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Biochemical and Biophysical Research Communications 329 (2005) 256–265
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Quantitative measurement of changes in adhesion force involving focal adhesion kinase during cell attachment, spread, and migration

Chia-Ching Wu[a], Hsiao-Wen Su[b], Chen-Chen Lee[a], Ming-Jer Tang[b], Fong-Chin Su[a,*]

[a] *Institute of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan*
[b] *Department of Physiology, National Cheng Kung University, Tainan, Taiwan*

Received 5 January 2005

Abstract

Focal adhesion kinase (FAK) is a critical protein for the regulation of integrin-mediated cellular functions and it can enhance cell motility in Madin–Darby canine kidney (MDCK) cells by hepatocyte growth factor (HGF) induction. We utilized optical trapping and cytodetachment techniques to measure the adhesion force between pico-Newton and nano-Newton (nN) for quantitatively investigating the effects of FAK on adhesion force during initial binding (5 s), beginning of spreading (30 min), spreadout (12 h), and migration (induced by HGF) in MDCK cells with overexpressed FAK (FAK-WT), FAK-related non-kinase (FRNK), as well as normal control cells. Optical tweezers was used to measure the initial binding force between a trapped cell and glass coverslide or between a trapped bead and a seeded cell. In cytodetachment, the commercial atomic force microscope probe with an appropriate spring constant was used as a cyto-detacher to evaluate the change of adhesion force between different FAK expression levels of cells in spreading, spreadout, and migrating status. The results demonstrated that FAK-WT significantly increased the adhesion forces as compared to FRNK cells throughout all the different stages of cell adhesion. For cells in HGF-induced migration, the adhesion force decreased to almost the same level (~600 nN) regardless of FAK levels indicating that FAK facilitates cells to undergo migration by reducing the adhesion force. Our results suggest FAK plays a role of enhancing cell adhesive ability in the binding and spreading, but an appropriate level of adhesion force is required for HGF-induced cell migration.
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Keywords: Adhesion force; Optical tweezers; Cytodetachment; AFM probe; MDCK cell; FAK protein; Hepatocyte growth factor

Cell adhesion is not only involved in various natural phenomena such as embryogenesis, wound healing, immune response, and metastasis [1], but is also the initial and critical step in the tissue engineering approach for maintenance of tissue structure and cell integration of biomaterial [2,3]. The cell membrane proteins and proteins of ECM are involved in cell-extracellular matrix (ECM) interaction. The integrin family, with different types of α and β components of cell membrane proteins, acts as an interface between the intra- and extracellular compartments, translates the attachment of external ligands to internal information which induces adhesion, spreading, or cell migration, and consequently regulates cell growth and differentiation by binding to proteins on the ECM.

Focal adhesion kinase (FAK), a 125-kDa cytoplasmic tyrosine kinase localized in focal adhesions, has been implicated to play an important role in regulating integrin-mediated cellular functions, including cell spreading [4,5], cell migration [6,7], cell cycle progression [8,9], and cell survival [10–12]. Overexpression of FAK in Madin–Darby canine kidney (MDCK) cells has demonstrated the enhancement of HGF-induced cell scattering within collagen gel upon HGF stimulation [13]. These enhancements suggest a link between HGF-increased integrin expression, FAK activation, and enhanced cell motility, and implicate a role for

* Corresponding author. Fax: +886 6 2343270.
E-mail address: fcsu@mail.ncku.edu.tw (F.-C. Su).

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doi:10.1016/j.bbrc.2005.01.126

FAK in the facilitation of growth factor-induced cell motility. However, the quantitative adhesion force and the related role for FAK mediated mechanism have rarely been investigated.

Several techniques including the optical tweezers [14,15], magnetic tweezers [16], micropipette-based suction and force transducers [17–23], atomic force microscope (AFM) [24,25], centrifugation [26], and shear flow [27,28] can be used to measure the adhesion force. Optical tweezers, based on the principle of force balance of optical momentum as the laser passes through the objective lens [3], can trap and manipulate objects including dielectric spheres [3], sperm cells [29], or DNA [30]. Using optical trapping techniques, the cell binding force on fibronectin substrate [31] or mechanical properties of single collagen molecules [32,33] can be quantitatively measured. Within pico-Newton (pN, 10^{-12} N) of manipulation force, the optical tweezers has opened a new era and provides a novel tool to micromanipulate cells and can be used to quantitatively measure the binding force of single cell to different types of ECM [34]. However, once the cell spread out due to adhesion on the substrate, the adhesion force will be out of the range that optical tweezers can be applied. The other method to investigate the quantified adhesion force of single cell was named cytodetachment, using glass probe [35,36], fiber-optic probe [37], or commercial AFM probe [38,39] to apply shear force on single cell and measure the detach force by calculating the deformation of probe.

The purpose of this study was to quantitatively investigate the effect of FAK on cell adhesion force during different stages of initial binding, spreading, long-term adhesion, and induced migration. The overexpression of FAK (FAK-WT), expression of FAK-related non-kinase (FRNK), and vector control of MDCK cells were used for both optical tweezers and cytodetachment technique to measure the adhesion force in different cell status of initial binding (5 s), beginning of spreading (30 min), spreadout (12 h), and migration. For measuring the initial binding force by optical trapping, we used three types of strategies to investigate the force of interaction between trapping object and binding subject, including suspending cell and glass coverslide (SC/G), trapped bead and initial binding cell (B/IC), or trapped bead and the apical surface of long-term spread cell (B/LC). Different strategies allow us to investigate the effect of FAK on binding formation between the two surfaces and subsequently strengthening adhesion in different stages of cell attachment. We also integrated the cytodetachment equipment on optical tweezer workstation by using commercial AFM probe to provide the force of the order of nano-Newton (nN, 10^{-9} N) for quantifying the adhesion force of cell in spreading and HGF-induced migrating status. Within the manipulation force order from pN to nN, the whole system enables to quantitatively investigate the interactions of cell adhesion ability. Our results in the present study demonstrated that the cell adhesion force interacts with the function of FAK in different physiological status.

Materials and methods

Cell culture. The MDCK II 3B5 with FAK-WT, FRNK, and vector control cells were provided by Prof. M.J. Tang and maintained in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Phoenix, Arizona) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Phoenix, Arizona). Briefly, MDCK cells were grown on culture dishes and transfected with overexpressing HA epitope-tagged wild-type FAK (FAK-WT), D395A, P712A/P715A, and Y925F for FAK non-kinase (FRNK), or vector for control using LipofectAMINE (Life Technologies, Phoenix, Arizona) following the manufacturer's instructions [13]. To generate cells stably expressing FAK overexpression and knockout, clones were selected in G418-containing medium (Sigma) and screened by immunoblotting with anti-HA.

For measurement of cell adhesion force in suspension cells, the cultured cells were raised twice with phosphate-buffered saline (PBS, Life Technologies, Phoenix, Arizona), detached with 1× trypsin–EDTA (Life Technologies, Phoenix, Arizona) solution at 37 °C for 4 min, and fresh culture medium to inhibit trypsin–EDTA was then added. After centrifugation at 1000*g* for 5 min, the cells were suspended by adding fresh DMEM and transferred into the Lab-Tek Chamber Slide System (Nunc A/S, Denmark) with the coverslide bottom for optical trapping or seeded on glass coverslide for cytodetachment experiments. The DMEM without phenol red (Life Technologies, Phoenix, Arizona) was used for optical trapping experiments to prevent the absorption of IR laser power.

Thin coating of type I collagen. The type I collagen from rat tail (BD Bioscience) was coated on coverslide and polystyrene (PS) bead with a concentration of 10 μg/cm^2. The number one glass microscope coverslide (1.2 mm thickness) was cut into the dimensions of 5.0 mm width and 20.0 mm length for fitting into the optical trapping Chamber Slide system. After cutting, the coverslide raised by 70% ethanol for overnight was follow by UV irradiation for 15 min. The sterilized coverslide were used to coat a thin layer of collagen for subculturing cells. The collagen-coated coverslide was used as the adhesion substrate for both suspended cells in optical tweezers and spread cells in cytodetachment experiments. The diluted collagen was coated overnight in PBS at 4 °C, followed by rinsing twice with PBS, and then added with 1% bovine serum albumin and incubated at room temperature for 1 h to block the non-coated sites [35].

The 10 μm of PS beads (Polyscience, Warrington, PA) uniform in size and spherical in shape was used for evenly coating collagen on PS beads. The beads were placed into a 2 ml centrifuge tube with estimated total surface areas to fit the collagen concentration similar to coverslide coating (10 μg/cm^2) and placed on the roller overnight at a speed of 6 s/circle at 4 °C. Subsequently, the beads were added with 1% bovine serum albumin (BSA) (Sigma, USA) and incubated at room temperature for 1 h to block the non-coated sites. After coating, the beads were diluted by PBS and added into Chamber Slide system for optical trapping. The collagen-coated beads were freshly made to prevent bead aggregation and synthesis of collagen cluster.

Force measurement by optical tweezers. For quantitatively measuring the initial binding force, the optical tweezers with 1064 nm wavelength (1.5 W, continuous wave diode Nd:YAG laser) (Cell Robotics, Albuquerque, NM) were mounted with the inverted optical microscope of TE300 (Nikon, Japan) which have a multi-port with side port allowing 100% of the laser light to pass through for combining with laser tweezer system. The 100× oil objective lens (CFI Plan Fluor with 1.3 N.A. and 0.2 mm working distance) was used for optical

trapping. The control of motorized stage movement, microscope focus, and image capture was synchronized and programmed by LabVIEW software. To make sure the repositioning accuracy of the motorized stage movements, the precision DC servomotors with shaft encoders and zero backlash gear heads to drive micrometer lead screws mechanism was used with a maximum travel speed of 250 μm/s in X or Y direction and 0.2 μm of reposition resolution. The monocolor CCD camera (SONY) was controlled by LabVIEW program with the speed of 100 frames/s to capture the image during optical trapping and cytodetachment.

The trapping force of beads and suspended MDCK cells was calculated by Stoke's law as $F = 6\pi\eta\gamma\upsilon$, which the trapped objects held steady in the focus point of laser field and the external dragging force was applied to the trapped object by moving the motorized stage, where F is the trapping force in pN; η is the coefficient of viscosity of solution (10^{-3} Ns/m^2 for water at 20 °C); γ is the radius of trapping object; and υ is the velocity when the bead was pulled out of the trap. The radii of optically trapped bead and MDCK cell were measured by grabbing the images during the trapping. By adjusting the laser power and speed of motorized stage, the trapping force of objects in median without any obstacle 3D surrounding area was estimated by linear curve fitting algorithm (Table 1). After calibration, the constant velocity (1 μm/s) of motorized stage movement with different levels of laser power (10% of power interval for 1.5 W laser) was used for measuring the initial binding forces. For preventing the suspending cell from undergoing apoptosis and cell damage owing to long duration of optical trapping, all the optical trapping experiments were performed within 30 min after trypsinization of cells and the system was maintained with 5% CO_2 supplement and at 37 °C temperature. The trapped cell was only used for one trial of detachment and the maximum binding force was determined as certain laser power that is able to detach the trapped object from the adhesive surface with 80% success efficiency among 200 trails for each strategy group.

The strategies for measurement of the initial binding force by optical tweezers were divided into three groups: trapping the suspending cell to approach glass coverslide (SC/G), trapping the bead to approach initial by attached cell (B/IC), and trapping the bead to approach the apical surface of long-term spread cell (B/LC). Comparison of the binding force between B/IC and B/LC groups would help us to identify the distribution of FAK as the adhesion binding receptors and its effect on the change of adhesion force. In the SC/G group, we designed to measure the initial binding force by trapping the suspending MDCK cell to approach the glass coverslide coated with type I collagen. The suspending cell was trapped by optical tweezers, approached the glass coverslide by moving the motorized stage (Fig. 1A), attached for 5 s (Fig. 1B), and then detached by using a different trapping power of the laser (Fig. 1C). The B/IC group was used to measure the binding force between trapped beads and initially attached MDCK cell. The MDCK cells and PS beads were added into the chamber with an approximate ratio of 1:1. First, the optical tweezer was used to trap MDCK cell binding to collagen-coated coverslide (Fig. 1D). Then, the bead was trapped and approached the initially attached MDCK cell from the opposite side of the glass binding surface for 5 s (Fig. 1E). Finally, the trapped bead was detached by a different laser power (Fig. 1F). In the B/LC group, a bead coated with collagen was also trapped and approached the apical side of a long-term seeded MDCK cell for evaluating the change of binding force and surface properties between initial and long-term seeded cells. The MDCK cells were cultured in DMEM on the collagen coated-coverslide in a culture dish for 12 h and then held vertically by self-designed slide holder on the bottom of Chamber Slide System for the experiment (Fig. 1G). The bead was trapped and approached the apical side of a well-spread cell for 5 s (Fig. 1H), and then we measured the detachment force by different power of laser (Fig. 1I).

Force measurement by cytodetachment. For long-term adhesion measurement, the cytodetachment technique was developed to quantify the adhesion of cells using the commercial AFM probe with an appropriate spring constant as a cyto-detacher. The cytodetachment setup was integrated with the laser tweezer workstation. The AFM probe (Polyscience, Warrington, PA) with spring constant ranging from 0.016 to 0.073 N/m was used for the quantitative measurement of the adhesion force between the substrate and cell. The AFM probe was clapped in the self-developed holder, which could be moved in the direction along the vertical Z axis, and fixed to the micromanipulator base of CellSelector (Cell Robotics, Albuquerque, NM) that could be manually moved in the X and Y directions by screwing the screw (Fig. 2A) as previously described [25]. The 40× objective lens (CFI Plan Fluor ELWD with 0.6 N.A. and 3.7–2.7 mm working distance) was used for all the cytodetachment experiments.

The MDCK cells were seeded on collagen coated glass coverslide and then turned vertically by glass holder mounted on the bottom of Chamber Slide system as optical trapping experiments. The 3D coordinated position of target MDCK cell was defined and the cell can travel by moving the motorized stage in the X–Y direction. The 3D position of the AFM probe was manually adjusted to near the surface of coverslide and aligned perpendicularly by verifying microscopy images in a different focus plane with the focus drive. Before applying AFM probe to detach the cell, the motorized stage was controlled by computer to move back and forward in the direction of cell detachment to make sure that there is no contact resistance which could affect the deformation of AFM probe. The single MDCK cell was detached with constant velocity (5 μm/s) of motorized stage and the deformation of AFM probe was measured by synchronizing image. The maximum detachment force was deducted by using Hook's law from the deflection of AFM probe as the spring constant was known Figs. 2B and 2C). After detachment, the AFM probe was manually drawn back from the detachment surface to make sure that the cell was totally detached and no titer existed between the cell and coverslide (Fig. 2D). An image analysis program was coded by MATLAB software (Math Works) with the following steps: mean filter, histogram equalization, edge filter, edge detection, and force reduction as in previous study [34], to detect the pixels of AFM probe deflection, and transformed to detach force (Fig. 2E).

The change of adhesion force can be measured among the different types of seeded MDCK cells in the beginning of spreading (seeded for 30 min), spreadout (seeded for 12 h), and migration (after HGF-induction). The MDCK cells were seeded with low density (about 1000 cells) on the coverslide for 30 min and 12 h to spread independently

Table 1
The size of suspending beads and MDCK cells trapped by optical tweezers for calibrating the trapping force

Bead/cell type	Size (mean ± SD, (n))	A	B	R^2
Collagen coated bead	10 ± 0.03 (114)	5.603	0.143	0.994
Control	15.71 ± 0.88 (143)	2.445	0.316	0.983
FAK-WT	17.26 ± 0.90 (138)	3.475	0.262	0.983
FRNK	16.75 ± 0.68 (155)	1.948	0.281	0.988

The polynomial factors and R^2 for the relationship between trapping force and laser power are shown as $Y = A + BX$, by fitting with linear first-order function of curve fitting. n, number of trails; A, value of Y axis as X is zero; and B, slop of linear polynomial.

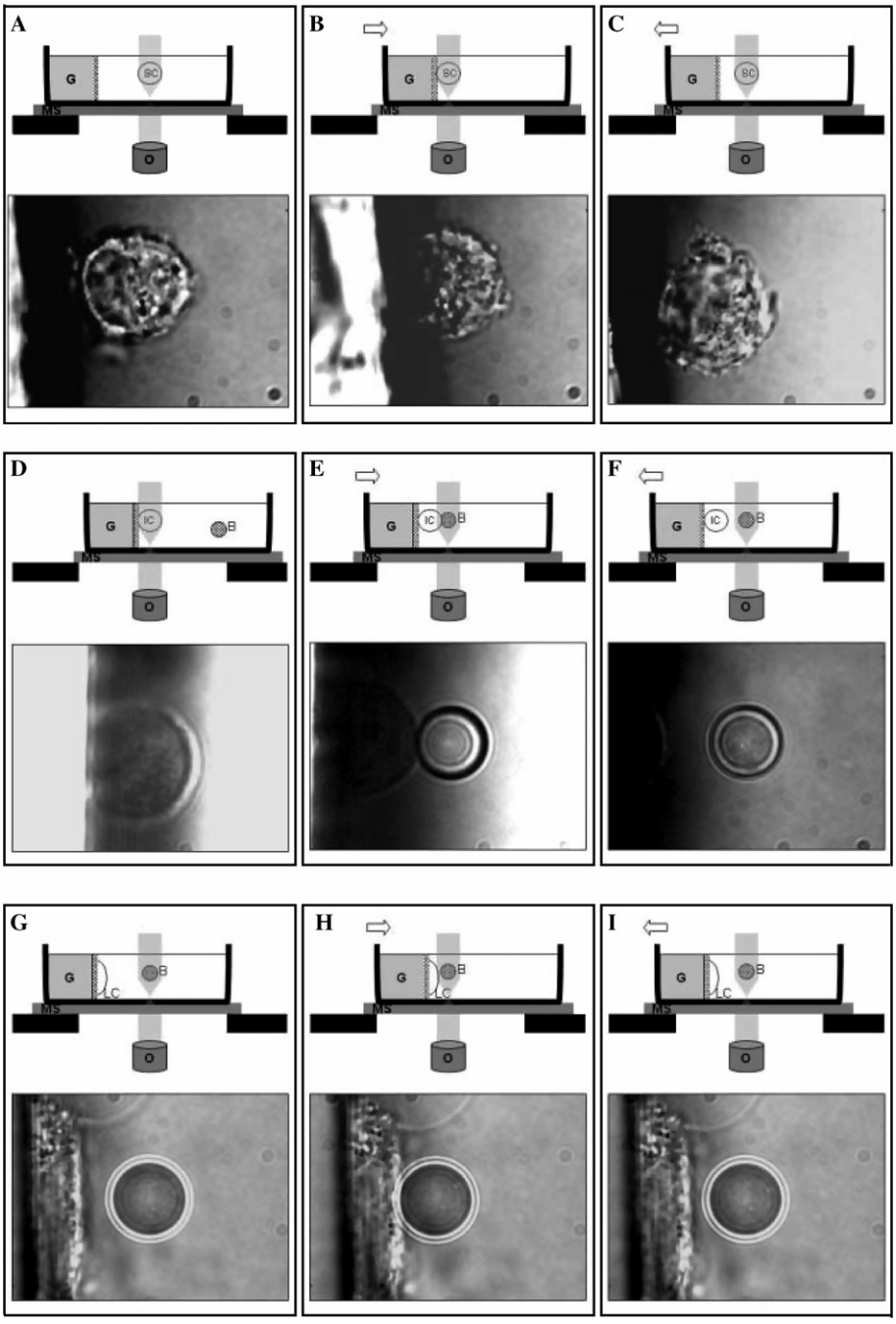

Fig. 1. The schematic drawing and microscopic images for SC/G (A–C), B/IC (D–F), and B/LC (G–I) groups for measuring the initial binding force by optical trapping. The suspending cell was trapped by laser tweezers and then approached the collagen coated glass surface by moving the motorized stage (A) in the SC/G group, or the trapped bead approached the opposite side of initial adhesive cell (D) or long-term spreadout cell (G) in B/IC or B/LC group, respectively. The binding occurred between cell and glass surface (B), or between cell and beads (E and H) by attachment for 5 s. The initial binding force was measured by different rupture forces by adjusting the laser power (C,F,I). O, 100× objective lens; MS, motorized stage; G, glass coverslide with collagen coating; B, polystyrene bead (10 μm) with collagen coating; SC, suspending cell; IC, initial binding cell; and LC, long-term spreadout cell.

and then mounted vertically in glass holder for cytodetachment experiments. For HGF-induction, cells (1×10^5 cells/ml) were allowed to grow as discrete colonies contained between 5 and 10 cells (12 h after seeding), the medium was replaced by fresh medium containing 5% serum and 10 ng/ml HGF (Sigma Medical, Irving, TX) for 12 h, and then the change of adhesion force was evaluated after 12 h of induction.

Statistical analysis. The linear curve fitting algorithm was used to calculate the trapping force of different laser power. The Student's *t* test and analysis of variance (i.e., ANOVA) were used to determine the significance of differences between two sets of experiments and between more than two sets of experiments, respectively. A value of $P < 0.05$ was considered statistically significant.

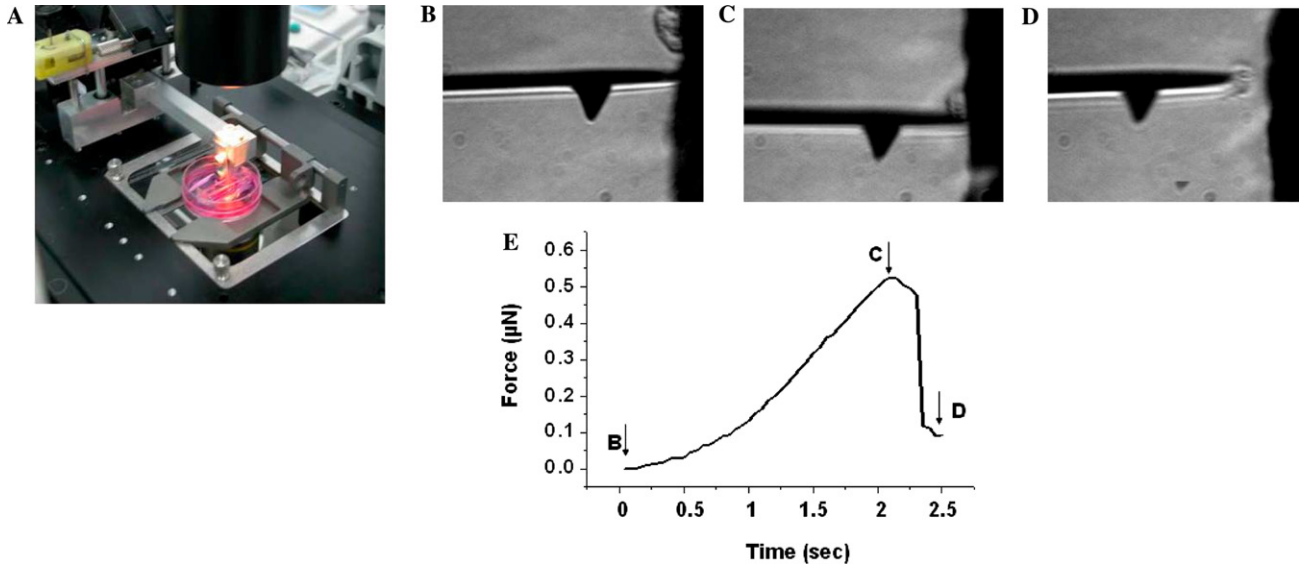

Fig. 2. The cytodetachment equipment used for measuring the adhesion force in the beginning of spreading (30 min), spreadout (12 h), and after HGF treatment cells. The AFM probe was fixed on the holder and approached the seeded cell (A), during detachment the adhesion force can be deduced from the deflection of AFM probe (B,C). After detachment, the MDCK cell detached completely from the collagen coated glass surface and was able to leave the contact surface as we drew back the AFM probe (D). The cell adhesion force curve is calculated by the deformation of AFM probe in detaching images (100 frames/s, such as the frame of time point in B–D with known spring constant using Hook's law (E).

Results

Quantitative measurement of trapping force in cells and beads

The initial step was to calibrate the magnitude of trapping force related to the percentage of laser power on different types of cells or beads. Both PS beads and MDCK cells with FAK-WT, FRNK, and control cells were trapped, respectively, and the trapping force was estimated by Stoke's law with 10% laser power increment (Fig. 3). The sizes of trapped cells were bigger than those of beads and resulted in an increase of trapped force as the same laser power applied on the trapping object. The measured trapping forces were fitted by linear regression with reliable R^2 values to obtain the relationship between the laser power and trapping force (Table 1).

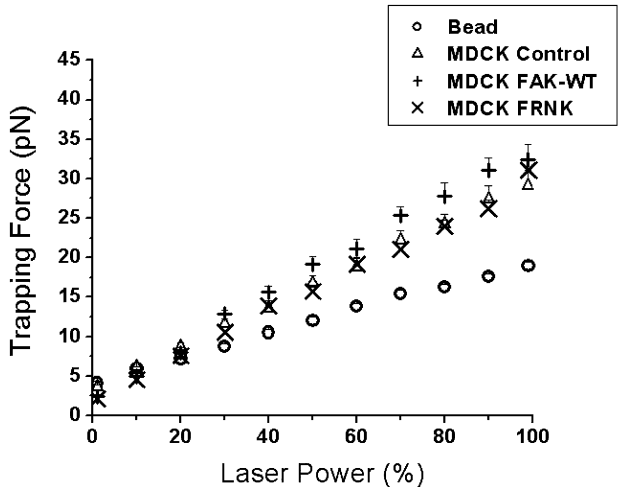

Fig. 3. The trapping force (pN, 10^{-12} N) was calculated by Stoke's law as trapping suspending bead or cell with differently moving velocity in medium without any obstacles. The trapping force showed a highly linear relationship between the percentage of laser power and trapped objects.

FAK increased the initial binding force for the cell to attach on the collagen substrate

Owing the using optical tweezers to investigate the initial binding force for different expression levels of FAK, the overexpression of FAK level caused significant increase of initial binding force than FRNK (Fig. 4). By trapping the cell to approach the glass surface (SC/G group), the binding force showed consistency with the FAK level in the order of FAK-WT, control, and FRNK cells. The quantitative binding forces were 31.07 ± 2.58 pN for FAK-WT, 24.26 ± 1.53 pN for control, and 15.70 ± 0.32 pN for FRNK cells, respectively.

The strategy of trapping cell approach to glass surface (SC/Ggroup) showed an increase of binding force than trapping bead approach to cells (B/IC and B/LC groups). The overexpression of FAK did not cause a significant difference as compared to control cells, but the binding force between beads and cells was significantly increased in FAK-WT as compared to FRNK cells in both B/IC and B/LC groups (Fig. 4). In the B/IC group, the knockout of FAK (FRNK) significantly reduced the

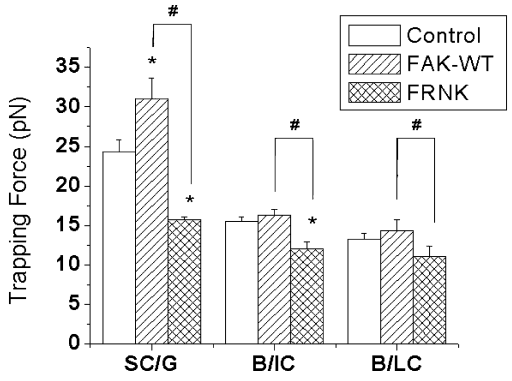

Fig. 4. The changes of initial binding force in MDCK control, FAK-WT, and FRNK cells by different optical trapping strategies. *Significant difference from control cells. #Significant difference overexpression of FAK (FAK-WT) and FAK non-kinase (FRNK) cells.

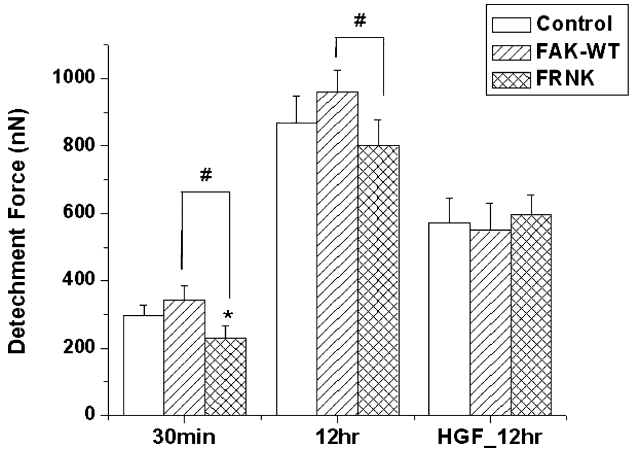

Fig. 5. The cell adhesion force measured by cytodetachment equipment at different experimental time points after the cell was seeded for 30 min, 12 h, and HGF treatment for 12 h. *Significant difference from control cells. #Significant difference overexpression of FAK (FAK-WT) and FAK non-kinase (FRNK) cells.

binding force as compared to FAK and control cells. The binding forces between control, FAK-WT, and FRNK cells were 15.55 ± 0.53, 16.27 ± 0.73, and 12.03 ± 0.90 pN, respectively, in B/IC group. In B/LC group with the cells cultured for 12 h, the binding force on the apical surface of cells demonstrated similar phenomena as the B/IC group which showed a significant increase of binding force in FAK-WT than FRNK. The binding forces between control, FAK-WT, and FRNK cells were 13.29 ± 0.75, 14.34 ± 1.45, and 11.07 ± 1.31 pN, respectively, in the B/LC group. Furthermore, the binding forces were decreased in every case for the trapped bead approaching the apical surface of long-term spread cell than the initial binding cell, but did not attain statistical significance.

Overexpression of FAK enhanced adhesion force in cell spreading as compared to FRNK

Once the cell attached to the collagen coated and began spreading out, the adhesion force increased of the order of nano-Newton. The adhesion force between a spreading cell and collagen coated glass surface was measured as the cell seeded for 30 min and 12 h by cytodetachment. The adhesion force obviously increased about three times as the cell seeded for 12 h than that seeded for 30 min (Fig. 5).

The overexpression of FAK significantly enhanced the adhesion force as compared to FAK non-kinase at time points of both 30 min and 12 h. After seeded for 30 min, the adhesion forces between control, FAK-WT, and FRNK cells were 297.4 ± 27.5, 343.2 ± 43.4, and 228.8 ± 36.6 nN, respectively. The non-kinase of FAK significantly reduced the adhesion force at the beginning of spreading at 30 min with more rounds of morphology than control and FAK cells, but did not reach significant difference as compared to control cells after seeding for 12 h, indicating that FAK is playing an important role for promoting cell spreadout. After seeded for 12 h, the adhesion forces were 869.4 ± 77.8, 961.0 ± 64.1, 800.0 ± 75.5 nN for control, FAK-WT, and FRNK cells, respectively.

Retaining adhesion force for HGF-induced cell migration

The MDCK cells were induced by HGF growth factor and began to scatter and migrate, similar to previous studies [13]. The morphology of cell colonies (before induction) and single cell (after HGF-induced 12 h) showed at both top and side view for three cell types (Fig. 6). As we added 10 ng/ml HGF for induction and measured the adhesion force after 12 h, the adhesion forces for control, FAK-WT, and FRNK cells that seeded on collagen coated glass decreased dramatically to 572.0 ± 73.2, 549.1 ± 80.1, and 594.9 ± 61.2 nN after 12 h of HGF-induction (Fig. 5). After HGF-induction for 12 h, the adhesion force was not significantly different between control, FAK-WT, and FRNK cells on collagen coated glass surface, indicating that the MDCK cells retained a basal level of adhesion force for HGF-induced migration. There was no significant difference in adhesion force for single MDCK cells seeded between 12 and 24 h without HGF treatment (data not shown). The reduction of adhesion force in migrating FAK-WT cells after HGF treatment indicated that the overexpression of FAK can promote the cell in undergoing migration.

Discussion

This is the first study that integrated two different adhesion force measurement techniques to quantitatively investigate different scale of adhesion force during cell attachment, spreading, and migration status. Furthermore, we applied these techniques study the role

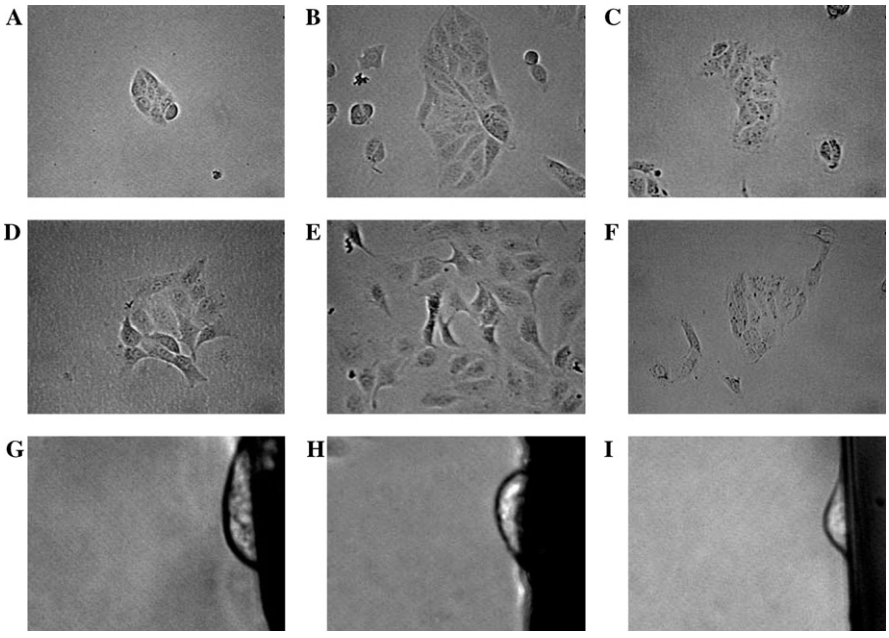

Fig. 6. The MDCK cell synthesized colonies after sended for 12 h (A–C, by 10× objective lens) and induced into migration and scattering with HGF treatment for 12 h (D–F, by 10× objective lens). The side view of migrating MDCK control, FAK overexpression (FAK-WT), and FAK non-kinase (FRNK) cells (G–I, by 40× objective lens).

of FAK and the effects of growth factor induction into changing of adhesion forces. FAK, the best-characterized adhesion sites as the coworker of integrin to link between extracellular matrix and the cytoskeleton, anchors to substrate for developing bonds with new matrix molecules for migration, matrix remodeling or traction force generation in the area of adhesion sites [40]. Phosphorylation of FAK is involved in early integrin-mediated signaling and increases linearly with the number of initial integrin–fibronectin binding in the human fibroblast [41]. The concentration of fibronectin positively correlated with the adhesive association rate and increased binding force in suspending fibroblast via vertically manipulating the cell to attach and detach from the bottom of culture dish by optical tweezers [31]. von Wichert et al. [42] placed the fibronectin coated bead (5.9 μm diameter) on the upper surface of spreading fibroblast (spreading for 15 min), incubated for another 15 min, used the optical tweezer to measure the resistance of re-trapping for beads' escape from the trap field (500 nm), and demonstrated that the resistance of re-trapping force was required by Shp2 to down-regulate the dynamics of focal adhesion complex and enhance residency times of integrin–cytoskeleton linkage formation.

Our study demonstrated that the overexpression of FAK increased the initial binding force in suspending MDCK cells (SC/G and B/IC groups), indicating that the FAK played a role for enhancing adhesion ability of the suspending cell to be adhesive on substrate. In our results, the initial adhesion force measured from the trapped cell, SC/G group, was higher than an trapped bead, B/IC groups. It may be due to the increase of the contact surface and might relate to the numbers of direct associate binding between collagen and membrane receptors. The advantages of using trapping strategy like SC/G group include direct measures of the interaction between a cell and the surface, and easy modification of the surface properties, especially for materials without optical transparency for laser tweezers, such as titanium or aluminum alloy that is commonly used in orthopedic implant. However, there are still some disadvantages that needed to be are dealt with including the geometry of cell deformation and possible damage while directly applying a laser on cells. Trapping bead to measure the binding force on cell surface (B/IC and B/LC groups) in this study provided authentic strategies for force application with more symmetrical shape and minimal deformation of PS beads and reduced the cell damages from heat absorption by side view trapping without passing the laser light through the cell. Furthermore, these strategies can also be used to reveal the variation of binding force during different cell physiological stages (cell cycle progression, polarization, or apoptosis) on different areas of the apical surface of the cell, such as the highest top of cell (performed in this study), lamellopodia, or filopodia. However, the requirement of optical transparency in trapping object might restrict the trapping material in future applications. There are still a lot of factors that are needed to be concerned in the biomaterial domain for adhesion that the attachment

phase occurs rapidly and involves short-term events like physicochemical linkages between cells and materials involving ionic forces, van der Walls forces, etc [2]. Several more detailed aspects also need to be discussed as we trapped cell by optical tweezers. From physical principle aspects, optical trapping occurred by moment's change of light and usually is Gaussian degradation from the center of trap. The morphology was changed to a more symmetrical ball shape and indicated applied centripetal forces as we trapped cells. The effects of the centripetal force related to cell adhesion are still unknown.

For cell spreading, the mutation of Tyr-397 impairs the ability of FAK to promote early spreading [7,43]. In present study, the adhesion force measured via cytodetachment was enhanced in FAK-WT cells at the early spreading (30 min), as well as spreadout cells after seeding 12 h, indicating that FAK promotes the cell-substrate adhesion ability. The optical tweezer results also showed significant increase of binding force on the apical surface of long-term spreadout FAK-WT cells (B/LC group). The non-significant change of binding force between B/IC and B/LC group indicated that the focal adhesion site is still able to synthesize on the apical surface of long-term spreadout cells and the distribution of FAK is even through out the entire cell membrane.

Cell migration involves complex interplay between the formation of cell-substrate adhesion, the exertion of propulsive forces, and the detachment of the adhesion sites [44]. The results of this study showed that the migrating MDCK cells induced by HGF have no significant difference in adhesion force between different FAK expression levels. Interesting, the overexpression of FAK caused an increase of cell migration and scattering with HGF treatment as in previous study [13]. The rate and direction of migration is determined largely by differences in size, lifespan, and traction forces among multiple protrusions. Based on the apparently enlarged focal adhesions, and prolonged lifespan of focal adhesions during cell spreading, it was speculated that FAK may be required for the turnover of focal adhesions. Another factor to effect the cell migration is the cell traction force, which was generated followed by synthesis of focal adhesion site, enabling the cell to pull itself forward. The FAK null fibroblast showed a decrease in migration speed, traction force, and loss of mechanosensing during migration as compared with cells expressing wild-type FAK [45]. FAK has major roles in promoting turnover and disassembly of focal adhesions [46,47] and has show the requirement of FAK/Src [7], Shp2 [42], and Rho family [48] to retract the cytoskeleton for generation of traction force. The result of this study demonstrates that the MDCK cells in migrating status showed a similar basal level of adhesion force, but further study might be needed to reveal the relationship between cell adhesion force and traction force.

The force scale measured by AFM probe was in the range of nano-Newton and much higher than the scale pico-Newton measured by optical tweezers. In addition, there are still a lot of distinctions between optical tweezers and cytodetachment. First of all, the applying force on the cell was normal force using the laser tweezers and shear force using cytodetachment technique. The micropipette technique applies a normal axial force on long-term adhesion cells, but it is difficult to detach an extended cell without damage of the cell due to concern about the sensitivity of force detection, size of micropipette's opening end, and cell spreading area [20,21]. The AFM probe we chose in present study provided a 50 μm wide cantilever as cyto-detacher to sufficiently cover all cell spreading area during the detachment. The disadvantage of side view approaching method in the present study is the inability to quantify the total adhesion force into stress distribution ($pN/\mu m^2$ and Pascal) and easily overestimated or underestimated from side view image. In the present study, we applied the detachment force in a random direction, regardless of the direction of cell migration. From a structural point of view, the cell alignment, direction of migration, and rearrangement of cytoskeleton could affect the cell adhesion force [49–51]. In future work, we could combine external force, such as shearing flow or stretch, to study the role of adhesion force in cell remodeling [52–54].

In summary, our study quantitatively demonstrated that FAK enhanced cell adhesion forces in initial attachment and spreading. The HGF-induced MDCK cell migration within a basal level of adhesion force, while FAK played a role to promote cell migration via decreasing adhesion force with HGF treatment. The combination of optical tweezers and cytodetachment methodology perform different scale of force and able to be applied to reveal adhesion force for cell mechanotransduction, force generation, and function tissue engineering.

Acknowledgments

This research was partially supported from the National Science Council Grant NSC 92-2320-B-006 -059, Taiwan and Biotechnology Center, National Cheng Kung University, Taiwan.

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