Mechanical properties of fibroblasts depend on level of cancer transformation

CrossMark

Yu.M. Efremov [a,*], M.E. Lomakina [b], D.V. Bagrov [a], P.I. Makhnovskiy [a], A.Y. Alexandrova [b], M.P. Kirpichnikov [a], K.V. Shaitan [a]

[a] Lomonosov Moscow State University, Faculty of Biology, Leninskie Gory 1/12, Moscow 111991, Russia
[b] Institute of Carcinogenesis, Blokhin Cancer Research Center, Kashirskoye shosse 24, Moscow 115478, Russia

ARTICLE INFO

ABSTRACT

Recently, it was revealed that tumor cells are significantly softer than normal cells. Although this phenomenon is well known, it is connected with many questions which are still unanswered. Among these questions are the molecular mechanisms which cause the change in stiffness and the correlation between cell mechanical properties and their metastatic potential. We studied mechanical properties of cells with different levels of cancer transformation. Transformed cells in three systems with different transformation types (monooncogenic *N-RAS*, viral and cells of tumor origin) were characterized according to their morphology, actin cytoskeleton and focal adhesion organization. Transformation led to reduction of cell spreading and thus decreasing the cell area, disorganization of actin cytoskeleton, lack of actin stress fibers and decline in the number and size of focal adhesions. These alterations manifested in a varying degree depending on type of transformation. Force spectroscopy by atomic force microscopy with spherical probes was carried out to measure the Young's modulus of cells. In all cases the Young's moduli were fitted well by log-normal distribution. All the transformed cell lines were found to be 40–80% softer than the corresponding normal ones. For the cell system with a low level of transformation the difference in stiffness was less pronounced than for the two other systems. This suggests that cell mechanical properties change upon transformation, and acquisition of invasive capabilities is accompanied by significant softening.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Many facts point at the importance of cell mechanical properties in tumorigenesis and cancer progression [1–3]. Low Young's modulus is a new recently discovered marker of many cancer cells [4,5]. It is established that tumor cells are softer than benign and healthy ones. However, there is no clear correlation between cell stiffness and their metastatic potential. Direct correlation was shown in some works [5–7], but reversed, non-significant or complicated correlation was observed in the others [8–11].

It was shown that mechanical properties of cells are mainly defined by actin cytoskeleton structure [12,13]. The actin cytoskeleton is a network of microfilaments responsible for cell shape, protrusion formation and motility. Cancer cells show distinct alterations in morphology and actin cytoskeleton structure compared to normal cells. They generally have a reduced number of stress fibers; the residual microfilament bundles are in disorder, and maturation of focal adhesions is disturbed [14,15]. Presumably, cytoskeleton characteristics of cancer cells play an important role in their migration activities, abilities of intravasation

and extravasation and thus determine their metastatic potential [16]. The same cytoskeleton characteristics may lead to essential changes in the mechanical properties of tumor cells. The ability of cancer cells to migrate and produce metastasis is one of the main reasons of tumor mortality [17].

Fibroblasts proved to be a good model for investigation of the mechanisms of cell movements and factors that influence cell mechanical properties [18–20]. They demonstrate that one of the main morphological types of cells (fibroblast phenotype) with well-pronounced actin stress fibers and associated focal adhesions has high motility and is strongly adherent. Fibroblasts display mesenchymal migration mode, characterized by elongated cell shape, protrusive activity and lamellipodium formation. Cancer transformation induces alterations in cytoskeleton structure, motility and mechanical properties of fibroblasts [19]. Molecular mechanisms of these alterations are not clear. Investigation of cells with different levels of transformation may help to reveal these mechanisms. In our work we used three cell systems with different types and levels of cancer transformation (by oncogene *N-RAS*, viral and cells of tumor origin — from mildest to strongest) to investigate its effect on the mechanical properties and actin cytoskeleton organization of fibroblasts.

Several experimental methods are suitable for stiffness measurements on living cells [21,22]. Force spectroscopy by AFM (atomic force microscopy) is one of them, which has been proven to be effective in

* Corresponding author at: Leninskie Gory 1/73, Moscow 111991, Russia. Tel./fax: +7 4959395738.
  E-mail address: yu.efremov@gmail.com (Y.M. Efremov).

investigation of tumor cells [23]. AFM can measure forces of tens/hundreds piconewtons under near physiological conditions [24,25]. In the current work we used AFM to measure the mechanical properties of transformed and normal fibroblasts. Cell morphology, cytoskeleton structure and focal adhesions were examined by fluorescent microscopy.

2. Materials and methods

2.1. Cell cultures

Three cell systems consisting of non-transformed cells and their derivates with different levels of cancer transformation were investigated. The first system was the Ras-transformation system: it included 10(3) cells, which are immortalized mouse fibroblasts [26], as a control cell line and 10(3)RAS cells obtained by the transfection of 10(3) cells with a construct containing constitutively active N-RAS^{asp13} gene [27]. The second system (SV40-transformation system) included non-transformed MRC-5 cells (human fetal lung fibroblasts) and their derivatives — the transformed cells MRC-5V1 and MRC-5V2 obtained by infection of the initial MRC-5 cells with SV40 virus [28]. The third system included 1036 human dermal fibroblasts as control and HT-1080 fibrosarcoma (malignant mesenchymal tumor derived from fibrous connective tissue) cell line [29].

All the studied cells were grown in DMEM medium (Paneco, Russia) supplemented with 10% fetal bovine serum (FBS) (PAA Laboratories, Austria) at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Fluorescent microscopy

For analysis of actin cytoskeleton and focal adhesions cells were fixed with 3.7% paraformaldehyde in PBS. Fixed cells were washed three times with PBS, permeabilized with 0.5% Triton X-100 in PBS for 3 min and then again washed three times with PBS for 5 min. The filamentous actin (F-actin) was labeled with the fluorescent dye Alexa Fluor 488 phalloidin (Molecular Probes, United States). Focal adhesions were labeled with two different antibodies: anti-paxillin antibody (Transduction Laboratories) and anti-vinculin antibody (hVIN-1 (Sigma, United States)). Then secondary TRITC anti-mouse antibodies (Sigma, Unite States) were applied. Both paxillin staining and vinculin staining revealed the same adhesion structures. Samples were mounted in Elvanol in PBS and examined under a fluorescent microscope Axioplan (Zeiss, Germany) with a 100× oil Plan-Neofluar objective. Images were obtained with an Olympus DP70 digital camera using the DP Controller software (Olympus, Japan).

2.3. Morphometry

To characterize the morphological alterations of the cells after the transformation we used ImageJ software (National Institutes of Health, Bethesda, MD). Cell area and perimeter were measured and circularity was calculated as:

$$4\pi \times \frac{[\text{Area}]}{[\text{Perimeter}]^2}.$$

Circularity describes the level of cell polarization. A value of unity indicates a perfect circle. As the value approaches zero, it indicates an increasingly elongated shape. To characterize the maturation level of focal adhesions we measured their number and length revealed by immunofluorescence.

2.4. AFM measurements

Before AFM experiments, the cells were seeded on glass cover slips and grown until 70–80% confluency. The AFM measurements were performed at 37 °C using a commercial atomic force microscope Solver Bio (NT-MDT, Russia) combined with an inverted optical microscope as described previously [30]. Just before the AFM measurements the growth medium was replaced by DMEM HAM/F12 medium with HEPES (Sigma, United States). Typically, 3–5 force curves were taken at 1 μm/s rate on each of the studied cells near its center, excluding the nucleus area. Tipless AFM probes CSG11 (NT-MDT, Russia) modified with a 9 μm diameter polystyrene bead were used. Typical length, width, and thickness of these cantilevers are 350, 35, and 1 μm, respectively, nominal force constant k = 0.03 N/m, resonance frequency in air is near 10 kHz. Compared to the ordinary AFM cantilever, the modified ones provided a rather accurate control of the probe geometry. Also they helped to rapidly obtain Young's modulus values, averaged over a rather big (~1 μm²) contact area.

The Hertz's contact model was applied for processing the first 500 nm of the converted force vs. indentation curve to calculate Young's modulus (E). For each cell line, 3 samples of cells were tested, 15–25 cells per sample. The mechanical response from cell surface is complicated and generally includes both elastic and viscous components. In this study, E should be regarded as effective value of local stiffness (apparent Young's modulus) rather than the elastic modulus. However, some studies have shown that at low tip approach velocities (below 1 μm/s) the contribution of cell viscosity is attenuated and elasticity is dominant [7,30,31]. Thus 1 μm/s velocity was chosen as the force curve acquisition speed.

The non-parametric Mann–Whitney U test was used to determine if the differences between the groups were significant. Analysis was performed using Statistica software, version 8.0 (StatSoft, United States). The Young's modulus values were described using both arithmetic (± standard deviation) and geometric means (*/multiplicative standard deviation).

3. Results and discussion

3.1. Morphology, cytoskeleton structure and focal adhesions

All the transformed cell lines showed clear morphological difference from the control ones (Figs. 1–3, Table S1). The non-transformed cells (10(3), MRC5, 1036) had typical fibroblast polarized morphology with well-pronounced actin stress fibers and matured focal adhesions. Transformation led to significant decrease of the cell area, which is a typical morphological alteration after transformation [15,32,33]. In the case of Ras-transformation cell polarity was slightly increased (circularity decreased), consistent with the previous results [33]. In the other cell systems (SV40-transformed and fibrosarcoma cells) transformation led to decrease of polarity due to an increase of edge activity and appearance of a lot of lamellipodia and ruffles all over the cell perimeter.

In all cells transformation led to lack of actin stress fibers. But while in the case of N-Ras-transformation one could see some residual actin bundles, there are significantly less of them for SV40-transformed cells and for tumor fibrosarcoma cells there are no bundles at all. (Fig. 1B, D, E, G). We also analyzed alteration of focal adhesion (FA) system after the transformation (Figs. 2, 3 and Table S2). For all cell systems significant decrease of FA size was noticed (by 50–60%). This reduction occurs due to disappearance of large matured FA and by appearance of new small focal complexes. Violation of maturation of the focal adhesions as well as disappearance of the stress fibers and cell area reduction were shown in tumor cells earlier [14,19,20]. It should be noticed that in our systems the changes associated with transformation increased from cells with mono-oncogene transformation (Ras-transformation) to cells after viral transformation (SV40-transformation system) and cells of tumor origin (HT1080 fibrosarcoma).

3.2. Mechanical properties

Representative force curves obtained for different cell lines are shown in Fig. 4. The first 500 nm part of each force curve was used for
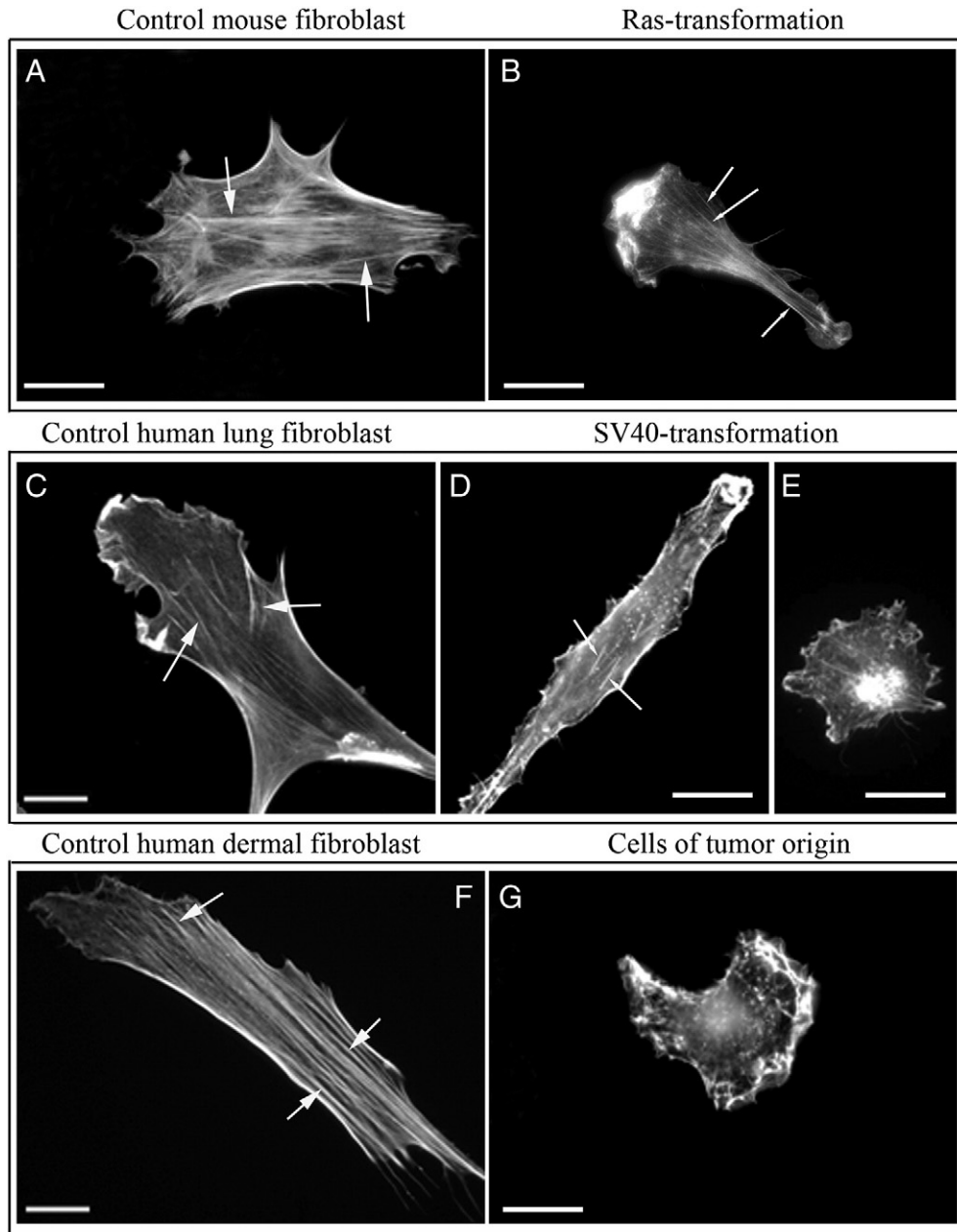
Fig. 1. Actin cytoskeleton of studied cells. Non-transformed fibroblasts: 10(3) (A), MRC-5 (C), 1036 (F) and their transformed derivates: mono-oncogenic Ras-transformation — 10(3)RAS (B); SV40-virus transformation, MRC-5V1 (D), MRC-5V2 (E) cell; cells of tumor origin HT-1080 (G). Non-transformed fibroblasts are polarized and have long actin stress fibers (marked by arrows). Transformed cells lack stress fibers; some of them could have residual actin bundles (marked by thin arrows on (B) and (D)) and have active edges with a lot of lamellipodia and ruffles all over the cell perimeter. Images obtained after fluorescent staining for F-actin, scale bar, 20 μm.
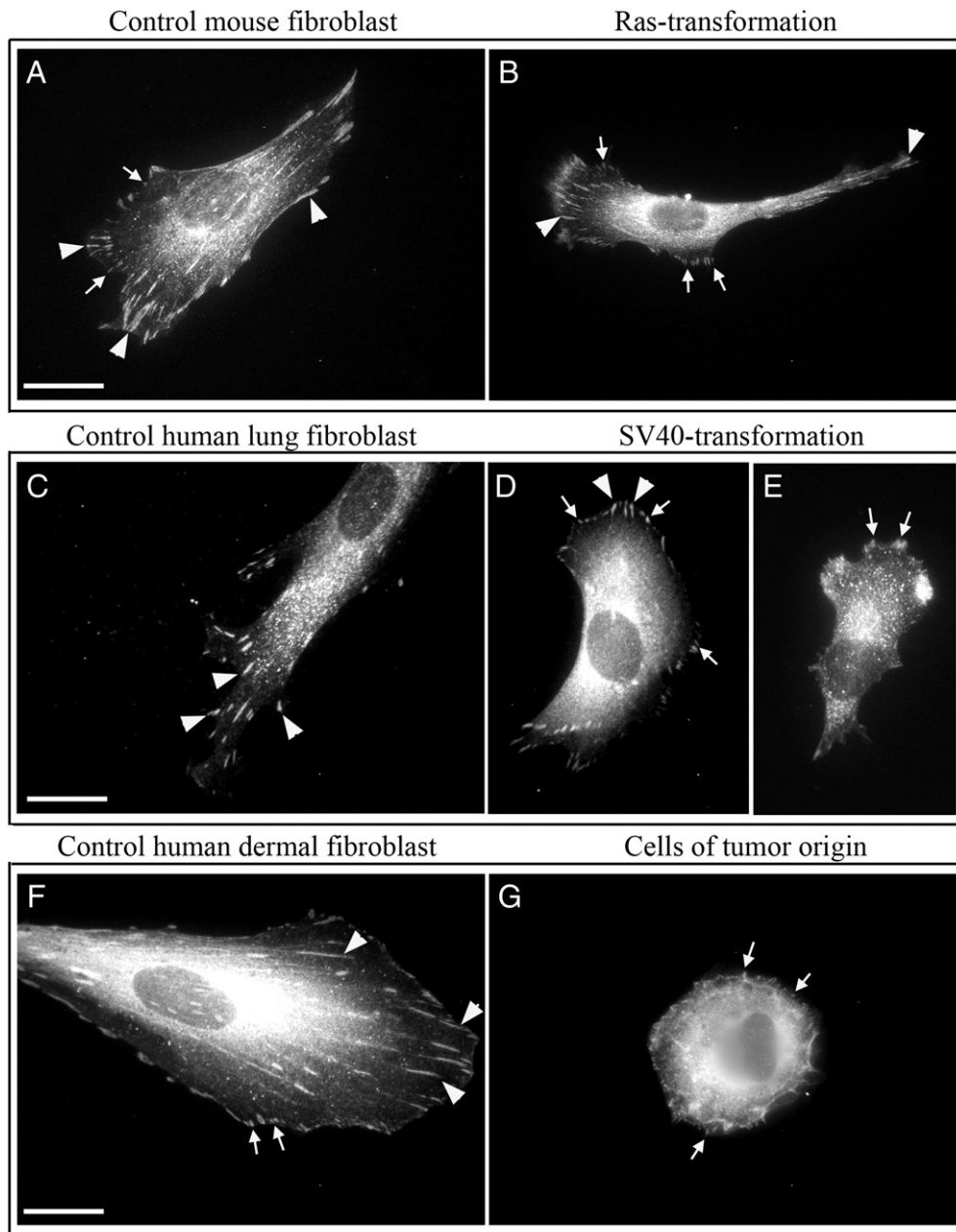
approximation with the Hertz model. It was done in order to avoid the substrate effect [34], which was observed at some force curves at indentations more than 1000–2000 nm (data not shown).

The Young's moduli of the studied cell lines are summarized in Table 1 and Fig. 5. These values fall within the range reported for other types of mammalian cells [35]. The reduced Young's modulus of transformed cells was registered in all the studied systems (by ~40% for 10(3)RAS, ~80% for MRC-5V1 and MRC-5V2, ~70% for HT-1080). In all cases the difference was significant ($p < 0.01$).

The distributions of Young's modulus values were close to log-normal for all studied cell types (Fig. S1). The multiplicative standard deviations were quite similar (~2). Log-normal distributions were previously shown for mechanical properties of adherent cells with different methods [6,7,36–41]. It may result from the log-normal distribution of actin cytoskeleton density or its level of crosslinking. The former was shown in several works [30,42], but its origin is still not completely clear.

Enhanced activation of the RAS-signaling pathways is one of the most frequent defects in human tumors [43]. It was previously shown that one-step single oncogene transformation of 10(3) mouse fibroblasts with *N-RAS* substantially modified cell morphology and cytoskeleton, providing the typical phenotype of transformed cells [27]. These cells still had some residual actin bundles and alterations of adhesion structures were the lowest between all studied cells. It should be noted also that Ras-transformation didn't lead to invasive phenotype in human breast epithelial cells [44]. Although *N-RAS* transfection in the studied cell system induces a pronounced morphological transformation of fibroblasts, they demonstrate the lowest change in the Young's modulus among the studied cell systems.

Control mouse fibroblast — Ras-transformation

Control human lung fibroblast — SV40-transformation

Control human dermal fibroblast — Cells of tumor origin

Fig. 2. Focal adhesion system of studied cells. Non-transformed fibroblasts: 10(3) (A), MRC-5 (C), 1036 (F) and their transformed derivates: mono-oncogenic Ras-transformation — 10(3) RAS (B); SV40-virus transformation, MRC-5V1 (D), MRC-5V2 (E) cell; cells of tumor origin HT-1080 (G). Non-transformed fibroblasts have large matured focal contacts (marked by arrowheads) and small focal complexes (marked by thin arrows). Transformed cells have reduced system of focal adhesions: while there are a few focal contacts in some cells (B, D) the others have only small focal complexes (marked by thin arrows). Images obtained after fluorescent staining for paxillin, scale bar, 20 μm.

Transformed cells in the other two cell systems (viral transformation of MRC-5 human lung fibroblast and tumor origin fibrosarcoma cell line HT-1080) demonstrate more evident morphological changes. Viral transformation induces several oncogenes, leading to malignization of cells [45]. HT-1080 cells derived from biopsy tissue of a fibrosarcoma are known to have very high invasiveness and tumorigenicity [46,47]. They have similar Young's modulus values as MRC-5V1 and MRC-5V2 transformed fibroblast.

Thus, the reduction of the Young's modulus in three studied cell systems correlated with the morphological alterations. One-step single oncogene transformed 10(3)RAS cells demonstrated less reduction than the two other cell systems with a higher level of transformation. Apparently, significant softening is connected with acquisition of invasive capabilities.

Our data, along with the works of other authors confirm that cell stiffness measured by AFM can be regarded as a biomarker for determination of cells at early stages of tumor progression [4–6]. It should be noted that cell properties in vivo and in vitro can be essentially different because of dimensionality (2D vs 3D), microstructure and stiffness of the extracellular matrix, molecular signals and other parameters. However, experiments conducted on biopsies [48] and tissue sections [23] collected from patients suffering from various cancers indicate that cancer cells have lower stiffness in natural tumor microenvironment as well as in vitro. It can be a consequence of the faster mitosis rate and dedifferentiation of cancer cells [49,50]. On the other hand, reduced stiffness could help cancer cells to move within interstitial cavities, crawl through extracellular matrix and through vascular walls [50,51].
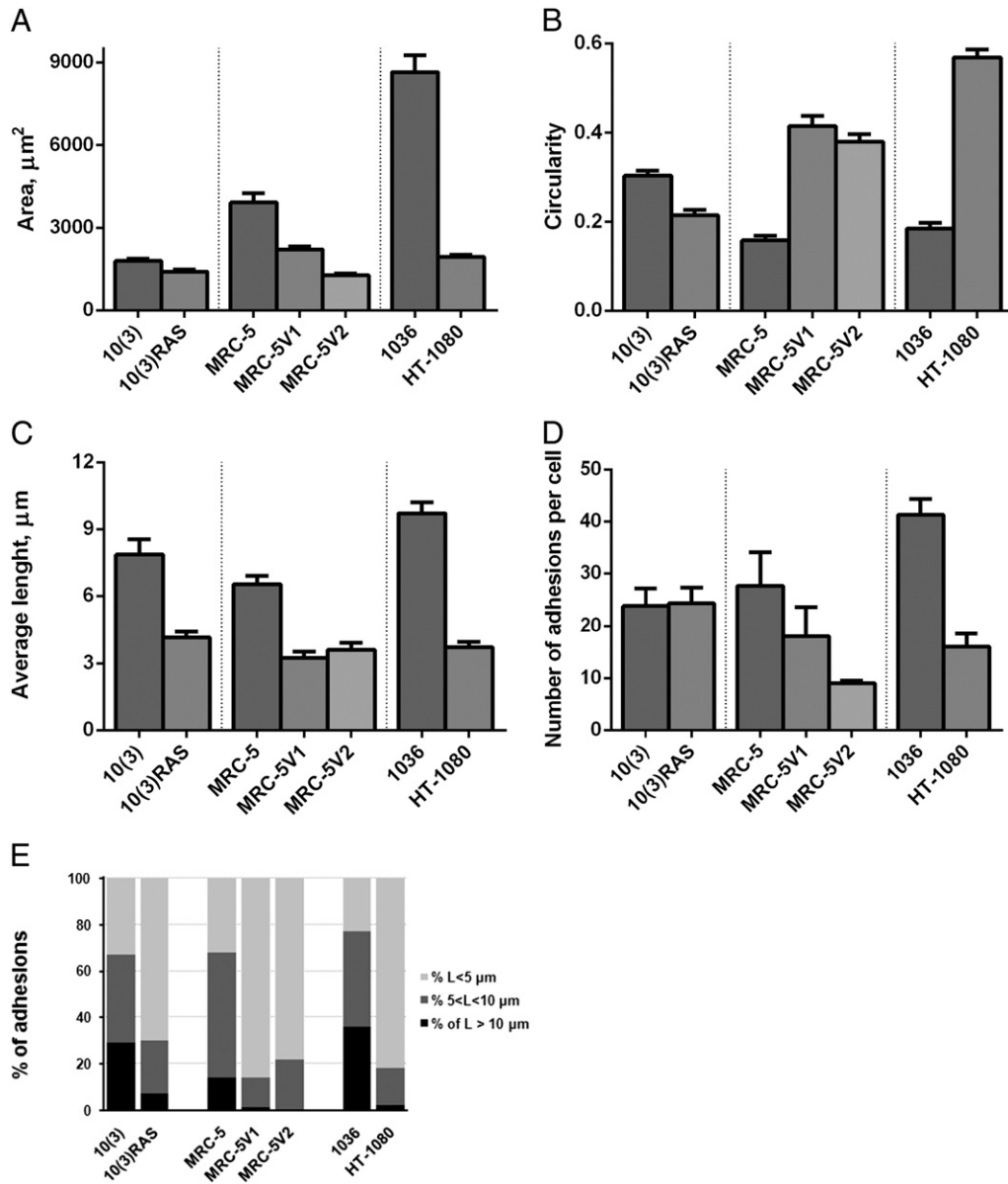
Fig. 3. Morphological characteristics of the studied cells. (A) Cell area, (B) circularity (cell polarization), (C) average length of adhesions. Difference between non-transformed and transformed cells is significant at $p < 0.01$. (D) Number of focal adhesions per cell, (E) size distribution of focal adhesions (L — length).
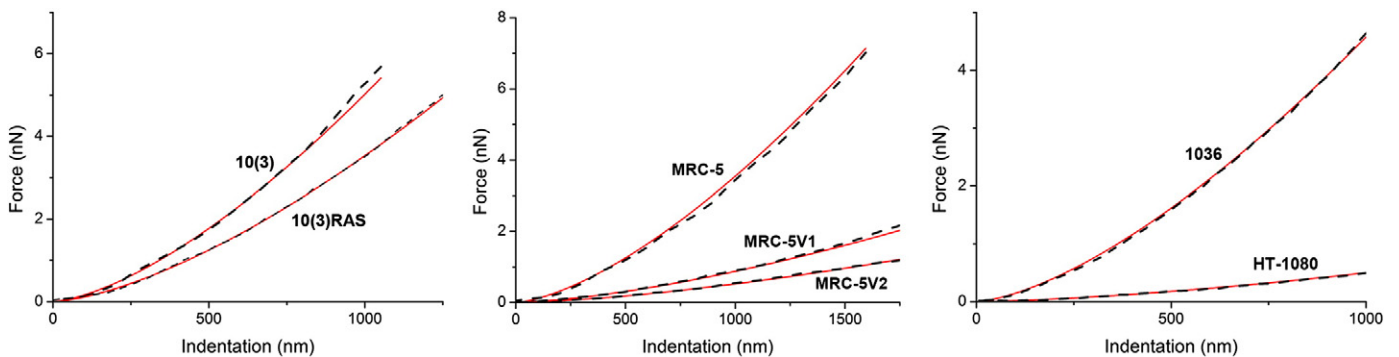
Fig. 4. Examples of force curves (black dashed lines) obtained for the studied cells. Solid red lines — Hertz model fitting. Although only the first 500 nm indentation range was used for fitting procedure, the resulted fitting curves are in good agreement at deeper indentations (over 1000 nm) which confirms the applicability of the Hertz model.

Table 1
Young's moduli calculated for the cell lines. n = total number of cells measured in 3 experiments.

Parameter	10(3) n = 75	10(3)RAS n = 75	MRC-5 n = 51	MRC-5V1 n = 54	MRC-5V2 n = 70	1036 n = 66	HT-1080 n = 66
Arithmetic mean, Pa	1810	1000	1720	360	380	930	310
Standard deviation, Pa	1280	720	1180	350	260	750	210
Geometric mean, Pa	1460	790	1280	250	310	700	250
Multiplicative standard deviation	1.9	2.1	2.3	2.3	1.9	2.2	1.9
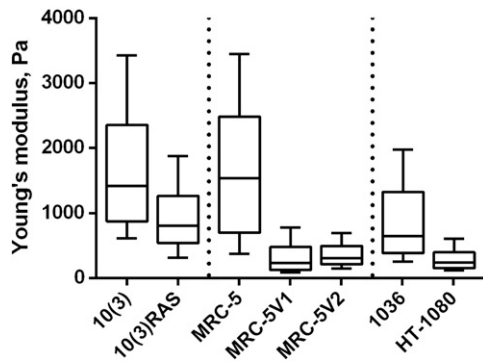
Fig. 5. Boxplot for Young's moduli calculated for the cell lines. The percentiles are 10%, 25%, 50%, 75% and 90%. Difference between the non-transformed and transformed cells is significant at $p < 0.05$.

4. Conclusions

In this study the Young's moduli of three different cell systems (fibroblasts with different levels of cancer transformation) were measured by force spectroscopy. The distribution of the Young's modulus values was found to be log-normal for all the studied cell lines. The Young's moduli of the transformed cells were significantly lower than those of the control ones. The decrease of Young's modulus was consistent with morphology changes and actin cytoskeleton rearrangement observed by fluorescent microscopy.

The changes in the Young's modulus of cells caused by a one-step single oncogene transformation were less pronounced than those for the other transformed cells (virus-transformed cells and cells of tumor origin). We suggest that cell stiffness declines with increase in the level of cancer transformation that could give benefits for tumor invasion. Further work is required to evaluate the generality of this suggestion, particularly to examine the correlation between cell stiffness and their metastatic potential.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2014.01.032.

References

[1] S. Kumar, V.M. Weaver, Mechanics, malignancy, and metastasis: the force journey of a tumor cell, Cancer Metastasis Rev. 28 (2009) 113–127.
[2] P. Katira, M.H. Zaman, R.T. Bonnecaze, How changes in cell mechanical properties induce cancerous behavior, Phys. Rev. Lett. 108 (2012) 028103.
[3] M. Makale, Cellular mechanobiology and cancer metastasis, Birth Defects Res. C 81 (2007) 329–343.
[4] M. Lekka, K. Pogoda, J. Gostek, O. Klymenko, S. Prauzner-Bechcicki, J. Wiltowska-Zuber, J. Jaczewska, J. Lekki, Z. Stachura, Cancer cell recognition—mechanical phenotype, Micron 43 (2012) 1259–1266.
[5] W. Xu, R. Mezencev, B. Kim, L. Wang, J. McDonald, T. Sulchek, Cell stiffness is a biomarker of the metastatic potential of ovarian cancer cells, PLoS One 7 (2012) e46609.
[6] V. Swaminathan, K. Mythreye, E.T. O'Brien, A. Berchuck, G.C. Blobe, R. Superfine, Mechanical stiffness grades metastatic potential in patient tumor cells and in cancer cell lines, Cancer Res. 71 (2011) 5075–5080.
[7] A.N. Ketene, E.M. Schmelz, P.C. Roberts, M. Agah, The effects of cancer progression on the viscoelasticity of ovarian cell cytoskeleton structures, Nanomed. Nanotechnol. Biol. Med. 8 (2011) 93–102.
[8] E.M. Darling, S. Zauscher, J. a Block, F. Guilak, A thin-layer model for viscoelastic, stress-relaxation testing of cells using atomic force microscopy: do cell properties reflect metastatic potential? Biophys. J. 92 (2007) 1784–1791.
[9] L. Bastatas, D. Martinez-Marin, J. Matthews, J. Hashem, Y.J. Lee, S. Sennoune, S. Filleur, R. Martinez-Zaguilan, S. Park, AFM nano-mechanics and calcium dynamics of prostate cancer cells with distinct metastatic potential, Biochim. Biophys. Acta 1820 (2012) 1111–1120.
[10] E.C. Faria, N. Ma, E. Gazi, P. Gardner, M. Brown, N.W. Clarke, R.D. Snook, Measurement of elastic properties of prostate cancer cells using AFM, Analyst 133 (2008) 1498–1500.
[11] G. Zhang, M. Long, Z.-Z. Wu, W.-Q. Yu, Mechanical properties of hepatocellular carcinoma cells, World J. Gastroenterol. 8 (2002) 243–246.
[12] M. Radmacher, Measuring the elastic properties of living cells by the atomic force microscope, Methods Cell Biol. 68 (2002) 67–90.
[13] D. a Fletcher, R.D. Mullins, Cell mechanics and the cytoskeleton, Nature 463 (2010) 485–492.
[14] Q.S. Li, G.Y.H. Lee, C.N. Ong, C.T. Lim, AFM indentation study of breast cancer cells, Biochem. Biophys. Res. Commun. 374 (2008) 609–613.
[15] J.M. Vasiliev, Cytoskeletal mechanisms responsible for invasive migration of neoplastic cells, Int. J. Dev. Biol. 48 (2004) 425–440.
[16] M.F. Olson, E. Sahai, The actin cytoskeleton in cancer cell motility, Clin. Exp. Metastasis 26 (2009) 273–287.
[17] P. Mehlen, A. Puisieux, Metastasis: a question of life or death, Nat. Rev. Cancer 6 (2006) 449–458.
[18] C. Rotsch, M. Radmacher, Drug-induced changes of cytoskeletal structure and mechanics in fibroblasts: an atomic force microscopy study, Biophys. J. 78 (2000) 520–535.
[19] S. Park, D. Koch, R. Cardenas, J. Käs, C.K. Shih, Cell motility and local viscoelasticity of fibroblasts, Biophys. J. 89 (2005) 4330–4342.
[20] M.S. Shutova, A.Y. Alexandrova, Normal and transformed fibroblast spreading: role of microfilament polymerization and actin–myosin contractility, Cell Tissue Biol. 4 (2010) 25–35.
[21] B.D. Hoffman, J.C. Crocker, Cell mechanics: dissecting the physical responses of cells to force, Annu. Rev. Biomed. Eng. 11 (2009) 259–288.
[22] K.E. Kasza, A.C. Rowat, J. Liu, T.E. Angelini, C.P. Brangwynne, G.H. Koenderink, D.A. Weitz, The cell as a material, Curr. Opin. Cell Biol. 19 (2007) 101–107.
[23] M. Lekka, D. Gil, K. Pogoda, J. Dulińska-Litewka, R. Jach, J. Gostek, O. Klymenko, S. Prauzner-Bechcicki, Z. Stachura, J. Wiltowska-Zuber, K. Okoń, P. Laidler, Cancer cell detection in tissue sections using AFM, Arch. Biochem. Biophys. 518 (2011) 151–156.
[24] Y.M. Efremov, D.V. Bagrov, E.V. Dubrovin, K.V. Shaitan, I.V. Yaminskii, Atomic force microscopy of animal cells: advances and prospects, Biophysics 56 (2011) 257–267.
[25] S. Kasas, G. Longo, G. Dietler, Mechanical properties of biological specimens explored by atomic force microscopy, J. Phys. D. Appl. Phys. 46 (2013) 133001.
[26] D.M. Harvey, A.J. Levine, p53 alteration is a common event in the spontaneous immortalization of primary BALB/c murine embryo fibroblasts, Genes Dev. 5 (1991) 2375–2385.
[27] M.E. Lomakina, A.Y. Alexandrova, Analysis of changes induced by oncogene N-RAS expression in pattern and distribution of pseudopodial activity of fibroblasts, Russ. J. Dev. Biol. 40 (2009) 222–231.
[28] L.I. Huschtscha, R. Holliday, Limited and unlimited growth of SV40-transformed cells from human diploid MRC-5 fibroblasts, J. Cell Sci. 63 (1983) 77–99.
[29] S. Rasheed, W.A. Nelson-Rees, E.M. Toth, P. Arnstein, M.B. Gardner, Characterization of a newly derived human sarcoma cell line (HT-1080), Cancer 33 (1974) 1027–1033.
[30] Y.M. Efremov, A.A. Dokrunova, D.V. Bagrov, K.S. Kudryashova, O.S. Sokolova, K.V. Shaitan, The effects of confluency on cell mechanical properties, J. Biomech. 46 (2013) 1081–1087.
[31] Y.B. Lu, K. Franze, G. Seifert, C. Steinhauser, F. Kirchhoff, H. Wolburg, J. Guck, P. Janmey, E.Q. Wei, J. Kas, A. Reichenbach, Viscoelastic properties of individual glial cells and neurons in the CNS, Proc. Natl. Acad. Sci. 103 (2006) 17759–17764.
[32] A.Y. Alexandrova, V.B. Dugina, H. Paterson, A.D. Bershadsky, J.M. Vasiliev, Motility of intracellular particles in rat fibroblasts is greatly enhanced by phorbol ester and by over-expression of normal p21N-ras, Cell Motil. Cytoskeleton 25 (1993) 254–266.

[33] S.A. Minina, A.Y. Aleksandrova, Y.M. Vasil'ev, Changes in the cell shape and actin cytoskeleton during the Ras oncogene-induced transformation: a possible role of Rho kinase, Dokl. Biol. Sci. 388 (2003) 83–85.

[34] E.K. Dimitriadis, F. Horkay, J. Maresca, B. Kachar, R.S. Chadwick, Determination of elastic moduli of thin layers of soft material using the atomic force microscope, Biophys. J. 82 (2002) 2798–2810.

[35] T.G. Kuznetsova, M.N. Starodubtseva, N.I. Yegorenkov, S.A. Chizhik, R.I. Zhdanov, Atomic force microscopy probing of cell elasticity, Micron 38 (2007) 824–833.

[36] S.E. Cross, Y.S. Jin, J. Rao, J.K. Gimzewski, Nanomechanical analysis of cells from cancer patients, Nat. Nanotechnol. 2 (2007) 780–783.

[37] S. Hiratsuka, Y. Mizutani, M. Tsuchiya, K. Kawahara, H. Tokumoto, T. Okajima, The number distribution of complex shear modulus of single cells measured by atomic force microscopy, Ultramicroscopy 109 (2009) 937–941.

[38] Y. Mizutani, M. Tsuchiya, S. Hiratsuka, K. Kawahara, H. Tokumoto, T. Okajima, Elasticity of living cells on a microarray during the early stages of adhesion measured by atomic force microscopy, Jpn. J. Appl. Phys. 47 (2008) 6177–6180.

[39] F.J. Alenghat, B. Fabry, K.Y. Tsai, W.H. Goldmann, D.E. Ingber, Analysis of cell mechanics in single vinculin-deficient cells using a magnetic tweezer, Biochem. Biophys. Res. Commun. 277 (2000) 93–99.

[40] B. Fabry, G.N. Maksym, S.A. Shore, P.E. Moore, R.A. Panettieri Jr., J.P. Butler, J.J. Fredberg, Selected contribution: time course and heterogeneity of contractile responses in cultured human airway smooth muscle cells, J. Appl. Physiol. 91 (2001) 986–994.

[41] B.D. Hoffman, G. Massiera, K.M. Van Citters, J.C. Crocker, The consensus mechanics of cultured mammalian cells, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 10259–10264.

[42] J. Guck, S. Schinkinger, B. Lincoln, F. Wottawah, S. Ebert, M. Romeyke, D. Lenz, H.M. Erickson, R. Ananthakrishnan, D. Mitchell, J. Käs, S. Ulvick, C. Bilby, Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence, Biophys. J. 88 (2005) 3689–3698.

[43] M. Barbacid, ras Genes, Annu. Rev. Biochem. 56 (1987) 779–827.

[44] A. Moon, M.S. Kim, T.G. Kim, S.H. Kim, H.E. Kim, Y.Q. Chen, H.R. Kim, H-ras, but not N-ras, induces an invasive phenotype in human breast epithelial cells: a role for MMP-2 in the h-ras-induced invasive phenotype, Int. J. Cancer 85 (2000) 176–181.

[45] T. Dayaram, S. Marriott, Effect of transforming viruses on molecular mechanisms associated with cancer, J. Cell. Physiol. 216 (2008) 309–314.

[46] W. Benedict, B. Weissman, C. Mark, E. Stanbridge, Tumorigenicity of human HT1080 fibrosarcoma X normal fibroblast hybrids: chromosome dosage dependency, Cancer Res. 44 (1984) 3471–3479.

[47] H.J. Cha, S.K. Bae, H.Y. Lee, O.H. Lee, H. Sato, M. Seiki, B.C. Park, K.W. Kim, Anti-invasive activity of ursolic acid correlates with the reduced expression of matrix metalloproteinase-9 (MMP-9) in HT1080 human fibrosarcoma cells, Cancer Res. 56 (1996) 2281–2284.

[48] M. Plodinec, M. Loparic, C.A. Monnier, E.C. Obermann, R. Zanetti-Dallenbach, P. Oertle, J.T. Hyotyla, U. Aebi, M. Bentires-Alj, R.Y.H. Lim, C.A. Schoenenberger, The nanomechanical signature of breast cancer, Nat. Nanotechnol. 7 (2012) 757–765.

[49] I. Sokolov, Atomic force microscopy in cancer cell research, in: H. Singh Nalwa, T. Webster (Eds.), Cancer Nanotechnology, American Scientific Publishers, Valencia, 2007, pp. 1–17.

[50] A. Fritsch, M. Höckel, T. Kiessling, K.D. Nnetu, F. Wetzel, M. Zink, J.A. Kas, Are biomechanical changes necessary for tumour progression? Nat. Phys. 6 (2010) 730–732.

[51] D. Wirtz, K. Konstantopoulos, P.C. Searson, The physics of cancer: the role of physical interactions and mechanical forces in metastasis, Nat. Rev. Cancer 11 (2011) 512–522.