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Probing the biophysical properties of primary breast tumor-derived fibroblasts

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Abstract

As cancer progresses, cells must adapt to a new and stiffer environment, which can ultimately alter how normal cells within the tumor behave. In turn, these cells are known to further aid tumor progression. Therefore, there is potentially a unique avenue to better understand metastatic potential through single-cell biophysical assays performed on patient-derived cells. Here, we perform biophysical characterization of primary human fibroblastic cells obtained from mammary carcinoma and normal contralateral tissue. Through a series of tissue dissociation, differential centrifugation and trypsinization steps, we isolate an adherent fibroblastic population viable for biomechanical testing. 2D TFM and 3D migration measurements in a collagen matrix show that fibroblasts obtained from patient tumors generate more traction forces and display improved migration potential than their counterparts from normal tissue. Moreover, through the use of an embedded spheroid model, we confirmed the extracellular matrix (ECM) remodeling behavior of primary cells isolated from carcinoma. Overall, correlating biophysical characterization of normal- and carcinoma-derived samples from individual patient along with patient outcome may become a powerful approach to further our comprehension of metastasis and ultimately design drug targets on a patient-specific basis.

1. Introduction

During tumor development, the microenvironment undergoes a number of changes including a significant increase in stiffness^{2,4}. Epithelial and stromal cell populations within the microenvironment modify their behavior in response to these new conditions, and prolonged

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Conflict of Interest Statement

T.A.A., F.B., S.P.C., M.C.L., D.R.K., S.S., S.V., S.J.S., and C.A.R. declare that they have no conflicts of interest.

Ethical Standards

All experiments using sample from human subject were carried out in accordance with Weill Cornell IRB guidelines. Human tissues were de-identified. No animals were used in this study.

exposure to this altered microenvironment is likely to permanently affect cells present in the tumor. Indeed, work performed on epithelial cell lines has repeatedly shown that cell contractility increases with metastatic potential^{19,27}. However, while epithelial and carcinoma cells have been characterized, we have yet to expand these findings to stromal cells present in the tumor microenvironment, including stromal fibroblasts, which are critically involved in many aspects of cancer progression^{13,23,42}. The effect of tumorigenesis on this cell population is of particular relevance considering the importance of epithelial-stromal interactions during mammary development, homeostasis, and malignancy^{33,34}.

Stromal fibroblasts enable tumor progression by promoting tumor growth, angiogenesis, and metastasis^{3,33}. Notably, stromal fibroblasts participate in extracellular matrix (ECM) remodeling that enables metastatic cells to escape the primary tumor^{8,13,24}. For example, fibroblasts can generate protease-dependent microtracks within the ECM that carcinoma cells can then use to migrate¹⁵. In addition, fibroblasts can use cell-matrix mechanocoupling to apply traction forces to individual ECM fibers, leading to increased matrix alignment^{17,41}, which can promote contact-guidance-facilitated 3D invasion³⁰. Both force-mediated and protease-mediated matrix remodeling events require the participation of cell contractility pathways^{15,17,41}. Since such fibroblast-mediated ECM remodeling enables cell invasion through the tumor stroma which ultimately leads to metastatic dissemination^{13,15,41}, the ability of stromal fibroblasts to remodel ECM, and thus, their biophysical properties, are of critical importance. Interestingly, it appears that the tissue origin of different fibroblasts might impact their contractility³⁶. Moreover, indirect evidence suggests that tumor-associated fibroblasts are different than their normal counterparts and may have heightened contractile potential^{18,25}. In that context, being able to perform biophysical measurements that define cell contractility, migration ability, and matrix remodeling fitness in normal- and tumor-associated fibroblasts might provide crucial clinical information regarding tumor progression.

In this work, we perform a biophysical characterization of primary stromal fibroblasts obtained from breast tumor tissue and the contralateral healthy breast tissue following double mastectomy. Notably, traction force microscopy measurements reveal that cells isolated from a breast tumor generate more traction than patient-matched cells isolated from the non-afflicted breast. Moreover, further investigation of the behavior of these tumor-associated mammary fibroblasts in 3D ECM scaffolds shows increased migration potential and the ability to form microtracks. Our results indicate that cellular adaptation to the tumorigenic microenvironment extends to the biophysical properties of stromal fibroblasts and suggest that carcinoma-associated changes in stromal fibroblast mechanobiology may further encourage tumor progression through metastasis-promoting behaviors including force generation, migration, and matrix remodeling.

2. Material and Methods

2.1. Cell culture and reagents

MDA-MB-231 highly metastatic breast adenocarcinoma cells (ATCC, Rockville, MD) and HMF3 human mammary fibroblasts (kind gift from Paraskevi Giannakakou, Weill Cornell

Medical Center) were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Life Technologies, Grand Island, NY). MCF10A cells mammary epithelial cells (ATCC) were maintained in Dulbecco's Modified Eagle's Media supplemented with 5% horse serum, 20 ng/mL EGF (Life Technologies), 10 mg/mL insulin, 0.5 mg/mL hydrocortisone, 100 ng/mL cholera toxin (Sigma-Aldrich, St. Louis, MO), and 1% penicillin-streptomycin. Bovine aortic endothelial cells (BAECs; VEC Technologies, Rensselaer, NY) were maintained in Medium 199 (Life Technologies) supplemented with 10% Fetal Clone III (HyClone, Logan, UT) and 1% each of MEM amino acids (Life Technologies), MEM vitamins (Mediatech, Manassas, VA), and penicillin-streptomycin. Primary cells were maintained in DMEM:F-12 supplemented with B-27 (Life Technologies), hEGF, insulin, human bFGF (Millipore, Billerica, MA), and bovine serum albumin as described by Marsden ²⁵. All cells were cultured at 37°C and 5% CO₂. Primary antibodies used were rabbit anti-vimentin (V6389, Sigma-Aldrich), mouse monoclonal anti-Keratin 8 (K8; C5301, Sigma-Aldrich), mouse monoclonal anti-EpCam Alexa 488 (5198; Cell Signaling Technology, Danvers, MA); goat monoclonal anti-VE cadherin (sc-6458; Santa Cruz Biotechnology, Santa Cruz, CA); and mouse monoclonal, anti-GAPDH (mAb374; Millipore). Secondary antibodies used were Alexa 488-goat anti-mouse IgG, Alexa 555-goat anti-mouse IgG and Alexa-594-goat anti-rabbit IgG (Life Technologies). Phosphate buffer saline (PBS) were purchased from Life Technologies; Triton X-100 was from (JT Baker, Phillipsburg, NJ); all other chemicals were from Sigma-Aldrich.

2.2. Primary Cell Isolation

De-identified tissue was obtained from double mastectomy patients at Weill Cornell/New York Presbyterian Hospital. Fibroblast cells were isolated as described previously by Marsden et al ²⁵. Briefly, the tissue was maintained on ice in 1x Hank's balanced salt solution (Life Technologies) for transport. In the laboratory, tissue was rinsed with 1x PBS and finely minced with a scalpel followed by overnight incubation at 37°C with 1x Collagenase/Hyaluronidase (07912; StemCell Technologies, Vancouver, BC) in complete DMEM:F-12. Undigested tissue was then removed by passing the resulting slurry sequentially through 100 µM and 40 µM cell strainers (Millipore) followed by centrifugation at 350g for 10 minutes to pellet the cells. Cells were plated in tissue culture flasks coated with 2% gelatin and cultured *in vitro* until reaching confluence. The fibroblast population was then isolated by differential centrifugation ³⁷.

2.3. 3D cell culture

Three-dimensional collagen matrices for cell migration experiments were prepared as previously described ⁶. Briefly, acid-extracted collagen I from rat tail tendon (Rockland Immunochemicals, Gilbertsville, PA) was diluted to 1.5 mg/mL with ice-cold media and neutralized to pH 7.0 with 1N NaOH. For single-cell migration experiments, cells were trypsinized and embedded sparsely within collagen solution prior to polymerization. To generate multicellular spheroids, cells were dissociated as for cell passage, resuspended in media containing 0.25% methylcellulose (StemCell Technologies), and plated at 25,000 cells/mL in non-adhesive round-bottom 96-well plates as previously described ⁷. After 24 h of spheroid formation, spheroids were isolated and individually embedded within collagen

solution prior to polymerization. Following 30 minutes of gel polymerization at 37°C, all collagen matrices were overlaid with culture medium.

2.4. Immunofluorescence

For immunofluorescence imaging, cells were seeded on coverslip coated with 2% gelatin and cultured overnight. Cells were washed twice with PBS, fixed for 10 min at room temperature with 3.7% paraformaldehyde in PBS, followed by permeabilization with 0.1% Triton X-100 (with 1% BSA in PBS) at room temperature for 10 min. They were then successively incubated overnight at 4 °C with anti-Keratin 8 (1/100 in PBS) and anti-vimentin (1/100 in PBS) followed with Alexa Fluor 488 anti-mouse IgG and Alexa Fluor 594 anti-rabbit IgG antibody for 1 h at room temperature. For EpCam staining, cells were directly incubated overnight at 4 °C with anti-EpCam Alexa 488. Images were captured on a Zeiss LSM700 confocal microscope.

2.5. Microscopy

Time-lapse phase contrast microscopy was used to monitor single cells migrating within 3D collagen matrices. Cell-seeded gels were maintained in a temperature, humidity, and CO₂-controlled enclosure and images were acquired every 10 minutes for 3 hours. Cell speed was calculated as the average of step-wise displacement divided by time lag and migration directionality was determined by dividing the net displacement linear path length of the cell by the total step-wise displacement path length.

Confocal reflectance microscopy was used to image collagen matrix organization associated with single cell and spheroid invasion. A Zeiss LSM700 confocal microscope equipped with a long working distance water-immersion C-Apochromat 40×/1.1 NA Zeiss objective was used as previously described⁶. To visualize 3D cell-generated tracks through collagen, voids in reflectance signal (indicating degraded matrix) were manually traced in sequential image slices. Maximum intensity z-projections and 3D-rendered images were compiled and pseudocolored using ZEN software.

2.6. Western Blotting

Total proteins were extracted with preheated (90°C) 2X sample buffer¹⁶. Twenty µg of protein extract were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and electro-transferred onto a polyvinylidene difluoride membrane. The blots were incubated with the primary antibody for 16 h at 4°C and then with the appropriate horseradish peroxidase-conjugated secondary antibody for 1 h at RT. The peroxidase signal was revealed with the SuperSignal West Pico kit (Thermo Scientific, Rockford, IL).

2.7. Traction Force Microscopy

Traction force microscopy was carried out as previously described²⁰. Briefly, polyacrylamide (PA) gels embedded with 0.5 µm diameter fluorescent beads (Life Technologies) and a Young's modulus of 5 kPa were made with a bisacrylamide:acrylamide ratio of 7.5:0.175 (Bio-Rad, Hercules, CA). The PA gels were coated with 0.1 mg/ml rat tail type I collagen (BD Biosciences, San Jose, CA) and cells were allowed to adhere for 24 hours. Phase contrast images of single cells were then taken immediately followed by

fluorescent images of the bead field at the gel surface. Cells were then removed with 0.05% trypsin/EDTA (Life Technologies) and a second fluorescent image of the bead field was captured. Bead displacements between the stressed and null state were calculated using the LIBTRC analysis library developed by M. Dembo (Dept. of Biomedical Engineering, Boston University) and used with the PA gel properties to calculate the most likely cellular traction vectors, T^{10} . The overall cellular force is the integral of the traction vector magnitudes over the total cell area³².

2.8. Statistical model of traction forces as a predictor of fibroblast malignancy

Traction forces were modeled as a predictor of cell type (malignant or healthy) using the additive logistic regression

$$\log \left(\frac{p_{ij}}{1-p_{ij}} \right) = c_i \beta_i + f(x_{ij}) \quad (1)$$

where p_{ij} is the probability that case i and sample j is a carcinoma cell, c_i equals one for case i and zero otherwise, and f is an unknown function of the traction forces x_{ij} . The unknown function f describes the relationship between traction forces and the log-odds of carcinoma, which is our primary interest. The intercepts β_1 , β_2 , and β_3 account for case-to-case variability, while f aggregates information across all 151 samples--and notably does not depend on the case.

The standard logistic regression assumption of linearity, i.e., $f(x_{ij}) = x_{ij}\beta$ for some unknown β , is restrictive and inadequate for the data. Therefore, we adopt a more sophisticated approach in which we model f as a smooth function of traction forces, so that (1) is a *generalized additive model*⁴⁰. The smoothness requirement guards against overfitting and produces flexible yet interpretable estimates. Specifically, we model f as a penalized cubic regression spline, which we may estimate--along with the other unknown parameters in (1)--using the penalized iteratively reweighted least squares algorithm in the R package *mgcv*³¹. Note that we could alternatively treat the case as a random effect and fit (1) as a generalized additive mixed model, but the resulting estimates are not sufficiently different to warrant such an increase in computational and conceptual complexity.

2.9. Statistical Tests

Traction force microscopy data were log transformed to satisfy equal distribution variances before analysis. Statistical analysis of both traction force microscopy data and migration data were done using analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) post-hoc analysis in JMP Pro 10.0.2.

3. Results

3.1. Isolated primary cells express fibroblast markers

To characterize the lineage of isolated cells, a number of epithelial and mesenchymal markers were used for Western blotting and immunofluorescence. Well-established cell lines of epithelial (MCF10a and MDA-MB-231), fibroblastic (HMF3) and endothelial

(BAEC) origin were selected as positive controls. These positive control cells have differential expression profiles that are characteristic of their origin (Fig. 1A). Notably, both the MCF10a and the MDA-MB-231 cell line contained keratin 8 (K8), an intermediate filament protein that is associated with all cells originating from simple and transitional epithelia¹⁴. The endothelial cell marker VE-cadherin was found only in BAECs while the mesenchymal marker vimentin was found to be expressed in all control cells (Fig. 1A). Interestingly, the isolated primary cells were found to express vimentin and displayed an organized vimentin intermediate filament network as determined by indirect immunofluorescence (Fig. 1B). Direct immunofluorescence of an additional epithelial marker, the epithelial cell adhesion molecule (EpCam), was found to be negative in the isolated primary cells (Fig. 1B). In addition, the isolated primary cells stained positive for the fibroblast marker FSP-1 (Fig. 1B). Protein expression and immunofluorescence staining patterns were found to be similar in primary cells isolated from either healthy or tumor tissue or from a different patient (data not shown). Moreover, the uniform staining observed for the different cell origin markers after the isolation of the primary cells suggest that there was no contamination from other cell types. Overall, these data suggest that the cells obtained through the isolation procedure were of fibroblastic origin.

3.2. Primary fibroblasts isolated from tumors generate increased traction forces

Since cancer-associated fibroblasts are known to affect the tumor microenvironment and promote cancer progression²⁴, and cellular forces have been linked to metastatic potential using epithelial cell line models¹⁹, we investigated whether primary stromal fibroblasts isolated from healthy and malignant breast tissue exhibited differential cellular traction forces. Cells obtained from the malignant and contralateral healthy breast tissue of two additional double mastectomy patients were confirmed to be fibroblastic (data not shown). Cell traction forces were measured using traction force microscopy on 5 kPa polyacrylamide gels coated with 0.1 mg/mL collagen to mimic the physical properties of the tumor microenvironment. As expected, fibroblastic cells from both healthy and tumor tissue exerted traction forces at cell extensions (Fig. 2A). For two of three cases studied, tumor-derived stromal fibroblasts exerted significantly greater traction stresses as compared to their patient-matched healthy tissue-derived counterparts (Fig. 2B). Interestingly, there was a considerable amount of patient-specificity that would not support the definition of a traction force “threshold” associated with malignancy. Nonetheless, all carcinoma-derived cells exerted traction forces approximately 50% greater than their patient-matched normal counterparts.

3.3. Statistical model of traction forces as a predictor of fibroblast malignancy

To further investigate the relationship between traction forces and malignancy, a generalized additive model (1) was used to infer a relationship between traction forces and the log-odds of carcinoma by estimating the unknown function of traction forces f (Fig. 2C). Since we account for case-to-case variation with the intercepts β_1 , β_2 , and β_3 , our estimate of f aggregates information across all 151 traction force values, and does not depend on the case.

An important feature of the generalized additive model (1) is that it allows us to compare the likelihood of cancer among cells within the same patient based *only* on the traction forces.

More specifically, for cells j and k within the *same* case i with traction forces $x + \Delta x$ and x , respectively, the odds ratio of cancer between the cells according to (1) is

$$\frac{p_{ij}/(1-p_{ij})}{p_{ik}/(1-p_{ik})} = \frac{\exp(\beta_i + f(x + \Delta x))}{\exp(\beta_i + f(x))} = \exp(f(x + \Delta x) - f(x)) \quad (2)$$

which notably does not depend on the case. In particular, comparisons of the odds of cancer between cells in the same patient can be made using only the vertical distances between the estimated function $\hat{f}$ evaluated at the traction forces $x + \Delta x$ and x . Therefore, cells with larger values of $\hat{f}$ are more likely to be cancerous than cells with smaller values of $\hat{f}$.

Most notably, $\hat{f}$ has a large positive slope with narrow confidence bands for traction forces below approximately 650 nN. This result suggests that traction forces in this region can significantly distinguish between carcinoma and normal cells, with smaller traction forces corresponding to smaller probabilities of carcinoma. Using the odds ratio in (2), the large positive slope implies that for pairs of cells from the same patient with traction forces less than 650 nN, small differences Δx between the traction forces may nonetheless provide enough information to identify which of the cells is more likely to be cancerous. However, for larger traction forces outside of this region, the relationship between traction force and the log-odds of cancer is more variable, and the distinction between carcinoma and normal cells is less clear. Since larger traction forces correspond to larger values of $\hat{f}$, the wider confidence bands suggest there is greater variability among cells which are more likely to be cancerous.

3.4. Primary cell migration and matrix remodeling in 3D collagen

To further characterize biophysical phenotype of primary stromal fibroblasts, cells were embedded within three-dimensional type I collagen matrices and their random motility was monitored using time-lapse microscopy. Collagen matrices were used to mimic the stromal extracellular matrix microenvironment within breast tissue as our group and others' have previously reported^{7,29,38}. Both normal and carcinoma-derived cells were able to migrate through a 1.5 mg/mL 3D collagen matrix (Fig. 3A). While carcinoma-associated cells migrated faster than their normal counterparts (Fig. 3B), this difference was not statistically significant, likely due to considerable variability of cell movement. Still, tumor-derived cells showed net displacements approximately twice those of normal cells, which was reflected by a significant increase in migration directionality (Fig. 3B).

Since efficient cell migration through restrictive 3D environments requires matrix remodeling to modify the ECM and overcome steric hindrance³⁸, matrix reorganization and degradation by patient-derived cells were assessed following migration experiments. A spheroid invasion assay was used as previously described study cell-matrix interactions during 3D migration in a controlled manner⁷. Naïve collagen matrix prior to invasion was isotropically organized and showed no preferential alignment (data not shown). However, as tumor-derived fibroblasts invaded past the original spheroid boundary (Fig. 4A, B; dashed line and "S") into the 3D collagen over 48 h, they induced significant remodeling of the collagen ECM, as indicated by reorientation of collagen fibers perpendicular to the spheroid

surface (Fig. 4B; arrowhead). This matrix reorganization was observed peripheral to invading cells (Fig. 4B; asterisks). In addition to mechanical reorganization of collagen fibers, cells migrating in 3D microenvironments can proteolytically degrade matrix to facilitate their movement and the movement of other cells³⁹. Single carcinoma-derived cells migrating in 3D matrix produced microtracks as they moved, as indicated by voids in signal within sequential slices from a confocal reflectance z-stack (Fig. 4C; outlines). Three-dimensional reconstruction of a cell-derived microtrack revealed a single cell at one end of the microtrack (Fig. 4C, D; asterisk) and a microtrack cross-sectional diameter of 15–20 μm (Fig. 4D).

4. Discussion

Breast cancer progression is associated with widespread cellular and extracellular changes in both the epithelial and stromal compartments of the affected tissue. While there has been considerable interest in characterizing differences between normal and malignant epithelium, there is increasing evidence that the stromal compartment is a major contributor to tumor progression³³. Notably, stromal fibroblasts, which constitute the predominant cell type in breast tissue stroma, are known to promote local tumor invasion and facilitate metastasis through biophysical extracellular matrix remodeling⁹. Therefore, it is critical to directly characterize the effect of breast tumorigenesis on biophysical behaviors of stromal fibroblast that contribute to disease progression. Here, we isolate primary human stromal fibroblasts from mammary tumors and contralateral healthy breast tissue following double mastectomy and show that stromal fibroblasts derived from breast tumors generate higher traction forces and are more migratory than their normal tissue-derived, patient-matched counterparts. Furthermore, we demonstrate that migration of carcinoma-derived fibroblasts through physiological 3D collagen matrix is associated with ECM reorganization and degradation.

Several groups have isolated human stromal fibroblasts from healthy and cancerous breast tissue, showing that tumor-derived fibroblasts promote tumor growth^{12,26} and generate invasion-enabling ECM¹³ more so than their normal tissue-derived counterparts. Consistent with these studies, our finding that tumor-derived fibroblasts exert greater traction forces and migrate more efficiently than fibroblasts from healthy stroma indicates that tumor progression affects stromal fibroblasts at least in part by modifying their biophysical properties.

Cellular biomechanics are of critical importance to tumor progression⁵ and were recently reported to vary widely between non-tumorigenic and metastatic mammary epithelial cells¹. Additionally, our group demonstrated that metastatic epithelial cell lines exert greater traction forces than their non-metastatic counterparts from the same tissue¹⁹. Similarly, we showed that non-metastatic and metastatic epithelial cells differ in their ability to induce ECM remodeling required for 3D migration⁷. Despite these findings and the importance of epithelial-stromal interactions during tumor invasion^{9,35}, such properties have not been directly measured for patient-derived stromal fibroblasts. Here, we focused on traction force generation, 3D cell migration, and matrix remodeling since together, cell force- and protease-mediated ECM remodeling during leader cell invasion provide follower cells with

migration-enabling paths of least resistance^{7,15,30}, and leader-follower behavior is a major presumed mechanism of stromal fibroblast function in driving cancer invasion and metastasis⁹. We show that, in addition to exerting increased traction forces, tumor-derived fibroblasts migrate more directionally, induce ECM realignment, and generate matrix-free microtracks. These results indicate that altered cellular biomechanics with respect to cell contractility and matrix remodeling within a tumor extends beyond epithelial cells, and increased contractility may be one way tumor-associated fibroblasts act to create a metastasis-promoting microenvironment.

It has been elegantly shown that increased matrix stiffness disrupts epithelial morphogenesis, promotes a malignant cell phenotype, and enables tumorigenesis^{21,27,30}. Importantly, desmoplastic matrix stiffening is a common feature of invasive breast carcinomas and stromal fibroblasts are thought to be both mediators of and sensitive to desmoplasia^{7,18,21,33}. We show here that tumor-derived fibroblasts exhibit an enhanced mechanical phenotype. Therefore, it is conceivable that 1) residence within a stiffened desmoplastic tumor-associated stroma conditions mechanical fibroblast behavior to match the tumor microenvironment and/or 2) resident stromal fibroblasts that are responsible for the desmoplastic response stiffen the tumor microenvironment to match their mechanical state¹¹. These theories are especially interesting since we observed significant patient specificity in traction force generation. Indeed, previous work has shown extensive intra-lesion (local variation) and inter-lesion (sample to sample variation) heterogeneity of stiffness in rodent^{22,27} and human tumor samples²⁸, suggesting that heterogeneity of tumor/stroma stiffness could provoke differential biomechanical phenotypes in stromal fibroblasts. The relationship between stromal ECM and stromal fibroblast mechanics, as well as the mechanisms driving tumorigenesis-mediated changes in stromal fibroblast biomechanics, remain to be fully defined.

In conclusion, we have shown that primary stromal fibroblasts derived from human mammary tumors exhibit enhanced biomechanical activity over their normal counterparts derived from the healthy contralateral mammary tissue. Carcinoma-associated fibroblasts exert increased traction forces, migrate more efficiently through 3D collagen, and induce substantial ECM remodeling including matrix reorganization and degradation, suggesting that tumor-associated fibroblasts are well suited to facilitate tumor invasion and promote metastasis. Our data demonstrate that biophysical properties of non-epithelial (stromal) cell types in the tumor microenvironment are sensitive to tumorigenesis and underscore the importance of considering the entire tumor microenvironment. Ultimately, by correlating such biophysical characterization of normal- and carcinoma-derived patient cells with patient outcome, we may be able to better understand metastasis and identify drug targets to prevent metastasis on a patient-specific basis.

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Figure 1.

Isolated primary cells are of fibroblastic origin. (A) Western blotting of total protein extracts of isolated primary cells from normal (N) and carcinoma (C) derived tissue showing VE-cadherin, keratin 8 and vimentin intermediate filaments content. Epithelial (MCF10a and MDA-MB-231), fibroblastic (HMF3) and endothelial (BAEC) cell lines were used as positive controls. GAPDH was used as loading control. (B) Immunofluorescence images of isolated primary cells and epithelial cell controls positive for vimentin intermediate filaments and FSP1 but negative for keratin 8 intermediate filaments and EpCam.

Figure 2.

Carcinoma-derived fibroblasts generate more traction force. (A) Representative traction force maps of primary stromal fibroblasts derived from normal (left) and tumor (right) mammary tissue. (B) Quantification of total traction force exerted by cells on 0.1 mg/mL collagen-coated polyacrylamide substrates with a Young's Modulus (E) = 5 kPa. Mean $\pm$ SE. * indicates $p < 0.05$. (C) Larger values of $\hat{f}$, the estimated function of traction forces, correspond to greater odds of carcinoma. Traction forces less than 650 nN are associated with a large positive slope of $\hat{f}$, which provides more significant differentiation between carcinoma and normal cells. The estimated function $\hat{f}$ (solid line) from (1) is plotted with 95% confidence bands (shaded region). Tick marks denote the sample values of traction forces.

Figure 3. Carcinoma-derived fibroblasts have more efficient 3D migration. (A) Cell tracks measured over 3 h from phase contrast image of both normal and carcinoma derived cells. (B) Quantification of cell speed, migration path length, and migration directionality obtained from three independent experiments. Mean $\pm$ SE. * indicates $p < 0.05$; ** indicates $p < 0.001$.

Figure 4.

3D ECM remodeling by carcinoma-derived fibroblasts. (A) 3D reconstruction from confocal reflectance sections of a spheroid (S) composed of carcinoma-derived primary fibroblasts invading into a 3D collagen scaffolds. Cells were allowed to invade into the ECM for a period of 48 h. The original spheroid boundary is indicated by the dashed line. (B) Phase contrast image of the invading cells (indicated by the asterisks) and the corresponding confocal reflectance image showing the collagen remodeling perpendicular to the spheroid boundary (arrow). (C) Confocal reflectance images of collagen showing a Z stack of the microtrack left in the wake of a single cell migration due to ECM remodeling and (D) the corresponding 3D reconstruction of the microtrack.