Mechanical Interactions Among Cytoskeletal Filaments

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Abstract—Mechanical properties of the cells are important in controlling cell shape, cell migration, and other functions. To understand how cytoskeletal (CSK) filaments interact with one another mechanically, mechanical properties of adherent endothelial cells were analyzed after treatment with CSK-disrupting drugs. CSK stiffness (the ratio of applied stress to strain, a measure of cell resistance to shape deformation), viscosity (an index of intracellular structural damping), and permanent deformation (a measure of “plasticity”) were measured with magnetic twisting cytometry, by which rotational stress was applied directly to integrin receptors with ferromagnetic beads coated with RGD-containing peptide. Treatment with cytochalasin D, which disrupts actin microfilaments inhibited stiffness by 50% and decreased permanent deformation from 70% to 50% but had almost no effect on viscosity. In contrast, nocodazole, a microtubule disrupter, had very little effect on inhibition of CSK stiffness, decreased viscosity by 25%, and had no effects on permanent deformation. Acrylamide, an intermediate filament disrupter, had little effect on inhibition of CSK stiffness, little effect on viscosity, and no effect on permanent deformation. Taxol, a drug that facilitates microtubule polymerization, increased stiffness by 10%, increased viscosity by 10%, and decreased permanent deformation from 70% to 50%. Combinations of cytochalasin D and nocodazole, cytochalasin D and acrylamide, or all three drugs resulted in a synergistic effect on inhibition of CSK stiffness and viscosity but not in permanent deformation. Inhibition of oxidative metabolism with potassium cyanide had no effects on stress-induced stiffening response. Inhibition of tyrosine phosphatase with phenylarsine oxide had no effect on stress-induced stiffening response. We conclude that higher order mechanical interactions of CSK filaments are important in determining the mechanical properties of the cell. (Hypertension. 1998;32:162-165.)

Key Words: microfilaments ■ microtubules ■ cytoskeleton ■ cytometry

One of the fundamental questions in cellular mechanics is how individual filamentous systems contribute to the overall behavior of cell shape stability, which has profound implications in regulating vascular tone. Control of cell and thus nuclear shape is critical for cell growth, motility, differentiation, and apoptosis.1-6 Loss of shape control is a hallmark of neoplastic transformation and is associated with deregulation of both cytoplasmic and nuclear functions.7,8 While soluble molecules such as growth factors are important in switching genes on and off, these molecules alone cannot determine cell shape stability. Because cytoskeletal (CSK) structural and mechanical alterations appear to be important in control of cell deformability,9,10 we set out to quantify changes in CSK mechanics using magnetic twisting cytometry after treatment with different drugs.11-13 We demonstrate here that the interplay between different CSK filament systems is required for control of cell mechanics.

Methods

Cell Culture

Bovine capillary endothelial cells isolated from adrenal cortex were cultured as described.3 Quiescent confluent monolayers were exposed to trypsin/EDTA and washed in DMEM containing 1% BSA. Cell aliquots were pelleted and resuspended in defined medium consisting of DMEM, transferrin (5 μg/mL, Collaborative Research), high-density lipoprotein (10 μg/mL, Bionetics Research Institute), 1% BSA, and basic fibroblast growth factor (2 ng/mL, Takeda Chemical Industries).

Magnetic Twisting Cytometry

Ferromagnetic microbeads (4.5 μm in diameter) were precoated with a synthetic RGD (Arg-Gly-Asp)-containing peptide (Peptite 2000, Telios) in a carbonated buffer (50 μg/mL protein per milligram beads) to facilitate protein absorption onto the beads.3 Endothelial cells were plated in defined medium at 3×104 cell per well on fibronectin-coated (500 ng/cm2; this coating density promotes maximum cell spreading)3 plastic dishes (96-well Removawells, Immunolon II, Dynatech) for 4 to 6 hours before twisting experiments. Beads were added to each well at 20 μg per well (~60 000 beads per well, average 2 beads per cell) for 15 minutes, and then unbound beads were washed away with 1% BSA/DMEM. The well was placed into the magnetic twisting cytometer and maintained at 37°C. A brief (10 μs) but strong (1000 G) magnetic pulse was applied to magnetize all surface-bound beads in the horizontal direction.11-13 A twisting torque then was applied with a weak vertical homogeneous magnetic field (0 to 30 G). The extent of bead rotation (angular strain) was measured by an in-line magnetometer that measured the magnitude of the bead magnetic vector in the horizontal direction. Stress was calibrated in a viscous standard, and angular strain was measured as the beads rotated in place in response to applied stress.12,13 Stiffness is defined as the ratio of stress to angular
strain. Apparent viscosity was calculated as the product of time constant after stress release and stiffness. Permanent deformation was calculated as the percent angular strain sustained after stress release.12

Results
To determine how individual CSK filament systems interact with each other mechanically, changes in CSK mechanics were measured after different filament systems were disrupted. Endothelial cells were plated on fibronectin for 6 hours before the experiments. RGD-coated ferromagnetic beads were then added to the well for 15 minutes, and unbound beads were washed away. A stress of 40 dyne/cm² was applied, and angular strain was measured. Cytochalasin D (0.1 μg/mL), which severs microfilaments and disrupts actin network,14 nocodazole (10 μg/mL), which inhibits microtubule formation,15 acrylamide (4 mmol/L), which disrupts intermediate filaments, or a combination of these agents was added to the cells for 15 to 60 minutes. Treatment with cytochalasin D inhibited stiffness by 50% (P<0.05), decreased permanent deformation from 70% to 50% (P<0.05), but had almost no effect on viscosity (P>0.05). In contrast, nocodazole inhibited stiffness by 20% (P<0.05), decreased viscosity by 25% (P<0.05), and had no effect on permanent deformation (P>0.05). Acrylamide inhibited stiffness by 15% (P<0.05) and viscosity by 10% (P<0.05) and had no effect on permanent deformation (P>0.05). A combination of cytochalasin D and nocodazole, cytochalasin D and acrylamide, or all 3 drugs resulted in added effects on inhibition of stiffness by 75% to 80% (P<0.05) and viscosity by 55% to 70% (P<0.05) but had no added effect on permanent deformation (P>0.05 compared with cytochalasin D effect) (Figure 1). Because the stiffness probed through nonadhesion molecules such as scavenger receptors was about 12 dyne/cm² (not shown), and assuming that stiffnesses are additive and the rest of the stiffness is CSK stiffness, these results show that about 95% of the CSK stiffness was inhibited when all 3 drugs were added simultaneously. Therefore, all 3 filament systems interact mechanically to contribute to the stiffness of the entire CSK and thus of the whole cell.

Discussion
Our results indicate that the apparent viscosity is an index of CSK structural damping (but not of intracellular fluid viscosity), which can be decreased by disrupting microtubules. This damping process depends on the mechanical interactions among all 3 filament systems. Interestingly, permanent deformation appears to be associated only with microfilaments and not with microtubules and/or intermediate filaments. Together with our earlier work indicating that permanent deformation could depend on chemical remodeling due to actions of various small CSK severing, cross-linking, and bundling proteins, we suggest that these remodeling processes that are associated with the microfilament structure are a source of permanent deformation.

Taxol, a drug that facilitates microtubule polymerization, increased stiffness by 10% (P<0.05), increased viscosity by 10% (P<0.05), and decreased permanent deformation from 70% to 50% (P<0.05). Addition of taxol and nocodazole together abolished the effects of nocodazole, and addition of taxol and cytochalasin D had the same effects as cytochalasin D alone (not shown). These results show that taxol specifically modulates microtubules. The resulting increases in stiffness and viscosity due to microtubule hyperpolymerization are consistent with our earlier results in cardiac muscle cells.16 Taxol might change permanent deformation via inter-
Stress-induced stiffening response (Figure 2). A recent article suggests that stress-induced stiffening response might be dependent on the process of oxidative metabolism. Treating endothelial cells with potassium cyanide (2.5 mmol/L for 15 minutes), an oxidative metabolism inhibitor, had no effect on inhibition of stress-induced stiffening response (Figure 2). A recent article suggests that stress-induced stiffening response might be dependent on tyrosine dephosphorylation. To test this possibility, we added RGD-coated beads for 15 minutes, washed away unbound beads, and then treated cells with a low dose of phenylarsine oxide (PAO, 5 nmol/L for 10 minutes). Surprisingly, inhibition of tyrosine phosphatase with PAO had no effect on stress-induced stiffening response (Figure 3). Treating the cells with a much higher dose of PAO (10 μmol/L for 10 minutes) did not have an effect on the stiffening response either (not shown). The reason for this discrepancy might lie in earlier experiments: the bead was added for seconds only before the force was applied. The focal adhesion complex was still in the process of being formed, ie, different linking proteins were still in the process of being recruited to the bead; thus, the force-dependent stiffening response observed in their experiments might reflect the strengthening process in the focal adhesion complex that depends on tyrosine dephosphorylation. In contrast, in our experiments, beads were allowed to bind to the cells for 15 minutes before twisting, and by this time the recruitment of the linking proteins to the focal adhesion is already almost complete. Thus, our observed stress-induced stiffening response might reflect the process of mechanical rearrangement of CSK filaments.

In summary, we have demonstrated that the mechanical properties of the cell depend on the mechanical interactions among all 3 CSK filament systems.

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