis.

Our data suggest that therapeutic targeting of stromal cells rather than (or as well as) cancer cells may be beneficial because they appear to have a dominant capacity to modulate and control epithelial morphogenesis and mitogenesis. The inhibition of tumor stroma may result in more efficient tumor regression and treatment by depriving the tumor cells of an essential structural and functional support system. In summary, we have established a novel coculture system that reconstitutes many of the functional interactions between breast...
epithelial and stromal cells. This assay system will provide us with a unique opportunity to characterize mechanisms regulating breast stromal-epithelial cell interactions.

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**References**


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