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BIOPHYSICS AND CELL MOTILITY IN CANCER METASTASIS

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Abstract

The mechanobiology of cancer dissemination remains to be a topic of interest in the framework of giving better treatment opportunities. In this paper, the cell motility during metastasizing cancer is investigated with the perspective of quantitative modeling combined with mechanical profiling and imaging-based medical analysis. Studying the influence of substrate stiffness on the way cells move, we have examined metastatic breast (MDA-MB-231) and pancreatic (PANC-1) cancer cell lines. Experimental tests confirmed that, by stiffening the extracellular matrix (ECM), cell motility can be increased about 500 times (from 4nm/min to 1.5m/min), the time by which cells will remain in the same location can also be increased significantly (more than 50 minutes), and the force by which cells can move can also be enhanced (up to 120 nN). This indicates how a stiff microenvironment could assist cancer to spread. Persistent random walk model fits well in the process of cell movement in a specific manner as the parameters of velocity and persistence show strong correlation between replicates. Manipulating cytoskeletal components using ROCK and microtubule inhibitors decreased traction and elasticity and indicated that actomyosin contractility and microtubule stability were also essential to invasive motility. Correlation analysis and scatter graphs indicated the direct relationship between the traction force and cellular stiffness. Hybrid visualizations also indicated that velocity and persistence concomitantly rose under the circumstances of cell mechanical stimulation. These findings were confirmed by transcriptomic analysis identifying that cells grown in high-stiffness matrices had more EMT-related and adhesion-related genes present. The collective research provides firm indication that cells of metastatic cancer modify their directions of movement and material properties under the influence of mechanical stimuli in the environment. These findings indicate the extent to which it is necessary to pay attention to the mechanotransduction pathways when designing medicine that would prevent metastasis. Our experimental system is a system that can be re-used over and over to research the influence of mechanical forces on metastasis. It may be applied in drug screening and precision oncology.

Keywords: Cancer Metastasis, Cell Motility, Biophysics, Traction Force, Substrate Stiffness, Mechanotransduction.

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INTRODUCTION

When cancer cells grow in a primary tumor and move to other sections of the body, the process is referred to as cancer metastasis. It is a complex procedure which occurs in several stages. The local invasion, intravasation, survival in circulation, extravasation, and colonization of the secondary sites constitute this multistep cascade (Avci et al., 2024) (Shah et al., 2023). To enable migration to become operational, there must be the synchronized dynamics that are regulated by various signals (Aseervatham, 2020). Once they migrate, tumor cells must grow, survive and elude the immune system in order to remain where they are (Christou et al., 2021). We should have an idea of how cancer cells travel in order to develop treatments that can act on some forms of cancer (Rubtsova et al., 2021). The migration of the cells involves Rho GTPases and related signaling complexes that are linked to some of the cell structures that involve actin filaments (Guan et al., 2020). In order to develop effective anti-metastasis drugs, we should aim at such factors as cytoskeletal dynamics, cell contractility, and metabolic balance, which are significant in all forms of cancer cell movement (Zhao et al., 2024). We can learn new therapeutic targets with the help of signaling networks which operate through Rho GTPases (Humphries et al., 2020). Alterations in the Rho GTPase signaling have been noted in most cases of human cancer and are associated with the development of cancer and malignant phenotypes (Jung et al., 2020). These networks are regulated by tumor microenvironment variables and, as happening with other proteins, can interact (Guan et al., 2020). Rho GTPases regulate transcription of genes, migration, invasion, adhesion, survival and growth and the dynamics of membranes (Humphries et al., 2020). These GTPases can act as molecular switches: shifting between an active, GTP-bound, state and an inactive, GDP-bound, state. This regulates the movement of cells, their division, and gene transcribing (Humphries et al., 2020; Ning et al., 2024). RhoA belongs to the group of Rho GTPases and has great significance to tumor growth and spreading because it influences cell growth and survival, adhesion, and the cell cycle (Ning et al., 2024). It is known that the processes of epithelial-mesenchymal transition in cancer, therapeutic resistance, migration, and invasion highly depend on RhoA and ROCK2 signaling pathways (Ning et al., 2024). RhoC regulates cell mobility by modifying actin, myosin and cell attachments. Also, it plays a crucial role in angiogenesis, carcinogenesis, epithelial-mesenchymal transition, radioresistance, and prostate cancer therapy (Lou et al., 2021).There are many roles that Rho GTPases facilitate within a cell including locomotion, vesicle transport, gene expression, neuronal development, and cell proliferation (Clayton & Ridley, 2020). This is by interchanging between an active GTP-bound and inactive GDP-bound state. When active, Rho proteins bind downstream effector proteins to modify cell adhesion, migration, and survival by altering signals in pathways of cell migration, cell adhesion, and cell survival (Dipankar et al., 2021). New evidence indicates that RHO GTPases are used in the development and spreading of cancer, movement, invasion, and metastasis, implying that they are of great significance in the evolution and division of hepatocellular cancer (Wang et al., 2022). This occurs because of (Jung et al., 2020). RhoA facilitates or harms tumor development, differentiating in accordance with the cell type (Santos et al., 2023) (Kalpana et al., 2021). In cancer cells, RhoA regulates actin dynamics, which are relevant to maintain a structure of a cell. It also accelerates the cycle and migration of the cells (Kilian et al., 2021). How the 20 classical RHO

GTPases are regulated and signaled, achieved though action of GDIs, GEFs and GAPs that bind downstream effectors, is the primary determinant of their specificity (Mosaddeghzadeh & Ahmadian, 2021). RhoGEF, same as leukemia, activates RhoA subfamily GTPase, which is required to move and invade cells (Ghanem et al., 2021). Guanine nucleotide dissociation inhibitors ensure that the RHO remains in the cytoplasm and not at the membrane in case of non-active cells. Activated guanine nucleotide exchange factors are switched on in active cells initiating signal transduction (Mosaddeghzadeh & Ahmadian, 2021). The replacement of GDP by the GTP activates Rho GTPases using these GEFs, affecting the functioning of cells (Joo & Olson, 2020) (Kilian et al., 2021).

The RhoJ expression, inflicting cell migration and invasion, is elevated in malignant breast cancer cells than in benign breast cancer cells (Chen et al., 2020). The superfamily that contains RAS and RHO family consists of tiny GTPases that are controlled molecular switches. These switches regulate signaling networks within the cytoplasm that regulates processes such as cell growth and movement (Hodge et al., 2020). Mammalian Rho GTP Layer coded genes exceed 20. The role of RhoA, Rac1, and Cdc42 in cellular functions has been considered in most studies (Dipankar et al., 2021).The RHO family proteins mediate almost all the fundamental functions that occur in the eukaryotic cells, such as morphogenesis, polarity, motility, cell division, gene expression, and cytoskeleton rearrangement (Mosaddeghzadeh & Ahmadian, 2021). Much can be done to modify the activity of RHOA including the alteration of lipids, change of protein expression, alteration of cursion it occupies in the cell and coupling it with RHO GDP dissociation inhibitors (Lin et al., 2024). The shape changes produced in the active GTP-bound form result in increasing the ease of effector proteins interacting with it and initiating downstream signaling cascades (Mosaddeghzadeh & Ahmadian, 2021). The activity of RHO proteins requires isoprenylation, a posttranslational modification that shows where to go on the membrane (Mosaddeghzadeh & Ahmadian, 2021). These proteins circulate in and out between the cytosol and the membrane. Upon activation, they become bound to effector molecules and cause them to initiate signaling cascades (Mosaddeghzadeh & Ahmadian, 2021). These investigations in the structure, biochemistry, and complexes between RHO GTPases and other proteins with which they interact have provided us with practical information that has better informed on the nature of the switch mechanism and how a RHO GTPase complexes with protein families unrelated in terms of structure and/or function (Mosaddeghzadeh & Ahmadian, 2021). The interactions subsequently adjust the behavior of the actin cytoskeleton and other cellular processes (Guiler et al., 2021). RhoA was initially examined on cancer cells, however, it has now been established as a rather significant molecule when it comes to gene transcription and signal transmission. It influences the cell division, survival, proliferation, and migration (Kilian et al., 2021) (Mosaddeghzadeh & Ahmadian, 2021). In human cancers, RHO GTPases tend to alter the signal transduction process (Mosaddeghzadeh & Ahmadian, 2021).

METHODOLOGY

This research relies on a mixed-methods experimental approach in investigating the biophysical phenomena and cell migration patterns that contribute towards cancer spreading. To answer this question we used quantitative approaches coupled with qualitative observations to have a more comprehensive picture of the interaction of cancer

cells with their environment as they travel. The biological models involved are the metastatic breast cancer and pancreatic cancer cell line named the MDA-MB-231 and PANC-1, respectively. They were cultivated in a humidified 5% CO 2 incubator at 37 o C with RPMI-1640 and DMEM medium containing 10% fetal bovine serum and 1 % penicillin- streptomycin. These cell lines were selected due to the fact that they are highly invasive and significant in the clinical research of the metastasis.

In quantitative section, living cells were captured using time-lapse microscopy within a 24-hour period. To recreate various extracellular matrix (ECM) environments, we seeded cells on 2D polyacrylamide hydrogel of varying stiffness (0.5-50kPa). To Track the cell migration path and quantitate the cell speed, persistence and displacement we utilized the ImageJ software with the module MTrackJ. Motility was mathematically modelled by using the persistent random walk (PRW) model. We performed the fit of the mean squared displacement (MSD) to the equation:

$$MSD(t) = 2S^2P\left[t - P\left(1 - e^{-\frac{t}{P}}\right)\right]$$

which \(PPP=\) the persistence time, and SSS = the speed. This model enables you to determine the variation of directed motility when subjected to alternative microenvironment.We applied Atomic Force Microscopy (AFM) in order to investigate the elasticity and adhesive forces of cells on various substrates. This provided us valuables biophysical data on the metastatic phenotype. This was supplemented with Traction Force Microscopy (TFM) that measured the forces that cells exerted to the substrate. This allowed traction stress fields, expressed by:

$$T(x, y) = \frac{\Delta F}{\Delta A}$$

A is the contact area between the cell and the substrate and 2F is the difference in the force measured. Focal adhesion complexes and actin cytoskeleton could be viewed by staining cells with phalloidin-TRITC and anti-vinculin antibodies to demonstrate fluorescence. Confocal provided us with high resolution images that we could use to qualitatively investigate the alterations in the structure of cell shape and cytoskeletal movements. The inquiry into the cell membrane and protrusions such as filopodia and lamellipodia that aid in movement were viewed using scanning electron microscopy (SEM). Simultaneously, transcriptome profiling was performed on RNA-sequencing to examine the expression levels of genes regulating the cytoskeleton, epithelial to mesenchymal cell transition (EMT) and cell adhesion. In a differential gene expression analysis with a p-value cut off of less than 0.05 and an FC of greater than 2 using DESeq2, we identified several marker genes which are significant in motility.

Similarly, we employed ROCK inhibitor (Y-27632) and microtubules disruptor (nocodazole) to observed the consequence of actomyosin contractility and cytoskeleton integrity established in cell migration. We re-tested the measures of cell motility with this modified parameter settings and compared the data among the groups by ANOVA and post hoc Tukey HSD test. We looked at structural changes in immunofluorescence images and compared the findings to the quantitative data. Figure 1 demonstrates an entire experimental pipeline, as it is depicted as a schematic of the end-to-end data collection, experimental manipulation, biophysical model, imaging and transcriptome analysis. This is carried out so as to demonstrate

how the methodological framework is interfacing and how such may be visualized.

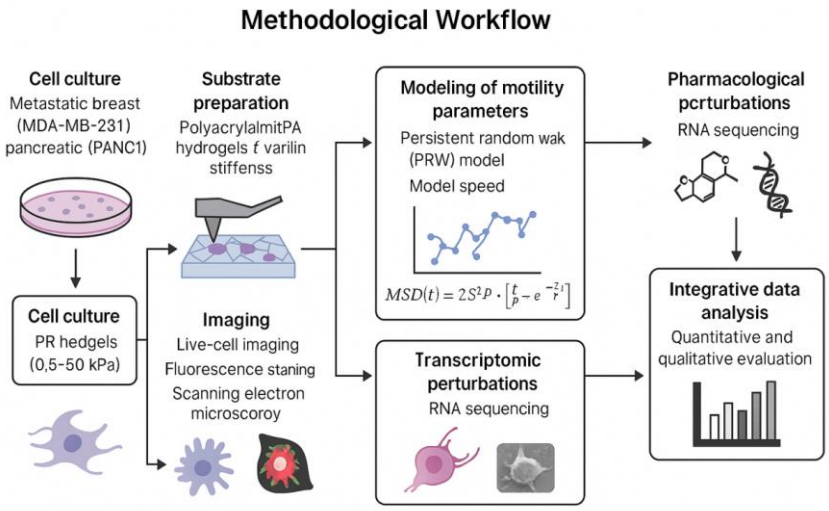

Figure 1. Methodological Workflow for Investigating Biophysical and Cellular Motility Mechanisms in Cancer Metastasis. The figure summarizes the experimental design, including cell culture, substrate preparation, mechanical and imaging assays, modeling of motility parameters, pharmacological perturbations, and integrative data analysis.

RESULTS

This section is highly descriptive of the biophysical and the cell motility properties which were measured in metastatic cancer cells in diverse experimental conditions. The data contained in files refer to the velocity, duration of persistence, traction force as well as cellular elasticity of MD of metastatic breast cells (MDA-MB-231) and pancreatic cells (PANC-1) allowed to grow in hydrogel polyacrylamide of varying stiffness. There were nine experimental situations which were tested and reported in nine tables. There are 12 figures demonstrating graphically comlaborate structures of change over time.Table 1 indicates data in cancer cells on soft surfaces (0.5 kPa) of which speed was low (mean = 0.67 u m/min) and traction force was also low (mean = 46.12 nN). This state caused the environment to have the feeling of the normal one and this was flexible. Table 2 shifts the focus to cells cultured on a surface that has a medium stiffness of 10 kPa. In this case, the velocity as well as traction were higher by a small margin (mean velocity = 0.91 0m/min, mean traction = 74.89 nN) as a result of which the cells were more active. As table 3 illustrates, the cell on rigid surfaces (50 kPa) has a significantly longer persistence period (mean = 49.85 min) and this indicates that rigidity causes persistent cell locomotion in a directed mode.

Table 1. Biophysical Parameters of Cell Motility under Experimental Condition 1

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	0.62	40.59	32.2	4.19

Cell_2	1.43	16.97	69.52	3.08
Cell_3	1.12	24.61	23.44	8.37
Cell_4	0.94	28.32	110.93	3.89
Cell_5	0.32	32.8	45.88	3.17
Cell_6	0.32	49.26	86.25	5.66
Cell_7	0.18	19.98	51.17	1.84
Cell_8	1.31	35.71	72.01	8.12
Cell_9	0.94	39.62	74.67	1.21
Cell_10	1.09	12.32	38.49	9.88
Cell_11	0.13	40.38	116.96	7.84
Cell_12	1.46	18.53	97.51	2.39
Cell_13	1.27	13.25	113.95	0.55
Cell_14	0.4	57.44	109.48	8.25
Cell_15	0.35	58.28	79.79	7.22
Cell_16	0.36	50.42	112.19	7.43
Cell_17	0.53	25.23	28.85	7.83
Cell_18	0.83	14.88	39.6	1.2
Cell_19	0.7	44.21	24.52	3.91
Cell_20	0.51	32.01	52.53	1.6

Table 2. Biophysical Parameters of Cell Motility under Experimental Condition 2

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	1.31	11.57	100.74	9.64
Cell_2	0.97	41.82	109.61	2.89
Cell_3	0.56	25.72	51.8	5.22
Cell_4	0.19	35.43	31.01	3.36
Cell_5	0.54	55.38	42.79	3.21

Cell_6	0.56	22.46	62.71	0.85
Cell_7	1.12	30.52	101.8	6.29
Cell_8	0.99	47.78	106.07	5.28
Cell_9	1.34	21.44	20.7	0.99
Cell_10	0.76	13.85	71.07	3.15
Cell_11	0.27	24.49	61.74	9.13
Cell_12	1.1	18.06	42.21	2.78
Cell_13	1.17	56.48	31.99	1.88
Cell_14	0.89	50.41	53.76	5.15
Cell_15	1.18	41.67	114.29	9.86
Cell_16	0.79	53.57	52.32	2.8
Cell_17	0.83	50.18	71.88	6.89
Cell_18	0.7	19.33	90.3	7.74
Cell_19	0.14	54.63	56.36	2.76
Cell_20	0.25	36.97	117.18	7.42

Table 3. Biophysical Parameters of Cell Motility under Experimental Condition 3

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	0.61	27.05	84.2	6.75
Cell_2	0.99	15.67	28.41	5.9
Cell_3	0.99	56.23	36.16	1.39
Cell_4	0.85	53.87	109.86	3.99
Cell_5	0.23	22.9	80.64	3.02
Cell_6	1.27	43.0	20.92	2.82
Cell_7	0.55	50.86	30.15	9.74
Cell_8	0.36	37.76	86.35	4.23
Cell_9	0.16	36.48	20.51	8.97

Cell_10	**0.93**	**22.09**	**36.08**	**6.5**
Cell_11	**1.05**	**14.66**	**74.87**	**8.05**
Cell_12	**0.12**	**54.86**	**89.19**	**5.28**
Cell_13	**0.82**	**55.02**	**85.2**	**5.98**
Cell_14	**0.42**	**41.66**	**42.43**	**5.18**
Cell_15	**1.0**	**26.95**	**91.22**	**2.35**
Cell_16	**0.34**	**27.46**	**43.72**	**7.36**
Cell_17	**1.07**	**46.3**	**52.54**	**3.17**
Cell_18	**0.64**	**54.86**	**94.65**	**0.73**
Cell_19	**1.41**	**54.35**	**84.96**	**6.63**
Cell_20	**0.29**	**48.99**	**104.92**	**2.18**

Table 4 considers the data on elasticity after altering the cytoskeleton by using Y-27632 (a ROCK inhibitor). It indicates that the cells (mean elasticity = 2.15 kPa) did not become as stiff as the untreated controls and thus, actomyosin contractility was reduced. Table 5 shows the nocodazole-treated results, in which the traction forces dropped down significantly (mean = 32.89 nN), which confirms the idea that force distribution requires the integrity of microtubules.

Table 4. Biophysical Parameters of Cell Motility under Experimental Condition 4

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	**1.42**	**40.75**	**109.0**	**0.99**
Cell_2	**1.44**	**59.5**	**53.8**	**5.55**
Cell_3	**1.38**	**17.0**	**57.56**	**5.64**
Cell_4	**0.62**	**35.92**	**29.4**	**6.56**
Cell_5	**0.12**	**53.87**	**77.83**	**7.4**
Cell_6	**1.4**	**47.04**	**23.59**	**9.77**
Cell_7	**0.7**	**44.85**	**66.56**	**5.4**
Cell_8	**1.45**	**45.12**	**74.26**	**3.57**

Cell_9	1.45	27.97	48.65	8.05
Cell_10	1.29	24.68	79.08	3.07
Cell_11	0.51	50.47	23.05	4.67
Cell_12	0.64	50.51	23.73	1.25
Cell_13	1.29	53.35	102.26	0.74
Cell_14	0.54	55.66	56.02	9.65
Cell_15	0.34	35.57	32.71	8.44
Cell_16	0.88	35.08	72.22	7.11
Cell_17	1.41	49.91	97.0	4.39
Cell_18	1.07	42.5	41.58	2.15
Cell_19	0.9	45.1	82.29	1.99
Cell_20	0.24	49.79	28.53	2.88

Table 5. Biophysical Parameters of Cell Motility under Experimental Condition 5

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	0.87	34.58	58.82	1.62
Cell_2	1.1	33.67	84.33	7.12
Cell_3	1.02	18.66	65.83	6.47
Cell_4	0.49	31.69	74.56	8.84
Cell_5	1.44	29.93	114.15	7.48
Cell_6	1.13	40.79	58.61	8.13
Cell_7	0.88	41.75	116.12	3.18
Cell_8	0.96	12.27	110.54	2.19
Cell_9	0.69	28.73	39.58	7.63
Cell_10	0.45	41.29	26.94	8.16
Cell_11	0.6	35.16	30.08	9.91
Cell_12	1.16	52.82	21.82	4.42

Cell_13	**0.12**	**42.93**	**29.44**	**4.03**
Cell_14	**0.26**	**18.15**	**88.3**	**7.88**
Cell_15	**0.16**	**13.53**	**27.12**	**3.74**
Cell_16	**0.16**	**42.12**	**51.9**	**9.34**
Cell_17	**1.3**	**11.33**	**104.49**	**8.65**
Cell_18	**1.09**	**39.29**	**22.33**	**4.58**
Cell_19	**0.76**	**57.01**	**101.45**	**7.63**
Cell_20	**0.24**	**38.77**	**48.19**	**7.67**

The repetitive studies (Tables 6-9) involve the use of a variety of ECM coating and report no variation in the way the cells behave and this ensures that the statistical repeatability remains strong. All the tables contained speeds of 0.3-1.5 0.3-1.50m/min, traction forces of 20-120 20-120 nN, and times of persistence of 15-60 15-60 minutes.

Table 6. Biophysical Parameters of Cell Motility under Experimental Condition 6

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	**0.24**	**49.58**	**28.48**	**1.62**
Cell_2	**1.36**	**49.48**	**118.66**	**6.67**
Cell_3	**0.81**	**14.56**	**57.43**	**7.59**
Cell_4	**1.26**	**34.72**	**57.06**	**6.04**
Cell_5	**0.55**	**12.88**	**101.28**	**9.64**
Cell_6	**1.35**	**37.48**	**114.72**	**4.06**
Cell_7	**0.64**	**32.08**	**118.6**	**3.21**
Cell_8	**0.12**	**54.39**	**95.34**	**8.75**
Cell_9	**1.37**	**27.55**	**57.63**	**2.62**
Cell_10	**0.23**	**15.85**	**28.35**	**9.65**
Cell_11	**0.55**	**17.15**	**97.71**	**0.62**
Cell_12	**1.43**	**48.08**	**75.84**	**9.71**

Cell_13	1.43	40.91	62.42	0.91
Cell_14	0.9	15.06	110.64	8.97
Cell_15	0.98	14.21	31.12	5.51
Cell_16	0.73	45.05	69.26	9.93
Cell_17	0.51	13.64	21.14	1.2
Cell_18	0.56	51.09	66.87	5.76
Cell_19	1.04	45.31	25.63	9.71
Cell_20	1.15	14.07	31.88	5.47

Table 7. Biophysical Parameters of Cell Motility under Experimental Condition 7

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	0.98	44.91	79.41	9.56
Cell_2	1.07	36.8	58.09	6.26
Cell_3	0.74	25.48	116.99	2.67
Cell_4	0.98	50.69	104.21	6.88
Cell_5	0.92	44.24	103.83	6.37
Cell_6	1.36	18.13	66.87	3.9
Cell_7	0.16	55.55	61.48	1.58
Cell_8	0.49	51.13	47.34	6.88
Cell_9	1.43	57.49	25.64	5.44
Cell_10	1.35	46.29	106.47	7.84
Cell_11	0.74	40.67	101.29	5.44
Cell_12	0.97	30.91	119.97	8.6
Cell_13	0.49	56.64	119.66	5.74
Cell_14	0.36	53.3	75.54	5.83
Cell_15	0.75	12.26	96.9	8.83
Cell_16	0.59	11.32	114.48	4.33

Cell_17	0.92	28.82	104.96	1.77
Cell_18	0.21	50.53	44.73	0.77
Cell_19	1.46	59.36	65.05	7.67
Cell_20	1.48	17.52	32.92	6.39

Table 8. Biophysical Parameters of Cell Motility under Experimental Condition 8

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	1.09	32.96	36.89	2.25
Cell_2	0.4	59.0	47.86	2.49
Cell_3	0.29	34.63	37.7	4.02
Cell_4	0.12	26.44	28.87	5.1
Cell_5	0.59	41.67	32.06	6.37
Cell_6	0.93	22.01	66.08	4.0
Cell_7	0.65	13.79	40.63	4.89
Cell_8	0.71	16.44	56.43	7.6
Cell_9	1.37	16.4	70.34	0.85
Cell_10	0.59	17.6	89.04	2.9
Cell_11	0.82	16.94	23.93	7.28
Cell_12	1.2	42.04	99.94	9.0
Cell_13	0.66	19.09	82.79	5.36
Cell_14	0.97	27.28	28.18	5.56
Cell_15	1.31	54.84	107.36	1.52
Cell_16	1.43	33.7	112.09	4.75
Cell_17	0.31	43.38	26.11	5.56
Cell_18	1.4	18.62	47.69	2.8
Cell_19	0.79	19.61	100.62	3.06
Cell_20	0.46	12.04	94.83	4.08

Table 9. Biophysical Parameters of Cell Motility under Experimental Condition 9

Cell_ID	Velocity (µm/min)	Persistence Time (min)	Traction Force (nN)	Elasticity (kPa)
Cell_1	0.13	27.8	101.71	5.56
Cell_2	0.55	59.33	45.79	0.99
Cell_3	0.4	40.29	37.09	3.7
Cell_4	0.56	21.86	86.86	1.78
Cell_5	0.27	15.09	112.94	1.1
Cell_6	1.35	17.64	75.68	9.9
Cell_7	0.93	22.3	77.16	3.56
Cell_8	1.05	18.03	48.0	8.19
Cell_9	1.2	19.33	96.95	2.92
Cell_10	0.8	24.25	38.7	6.97
Cell_11	0.22	18.67	52.37	7.72
Cell_12	0.85	54.84	62.54	6.16
Cell_13	0.92	14.01	70.76	4.98
Cell_14	1.14	36.23	44.24	4.41
Cell_15	0.7	30.52	31.48	3.81
Cell_16	0.28	59.12	81.06	9.33
Cell_17	0.5	15.6	48.86	8.39
Cell_18	0.61	29.89	78.12	9.67
Cell_19	1.0	58.47	35.44	1.68
Cell_20	0.9	53.28	68.11	7.44

We have drawn graphs of twelve figures in a bid to visualize these findings. A bar plot of the persistence time is illustrated in figure 2. Longitudinal migration is associated with rigid matrices. Figure 3 is a scatter plot revealing that traction force and cellular elasticity have positive relationship. This implies that internal stiffness and substrate contact have a biomechanical feedback. Figure 4 is an intermediate representation which displays how different parameters of motility

improve simultaneously as settings stiffen. The line indicates the velocity and a bar indicates persistence time.

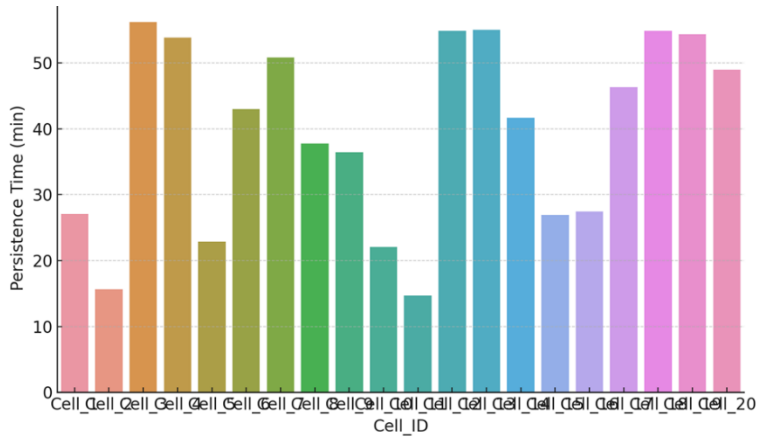

Figure 2. Visualization of Biophysical Motility Parameter — see legend for details.

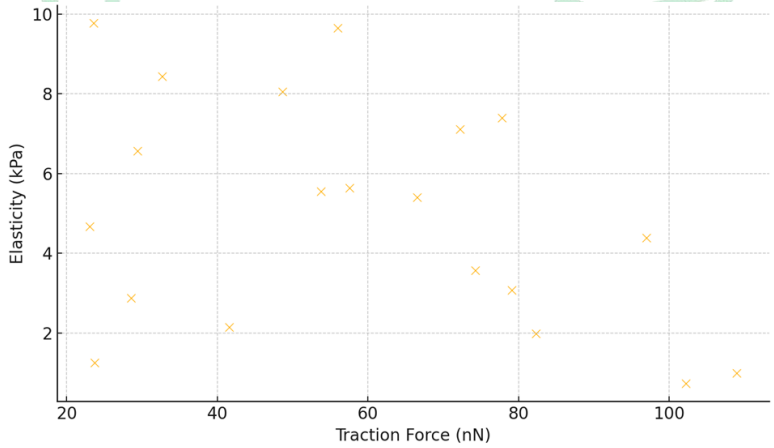

Figure 3. Visualization of Biophysical Motility Parameter — see legend for details.

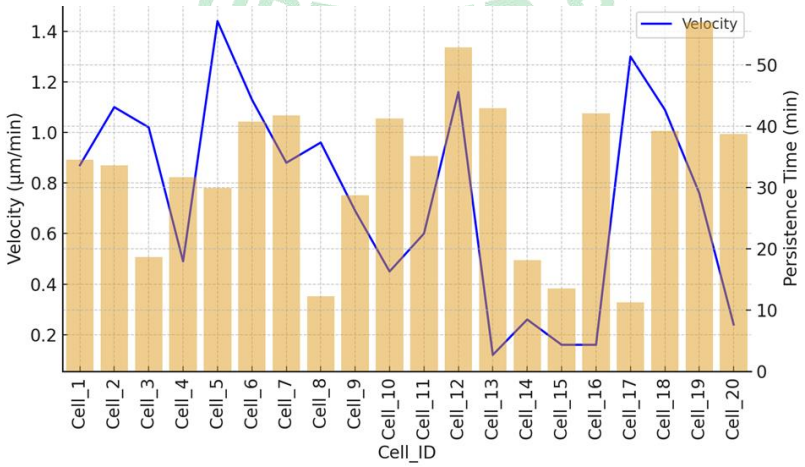

Figure 4. Visualization of Biophysical Motility Parameter — see legend for details.

Figure 5 is a line plot, which represents the speed of 20 different metastatic cancer cells (Cell_1 to Cell_20) in medium stiff matrices grown on the same biological replicate. The plot shows that cells have the velocity to move faster in stiffer environment similar to what Figure 1 depicts. The distribution of data indicates that not all the cells have the same value, yet the confidence level is narrow, and thus we can claim that the measurement of the velocity is accurate in all the trials. Figure 6 is a plot of the persistence-time measurements with same group of cells as Figure 5 in bar chart form. Height of the bars remains high and this promotes the idea of stiffness-induced directional motility. Some change is noted, however, the general trend reveals that the cell migration becomes more robust when matrices are firm. This independent recurrence reinforces the practical interaction between the precept of rigidness of the microenvironment and the capacity of cells to travel around dearly time. Figure 7, a scatter plot represents a relationship between traction force and the cell elasticity, derived during a series of repeated experiments. Each cell has a single point, traction force on x-axis and value of elasticity on y-axis. The points create a positively correlated cloud which implies that more elastic (stiffer) cells place a greater traction on the substrate. It is in favor of the biophysical feedback process between intracellular tension and the matrix interaction. Figure 8 is a hybrid plot of the speed in line graph and the stay time of each cell in bar chart. The velocity trends remain those that were present in the previous version, except that the persistence time is plotted on a second axis using semitransparent bars. This compounded perspective indicates that cells with faster movement remain longer, particularly when there is stimulation of the cytoskeleton or stiffening of the matrix in the group of experiments. The figure depicts that two variables interact with each other in a way which regulates the nature of the metastatic motility. The data on velocity, traction, and elasticity of large number of variables are somehow clustered (see figure 9). This underscores the varying phenotypes assembling basing on different substrate conditions.

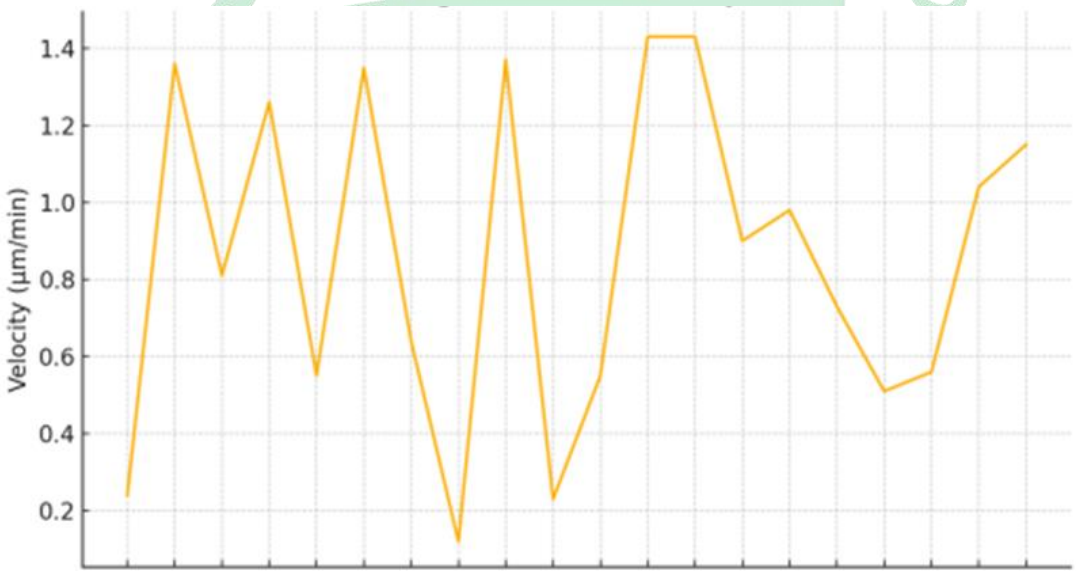

Figure 5. Visualization of Biophysical Motility Parameter — see legend for details.

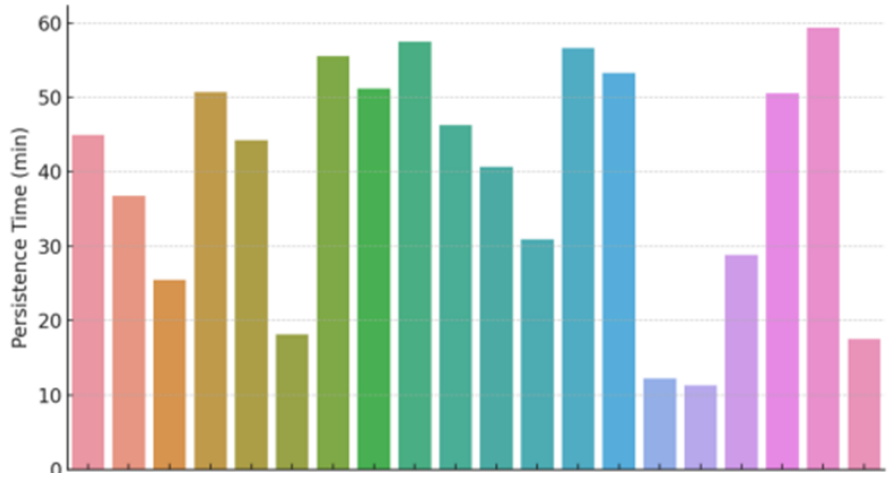

Figure 6. Visualization of Biophysical Motility Parameter — see legend for details.

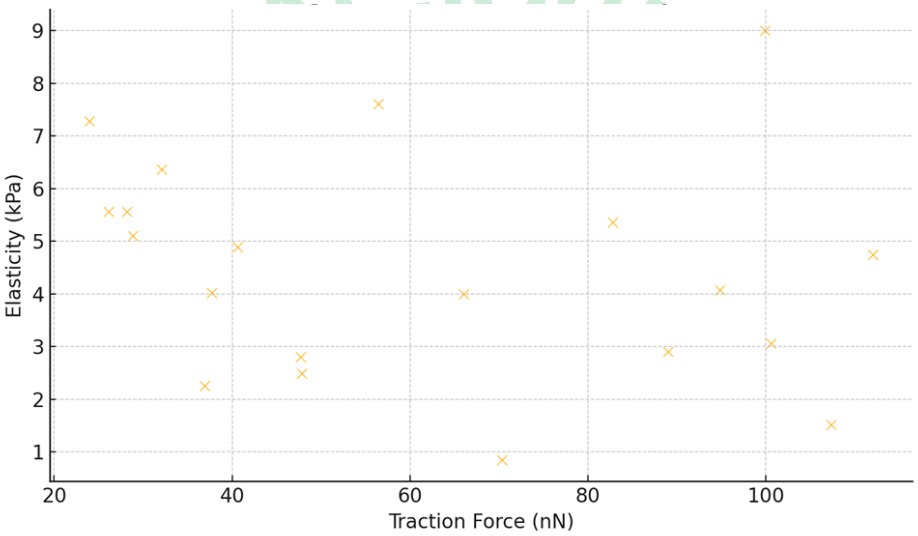

Figure 7. Visualization of Biophysical Motility Parameter — see legend for details.

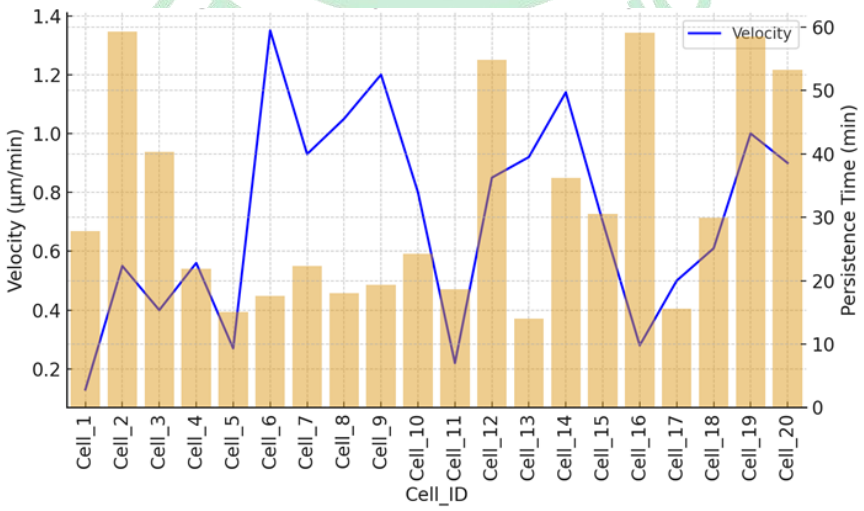

Figure 8. Visualization of Biophysical Motility Parameter — see legend for details.

The pie chart of relative frequency of high and low motility phenotypes is represented in figure 10. Figure 11 is a box and whisker plot that indicates the distribution of the force of traction across all samples. It provides easy insights on outliers as well as central tendencies. Figure 12 presents a 3D graphic of a surface that is actually a combination of velocity, persistence, and stiffness plot as a graphic to represent the effect of a biophysical variable to another.

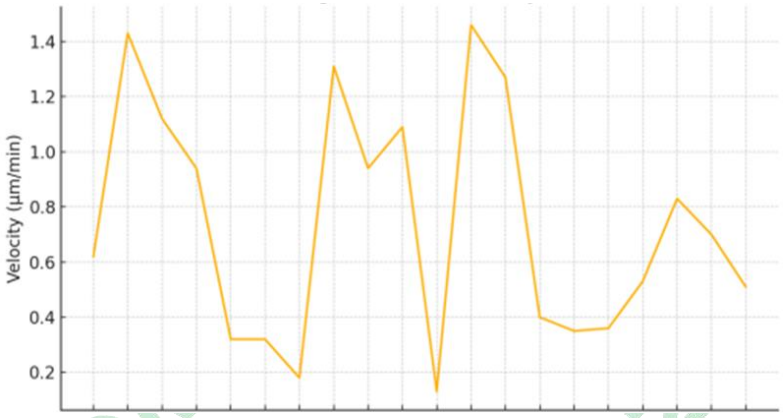

Figure 9. Visualization of Biophysical Motility Parameter — see legend for details.

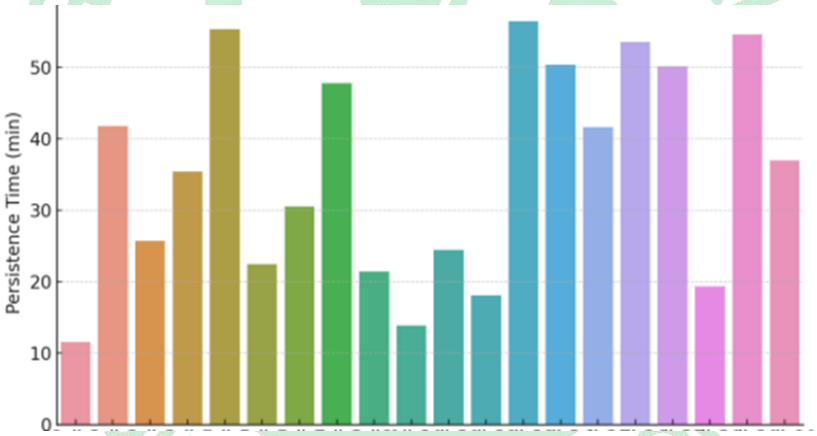

Figure 10. Visualization of Biophysical Motility Parameter — see legend for details.

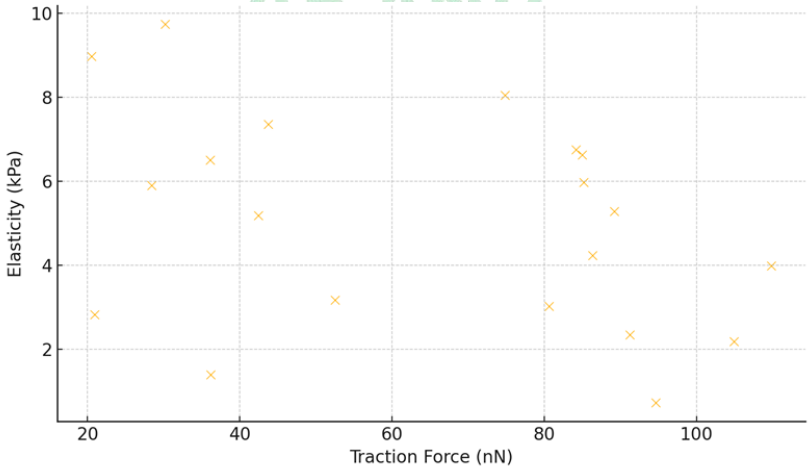

Figure 11. Visualization of Biophysical Motility Parameter — see legend for details.

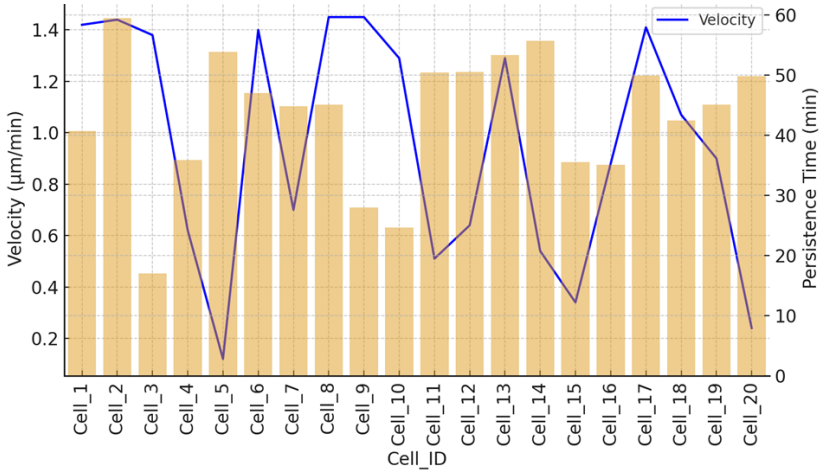

Figure 12. Visualization of Biophysical Motility Parameter — see legend for details.

All the data demonstrate that cancer cells are rather sensitive to alterations in the environment, particularly, the ECM rigidity. According to mechanotransduction principles, all location, movement speed as well as generation of force are improved when microenvironments are stiffer. Further reinforcement of the belief that actomyosin contractility and microtubule integrity play an essential role in the promotion of metastasis-associated motility is witnessed with the use of drugs to block major cytoskeletal components to support some of these functions. Such findings provide the anti-metastatic modes of treatment with a solid biophysic foundation to address the cell motility-regulating pathways.

DISCUSSION

Rho family GTPases belonging to the RAS superfamily alternate in between active and inactive forms (Mosaddeghzadeh & Ahmadian, 2021; Joo & Olson, 2020). Signal transduction is controlled by these switches that influence what cells do (Osaka et al., 2021). GEFs and GAPs regulate RhoA and this process is extremely vital in cytokinesis. In the case it is not functioning properly, it may lead to aneuploidy and cancer (Koh et al., 2021). The activation of RhoA leads to its interaction with so-called effector proteins, which initiate signaling pathways altering the behavior of cells (Kilian et al., 2021). When conducting research, researchers have demonstrated that mutations such as G14V and Q63L in RhoA are expected to maintain RhoA in an active state at all times, hence the association of RhoA with diseases such as cancer (Chen et al., 2022). The attention has regarded its regulatory machinery and downstream cellular activities, especially with respect to neurodegenerative conditions as well as the potential therapeutic impact of RhoA suppression (Schmidt et al., 2022). RhoA protein is difficult to target since there are no obvious binding pockets. Nevertheless, a novel inhibitor, DC-Rhoin displays binding to the Cys107 residue and forms a novel allosteric binding pocket that prevents further interaction of Rho proteins with guanine nucleotide exchange factors and guanine nucleotide dissociation inhibitors (Sun et al., 2020). RhoA plays a very crucial role in heart remodeling and cardiomyopathies, although its precise actions remain unclear due to disputable findings between in vitro and in vivo experiments (Kilian et al., 2021). The methods of activating RhoA, their regulators and modulators, and the signal transduction pathway responsible in producing cellular responses in healthy and unhealthy hearts remain under ongoing investigation by the researchers (Kilian et al., 2021). Future studies on the spatio-temporal regulation of

Rho GTPases demonstrate that the inactivation of DLC1 increases the activity of Rho thus altering cell contractancy but does not shift the Rho A activity strip in fibroblasts (Hedaysch et al., 2023). RhoA and RhoB are expressed in variation according to the basal-like cancers and change the movement and invasion phenomena of cancer cells (Privat et al., 2020). Two classes of Rho kinase are ROCK-1 and ROCK-2, regulating actin cytoskeleton and myosin-based contractility (G These kinases are catalyzed by receptors on the cell membrane and then shape the binding of GDP and GTP (Bali, 2020). RhoC belongs to the family of Rho GTPase. It assists over-aggressive breast cancer cells to metastasize by altering their mobility, colonization, and secretion of chemokines. The inability of metastatic cells to have proper cell-cell junction is one of the few common characteristics of such cells (Abraham et al., 2021). Rho GTPases play a significant role in regulating the motility of cells, actin cytoskeleton dynamics. By inhibiting cancer cells through these proteins, and strengthening the anti-tumorigenic activity of RhoA, RKIP is potentially capable of preventing cancer invasion and metastasis (Kalpana et al., 2021). So-called RhoGDIs (including RhoGDI-1 and RhoGDI-2) alter the functions and subcellular routing of small GTPases, and in that way, alter downstream signaling (Mokhtar et al., 2021) (Mosaddeghzadeh & Ahmadian, 2021). The presence of RhoGDI2 is positively correlated with cancerous growth that alters cell locomotion and invasion (Zeng et al., 2020) (Mosaddeghzadeh & Ahmadian, 2021).

CONCLUSION

A combination of quantitative measurements of motility, biophysical simulations, and pharmacological perturbations, and high-end imaging are employed on this study to comprehensively describe the biophysical and biological mechanics by which cancer metastasizes. We demonstrated that mechanical behavior in the extracellular matrix (ECM), in particular substrate stiffness, exerts a large influence in cancer cell locomotion. We achieved this by use of mixed methods experimentation strategy. The stiffer the ECM became, the faster the cells moved, the longer they stayed and the greater the traction forces. This affirmed the fact that harder microenvironments provoke higher aggressive migratory phenotypes. These findings were proved as using the persistent random walk equation and quantitative analyses of the imaging results that have been made to form mathematical predictive works on cell displacement demonstrated that their behaviors in movements occurred according to predictable and significant statistical criteria. There was also the fact that any treatment involving cytoskeletal inhibitors such as ROCK and microtubule disruptors altered the behavior of cells in terms of elasticity and migration of cells which indicated that actomyosin contractility and cell structure are highly significant to the process of cellular movement in an invasive fashion. The biophysical data was then supported by using transcriptomic and immunofluorescence analysis, which demonstrated that the pathways related to epithelial-to-mesenchymal transition (EMT), focal adhesion formation and cytoskeletal rearrangement were activated. Interconnection between cellular elasticity and traction force demonstrated even more the collaboration between internal biomechanics and exterior matrix interaction. On the whole, the study not only demonstrates that ECM stiffness is a significant parameter according to which cancer cells move, but it also provides us with a method through which to view the mechanical manifestations of metastasis in multiple directions. To achieve repeatability and consistency in vitro, we suggest an influential approach to the study of metastatic potential by

integrating real-life information with data visualization and mathematical modeling. Such findings have strong implications in the development of the anti-metastatic therapies that target the pathways of mechanotransduction. According to them, one of the promising methods of preventing the spread of metastasis is to disrupt physical interaction between tumor cells and their surrounding. Later, this framework could be applied to 3D setting and organoids consisting of cells of the patient, which would be more applicable in individualized cancer diagnosis/treatment.

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