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Physical properties of mesenchymal stem cells are coordinated by the perinuclear actin cap

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ABSTRACT

Mesenchymal stem cells (MSCs) have been extensively investigated for their applications in regenerative medicine. Successful use of MSCs in cell-based therapies will rely on the ability to effectively identify their properties and functions with a relatively non-destructive methodology.

In this study, we measured the surface stiffness and thickness of rat MSCs with atomic force microscopy and clarified their relation at a single-cell level. The role of the perinuclear actin cap in regulating the thickness, stiffness, and proliferative activity of these cells was also determined by using several actin cytoskeleton-modifying reagents. This study has helped elucidate a possible link between the physical properties and the physiological function of the MSCs, and the corresponding regulatory role of the actin cytoskeleton.

1. Introduction

Mesenchymal stem cells (MSCs) are a heterogeneous population of stem/progenitor cells with the pluripotent capacity to differentiate into mesodermal and non-mesodermal cell lineages. They have generated a great deal of interest owing to their potential use in regenerative medicine and tissue engineering [1,2]. In order to maximize the potential of MSCs for biomedical applications, a more comprehensive characterization of MSCs is required. To achieve this goal, the development of a direct, relatively non-destructive method for measuring physical properties, which reflect the fate and physiological state of MSCs, is necessary. Population thickness (height) of adhered human MSCs may be related to various cell functions, such as proliferation activity and cell cycle [3,4]. However, a more comprehensive view of the physical properties of MSCs is required.

Mechanical properties such as cytoskeleton organization and elasticity, membrane tension, cell shape, and adhesion strength may play important roles in stem-cell fate and differentiation [5–7]. A change in mechanical properties, and in particular, in the stiffness (elasticity) of tissue cells, has been recognized as an indication of cancer [8]. Several techniques have been successfully employed to study cell mechanical properties, including micropipette aspiration, magnetic twisting cytometry, optical traps, and atomic force microscopy (AFM) [9–11]. In particular, AFM can be used to analyze live cells [12,13] and to investigate their mechanical properties in physiological conditions in a relatively non-destructive manner [14].

Surface mechanical properties of a cell are dominantly defined by the actin cytoskeleton [15–18]. Stress fibers are specific determinants of cell mechanics [19], and cortical actin promotes cortical rigidity [20,21]. Dominant types of actin cytoskeleton differ by cell types as well as position. Therefore, the key determinants for cell mechanics probably vary across different cell types, and it is necessary to determine the character of cell mechanics in each cell type.

Previously, we reported that the elastic modulus of human MSCs decreased dramatically by actin de-polymerization, whereas the cell thickness increased [22]. The elasticity and the thickness of an actin de-polymerized MSC and a bare nucleus were almost the same. Thus, regulatory factors of nuclear thickness and cell elasticity are possibly the same, and these may be related to the actin cytoskeleton. Recently, Khatau et al. reported that a perinuclear actin cap, which is an actin filament structure that forms a cap or dome above the apical surface of the nucleus, tightly regulates the nuclear shape of adherent fibroblasts [23]. In this study, we examined the regulatory effect of the perinuclear actin cap on thickness and stiffness of adherent rat MSCs by using several actin cytoskeleton-modifying drugs.

Abbreviations: MSC, mesenchymal stem cell; AFM, atomic force microscopy; FBS, fetal bovine serum; CLSM, confocal laser scanning microscopy.

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2. Materials and methods

2.1. Materials

The AFM probe (ATEC-CONT; spring constant: 0.02–0.75 N/m) was purchased from Nanosensors (Neuchatel, Switzerland). Cell culture media was purchased from Nacalai Tesque (Kyoto, Japan), and fetal bovine serum (FBS) was purchased from JRH Biosciences (Lenexa, KS). Antibiotics were purchased from Sigma–Aldrich (St. Louis, MO). Fisher 344 male rats were purchased from Japan SLC (Shizuoka, Japan). ATP bioluminescence assay kit was purchased from Toyo Ink (Tokyo, Japan). Other reagents were purchased from Sigma–Aldrich, Wako Pure Chemical Industries Ltd. (Osaka, Japan), or Invitrogen (Carlsbad, CA).

2.2. Preparation and culture of rat MSCs

Rat MSCs were isolated and primarily cultured as described previously [24]. In brief, bone marrow cells were obtained from the femoral shafts of 7-week-old male Fisher 344 rats. The cells were obtained from at least 2 rats and mixed. The culture medium was Eagle's minimal essential medium (with Earle's Salt and L-glutamine) containing 15% FBS and antibiotics (100 U/mL penicillin G, 100 μg/mL streptomycin sulfate, and 0.25 μg/mL amphotericin B). The medium was renewed twice a week, and cells at passages 2–6 were used in this study.

Y-27632 (10 μM), blebbistatin (5 μM), cell permeable C3 transferase (20 ng/mL), calyculin A (0.15 nM), and wiskostatin (1 μM) were used to analyze inhibition or acceleration of the actin cytoskeleton. Cells were cultured in the medium containing these reagents for 2 days and then manipulated by AFM.

2.3. AFM measurements

Rat MSCs cultured on 35-mm culture dishes in the medium were manipulated by AFM (Nanowizard I; JPK Instruments AG, Berlin, Germany) at room temperature. The probe was indented into the cells up to 50 nN at 10 μm/s. The Young's modulus of the cell was calculated according to the Hertz model [25]. Although the Hertz model is accommodated in an elastic body, various kinds of cellular stiffness have been evaluated by this model as Young's modules [26]. The force–distance curve at the region up to 400 nm of cell surface indentation was fitted to the model (Fig. 1A). Although the ATEC-CONT is a tetrahedral probe, the edge of the probe is conoidal. Thus, the following equation was used in the model of indentation:

$$F = \frac{E}{(1-\nu^2)}\frac{2\tan\alpha}{\pi}I^2$$

where F = force, I = depth of indentation, α = semi-opening angle of the cone (5°), ν = Poisson's ratio (0.5), and E = Young's modulus.

Cell thickness was derived from the length, from the cell contact point to the substrate (Fig. 1A). All experiments were performed in more than 10 cells, and each cell was examined at 9 different points within a size-defined grid on the nuclear region of the membrane. In this study, we assumed that the distribution of Young's moduli and the thickness of cells were in accordance with the

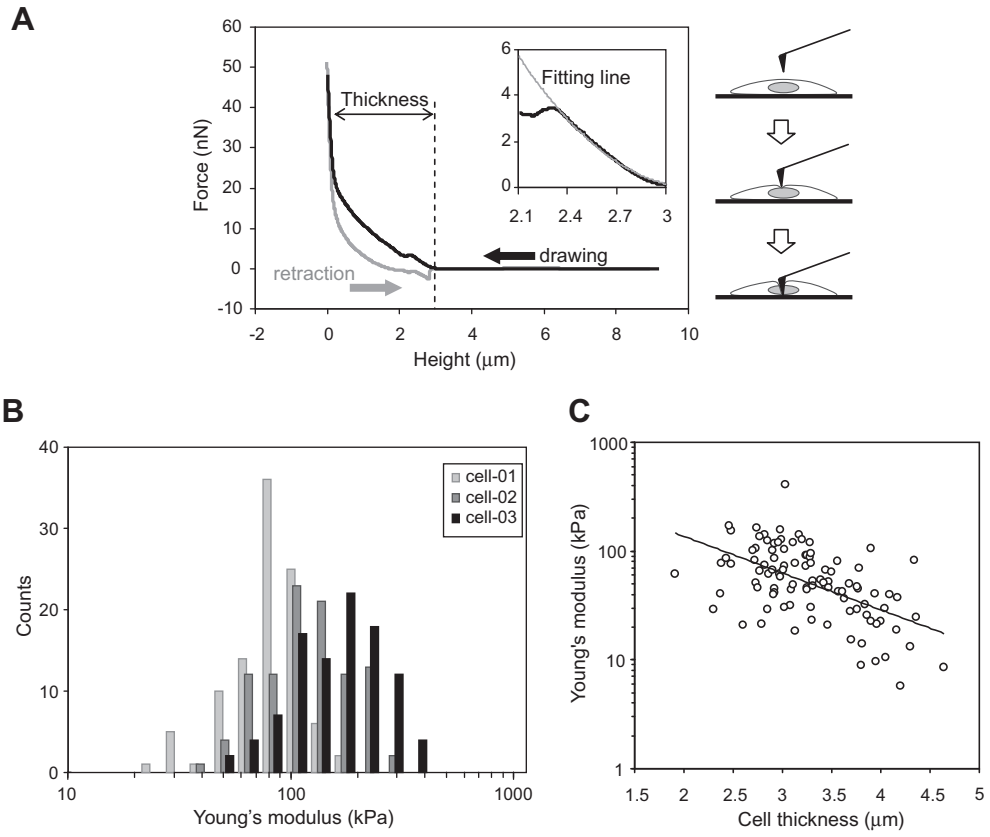


Fig. 1. Measurement of the cell stiffness and cell thickness of MSCs. (A) Details of the method of measuring cell stiffness and thickness by AFM. Typical force–distance curves obtained from the indentation of and pulling up from the surface of rat MSCs are shown at the left, and the schema for the AFM manipulation is shown at the right. Cell thickness is represented as the distance between the contact point of cell surface and substrate. Stiffness is calculated from the force curve at the region of indentation (up to 400 nm) by fitting it to the Hertz model (inset). (B) Distribution of Young's modulus of the same MSCs. Young's modulus of each cell (total of 3 cells) was measured 100 times repeatedly. The distribution pattern of Young's modulus of rat MSCs shows a log-normal pattern. (C) Relationship between the median value of Young's modulus and cell thickness in rat MSCs. Each data point is for one cell (total of 100 cells). The line shows approximated curve for the points (R = 0.35, P = 0.0004).

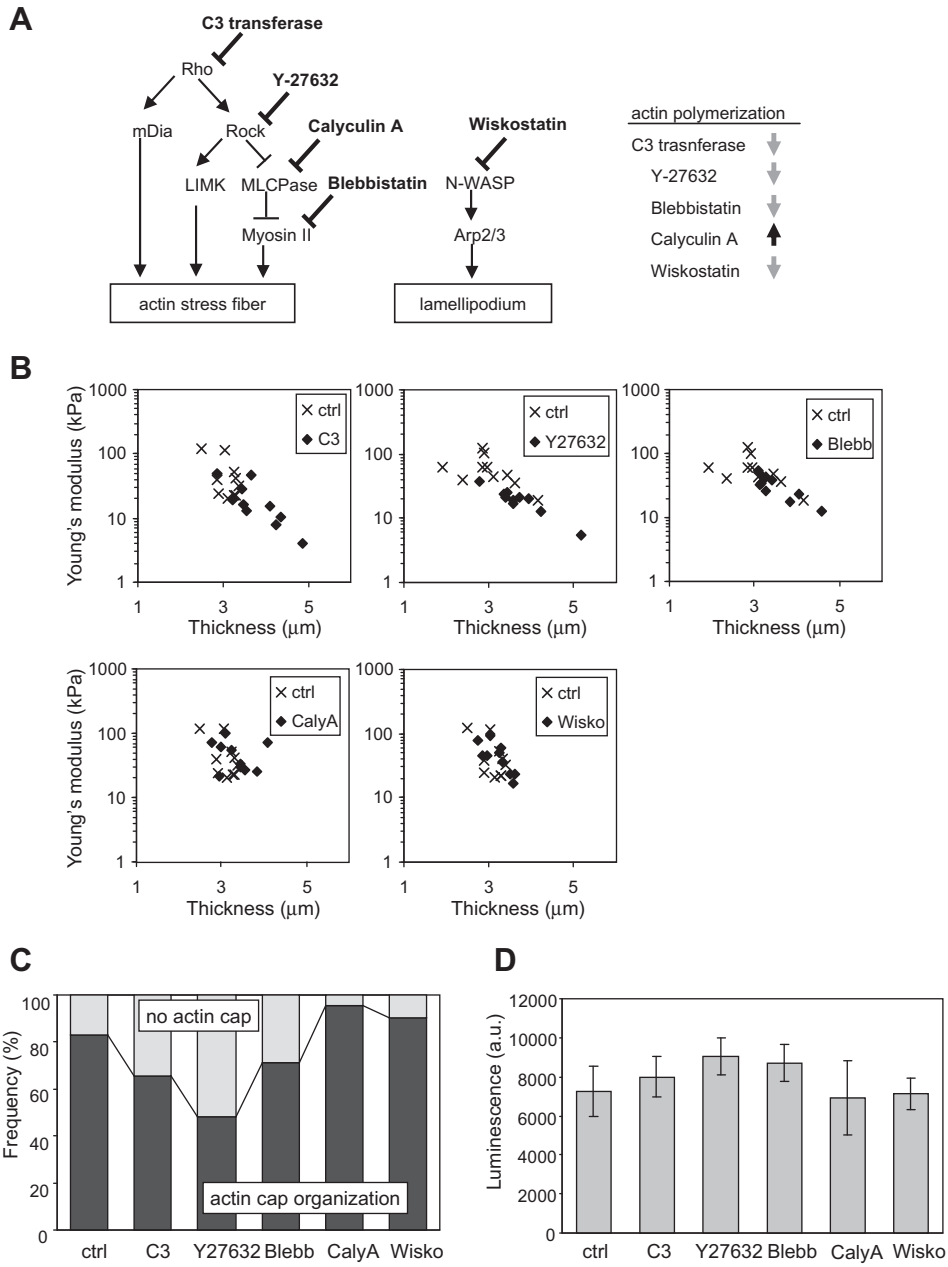


Fig. 2. Role of the actin cytoskeleton in regulating the cell properties of rat MSCs. (A) Schematics of effects of the used reagents on the actin cytoskeleton. C3 transferase, Y-27632, and blebbistatin inhibit actin polymerization and stress fiber formation. In contrast, calyculin A inhibits myosin light chain phosphatase and leads to actin polymerization, resulting in stress fiber and cortical actin formation. Wiskostatin inhibits lamellipodium formation. (B) Influence of the agents on Young's modulus and thickness of rat MSCs. Each point indicates a data point from each cell (a total of 10 cells for each condition). C3 transferase (C3), Y-27632 (Y27632), and blebbistatin (Blebb) decreased surface rigidity of rat MSCs and increased cell thickness. On the other hand, calyculin A (CalyA) and wiskostatin (Wisko) had little impact on these physical properties of MSCs. (C) Organization of pericellular actin cap of rat MSCs following treatment with the agents. The frequencies of organization of perinuclear actin cap are shown. The presence of the perinuclear actin cap at each condition was determined from more than 23 cells. The typical images for organization and disruption or not of the actin cap are shown in Supplementary Fig. S1. C3 transferase (C3), Y-27632 (Y27632), and blebbistatin (Blebb) decreased actin cap organization; calyculin A (CalyA) and wiskostatin (Wisko) increased actin cap organization. (D) Proliferation activity of rat MSCs cultured with various reagents. The cell number at 6 days was evaluated by measuring the cellular ATP contents with chemiluminescence system. C3 transferase (C3), Y-27632 (Y27632), and blebbistatin (Blebb) slightly increased the cell numbers; on the other hand, calyculin A (CalyA) and wiskostatin (Wisko) did not influence the proliferation.

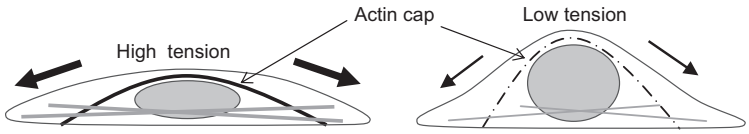
log-normal distribution, and a median value of 9 points was adopted for the Young's modulus and the thickness of each cell.

2.4. Cell proliferation assay

Approximate cell numbers were determined using an ATP bioluminescence assay kit according to the manufacturer's instructions. Rat MSCs were plated at a density of 1×10^3 cells/well into white 96-well culture plates and cultured in the culture medium containing the cytoskeleton-modifying reagent for 6 days. The ATP bioluminescence reagent was added to the wells, and the cultured cells were shaken for 1 min and then incubated for another 10 min at 25 °C. Luminescence was measured with Synergy HT (BioTek, Winooski, VT).

2.5. Evaluation of perinuclear actin cap

The perinuclear actin cap was evaluated by staining the actin filaments of rat MSCs. Cells cultured for 2 days with the cytoskeleton-modifying reagent were fixed with 4% paraformaldehyde,



	Normal MSC	Stress fibers- attenuated MSC
Surface stiffness	Rigid	Elastic
Thickness	Low	High
Actin cap	Developed	Decreased
Tension source	Stress fibers	Isotropic cortex tension
Proliferation activity	Normal	Increased

Fig. 3. Overview of the physical properties of MSCs. In normal MSCs, developed perinuclear actin caps generate highly surface tension and press tightly against the nucleus, giving the cells a rigid surface stiffness and flat thickness. In contrast, in stress fibers-attenuated MSCs, the surface tension was decreased and the cell surface was boosted by the nucleus, resulting in the cells with elastic surfaces and a high thickness. Furthermore, elastic and tall cells tended to also have high proliferation activities.

permeabilized with 0.5% Triton X-100, and then stained with FITC-labeled phalloidin for actin filaments and propidium iodide for nucleus. Specimens were observed by confocal laser scanning microscopy (CLSM; FV-1000; Olympus, Tokyo, Japan) in 0.5 μm serial sections. The presence of the perinuclear actin cap was determined from the status of actin filaments over the nucleus in more than 23 cells for each condition (Supplementary Fig. S1).

3. Results

3.1. Relationship between stiffness and thickness of rat MSCs

First, we examined the relation between every AFM-measured Young's modulus and the thickness of each rat MSC (Supplementary Fig. S2). Compared with the thickness, the measured Young's modulus varied widely for each AFM manipulation (Supplementary Fig. S2). This was due to the fact that cellular mechanical properties are local and can change dramatically based on the location being probed [27]. Since it was difficult to determine the cell stiffness from these scattered Young's moduli, we examined the distribution of Young's moduli of rat MSCs in single cells and found that the distribution of Young's moduli corresponded to a log-normal pattern of distribution (Fig. 1B). Therefore, we selected the median value of the widely spread Young's moduli of each cell in this study, because the median provides a better estimate of the target value than the mean in a log-normal distribution.

Thickness versus Young's modulus of each adherent rat MSC was then plotted (Fig. 1C). Analyses of 100 cells revealed that Young's modulus of various adherent rat MSCs varied largely, and that it had a weak inverse correlation with cell thickness in each cell (Fig. 1C). The correlation coefficient R between these 2 factors was 0.35 ($P = 0.0004$). In short, flat, adherent MSCs tended to be rigid, whereas tall MSCs showed a relatively elastic property.

3.2. Regulation of cell stiffness and thickness of rat MSCs by actin cytoskeleton

An intact actin cytoskeleton contributes a major part of cell stiffness, yet there are several forms of actin cytoskeleton in a cell, e.g., stress fibers, lamellipodia, filopodia, and cortical actin. Here, we examined the effect of actin stress fibers and lamellipodia on stiffness and thickness of adherent rat MSCs, using several pharmacological agents that affect the actin cytoskeleton (Fig. 2A). We used gentle concentrations of all agents to avoid inducing obvious morphological changes (Supplementary Fig. S3).

Rho inhibitor C3 transferase, ROCK inhibitor Y-27632, and myosin II ATPase inhibitor blebbistatin prevent and attenuate stress fiber formation [28–30]. The Young's moduli of the cells treated with these agents decreased relatively while their thickness increased (Fig. 2B). On the other hand, the properties of the cells treated with myosin light chain phosphatase inhibitor calyculin A [31], which activates actomyosin formation and enhances actin polymerization in stress fibers, lamellipodia formation, and cortical actin, were hardly altered (Fig. 2B). Moreover, cells treated with the N-WASP inhibitor wiskostatin, which inhibits lamellipodia formation [32], showed no changes in either the Young's modulus or thickness (Fig. 2B). Thus, the cell stiffness and thickness of adherent rat MSCs were strongly affected by attenuated actin stress fibers, but were barely influenced by activation of actomyosin formation or inhibition of lamellipodia formation.

3.3. Perinuclear actin cap correlation with cell thickness and stiffness of rat MSCs

Actin filament structures that form a cap or dome located above the apical surface of the nucleus, referred to as the perinuclear actin cap, regulate the nuclear shapes of adherent fibroblasts [23]. The perinuclear actin cap is specifically disorganized or eliminated by the inhibition of actomyosin contractility. To identify a possible role of the perinuclear actin cap in regulation of stiffness and thickness of rat MSC, we examined the changes in the actin cap organization in rat MSC under the presence of each actin cytoskeleton-modifying agent by observing with CLSM.

The majority of rat MSCs had a well-developed perinuclear actin cap under normal culture conditions (Fig. 2C and Supplementary Fig. S1), rendering a flat shape to rat MSCs. Analysis of collected images demonstrated that treatment with actin stress fiber-formation inhibitors, namely, C3 transferase, blebbistatin, and particularly Y-27362, led to an increase in the number of cells with no actin cap as compared to the control cells (Fig. 2C and Supplementary Fig. S1). In contrast, more MSCs possessed an organized actin cap after treatment with calyculin A and wiskostatin (Fig. 2C). Attenuation of actin stress fibers results in reduction of perinuclear actin cap organization, which corresponds to the data of the cell thickness and stiffness of rat MSCs (Fig. 2B, C). On the other hand, augmentation of actin cap organization had little impact on the cell thickness and stiffness of rat MSCs (Fig. 2B, C).

3.4. Actin cytoskeleton affects proliferation activity of rat MSCs

Population thickness of adhered human MSCs may be related to proliferation activity at the donor level [3]. We thus examined the possible affect of actin cytoskeleton on proliferation activity of rat MSC population to determine whether cell stiffness relates with proliferation. Adding the agents to the cell culture media revealed that Y-27632, C3 transferase, and blebbistatin, which attenuate the actin stress fibers and the perinuclear actin cap, slightly increased the growth activity of rat MSCs (Fig. 2D). On the other hand, upregulation of perinuclear actin cap formation, as caused by treating the MSCs with wiskostatin or calyculin A, had little effect on cell

proliferation (Fig. 2D). These data consist with the results of cell thickness and stiffness. We therefore propose that actin cap organization regulates the proliferation activity and cell thickness and stiffness of rat MSC.

4. Discussion

In this study, we experimentally demonstrated two major points relevant to surface stiffness of substrate-adhering rat MSCs. One is that cell stiffness and cell thickness showed an inverse correlation at a single-cell level (Fig. 1C). The second is that the perinuclear actin cap organization regulates the cell stiffness, thickness, and possibly proliferation activity of rat MSCs (Fig. 2). A schematic diagram of our findings is shown in Fig. 3. Originally, MSCs adhere flat on a given substrate, and their actin stress fibers and perinuclear actin cap are well developed. A developed actin cap flattens out the nucleus and increases surface stiffness. On the other hand, cells with diminished stress fibers also lose their actin cap, with a resulting decrease in surface stiffness, and the nucleus boosts the cell height according to the plasticity of nuclear lamin A [23]. Thus, although surface stiffness and cell thickness are essentially different physical properties of cells, the perinuclear actin cap coordinates these properties in MSCs. However, these cell properties were not affected by augmentation of the actin cap organization after treatment with either calyculin A or wiskostatin (Fig. 2). In particular, calyculin A is an activator for actomyosin formation, and it increases surface stiffness in *Drosophila* embryonic S2R+ cells by accelerating cortical actin formation [21]. Cortical actin determines isotropic cortical tension of non-adherent or mitotic cell [33], and the surface stiffness of some cells is mainly determined by this cortical actin formation [21]. Thus, it is believed that anisotropic surface tension that arises from the perinuclear actin cap is higher than the isotropic cortical tension in MSCs, which would explain why behavior of the stiffness of rat MSCs differed from the above cell. In addition, further-developed stress fibers and actin cap of MSCs may induce partial buckling of the actin cytoskeleton [34] or fluidization of cell [35]; the physical properties are unaffected by augmentation of the actin cap formation. Interestingly, the stress fiber-attenuated MSCs tended to show a high proliferation activity (Fig. 2). This result corresponds with the previous study relating cell thickness with proliferation activity in human MSCs [3,4]. Our results additionally show that the states of the actin cap and actin stress fibers in MSCs are potent regulators of the proliferation activity of each cell.

It is well known that mechanical properties of cell environments control cell life [36,37]. In particular, commitment of differentiation lineage of MSCs is specified by matrix elasticity which is sensed by actomyosin contraction [38]. Furthermore, the surface stiffness of MSCs changes depending on the substrate elasticity. The surface stiffness of MSCs also changes during cell differentiation [39]. Our present study reveals that the surface stiffness of MSCs is regulated by actin stress fibers, including the perinuclear actin cap, and is related with proliferation activity. Thus, the mechanical properties of MSCs are potent indicators for their cell behavior and physiological functions. The evaluation of cell quality and estimation of cell activities of MSCs are essential cytotechnology for applications in regenerative medicine and tissue engineering. We predict that in the future non-destructive and high-speed methods of measuring mechanical properties of MSCs will become an essential part of the cytotechnology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.04.022.

References

[1] F.P. Barry, J.M. Murphy, Mesenchymal stem cells: clinical applications and biological characterization, Int. J. Biochem. Cell. Biol. 36 (2004) 568–584.
[2] Z.J. Liu, Y. Zhuge, O.C. Velazquez, Trafficking and differentiation of mesenchymal stem cells, J. Cell. Biochem. 106 (2009) 984–991.
[3] Y. Katsube, M. Hirose, C. Nakamura, H. Ohgushi, Correlation between proliferative activity and cellular thickness of human mesenchymal stem cells, Biochem. Biophys. Res. Commun. 368 (2008) 256–260.
[4] A. Tokumitsu, S. Wakitani, M. Takagi, Noninvasive estimation of cell cycle phase and proliferation rate of human mesenchymal stem cells by phase-shifting laser microscopy, Cytotechnology 59 (2009) 161–167.
[5] J. Settleman, Tension precedes commitment-even for a stem cell, Mol. Cell 14 (2004) 148–150.
[6] R. McBeath, D.M. Pirone, C.M. Nelson, K. Bhadriraju, C.S. Chen, Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment, Dev. Cell 6 (2004) 483–495.
[7] V.E. Meyers, M. Zayzafoon, J.T. Douglas, J.M. McDonald, RhoA and cytoskeletal disruption mediate reduced osteoblastogenesis and enhanced adipogenesis of human mesenchymal stem cells in modeled microgravity, J. Bone Miner. Res. 20 (2005) 1858–1866.
[8] S.E. Cross, Y.S. Jin, J. Rao, J.K. Gimzewski, Nanomechanical analysis of cells from cancer patients, Nat. Nanotechnol. 2 (2007) 780–783.
[9] J. Dai, M.P. Sheetz, Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers, Biophys. J. 68 (1995) 988–996.
[10] K.J. Van Vliet, G. Bao, S. Suresh, The biomechanics toolbox: experimental approaches for living cells and biomolecules, Acta Mater. 51 (2003) 5881–5905.
[11] H. Huang, R.D. Kamm, R.T. Lee, Cell mechanics and mechanotransduction: pathways, probes, and physiology, Am. J. Physiol. Cell Physiol. 287 (2004) C1–11.
[12] T. Kihara, C. Nakamura, M. Suzuki, S.W. Han, K. Fukazawa, K. Ishihara, J. Miyake, Development of a method to evaluate caspase-3 activity in a single cell using a nanoneedle and a fluorescent probe, Biosens. Bioelectron. 25 (2009) 22–27.
[13] T. Kihara, N. Yoshida, T. Kitagawa, C. Nakamura, N. Nakamura, J. Miyake, Development of a novel method to detect intrinsic mRNA in a living cell by using a molecular beacon-immobilized nanoneedle, Biosens. Bioelectron. 26 (2010) 1449–1454.
[14] K.D. Costa, Imaging and probing cell mechanical properties with the atomic force microscope, Methods Mol. Biol. 319 (2006) 331–361.
[15] A.M. Collinsworth, S. Zhang, W.E. Kraus, G.A. Truskey, Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation, Am. J. Physiol. Cell Physiol. 283 (2002) C1219–1227.
[16] W.R. Trickey, T.P. Vail, F. Guilak, The role of the cytoskeleton in the viscoelastic properties of human articular chondrocytes, J. Orthop. Res. 22 (2004) 131–139.
[17] I. Titushkin, M. Cho, Distinct membrane mechanical properties of human mesenchymal stem cells determined using laser optical tweezers, Biophys. J. 90 (2006) 2582–2591.
[18] H. Kagiwada, C. Nakamura, T. Kihara, H. Kamiishi, K. Kawano, N. Nakamura, J. Miyake, The mechanical properties of a cell, as determined by its actin cytoskeleton, are important for nanoneedle insertion into a living cell, Cytoskeleton (Hoboken) 67 (2010) 496–503.
[19] L. Lu, S.J. Oswald, H. Ngu, F.C. Yin, Mechanical properties of actin stress fibers in living cells, Biophys. J. 95 (2008) 6060–6071.
[20] A.S. Maddox, K. Burridge, RhoA is required for cortical retraction and rigidity during mitotic cell rounding, J. Cell Biol. 160 (2003) 255–265.
[21] P. Kunda, A.E. Pelling, T. Liu, B. Baum, Moesin controls cortical rigidity, cell rounding, and spindle morphogenesis during mitosis, Curr. Biol. 18 (2008) 91–101.
[22] T. Sugitate, T. Kihara, X.-Y. Liu, J. Miyake, Mechanical role of the nucleus in a cell in terms of elastic modulus, Curr. Appl. Phys. 9 (2009) e291–e293.
[23] S.B. Khatau, C.M. Hale, P.J. Stewart-Hutchinson, M.S. Patel, C.L. Stewart, P.C. Searson, D. Hodzic, D. Wirtz, A perinuclear actin cap regulates nuclear shape, Proc. Natl. Acad. Sci. USA 106 (2009) 19017–19022.
[24] T. Kihara, M. Hirose, A. Oshima, H. Ohgushi, Exogenous type I collagen facilitates osteogenic differentiation and acts as a substrate for mineralization of rat marrow mesenchymal stem cells in vitro, Biochem. Biophys. Res. Commun. 341 (2006) 1029–1035.
[25] H. Hertz, Über die berührung fester elastischer Körper, J. reine und angewandte Mathematik 92 (1881) 156–171.

[26] C. Rotsch, K. Jacobson, M. Radmacher, Dimensional and mechanical dynamics of active and stable edges in motile fibroblasts investigated by using atomic force microscopy, Proc. Natl. Acad. Sci. USA 96 (1999) 921–926.
[27] H. Huang, J. Sylvan, M. Jonas, R. Barresi, P.T. So, K.P. Campbell, R.T. Lee, Cell stiffness and receptors: evidence for cytoskeletal subnetworks, Am. J. Physiol. Cell Physiol. 288 (2005) C72–80.
[28] P. Chardin, P. Boquet, P. Madaule, M.R. Popoff, E.J. Rubin, D.M. Gill, The mammalian G protein rhoC is ADP-ribosylated by *Clostridium botulinum* exoenzyme C3 and affects actin microfilaments in Vero cells, EMBO J. 8 (1989) 1087–1092.
[29] M. Uehata, T. Ishizaki, H. Satoh, T. Ono, T. Kawahara, T. Morishita, H. Tamakawa, K. Yamagami, J. Inui, M. Maekawa, S. Narumiya, Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension, Nature 389 (1997) 990–994.
[30] A.F. Straight, A. Cheung, J. Limouze, I. Chen, N.J. Westwood, J.R. Sellers, T.J. Mitchison, Dissecting temporal and spatial control of cytokinesis with a myosin II Inhibitor, Science 299 (2003) 1743–1747.
[31] H. Ishihara, H. Ozaki, K. Sato, M. Hori, H. Karaki, S. Watabe, Y. Kato, N. Fusetani, K. Hashimoto, D. Uemura, et al., Calcium-independent activation of contractile apparatus in smooth muscle by calyculin-A, J. Pharmacol. Exp. Ther. 250 (1989) 388–396.
[32] J.R. Peterson, L.C. Bickford, D. Morgan, A.S. Kim, O. Ouerfelli, M.W. Kirschner, M.K. Rosen, Chemical inhibition of N-WASP by stabilization of a native autoinhibited conformation, Nat. Struct. Mol. Biol. 11 (2004) 747–755.
[33] J. Fouchard, D. Mitrossilis, A. Asnacios, Acto-myosin based response to stiffness and rigidity sensing, Cell Adh. Migr. 5 (2011) 16–19.
[34] O. Chaudhuri, S.H. Parekh, D.A. Fletcher, Reversible stress softening of actin networks, Nature 445 (2007) 295–298.
[35] X. Trepat, L. Deng, S.S. An, D. Navajas, D.J. Tschumperlin, W.T. Gerthoffer, J.P. Butler, J.J. Fredberg, Universal physical responses to stretch in the living cell, Nature 447 (2007) 592–595.
[36] D.E. Discher, P. Janmey, Y.L. Wang, Tissue cells feel and respond to the stiffness of their substrate, Science 310 (2005) 1139–1143.
[37] D.E. Ingber, Cellular mechanotransduction: putting all the pieces together again, FASEB J. 20 (2006) 811–827.
[38] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689.
[39] E.M. Darling, M. Topel, S. Zauscher, T.P. Vail, F. Guilak, Viscoelastic properties of human mesenchymally-derived stem cells and primary osteoblasts, chondrocytes, and adipocytes, J. Biomech. 41 (2008) 454–464.