Biochemical and Biophysical Research Communications 406 (2011) 229–233

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Glomerular podocytes: A study of mechanical properties and mechano-chemical signaling

Alexander Eekhoff [a], Navid Bonakdar [a], José Luis Alonso [b], Bernd Hoffmann [c], Wolfgang H. Goldmann [a,*]

[a] Center for Medical Physics and Technology, Biophysics Group, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany
[b] Massachusetts General Hospital/Harvard Medical School, Charlestown, MA 02129, USA
[c] Forschungszentrum Jülich GmbH, Institute of Complex Systems 7, Biomechanics, Jülich, Germany

ARTICLE INFO

Article history:
Received 3 February 2011
Available online 15 February 2011

Keywords:
Podocytes
Cell mechanics and signaling
Magnetic tweezer
Magnetic twisting cytometry
Cell stretcher
Actin cytoskeleton
AT1 receptor
Angiotensin II
Calcium

ABSTRACT

Kidney glomeruli function as filters, allowing the passage of small solutes and waste products into the urinary tract, while retaining essential proteins and macromolecules in the blood stream. These structures are under constant mechanical stress due to fluid pressure, driving filtration across the barrier. We mechanically stimulated adherent wildtype podocytes using the methods of magnetic tweezer and twisting as well as cell stretching. Attaching collagen IV-coated or poly-L-lysine-coated magnetic beads to cell receptors allowed for the determination of cellular stiffness. Angiotensin II-treated podocytes showed slightly higher stiffness than untreated cells, the cell fluidity (i.e. internal dynamics) remained similar, and showed an increase with force. The bead detachment (a measure of the binding strength) was higher in angiotensin II-treated compared to untreated podocytes. Magnetic twisting confirmed that angiotensin II treatment of podocytes increases and CDTA treatment decreases cell stiffness. However, treatment with both angiotensin II and CDTA increased the cell stiffness only slightly compared to solely CDTA-treated cells. Exposing podocytes to cyclic, uniaxial stretch showed an earlier onset of $ERK^{1/2}$ phosphorylation compared to MEF (control) cells. These results indicate that angiotensin II might free intracellularly stored calcium and affects actomyosin contraction, and that mechanical stimulation influences cell signaling.

1. Introduction

Chronic kidney disease (CKD) is among the leading health problems worldwide and options for treatment are limited to dialysis and kidney transplantation. Only recently, CKD as well as proteinuria have been attributed to the dysfunction of the glomeruli and the damage and loss of podocytes [1]. Glomerular podocytes are highly specialized epithelial cells with a complex cytoarchitecture that cover the outer layer of the glomerular basement membrane. Podocytes consist of cell bodies, major processes, and most prominently of foot processes of ~12 μm length and ~200 nm width which culminate between adjacent cells [2]. Specialized structures known as slit diaphragms function as modified adherens junctions connecting the podocyte foot processes. Since blood filtration is accomplished through a membrane comprising of three layers: endothelial cells, the glomerular basement membrane, and podocytes as an outer layer, podocytes represent the weak spot of the glomeruli. To date these cells are the focus to treat CKD disease and proteinuria.

Podocytes react in a stereotypic pattern to various damaging events, e.g. effacement (= loss) of foot processes results in reduced filtration and leakage of proteins (proteinuria) [3–5]. Foot processes are shaped by the actin cytoskeleton and adhere to the glomerular basement membrane. Mutation or loss of actin-associated proteins, focal adhesion proteins, and extracellular matrix (ECM) proteins of podocytes cause glomerular disease and renal failure in humans and transgenic mice [6]. Proteins essential for podocytes' viscoelastic and signaling properties include actin, myosin, α3/β1 integrin, $ERK^{1/2}$, Cas^{130}, and collagen IVα3, α4, α5 [7]. However, the molecular mechanisms that govern formation, maintenance, or effacement of foot processes are largely unknown.

It has previously been reported by several research groups that the polypeptide angiotensin II (Ang II) influences podocytes on many levels: the glomerular function (that can lead to CKD and proteinuria), the internal calcium release, and the cytoskeleton [8,9]. Other researchers described the effect of physiological hydrostatic pressure or external stimulus (stretch) on podocytes which react to Ang II treatment with a change in cell proliferation and $pERK^{1/2}$ signaling as well as with an increase in $[Ca^{2+}]_i$ [6,10,11]. The aim of this study is to analyze podocyte mechanics. Using

* Corresponding author. Address: Friedrich-Alexander-University of Erlangen-Nuremberg, Center for Medical Physics and Technology, Biophysics Group, Henkestrasse 91, 91052 Erlangen, Germany. Fax: +49 9131 85 25601.
E-mail address: wgoldmann@biomed.uni-erlangen.de (W.H. Goldmann).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.02.022

various biophysical methods, we elucidated the complex interplay of cell stiffness, fluidity, and binding strength upon angiotensin (AT1) receptor stimulation by Ang II and calcium depletion by CDTA as well as the influence of mechanical stimulation on cell signaling.

2. Materials and methods

2.1. Cell culture, calcium, and angiotensin II

The immortalized mouse podocytes were a kind gift from Dr. J. Reiser, Miller School of Medicine, University of Miami [12]. Frozen podocytes were thawed at 37 °C and then incubated in RPMI 1640 (Biochrom), 10% FBS, 100 Units/mL Penicillin and 100 µg Streptomycin and γ-interferon (40 units/mL in the first two passages, 20 units/mL after the second passage; Gibco) at 33 °C. At ~80% confluency, the cells were passaged. For differentiation, the cells were incubated at 37 °C for 10 days in above medium without γ-interferon (modified [12]), and for proper cell attachment the flasks were collagen IV-coated (25 µg/mL; Sigma). Prior to experimentation, cells were serum-starved for 3 h. Magnetic tweezer and magnetic twisting measurements in the presence of 100 nM and 10 µM angiotensin II (Sigma), respectively, were performed 3 h after the compound addition. The calcium chelator CDTA (10 mM; Merck) was only used in magnetic twisting experiments.

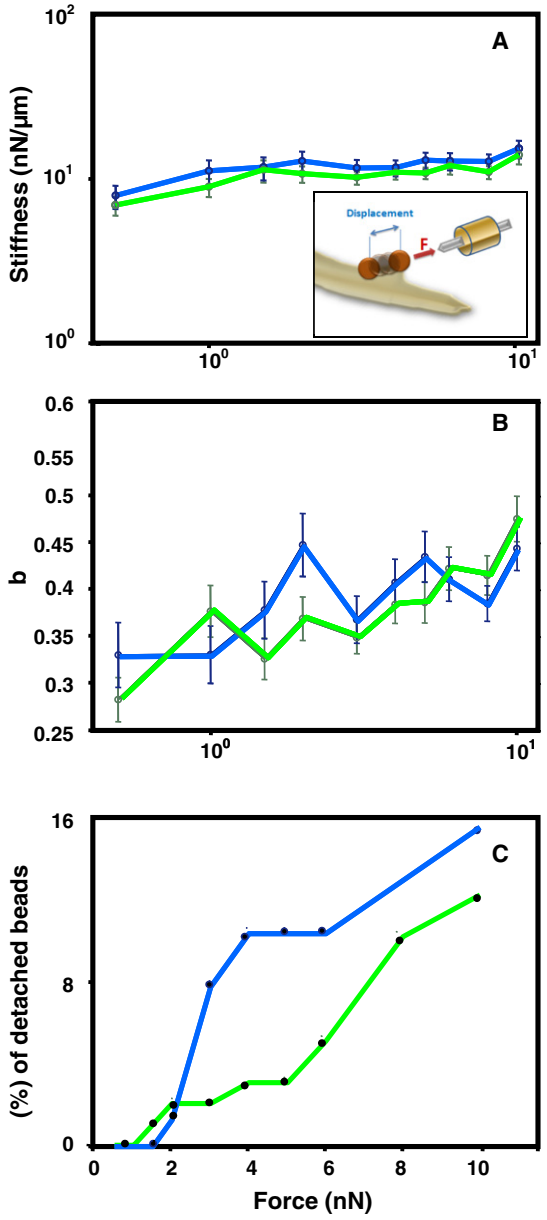

Fig. 1. Effect of angiotensin II on cell stiffness, fluidity, and binding strength using the magnetic tweezer. (A) Cell stiffness and (B) cell fluidity of untreated and with 100 nM angiotensin-treated podocytes were determined. (A, inset) shows the principle of a magnetic tweezer. The percentage of beads detached from the cells *vs.* pulling force are shown in (C). The adhesion strength was significantly lower at 100 nM angiotensin II-treated compared to untreated podocytes. Conditions: $n = 86$ of angiotensin II-treated cells, blue; and $n = 65$ of untreated cells, green; (mean value, ±S.E.). Note, that the *x*-axes in (A + B) are shown on a logarithmic and in (C) on a normal scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Magnetic tweezer

The principle of the magnetic tweezer device used was previously described in [13]. Single cells are deformed by generating a local magnetic field that attracts super-paramagnetic beads bound to the cells (Fig. 1A, inset). These beads (4.5 µm, Dynabeads M450; Invitrogen) at a concentration of 1×10^7 beads/mL are coated with collagen IV (100 µg/ml; Sigma) in PBS at 4 °C overnight. Beads are then washed three times in PBS and stored in this buffer at 4 °C. Before measurements, the beads are sonicated to avoid clustering. About 2×10^5 beads per 35 mm dish are added, and cells are incubated with the beads for 30 min at 5% CO_2 and 37 °C. Thereafter, the medium is exchanged with freshly, prewarmed medium to remove unbound beads. Measurements are performed on a heated inverted microscope stage at 40× magnification (NA 0.6) without CO_2. The measuring time was limited to 30 min per dish. When a force step with an amplitude ΔF was applied to a cell-bound bead, it moved with a displacement $d(t)$ towards the tweezer needle tip. The ratio $d(t)/\Delta F$ defines the creep response $J(t)$. $J(t)$ of the cells follows a power law: $J(t) = a(t/t_0)^b$, where the pre-factor a and the power law exponent b are both force-dependent, and the reference time t_0 is set to 1 s. The values for a and b are determined by a least-squares fit. The pre-factor a (units of µm/nN) characterizes the elastic cell properties and corresponds to compliance (i.e. inverse of stiffness). b reflects the dynamics of the force-bearing structures of the cell that are connected to the bead. Note, that a power law exponent of $b = 0$ indicates a purely elastic solid and $b = 1$ a purely viscous fluid. In cells, the power law exponent usually falls in the range between 0.1 and 0.5, whereby higher values have been linked to a higher turnover rate of cytoskeletal structures [14]. Higher b values are often associated with reduced cell stiffness.

2.3. Bead detachment

The detachment of beads that are bound to cells for 30 min is measured during force application ranging between 0.5 and 10 nN. The percentage of all detached beads in relation to the detachment force is used as a measure for the bead binding strength to the cell.

2.4. Magnetic twisting cytometry with optical detection (OMTC)

Details of the magnetic twisting device are described in [15,16]. Controlled mechanical stresses are applied directly to cell surface receptors using ferromagnetic microbeads precoated with poly-L-lysine. The beads are prepared by gentle shaking for 2 h at room temperature in a 0.2 M sodium phosphate buffer at pH 7.4, then washed three times in PBS (80 mM Phosphate, 1.37 M NaCl, pH 7.4; BostonBioproducts) for at least 1 h prior to use. Adherent podocytes spread for 4 h on collagen IV-coated 35 mm ∅ dishes. Poly-L-lysine-coated beads were bound for 7 min to the cells in