Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: April 30, 2023

Citation: Okamoto M., 2023. Cellular morphology and functional characteristics of breast cancer cells, Medical Research Archives, [online] 11(4). https://doi.org/10.18103/mra.v11i4.3779

DOI: https://doi.org/10.18103/mra.v11i4.3779

ISSN: 2375-1924

REVIEW ARTICLE

Cellular morphology and functional characteristics of breast cancer cells

Masami Okamoto*

Advanced Polymeric Nanostructured Materials Engineering, Graduate School of Engineering, Toyota Technological Institute, 2-12-1 Hisakata, Tempaku, Nagoya 468 8511, Japan

*okamoto@toyota-ti.ac.jp

Abstract

When traction forces are generated in the cells underlying extracellular matrix (ECM) including artificial scaffolds, the cells feel essentially stiffness of the surrounding microenvironment and respond to applied forces and exert forces in the matrix, in which the traction forces can change cellular morphology and cytoskeletal structure. To date, analysis of cell morphology, including quantitative measurements such as cytoplasm roundness, cytoplasm elongation factor, nuclear elongation factor, ratio nuclear area to cytoplasm area ratio (A_N/A_C), nuclear dimension, and nuclear height, has been widely used in cancer diagnostics and hematology. Increasing evidence suggests that the extracted morphological features such as cell area and the length of major and minor axes, could also be used to analyze the dynamic changes of cells in diseases of the nervous system and cellular stress related phenomena. Furthermore, multivariate analyses of morphological data suggest that quantitative cytology may be a useful adjunct to conventional tests for the selection of new substances.

Thus, understanding the interaction between microenvironment and cancer cells via cellular morphology is critical subject to tackle metastatic spread of cancer cells and its many associated issues. In this topic, the cellular morphological parameters and functional characteristics of cancer cells are summarized.

Keywords: breast cancer cells, cellular morphology, metastasis, diagnosis

1. Introduction

Mechanotransduction is well known as a key role in physiological processes in regeneration, homeostasis, aging, and disease[1]. Figure 1 shows the direct physical signal transmission pathway and indirect mechanochemical signal transduction from the extracellular matrix (ECM)[2-4]. *Via* actin organization the direct mechanical stimuli from ECM are transmitted, at the same time indirect biochemical signals are generated, where the Ras-Raf-MEK-ERK signaling is well known as the regulation of cell fate and proliferation of mesenchymal stem cells (MSCs)[5-7]. Changing in the material features of ECM influences the force balance between cells and ECM.

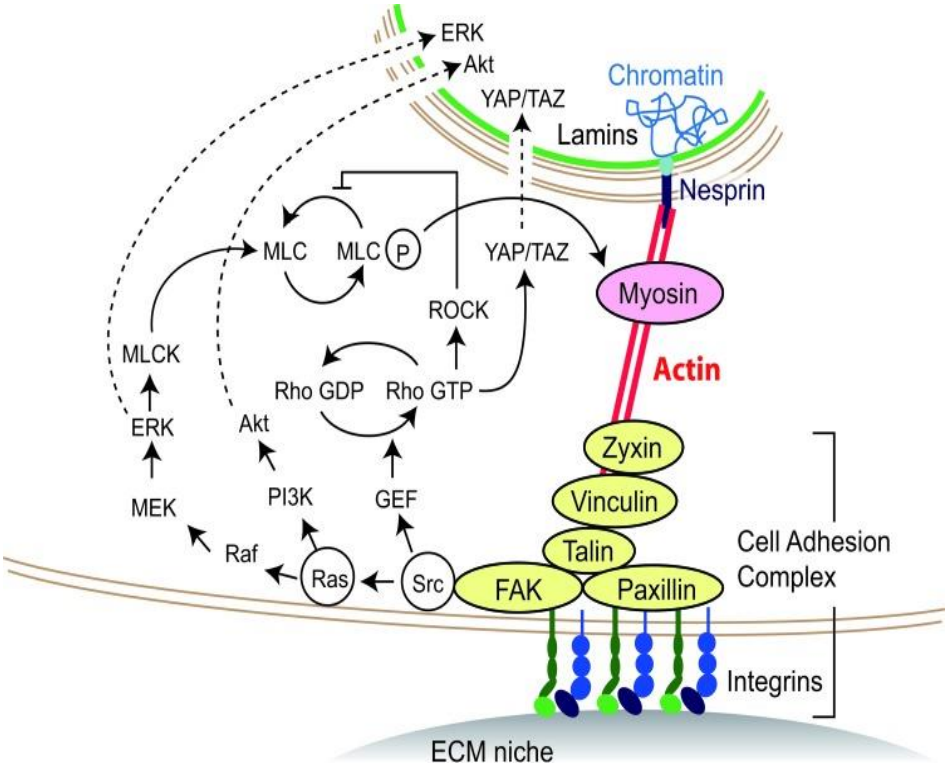

Fig. 1. Direct physical signal transmission pathway and indirect mechanochemical signal transduction from the ECM[2-4]. Via integrin transmembrane molecules into the cytoplasm mechanical stimuli are transmitted (right). (Copyright 2015. Reproduced[3]).

Such forces can change nuclear morphology and cytoskeletal structure due to the traction force generation[8], which influence cell response and cell fate (chromatin remodeling and DNA unzipping[9]. When the traction forces are generated in the cells underlying substrate the cells feel stiffness essentially[10]. The cells apply greater traction forces to develop more stable and distinct focal adhesion (FA). Owing to the well-organized actin fibers, the cells spread more extensively on the surfaces with rigid property than on soft (compliant) surfaces (Figure 2).

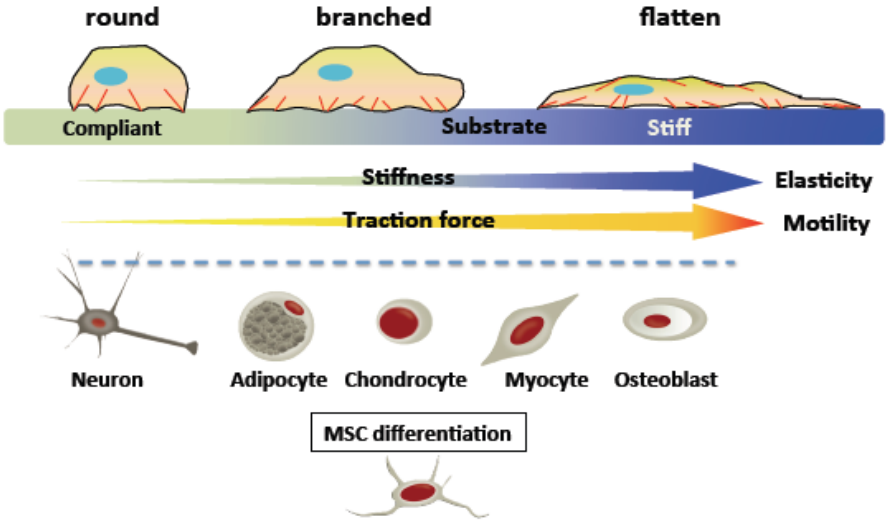

Fig. 2. Cells apply traction forces their underlying substrate, essentially "*feeling*" this stiffness[2]. In general, cells generate greater traction forces, establish more stable and distinct focal adhesion (FA), and form more defined actin stress fibers (red) and spread more extensively on rigid substrates than on compliant substrates. Soft substrates are beneficial for a neurogenic or adipogenic differentiation, stiff substrates are beneficial for an osteogenic differentiation, and substrates of an intermediate stiffness favor a myogenic lineage commitment, for "tissue cells feel and respond to the stiffness of their substrate"[6, 7].

When cells embedded within a soft (collagen) 3D scaffold without arginine-glycine-aspartate (Arg-Gly-Asp) (RGD) sequences, the FA proteins could not generate aggregates. However, the FA dispersed through the cytoplasm widely. *Via* mechanotransductive processes, these proteins are still indirectly affected in speed of motility and modulating cell traction despite the lack of stable FA[11].

To date, extensive efforts have been made in this complex interrelation between FA formation (F-actin network), traction forces, and extracellular cues *via* nanoscale surface topography on the substrates to precisely control stem cell fate.

2. Cancer invasion

The cell-ECM interactions regulate signaling and gene expression that underlie cellular processes in migration and cancer invasion. Understanding the interaction between microenvironment and cancer cells is also critical subject to progress in the cancer treatment[12,13]. As mentioned in the mechanotransduction, cellular microenvironment plays a pivotal role to form tissues and maintain homeostasis by interacting each other. Cancer destroys the normal balanced in the microenvironment *via* the induction of aberrant ECM reconstruction. These disruptions induced gene expression, proliferation, and migration to promote cancer malignancy[14].

Especially, ECM seems to have a crucial role for cancer progression because stiffening and aligned ECM is observed in the vicinity of tumors. During cancer progression cancer cells and fibroblasts mainly reconstruct the

aberrant ECM. The cells do not simply recreate the ECM and the remodeled ECM provides biochemical and biophysical cues to the neighboring cells (cancer cells and stromal cells) to promote cancer progression[15-18]. Thus, the cell-ECM interactions should be elucidated to understand mechanism of the cancer progression. To solve this profound subject, artificial ECM is required which mimics *in vivo* environment.

Cancer cells also receive mechanical cues from artificial ECM[19]. It has been reported that rigid substrates progress malignant phenotypes *via* integrin dependent regulation[20,21]. Furthermore, several researchers suggested that the stiffness of substrate is highly associated with the cellular apoptosis, growth and motility[22-24]. Generally the cancer cells prefer stiffer substrates and the proliferation and motility were enhanced when they were cultured on stiffer substrate. In addition, Ishihara *et al.* reported that the transcription factor, nuclear factor κB (NF-κB) is activated by stiff substrate *via* actomyosin contractions[25]. As NF-κB is associated with the cellular proliferation, adhesion and apoptosis[26], its activation should be inhibited. Collagen fibers nearby tumor tend to be aligned and cancer cells reorganize collagen fibers to be aligned. This collagen alignment contributes the tumor progression[27,28].

The capability of metastatic cancers to shift motility modes is one of the main features of invasion. The tumor microenvironment is able to elicit epithelial-mesenchymal transition (EMT), where an epigenic program leads epithelial cells to lose their cell-cell and cell-ECM interactions to undergo cytoskeleton reorganization and to gain morphological and functional characteristics of mesenchymal cells.

Artificial nanofiber substrates can induce EMT for some breast and lung cancer cells[29-31]. Considering these findings, designing substrates properties have possibilities to control not only EMT but also vascularization and inflammation phenomena. In addition, these phenomena are important not only for cancer progression but also tissue regeneration[32-36]. However, the cell-ECM interactions have not been elucidated yet. The cancer therapies with poor prognosis prompt us to conduct a novel study on an effective cancer therapy. That is, breakthroughs in effective cancer therapies are imperative.

3. Cellular morphology and metastatic features of cancer cells

Okamoto group reported new findings in 2017[37]. Their study was aimed to assess the combination of both surface topographies (fiber alignments) and different stiffness of the polymeric substrates poly(*L*-lactic acid) (PLLA) with an elastic modulus of 2 GPa and poly(*ε*-caprolactone) (PCL) with an elastic modulus of 0.15 GPa) to evaluate the effect on the cellular morphologies, proliferation, motility and gene expression regarding EMT of two different types of human breast cancer cells (MDA-MB-231 and MCF-7). The cellular morphologies (roundness and nuclear elongation factor), E-cadherin and vimentin expression, and cellular motility in terms of cellular migration speed, persistent time and

diffusivity were discussed comprehensively. They have demonstrated that the microenvironment of cell culture substrates influences cancer progression and metastatic potential.

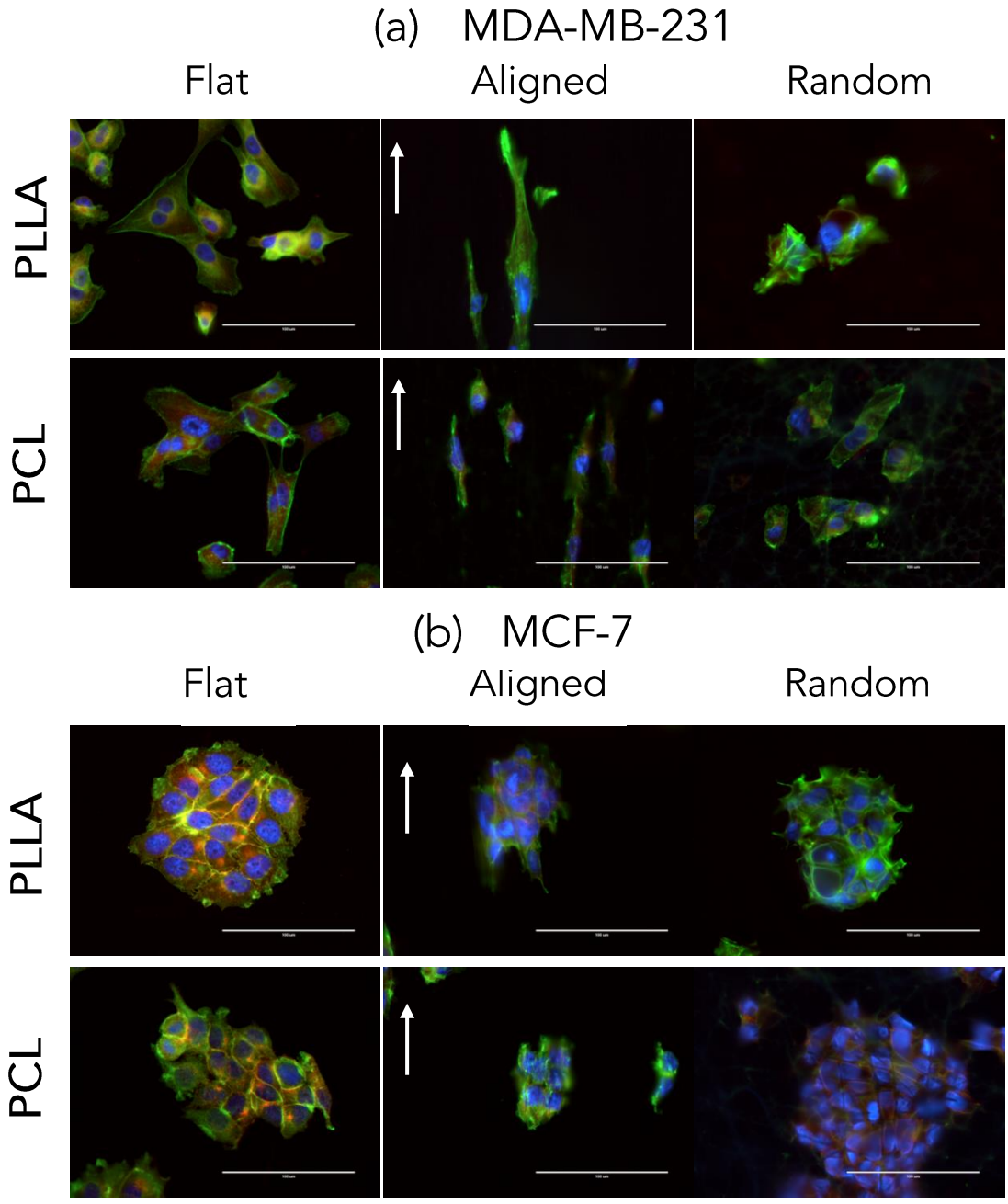

Fig. 3. Morphological comparison of different type of breast cancer cell lines cultured on different substrates for 3 days. (a) MDA-MB-231 and (b) MCF-7. Immunofluorescence of breast cancer cells was imaged with Hoechest 33342 (blue), phalloidin (green), and E-cadherin antibody (red). Scale bar 100 μm. Arrows indicate the aligned fiber direction of the substrate. (Copyright 2017. Reproduced[37]).

Figure 3 shows cellular morphology of MDA-MB-231 and MCF-7 breast cancer cells cultured on different substrates (flat substrate, designated as F-; aligned fiber substrate, A-; random fiber substrate, R-) at day 3[37, 38]. The corresponding morphological parameters are summarized in Figure 4. For MCF-7 cells incubated on all substrates, E-cadherin (red fluorescence) was detectable and localized at the intercellular boundaries (borders), indicating the multicellular aggregate (colonization) of the cells (Fig. 3(b)). On the other hand, such aggregation of MDA-MB-231 cells is not observed (Fig. 3(a)), but the cells are elongated and arranged to fiber direction when the cells are incubated on the aligned fibers substrates. For MDA-MB-231 cells under normoxic condition (20% O_2), both cytoplasm and nuclear elongation factors show much higher values in both the PLLA and PCL substrates (Fig. 4(b) and (c)) as compared to those of MCF-7 cells (data not shown: see Figure 4 in ref.[38]). The similar trend is observed in hypoxic condition (Fig. 4(b') and (c')).

The nuclear elongation factor (Fig. 4(c) and (c')) and the nuclear area to cytoplasm area ratio (A_N/A_C) (Fig. 4(d) and (d')) represent the extent of stresses are transmitted to nucleus. With this in mind, MDA-MB-231 cultured under normoxic condition on A-PLLA exhibited more elongated and spread morphologies. For MDA-MB-231 cells cultured on both R-substrates under both oxygen concentration conditions (1% and 20% O_2), smaller value of nuclear elongation factor was observed. This indicates that the cell stretching is restricted with R-PCL substrate and the more circular morphology of the cells is overwhelming (Fig. 4(a), (c), (a') and (c')). As seen in Fig. 4, the hypoxic treatment had no remarkable effect on both cell's morphologies.

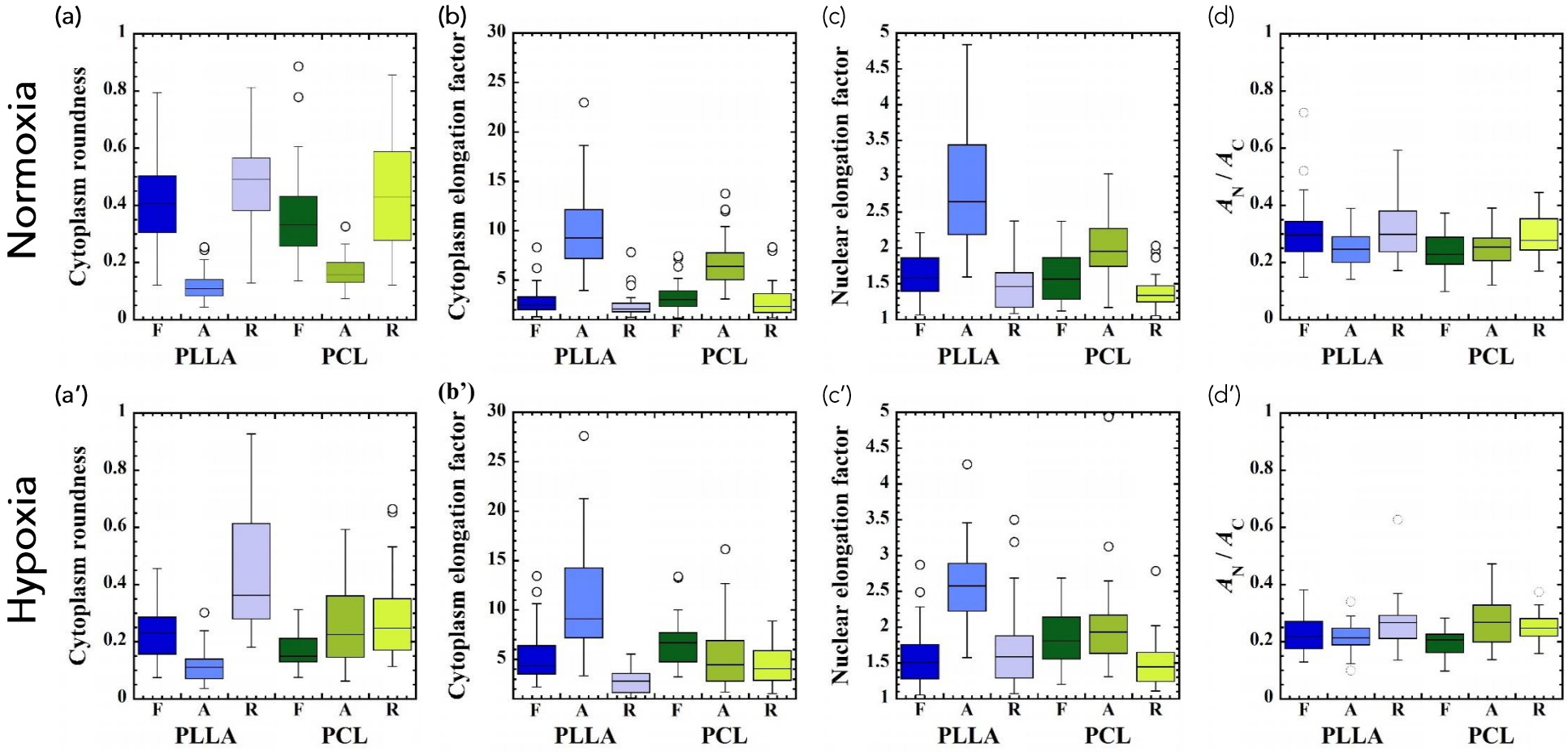

Fig. 4. Quantification of the cellular morphologies of cytoplasm roundness ((a) and (a')), cytoplasm elongation factor ((b) and (b')), nuclear elongation factor ((c) and (c')) and nuclear area (A_N) to cytoplasm area (A_C) ratio (A_N/A_C) ((d) and (d')) as boxplots for MDA-MB-231 cultured under normoxic and hypoxic conditions at day 7. PLLA, poly(L-lactic acid); PCL, poly(ε-caprolactone). (Copyright 2019. Reproduced[38]).

The cellular morphological parameters are chosen to clarify the relationship between morphology induced by physical properties of substrates and gene expression of typical EMT markers. Relative TGFB (Figure 5), HIF1A (Figure 6), and Vimentin (Figure 7) expressions are plotted as a function of morphological parameters. For MDA-MB-231 cells under both oxygen concentration conditions, linear relations between four cellular morphologies (cytoplasm roundness, cytoplasm elongation factor, nuclear elongation factor, and ratio A_N/A_C and TGFB and HIF1A expression are found on PLLA substrate with different stiffness, indicating the elongation of cells induces the expression of both genes. In addition, the significant increasing of slope in the linear relation under hypoxia, this evidence suggests hypoxia upgrades EMT in MDA-MB-231 cells on PLLA substrate.

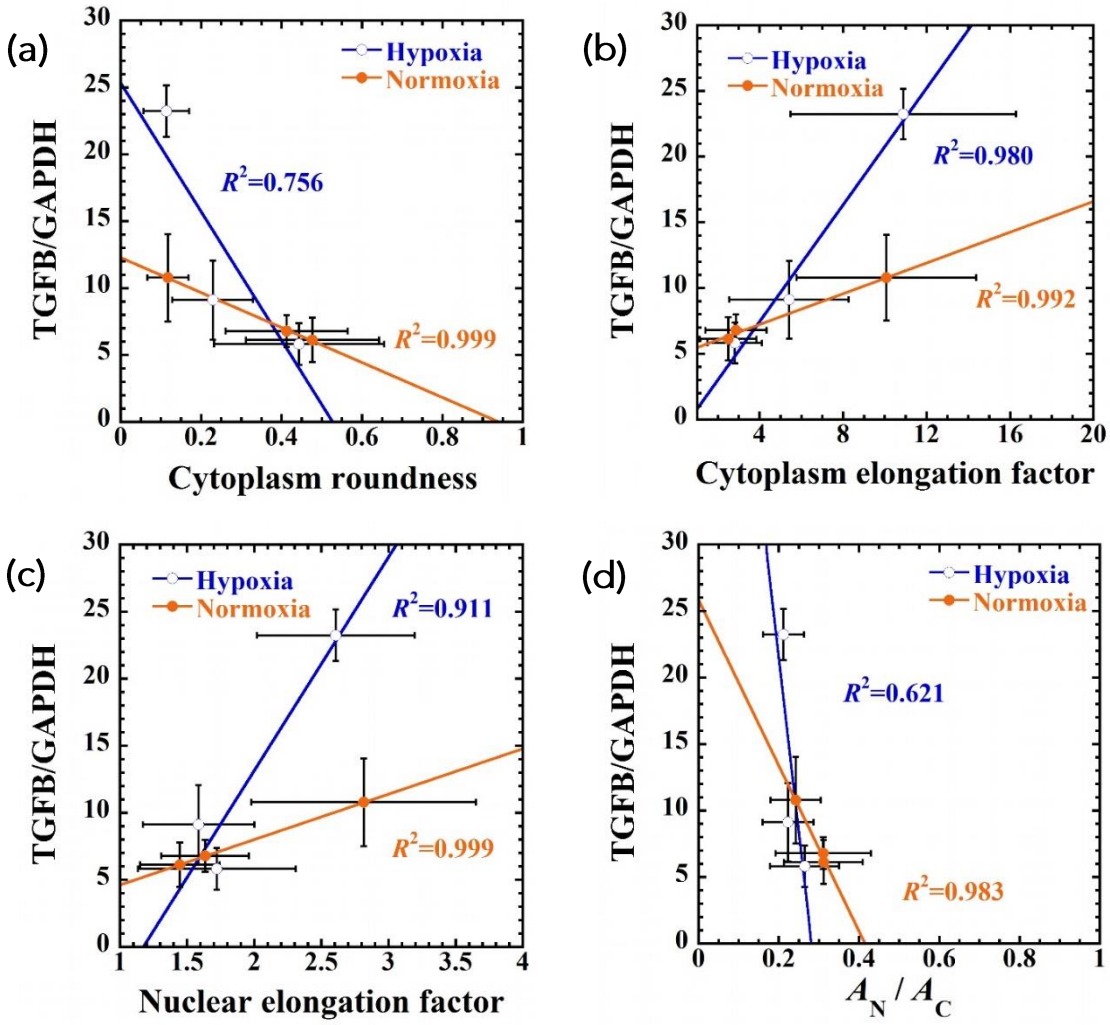

Fig. 5. Relationships between gene expression of TGFB and morphological parameters (cytoplasm roundness (a), cytoplasm elongation factor (b), nuclear elongation factor (c), and nuclear area to cytoplasm area ratio (A_N / A_C) (d)) for MDA-MB-231 cultured on three type of PLLA substrates under normoxic and hypoxic conditions at day 7.GAPDH, glyceraldehyde-3-phosphatase dehydrogenase. (Copyright 2019. Reproduced[38]).

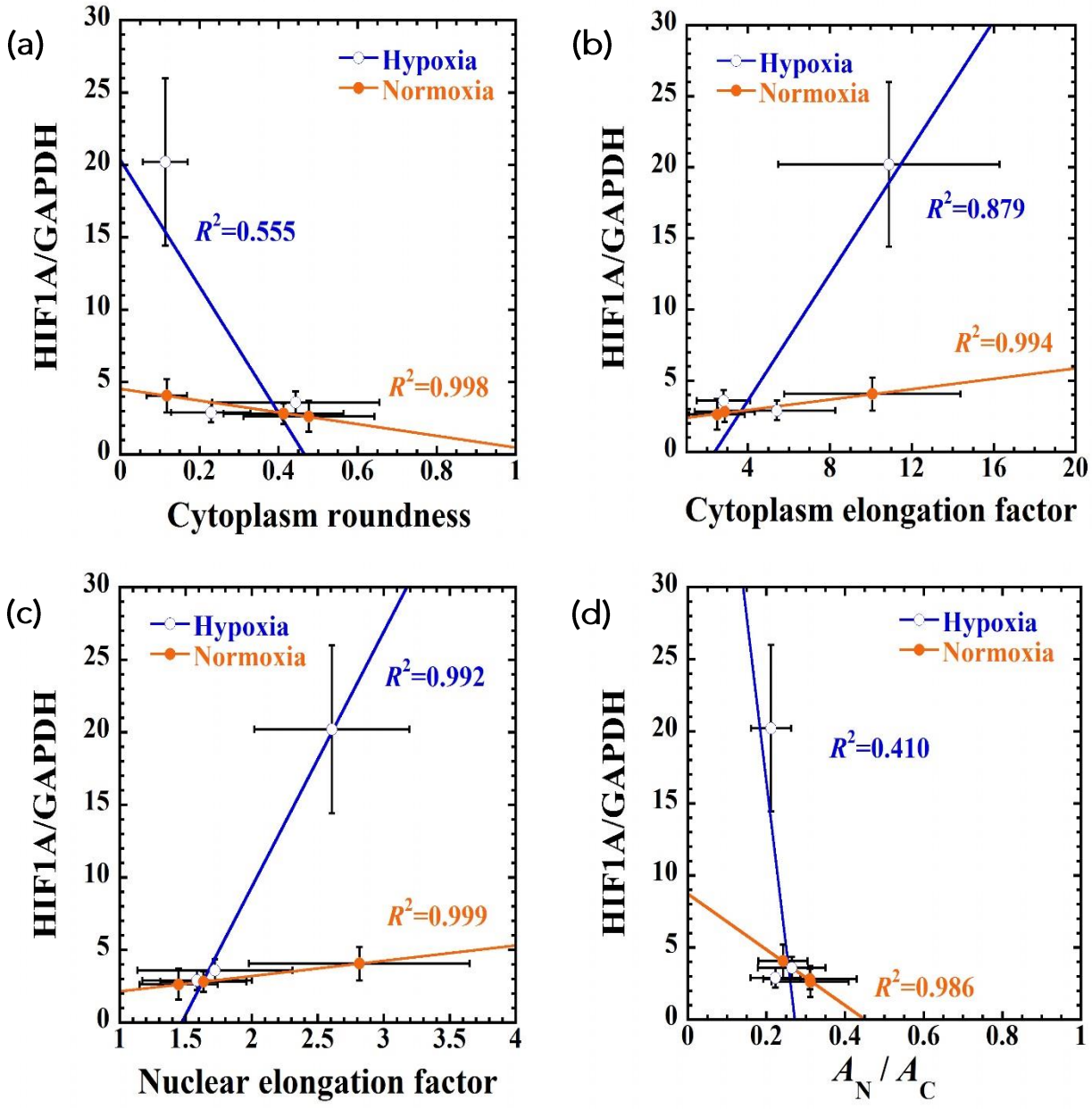

Fig. 6. Relationships between gene expression of HIF1A and morphological parameters (cytoplasm roundness (a), cytoplasm elongation factor (b), nuclear elongation factor (c), and nuclear area to cytoplasm area ratio (A_N / A_C) (d)) for MDA-MB-231 cultured on three type of PLLA substrates under normoxic and hypoxic conditions at day 7. GAPDH, glyceraldehyde-3-phosphatase dehydrogenase. (Copyright 2019. Reproduced[38]).

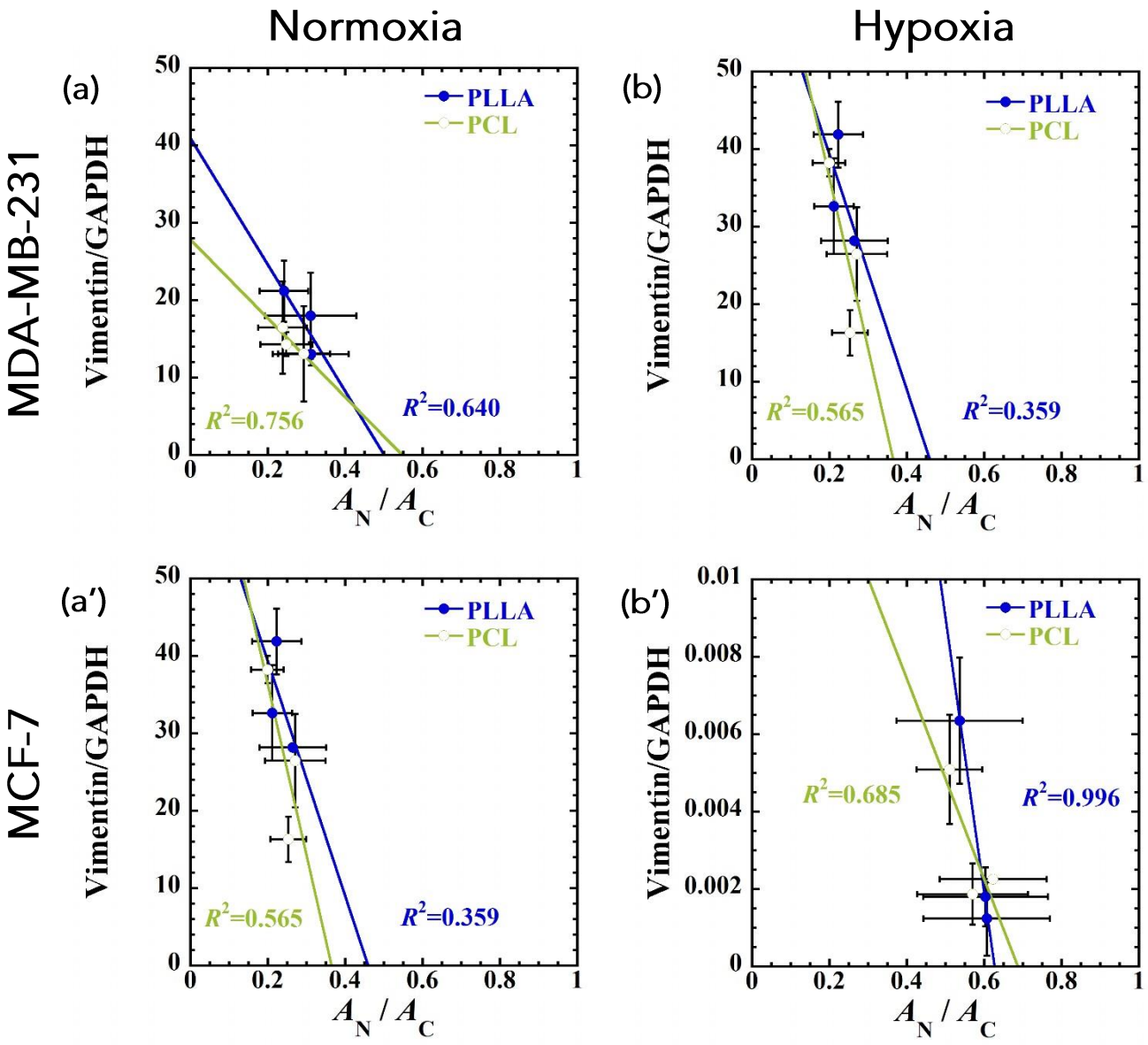

Fig. 7. Relationships between gene expression of Vimentin and morphological parameter (ratio of A_N and A_C) for MDA-MB-231 (a, b) and MCF-7 (a', b') cultured on six different substrates under normoxic and hypoxic conditions at day 7. GAPDH, glyceraldehyde-3-phosphatase dehydrogenase. (Copyright 2019. Reproduced[38]).

A significant level of repression in Vimentin expression with increasing of area ratio (A_N/A_C) is observed (Fig. 7) in both MDA-MB-231and MCF-7 cancer cells under both oxygen concentration conditions. The slope under hypoxic condition reflects the upregulation of EMT phenomenon. Interestingly, the cells on the PCL substrates can downregulate Vimentin expression compared with on the PLLA substrates. Schrader *et al.* demonstrated that the Vimentin expression was upregulated with

increasing stiffness of substrate[39]. In their study, the PLLA substrate is more favorable to enhance Vimentin expression for both cancer cells because of the synergetic effect of fiber alignment and stiffness.

For MDA-MB-231 cells on PLLA substrate, they reported that relative CDH1 (E-cadherin) expression has no relation to the morphological parameters or the elongation of cells. This behavior is consistent with the results of MCF-7 cells incubated on PLLA substrate, where the elongation of cells inhibits the repressions of E-cadherin[38]. In addition, the expression of CDH2 (N-cadherin) in MDA-MB-231 cells on PLLA substrate exhibits similar relation in the elongation of cells. Given the fact that both cadherin expressions in cancer cells may be not responsible for the cellular morphologies although the upregulation of CDH2 and downregulation of CDH1 are undergoing in both cancer cells of the PLLA substrate.

In each of the substrate conditions tested in this study, the topographical effect on EMT is much more beneficial for mesenchymal type (MDA-MB-231) cells than epithelial type (MCF-7) cells, in which significant changes of SNAI2 and ZEB1 are evident for MDA-MB-231 cells (Figure 8). The transcription factors associated with EMT (SNAI2 and ZEB1) are activated by both hypoxic condition and elongation of cells. They also found that the elongation of cells promotes the upregulation of the transcription factors in MDA-MB-231 cells.

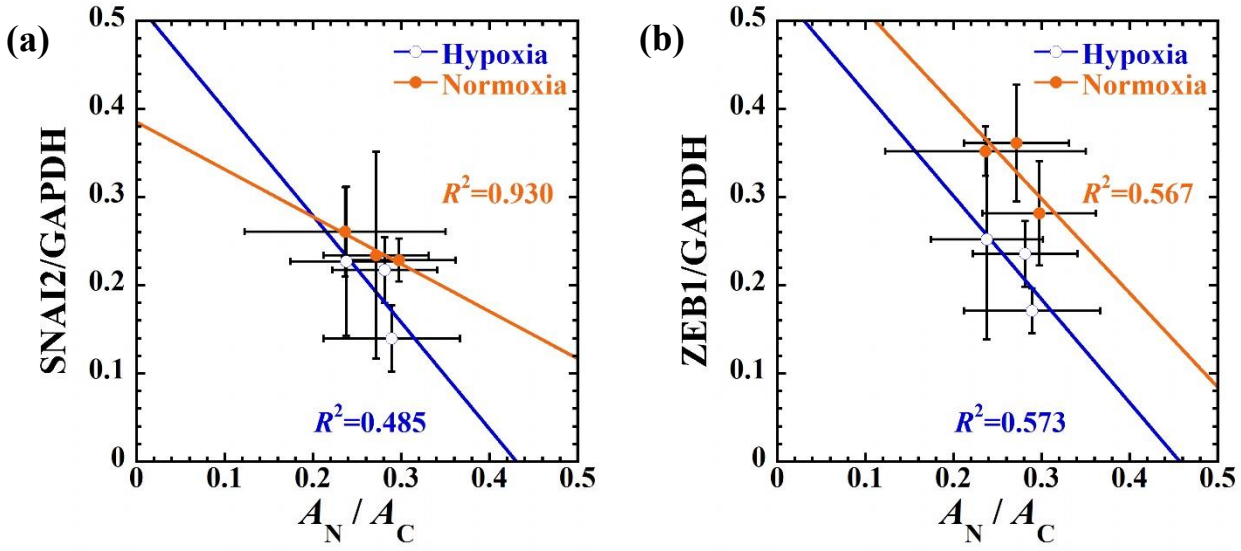

Fig. 8. Relationships between gene expression of SNAI2 (a) and ZEB1 (b) and morphological parameter (ratio of A_N and A_C) for MDA-MB-231 cultured on three type of PLLA substrates under normoxic and hypoxic conditions at day 3. GAPDH, glyceraldehyde-3-phosphatase dehydrogenase. (Copyright 2019. Reproduced[38]).

4. Morphological change of cancer cells and cancer diagnosis

With digital holographic microscopy (DHM) the morphological analysis and dynamic behavior of cancer cells without contrast agents was reported[40]. The suitable technique for gynecologic cervical samples instead of cytology Papanicolaou (Pap) test was demonstrated[41]. The normal cells images of Pap test showed superficial cells with small nuclei. In contrast, the abnormal cell images revealed superficial cells with larger, irregular nuclei. Benzerdjeb group identified the DHM criteria that could differentiate the abnormal cells. The best diagnostic criteria were the greatest nuclear dimension (ND), maximum nuclear height (MHN), and nuclear-to cytoplasmic ratio (A_N/A_C)). By using a cutoff value for the ND and A_N/A_C we may establish to build an automated algorithm for the selection of abnormal cells.

Combining with machine learning for data analysis DHM images are a powerful tool to distinguish SW480 colon cancer cells in suspension from white blood cells[42]. In addition, DHM analysis with deep learning neural networks is to discriminate between iso genic cell lines of different metastatic stage[43], and distinguish healthy and nutrient-deprived cancer cells[44]. Furthermore, there is the potential for cancer cell cycle tracking to identify cancer cell behaviors such as morphologically distinct motility patterns. This leads to targeting mechanisms of cancer growth and anticancer drug susceptibility[45].

Assessing cell morphology in relation to contractile and stress-resistant cytoskeleton has linkage between phase signal from the cell border, and/or with nucleus. As discussed in section 3, cell morphology of different cancer phenotypes provides a quantitative method to distinguish different effects of cancer biomarkers.

5. Cancer cell migration

The relation of the morphological change of cell to EMT biomarkers may support the mechanism of cancer migration and invasiveness. The induction of EMT is believed to promote tumor cell motility and invasion. Using polymeric gel substrates with different viscoelasticity as microenvironment of cell culture substrates that influences cancer progression and metastatic potential, Okamoto group have examined the effect of the viscoelasticity on the direct relation between cellular motility and mesenchymal properties with induction of EMT in MDA-MB-231 cells. The MDA-MB-231 cells cultured on gel substrates showed different cellular morphologies and these morphological differences were robustly correlated with cellular migration speed (S) and damping coefficient ($\tan\delta$) of gel substrates (not substrate stiffness: elastic modulus). The linkage between the acquisition of the mesenchymal phenotype in the cells through the induction of EMT mediated by stiffer substrate and the promotion of the cellular motility were not observed[46].

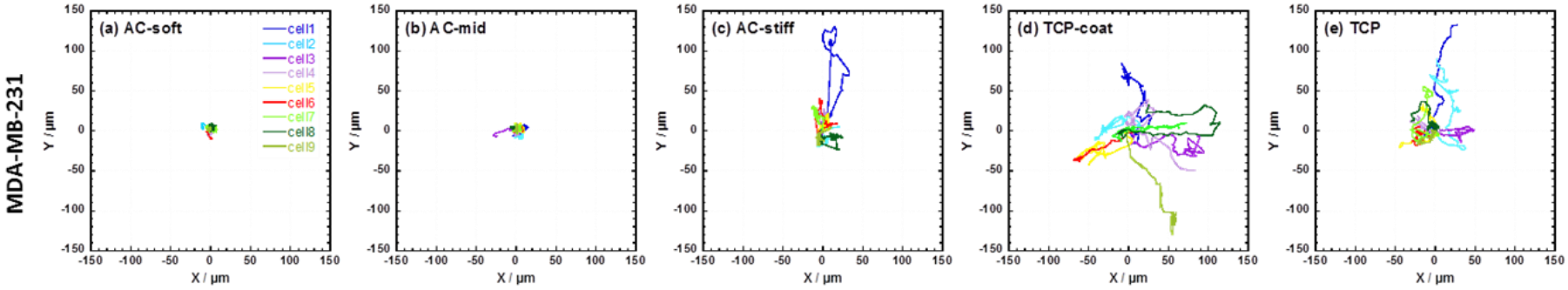

Fig. 9. Representative trajectory of MDA-MB-231 cells cultured on five different gel substrates under hypoxic condition over 16 h at day 3. The data were obtained in 300 x 300 μm^2. (Copyright 2019. Reproduced[46]).

The representative recorded trajectories of both breast cancer cells cultured on different substrates of acrylamide-based copolymer (AC) gels with different storage moduli ranged from 32 to 1,510 Pa and type-I collagen coated plates ((TCP)-coat) under hypoxic condition over 16 h at day 3 point are shown in Figure 9. For MDA-MB-231cells on AC-soft (storage modulus (*G'*) of 32 Pa) and AC-mid (*G'* of 174 Pa) substrates, their migrations are restricted compared to those on AC-stiff (*G'* of 1,510 Pa), TCP-coat, and TCP (*G'* of 1.4 GPa), while the cells migrated in random directions. As expected, increasing in stiffness of the substrates leads cells to move generally (Fig. 9(c)) and farther away with higher motility (Fig. 9(d)) regardless any direction. Another interesting feature is that both cells cultured on TCP have some adverse effect on migration with a significant decrease in displacement than that on TCP-coat. This trend is supported by the substrate surface that coated with type-I collagen.

Cellular migration speed (*S*) iwas plotted as a function of morphological parameters of cytoplasm roundness, A_N/A_C, and nuclear elongation factor. For MDA-MB-231 cells in hypoxia, linear relations between three cellular morphologies and migration speed are found on all substrates with different stiffness (except TCP), indicating the elongation of cells enhances the cellular motility (Figure 10). The decreasing in cytoskeletal stiffness (A_N/A_C), which is equal to the increasing in cellular spreading factor, is also beneficial to understand the metastatic potential under both oxygen concentration conditions[46].

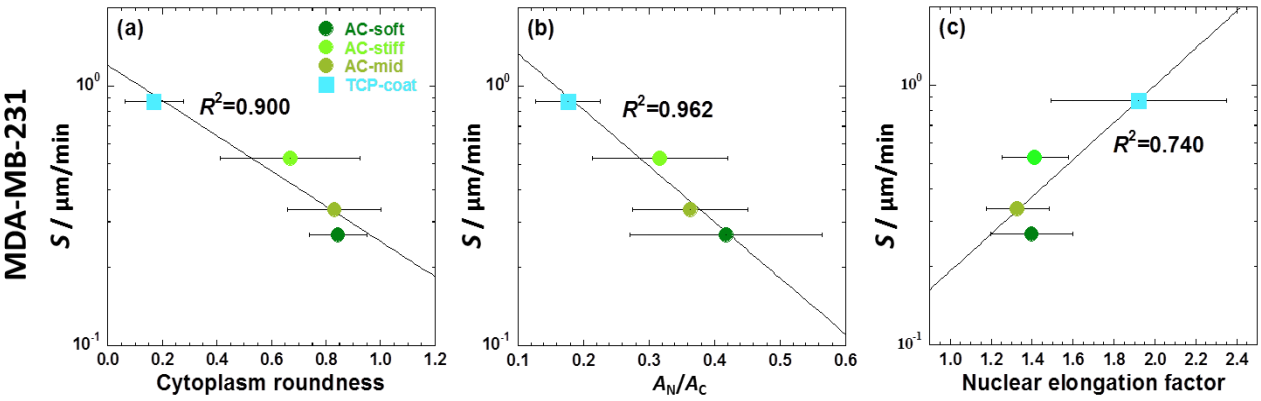

Fig. 10. Relationships between cellular migration speed (*S*) and morphological parameters of cytoplasm roundness (a), A_N/A_C (b), and nuclear elongation factor (c) for MDA-MB-231 at day 3 cultured under hypoxic condition on four different substrates. (Copyright 2019. Reproduced[46]).

On the other hand, the migration speed (*S*) in MDA-MB-231 cells under hypoxia was significantly upregulated with decreasing in damping coefficient (tanδ) (Figure 11(a)). Although the stiff substrate increased cellular motility, as discuss in many papers, their results found that the migration speed is not robustly correlated with substrate stiffness

(relative storage modulus (G'/ρ) with substrate density (ρ) in Figure 11(d)). This result suggests that the damping is the driving force of the cellular migration in rather more-invasive MDA-MB-231 cells. In addition, interestingly, tanδ promotes the persistent time (P) in a log-log linear relation compared to G'/ρ (Figure 11(e)). This behavior is consistent with the results of the cellular diffusivity (D) (Figure 11(c) and (f))[46].

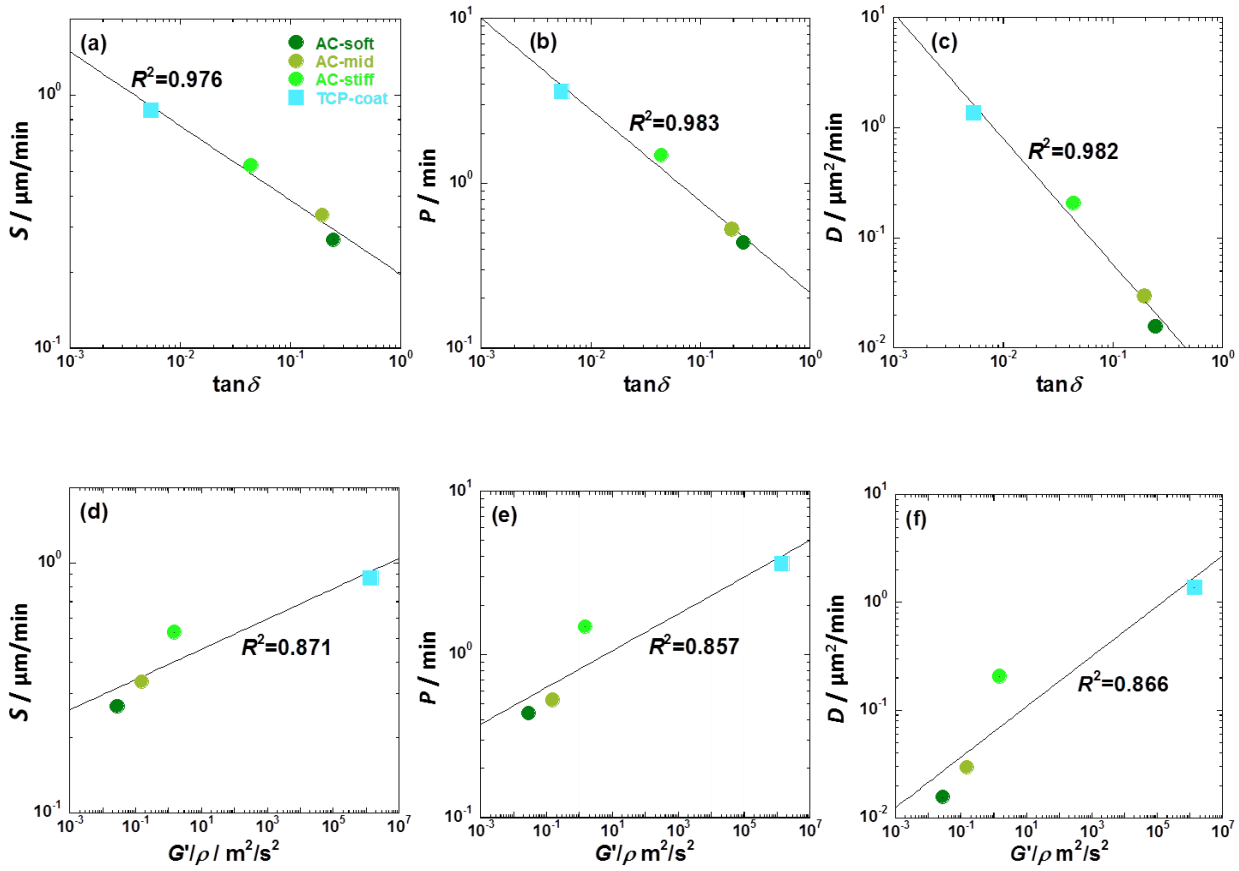

Fig. 11. Relationships between cellular migration speed (S) ((a) and (d)), persistent time (P) ((b) and (e)), and cellular diffusivity (D) ((c) and (f)) against damping coefficient (tanδ) and/or relative storage modulus (G'/ρ) for MDA-MB-231 cells at day 3 cultured on four different substrates in hypoxia. (Copyright 2019. Reproduced[46]).

The extensive studies have been made in mechanotransduction via surface topography and stiffness on the substrates, in which the cells respond to applied forces and exert forces in the substrate (ECM)[8,9,47,48]. Essentially, the cells feel stiffness, however, tanδ value might be more favorable to understand the basic of cell-ECM interactions and mechanotransduction. For this reason, tanδ value has robust correlation to the morphological parameters of cytoplasm roundness (deformability) and cellular spreading factor (A_N/A_C) in MDA-MB-231 cells in hypoxia[46].

In their study, the HIF1A level under hypoxic condition was markedly increased in MDA-MB-231 cells incubated on stiffer substrates (AC-stiff and TCP-coat). The cells incubated on TCP-coat showed significant increase in TGFB expression and SNAI2. The AC gel substrates were more favorable to enhance vimentin expression for MDA-MB-231 cells in comparison with that incubated on stiffer substrate (TCP-coat), indicating the vimentin expression has no effect on *S*.

So far, the induction of EMT is believed to promote tumor cell motility and invasion. In another study, Tsuji *et al.* reported collective migration for the role of EMT in cancer invasion and metastasis, where EMT cells are responsible for invasion and both EMT and non-EMT cells are responsible for intravasation[49]. Okamoto group's findings provide further indication that the linkage to the EMT phenotype and their motility mediated by substrate viscoelasticity may have a broad implication in cancer invasion and metastasis in therapeutic failure and relapse.

6. Conclusions and perspectives

Breast cancer is one of the most common malignancies in women and is the higher incidence and mortality rates among all female malignant tumors[50]. The average 5-year survival rate of breast cancer is reported to be around 55% due to the poor outcome of the therapy for metastatic disease in the resistance to radiation and chemotherapy[51]. The cancer therapies with poor prognosis prompt us to conduct a novel study on an effective cancer therapy.

Microenvironment-mediated tumor progression in the artificial substrates explore the design criteria to understand the cancer progression mechanism and metastatic potential. Cancer cells express multiple biophysical cues during migration, and epigenic program of phenotype transition. Quantifying and probing these biophysical cues as a morphological changes of cancer cells is utilized for cancer therapies and diagnosis as well as hematology[52].

In recent years, the extracted morphological features could also be used to analyze the dynamic changes of cells in diseases of the nervous system and cellular stress related phenomena[53]. In addition, multivariate analyses of morphological data suggest that quantitative cytology is a useful adjunct to conventional tests for the selection of new substances[54]. Because biophysical cues exist long before cancer develops, they are most useful in determining whether cells will metastasize. Knowledge of potential cancerous transformations in the body may enable clinicians to initiate preventative measures in the form of lifestyle changes and chemotherapy.

Corresponding Author
M. Okamoto
Advanced Polymeric Nanostructured Materials Engineering, Graduate School of Engineering, Toyota Technological Institute, 2-12-1 Hisakata, Tempaku, Nagoya 468 8511, Japan
E-mail: okamoto@toyota-ti.ac.jp

Conflicts of Interests
Author declares that there are no conflicts of interests.

Acknowledgments
This work was supported by the KAKENHI of the Ministry of education, Sports, science and Technology, Japan.

Funding information
Ministry of education, Sports, science and Technology, Japan.

ORCID ID
Masami Okamoto
http://orcid.org/0000-0002-5732-1652

References

1. Przybyla L, Muncie J.M, Weaver V.M. Mechanical Control of Epithelial-to-Mesenchymal Transitions in Development and Cancer. *Annu. Rev. Cell Dev. Biol.* 2016;32:527-554.

2. Wang N, Tytell J.D, Ingber D.E. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nat. Rev. Mol. Cell Biol.* 2009;10:75-82.

3. Miyoshi H, Adachi T. Topography design concept of a tissue engineering scaffold for controlling cell function and fate through actin cytoskeletal modulation. *Tissue Eng. B.* 2014;20:609-627.

4. Sun Y.B, Chen C.S, Fu J.P. Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment. *Annu, Rev. Biophys.* 2012;41:519-542.

5. Muller P, Langenbach A, Kaminski A. Modulating the Actin Cytoskeleton Affects Mechanically Induced Signal Transduction and Differentiation in Mesenchymal Stem Cells. *J. Rychly, PloS One.* 2013;8:e71283.

6. Zhang R, Ma P.X. Porous poly(l-lactic acid)/apatite composites created by biomimetic process. *J. Biomed. Mater. Res.* 1999;45:285-293.

7. Wen J.H, Vincent L.G, Fuhrmann A, Choi Y.S, Hribar K.C, Taylor-Weiner H, Chen S.C, Engler A.J. Interplay of matrix stiffness and protein tethering in stem cell differentiation. *Nat. Mater.* 2014;13(10):979-987.

8. Dalby M.J, Gadegaard N, Tare R, Andar A, Riehle M.O, Herzyk P, Wilkinson C.D.W, Oreffo R.O.C. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nat. Mater.* 2007;6:997-1003.

9. Mih J.D, Marinkovic A, Liu F, Sharif A.S, Tschumperlin D.J, Matrix stiffness reverses the effect of actomyosin tension on cell proliferation. *J Cell Sci.* 2012;125:5974-5983.

10. Pelham R.J, Wang Y.L. Cell locomotion and focal adhesions are regulated by substrate flexibility. *PNAS (Proc. Natl. Acad. Sci. U.S.A.).* 1997;94(25):13661-13665.

11. Fraley S.I, Feng Y, Krishnamurthy R, Kim D-H., Celedon A, Longmore G.D, Wirtz D. A distinctive role for focal adhesion proteins in three-dimensional cell motility. *Nat. Cell. Biol.* 2010;12:598-604.

12. McAllister S.S, Weinberg R.A. The tumour-induced systemic environment as a critical regulator of cancer progression and metastasis. *Nature Cell Biology.* 2014;16:717-727.

13. Taddei M.L, Giannoni E, Comito G, Chiarugi P, Microenvironment and tumor cell plasticity: An easy way out. *Cancer letters.* 2013;341:80-96.

14. Park C.C, Bissell M.J, Barcellos-Hoff M.H. The influence of the microenvironment on the malignant phenotype. *Molecular Medicine Today.* 2000;6:324-329.

15. Gikes D.M, Semenza G.L, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nature Reviews.* 2014;14:430- 439.

16. Xiong G.F, Xu R. Function of cancer cell-derived extracellular matrix in tumor progression. *Journal of cancer metastasis and treatment.* 2016;2:357-364.

17. Insua-Rodriguez J, Oskarsson T, The extracellular matrix in breast cancer. *Advanced drug delivery reviews.* 2016;97:41-55.

18. Lu P, Weaver V.M, Werb Z. The extracellular matrix: A dynamic niche in cancer progression. *Journal of Cell Biology.* 2012;196:395-406.

19. Humphrey J.D, Dufresne E.R, Schwartz M.A. Mechanotransduction and extracellular matrix homeostasis. *Nature Reviews* 2014;15:802-811.

20. Butcher D.T, Alliston T, Weaver V.M. A tense situation: forcing tumour progression. *Nature Reviews Cancer* 2009;9:108-122.

21. Paszek M.J, Zahir N, Johnson K.R, Lakins J.N, Rozenberg G.I, Gefen A, Reinhart-King C.A, Margulies S.S, Dembo M, Boettiger D, Hammer D.A, Weaver V.M. Tensional homeostasis and the malignant phenotype. *Cancer cell.* 2005;8:241-254.

22. Pelham R.J, Wang Y.L. Cell locomotion and focal adhesions are regulated by substrate flexibility. *PNAS* 1997;94:13661.

23. Wang H-B, Dembo M, Wang Y-L. Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *American journal of physiological cell physiology.* 2000;279:1345-1350.

24. Ulrich T.A, De Juan Pardo E.M, Kumar S. The Mechanical Rigidity of the Extracellular Matrix Regulates the Structure, Motility, and Proliferation of Glioma Cells. *Cancer Research.* 2009;69:4167-4174.

25. Ishihara S, Yasuda M, Harada I, Mizutani T, Kawabata K, Haga H. Substrate stiffness regulates temporary NF-κB activation via actomyosin contractions. *Experimental Cell Research.* 2013;319:2916-2927.

26. Bharti A.C, Aggarwal B.B. Nuclear factor-kappa B and cancer: its role in prevention and therapy. *Biochemical pharmacology.* 2002;64:883-888.

27. Provenzano P.P, Eliceiri K.W, Campbell J.M, Inman D.R, White J.D, Keely P.J. Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC medicine.* 2006;4:38.

28. Riching K.M, Cox B.L, Salick M.R, Pehlke C, Riching A.S, Ponik S.M, Bass B.R, Crone W.C, Jiang Y, Weaver A.M, Eliceiri K.W, Keely P.J. 3D Collagen Alignment Limits Protrusions to Enhance Breast Cancer Cell Persistence. *Biophysical Journal.* 2014;107:2546-2558.

29. Foroni L, Vasuri F, Valente S, Gualandi C, Focarete M.L, Caprara G, Scandola M, D'Errico-Grigioni A, Pasquinelli G. The role of 3D microenvironmental organization in MCF-7 epithelial–mesenchymal transition after 7 culture days. *Experimental Cell Research.* 2013;319:1515-1522.

30. Girard Y.K, Wang C, Ravi S, Howell M.C, Mallela J, Alibrahim M, Green R, Hellermann G, Mohapatra S.S, Mohapatra S. A 3D Fibrous Scaffold Inducing Tumoroids: A Platform for Anticancer Drug Development. *Plos One.* 2013;8:e75345.

31. Saha S, Duan X, Wu L, Lo P, Chen H, Wang Q. Electrospun fibrous scaffolds promote breast cancer cell alignment and epithelial-mesenchymal transition. *Langmuir.* 2012;28:2028-2034.

32. Kalluri R, Weinberg R.A. The basics of epithelial-mesenchymal transition. *The journal of clinical investigation*. 2009;119:1420-1428.

33. Moustakas A, Heldin C-H. Signaling networks guiding epithelial–mesenchymal transitions during embryogenesis and cancer progression. *Cancer Science*. 2007;98:1512-1520.

34. Tidball J.D. Inflammatory processes in muscle injury and repair. *American Journal of Physiology Regulatory Integrative and Comparative Physiology*. 2005;288:R345-R353.

35. Eming S.A, Krieg T, Davidson J.M. Inflammation in wound repair: molecular and cellular mechanisms. *Journal of Investigative Dermatology*. 2007;127:514-525.

36. Midwood K.S, Williams L.V, Schwarzbauer J.E, Tissue repair and the dynamics of the extracellular matrix. *The International Journal of Biochemistry & Cell Biology*. 2004;36:1031-1037.

37. Domura R, Sasaki R, Okamoto M, Hirano M, Kohda K, Napiwocki B, Turng L-S. Comprehensive study on cellular morphologies, proliferation, motility, and epithelial–mesenchymal transition of breast cancer cells incubated on electrospun polymeric fiber substrates. *J Mater Chem B*. 2017;5:2588-2600.

38. Sasaki R, Domura R, Okamoto M. Cellular morphologies, motility, and epithelial–mesenchymal transition of breast cancer cells incubated on electrospun polymeric fiber substrates in hypoxia. *Mater Today Chem*. 2019;11:29-41.

39. Schrader J, Gordon-Walker T.T, Aucott R.L, Van Deemter M, Quaas A, Walsh S, Benten D, Forbes S.J, Wells R.G, Iredale J.P. Matrix stiffness modulates proliferation, chemotherapeutic response, and dormancy in hepatocellular carcinoma cells. *Hepatology*. 2011;53:1192-1205.

40. Park Y, Depeursinge C, Popescu G. Quantitative phase imaging in biomedicine. *Nat. Photon*. 2018;12:578-589.

41. Benzerdjeb N, Garbar C, Camparo P, Sevestre H. Digital holographic microscopy as screening tool for cervical cancer preliminary study. *Cancer Cytopathol*. 2016;124:573-580.

42. Chen CL, Mahjoubfar A, Tai LC, Blaby IK, Huang A, Niazi KR, Jalali B. Deep learning in label-free cell classification. *Sci Rep*. 2016;6:21471.

43. Roitshtain D, Wolbromsky L, Bal E, Greenspan H, Satterwhite LL, Shaked NT. Quantitative phase microscopy spatial signatures of cancer cells. *Cytometry A*. 2017;91A:482-493.

44. Strbkova L, Zicha D, Vesely P, Chmelik R. Automated classification of cell morphology by coherence-controlled holographic microscopy. *J Biomed Opt*. 2017;22:221-229.

45. Dickson MA, Schwartz GK. Development of cell-cycle inhibitors for cancer therapy. *Curr Oncol*. 2009;16:36–43.

46. Ishikawa Y, Sasaki R, Domura R, Okamoto M. Cellular morphologies, motility, and epithelial–mesenchymal transition of breast cancer cells incubated on viscoelastic gel substrates in hypoxia. *Mater Today Chem.* 2019;13:8-17.

47. Chaudhuri P.K, Pan C.Q, Low B.C, Lim C.T. Topography induces differential sensitivity on cancer cell proliferation via Rho-ROCK-Myosin contractility. *Sci. Rep.* 2016;6:19672.

48. Wu J, Chu C-C. Block copolymer of poly(ester amide) and polyesters: Synthesis, characterization, and in vitro cellular response. *Acta Biomater.* 2012;8:4314-4323.

49. Tsuji T, Ibaragi S, G-F. Hu. Epithelial-mesenchymal transition and cell cooperativity in metastasis. *Cancer Res.* 2009;69:7135-7139.

50. Allemani C, Weir H.K, Carreira H, Harewood R, Spika D, Wang X.S, Bannon F, Ahn J.V, Johnson C.J, Bonaventure A, Marcos-Gragera R, Stiller C, Azevedo E Silva G, Chen W.Q, Ogunbiyi O.J, Rachet B, Soeberg M.J, You H, Matsuda T, Bielska-Lasota M, Storm H, Tucker T.C, Coleman M.P. Global surveillance of cancer survival 1995–2009: analysis of individual data for 25 676 887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet.* 2015;385:977-1010.

51. Hanahan D, Weinberg R.A. Hallmarks of Cancer: The Next Generation. *Cell.* 2011;144(5):646-674.

52. Stacy N.I, Isaza R, Wiedner E. First report of changes in leukocyte morphology in response to inflammatory conditions in Asian and African elephants (Elephas maximus and Loxodonta africana). *PLoS One.* 2017;12(9):e0185277.

53. Pekny M, Pekna M, Messing A, Steinhauser C, Lee J.M, Parpura V, Hol E.M, Sofroniew M.V, Verkhratsky A. Astrocytes: a central element in neurological diseases. *Acta Neuropathol.* 2016;131(3):323-345.

54. Chen S, Zhao M, Wu G, Yao C, Zhang J. Recent advances in morphological cell image analysis. *Comput Math Methods Med.* 2012:2012101536.