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Spontaneous migration of cancer cells under conditions of mechanical confinement†

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Abstract

When cancer cells spread away from the primary tumor, they often follow the trajectories of lymphatic vessels, nerves, white matter tracts, or other heterogeneous structures in tissues. To better understand this form of guided cell migration we designed a series of microfluidic devices that mechanically constrain migrating cancer cells inside microchannels with cross-section comparable to cell size. We observed unexpectedly fast and persistent movement in one direction for several hours of cancer cells of different types. The persistent motility occurs spontaneously, in the absence of external gradients, suggesting the presence of intrinsic mechanisms driving cancer cell motility that are induced in conditions of mechanical confinement. To probe the mechanisms responsible for this behavior, we exposed cancer cells inside channels to drugs targeting the microtubules, and measured a significant reduction in the average migration speed. Surprisingly, a small number of cells appeared not to be affected by the treatment and displayed fast and persistent migration, comparable to the untreated cells. The new matrix-free, 3D-confined motility assay replicates critical interactions that cancer cells would normally have inside tissues, is compatible with high-content, high-throughput analysis of cellular motility at single cell level, and could provide useful insights into the biology of cancer cell migratory phenotype.

Introduction

Cancer cells can move out of the primary tumor and invade proximal and distant tissues where they can form metastases, which are ultimately responsible for 90% of cancer associated deaths.¹ It is generally accepted that the journey away from the primary tumor requires the cancer cells to interact with other cells, degrade matrix, or proliferate before they can establish thriving metastases.² At the same time, new evidence is emerging suggesting that the migration of the cancer cells forms the primary tumor or secondary metastasis and through tissues is favored along preexisting paths in the form of blood or lymphatic vessels,^{3,4} collagen fibers,⁵ white matter tracts,⁶ or normal routes of peritoneal fluid flow.⁷ This form of guided cancer cell migration appears to be important in the dissemination of cancer cells away from several types of malignant tumors. Careful studies of guided cell migration could provide useful insights into the biology of cancer cell migratory phenotype and create opportunities for new therapeutic approaches.

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Insight, innovation, integration

To study the invasion of cancer cells from primary tumors, we designed a microfluidic device that confines cells mechanically in channels of size comparable to cell size. When migration of various cancer cell lines was restricted in one dimension, we observed spontaneous, fast, and persistent migration of individual cells for several hours in the absence of external chemical gradients. This motility phenotype may be relevant to the preferential invasion of different tumor cells along lymph vessels, collagen fibers, or white matter tracts. The new assay is compatible with high-content, highthroughput analysis of cellular motility and could become a screening tool for studying cancer cells invasion.

Several experimental systems are currently available to study the migration of cancer cells. Migration of cancer cells is usually induced by soluble gradients and the migration space is assumed to be homogenous. Most of these assays are only partially relevant for guided cancer cell invasion due to the absence of a structured component. In many systems, the quantification of cell migration at single cell level is difficult, making the comparison between conditions and treatments less precise. For example, although *in vivo* measurements rely on sophisticated imaging systems to track individual cells moving away from the primary tumor site,^{8,9} precise quantification of the migration is complicated by the natural variability and complexity of the microenvironment experienced by the moving cells¹⁰ and by the close interaction with other cells, especially monocytes and lymphocytes.⁸ Although *in vitro* assays usually employ simpler matrices, predetermined cell types, and provide better control of these conditions, most are end-point assays and provide no detail on the actual cell movement, *e.g.* the Boyden chamber assay or recent modifications of it,^{11,12} or gel invasion assays from isolated cells^{13,14} or tumor spheroids.^{15,16} Moreover, these assays rely on cell count after a defined period of time and have to take into account the proliferation of cells that occurs during the assay and subtract that from the counting of migrating cancer cells,¹⁷ thus making the quantification of cellular motility less accurate.¹⁸ While detailed recording and analysis of moving cells are possible in some *in vitro* systems,¹⁹ these experiments are performed on flat surfaces, *e.g.* wound healing assay,²⁰ and extrapolating their relevance from 2D to the behavior of cancer cells in 3D tissues is challenging.^{21,22}

Recent developments in microfluidic technologies allowed for the observation of individual cell migration with high spatial and temporal resolution. Following the first demonstration of neutrophil chemotaxis in linear interleukin 8 gradients,²³ several studies reported observations of other cells migration towards different chemokines,^{24–27} gradient profiles,²⁸ or combinations of different chemokine gradients.^{24,29} However, when applied to cancer cells, most of these assays focused on the role of soluble gradients in directed cancer cell motility.^{30–33} In the absence of soluble gradients, other directional clues, in the form of adhesion gradients³⁴ or patterned surfaces,^{35–37} have been used for the study of migratory cells.

To overcome the shortcomings of current cancer cell invasion assays and provide precise measurement of cancer cell motility at single cell level and in high-throughput format, we designed a microfluidic system that confines cells into channels of cross-section comparable to cell size. This system enabled us to simultaneously track the migration of hundreds of individual cancer cells. We observed unexpected persistence in the migration of individual cancer cells which are capable of moving continuously in one direction for more than 12 h in the absence of any external chemical gradients. We observed that a small number of cells are still able to migrate through the channels following the exposure of the cancer cells to

microtubule destabilizing and stabilizing drugs at concentrations higher than those required for blocking proliferation. Overall, the new experimental system for measuring the invasion of cancer cells from the primary tumors could enable new studies for better understanding of cancer dissemination and identifying targets for drugs to block cancer cell motility and alter the natural course of malignant diseases.

Materials and methods

The proposed assay for cancer cell invasion consists of an array of channels with cross-section comparable to the size of the cancer cells. One central well is connected to the media around and above the device through four arrays of 50 parallel channels. Each channel has a rectangular cross-section, height either 3 or 12 μm , and width 6, 10, 12, 15, 18, 25, 30, 50, 75 or 100 μm . The walls of the channels are coated with extracellular matrix proteins. Several devices are arrayed at the bottom of a multi-well plate and the array of channels can be imaged using a microscope with an automated stage.

Microfluidic design

Microfluidic devices were manufactured by casting polydimethyl siloxane (PDMS, Dow Corning, Midland, MI) on a microstructured mold. The microstructured mold was fabricated using standard photolithographic technologies. For this purpose, a silicon wafer was coated with a 3 or 12 μm thin layer of photoresist (SU8, Microchem, Newton, MA) and processed following the standard protocol as recommended by the manufacturer. A second, thicker layer of approximately 50 μm was then photopatterned on the same wafer and aligned with respect to the first layer in order to define the central well and peripheral connections between channels. The mold was placed in a Petri dish and covered with PDMS freshly prepared according to the manufacturer's instructions. After baking for 8 h at 65 °C, the cast PDMS was removed from the mold, one well for each device was punched using a 2 mm puncher, and each device was cut using a 5 mm puncher. After exposure for 20 s to oxygen plasma in a plasma asher (March, Concord, CA), the devices are individually bonded on the coverslips at the bottom of 96-well plates (Mattek, Ashland, MA).

Surface modifications and hydrogel filling of channels

Immediately after bonding, and while the PDMS was still hydrophilic, 2 μL of a solution of 2 $\mu\text{g mL}^{-1}$ collagen IV were added inside the center well of each device. The strong capillary force ensured the collagen solution quickly filled the channels. Excess collagen could be later washed away by adding 5 μL of phosphate buffer (PBS) to the central well of the devices.

Cell lines

Seven cancer cell lines of human origin were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured following the specific protocols recommended by ATCC (Table 1). Before the migration assay, cells growing in cell culture flasks were washed with PBS, lifted from the surface using trypsin (0.02% trypsin-EDTA in PBS, Sigma) or 10 mM calcium chelator solution in buffer (EDTA, Sigma Aldrich, St. Louis, MO), suspended into 10 mL of media, centrifuged, and re-suspended into media at 10^6 cells mL^{-1} . Cells were then seeded in the central well of each device, by directly pipetting 3–4 μL of the cell suspension into each well. After loading the cells, 3 mL of corresponding media were added to each well of the multi-well plate, completely covering the devices.

Mouse fibroblast cell line (3T3, ATCC) was cultured in DMEM media with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (PenStrep). For experiments, cells

were seeded in the central well of each device following protocols identical to those for the cancer cells.

To test the effect of drugs on cell migration, several concentrations of Taxol (0.16, 1.6, and 16 μM , Sigma) and nocodazole (12 nM, 0.12 and 1.2 μM , Sigma) were prepared in culture media and mixed with the cell suspension and media before seeding the cells.

Time lapse imaging

To record the migration of cancer cells inside channels, we placed the multi-well plate on the motorized stage of a Zeiss Axiovert microscope fitted with an environmental chamber. The environment was set at 37.7 °C and 5% CO₂. Cells were imaged using 10 $\times$ objective and phase contrast. Three separate images from each array were acquired every 6 min for 24–48 h. No fluorescent dyes were used in these experiments.

Image analysis and statistical analysis

To quantify the migration of individual cells, we analyzed time-lapse images using the manual tracking function in the Image J software. The middle of cells was tracked through the series of frames and only cells entering the channels were tracked. We only considered cells migrating for more than 20 μm inside the channels, and with no interactions with other cells in front of them. Also, cells that divided during the experiment were excluded from the analysis. At least 20 cells migrating through the channel array were tracked for the majority of experimental conditions. Inside wider channels only the first cells to enter the channels were tracked. Average velocity over more than 10 h was calculated from temporary velocities calculated over each 6 or 10 min interval and presented as mean and standard error of the mean.

Final analysis of the data was performed in Excel and Sigma Plot. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. Individual dots represent outliers. To test for normal distribution of the velocity values we used Pearson's chi-square (χ^2) to estimate if the observed frequency distribution differs from a normal distribution with the same mean and standard deviation. Comparisons between normal-distributed populations were performed using T-test and 5% confidence interval.

Results

The newly developed motility assay for cancer cells enabled us to run several independent migration assays in 12, 24, or 96 well array format (Fig. 1). Single cell tracking of MDA-MB 231 breast cancer cells migrating inside collagen-coated, 12 $\times$ 15 $\times$ 600 μm channels revealed unexpected migration persistence for several hours in one direction. More than 80% of the cells entering the channels in the first 6 h moved from one end to the other of the channels without stopping or changing direction. We observed several cells turning back after reaching the distal end of the channel and then migrating towards the center well. Interestingly, the migration of these cells had the same velocity in both directions (Fig. 2). As more cells progressively enter the channels during the experiment, these cells interact with cells already present in the channels, complicating the analysis of cell migration.

To determine the optimal size of the channels, we measured the average migration speed of MDA-MB 231 breast cancer cells in channels of rectangular cross-section, that are 3 or 12 μm in height, and have widths ranging from 6 to 100 μm . Motility inside the 3 μm channels was the fastest for cells in the 25 μm wide channels, with an average of 50 $\mu\text{m h}^{-1}$ (Fig. 3). Motility inside smaller channels was slower, with an average of 20 $\mu\text{m h}^{-1}$, but maintained

the persistent characteristics. Motility inside the channels that were wider than 25 μm was less persistent, with frequent stops and cells passing each other. Average motility inside the 12 μm tall channels appeared not to be altered by the width of the channels. While cells completely filled channels narrower than 15 μm , they preferentially migrated on the side of the channels larger than 25 μm (see supplementary movies[†]). The migration was persistent as long as the cells remained on the side of the channels, and become more random whenever cells moved towards the center of the channels.

To probe the role of cell adhesion to the channel walls we measured the migration of MDA-MB 231 cells through channels coated with collagen IV, fibronectin or without any preliminary protein coating before the experiment. We observed no significant difference in the average migration speed over 24 h between the collagen and fibronectin coated channels (average migration speed 62.2 ± 4.6 and 46.3 ± 7.5 $\mu\text{m h}^{-1}$; $N=22$ and $N=17$, respectively, $p=0.07$). A statistically significant slower velocity was recorded inside channels not coated with extracellular matrix protein (average migration speed 19.7 ± 2.4 $\mu\text{m h}^{-1}$, $N=11$, $p<0.05$), although it is very likely that proteins from the serum in the media would provide a coating immediately after cell loading (supplementary Fig. 1[†]).

To test if the fast and persistent migration is a general characteristic of cancer cells, we measured motility in several human cancer cell lines inside empty $12 \times 15 \times 600$ μm channels, coated with collagen IV (Fig. 4). The motility of cells originating from prostate, breast, lung, colon and brain was measured for at least 12 h in at least three arrays of channels. Motility was the highest for the NCI-H446 lung cancer (average speed 85.7 ± 6.7 $\mu\text{m h}^{-1}$, $N=40$) and PC3 prostate cancer (average speed 67.8 ± 4.9 $\mu\text{m h}^{-1}$, $N=40$) and lower in other cell lines: breast adenocarcinoma MDA-MB 231 (average speed 52.1 ± 1.9 $\mu\text{m h}^{-1}$, $N=102$), lung carcinoma H1650 (average speed 49.5 ± 3.6 $\mu\text{m h}^{-1}$, $N=40$), prostate carcinoma LnCaP (average speed 28.0 ± 3.4 $\mu\text{m h}^{-1}$, $N=24$), glioblastoma U87 (average speed 36.4 ± 2.5 $\mu\text{m h}^{-1}$, $N=20$), and colon adenocarcinoma H29 (average speed 7.5 ± 1.1 $\mu\text{m h}^{-1}$, $N=13$). Interestingly, a small number of cancer cells in different cell lines displayed average velocities that were in excess of 100 $\mu\text{m h}^{-1}$ and were maintained for the entire length of the channels. Also of interest, the motility of mouse 3T3 fibroblasts through the same channels had average speed (42 ± 6.7 $\mu\text{m h}^{-1}$, $N=12$) comparable with cancer cells of epithelial origin. However, fibroblasts displayed more frequent changes in direction and extended pauses, sometimes for more than 12 h (supplementary Fig. 2[†]).

To probe the mechanisms responsible for the spontaneous and persistent cell migration under mechanical confinement inside channels, we exposed the cells to drugs that alter microtubule dynamics (Fig. 5). We observed a reduction in the speed of cancer cell migration through the channels in the presence of nocodazole of increasing concentration. Compared to control MDA-MB 231 cells that migrate at an average 43.4 ± 3.0 $\mu\text{m h}^{-1}$ ($N=44$), cells exposed to 12 nM nocodazole migrate at a comparable speed (46.7 ± 2.7 $\mu\text{m h}^{-1}$, $N=44$, $p=0.41$) and only cells exposed to higher concentrations show a statistically significant reduction in average migration speed compared to controls (120 nM nocodazole, 35.6 ± 1.8 $\mu\text{m h}^{-1}$, $N=51$, $p=0.03$, and 1.2 μM nocodazole, 13.9 ± 1.5 $\mu\text{m h}^{-1}$, $N=27$, $p<0.01$). Similarly, after treatment with Taxol, moving cells did not display significant alteration of their average migration speed at lower concentrations (160 nM Taxol, 45.2 ± 2.7 $\mu\text{m h}^{-1}$, $N=42$, $p=0.65$, and 1.6 μM Taxol, 48.8 ± 2.5 $\mu\text{m h}^{-1}$, $N=46$, $p=0.17$), but moved significantly slower at higher concentration (16 μM Taxol, 13.3 ± 1.0 $\mu\text{m h}^{-1}$, $N=51$, $p<0.01$). The effects of both Taxol and nocodazole appear to be comparable on cells already in the channels at the beginning of the experiments, as well as cells entering the

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channels at a later time. One could also notice that even at the maximum concentrations of Taxol and nocodazole (16 and 1.2 μM , respectively) a few cells managed to move faster than the average speed of untreated cells ($55 \mu\text{m h}^{-1}$).

Discussion

We developed a novel assay for cancer cell invasion that allowed us to directly observe and quantify cancer cell migration at single cell level, with very high spatial and temporal resolution. We made the unexpected observation that when cancer cells from different human cell lines are mechanically constrained inside channels of size comparable with cell size, they move spontaneously, in the absence of an external gradient. The motility was continuous and persistent in one direction for several hours, unlike the classical motility on flat surfaces, when motile and stationary phases alternate frequently.³⁸ We observed a large dispersion of the motility abilities of cells from different cancer cell lines and within the same cell line, with some cells moving faster than $100 \mu\text{m h}^{-1}$, comparable to some of the fastest human cancer cells on plasma-treated, flat cover glass.³⁹ In contrast to cells on a flat surface, which could maintain their speed only for short periods of time (on the order of minutes), in our assay cancer cells mechanically confined in channels are capable of persistently moving at high speed for several hours.

The persistent cell migration along predefined tracks has relevance to *in vivo* situations when cancer cells spread away from the primary tumor. One common situation for many malignant tumors is the migration of cancer cells along lymphatic vessels^{3,4} reaching the lymph nodes. In the clinical context, spreading of the cancer cells to the sentinel lymph nodes is usually a non-favorable prognostic sign prompting the need for aggressive surgical, chemical, and radiation therapy. Inside vascular capillaries, cancer cells are not only passively deformed, but also their active movement could be involved in the passage through the capillary bed and possibly in the formation of distant metastasis.⁴⁰ Extravascular migration of cancer cells along the periphery of blood vessels,^{41,42} lymphatics⁴³ or nerves⁴⁴ is supported by histopathology evidence and direct *in vivo* imaging of fluorescently tagged cancer cell migration. Some cancers, like the glioblastoma, migrate preferentially along white matter tracts in the brain and spread early in the evolution of the tumor.⁶ This represents a major clinical problem and even the extensive resection of the primary tumor leaves a large number of cells in distant parts of the brain that will continue the cancerous process. Intraperitoneal tumors like ovarian, gastro-intestinal, or pancreatic cancer often spread throughout the peritoneal cavity, following the virtual space between juxtaposed peritoneal surfaces and normal routes of peritoneal fluid flow.^{45,46} The transcoelomic route involves the migration of cancer cells between the mesothelial cell layers, and together with the lymphatic vessels is responsible for the dissemination of the majority of gastro-intestinal and ovarian carcinomas and sarcomas.^{7,47} Overall, better understanding of and the ability to control the migration of cancer cells along different structures inside tissues could have clinical implications for the treatment of many invasive cancers.

Our motility assay provides a simplified model of 3D mechanical interactions and controlled conditions during cell migration. When cells migrate inside the channel, they contact the extracellular matrix proteins throughout their entire circumference in a manner comparable to the interaction with a regular 3D environment. In addition, the rigid channel walls provide the mechanical support for cell migration, which would otherwise depend on heterogeneous structures in tissues. Because the extracellular matrix proteins are only present on the walls, the motility of the cells along the channel is not restricted by their abilities to degrade the matrix. These conditions enable reproducible measurement of the full migration potential of the cancer cells and document the relation between channel size and cell invasion. Previous studies have suggested that in fact the porosity of the gel could be more important than the

nature of the gel.⁴⁸ The ability of the cells to move along the main axis inside channels of uniform cross-section size, and represents a more controlled situation compared to the squeezing through pores of heterogeneous size in the gel.

The uniform cell migration along a predefined axis of the channel has practical benefits for quantification of cancer cell motility. It facilitates tracking and makes the position of cancer cells predictable during migration. In combination with the parallelization of the assay in multi-well plates, the new assay could become a productive tool for motility screening at single cell resolution. Such abilities are critically important for studying cancer cells, where metastasis is generally the result of single or few cell migration and proliferation abilities, rather than the result of average cancer cell properties. These advantages are even more important when migration assays are scaled up, and intense efforts are currently dedicated to the development of high-throughput methods for screening large libraries of cells and potential drugs.

The fast and persistent migration of cancer cells through matrix-protein-coated channels in the absence of external soluble gradients represents, to the best of our knowledge, a unique feature for our experimental system. Emerging evidence suggests that different cell types show distinct migratory phenotypes inside channels. For example, differentiated HL-60 cells (a common neutrophil model) display uniform migration through small but not through large channels, following chemoattractant gradients.⁴⁹ Mouse, bone-marrow derived dendritic cells display random patterns of migration inside 4 μm diameter channels.⁵⁰ Mouse 3T3 fibroblasts were able to enter the channels, however they migrated slower than the cancer cells and displayed frequent changes in direction and pauses for extended periods of time during their migration. The average speed of fibroblasts in our device is faster than previously reported values on 2D surfaces, and in the low range of values reported for fibroblasts moving along narrow adhesive 1D patterns.^{36,37}

Surprisingly, after exposing cancer cells to Taxol at concentrations higher than those required to stop proliferation⁵¹ of cancer cells, a small number of cells are still able to migrate through the channels. Although the number of such cells is low, it is tempting to speculate that unresponsive cells like these remain able to traverse large distances in tissues and establish metastasis despite treatment. The effect of these cells on the average velocity is small and could be missed using current bulk chemotaxis assays, but their clinical and biological importance may be higher than the average cells. Our results also point towards a role for the microtubules in the persistent migration. Microtubules are already known to play a critical role in epithelial cell polarization⁵² and in the stochastic behavior of polarizing cells.⁵³ It is possible that the mechanical confinement of the cancer cell results in reorganization of the cytoskeleton along the axis of the channel and this drives the internal polarization required for persistent migration. This effect is comparable to the effect of polarized microtubule distribution in fibroblasts spreading on 3D matrixes⁵⁴ or along 1D topographies,^{36,37} and may represent a reminiscence of the original polarization in normal epithelial cells.⁵² Other mechanisms directing the preferential polymerization of actin in the direction of cell movement may be involved as well, as suggested by recent biophysical models.⁵⁵ Overall, the observations of cell migration in a mechanically confined environment of microscale channels suggest that the motility of individual cancer cells is the result of robust mechanisms and these may sometimes not be affected by the current regimens of chemotherapy.

Conclusions

We have developed a cell motility assay that allows us to precisely quantify the migration of individual cancer cells inside channels either coated or filled with extracellular matrix

proteins. The new assay can be used to screen for migration abilities in conditions relevant to *in vivo* conditions experienced by cells after leaving the primary tumor. Compared to the tremendous progress over the past two decades in uncovering efficient drugs against cancer cell proliferation, our options to specifically control cancer cell migration are still very limited. Considering that metastases are the major cause of death in cancer patients, new technologies for investigating cancer cell invasion are poised to play a central role in developing new therapeutic approaches for preventing or delaying the formation of metastasis, and extending the life of cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Single cell motility analysis and schematics of the microfluidic device

(a) Cancer cell migration is observed inside channels formed between a PDMS piece and a glass coverslip. (b) Multiple devices are fixed in the wells of a 96-well plate for high-throughput motility screening. One such device is presented in more detail in the inset.

Fig. 2. Persistent migration of cancer cells under mechanical confinement inside the channels
 (a) MDA-MB 231 breast cancer cells move persistently away from the seeding chamber. Many cells that reverse their direction maintain the same speed of migration as before. (b) Sequential frames over 4 h show the displacement of two cells, one with amoeboid and one with mesenchymal morphology, inside the same channel.

Fig. 3. Average motility of MDA-MB 231 breast cancer in channels with different cross-section area

(a) Inside 3 μm tall channels (filled circles), the velocity of migration increases with the increasing cross-section of the channels, reaching an optimum at 25 μm width, and then decreases progressively inside wider channels. In 12 μm tall channels (empty squares), migration appears not to be altered by the width of the channel for the range of dimensions tested. While the cells completely fill the smaller channels, they are preferentially distributed towards the sides inside the wider channels. (b) Confocal imaging of MDA-MB 231 loaded with cell tracker dye orange (CMRA, Invitrogen) shows that the majority of moving cells are in contact with all four walls of the channel, and that a smaller number of cells will only touch three or two of the walls.

Fig. 4. Average motility of cells from different cell lines

The migration of seven cell lines was tested in channels with rectangular, $12 \pm 15 \mu\text{m}$ cross-section. Cell lines known to be more aggressive display highest motility. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

Fig. 5. The effects of Taxol and nocodazole on MDA-MB 231 breast cancer line

(a) Taxol inhibited persistent motility at concentrations above 1.6 μM and nocodazole at concentrations above 120 nM. Only nocodazole at concentrations above 1.2 μM and Taxol above 16 μM appear to inhibit the migration of the fastest moving cells. (b) Individual tracks of cell displacement in the presence of nocodazole (1.2 μM) and Taxol (16 μM) show slower and less persistent movement of the treated cells that enter the channels.

Table 1

Cancer cell lines tested in the new confined-motility assay

Designation	Source	Culture media (as recommended by ATCC)
H1650	Lung adenocarcinoma	RPMI1640 with 10% FBS and 1% PenStrep
H446	Lung carcinoma	RPMI1640 with 5% FBS and 1% PenStrep
PC3	Prostate adenocarcinoma	F-12K with 10% FBS and 1% PenStrep
LnCaP	Prostate carcinoma	RPMI1640 with 10% FBS and 1% PenStrep
MDA-MB 231	Breast adenocarcinoma	DMEM with 10% FBS and 1% PenStrep
U-87 MG	Glioblastoma	EMEM with 10% FBS and 1% PenStrep
HT-29	Colorectal adenocarcinoma	McCoy's 5a with 10% FBS and 1% PenStrep