Breast Cancer Subtype-Specific Interactions with the Microenvironment Dictate Mechanisms of Invasion

Tuyen T. Dang, Amanda M. Prechtl, and Gray W. Pearson

Abstract

Most ductal breast carcinoma cells are weakly invasive in vitro and in vivo, suggesting that components of their microenvironment may facilitate a transition from in situ to invasive stages during progression. Here, we report that coculture of mammary fibroblasts specifically triggers invasive behavior in basal-type breast cancer cells through a ligand independent mechanism. When cultured alone in organotypic culture, both basal- and luminal-type breast cancer cells formed noninvasive spheroids with characteristics of ductal carcinoma in situ (DCIS). In contrast, when cocultured with mammary fibroblasts, basal-type spheroids exhibited invasive character whereas the luminal-type spheroids retained a benign and noninvasive duct-like architecture. Real-time imaging and functional studies revealed that the specificity of invasion was linked to a unique capacity of basal-type breast cancer cells to move within spheroids. Mammary fibroblasts induced invasion by triggering basal-type breast cancer cells to convert from a noninvasive program of mammary epithelial morphogenesis to an invasive program of sprouting endothelial angiogenesis. Contrary to the existing invasion models, soluble ligands produced by the fibroblasts were not sufficient to trigger invasion. Instead, basal-type invasion relied upon a Cdc42-dependent reorganization of collagen fibers in the extracellular matrix by fibroblasts. Inhibiting basal-type cell movement with clinically relevant drugs blocked invasion both in organotypic culture and in animals, suggesting a new treatment strategy for early-stage patients. Together our findings establish that fibroblast recruitment by basal-type breast cancer cells into early-stage tumors is sufficient to trigger their conversion from a benign, noninvasive DCIS-like stage to a malignant invasive stage. Furthermore, our findings suggest that different subtypes of breast cancer may require distinct types of contributions from the microenvironment to undergo malignant progression. Cancer Res; 71(21); 6857–66. ©2011 AACR.

Introduction

The progressive loss of epithelial architecture underlies the development of invasive breast cancer (1). Neoplastic cells first accumulate within the lumen of the ductal epithelial bilayer and later migrate out of the ducts and into the breast stroma (2). In addition, the fibrous connective tissue is reorganized around invasive tumors (3), sometimes into linear paths that intersect with blood vessels (4). So, rather than solitary neoplastic cells charting their own distinct paths out of ducts and into blood vessels, invasive tumors contain a structural framework that supports the dissemination of tumor cells on identical paths along collagen tracks (5).

The contribution of tumor cells to the induction of invasion may vary from patient to patient depending on the heterogeneity of primary breast tumors, which can be divided into 5 different subtypes (6). The claudin-low subtype has the characteristics of fibroblasts (7) and can independently accomplish the tasks required for invasion, including traversing a model basement membrane (8) and reorganizing collagen fibers into linear paths for later tumor cells to follow (9). However, the claudin-low subtype accounts only for 10% of diagnosed breast cancers (10). The remaining breast cancer subtypes are poorly invasive when cultured alone (8, 11). Thus, the vast majority of breast cancers likely require nontumor cells in the microenvironment to trigger invasion and create a structural system that supports metastatic dissemination (12, 13). Determining the nature of the cooperation between tumor cells and the microenvironment that leads to invasion could assist in revealing the risk of metastatic recurrence after surgery and may identify therapeutic strategies that could thwart the spread of the disease.

Materials and Methods

Cell culture and reagents

MCFDCIS cells were purchased from Asterand. T47D and MCF7 cells were obtained from the American Type Culture

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Collection. HCC1143, HCC1806, HCC1954, HCC1569, and HCC1428 cells (14) were a gift from Michael Peyton and John Minna [University of Texas Southwestern Medical Center (UTSW)] and validated by Powerplex genotyping before use. Cells were cultured as described (8, 14). Mammary fibroblasts were isolated from both benign breast tissue and invasive ductal breast carcinoma cases treated at UTSW. The tissue samples were minced with a scalpel and digested with 0.25 mg/mL collagenase I overnight in a rotary shaker. Mammary fibroblasts were then separated from the tissue samples by differential centrifugation. The fibroblasts were grown in 10% FBS/DMEM-F12 and used within the 17 passages after initial isolation. Cell lines stably expressing pCLNRX-H2B:GFP and pBABE mCherryCAAX were generated as described (15). pBOB-GFP (16) and PGK-H2BmCherry (17) lentivirus were generated as described (15). Immunofluorescence staining (Falcon) and live imaging (Nunc) as described (15, 18) with the modifications described in the Supplementary Methods. Organotypic cultures were immunostained or imaged on the indicated day of growth.

Quantification of invasion

In all experiments, a spheroid was classified as invasive if at least 3 cells collectively invaded away from a main mass of 15 or more cells. A total of 50 spheroids were counted per condition in 3 independent experiments.

Organotypic culture

In each organotypic culture experiment, the breast cancer cells and fibroblasts were plated in 8-well chamberslides for immunofluorescence staining (Falcon) and live imaging (Nunc) as described (15, 18) with the modifications described in the Supplementary Methods. Organotypic cultures were immunostained or imaged on the indicated day of growth.

Fibroblast conditioned media

Fibroblast conditioned medium was generated by plating fibroblasts in 10% FBS/DMEM-F12. The next day, the fibroblasts were washed twice with PBS and cultured in serum-free DMEM-F12. After 48 hours, the fibroblast conditioned medium was removed and supplemented with 2% Matrigel and 5% horse serum before addition to day 2 organotypic cultures.

Transfection of fibroblasts

A total of 20,000 mammary fibroblasts per well in a 96-well plate were reverse transfected with RNAiMax (Invitrogen), using ON-TARGETplus SMART pools (Thermo Fisher Scientific). Nontargeting control (001810-10-05) or Cdc42 (J-005057-05: CGGAAUAUGUACCGACUGU; J-005057-06: GCAGUCACA-GUAUAUGAU; J-005057-07: GAUGACCCUCUACUAUUG; and J-005057-08: CUGCAAGGCAAGGAGUA) siRNAs were used at a final concentration of 100 nmol/L. Cdc42 targeting was confirmed with 2 individual siRNAs from the siGENOME SMART pool (005057-05 and 005057-07). Mammary fibroblasts were harvested 24 hours after transfection, and 2,500 fibroblasts were re-plated in organotypic culture for analysis of fibroblast-induced reorganization of collagen I (5 days after transfection) or fibroblast-induced tumor cell invasion (7 days after transfection).

Real-time imaging of organotypic cultures, immunofluorescence, and immunoblot analysis

Cultures were imaged with a PerkinElmer Ultraview ERS spinning disk confocal microscope enclosed in a 37°C chamber supplemented with humidified CO2 (Solent) and a CCD camera (Orca AG; Hamamatsu). Western blotting and immunostaining were carried out as described (15). Immunofluorescence images were acquired on a Nikon Eclipse TE 2000-E confocal microscope. For details, see Supplementary Methods.

Xenografts

Female severe combined immunodeficient mice (purchased from an in-house vendor) between 6 and 8 weeks of age were injected in the number 4 fat pad with 50,000 MCFDCIS cells and 200,000 mammary fibroblasts in a 50% Matrigel/DMEM-F12 mixture. Mice were treated daily starting 14 days after fat pad injection with diluent or 25 mg/mL PD0325901 by oral gavage and sacrificed on day 21 with CO2 according to UTSW Institutional Animal Care and Use Committee guidelines. Tumors were removed, fixed in formalin, and embedded in paraffin for immunohistochemistry by standard protocols. Paraffin-embedded tumor slices were stained with the indicated antibodies according to the manufacturer’s protocol.

Results

Fibroblast-induced invasion is dependent on breast cancer intrinsic subtype

Mammary fibroblasts are prominent components of the tumor microenvironment and accumulate around noninvasive ductal carcinoma in situ (DCIS) lesions, which are a direct precursor of invasive breast cancer (1, 19). The ability of mammary fibroblasts to accelerate the transition from DCIS to invasive breast cancer in a xenograft model suggests a functional role for fibroblasts in promoting invasion (20). To determine how fibroblasts induce breast cancer invasion, we developed an organotypic coculture model. Organotypic culture models can be used to study the initial steps of invasion because the breast cancer cells form multicellular spheroids that reconstitute many features of the primary tumor (18). We first investigated the interaction between MCFDCIS cells and mammary fibroblasts to determine whether our organotypic model could recapitulate the induction of invasion that is observed in vivo. Consistent with their behavior in the xenograft model (Fig. 1A), MCFDCIS cells formed multicellular DCIS-like spheroids that were induced to invade by mammary fibroblasts in organotypic culture (Fig. 1B and C and Supplementary Fig. S1). With our organotypic coculture model established, we identified 7 breast cancer cell lines that formed noninvasive spheroids with characteristics of human DCIS and determined whether fibroblasts could induce their invasion. A subgroup of 4 breast cancer cell lines was induced to invade by mammary fibroblasts, indicating that they harbored a unique set of characteristics that permitted fibroblast-induced invasion (Fig. 1D and E). Consistent with this possibility, the 4 invasive breast cancer cell lines had a basal-like mRNA signature whereas the 3 cell lines that were noninvasive when cocultured with fibroblasts were classified as luminal (8, 21).
Our results suggest that fibroblast-induced invasion could contribute to poor clinical outcome of patients with basal-type breast cancer (22).

\textbf{Mammary fibroblasts induce the sprouting invasion of motile neoplastic cells}\n
Although our data indicated that there was a correlation between the basal intrinsic subtype and the ability of fibroblasts to induce invasion, the mechanism of invasion remained unknown. The growth of cells beyond the confines of the basement membrane can occur either through proliferative expansion or through migratory collective invasion (23). To determine whether invasion was driven by proliferative expansion or motile collective invasion, we investigated the behavior of MCFDCIS spheroids and mammary fibroblasts in real time at single-cell resolution. We found that MCFDCIS spheroids contained motile cells that could exchange cell–cell interacting partners while migrating within the duct-like spheroid (Supplementary Movies S1 and S2). These motile cells did not become invasive over time and remained confined within a

\begin{figure}
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\caption{Fibroblasts specifically induce basal-type breast cancer cell invasion. A, MCFDCIS xenografts were removed on day 14 or day 21 of tumor growth. Paraffin-embedded tumor sections were immunostained with anti–smooth muscle actin antibody (brown), which stains the myoepithelial cell layer and mammary fibroblasts, and counterstained with hematoxylin (blue). Representative examples from 8 mice per condition are shown. Scale bars, 200\,\mu m. B, confocal images of organotypic cultures grown for 8 days and immunostained with α-laminin-5 antibody (red) and counterstained with Hoechst (blue, nuclei) are shown. Scale bars, 100\,\mu m. The white arrow identifies an example area of invasion. C, quantification of the frequency of invasion induced by mammary fibroblasts over time in MCFDCIS cocultures. See Materials and Methods for details. Data are the mean ± SD. The quantification includes results with mammary fibroblasts isolated from 3 different patients. No difference in frequency of invasion was observed comparing fibroblasts from matched benign and neoplastic tissues. D, organotypic cultures were grown for 8 days and fixed with formalin. H2B:GFP (HCC1143, HCC1569, and T47D) or H2B:mCherry (HCC1806 and HCC1954) expression in the breast cancer cells or Hoechst staining (MCF7) is shown (nuclei, white). Scale bars, 100\,\mu m. Solid white arrows indicate example areas of invasion. E, quantification of the frequency of invasion induced by mammary fibroblasts. Data are the mean ± SD. **, P < 0.01 versus no fibroblast control by the t test. C, control; MF, mammary fibroblast.}
\end{figure}
laminin-5–based basement membrane (Fig. 1B). This noninvasive motility is similar to the movement that is induced by the activation of the mitogen-activated protein (MAP) kinases extracellular signal-regulated 1/2 (ERK1/2) in MCF10A mammary epithelial spheroids (15, 24) and during the branching morphogenesis of mouse mammary epithelial organoids (25). We term this motility within multicellular lesions "intraspheroid motility" to distinguish it from single-cell migration.

During fibroblast-induced invasion, the MCFDCIS cells remained adherent to each other as the first invasive cell extended away from the spheroid while converting from an orientation of lateral and apical contacts to a tip-to-tail orientation (Fig. 2A and Supplementary Movie S3) analogous to sprouting invasion that occurs during the development of vascular endothelium (26). The leading cell was then followed by additional motile cells from the primary spheroid (Fig. 2B and C, Supplementary Fig. S2, and Supplementary Movies S4, S5, S6, and S7). Sprouting invasion was the mechanism of invasion for all spheroids. We did not observe single cells dissociating from any of the spheroids either during the initiation of invasion or after cells left the spheroids and entered the organotypic stroma. Although single-cell motility requires only directional propulsion (26), our live-imaging experiments revealed that the invading cells were both motile and modulated cell–cell contacts while changing interacting partners, indicating that the main features of intraspheroid motility were directly contributing to invasion (Fig. 2C, Supplementary Fig. S2, and Supplementary Movie S7). Invasion did not correlate with the size of the spheroid, as small lesions could rapidly invade toward fibroblasts (Supplementary Movie S8). These results show that fibroblasts induce motile collective invasion.

**Intraspheroid motility is a unique property of basal-type breast cancer cells**

To determine whether intraspheroid motility was the basis for the selectivity of fibroblast-induced invasion, we imaged spheroids from 2 basal-type and 2 luminal-type cell lines in real time. Figure 2. Mammary fibroblasts induce motile neoplastic cells to collectively invade. A, confocal slices of MCFDCIS-mCherryCAAX cells (plasma membrane, red) cultured alone (left) or with mammary fibroblasts expressing GFP (green, right) are shown at 1.5-hour intervals over 4.5 hours total. The white arrow indicates where the MCFDCIS cell begins sprouting invasion away from the main spheroid. B, confocal slices of MCFDCIS-H2B:mCherry cells (nuclei, red) cultured alone or with mammary fibroblasts are shown at 2-hour intervals over 8 hours total. The white arrow indicates where a MCFDCIS cell begins branching away from the original invasive track of cells. C, confocal slices of MCFDCIS-H2B:mCherry cells (red) cultured alone (top) or with mammary fibroblasts (bottom) are shown at 4.5-hour intervals over 13.5 hours total. The solid white arrow shows a different MCFDCIS cell invading away from the main spheroid forming the existing invasive projection of cells. Scale bar for all panels, 20 μm. The results are representative of more than 30 spheroids imaged per condition over 3 independent experiments.
time. The HCC1806 and HCC1954 spheroids displayed intraspheroid motility, whereas the HCC1428 and T47D spheroids contained proliferating cells that were not motile (Fig. 3A, Supplementary Fig. S3, and Supplementary Movies S9, S10, S11, and S12). When cocultured with fibroblasts, the HCC1954 and HCC1806 spheroids converted to sprouting invasion and migrated toward fibroblasts, identical to results obtained in the MCFDCIS coculture model (Fig. 3B and Supplementary Movies S13, S14, S15, and S16). In contrast, coculturing the HCC1428 or T47D spheroids with fibroblasts did not induce either movement of the breast cancer cells or sprouting invasion (Fig. 3B and Supplementary Movies S17, S18, S19, and S20). Taken together, our findings suggest that the induction of invasion requires intraspheroid motility and that intraspheroid motility is a unique characteristic of basal-type breast cancer cells.

The ability of cells in the basal-type spheroids to move and change cell–cell interacting partners suggested that there was a reduction in the expression of cell–cell adhesion proteins in the basal-type cells, which could serve as biomarkers to identify motile cells with the potential for fibroblast-induced invasion. We analyzed the expression of E-cadherin by Western blotting and found that there was a modest decrease in the expression of E-cadherin in the basal-type HCC1954 and HCC1569 cells compared with the luminal-type HCC1428 cells (Supplementary Fig. S4A and S4B). These results are similar to a previous finding of E-cadherin in the basal-type HCC1954 and HCC1569 cells (8). Although the marginal decrease in E-cadherin expression would likely be difficult to determine by immuno-
histochemical staining of patient samples, reduced E-cadherin expression can correlate with a loss of the majority of E-cadherin at the sites of cell–cell contact (15), which would be detectable. However, no pronounced difference in E-cadherin localization was observed when comparing luminal HCC1428 and basal HCC1954 spheroids (Supplementary Fig. S4C). Furthermore, we found that E-cadherin was expressed in the invading HCC1954 cells and that any detectable E-cadherin remained localized to the sites of cell–cell contact when cocultured with fibroblasts (Supplementary Fig. S4C). Although these results indicate that changes in E-cadherin expression or localization may not be useful tools to predict the risk of fibroblast-induced invasion, it is possible that a modest or transient change in E-cadherin expression at cell–cell contacts occurs specifically in the invading basal-type cells. Consistent with the sustained expression and localization of E-cadherin that was observed, the invading subpopulation of basal-type cells also retained their epithelial character, as indicated by the expression of epithelial cell adhesion molecules (Supplementary Fig. S4D; ref. 27). To confirm these findings in vivo, we immunostained MCFDCIS xenografts and found that E-cadherin was expressed at similar levels and at the sites of cell–cell contact in the DCIS and invasive lesions, indicating that fibroblasts do not trigger invasion through first inducing a reduction or relocalization of E-cadherin in a large fraction of the DCIS cells in vivo (Supplementary Fig. S4E). Taken together, our results indicate that evaluating the expression or localization of E-cadherin or other epithelial differentiation markers will not be sufficient to determine whether

Figure 3. Only basal-type breast cancer cells are capable of intraspheroid motility and invasion. A, quantification of the speed and displacement of cells per 14 hours. The low-level speed and displacement of the luminal-type spheroids is due to cell division and stochastic movement resulting from modest stage drift. Vertical scatter plots of the mean speed and displacement of 15 spheroids per cell line over 3 independent experiments are shown. Horizontal bars are the mean for each cell line. Error bars are ± SD. **, P < 0.001 compared with HCC1428 by the Mann–Whitney U test. B, time-lapse confocal slices of the indicated breast cancer spheroids cultured alone or with mammary fibroblasts. H2B:GFP (nuclei, white) expression is shown. The position of 2 cells in each spheroid is indicated by solid and dashed white arrows. Scale bars, 20 μm. The results are representative of 30 spheroids imaged per condition over 3 independent experiments.
breast cancer cells are capable of intraspheroid motility or at risk for fibroblast-induced collective invasion.

**Fibroblasts reorganize collagen fibers to induce collective invasion**

Hepatocyte growth factor can induce MCFDCIS invasion in organotypic culture (28) and fibroblasts can trigger distinct gene expression changes in basal-type breast cancer cells compared with luminal-type breast cancer cells (29), suggesting that paracrine signals from the fibroblasts were inducing invasion. However, we found that the conditioned medium from mammary fibroblasts was not sufficient to induce MCFDCIS invasion in organotypic culture (Fig. 4A and B). Although these results did not rule out a role for paracrine signaling in promoting invasion, they did indicate an additional requirement for fibroblast-induced invasion. The collagen in our organotypic cocultures was reorganized from a lattice-like mesh into linear fibers, which suggested that the fibroblasts reorganized collagen to induce invasion (Fig. 4C). Collagen fibers surrounding collectively invading mammary tumor cells in patient samples (3) and transgenic mice (30) are reorganized, which indicated that our organotypic culture model was reconstituting a feature of tumor invasion in vivo. To determine whether collagen reorganization occurred before invasion, we imaged the MCFDCIS cells, fibroblasts, and fluorescein isothiocyanate–collagen in real time before MCFDCIS invasion. The collagen was indeed reorganized in areas surrounding the fibroblasts before the tumor cells invaded, as indicated by the increased local concentration of collagen (Fig. 4D and Supplementary Fig. S5), which is the result of the interaction and contraction of collagen fibers by fibroblasts (31). The MCFDCIS cells exclusively invaded toward fibroblasts located within areas of reorganized collagen (Fig. 4D, Supplementary Fig. S5 and Supplementary Movies S21 and S22). The leading MCFDCIS cell remained attached to the collectively invading group of cells (Supplementary Movies S23 and S24), consistent with our earlier results (Fig. 2, Supplementary Fig. S2, and Supplementary Movies S5, S6, and S7). Rho-family small GTP-binding proteins (G-proteins) regulate fibroblast-mediated collagen reorganization (31), so we transfected mammary fibroblasts with siRNAs that target the small G-protein Cdc42 to determine whether collagen reorganization was required for invasion. Reduced expression of Cdc42 (Supplementary Fig. S6) was sufficient to prevent cell elongation and reduced the degree of reorganization of the collagen fibers in fibroblasts (Fig. 4E). Correspondingly, invasion was reduced when HCC1954 cells were cocultured with fibroblasts transfected with Cdc42 siRNAs (Fig. 4F and G). These results show that fibroblast-mediated collagen reorganization is necessary for basal-type breast cancer invasion in organotypic culture.

**Therapeutic targeting of intraspheroid motility blocks fibroblast-induced invasion**

The fact that fibroblasts did not induce invasion through the de novo stimulation of an epithelial-mesenchymal transition suggested that therapeutic targeting of the intrinsic intraspheroid motility of the basal-type breast cancer cell spheroids could prevent fibroblast-induced invasion. We previously found that the activation of the ERK1/2 MAP kinases was sufficient to induce the intraspheroid motility of MCF10A human mammary epithelial cells in organotypic culture (15, 24), suggesting that ERK1/2 activity may be necessary for intraspheroid motility in the basal-type breast cancer cells. To determine whether ERK1/2 activity was required for intraspheroid motility and sprouting invasion, we treated MCFDCIS cocultures with the MEK1/2 inhibitor PD0325901. We found that within 48 hours of treatment with the MEK1/2 inhibitor, both the velocity and displacement of cell movement were reduced (Fig. 5A and B and Supplementary Movies S25 and S26). In addition, treatment with either PD0325901 or the epidermal growth factor receptor inhibitor erlotinib blocked fibroblast-induced invasion of both MCFDCIS and HCC1806 spheroids (Fig. 5C and D and Supplementary Fig S7). Treatment of mammary fibroblasts with PD0325901 or erlotinib did not alter fibroblast morphology or block collagen reorganization (Supplementary Fig. S8), indicating that the inhibition of invasion was due to the suppression of cell movement and not by antagonizing the proinvasive action of the fibroblasts. In comparison, treatment with the Rho kinase inhibitor Y27632 reduced fibroblast-induced collagen reorganization (Supplementary Fig. S8), similar to previous findings (32).

To determine whether ERK1/2 activity was necessary for the fibroblast-induced invasion of MCFDCIS cells in vivo, mice were treated daily with either vehicle or 25 mg/mL of the MEK1/2 inhibitor PD0325901 (33) between day 14 and day 21 of tumor growth. In mice treated with vehicle, mammary fibroblasts induced MCFDCIS invasion by day 21 (Fig. 5E and Supplementary Fig. S8). In contrast, the tumors in mice treated with the MEK1/2 inhibitor were both noninvasive and dramatically reduced in size (Fig. 5E and F and Supplementary Fig. S9). The small lesions in the MEK1/2 inhibitor treated mice were not invasive; however, we were not able to conclusively determine whether the blockade of invasion was related to the suppression of movement or the general growth and survival of tumor cells. Nevertheless, our data show that targeting ERK1/2-dependent signaling pathways can prevent the progression of tumors past small duct-like nodules. Taken together, our findings using organotypic culture and xenograft models indicate that there are overlapping molecular requirements for movement and collective invasion. These results suggest that clinically approved drugs may be able to delay or prevent the progression of early-stage tumors into invasive breast cancer.

**Discussion**

Here, we find that the reorganization of collagen fibers by mammary fibroblasts creates avenues for invasion that can be exploited by the motile breast cancer cells in our organotypic culture model. The movement observed in the breast cancer cells seemed to be triggered by the induction of a preexisting biological program for mammary gland development (25). The concept of a preexisting biological program contributing to the metastatic cascade has been
Figure 4. Cdc42 expression in fibroblasts is required for collagen reorganization and basal-type breast cancer invasion. A, organotypic cultures were grown for 6 days, immunostained with α-laminin-5 antibody (basement membrane, red), and counterstained with Hoechst (nuclei, blue). Conditioned medium from the mammary fibroblasts was added on day 2 and day 4. The white arrow identifies an area of invasion. Scale bars, 100 μm. B, quantification of the frequency of invasion. Data are the mean ± SD. *, P < 0.05 versus no fibroblast control by the t-test. C, confocal slices of organotypic cultures immunostained with anti-collagen I (red) and anti-E-cadherin (green) antibodies and counterstained with Hoechst (nuclei, blue) are shown. A solid white arrow indicates the direction of invasion, and a dotted white arrow shows a nearby fibroblast. Scale bars, 100 μm. D, confocal slices of MCFDCIS-mCherryCAAX cells (red) cultured alone or with mammary fibroblasts. Fluorescein isothiocyanate (FITC)-labeled collagen I is shown in green. The invasion of MCFDCIS-mCherryCAAX cells at 2-hour intervals over 6 hours total is shown. The area of collagen remodeling, indicated by increased fluorescent signal intensity, is outlined with a white rectangle. Photobleaching decreases the FITC-collagen signal over time. The solid white arrow indicates where the MCFDCIS cell begins sprouting invasion away from the main spheroid. The dotted white arrow indicates a branch point in the invasion as the MCFDCIS cells spread through the perpendicular region of reorganized collagen containing a fibroblast. Scale bars, 20 μm. The results are representative of 30 spheroids imaged per condition over 3 independent experiments. E, confocal slices of transfected fibroblasts after 4 days in organotypic culture were immunostained with anti-collagen I antibody (green) and counterstained with phalloidin (F-actin, red) and Hoechst (nuclei, blue). Scale bars, 100 μm. F, confocal slices of HCC1954 spheroids cocultured with fibroblasts transfected with the indicated siRNA pools are shown. Cultures were fixed and stained with phalloidin (F-actin, red) and Hoechst (nuclei, blue) after 6 days of growth. Dashed white arrows identify areas containing fibroblasts. G, quantification of the frequency of invasion. The mean ± range of at least 2 independent experiments is shown.
previously suggested (34). However, instead of a dramatic switch from an epithelial to a fibroblast-like phenotype, which occurs during embryonic development (34), a tissue-specific mammary gland branching morphogenesis program was engaged that permits movement within multicellular clusters and the exchange of cell–cell interacting partners. A similar type of intraspheroid motility has been observed during the development of additional epithelial tissues, such as the salivary gland (35), kidney (36), and lung (37). Furthermore, intraspheroid motility is an evolutionarily conserved feature of development that is observed during the morphogenesis of nonepithelial tissues in mammals (26). Thus, the direct modulation of intraspheroid motility by tumor-associated fibroblasts may trigger invasion in multiple tumors types. Consistent with this possibility, motile squamous carcinoma cells can invade into tracks created by fibroblasts (38). Like breast cancer, squamous cancer can be divided into intrinsic subtypes (39). However, whether the fibroblasts induced the invasion of a specific subtype of squamous cancer was not determined (38). Further investigation is needed to determine whether intraspheroid motility is a unique feature of intrinsic subtypes in additional carcinomas that makes neoplastic cells exquisitely sensitive to fibroblast-induced invasion out of ductal epithelium.

Our results suggest that the recruitment of fibroblasts to basal-type DCIS tumors is sufficient to trigger invasion. In comparison, luminal-type tumors may require additional
contributions from the microenvironment, such as secreted factors from macrophages, to promote the intraperihepatic motility that is necessary for invasion. If a more complex series of events is necessary for luminal-type invasion, basal-type tumors may progress to invasion more rapidly than luminal-type tumors. An accelerated transition to invasive breast cancer is consistent with the lack of correlation between primary tumor size and lymph node involvement in patients with basal-type breast cancer (40), as well as a greater risk of future metastasis (22). Further investigation is needed to precisely define the contribution of fibroblast-induced invasion of basal-type breast cancer cells to these known poor clinical phenotypes and develop new intervention modalities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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