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Altering the cellular mechanical force balance results in integrated changes in cell, cytoskeletal and nuclear shape

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Summary

Studies were carried out with capillary endothelial cells cultured on fibronectin (FN)-coated dishes in order to analyze the mechanism of cell and nuclear shape control by extracellular matrix (ECM). To examine the role of the cytoskeleton in shape determination independent of changes in transmembrane osmotic pressure, membranes of adherent cells were permeabilized with saponin (25 μg/ml) using a buffer that maintains the functional integrity of contractile microfilaments. Real-time videomicroscopic studies revealed that addition of 250 μM ATP resulted in time-dependent retraction and rounding of permeabilized cells and nuclei in a manner similar to that observed in intact living cells following detachment using trypsin-EDTA. Computerized image analysis confirmed that permeabilized cells remained essentially rigid in the absence of ATP and that retraction was stimulated in a dose-dependent manner as the concentration of ATP was raised from 10 to 250 μM. Maximal rounding occurred by 30 min with projected cell and nuclear areas being reduced by 69 and 41%, respectively. ATP-induced rounding was also accompanied by a redistribution of microfilaments resulting in formation of a dense net of F-actin surrounding retracted nuclei. Importantly, ATP-stimulated changes in cell, cytoskeletal, and nuclear form were prevented in permeabilized cells using a synthetic myosin peptide (IRICRKG) that has been previously shown to inhibit actomyosin filament sliding in muscle. In contrast, both the rate and extent of cell and nuclear rounding were increased in permeabilized cells exposed to ATP when the soluble FN peptide, GRGDSP, was used to dislodge immobilized FN from cell surface integrin receptors. GRGDSP had little effect on cell or nuclear shape in the absence of ATP and, hence, in the absence of cytoskeletal tension. These data suggest that large-scale changes in cell and nuclear shape result from the action of mechanical tension that is generated within the cytoskeleton via an actomyosin filament sliding mechanism, transmitted across integrin receptors and physically resisted by immobilized adhesion sites within the extracellular matrix. Rapid and coordinated changes of cell, cytoskeletal and nuclear form result when this cellular force balance is altered.

Key words: extracellular matrix, integrin, actomyosin interactions, fibronectin, microfilaments, cytomechanics.

Introduction

One of the most fundamental problems in tissue morphogenesis is the question of how local changes in extracellular matrix (ECM) produce alterations of cell and nuclear form. ECM-dependent shape control is critical for cell growth (Folkman and Greenspan, 1975; Gospodarowicz et al., 1978; Ingber, 1990; Mooney et al., 1992), differentiation (Emerman and Pitelka, 1977; Bissell et al., 1982; Watt et al., 1988; Mooney et al., 1992), establishment of cell polarity (Ingber et al., 1986), and development of tissue pattern (Ingber et al., 1981; Ingber and Folkman, 1989). Changes in cell shape are commonly associated with alterations of nuclear structure (Ingber et al., 1987) which, in turn, appear to be required for changes in nuclear functions, including gene expression, nuclear transport and initiation of DNA replication (Nicolini et al., 1986; Feldherr and Akin, 1990; Pienta et al., 1991). Loss of shape control is a hallmark of neoplastic transformation and is associated with deregulation of both cytoplasmic and nuclear functions (Folkman and Greenspan, 1975; Ingber et al., 1981; Wittelsberger et al., 1981; Pienta et al., 1991). ECM molecules, such as fibronectin (FN), produce cell shape changes via binding to cell surface integrin receptors which span the membrane and interconnect with actin microfilaments inside the cell (Hynes, 1987; Rouslahti and Pierschbacher, 1987). Structural coupling between ECM, integrins and actin microfilaments suggests that the effects of ECM on cell shape are likely mediated via alterations in the cytoskeleton. In fact, great advances have been made in terms of identifying cytoskeletal components (e.g. talin, vinculin, α-actinin, paxillin) that interlink integrins with

microfilaments (Burridge, 1986). However, the mechanism by which local ligation of integrins results in cytoskeletal reorganization and global changes of cell shape remains unclear. Even less is known about how ECM promotes coordinated changes in cell and nuclear structure that are commonly observed in living cells.

Many past studies on cell shape control focused on identification of specific ECM receptors that mediate cell attachment and spreading. Other experiments characterized the chemical and mechanical properties of isolated cytoskeletal filaments and extracts. However, studies with isolated molecules do not permit analysis of the role of *cell-generated forces* in shape determination and thus, they can not fully explain how dynamic and integrated changes of shape take place within living cells. For example, internal hydraulic pressures which result from osmotic differences across the cell surface have been suggested to be responsible for shape changes during cell migration (Oster and Perelson, 1987; Bereiter-Hahn and Strohmeier, 1987). Cytoskeletal tension also is likely to be critical for ECM-dependent shape control given that cells exert tractional forces on their adhesion sites (Harris et al. 1980; Lotz et al., 1989; Heath and Holifield, 1991) and exhibit different shapes depending on whether or not the ECM can deform in response to this force (Emerman and Pitelka, 1978; Ingber and Jamieson, 1985; Li et al., 1987; Ingber and Folkman, 1989; Opas, 1989; Mochitate et al., 1991). Thus, any analysis of cell shape control by ECM must take into account both the cellular mechanism of force generation and the ability of the adhesive substratum to establish a mechanical force balance.

Actomyosin interactions have been implicated in cytoskeletal tension generation and shape control in cultured cells, based primarily on analogy with skeletal muscle (Hoffmann-Berling, 1954; Korn, 1978; Hynes et al., 1987). However, the molecular basis of tension generation has not been clearly demonstrated because experimental perturbation of actomyosin interactions commonly results in disassembly of actin and myosin filaments in non-muscle cells. Recently, a method for permeabilizing the membranes of cultured cells was developed which maintains the functional integrity of the actin cytoskeleton (Wysolmerski and Lagunoff, 1990). Cells permeabilized in this manner lack plasma membrane integrity and, thus, differences in transmembrane osmotic pressure. Yet, these intact cytoskeletal preparations retain the ability to generate tension and change shape in the presence of calcium and ATP. Here we use this permeabilization technique to elucidate the biomechanical basis of cell shape control by ECM. We show that changes in cell and nuclear shape result from the action of cytoskeletal tension that is generated via an actomyosin filament sliding mechanism, transmitted across transmembrane integrin receptors, and physically resisted by immobilized binding sites within the underlying ECM. A shift in this cellular force balance, in either living or membrane-permeabilized cells, results in integrated changes in cell, cytoskeletal and nuclear form.

Materials and methods

Culture system

The capillary endothelial cells used in these studies were isolated from bovine adrenal cortex and maintained in culture as previously described (Ingber, 1990). Serum-deprived, confluent endothelial monolayers were dissociated into single cells by brief exposure to trypsin-EDTA (Gibco, Grand Island, NY), washed in Dulbecco's Modified Eagle's Medium containing 1% bovine serum albumin (BSA; Fraction V, Armour Pharmaceuticals, Tarrytown, NY), and cultured in chemically defined medium (Ingber, 1990) for approximately 3.5 h prior to experimental manipulation. Cells were plated at low density (5×10^3 cells/cm^2) onto 35 mm Petri dishes (no. 1008 plates; Falcon Labware, Oxnard, CA) or LabTek 8 well multichamber slides coated with a density of human serum FN (Boehringer-Mannheim Biochemicals, Indianapolis, IN) that we have shown to be saturating for cell attachment and spreading (2.5 μg/cm^2). Our methods for FN coating have been previously described in detail (Ingber and Folkman, 1989; Ingber, 1990).

Cell permeabilization-retraction system

The procedure we used for permeabilizing adherent endothelial cells and generating tension within cytoskeletal preparations was based on a modification of a previously published technique (Wysolmerski and Lagunoff, 1990). In brief, cells adherent to FN-coated dishes were washed once in a cytoskeletal stabilization buffer (50 mM KCl, 10 mM imidazole, 1 mM EGTA, 1 mM $MgSO_4$, 0.5 mM dithiothreitol, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 0.1 mM PMSF, and 20 mM PIPES, pH 6.5) which preserves contractile microfilaments in a functional form and then were incubated in the same buffer containing saponin (25 μg/ml; Sigma, St. Louis, MO) for 8 min at 37°C. In experiments in which quantitation of cell and nuclear size changes were carried out, 2 M glycerol was also included to ensure that the microtubular cytoskeleton was stabilized; similar results were obtained with both methods.

Cells were washed once in buffer without saponin and then retraction was induced by incubation in a tension-generation buffer (250 μM $CaCl_2$, 50 mM KCl, 2 mM $MgSO_4$, 1 mM EGTA, 0.5 mM dithiothreitol, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 0.1 mM PMSF, 2 M glycerol, and 25 mM PIPES, pH 7.0) with or without ATP (0-250 μM). In studies analyzing the role of actomyosin interactions, the synthetic myosin peptide, IRICRKG (50-250 μg/ml; synthesized using an Applied Biosystems Peptide Synthesizer) or the same concentration of a control peptide containing a single amino acid change (IRICEKG) was included in the ATP-containing retraction buffer. In experiments designed to define the role of integrins in shape control, the active FN peptide, GRGDSP, or inactive GRGESP (both 1.5 mM; Telios, La Jolla, CA), was added in the retraction buffer with or without ATP. In parallel studies comparing effects of detachment of living cells from FN-coated dishes, non-permeabilized cells were plated for 3.5 h on FN-coated dishes, washed once in PBS without calcium, and then exposed to a mixture of 0.05% trypsin/0.53 mM EDTA (Gibco). To examine the effects of preventing binding of integrins to immobilized FN in living cells, GRGDSP (0.03 mM) was included in the chemically defined medium during cell plating. This low concentration permits capillary endothelial cell attachment to FN but prevents subsequent cell spreading.

Morphological studies

For video analysis, cells were maintained at 37°C in retraction buffer using a SAGE air curtain incubator and images were recorded in real-time using a Nikon Diaphot inverted microscope in conjunction with a MTI television camera and a GYYR time-lapse video recorder. Photomicrographs were either taken off the television screen (when video recording was utilized) or directly on the photomicroscope. To better visualize changes in nuclear form and actin filament organization after experimental manipu-

lation, cells were fixed in formalin and stained sequentially with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI; 5 μg/ml; Molecular Bioprobes, Eugene, OR) and rhodaminated-phalloidin (200 nM; Sigma), which visualize DNA and F-actin, respectively. Images were recorded on Kodak Tri-X-Pan film using a Zeiss photomicroscope II equipped with epifluorescence optics.

For quantitation of changes in cell and nuclear size, permeabilized cells were incubated at 37°C under 10% CO_2 in a Forma Scientific culture incubator. After 10 to 30 min, cells were fixed in formalin, dried, and stained with Coomassie blue for cell size analysis. To visualize nuclear borders better for nuclear size measurements, cells were fixed in 1% paraformaldehyde/PBS for 15 min and washed sequentially with cold 10% glacial acetic acid in methanol for 10 min, water for 1 min, and 1 M NaOH for 1 min prior to staining in no. 3 Gill's Hematoxylin. Changes in cell and nuclear size (projected areas) were quantitated using computerized image analysis as previously described (Ingber, 1990); standard error was consistently less than 10% of the mean.

Results

Permeabilized cells and nuclei spontaneously retract in the presence of ATP

Studies were carried out with saponin-permeabilized endothelial cells to try to understand the biomechanical basis of dynamic cell shape changes, such as the spontaneous retraction and rounding of the cell and nucleus that occur when living cells are enzymatically released from their ECM attachments (Fig. 1a-d). Membrane permeabilization of spread cells adherent to FN resulted in loss of refractility but had little effect on cell form and, hence, both the cells and nuclei remained well extended when ATP was not present (Fig. 1e). In contrast, real-time video microscopic analysis revealed that spontaneous retraction of permeabilized cells and nuclei occurred within 10 to 30 min following addition of 250 μM ATP (Fig. 1e-h). Cell contraction was initiated by retraction of the cell periphery, with cytoplasmic and nuclear shrinkage following closely behind (Fig. 1e-h), as observed in living cells during trypsinization (Fig. 1a-d). The tight temporal and spatial coupling between cell and nuclear shape changes was confirmed using computerized image analysis (Fig. 2). The average projected cell and nuclear areas, respectively, decreased by 34 and 15% within 10 min following addition of ATP, with the maximal reduction in size (69% for cell area; 41% for nuclear area) being observed at 30 min.

A saturating concentration of ATP (250 μM) was used in our initial studies. However, dose-dependent stimulation was observed over a wide range of ATP concentrations in the micromolar range (Fig. 3). No retraction occurred in the absence of either ATP (Fig. 2) or calcium (not shown). The same permeabilization buffer has been previously shown to support cytoskeletal tension generation and partial retraction of cell borders within dense endothelial cell monolayers (Wysolmerski and Lagunoff, 1990). In our sparse cultures, some cells similarly underwent partial retraction whereas others became completely round (Fig. 4a).

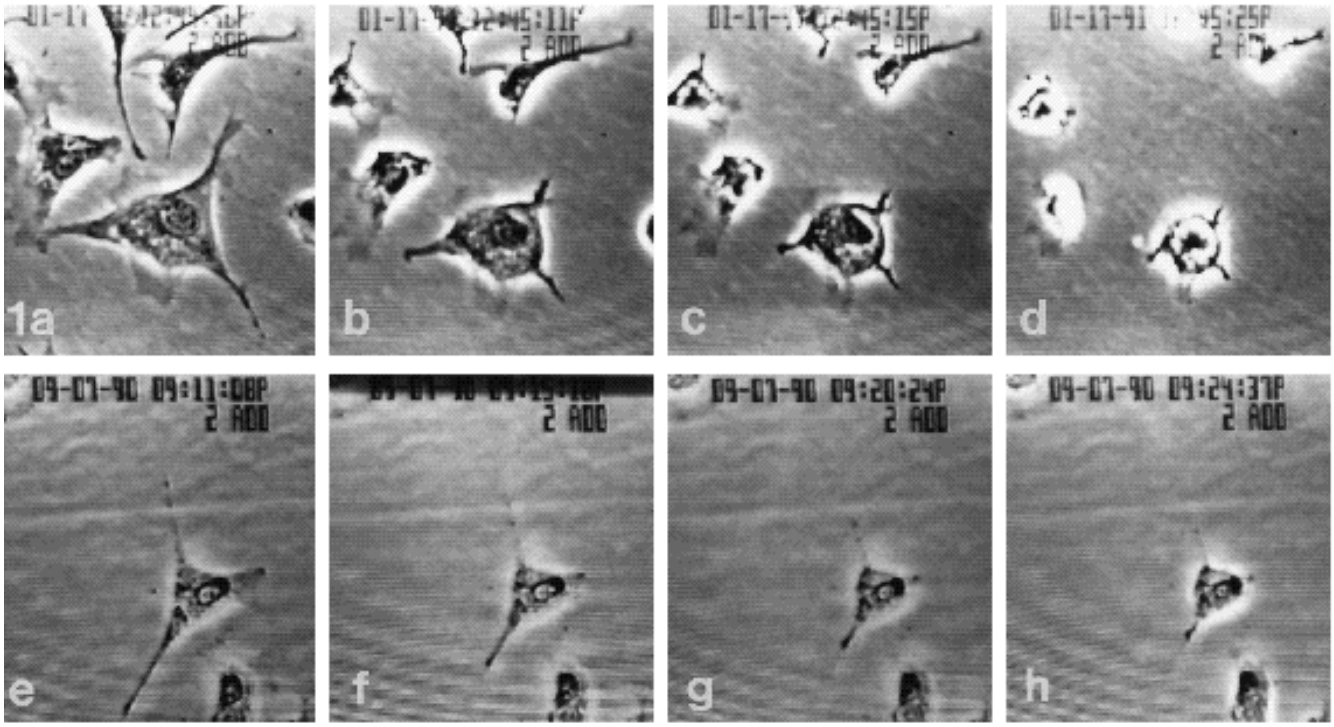

Fig. 1. Time-lapse video microscopic analysis of cell and nuclear retraction in intact (a-d) and membrane-permeabilized (e-h) capillary endothelial cells cultured on FN-coated dishes. (a-d) Adherent cells were washed in calcium-free PBS and exposed to a mixture of trypsin and EDTA. The extended cell processes pull back first, with cytoplasmic and nuclear retraction following closely behind. (e-h) Cytoskeletal tension was generated in membrane-permeabilized cells by addition of 250 μM ATP as described in Materials and methods. Similar coordinated cell and nuclear retraction is observed, although over a slower time course (13 min compared to 39 s).

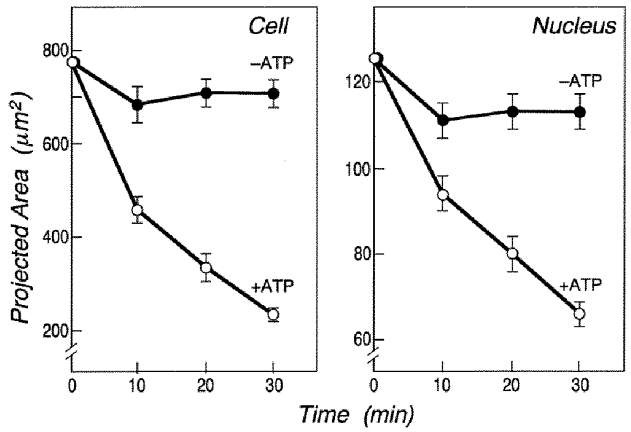

Fig. 2. Time-dependence of cell and nuclear shape changes in permeabilized cells. Cells adherent to FN were incubated for varying times in retraction buffer supplemented with (open circles) or without (closed circles) 250 μM ATP. Cell and nuclear size (project cell and nuclear areas, respectively) were quantitated using computerized image analysis.

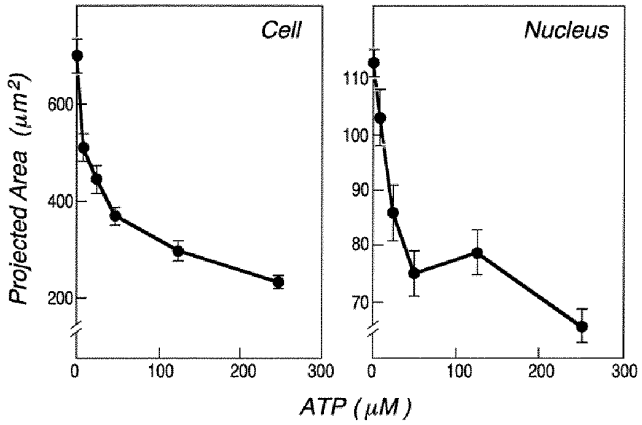

Fig. 3. Concentration-dependence of ATP-stimulated cell and nuclear retraction in permeabilized cells. Methods were as described for Fig. 2, except that the concentration of ATP was varied from 0 to 250 μM in the retraction buffer, and cell and nuclear areas were measured after 30 min.

A soluble myosin peptide inhibits tension generation and prevents shape control

The cell retraction that we observed in response to addition of ATP could be driven by increased cytoskeletal tension or by ATP-induced chemical modifications that result in filament breakage and cytoskeletal lattice disruption. Importantly, ATP-induced cell and nuclear rounding (Fig. 4a) were inhibited in permeabilized endothelial cells when a synthetic myosin heptapeptide, IRICRKG (250 μg/ml), was included in the retraction buffer (Fig. 4b). This peptide has been previously shown to inhibit actomyosin rigor complex formation and, hence, tension generation in cytoskeletal filaments isolated from skeletal muscle (Suzuki et al., 1987). Morphometric analysis confirmed that IRICRKG inhibited cell rounding by more than 55% and completely prevented nuclear retraction, even when added at concentrations as low as 100 μg/ml (Fig. 5). A control heptapeptide with a single amino acid change (IRICEKG) did not significantly inhibit cell or nuclear retraction at this concentration (Fig. 5).

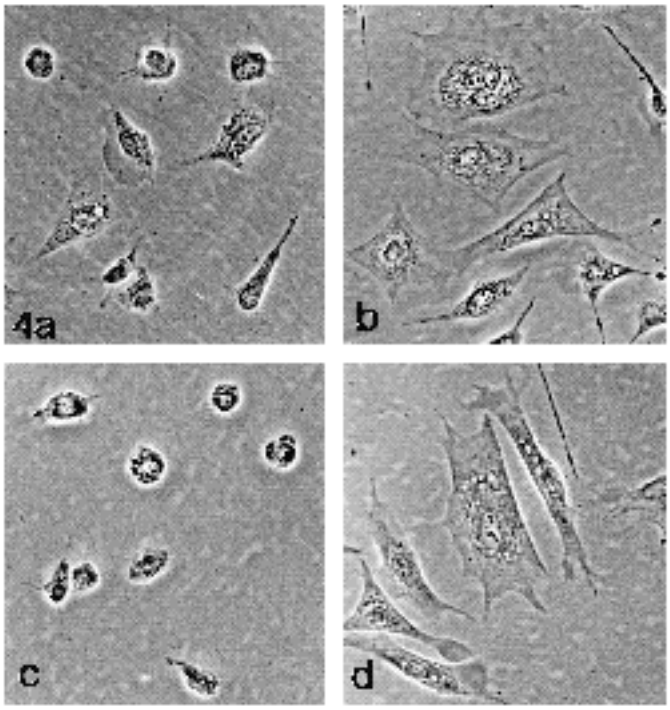

Fig. 4. Role of actomyosin interactions and cell-ECM adhesions during retraction of membrane-permeabilized cells.
(a) Permeabilized cells retract to varying degrees when exposed to retraction buffer containing 250 μM ATP. (b) Retraction was completely inhibited when the synthetic myosin peptide, IRICRKG, was included in the retraction buffer. (c) Addition of GRGDSP augmented cell retraction and rounding in buffer containing ATP. (d) GRGDSP had no effect when ATP was removed from the buffer. All photographs were taken 30 min after addition of ATP and/or peptide.

Disruption of integrin-FN contacts augments cell retraction but only in the presence of ATP

Our finding that permeabilized cells and nuclei retracted when cytoskeletal tension increased to high levels suggested that the cell's ECM attachments may play an important load-bearing function. The capillary endothelial cells we studied use integrins $\alpha_5\beta_1$ and $\alpha_V\beta_3$ to attach to immobilized FN (Schwartz et al., 1991a,b). Integrin β chains physically interconnect FN with contractile microfilaments via binding interactions with different actin-associated proteins (e.g. talin, vinculin, α-actinin) on the inner surface of the plasma membrane (Burridge, 1986). To determine whether ECM controls cell shape by resisting cytoskeletal tension that is transmitted across integrins, permeabilized cells were incubated in ATP-containing retraction buffer that was supplemented with the soluble FN hexapeptide, GRGDSP (1.5 mM). GRGDSP has been previously shown to induce rounding in living cells by dislodging integrin receptors from their RGD binding sites within immobilized FN (Hayman et al., 1985). Addition of

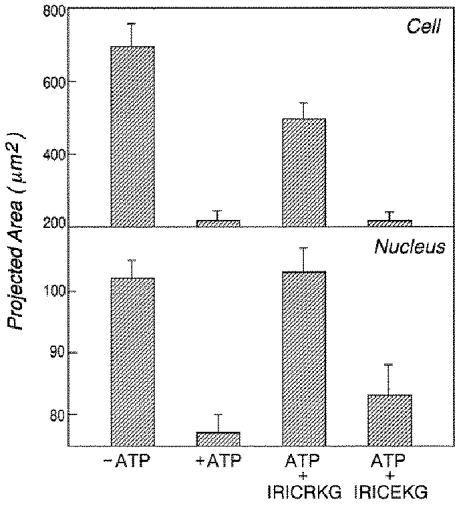

Fig. 5. Inhibition of ATP-induced cell and nuclear retraction using myosin peptides to inhibit actomyosin rigor complex formation. Permeabilized cells and nuclei were incubated for 30 min in retraction buffer with (+) or without (−) 250 μM ATP. The active myosin peptide (IRICRKG) and the control peptide (IRICEKG) were both used at 100 μg/ml in the same ATP-containing buffer. IRICRKG, but not IRICEKG, inhibited ATP-induced cell and nuclear retraction.

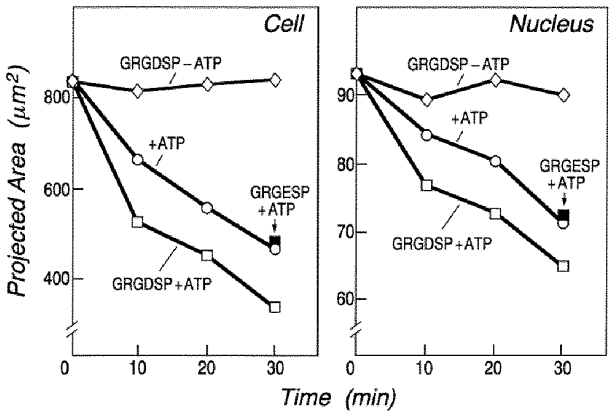

Fig. 6. Enhancement of the rate and extent of cell and nuclear retraction in permeabilized cells by disrupting integrin-FN contacts. Permeabilized cells were incubated for various times in retraction buffer supplemented with 250 μM ATP alone (open circles) or the same concentration of ATP supplemented with either GRGDSP (open squares) or GRGESP (closed squares; both at 1.5 mM). In one experiment, a similar concentration of GRGDSP was included in retraction buffer in the absence of ATP (open diamonds).

GRGDSP further increased the rate and extent of cell and nuclear rounding in permeabilized cells when included in the ATP-containing retraction buffer (Fig. 4c and 6). A similar hexapeptide with a single amino acid change (GRGESP) which does not interfere with FN-integrin binding (Hayman et al., 1985) did not significantly alter cell or nuclear form when added in the same buffer (Fig. 6). Interestingly, neither GRGDSP (Figs 4d and 6) nor trypsin-EDTA (not shown) had any effect on the shape of permeabilized cells or nuclei when added in the absence of ATP.

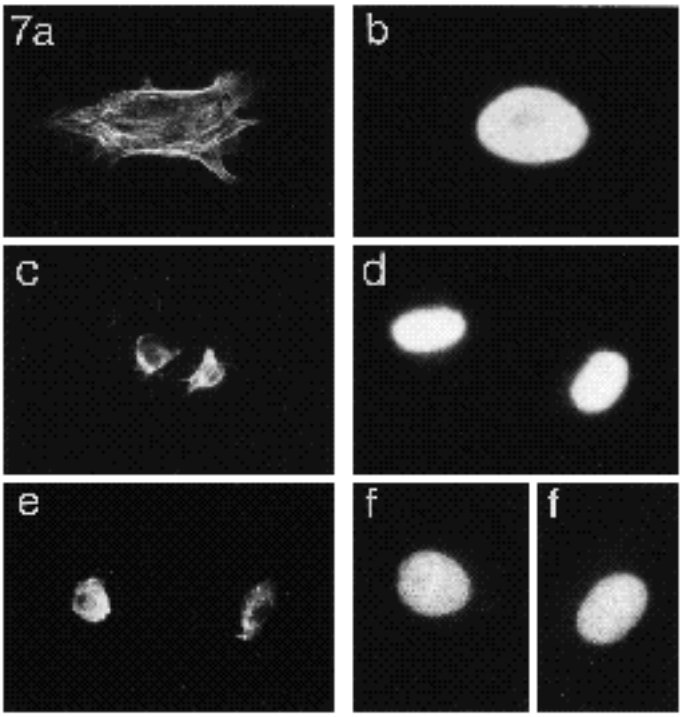

Fig. 7. Effects on actin bundle organization (a,c,e) and nuclear size (b,d,f) within individual endothelial cells. (a,b) Intact (non-permeabilized) cells adherent to FN. (c,d) Membrane-permeabilized cells exposed to retraction buffer containing 250 μM ATP. Note the absence of any residual actin staining in areas of the dish surface surrounding the retracted cells. (e,f) Intact (non-permeabilized) cells plated in the presence of soluble GRGDSP (0.03 mM). Identification of changes in nuclear size and F-actin distribution within the same cells was accomplished by sequential staining with DAPI and rhodaminated-phalloidin. The photographs of nuclei are printed at a magnification 3 times larger than those showing cellular F-actin.

Cytoskeletal tension drives cytoskeletal organization

Experiments using membrane-permeabilized endothelial cells also revealed that the well-developed actin bundles characteristic of spread cells (Fig. 7a) retracted and reorganized into a dense fibrillar net upon addition of 250 μM ATP (Fig. 7c). No residual actin staining was observed in regions on the culture dish surface surrounding the retracted cell, suggesting that increased cytoskeletal tension generation did not result in physical tearing of the actin cytoskeleton along the cell periphery. Fluorescence analysis of the same cells using DAPI confirmed that alteration of the actin filament lattice was also accompanied by nuclear retraction (Fig. 7b,d). Furthermore, living cells exhibited similar F-actin staining and small nuclei when they were grown in the presence of a low concentration of GRGDSP (Fig. 7e,f), which interferes with integrin binding to immobilized FN and thereby prevents the initial transfer of mechanical loads from the cytoskeleton to the ECM.

Discussion

Tension is generated in non-muscle cells via an actomyosin filament sliding mechanism

An important first step in understanding the mechanical basis of cell shape control by ECM would be direct demonstration of a requirement for cytoskeletal tension. To do this, we used conditions in which transmembrane osmotic pressure differences would not play a significant role. Specifically, we studied the mechanism of force generation in saponin-permeabilized cells. Capillary endothelial cells cultured on FN-coated dishes were utilized because their growth and differentiation are highly sensitive to shape modulation by ECM (Ingber et al., 1987; Ingber and Folkman, 1989; Ingber, 1990).

The permeabilization method we used has been previously shown to maintain the functional integrity of the actin cytoskeleton in endothelial monolayers, such that partial retraction of cell borders can be induced by the addition of calcium and ATP (Wyslomerski and Lagunoff, 1990). We have extended these studies by demonstrating that coordinated changes in cell and nuclear shape can be induced in microvascular endothelial cells cultured at a low plating density using a similar permeabilization/retraction protocol. In addition, we showed that cell and nuclear retraction induced by ATP can be inhibited using the active myosin peptide, IRICRKG, but not by a control peptide containing a single amino acid change (IRICEKG). The active peptide has been previously shown to compete for binding of the SH1 portion of myosin to F-actin and thereby to inhibit formation of the actomyosin rigor complex within cytoskeletal filaments isolated from skeletal muscle (Suzuki et al., 1987). Thus, the mechanical forces which are responsible for changes of cell and nuclear shape in endothelial cells appear to be generated by the same molecular binding interactions that mediate actomyosin filament translocation in muscle. This possibility is supported by the finding that retraction of permeabilized large vessel endothelial cell monolayers depends on myosin light-chain kinase phosphorylation of myosin light chains, as does smooth muscle contraction (Wyslomerski and Lagunoff, 1990).

Importantly, changes in cell and nuclear shape were also accompanied by restructuring of the actin cytoskeleton. The long actin bundles seen within normal extended cells became replaced by a condensed actin net which surrounded the retracted nucleus. This change in the distribution of actin filaments did not appear to be due to breakage or tearing of cell processes, rather it resulted from coordinated restructuring of the entire cytoskeletal lattice. These findings are consistent with past studies which show that cell rounding during trypsinization results from reorganization rather than depolymerization of F-actin (Bereiter-Hahn et al., 1990). Similar rapid cell rounding and disruption of actin filament bundles also result when stressed collagen gels containing adherent fibroblasts are released from their fixed attachments and allowed to relax (Mochitate et al., 1991).

Cell and nuclear shape changes result from modulation of a cellular force balance

In our experiments, we used a variety of methods to shift forces from the ECM onto internal cytoskeletal struts including enzymatic removal of adhesions (trypsin-EDTA), chemical competition for integrin binding sites (soluble GRGDSP), and increasing cytoskeletal tension above normal physiological levels (adding micromolar concentrations of ATP). Importantly, all of these techniques produced coordinated changes in cell, cytoskeletal and nuclear form, and similar effects were observed in living and permeabilized cells. In addition, we found that cytoskeletal tension must be applied to integrins in order for soluble GRGDSP or trypsin-EDTA to produce cell and nuclear retraction. In other words, changes in integrin occupancy alone (i.e. in the absence of cell tension) were not sufficient to induce shape changes.

Taken together, these data strongly suggest that ECM and integrins regulate cell shape based on a biomechanical mechanism, rather than one which is exclusively chemical in nature. While transmembrane pressure differences may be important during formation of local cell protrusions during migration (Bereiter-Hahn and Strohmeier, 1987; Oster and Perelson, 1987), our results with permeabilized cells clearly demonstrate that plasma membrane continuity is not necessary for global changes of cell form. Instead, cytoskeletal tension provides the driving force behind these shape changes. Specifically, cell and nuclear shape appear to be determined through a dynamic balance of mechanical forces that are generated within contractile microfilaments, transmitted across transmembrane integrin receptors, and resisted by immobilized RGD-binding sites within the ECM. When the balance of forces shifts such that mechanical loads are transferred from ECM contacts onto internal cytoskeletal elements, cell, cytoskeletal and nuclear retraction result. Conversely, inhibition of outward transfer of force (e.g. using soluble GRGDSP during plating) prevents cytoskeletal reorganization as well as cell and nuclear extension. All of these findings support the concept that integrins and ECM normally control both cell and nuclear shape by physically resisting cytoskeletal tension (Ingber et al., 1981; Ingber and Jamieson, 1985).

Tension as an integrator of cell, cytoskeletal and nuclear structure

How can a change in the mechanical force balance produce integrated changes in cell, cytoskeletal and nuclear form? To answer this question we must first understand how changes in mechanical parameters alter cytoskeletal organization. One possibility is that cytoskeletal reorganization is based on a "solation-contraction coupling" mechanism in which changes in actin network organization are *driven* by localized disruption of cytoskeletal gel structure (Kolega et al., 1991). Alternatively, it is possible that actin network disruption and reorganization may result from the action of increased cytoskeletal tension (i.e. "contraction-solation coupling"). It is difficult to discriminate between these two possibilities in living cells adherent to a substratum because they continually generate tension in their cytoskeleton and exist in a state of isometric tension (Harris et al., 1980; Ingber and Jamieson, 1985; Joshi et al., 1985).

While solation-contraction coupling may occur locally, our data with membrane permeabilized cells clearly show that increased cytoskeletal tension can drive actin network

reorganization and induce large-scale changes of cell shape, such as cell rounding. These results are consistent with the use of contraction-solation coupling and thus, with a thixotropic liquid crystal model of the cytoskeleton (Kerst et al., 1990). In this model, filament streaming results from the action of force on a filamentous lattice rather than from local changes in the solubility of individual components. While actin filaments were examined in this study, it is possible that other cytoskeletal filaments, such as microtubules or intermediate filaments, also bear some of this transferred load (Joshi et al., 1985; Danowski, 1989; Hollenbeck et al., 1989).

Force-induced restructuring of the cytoskeleton was accompanied by coordinated changes in nuclear shape in both living and membrane permeabilized cells. This observation is consistent with the concept that the nucleus is a dynamic structure and that it is structurally integrated with other elements of the cytoskeleton (Ingber and Jamieson, 1985; Pienta et al., 1991). While it is possible that the nucleus is passively compressed by a surrounding actin network, this mechanism is not supported by our data. A contractile actin shell of ever-decreasing diameter would be expected to first impinge on the most outermost protrusions of the extended nucleus before significant nuclear shrinkage would occur. Thus, loss of nuclear asymmetry (i.e. nuclear rounding) should result before significant decreases in nuclear diameter and inter-nucleolar spacings are observed. Instead, we observed coordinated shrinkage of the entire nucleus throughout the course of cell retraction with full rounding of the nucleus only being seen at later times (Fig. 1). It is possible that actin and myosin that are found in nuclei (Capco et al., 1982; Berrios and Fisher, 1986) could interact, generate centripetal tension in the presence of ATP, and thus actively promote nuclear retraction. However, the functional significance of nuclear actin and myosin remains to be demonstrated. Alternatively, studies with nucleated cell models that are built according to the rules of tensegrity (tensional integrity) architecture (Ingber and Jamieson, 1985) suggest that the nucleus may passively extend when the cell spreads due to redistribution of mechanical forces across structural interconnections that have been shown to interlink the nuclear matrix, cytoskeletal filaments and the cell surface (Wolosewick and Porter, 1979; Fey et al., 1984; Pienta et al., 1991). If cells use this type of tensional integrity system for structural integration then the "pre-stressed" cell and nucleus would be expected to retract spontaneously when the cell's anchors are released.

Our studies with both living and membrane-permeabilized cells similarly suggest that temporal and spatial coordination between cell and nuclear shape changes may be orchestrated through maintenance of structural and tensional continuity throughout the cell and nucleus. Thus, when cytoskeletal forces overcome the mechanical resistance of the ECM substratum, rapid and coordinated restructuring of the cell, cytoskeleton and nucleus results. Conversely, ECM molecules may promote extension of the entire cell by physically resisting tension that is transmitted throughout the cytoskeletal-nuclear matrix lattice and across integrin receptors, such that the net force vector shifts outward onto the attachment substratum. Utilization of this type of cellular force balance for shape control may explain why poorly adhesive rigid surfaces and highly adhesive malleable substrata both promote cell retraction and rounding rather than cell extension (Emerman and Pitelka, 1978; Harris et al., 1980; Ingber and Jamieson, 1985; Li et al., 1987; Ingber and Folkman, 1989; Opas, 1989; Ingber, 1990; Mochitate et al., 1991; Mooney et al., 1992).

Implications for development

In morphogenesis, ECM-dependent changes of cell and nuclear shape regulate cell sensitivity to soluble growth factors and thereby govern whether a cell will grow, differentiate, or involute in the local tissue microenvironment (Watt et al., 1988; Ingber and Folkman, 1989; Mooney et al., 1992). Past studies on control of tissue development focused on identification of specific molecules, receptors and chemical signaling pathways which mediate these effects. In contrast, the present work suggests that much of the pattern-regulating information that is transmitted to cell by the ECM may be physical in nature. Hence, the guiding role that the ECM plays during tissue development may depend on its mechanics as well as its chemistry. These findings also support the concept that integrins may provide a molecular conduit for force transmission across the cell surface as well as a mechanism for transduction of mechanical signals into a biochemical/cytoskeletal response (Ingber, 1991). Thus, complete understanding of the mechanism by which ECM molecules control cell form and function may not be obtained exclusively by chemical or molecular analysis. Elucidation of the basic principles of cellular architecture also will be required.

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