FEBS Letters 587 (2013) 763–769

FEBS Letters

journal homepage: www.FEBSLetters.org

Activation of beta 1 but not beta 3 integrin increases cell traction forces

Grace L. Lin [a], Daniel M. Cohen [a], Ravi A. Desai [a], Mark T. Breckenridge [a], Lin Gao [a], Martin J. Humphries [b], Christopher S. Chen [a,*]

[a] Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA
[b] Faculty of Life Sciences, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Manchester, UK

ARTICLE INFO

Article history:
Received 13 August 2012
Revised 3 January 2013
Accepted 28 January 2013
Available online 8 February 2013

Edited by Michael R. Bubb

Keywords:
Integrin
Adhesion
Cytoskeletal tension
Cell traction force
Micropost array
Cell spreading

ABSTRACT

Cell-generated traction forces induce integrin activation, leading to focal adhesion growth and cell spreading. It remains unknown, however, whether integrin activation feeds back to impact the generation of cytoskeletal tension. Here, we used elastomeric micropost arrays to measure cellular traction forces in wildtype and integrin-null cells. We report that activation of β_1 but not β_3 integrin, by either increasing density of immobilized fibronectin or treating with manganese, elicited fibroblast spreading and cytoskeletal tension. Furthermore, this force generation required Rho kinase and myosin activity. These findings suggest that integrin activation and cell traction forces comprise a bi-directional signaling unit of cell adhesion.
© 2013 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

The binding of integrins to extracellular matrix (ECM) initiates cell adhesion, which can be described as a series of processes including cell spreading against the underlying matrix, assembly of focal adhesions (FAs), and generation of actomyosin-mediated cytoskeletal tension against these adhesions [1]. Each of these processes appears to be linked through several pathways. For example, the degree of cell spreading against a micropatterned substrate regulates RhoA activity and cytoskeletal tension [2,3], and this cytoskeletal tension is important for adhesion assembly [4,5]. Conversely, it has been shown that the clustering of integrins required for adhesion assembly is critical to support cell spreading and tension generation [6,7]. Because cell spreading, adhesion assembly, and cytoskeletal tension each have been shown to regulate many cellular functions including proliferation, differentiation, and migration, understanding how these processes are regulated is an important question.

Integrin receptors undergo conformational activation from a low affinity to high affinity state [8,9], and these changes in integrin activity may contribute to the regulation of cell spreading and FA assembly. Indeed, direct activation of integrins via manganese (Mn^{2+}) [10] or conformation-modulating antibodies [11] appears to enhance cell spreading and adhesion assembly [12,13]. Although numerous studies have linked integrin activation to FA growth and superior cell adhesion and spreading on ECM, it is unclear whether integrin activation can also directly regulate cytoskeletal tension generation.

In this study, we found that β_1 integrin activation via increased fibronectin (FN) density or Mn^{2+} leads to enhanced generation of cellular traction forces. We measured these forces by culturing cells on FN-functionalized arrays of uniformly spaced elastomeric microposts, a system we developed previously to enable studies of traction force dynamics [5,14]. Our data indicate that the activation state of integrins is intimately connected to basic adherent cell behaviors like contractility, which has implications for improving our understanding of the regulation of cell shape, mechanics, and function.

Abbreviations: β1KO, β_1-integrin null mouse embryonic fibroblast; β3KO, β_3-integrin null mouse embryonic fibroblast; BSA, bovine serum albumin; ECM, extracellular matrix; FA, focal adhesion; FN, fibronectin; LPA, lysophosphatidic acid; mPAD, micropost array detector; PDMS, polydimethylsiloxane; ROCK, Rho kinase; wtMEF, wildtype mouse embryonic fibroblast

* Corresponding author. Address: Department of Bioengineering, University of Pennsylvania, 510 Skirkanich Hall, 210 South 33rd Street, Philadelphia, PA 19104, USA. Fax: +1 (215) 746 1752.

E-mail addresses: grlin@mail.med.upenn.edu (G.L. Lin), cohendm@seas.upenn.edu (D.M. Cohen), rdesai@seas.upenn.edu (R.A. Desai), markbrec@seas.upenn.edu (M.T. Breckenridge), lingao@seas.upenn.edu (L. Gao), martin.humphries@manchester.ac.uk (M.J. Humphries), chrischen@seas.upenn.edu (C.S. Chen).

0014-5793/$36.00 © 2013 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.febslet.2013.01.068

2. Materials and methods

2.1. Cell culture

Wildtype and β_3 integrin-null MEFs were provided by Dr. Richard Assoian (University of Pennsylvania) and Dr. Richard Hynes (MIT), respectively. β_1 Integrin-null MEFs were maintained as previously described [15]. All cells were cultured in 10% FBS/DMEM (Atlanta Biologicals).

2.2. Reagents and antibodies

Reagents were obtained as follows: fibronectin (BD); vitronectin (Sigma); lysophosphatidic acid (Avanti Polar Lipids); Y27632 (Tocris Bioscience); blebbistatin (Calbiochem); FN blocking antibody 16G3 (20 μg/ml; gift of Dr. Martin Schwartz, University of Virginia); β_1 integrin blocking antibody BMC5 and rat control IgG (10 μg/ml; Chemicon); anti-β_1 integrin (BD); anti-GAPDH (Ambion); anti-active-β_1 integrin (clone 9EG7, BD); anti-vinculin (hVin1, Sigma–Aldrich); adenoviral sh-α_5 integrin and scrambled sequence (gift of Dr. Rebecca Wells, University of Pennsylvania).

2.3. Cell attachment assay

Plates were coated overnight at 4 °C with FN in triplicate (BD Biosciences) and blocked with 50 μg/ml BSA/PBS. Cells were seeded, gently rinsed after 1 h with warm PBS, and quantified using CyQuant (Invitrogen Molecular Probes).

2.4. Substrate preparation

Micropost array detectors (mPADs) were fabricated using PDMS-based replica-molding as previously described [5,16]. Microcontact printing FN on these or flat substrates, with either continuous or 625 μm^2 islands, was performed as described previously [17]. FN concentrations of 0.0625 or 4.0 μg/ml FN in 50 μg/ml BSA are designated as low or high FN density, respectively.

2.5. Western blotting

Cells were lysed in Laemmli sample buffer (Bio-Rad), separated via SDS–PAGE, transferred to PVDF, immunoblotted, and detected using SuperSignal West Dura detection kit (Thermo Scientific).

2.6. Immunofluorescence, cell imaging, and quantitative analysis of focal adhesions and strain energies

For immunofluorescence, cells were fixed with 3.7% paraformaldehyde (Electron Microscopy Sciences), permeabilized with 0.1% Triton X-100, and labeled using primary and then secondary antibodies. Quantitative analyses of adhesions and cell area were performed using a custom-developed MATLAB program [18]. For mPAD experiments, cells were labeled with CellTracker Green CMFDA (Invitrogen Molecular Probes). Quantitative analyses of cell area and total cell strain energies on mPADs were performed as previously described [5].

2.7. Knockdown of α_5 integrin

MEFs were infected with adenovirus encoding either shRNA directed against α_5 integrin or a scrambled sequence [19] at a MOI of 50. Cells were trypsinized at 48 h post-infection and seeded on mPAD substrates.

2.8. Statistical analysis

For each box-and-whisker plot, 15 or more cells per condition were imaged and analyzed across 3 or more experiments. Statistical comparisons between experimental conditions used either Mann–Whitney-U tests or Wilcoxon signed-rank tests, as indicated in individual figure legends. For all tests, statistical significance was assigned at P-value $\leqslant 0.05$ (ns: non-significant, $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$).

3. Results

3.1. Integrin activation enhances cell spreading and traction force

We first confirmed that increasing the density of immobilized FN and exposure to Mn^{2+} enhanced integrin activation [20,21] in our system. Wildtype mouse embryonic fibroblasts (wtMEFs) were plated on substrates coated with a range of FN densities, cultured in the presence or absence of 1 mM Mn^{2+} for 1 h, and then immunostained for activated β_1 integrin. In the absence of Mn^{2+}, cells cultured on low FN density exhibited small peripheral β_1 integrin-positive adhesions (Fig. 1A). In contrast, Mn^{2+} treatment of cells on low FN resulted in increases in spread cell area and the number and size of β_1 integrin-positive adhesions (Fig. 1A and B). On high FN densities, cells displayed greater cell spreading and adhesion number and size relative to untreated cells on low FN, and Mn^{2+} treatment did not promote additional spreading (Fig. 1A and B). As a functional measure of integrin-mediated adhesion, we show that cell attachment was improved by increasing FN density and/or Mn^{2+} treatment (Fig. 1C). Together, these data confirm that shifting the equilibrium towards ECM-engaged integrin, by either increasing FN density or conformational activation of integrin by Mn^{2+}, promotes cell attachment, spreading, and adhesion assembly.

We next examined whether integrin activation impacts cytoskeletal tension, by using elastomeric micropost array detector substrates (mPADs) to measure cell traction forces [5]. wtMEFs attached to and spread on the posts (Fig. 1D – top left panel). Cell spreading correlated with FN density on mPADs similarly to flat substrates, and the deficiency in spreading on low FN was rescued by the addition of Mn^{2+} (Fig. 1D – top right panel and E). Importantly, we observed that Mn^{2+}-induced integrin activation triggered enhanced cell traction forces on low FN, and increasing FN density also increased traction force generation (Fig. 1D – bottom panels and F). Moreover, this enhanced traction force production was blocked by the addition of a FN blocking antibody, 16G3 (Fig. 1G), demonstrating that Mn^{2+}-triggered forces require the formation of new integrin–FN bonds. These data show that increasing the amount of ECM-engaged integrin leads to a net increase in traction forces.

3.2. β1KO MEFs have defects in Mn^{2+}-induced spreading and traction force generation.

Although Mn^{2+} activates integrins indiscriminately, we hypothesized that specific integrin subtypes might be important for mediating the changes in cell spreading and traction force generation in our system. Therefore, we tested the responses of MEFs carrying a deletion of the β_1 gene. As originally reported [15], expression of β_1 integrin is undetectable in these cells, as illustrated here by the absence of reactivity in a anti-β_1 Western blot (Fig. 2A). β1KOs showed reduced attachment to FN (Fig. 2B), relative to the robust attachment curves seen for wtMEFs (Fig. 1C). To assess whether β1KO cells could respond to Mn^{2+}, we assayed whether Mn^{2+} could induce cell spreading and FA assembly on low FN. While β1KOs

Download English Version:

https://daneshyari.com/en/article/10870985

Download Persian Version:

https://daneshyari.com/article/10870985

Daneshyari.com