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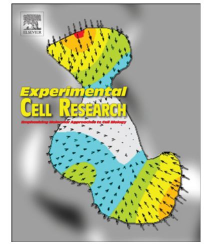

Research Article

Determining the mechanical properties of plectin in mouse myoblasts and keratinocytes

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ABSTRACT

Plectin is the prototype of an intermediate filament (IF)-based cytolinker protein. It affects cells mechanically by interlinking and anchoring cytoskeletal filaments and acts as scaffolding and docking platform for signaling proteins to control cytoskeleton dynamics. The most common disease caused by mutations in the human plectin gene, epidermolysis bullosa simplex with muscular dystrophy (EBS-MD), is characterized by severe skin blistering and progressive muscular dystrophy. Therefore, we compared the biomechanical properties and the response to mechanical stress of murine plectin-deficient myoblasts and keratinocytes with wild-type cells. Using a cell stretching device, plectin-deficient myoblasts exhibited lower mechanical vulnerability upon external stress compared to wild-type cells, which we attributed to lower cellular pre-stress. Contrary to myoblasts, wild-type and plectin-deficient keratinocytes showed no significant differences. In magnetic tweezer measurements using fibronectin-coated paramagnetic beads, the stiffness of keratinocytes was higher than of myoblasts. Interestingly, cell stiffness, adhesion strength, and cytoskeletal dynamics were strikingly altered in plectin-deficient compared to wild-type myoblasts, whereas smaller differences were observed between plectin-deficient and wild-type keratinocytes, indicating that plectin might be more important for stabilizing cytoskeletal structures in myoblasts than in keratinocytes. Traction forces strongly correlated with the stiffness of plectin-deficient and wild-type myoblasts and keratinocytes. Contrary to that cell motility was comparable in plectin-deficient and wild-type myoblasts, but was significantly increased in plectin-deficient compared to wild-type keratinocytes. Thus, we postulate that the lack of plectin has divergent implications on biomechanical properties depending on the respective cell type.

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Introduction

Plectin, a giant (>500 kDa), highly versatile cytolinker protein, is capable of connecting the different cytoskeletal filament systems (intermediate filaments (IFs), actin filaments, and microtubules) with each other to form network-like arrays. Plectin plays a crucial role by anchoring IFs to organelles and to extracellular matrix (ECM)-adhesion complexes at the cytoplasmic membrane, thereby ensuring mechanical stability [1]. As plectin is expressed in practically all cell types, mutations in the human plectin gene (*PLEC*) result in a pleiotropic phenotype and simultaneously affect several tissues, primarily skin, muscle and nerve [2,3]. Plectin's function in strengthening cells against mechanical stress is unequivocally demonstrated by the severe tissue fragility of patients suffering from the most common plectin mutation-associated disease, epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) [4]. In the multilayered epithelium of the skin (epidermis), plectin is an essential component of a specialized multiprotein complex forming hemidesmosomes, which provide mechanical stability to basal epidermal keratinocytes by anchoring keratin IFs to the ECM. Plectin is also prominently expressed in striated and smooth muscle cells, but its importance for the structure and function of muscle became only evident when patients with plectin-related EBS were found to suffer, in addition to skin blistering, from a late onset of muscular dystrophy. In skeletal muscle, distinct plectin isoforms are crucial for the integrity of myofibers by specifically targeting and anchoring desmin IFs to Z-disks, costameres, mitochondria and the nuclear/ER membrane system [5,6]. Recent studies have opened up new perspectives on plectin's cytolinker function that go far beyond the originally proposed role as a reinforcing element of the cellular cytoarchitecture, as plectin was shown to serve as a scaffold for various proteins involved in signaling, and to interact with a multitude of direct and indirect binding partners [2].

We hypothesize that a disruption of the plectin-mediated cytoskeletal crosslinking and anchorage to the cytoplasmic membrane-located ECM-adhesion complexes should lead to changes in biomechanical properties in terms of mechanical vulnerability, cell stiffness, dynamics, motility and force production. We used wild-type (plectin$^{+/+}$) and plectin-deficient (plectin$^{-/-}$) mouse myoblasts as well as keratinocytes and compared their mechanical behavior to dissect the differential influence of plectin deficiency in these cell lines. Our data provide evidence that the lack of plectin has divergent implications on cellular biomechanical properties depending on the cell type.

Materials and methods

Cells and cell culture

Immortalized (p53-deficient) plectin$^{+/+}$ and plectin$^{-/-}$ mouse myoblasts [7] and keratinocytes [8] were used. Myoblasts were cultured in F-10 growth medium (GibcoBRL) supplemented with 20% FCS, 1.5% penicillin/streptomycin (Biochrom AG) and 0.1% essential growth factor (rhFGF, Promega). Keratinocytes were cultured in basal keratinocyte growth medium without calcium (Lonza) supplemented with 2% calcium-free (Chelex 100-treated) FCS, 1% insulin-transferrin-selenium (Gibco), 0.4% bovine pituitary extract (Lonza) and 5 μM calcium.

Cell stretching

Cell stretch experiments were carried out on flexible polydimethylsiloxan (PDMS, Sylgard) substrates that were molded into the shape of a cell culture well with 4.0 cm^2 internal surface [9]. The stretcher device consisted of a linear stage for uniaxial stretch and was driven by a computer-controlled stepper motor. The substrates were coated with 5 μg/ml fibronectin in PBS overnight at 4 °C, and 10.000 cells were seeded 24 h prior to experiments. Uniaxial, cyclic stretching was performed in the incubator under normal cell culture conditions (37 °C, 5% CO_2, 95% humidity) for 1 h and 0.25 Hz at 30% stretch amplitude [10].

Magnetic tweezer rheology

We used a magnetic tweezer device as described in Ref. [11]. For measurements, 2×10^5 cells were seeded overnight in a 35 mm diameter tissue culture dish. Thirty minutes before the experiments, cells were incubated with fibronectin-coated paramagnetic beads of 4.5 μm diameter (Invitrogen). A magnetic field was generated using a solenoid with a needle-shaped core (HyMu80 alloy, Carpenter, Reading, PA). The needle tip was placed at a distance of 20–30 μm from a bead bound to the cell using a motorized micromanipulator (Injectman NI-2, Eppendorf). During measurements, bright-field images were taken by a CCD camera (ORCA ER, Hamamatsu) at a rate of 40 frames/s. The bead position was tracked on-line using an intensity-weighted center-of-mass algorithm. Measurements on multiple beads per well were performed at 37 °C for 1 h, using a heated microscope stage on an inverted microscope at 40× magnification (NA 0.6) under bright-field illumination [10].

Traction force microscopy

Traction measurements were performed on 6.1% acrylamide/bisacrylamide (ratio 19:1) gels (Young's modulus 12.8 kPa, thickness 300 μm) with 0.5 μm green fluorescent beads embedded at the top surface [12,13]. Gels were coated with 5 μg/ml fibronectin at 4 °C overnight. Cells were seeded at a density of 5.000 cells cm^{-2} and incubated under normal growth conditions overnight. During measurements, cells were maintained at 37 °C and 5% CO_2 in a humidified atmosphere. Cell tractions were computed with an unconstrained fast Fourier traction cytometry method [14] and measured before and after treatment with 80 μM cytochalasin D to relax the traction forces [10].

Migration assay

Fifty thousand cells were seeded in 35 mm diameter dishes coated with 50 μg/ml collagen type I. After 30 min, cells were placed in a microscope incubation chamber (37 °C, 5% CO_2, 95% humidity), and phase contrast images were recorded every minute for 2 h (10× magnification). Cell movements were computed using a Fourier-based difference-with-interpolation image analysis [13]. Cells moved spontaneously with a mean square displacement (MSD) that also followed a power law with time, $MSD=D\ (t/t_0)^{\beta}$, where t_o is the time interval of the image

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