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Oncogenic Ras-Transformed Human Fibroblasts Exhibit Differential Changes in Contraction and Migration in 3D Collagen Matrices

Gustavo C. Menezes^{1,2}, Miguel Miron-Mendoza², Chin-Han Ho², Hongmei Jiang², and Frederick Grinnell^{2,3}

¹Universidade Federal do Rio de Janeiro, Instituto de Biofisica Carlos Chagas Filho, Rio de Janeiro, Brazil

²Department of Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9039, USA

Abstract

Tractional force exerted by tissue cells in 3D collagen matrices can be utilized for matrix remodeling or cell migration. The interrelationship between these motile processes is not well understood. The current studies were carried out to test the consequences of oncogenic Ras (H-Ras^{V12}) transformation on human fibroblast contraction and migration in 3D collagen matrices. Beginning with hTERT-immortalized cells, we prepared fibroblasts stably transformed with E6/E7 and with the combination E6/E7 and H-Ras^{V12}. Oncogenic Ras-transformed cells lost contact inhibition of cell growth, formed colonies in soft agar and were unable to make adherens junctions. We observed no changes in the extent or growth factor dependence of collagen matrix contraction (floating or stress-relaxation) by oncogenic Ras-transformed cells. On the other hand, transformed cells in nested collagen matrices lost not only growth factor selectivity, but also cell matrix density-dependent inhibition of migration. These findings demonstrate differential regulation of collagen matrix contraction and cell migration in 3D collagen matrices.

Keywords

Fibroblast; Collagen; Contraction; Migration; Mechanoregulation; Oncogenic Ras; Adherens Junctions

INTRODUCTION

Fibrous connective tissue provides mechanical support and frameworks for the other tissues of the body. Type 1 collagen is the major protein component of fibrous connective tissue. Fibroblasts are the cell type primarily responsible for collagen biosynthesis and remodeling. During mechanical remodeling of fibrous connective tissues, collagen and other ECM molecules can stretch, slip, and undergo stable reorganization [1]. Such remodeling has been implicated in diverse aspects of normal physiology and pathology including wound repair [2,

³Correspondence; Email = frederick.grinnell@utsouthwestern.edu, tel = 214-648-2181; fax = 214-648-6712.

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3], fibrosis [4–6], scar formation [7,8], tumorigenesis [9,10], and aging [11]. Matrix remodeling also is an important design feature in tissue engineering [12–14].

Unlike conventional 2D surfaces, with which most research on cell-matrix interactions has been carried out, 3D collagen matrices exhibit mechanical properties that resemble connective tissues [15–17]. When fibroblasts interact with 3D collagen matrices, adhesion sites are limited to matrix fibrils. The cells can penetrate into the matrix, and they can reorganize and contract the matrix. Given this diversity, research on fibroblasts and other cells interacting with 3D matrices can be used to replicate and analyze a broad range of cell-matrix interactions under different biomechanical conditions [18,19].

Fibroblasts can contract collagen matrices by more than one mechanism. Floating collagen matrix contraction leads to contraction of the matrix that depends on cell ruffling [20–22], whereas stress-relaxation matrix contraction depends on cell contraction [23–25]. Tractional force exerted by tissue cells in 3D collagen matrices can be utilized for matrix remodeling or cell migration [26]. However, the interrelationship between these motile processes is not well understood. Recently, we developed a new model of nested collagen matrices to study fibroblast migration in the 3D tissue like environment [27]. Using this model, we found that different sets of physiological growth factors stimulate human fibroblast migration and contraction suggesting that these processes are under separate regulatory control [28].

SV40-transformed human fibroblasts and rat sarcoma cells exhibit reduced contraction of floating collagen matrices [29,30], and Ras-transformed fibroblasts show increased migration on 2D surfaces [31–34]. Given the foregoing differences, we reasoned that a more systematic comparison of control and transformed human fibroblasts might provide new insights into the regulation of contraction and migration of cells interacting with 3D matrices. Beginning with hTERT-immortalized human fibroblasts, we prepared cell lines stably transformed with E6/E7 and with the combination E6/E7 and H-Ras^{V12}. Oncogenic Ras-transformed cells lost contact inhibition of cell growth, formed colonies in soft agar and were unable to make adherens junctions. Compared to control human fibroblasts, no changes in extent or growth factor dependence were observed in contraction of collagen matrices (floating or stress-relaxation) by oncogenic Ras-transformed cells. On the other hand, transformed cells in nested collagen matrices lost not only growth factor selectivity, but also cell matrix density-dependent inhibition of migration. These findings indicate that collagen matrix contraction and cell migration are differentially regulated.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle medium (DMEM), CO₂-independent DMEM, Opti-MEM and 0.25% trypsin/EDTA solution were purchased from Invitrogen, Gaithersburg, MD. Fetal bovine serum (FBS) was purchased from Gemini, West Sacramento, CA. Type I collagen (3 mg/ml, Vitrogen) was purchased from Cohesion Technologies, Palo Alto, CA. Fatty acid-free bovine serum albumin (BSA), lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P), and epidermal growth factor (EGF) were acquired from Sigma-Aldrich, St. Louis, MO. Platelet-derived growth factor (BB isotype) (PDGF) was obtained from Upstate Biotechnology, Lake Placid, NY. SV589 cells (SV40-transformed human fibroblasts, originally Human Genetic Cell repository GM-00639) -- were provided by Dr. Richard Anderson (UT Southwestern).

Cell culture

Use of human foreskin fibroblasts was approved by the University Institutional Review Board (Exemption #4). BR5 cells are hTERT-immortalized, early passage human foreskin fibroblasts [35]. BR5 and SV589 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C in a 5% CO₂ humidified incubator. Experimental incubation media was DMEM with 10% FBS or 5 mg/ml BSA and growth factors as indicated in the figure legends.

Anchorage independent cell growth was tested in soft agar [36]. Briefly, cells in DMEM plus 10% FBS in 0.35% (w/v) agar were plated on top of a 0.5% agar layer in a 6-well plate. 1 ml DMEM containing 10% FBS was added weekly to each well and the incubations continued for two weeks.

Transfection of BR5 cells with HPV16 E6/E7 and H-Ras^{V12}

Initial experiments to prepare oncogenic Ras-transformed BR5 cells were unsuccessful because the cells became growth-arrested. To overcome growth-arrest, BR5 cells first were treated to stably express human papillomavirus E6 and E7, which causes the degradation of p53 and inactivation of pRb and thus bypasses oncogenic Ras-induced growth-arrest [37–41].

Stable transfection of BR5 cells with HPV16 E6 and E7 and with H-Ras^{V12} was accomplished as has been described [42,43]. Briefly, proviral plasmid DNA containing the E6 and E7 genes was transfected into Phoenix E cells by calcium phosphate precipitation. Viral supernatants from transfected cells were used to infect PA317 cells to generate stable lines expressing unarranged retrovirus. Following infection and a 7-day drug selection in 1 mg/ml G-418, the supernatant of antibiotic-selected PA317 was harvested, passed through a 0.45 µm filter and added to a BR5 culture in the presence of 8 µg/ml polybrene. Infected cells were selected for 7 days in DMEM containing 10% FBS and 1 mg/ml G-418. H-Ras^{V12} retroviral supernatants were obtained by transiently transfecting 293T cells with pCLAmpho retrovirus-packaging vector and pBabe-hygro-H-Ras^{V12} using Opti-MEM and FuGENE6 (Roche, Basel, Switzerland) as carriers of vectors. Forty-eight hours after transfection, 293T supernatant was harvested, passed through a 0.45 µm filter and added to BR5-E6/E7 cultures in the presence of 8 µg/ml polybrene. Infected cells were selected for 10 days in DMEM containing 10% FBS and 50 µg/ml hygromycin. Phoenix E and PA317 cell lines, plasmids pLXSN and pLXSN-E6/E7, and cDNA primers for HPV-16 E6 and E7 were provided by the Wright/Shay Laboratory (UT Southwestern). pCLAmpho, pBabe-hygro-H-Ras^{V12} plasmids, and 293T cells were provided by Dr. Michael White (UT Southwestern). Throughout the manuscript, for simplicity, BR5-E6/E7 cells are designated BR5-E cells and BR5-E6/E7-H-Ras^{V12} cells are designated BR5-E-Ras.

RT-PCR and western blots

Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis was used to assess E6 and E7 mRNA expression. Primers were designed for either E6 or E7 specific amplification: Forward E6 (nucleotides 201–220): 5'- GCAAGCAACAGTTACTGCGA-3', Reverse E6 (nucleotides 503–522): 5'- CAACAAGACATACATCGACC-3'; Forward E7 (nucleotides 652–671): 5'- AGCTCAGAGGAGGAGGATGA-3', Reverse E7 (nucleotides 835–854): 5'- GGTTTCTGAGAACAGATGGG-3'. PCR was performed with Platinum Pfx DNA Polymerase (Invitrogen, Gaithersburg, MD) using GeneAmp PCR system 9700 (Applied Biosystem, Foster City, CA). RT-PCR with GAPDH primer was carried out as an internal control for RT-PCR. To ensure that the assay was in the linear range, the cycle number and amounts of RNA were varied.

Immunoblotting analysis was carried out as described previously [20,35]. Primary antibodies used were anti-actin and anti- β tubulin from Sigma and anti-P53 and anti-H-Ras(259) from Santa Cruz Biotechnology, Santa Cruz, CA. Secondary HRP-conjugated antibodies were obtained from ICN Biomedical or MP Biomedical, Solon, OH.

Matrix contraction and cell migration

Data are presented as averages $\pm$ standard deviations for duplicate or triplicate samples. All experiments were carried out two or more times. Methods for preparing collagen matrix cultures and measuring floating collagen matrix contraction, stressed-released matrix contraction, and cell migration in nested collagen matrices have been described previously [20,27,28,35]. Briefly, to measure floating matrix contraction, collagen matrices (200 μ l, 1.5 mg/ml collagen, 2×10^5 cells/matrix) were polymerized for 1 hr and then released from the culture surface and incubated 4 hr floating in basal medium (DMEM + 5 mg/ml BSA) and growth factors as shown. To measure stressed-released matrix contraction, collagen matrices as above were cultured overnight in DMEM/10% FBS, rinsed, and then released from the culture surface and incubated 1 hr in basal medium and growth factors as shown. At the end of the incubations, samples were fixed with 3% paraformaldehyde in phosphate buffered saline. Overall appearance of nested collagen matrices was recorded using an Epson 4870 photo scanner, and matrix area measured using MetaVue imaging software (Molecular Devices, Sunnyvale, CA). Extent of matrix contraction was calculated by subtracting final matrix area from the starting area ($\sim 113 \text{ mm}^2$).

To measure cell migration using nested collagen matrices, floating matrices were precontracted for the times indicated in DMEM/10% FBS after which the cell-containing contracted matrices (dermal equivalents) were re-embedded in 200 μ l cell-free outer collagen matrices and then incubated for an additional 16 hr in basal medium and growth factors as shown. At the end of the incubations, samples were fixed and stained with 8 μ g/ml propidium iodide (Molecular Probes, Inc., Eugene, OR) in the presence of 20 μ g/ml RNase (DNase-free) Roche, Basel, Switzerland to detect cell nuclei. Cell migration index was calculated by counting the average number of nuclei of cells that had migrated out of dermal equivalents in four 10X microscopic fields selected arbitrarily. Each field included the border of the dermal equivalent detected by dark field microscopy and the furthest moving cells (detected by nuclear staining with propidium iodide).

To analyze cell migration by scrape-wounding, cells were incubated overnight on collagen-coated coverslips in DMEM/BSA and 0.1% FBS. The samples were scrape-wounded with a pipette tip, and then incubated an additional 24 hr in basal medium and growth factors as shown. At the end of the incubations, samples were fixed and then stained with propidium iodine and with phalloidin to detect actin.

Immunofluorescence, phase contrast, and time-lapse microscopic methods

Immunofluorescence microscopy was accomplished as described previously [20,27,35]. Cells were formaldehyde-fixed and Triton-X-100 permeabilized. Primary antibodies used were against H-Ras(259) and β -catenin (Zymed). For actin staining, we used Alexa Fluor® 488 or Alexa Fluor® 594-conjugated phalloidin (Molecular Probes, Inc). Samples were mounted on glass slides with Fluoromount G (Southern Biotechnology Associates, Birmingham, AL). Microscopic images were collected at 22° with a Nikon Elipse 400 fluorescent microscope using 10 $\times$ /0.45, 20 $\times$ /0.75, and 40 $\times$ /0.75 Nikon Plan Apo infinity corrected objectives, Photometrics SenSys camera and MetaView acquisition software. Subsequent image processing was carried out using Adobe Photoshop.

Time lapse microscopy was carried out as described previously [44]. Nested collagen matrices in 24 well culture dishes were placed in an environmental chamber at 37°C. Microscopic images were collected using a Zeiss Axiovert 200M inverted microscope equipped with an A-PLAN 10×/0.25 PH1 Zeiss objective and a Nikon DXM1200F camera. Z stack images were acquired at 20 µm steps and 5–10 min intervals using Metamorph software. The Z stack range was 800 µm to insure complete coverage of the range of cell movement, which typically occurred within 100–200 µm. Z planes in which cells were most in focus were reconstructed into single 2D videos.

RESULTS

Transformation of BR5 cells with H-Ras^{V12}

As described in Materials and Methods, preparation of oncogenic Ras-transformed BR5 cells required prior cellular expression of human papillomavirus E6 and E7. Figure 1A shows by RT-PCR that BR5 cells transformed with E6 and E7 (BR5-E) expressed both E6 and E7 messenger RNAs. Figure 1B demonstrates by immunoblotting that levels of P53 were much lower in BR5-E compared to BR5 cells. The results in Figure 1C show that BR5 and BR5-E had lower levels of Ras protein compared to BR5-E-Ras cells, and immunofluorescence analysis (Figure 1D) demonstrated that increased Ras expression occurred in most cells and not just in a subpopulation.

Figure 2A shows that BR5 and BR5-E cells had a similar flattened appearance and tended to remain as a monolayer as cell cultures became confluent. BR5-E-Ras cells were less flattened and more likely to overgrow each other. Loss of normal growth regulation was shown by the ability of BR5-E-Ras cells but not BR5 or BR5-E cells to form colonies when growing on agar (Figure 2B) and to continue cell division beyond confluency (Figure 2C), which for BR5 cells occurred at $\sim 3 \times 10^6$ cells/T75 culture flask.

As demonstrated by Figure 3, BR5-E-Ras cells had less well defined actin-staining stress fibers compared to control cells. Fibroblast adherens junctions were readily visualized at sites of cell-cell overlap by immunostaining for β -catenin as has been demonstrated by others [45–47]. These junctions were positive for N-cadherin (not shown). In oncogenic Ras transformed cells, β -catenin distribution was diffuse suggesting that adherens junctions were mostly absent.

Collagen matrix contraction activity of oncogenic Ras-transformed human fibroblasts

Experiments then were carried out to determine the ability of control and oncogenic Ras-transformed human fibroblasts to contract collagen matrices. Figure 4 compares BR5, BR5-E, and BR5-E-Ras in floating matrix contraction (FMC) and stressed-released matrix contraction (SMC). As described in the Introduction, floating matrix contraction depends on matrix remodeling by cell ruffling, whereas stressed-released matrix contraction depends on matrix remodeling by cell contraction. The results demonstrated that neither the extent nor the growth factor dependence of contraction differed for oncogenic Ras-transformed cells compared to controls.

To control for possible differences as a result of cell concentration, we compared BR5 and BR5-E-Ras cells over a 20 fold cell-concentration range. Similar contraction and growth factor dependence profiles were observed (Supplemental Figure 1). As an additional control, we compared BR5 cells with SV40-transformed human fibroblasts (SV589 cells). Consistent with previous work [30], contraction of SV40-transformed cells was decreased compared to control human fibroblasts (Supplemental Figure 2).

Migration of oncogenic Ras-transformed human fibroblasts in nested collagen matrices

We used nested collagen matrices to analyze migration of oncogenic Ras transformed and control fibroblasts in the 3D matrix environment. To prepare nested matrices, dermal equivalents (fibroblast-contracted floating collagen matrices) were embedded in cell-free, outer collagen matrices. Figure 5 presents representative phase contrast images from time-lapse videos of BR5 and BR5-E-Ras migration in PDGF-containing medium (Supplemental Video 1 and Supplemental Video 2). Viewed from above, the dermal equivalent appears dense (lower portion of the microscopic field) and the surrounding outer matrix more translucent. The overall shape of migrating cells and pattern of migration was similar for BR5 or BR5-E-Ras with a wave of highly elongated cells beginning to migrate out of the dermal equivalent into the outer matrix after a lag phase of several hours.

Migration of fibroblasts in nested collagen matrices can be quantified with propidium iodide (PI)-stained samples by counting the number of cell nuclei in a defined region of the outer matrix [27]. Figure 6A shows typical PI stained images of BR5 and BR5-E-Ras migration after 24 hr of migration, and Figure 6B presents quantification of the results. In these experiments, the inner dermal equivalents had been precontracted for different times varying from 6 hr to 48 hr. Previously, we observed that human fibroblasts in nested collagen matrices exhibit cell matrix density-dependent inhibition of migration. That is, at longer times of precontraction, cell matrix density increases and cell migration decreases [44]. Figure 6 shows that BR5-E-Ras cells no longer exhibit cell matrix density-dependent inhibition of migration.

Growth factor dependence of oncogenic Ras-transformed human fibroblast migration

With normal human fibroblasts, platelet-derived growth factor is much more promigratory compared to other physiological agonists; whereas sphingosine-1-phosphate is inhibitory [28]. Figure 7 shows the extent of cell migration after 24 hr in the presence of several different growth factors. Consistent with previous findings, PDGF was much more promigratory for BR5 cells than the other growth factors tested, and S1P inhibited migration. BR5-E-Ras cells not only showed greater migration overall but, in addition, a decreased PDGF growth factor requirement for migration. However, the oncogenic Ras transformed cells remained sensitive to inhibition of migration by S1P. The findings in Figure 7 were based on PI-stained cell preparations as described for Figure 6. Supplemental Video 3 and Supplemental Video 4 show migration of BR5 and BR5-E-Ras cells in serum-containing medium. Consistent with the quantitative measurements, BR5 cells show little migration in serum-containing medium. Migrating BR5-E-Ras cells tended to be less elongated in serum-containing medium compared to PDGF-containing medium (Supplemental Video 1 and Supplemental Video 2).

Oncogenic Ras-transformed fibroblast migration in 2D after scrape wounding

To learn if the differences in Figure 7 were specific to the 3D environment, some experiments also were carried out using scrape-wounded 2D human fibroblast cultures. Figure 8 shows representative images of cells 24 hr after scrape wounding, double-stained for actin (green) and propidium iodide (red). It can be seen from the photomicrographs that compared to controls, BR5-E-Ras cells exhibited a decreased PDGF growth factor requirement for migration.

DISCUSSION

The current studies were carried out to test the consequences of human fibroblast transformation with oncogenic Ras on cell-matrix contraction and cell migration in 3D collagen matrices. Beginning with hTERT-immortalized human fibroblasts (BR5), we prepared E6/E7 and E6/E7- H-Ras^{V12} stably transformed cell lines. Compared to control BR5 and BR5-E6/E7 cells, oncogenic Ras expressing fibroblasts lost contact inhibition of cell growth and formed

colonies in soft agar. We observed no changes in the extent or growth factor dependence of collagen matrix contraction (floating or stress-relaxation). However, oncogenic Ras-transformed cells in nested collagen matrices lost not only growth factor selectivity, but also cell matrix density-dependent inhibition of migration.

Mechanical force exerted by tissue cells can be used to remodel and contract the extracellular matrix or for cell migration [26]. Some evidence favors a close relationship between cell migration and contraction [48–51], but differences in regulation have been reported as well [52–54]. Complicating interpretation of the foregoing results, mechanisms of cell migration are more diverse in 3D environments compared to 2D surfaces [44,55–57]. The current findings with oncogenic Ras transformed human fibroblasts along with our recent observations on selectivity of growth factor stimulation [28] support the idea that matrix contraction and cell migration are differentially regulated.

Oncogenic Ras transformation influences diverse downstream targets including cell motile functions [58,59], and a requirement for Ras activation has been implicated in PDGF-stimulated cell migration [60]. Therefore, one explanation for the loss of growth factor selectivity by oncogenic Ras-transformed cells might have been general downstream activation of PDGF signaling pathways. However, this explanation seems unlikely since BR5-E-Ras cells retained growth factor specificity for collagen matrix contraction, e.g., contraction of floating matrices in basal medium was similar for BR5-E-Ras and control cells.

One intriguing possibility is that loss of growth factor selectivity and cell matrix density-dependent inhibition of migration are related to each other and depend on the loss of adherens junctions in oncogenic-Ras transformed cells. Fibroblast adherens junctions mediated by N-cadherin play diverse roles in fibrous connective tissues [61]. Differentiation of fibroblasts to myofibroblasts during wound repair under the influence of transforming growth factor β has been reported to involve a switch in cadherin types from N to OB, and OB cadherin cell-cell adherens junctions are believed to help stabilize myofibroblasts exerting contractile force [47,62]. Although often thought of as isolated cells in connective tissue, fibroblasts in 3D collagen matrices form adherens junctions [63] and gap junctions [64]. Disrupting adherens junctions without interfering with cell motile machinery would be expected to increase overall cell migration and to decrease the impact of increased cell matrix density, which occurs when dermal equivalents are precontracted for longer times. Of course, other factors might be equally important such as increased expression of matrix metalloproteinases [65,66]. Whatever the precise mechanism responsible for Ras activation of migration, S1P retains the ability to exert an inhibitory effect.

Differential regulation of cell migration and matrix contraction helps explain fibroblast/myofibroblast function during wound repair. Regardless whether wound myofibroblasts arise from fibroblasts, fibrocytes or epithelial-mesenchymal transition (EMT) [4,5,67,68], these cells exhibit potentially conflicting migratory and contractile behavior. Strong tractional forces can retard migration, and localized increases in matrix density resulting from contraction can present a steric hindrance to migration. However, differential regulation of cell migration and matrix contraction could permit the two processes to occur independently of each other thereby avoiding potential interference.

Differential regulation of migration and contraction also may be important for epithelial cell invasiveness, since the tumor environment frequently contains both migratory malignant cells and contractile myofibroblasts [10,69,70]. Epithelial cell invasiveness depends at least in part on loss of E-cadherin adherens junctions during epithelial-mesenchymal transition [71–73]. Therefore, EMT can give rise to cells that are highly migratory or highly contractile.

Finally, it should be noted that, although not evident with oncogenic Ras transformed fibroblasts, SV40 transformed cells showed decreased collagen matrix contraction as was reported previously [30]. Cell transformation by SV40 also influences multiple downstream cell signaling pathways [74,75]. Identification of the regulatory mechanisms that account for loss of cell-matrix contraction following SV40 transformation remains an important subject for future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. BR5 transfection with E6/E7 and H-RasV¹²

(A) RNA extracts of BR5 and BR5-E cells were subjected to RT-PCR to determine E6 and E7 mRNA expression. GAPDH was employed as loading control. (B) Protein extracts of BR5 and BR5-E cells were immunoblotted for p53 protein. Actin was employed as loading control. (C) Protein extracts of BR5, BR5-E and BR5-E-Ras cells were immunoblotted for p21Ras. β -tubulin was employed as loading control. (D) BR5, BR5-E and BR5-E-Ras cells were cultured overnight on glass coverslips, fixed and immunostained for p21Ras. Compared to controls, BR5-E-Ras cells expressed higher levels of p21Ras and most BR5-E-Ras cells appeared to be transformed. Bar = 25 μ m.

A **B** **C**

Figure 2. Growth properties of BR5, BR5-E, and BR5-E-Ras cells

(A) BR5, BR5-E and BR5-E-Ras cells were cultured to confluence on collagen-coated glass slides and imaged by phase-contrast microscopy. Bar = 50 μ m. (B) BR5, BR5-E and BR5-E-Ras cells were incubated in soft-agar-containing 6-well dishes for 2 weeks. At the end of the incubations, samples were fixed and the number of colonies counted in 10 separate fields per dish. Data show the averages $\pm$ SD from three separate experiments. (C) BR5, BR5-E and BR5-E-Ras fibroblasts were seeded in T75 flasks at starting concentrations of 1.0, 1.5 or 3.0 $\times 10^6$ cells and culture for 3 days. At the end of the incubations, cells were recovered by trypsinization and cell numbers counted. Data show the averages $\pm$ SD from three separate experiments.

Compared to controls, BR5-E-Ras cells appeared less well spread compared to controls, exhibited growth in soft agar and loss of contact inhibition. Bar = 50 μ m.

Figure 3. Distribution of stress fibers and adherens junctions in BR5 and BR5-E-Ras cells
BR5 and BR5-E-Ras cells were cultured for 2 days on glass coverslips, fixed and stained to detect actin and β -catenin. Compared to controls, BR5-E-Ras cells displayed less prominent actin stress fibers and exhibited few β -catenin-staining adherens junctions. Bar = 50 μ m.

Figure 4. Contraction of collagen matrices BR5, BR5-E, and BR5-E-Ras cells

BR5, BR5-E and BR5-E-Ras cells were harvested and polymerized in collagen matrices. To determine floating collagen matrix contraction (FMC), matrices were released for 4 hr in medium containing growth factors and serum as indicated after which the samples were fixed and matrix areas measured. Matrix contraction values (starting – final) shown are the averages $\pm$ SD for duplicate samples. To determine stressed-released collagen matrix contraction (SMC), matrices were cultured 24 hr in serum-containing medium, rinsed and released for 1 hr in medium containing growth factors and serum as indicated, and then the samples were fixed and matrix areas measured. Matrix contraction values (starting – final) shown are the averages $\pm$ SD for triplicate samples. BR5, BR5-E, and BR5-E-Ras cells exhibited similar collagen matrix contraction activity.

Figure 5. Migration of BR5 and BR5-E-Ras in nested collagen matrices

Representative images of the times indicated from time-lapse microscopic videos of BR5 cells (Sup_1.mov) and BR5-E-Ras cells (Sup_2.mov) in nested collagen matrices in medium containing PDGF. Dermal equivalents were precontracted for 6 hr in serum-containing medium. Migration was imaged over 15 hr. Individual cells migrated from the dermal equivalent (dark region at the bottom) into the outer matrix after a lag phase of several hours. BR5 and BR5-E-Ras cells showed the same overall pattern of migration. Bar = 120 μ m.

Figure 6. Cell migration in nested matrices prepared with dermal equivalents precontracted for different times

(A) Nested collagen matrices were prepared with dermal equivalents precontracted in serum-containing medium for the times indicated. Samples were incubated 24 hr in medium containing PDGF and stained to detect cell nuclei. Bar = 120 μ m. (B) Same as “A.” Cell migration index values shown are the averages $\pm$ SD of duplicate samples. Migration of BR5-E-Ras cells appeared to be relatively unaffected by the prolonged, precontraction period whereas migration of BR5 cells was reduced markedly.

Figure 7. Growth factor and serum stimulation of BR5 and BR5-E-Ras in nested collagen matrices Nested collagen matrices were prepared with dermal equivalents precontracted 10 hr in serum-containing medium. Migration was measured after 24 hr in medium containing growth factors and serum as indicated. Cell migration index values shown are the averages $\pm$ SD of duplicate samples from three separate experiments. Unlike control cells for which PDGF selectively stimulated migration, BR5-E-Ras exhibited migration with or without added growth factors except for S1P, which inhibited migration of control and oncogenic Ras-transformed cells.

Figure 8. Migration of BR5, BR5-E, and BR5-E-Ras cells after scrape wounding

BR5, BR5-E and BR5-E-Ras were cultured overnight, scrape wounded using a pipette tip, and further incubated 24 hr in medium containing growth factors and serum as indicated. At the end of incubation, samples were fixed and stained with to detect actin (green) and cell nuclei (red). Compared to control cells, BR5-E-Ras showed increased migration under all conditions. Bar = 100 μ m.