J Cancer Res Clin Oncol (2012) 138:859–866
DOI 10.1007/s00432-012-1159-5

ORIGINAL PAPER

# Higher cell stiffness indicating lower metastatic potential in B16 melanoma cell variants and in (−)-epigallocatechin gallate-treated cells

**Tatsuro Watanabe · Hiromi Kuramochi · Atsushi Takahashi · Kazue Imai · Naoko Katsuta · Tomonobu Nakayama · Hirota Fujiki · Masami Suganuma**

Received: 21 December 2011 / Accepted: 12 January 2012 / Published online: 2 February 2012


**Abstract**
*Purpose* To understand how nanomechanical stiffness affects metastatic potential, we studied the relationship between cell migration, a characteristic of metastasis, and cell stiffness using atomic force microscopy (AFM), which can measure stiffness (elasticity) of individual living cells.
*Methods* Migration and cell stiffness of three metastatic B16 melanoma variants (B16-F10, B16-BL6, and B16-F1 cells), and also effects of (−)-epigallocatechin gallate (EGCG), were studied using Transwell assay and AFM.
*Results* Migration of B16-F10 and B16-BL6 cells was 3 and 2 times higher than that of B16-F1 cells in Transwell assay, and cell stiffness determined by AFM was also different among the three variants, although they have similar morphologies and the same growth rates: Means of Young's modulus were 350.8 ± 4.8 Pa for B16-F10 cells, 661.9 ± 16.5 Pa for B16-BL6 cells, and 727.2 ± 13.0 Pa for B16-F1 cells. AFM measurements revealed that highly motile B16-F10 cells have low cell stiffness, and low motile and metastatic B16-F1 cells have high cell stiffness: Nanomechanical stiffness is inversely correlated with migration potential. Treatment of highly motile B16-F10 cells with EGCG increased cell stiffness 2-fold and inhibited migration of the cells.
*Conclusions* Our study with AFM clearly demonstrates that cell stiffness is a reliable quantitative indicator of migration potential, and very likely metastatic potential, even in morphologically similar cells. And increased cell stiffness may be a key nanomechanical feature in inhibition of metastasis.

**Keywords** Atomic force microscopy · Cell stiffness · Green tea · Metastasis · Young's modulus

T. Watanabe and H. Kuramochi contributed equally to this work.

T. Watanabe · A. Takahashi · M. Suganuma (✉)
Research Institute for Clinical Oncology, Saitama Cancer Center, Kitaadachi-gun, Saitama 362-0806, Japan
e-mail: masami@cancer-c.pref.saitama.jp

T. Watanabe
e-mail: watanabet@cancer-c.pref.saitama.jp

A. Takahashi
e-mail: takahashi.atsushi@pref.saitama.lg.jp

H. Kuramochi · T. Nakayama
National Institute for Materials Science, International Center for Materials Nanoarchitectonics, Ibaraki, Japan
e-mail: KURAMOCHI.Hiromi@nims.go.jp

T. Nakayama
e-mail: nakayama.tomonobu@nims.go.jp

A. Takahashi
Graduate School of Science and Engineering, Saitama University, Sakura-ku, Saitama, Japan

K. Imai
Department of Radiobiology and Molecular Epidemiology, Radiation Effects Research Foundation, Hiroshima, Japan
e-mail: kimai@rerf.or.jp

N. Katsuta
Nanotechnology Innovation Center, National Institute for Materials Science, Ibaraki, Japan
e-mail: anko@tj8.so-net.ne.jp

H. Fujiki
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan
e-mail: hfujiki@ph.bunri-u.ac.jp

**Abbreviations**

AFM Atomic force microscopy
EGCG (−)-Epigallocatechin gallate
MβCD Methyl-β-cyclodextrin

## Introduction

Inhibition of metastasis is a key subject of cancer research. To approach this subject, we wanted to develop a new parameter to determine the metastatic potential of cancer cells. Recently, various biophysical nanotechniques—including atomic force microscopy (AFM), a microfluidic optical stretcher, and a magnetic tweezer system—have made it possible to measure nanomechanical properties of cancer cells with high sensitivity (Fritsch et al. 2010; Bao and Suresh 2003; Remmerbach et al. 2009; Swaminathan et al. 2011). AFM has the advantage of being able to quantitatively determine cell stiffness (elasticity) of individual living cells in the physiological condition (Binnig et al. 1986; Suresh 2007). Studies with AFM have revealed that metastatic cells obtained from the body fluids of patients with lung, breast, and pancreas cancers have significantly lower cell stiffness than normal mesothelial cells from those body fluids (Cross et al. 2007). If cell stiffness is a new reliable quantitative indicator of metastasis, cancer diagnosis and treatment will be greatly improved. To clarify the significance of cell stiffness in metastatic potential, we used AFM to determine how cell stiffness differs among variants with differing metastatic potentials but similar morphology, and how inhibiting metastasis modulates cell stiffness. Since motility of cancer cells is a critical characteristic in metastasis (Steeg 2006), the relationship between migration and cell stiffness using Transwell assay and AFM was studied. We previously reported that (−)-epigallocatechin gallate (EGCG) in drinking water inhibited both lung colonization of B16-F10 cells and spontaneous metastasis of B16-BL6 cells from foot pad into the lungs of C57BL/6 mice (Taniguchi et al. 1992). EGCG is the main constituent of green tea and is known to be an effective cancer preventive beverage: 10 cups of green tea daily prevented 50% recurrence of colorectal adenomas in humans in phase II clinical trials (Shimizu et al. 2008). In addition, some clinical trials have demonstrated that green tea catechins are effective on cancer prevention in prostate, oral, and cervical premalignant regions, although human epidemiological studies with green tea have been inconclusive (Bettuzzi et al. 2006; Tsao et al. 2009; Connors et al. 2011; Singh et al. 2011; Yang and Wang 2010). EGCG is a suitable tool to reveal the significance of cell stiffness in inhibition of metastasis. So, three metastatic B16 mouse melanoma cell variants and EGCG-treated cells were used in this study using AFM.

Three B16 melanoma cell variants (B16-F10, B16-BL6, and B16-F1) have similar morphologies and growth rates. But injections of these three cell variants into mouse tail vein showed clear differences in their colony formation in the lungs of C57BL/6 mice: B16-F10 cells produced the highest number of foci; B16-BL6 cells, a medium number of foci; and B16-F1 cells, the lowest number (Fidler 1973; Poste et al. 1980). Our studies with Transwell assay and AFM revealed that highly motile B16-F10 cells and intermediate motile B16-BL6 cells have significantly lower cell stiffness than low motile B16-F1 cells. And treatment with EGCG dose-dependently induced increases in cell stiffness (up to rigid elasticity) and inhibited the migration of B16-F10 cells. This paper is the first report that cell stiffness as measured by AFM is a key nanomechanical feature indicating metastatic potential. In this paper, we discuss the mechanism of inducing increases in cell stiffness with EGCG, in relation to alteration of cell membrane organization.

## Materials and methods

### Cell lines and reagents

B16-F10, B16-BL6, and B16-F1 cell variants were kindly provided by Dr. Shun'ichiro Taniguchi at Shinshu University in Japan in 2009 and were stored in liquid nitrogen. The variants were grown in DMEM (Nissui, Tokyo) with 10% fetal bovine serum (JRH Bioscience, KS) and used within 2 months after resuscitation. Their origins and properties were reported by Fidler (1973) and Poste et al. (1980), and their morphologies, growth curves, and motilities had been tested in 2010. EGCG (more than 99% purity) isolated from Japanese green tea leaves was used for the experiments (Fujiki and Okuda 1992). (−)-Epicatechin (EC) and methyl-β-cyclodextrin (MβCD) were purchased from Funakoshi, Tokyo and Wako Pure Chemical Industries, Osaka.

### Migration potential by Transwell assay

The migration potential of B16 cell variants was determined in a Transwell cell culture chamber (Becton–Dickinson, NJ). Cells ($1 \times 10^4$/100 μl) in serum-free DMEM containing 0.1% BSA were added to the insert well with 8-μm-pore filter and then incubated with fibronectin (0–10 μg/ml) (Becton–Dickinson) in lower chamber for 4 h at 37°C. The cells that migrated to the reverse side of filter were stained with 0.4% Trypan blue and then counted (Liotta et al. 1986). B16-F10 cells in the upper chambers were incubated with various concentrations of EGCG or EC, and with 5 μg/ml fibronectin in lower chambers for 4 h. Inhibition of cell migration was expressed as % of

control, and the values are means of 3–5 independent experiments conducted in duplicate.

### Cell stiffness by AFM

MFP-3D-Bio-J AFM (Asylum Research, CA) with a sharpened silicon nitride cantilever (TR400PSA, 0.08 N/m of spring constant, Olympus, Tokyo) was used for the experiments. The spring constant of the cantilever was calculated by the thermal fluctuation method (Levy and Maaloum 2002). The cells ($5 \times 10^4$ cells) were seeded on a 6-cm dish, and after 2 days, seven force-curves per cell were obtained by the determination of nuclear region on the cell. Force-curves were recorded at 1 Hz for determination of Young's modulus (*E:* Pa) (Cross et al. 2007). *E* value was calculated by fitting with the Hertz model. The half angle of the probe on cantilever was determined to be 17.5°, and the Poisson ratio of the cell was taken to be 0.5 (Trickey et al. 2006). The effects of EGCG, EC, and M$\beta$CD on stiffness of B16-F10 cells were measured by AFM 4 h after incubation. The mean value of Young's modulus was obtained from log-normal fitting curve.

### Statistics

Statistical analysis for differences in mean value of Young's modulus was conducted using nonparametric analysis with Wilcoxon–Mann–Whitney, and the migration was analyzed by a two-sample independent Student's *t*-test.

## Results

### Migration of three B16 melanoma cell variants

The migration potentials of B16-F10, B16-BL6, and B16-F1 cell variants were determined by Transwell assay. The numbers of migrated cells into the reverse side of filters increased dose-dependently based on concentrations of fibronectin and showed that B16-F10 cells were the most motile, B16-BL6 cells medium motile and B16-F1 cells the least motile (Fig. 1). When the numbers of migrated cells were compared in the presence of 5 μg/ml fibronectin, B16-F10 and B16-BL6 cells were threefold and twofold more motile than B16-F1 cells. Our results are consistent with previously reported data (Fidler 1973; Poste et al. 1980; Duncan et al. 1998).

### Cell stiffness of three B16 melanoma cell variants

Determination of AFM was conducted at the precise center position of nucleus in a cell, and representative force-curves for three cell variants are shown in Fig. 2a. The force-curves provided slopes corresponding to the means of Young's modulus, and the difference in the slopes indicated varying cell stiffness among the three cell variants. Young's moduli were obtained from 475 to 713 force-curves for 85–127 cells of each variant and provided a histogram showing their cell stiffness (Fig. 2b; Table 1): Histogram shows a narrow spiked peak with mean value of Young's modulus 350.8 ± 4.8 Pa for B16-F10 cells, an intermediate peak with mean value of Young's modulus 661.9 ± 16.5 Pa for B16-BL6 cells, and a broader peak with mean value of Young's modulus 727.2 ± 13.0 Pa for B16-F1 cells. This means that the Young's modulus of B16-F10 cells has significantly lower cell stiffness, that is, soft elasticity, than those of B16-BL6 ($p < 0.0001$) and B16-F1 ($p < 0.0001$). Also, B16-BL6 cells show lower cell stiffness, that is, soft elasticity, than B16-F1 ($p = 0.005$). The mean of Young's modulus was found to be inversely correlated with the number of migrated cells at 5 μg/ml fibronectin (Table 1). Furthermore, the three cell variants had much lower Young's modulus (Pa) than normal mouse

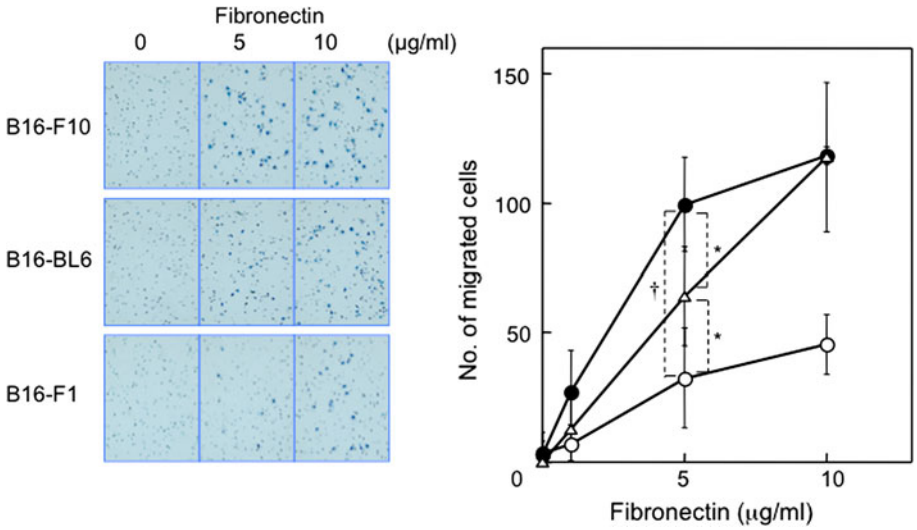


**Fig. 1** Migration of three B16 variants. Photos on *left side* show representative membrane attached migrated cells. B16-F10 (*filled circle*), B16-BL6 (*open triangle*), and B16-F1 cells (*open circle*)were incubated in the insert well with various concentrations of fibronectin in the lower chamber for 4 h at 37°C. Average of four independent experiments was plotted with SD value. *$p < 0.01$; †$p < 0.0001$

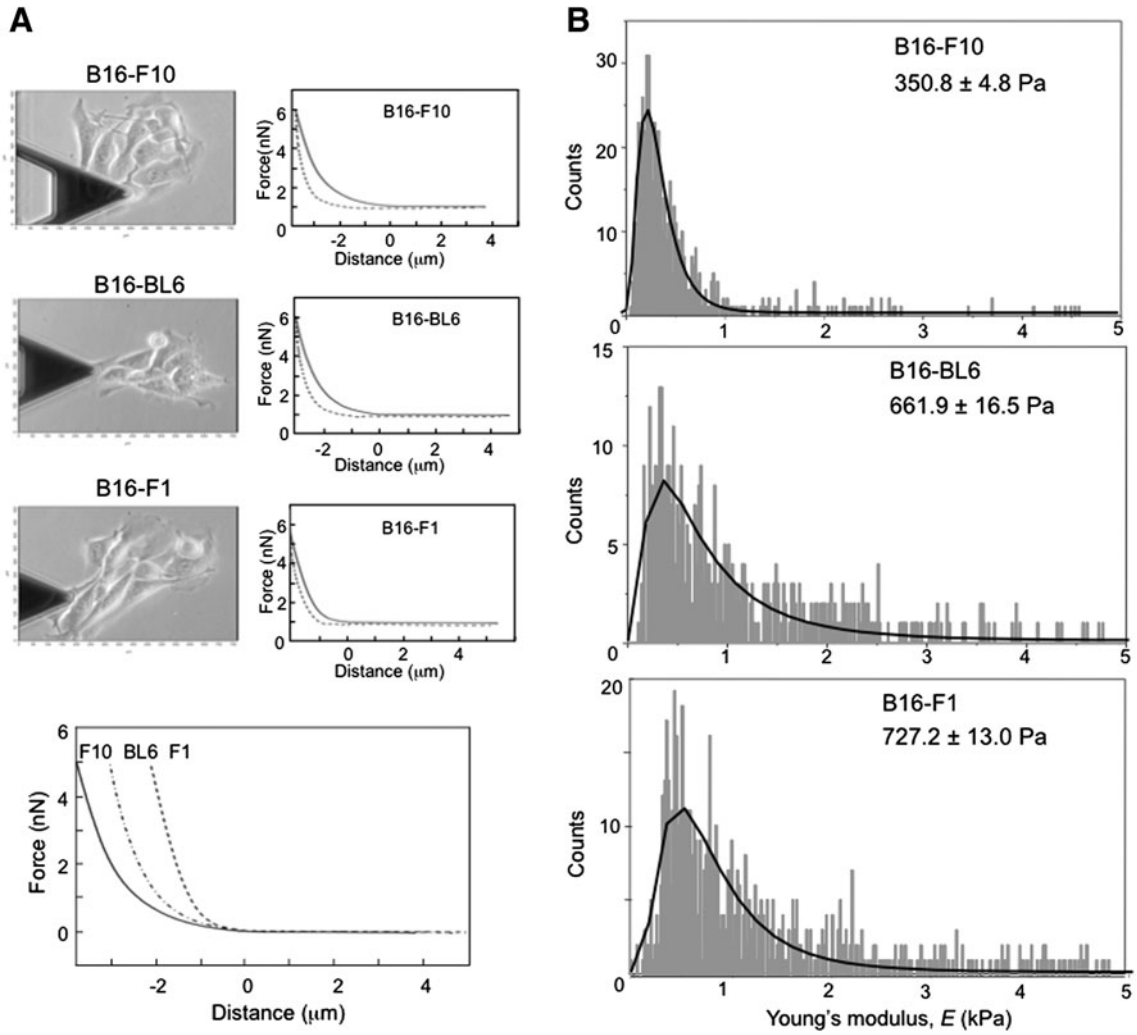


**Fig. 2** Cell stiffness of three B16 variants. **a** AFM cantilever (*black trigona*) located on the nucleus in the cell. Extension curve (*solid line*) of the cell is used for calculation of Young's modulus. **b** Histograms of Young's modulus obtained from 550 curves (B16-F10 cells), 475 curves (B16-BL6 cells), and 713 curves (B16-F1 cells). *Black line* indicates fitting curve obtained from log-normal distribution

fibroblasts (BALB/3T3)—a mean of 1,370 Pa—indicating that the melanoma cell variants have lower cell stiffness than normal cells, that is, soft elasticity. Thus, low cell stiffness is apparently a key characteristic of higher motility: We think that cell stiffness determined by AFM quantitatively indicates discrete migration potential—and probably metastatic potential—of individual cancer cells, even cells that are morphologically similar.

## Increasing stiffness of B16-F10 cells with EGCG associated with inhibition of migration

Treatment of highly motile B16-F10 cells with EGCG reduced the numbers of migrated cells into reverse sides of filters in the presence of 5 μg/ml fibronectin. Specifically, treatments with 50, 100, and 200 μM EGCG dose-dependently reduced the migration of B16-F10 cells to 57.1, 30.3, and 12.6%, respectively (Fig. 3), without affecting viability of the cells (103.6, 107.1, and 95.2%, respectively). These results supported our previous evidence showing that EGCG in drinking water inhibited the metastasis of B16-F10 cells into the lungs of C57BL/6 mice (Taniguchi et al. 1992), as mentioned above. EC, an inactive green tea catechin, showed only marginal inhibition (Fig. 3).

Next, the stiffness of B16-F10 cells treated with EGCG for 4 h was determined by AFM: Histogram shows that EGCG dose-dependently shifted Young's modulus to the high Pa side, indicating an increase in cell stiffness, that is, toward rigid elasticity (Fig. 4). The mean of Young's modulus for non-treated B16-F10 cells was 441.0 ± 8.1 Pa: Treatment with 50 μM EGCG elevated the mean of Young's modulus to 579.5 ± 27.0 Pa, with 100 μM EGCG to 680.0 ± 27.3 Pa, and with 200 μM EGCG to

**Table 1** Relationship between cell migration and cell stiffness in B16 melanoma cell variants

| | Migration | Cell stiffness | | | | | |
|---|---|---|---|---|---|---|---|
| | No. of migrated cells* | Mean value of Young's modulus (*E*: Pa ± SD) | No. of force-curves/ No. of examined cells | Distribution of cell stiffness (% of cells) | | | Maximum value (kPa) |
| | | | | < 1 kPa | 1 – 2 kPa | 2 – 5 kPa | 5 kPa < | |
| B16-F10 | 99.6 ± 18.3 | 350.8 ± 4.8 | 550/96 | 87.8 | 6.4 | 5.6 | 0.2 | 5.08 |
| B16-BL6 | 63.9 ± 19.1 | 661.9 ± 16.5 | 475/85 | 55.8 | 20.4 | 17.9 | 5.9 | 18.03 |
| B16-F1 | 32.6 ± 19.2 | 727.2 ± 13.0 | 713/127 | 51.5 | 21.4 | 18.2 | 8.8 | 22.30 |

* Fibronectin at 5μg/ml

* $p < 0.0001$

‡ $p < 0.01$

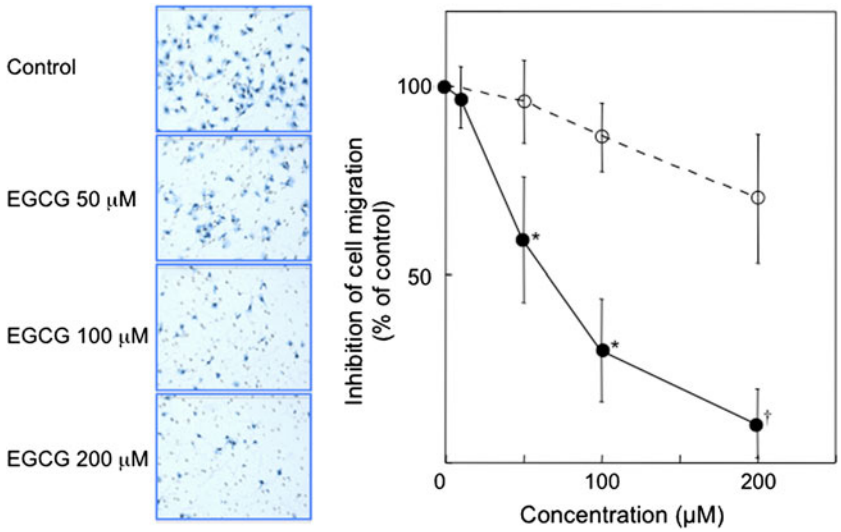


**Fig. 3** Inhibition of B16-F10 cell migration with EGCG. Photos on *left side* show representative membranes attached migrated cells treated with EGCG. Inhibition of migration with EGCG (*filled circle*), but not with EC (*open circle*). Number of migrated cells in non-treated cells was expressed as 100%. Average of five independent experiments was plotted with SD value. *$p < 0.001$; †$p < 0.0001$

804.5 ± 20.1 Pa (Fig. 4). Thus, treatment with EGCG increased Young's modulus of B16-F10 cells to the level of low motile B16-F1 cells. But, the treatment with 100 μM inactive EC did not show any increase in mean of Young's modulus, to be only 359.6 ± 28.0 Pa. Thus, the treatment with EGCG induced increased cell stiffness, that is, rigid elasticity, without causing any morphological change or growth inhibition. The results clearly demonstrated that increasing cell stiffness (rigid elasticity) is associated with reduction of migration, probably inhibition of metastasis.

## Increasing cell stiffness by alteration of membrane organization induced with M*β*CD

Considering the mechanism of EGCG, we previously reported that EGCG interrupts the interaction of ligands with their receptors on cell membrane, which we named the sealing effects of EGCG (Yoshizawa et al. 1992; Fujiki 2005). This is associated with results showing that EGCG causes reduction of detergent-insoluble membrane domain, that is, a decrease of lipid raft (Yoshizawa et al. 1992; Fujiki 2005; Adachi et al. 2007). We next studied whether another reagent that generates an EGCG-like effect on membrane organization could also enhance cell stiffness. M*β*CD is a typical reagent for inducing alteration of membrane organization (Yancey et al. 1996; Adachi et al. 2007), and as we expected, treatment with 10 mM M*β*CD for 1 h increased Young's modulus threefold (Fig. 5a). M*β*CD dose-dependently inhibited migration of B16-F10 cells without affecting cell viability. The results suggest that the alteration of membrane organization is a key mechanism in increasing cell stiffness (changing to rigid elasticity), resulting in inhibition of cell migration.

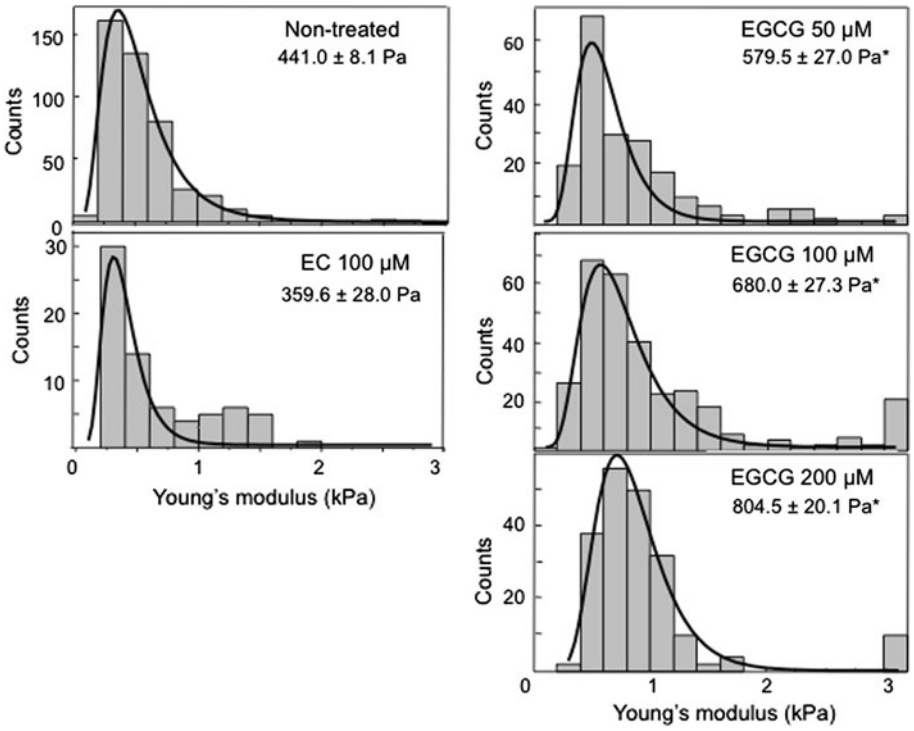


**Fig. 4** Increase of cell stiffness with EGCG. Histograms of Young's modulus obtained from 447 curves (non-treated B16-F10 cells), 192 curves (50 μM EGCG), 301 curves (100 μM EGCG), 102 curves (200 μM EGCG), and 72 curves (100 μM EC) after treatment for 4 h, as described in "Materials and methods". $*p < 0.001$

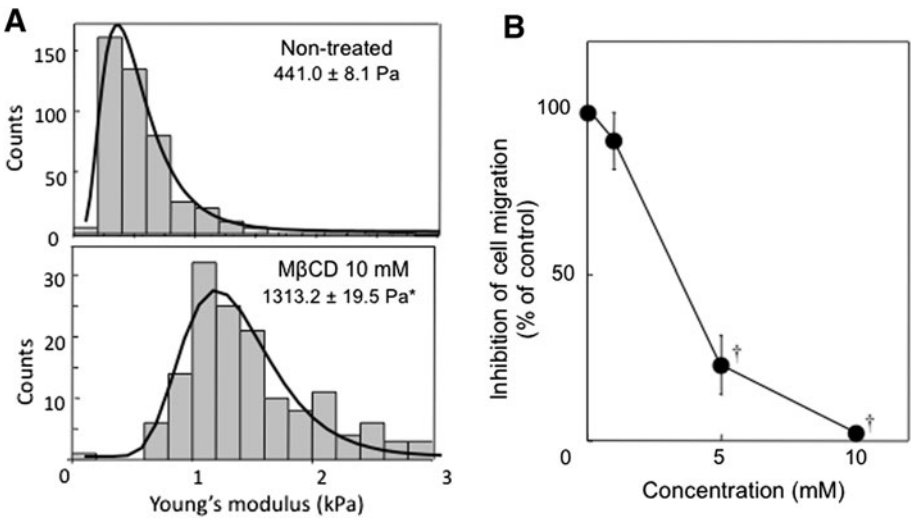


**Fig. 5** Increase of cell stiffness with M$\beta$CD associated with inhibition of migration in B16-F10 cells. **a** Histograms of Young's modulus obtained from 447 curves (non-treated B16-F10 cells) and 161 curves (10 mM M$\beta$CD) after treatment for 4 h, as described in "Materials and methods". $*p < 0.001$. **b** Inhibition of migration with M$\beta$CD. Number of migrated cells in non-treated cells was expressed as 100%. Average of three independent experiments was plotted with SD value. $\dagger p < 0.0001$

## Discussion

This manuscript reports that cell stiffness (measured by AFM) can predict cell migration, and probably metastatic potential, among cell variants with similar morphologies and growth rates: Lower cell stiffness (soft elasticity) with low Young's modulus is associated with higher migration potential, although it was recently reported that cell elasticity (stiffness) was closely related to cell morphology (Guo et al. 2012). Previous biomechanical investigations using AFM reported that cancer cells have a lower Young's modulus—i.e., soft elasticity—than normal/benign cells, for example, bladder cancer cells vs. normal bladder cells, breast cancer cells (MCF-7) versus non-malignant breast cells (MCF-10A) (Lekka et al. 1999; Li et al. 2008). Our study also demonstrates that three B16 cell variants had significantly lower means of Young's modulus—lower cell stiffness (softer elasticity)—than normal BALB/3T3 cells, and that the mean of Young's modulus is an indicator of migration potential, and probably metastatic potential. The results are well supported by investigation with a magnetic tweezer system: Cancer cells with high migration potential

were less stiff than cells with low migration potential (Swaminathan et al. 2011). Thus, we think that cell stiffness is an important quantitative diagnostic marker of metastatic property as well as diagnosis of cancer (Cross et al. 2009; Suresh 2007).

Histogram of Young's modulus clearly indicates the qualitative difference between high metastatic B16-F10 cells and low metastatic B16-F1 cells: B16-F10 cells showed narrower distribution of Young's modulus, and B16-F1 cells presented a broad distribution. B16-F10 cells were originally selected from B16-F1 cells by 9 times repeated in vivo selection, and as a result, B16-F10 cells possess an increased colonizing ability in the lungs after i.v. injection (Fidler 1973). So we assume that cells with low Young's modulus are accumulated during in vivo selection, and that broad distribution of Young's modulus in B16-F1 cells reflects either the multi-clonality of cancer cells or the presence of subpopulations with different cell motilities, and probably discrete metastatic potentials. AFM is the first method of quantitatively measuring individual cell features, and we think that the discrete characteristics of cancer cells proposed by Tomizo Yoshida in 1955 have been proved by AFM (Yoshida et al. 1955).

Our study with EGCG indicated that increased cell stiffness is a nanomechanical feature associated with inhibition of cell migration, and probably metastasis. Treatment with green tea extract made tumor cells obtained from cancer patients more stiff (Cross et al. 2011), and similar investigations revealed that transfection of a metastasis suppressor gene (breast cancer metastasis suppressor 1, BRMS1) increased Young's modulus of metastatic human breast cancer cell line (MDA-MB-435) 3.8 fold and suppressed metastasis (Wu et al. 2010). These results suggest that cell stiffness can be altered by treatment with inhibitors of metastasis, such as EGCG, and that an increase in nanomechanical stiffness along with high Young's modulus, that is, rigid elasticity, is associated with inhibition of cell migration. Curcumin is a cancer preventive compound and inhibits metastasis (Anand et al. 2008; Menon et al. 1999). We recently found that curcumin also significantly increased the cell stiffness of B16-F10 cells and inhibited migration of the cells (manuscript in preparation).

As for the potential mechanism of changing cell stiffness, we demonstrated an alteration of membrane organization, although cell stiffness is often discussed in relation to the reorganization of cytoskeletal F-actin (Costa 2003; Cross et al. 2011). The increase in cell stiffness after treatment with EGCG is related to the sealing effects of EGCG, which inhibit the interaction of ligands with their receptors by alteration of cell membrane organization (Yoshizawa et al. 1992; Fujiki 2005; Adachi et al. 2007). Treatment of cells with EGCG inhibited EGF binding to its receptor and the activation of EGF receptor through alteration of membrane organization. M$\beta$CD is reported to inhibit EGF receptor activation by depletion of cholesterol from the membrane, similar to EGCG does (Yancey et al. 1996; Adachi et al. 2007). Since cell stiffness determined by AFM is a general feature of membrane, cytosol, nucleus, and cytoskeleton, we think that AFM can detect early biochemical reactions on cell membrane induced by EGCG and M$\beta$CD, as changes in cell stiffness. Since a difference in membrane lipid composition was found among B16 melanoma cell variants (Schroeder and Gardiner 1984), lipid composition in the membrane might affect cell stiffness as well as metastatic potential.

We here report for the first time that low cell stiffness with low Young's modulus is associated with high cell migration, and that elevating cell stiffness is a new nanomechanical feature leading to inhibition of cell migration, and very likely metastasis. The study of cell stiffness by AFM will soon open a new era for more positive cancer prognosis.

**Acknowledgments** We thank Drs. James K. Gimzewski and Shivani Sharma, University of California, and Drs. Kunihiko Okajima and Mariko Ago, Tokushima Bunri University, for their fruitful discussion. We also thank Dr. Shun'ichiro Taniguchi for the supply of B16 variants, and Mr. Takahisa Matsuzaki, Ms. Kaori Suzuki and Ikuko Shiotani, Research Institute for Clinical Oncology, Saitama Cancer Center, for their technical assistance. This work was supported by the Smoking Research Fundation, Urakami Foundation, and World Premier International Research Center Initiative on Materials Nanoarchitectonics (H. K. and T. N.). This work was supported in part by the Nanotechnology Network Japan Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

**Conflict of interest** The authors did not have any conflicts of interest to disclose.

## References

Adachi S, Nagao T, Ingolfsson HI, Maxfield FR, Andersen OS, Kopelovich L, Weinstein IB (2007) The inhibitory effect of (−)-epigallocatechin gallate on activation of the epidermal growth factor receptor is associated with altered lipid order in HT29 colon cancer cells. Cancer Res 67:6493–6501

Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB (2008) Curcumin and cancer: an "old-age" disease with an "age-old" solution. Cancer Lett 267:133–164. doi:10.1016/j.canlet.2008.03.025

Bao G, Suresh S (2003) Cell and molecular mechanics of biological materials. Nat Mater 2:715–725

Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A (2006) Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res 66:1234–1240

Binnig G, Quate CF, Gerber C (1986) Atomic force microscope. Phys Rev Lett 56:930–933

Connors SK, Chornokur G, Kumar NB (2011) New insights into the mechanisms of green tea catechins in the chemoprevention

of prostate cancer. Nutr Cancer. doi:10.1080/01635581.2012.630158
Costa KD (2003) Single-cell elastography: probing for disease with the atomic force microscope. Dis Markers 19:139–154
Cross SE, Jin Y-S, Rao JY, Gimzewski JK (2007) Nanomechanical analysis of cells from cancer patients. Nat Nanotechnol 2:780–783
Cross SE, Jin Y-S, Rao JY, Gimzewski JK (2009) Applicability of AFM in cancer detection. Nat Nanotechnol 4:72–73
Cross SE, Jin Y-S, Lu Q-Y, Rao JY, Gimzewski JK (2011) Green tea extract selectively targets nanomechanics of live metastatic cancer cells. Nanotechnology 22:215101–215110
Duncan LM, Deeds J, Hunter J, Shao J, Holmgren LM, Woolf EA, Tepper RI, Shyjan AW (1998) Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. Cancer Res 58:1515–1520
Fidler IJ (1973) Selection of successive tumour lines for metastasis. Nat New Biol 242:148–149
Fritsch A, Hockel M, Kiessling T, Nnetu D, Wetzel F, Zink M, Kas JA (2010) Are biomechanica changes necessary for tumour progression? Nat Phys 6:730–732
Fujiki H (2005) Green tea: health benefits as cancer preventive for humans. Chem Rec 5:119–132
Fujiki H, Okuda T (1992) (−)-Epigallocatechin gallate. Drugs Future 17:462–464
Guo Q, Xia Y, Sandig M, Yang J (2012) Characterization of cell elasticity correlated with cell morphology by atomic force microscope. J Biomech 45:304–309
Lekka M, Laidler P, Gil D, Lekki J, Stachura Z, Hrynkiewicz AZ (1999) Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy. EBJ 28:312–316
Levy R, Maaloum M (2002) Measuring the spring constant of atomic force microscope cantilevers: Thermal fluctuations and other methods. Nanotechnology 13:33–37
Li QS, Lee GY, Ong CN, Lim CT (2008) AFM indentation study of breast cancer cells. Biochem Biophys Res Commun 374:609–613. doi:10.1016/j.bbrc.2008.07.078
Liotta LA, Mandler R, Murano G, Katz DA, Gordon RK, Chiang PK, Schiffmann E (1986) Tumor cell autocrine motility factor. Proc Natl Acad Sci USA 83:3302–3306
Menon LG, Kuttan R, Kuttan G (1999) Anti-metastatic activity of curcumin and catechin. Cancer Lett 141:159–165
Poste G, Doll J, Hart IR, Fidler IJ (1980) In vitro selection of murine B16 melanoma variants with enhanced tissue-invasive properties. Cancer Res 40:1636–1644
Remmerbach TW, Wottawah F, Dietrich J, Lincoln B, Wittekind C, Guck J (2009) Oral cancer diagnosis by mechanical phenotyping. Cancer Res 69:1728–1732
Schroeder F, Gardiner JM (1984) Membrane lipids and enzymes of cultured high- and low-metastatic B16 melanoma variants. Cancer Res 44:3262–3269
Shimizu M, Fukutomi Y, Ninomiya M, Nagura K, Kato T, Araki H, Suganuma M, Fujiki H, Moriwaki H (2008) Green tea extracts for the prevention of metachronous colorectal adenomas: a pilot study. Cancer Epidemiol Biomarkers Prev 17:3020–3025
Singh BN, Shankar S, Srivastava RK (2011) Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. Biochem Pharmacol 82:1807–1821. doi:10.1016/j.bcp.2011.07.093
Steeg PS (2006) Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 12:895–904. doi:10.1038/nm1469
Suresh S (2007) Nanomedicine: elastic clues in cancer detection. Nat Nanotechnol 2:748–749
Swaminathan V, Mythreye K, O'Brien ET, Berchuck A, Blobe GC, Superfine R (2011) Mechanical stiffness grades metastatic potential in patient tumor cells and in cancer cell lines. Cancer Res 71:5075–5080
Taniguchi S, Fujiki H, Kobayashi H, Go H, Miyado K, Sadano H, Shimokawa R (1992) Effect of (−)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. Cancer Lett 65:51–54
Trickey WR, Baaijens FP, Laursen TA, Alexopoulos LG, Guilak F (2006) Determination of the Poisson's ratio of the cell: recovery properties of chondrocytes after release from complete micropipette aspiration. J Biomech 39:78–87
Tsao AS, Liu D, Martin J, Tang XM, Lee JJ, El-Naggar AK, Wistuba I, Culotta KS, Mao L, Gillenwater A, Sagesaka YM, Hong WK, Papadimitrakopoulou V (2009) Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions. Cancer Prev Res (Phila) 2:931–941
Wu Y, McEwen GD, Harihar S, Baker SM, DeWald DB, Zhou A (2010) BRMS1 expression alters the ultrastructural, biomechanical and biochemical properties of MDA-MB-435 human breast carcinoma cells: an AFM and Raman microspectroscopy study. Cancer Lett 293:82–91. doi:10.1016/j.canlet.2009.12.016
Yancey PG, Rodrigueza WV, Kilsdonk EP, Stoudt GW, Johnson WJ, Phillips MC, Rothblat GH (1996) Cellular cholesterol efflux mediated by cyclodextrins. Demonstration of kinetic pools and mechanism of efflux. J Biol Chem 271:16026–16034
Yang CS, Wang X (2010) Green tea and cancer prevention. Nutr Cancer 62:931–937. doi:10.1080/01635581.2010.509536
Yoshida T, Isaka H, Nakamura K, Odashima S, Satoh H (1955) Studies on the ascites hepatoma. Trans Soc Pathol Jpn 44:407–426
Yoshizawa S, Horiuchi T, Suganuma M, Nishiwaki S, Yatsunami J, Okabe S, Okuda T, Muto Y, Frenkel K, Troll W, Fujiki H (1992) Penta-*O*-galloyl-*β*-D-glucose and (−)-epigallocatechin gallate. ACS Symp Ser 507:316–325