Biochemical and Biophysical Research Communications 436 (2013) 246–251

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Influence of focal adhesion kinase on the mechanical behavior of cell populations

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ARTICLE INFO

Article history:
Received 17 May 2013
Available online 30 May 2013

Keywords:
Focal adhesion kinase
Cadherins
Cell–cell contacts
Mouse embryonic fibroblasts
Collective cell migration

ABSTRACT

Mechanical forces play an important role in the organization, growth, maturation, and function of living tissues. At the cellular level, the transmission of forces from outside the cell through cell-matrix and cell–cell contacts are believed to control spreading, motility, maturation as well as intracellular signaling cascades that may change many characteristics in cells. We looked at cell populations of mouse embryonic fibroblasts that are deficient of focal adhesion kinase (FAK) and examined their mechanical profile. We observed that the lack of FAK induces a mesenchymal-epithelial switch including the regulation of adherens junctions *via* E-cadherin, leading to increased cell–cell-cohesion. Our results show that the absence of FAK influences the macroscopic cell colony spreading in two (2D) and three (3D) dimensions as well as the velocity fields of the tissue, the single cell persistence and correlation length, changing from an independent to a collective mode of migration. Additionally, the single cell size in the sheet decreases significantly.

1. Introduction

After adhesion to the extracellular matrix, cells form a highly complex cytoskeleton whose major components are actin and myosin connecting to focal adhesions (FAs). The highly regulated interplay of actin stress fibers and FAs essentially defines the mechanics of cells and thus their motility, morphology, and function [1–3]. Focal adhesion kinase (FAK) is a central protein of FAs and is known to have a regulatory role on several cytoskeletal proteins. Among these are integrin activation, the linkage from FAs to actin, FA turnover, and actomyosin contractility [4–7]. The mechanical stability is dependent on the proper integration and regulation of all these cellular processes [8,9]. To date, it has not been unambiguous how FAK influences the overall cellular mechanics, which is defined by the interaction of all the different cytoskeletal components.

We have recently examined the mechanical properties of single wildtype (FAK+/+) and FAK-deficient (FAK−/−) mouse embryonic fibroblasts (MEFs) using a magnetic tweezer, 2D traction, -and atomic force microscopy [10]. We showed that MEF FAK−/− cells, when sparsely seeded on the extracellular matrix, were more easily deformed, cytoskeletal dynamics was dramatically increased, and cytoskeletal remodeling processes were less organized compared to MEF FAK+/+ cells. Western blot analysis revealed that FAK−/− cells had increased activity of myosin light chain kinase (MLCK), an activator of actomyosin contractility. These observations implied instability of the force-transmitting connection of the cytoskeletal network in MEF FAK−/− cells, which was reflected in increased cytoskeletal dynamics. Immunofluorescence analysis indicated that the actin cytoskeleton had an altered spatial distribution in MEF FAK−/− compared to MEF FAK+/+ cells, which was assumed to contribute to the mechanical changes [11–14]. We concluded that the mechanical changes of MEF FAK−/− cells were caused by an unstable and unorganized cytoskeleton.

Previously, it was reported that FAK affects the cytoskeleton not only *via* the focal adhesions (FAs) but also *via* the adherens junctions (AJs) in densely seeded cells, both of which exhibit many striking similarities [15,16]. For instance, they consist of dense clusters of transmembrane receptors (integrins/cadherins) and large numbers of similar signaling and structural molecules that provide a highly dynamic and responsive mechanical link to the actin cytoskeleton [17]. It was previously shown that loss of FAK in MEF cells led to an epithelial phenotype where cells expressed E-cadherin, Cytokeratin-18, and Desmoplakin [18]. Furthermore, FAK rescue of MEF FAK−/− cells restored the mesenchymal phenotype *via* protein kinase B (AKT) signal transduction and regulation of Snail1, a key regulator of the epithelial to mesenchymal transition (EMT) [18].

Abbreviations: EMT, epithelial to mesenchymal transition; AJs, adherens junctions; FAs, focal adhesions.

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http://dx.doi.org/10.1016/j.bbrc.2013.05.084

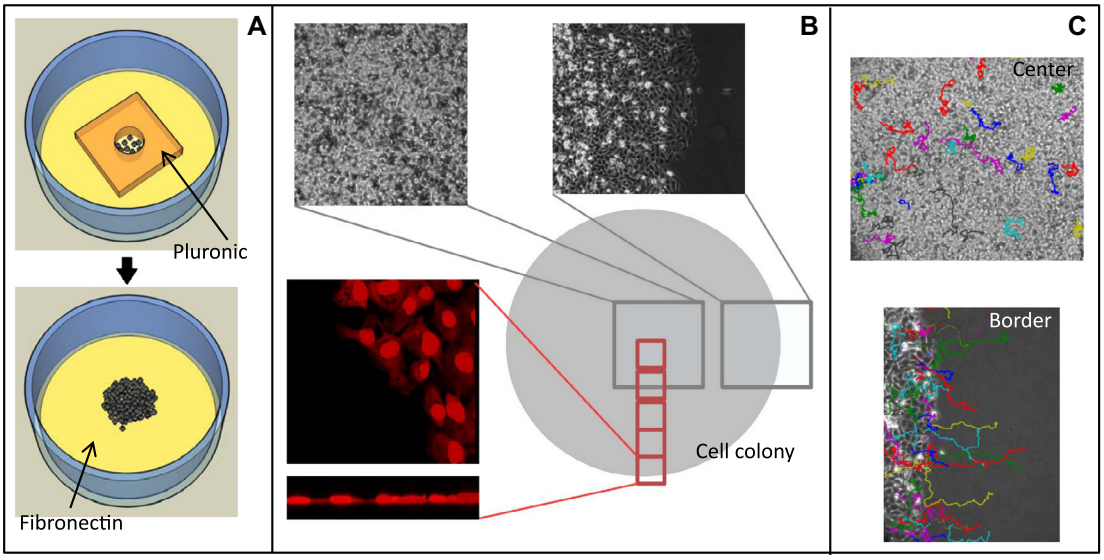
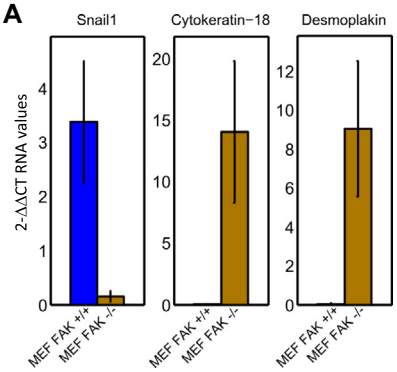


Fig. 1. Cells were seeded in a circular mask on a flat substrate coated with fibronectin and then allowed to form a monolayer. The mask was removed after 24 h (A). Single cells at the center and at the cellular front were tracked every 3 min for 24 h at 5× magnification. The 3D surface profile of the colony was reconstructed from confocal fluorescent image stacks (B). Examples of trajectories of individual cells at the center and cellular front are shown in color (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Based on these results, we elucidated the mechanical properties of MEF FAK−/− cells in a collective environment by using a combined macroscopic growth and microscopic migration assay in cell colonies. Cells were grown to confluency in a circular PDMS mask and continuously tracked by phase contrast -and fluorescence microscopy. The 3D shape of the cell colony was measured by confocal microscopy. Results indicated a different growth pattern in 2D and 3D for MEF FAK+/+ cells and MEF FAK−/− cells. MEF FAK+/+ cells showed a rapid increase in circular colony diameter of no more than 1–2 cell layers over 24 h; in contrast the colony diameter of FAK-deficient cells was smaller, of irregular shape, and dome-like structure of about eight layers. Furthermore, the migration pattern at the colony center and border of these cell lines was different. Due to the lack of cell–cell contacts, MEF FAK+/+ cells moved independently and at lower persistence compared to MEF FAK−/− cells, which migrated collectively at lower speed. In addition, the single cell size of MEF FAK−/− cells was decreased up to a factor of four when cells were in contact within cellular sheets.

Table 1
Semi-quantitative real time gene expression analyses showing a comparison of 2-ΔΔCT values between MEF FAK+/+ and MEF FAK−/− cells for the mouse genes Snail1, Cytokeratin-18 and Desmoplakin. (A) Graph shows the mean 2-ΔΔCT values for RNA as represented in (C) below of two independent cell cultures of MEF FAK+/+ and MEF FAK−/− cells. Note that MEF FAK−/− cells show low gene expression of Snail1 and high expression of Cytokeratin-18, thus confirming a more epithelial phenotype. In contrast, MEF FAK+/+ cells showed no or very low expression of both Cytokeratin-18 and Desmoplakin and high expression of the EMT regulator Snail1, supporting a mesenchymal phenotype. (B) Table shows mouse primer sequences designed from the NCBI mouse data base. Accession numbers are the following: Snai1 -31981483:64-858 Mus musculus (mSnai1) Snail homolog 1; Cytokeratin-18 – 254540067:64-1335 Mus musculus keratin 18 (mKrt18); Desmoplakin -190194417:296-8947 Mus musculus desmoplakin (mDsp). C) Table shows the mean 2-ΔΔCT values and s.e.m. for RNA (n=2) of Snail1, Cytokeratin-18, and Desmoplakin for MEF FAK+/+ and MEF FAK−/− cells.

A



B

Primer sequences

mSnai1-sybr-TF	5'TCAACTGCAAATATTGTAAGGA
mSnai1-sybr-BR	5' ACAGGTCGTGCAGACACAAG
mKRT18-sybr-TF	5' AACTGAGAACAGGAGACTGGAGAG
mKRT18-sybr-BR	5' TCTTCGATGATCTTGAAGTAGTGG
mDsp-sybr-TF	5' ATGATTGACATCGAGAAGATCAGA
mDsp-sybr-BR	5' TCTTGATGAAGTCTTGGTAATGGA

C

Cell type/ mean 2-ΔΔCT RNA values	Snail 1	Cytokeratin-18	Desmoplakin
MEF FAK +/+	3.38 ±1.13	0.02 ±0.01	0.02 ±0.01
MEF FAK -/-	0.15 ±0.10	14.06 ±5.76	9.04 ±3.49