Mechanical distension modulates pulmonary alveolar epithelial phenotypic expression in vitro

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Mechanical distension modulates pulmonary alveolar epithelial phenotypic expression in vitro. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L196–L202, 1998.—The pulmonary alveolar epithelium is composed of two distinct types of cells, type I and type II cells, both of which are critical for normal lung function. On the basis of experiments of both nature and in vivo studies, it has been hypothesized that expression of the type I or type II phenotype is influenced by mechanical factors. We have investigated the effects of mechanical distension on the expression of specific markers for the type I and type II cell phenotypes in cultured alveolar epithelial cells. Rat alveolar type I cells were tonically mechanically distended in culture. Cells were analyzed for a marker for the type I phenotype (rTI40, an integral membrane protein specific for type I cells) and for markers for the type II phenotype [surfactant protein (SP) A, SP-B, and SP-C] as well as for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Mechanical distension caused a 68 ± 25% (n = 3) increase in mRNA content of rTI40 relative to undistended controls. In contrast, mechanical distension resulted in a decrease in mRNA content of SP-B to 35 ± 19% (n = 3) and of SP-C to 20 ± 6.7% (n = 3) of undistended controls. There was no effect on mRNA content of SP-A or GAPDH. The differences in mRNA content of SP-B and SP-C were found to be primarily due to changes at the transcriptional level by nuclear run-on assays. The effects on rTI40 appear to be due to posttranscriptional events. These data show that mechanical distension influences alveolar epithelial phenotypic expression in vitro, at least in part, at the transcriptional level.

type II cell; type I cell; mechanical stretch; gene expression

The alveolar epithelium is composed of two morphologically distinct types of cells. Type I cells cover >95% of the alveolar surface, providing both the tight barrier and the short diffusion pathway between the air and blood components that are essential for efficient gas exchange. Type II cells synthesize, secrete (8), and recycle (39) surfactant components, which are responsible for lowering surface tension at the alveolar air-liquid interface, preventing alveolar collapse. Type II cells also produce immune effector molecules (36), transport ions (21), and act as stem cells in alveolar repair after injury (12).

The development and maintenance of normal alveolar epithelial phenotype are felt to be critical for normal lung function. Factors that may be involved in the regulation of expression of differentiated alveolar epithelial phenotypes include cell shape (34, 35), extracellular matrix (35), cell-to-cell interactions (35), growth factors (2), and soluble factors (29). It has been suggested also that mechanical factors may be important in modulating alveolar epithelial phenotypic expression. Experiments with fetal model systems have shown that mechanical forces play an important role in regulating lung growth (24) and suggested that these forces may be involved in determining alveolar epithelial phenotype (1). Mechanical factors also affect alveolar epithelial cells in the mature lung (22). Mechanical distension is felt to be critical for compensatory lung growth after a partial pneumonectomy; stretch-induced changes during this process result in an increase in the cellular content of adenosine 3′,5′-cyclic monophosphate (cAMP) and activation of cAMP-dependent protein kinase in vivo (33).

Despite in vivo evidence that mechanical forces play an important role in both the developing as well as the mature lung (1, 22, 33), there is little information about how mechanical factors affect alveolar epithelial phenotypic expression in vitro. This report represents the first description of the effects of mechanical distension on the expression of molecular markers for the type I and type II cell phenotypes in vitro. Our results suggest that, in our model system, mechanical forces are potent regulators of alveolar epithelial phenotypic expression.

METHODS

Isolation of alveolar type II cells. Alveolar type II cells were isolated from the lungs of pathogen-free adult male Sprague-Dawley rats weighing 180–200 g (Charles River, Hollister, CA) by previously described methods (7). Porcine pancreatic elastase was purchased from Boehringer Mannheim (Indianapolis, IN). We found that with some lots of elastase, preparations of type II cells contained 1–10% type I cells as contaminants. To remove type I cells from type II cell preparations, we used a depletion strategy utilizing a monoclonal antibody against rTI40 (11), an apical membrane protein of the type I cell, and goat anti-mouse immunoglobulin G coupled to magnetic beads (Miltenyi Biotec, Auburn, CA). Cells were separated by sorting through magnetic columns. This method yielded populations of cells that were 92 ± 2% (n = 6) type II cells, containing <0.5% type I cells by indirect immunofluorescence. Reverse transcription-polymerase chain reaction (RT-PCR) and Southern blot analysis for rTI40 in preparations depleted of type I cells were negative.

Cell culture and mechanical distension. Cells were cultured on elastic membranes and distended by previously described methods (38). Briefly, type II cells were cultured for 20–24 h on circular silicone membrane dishes coated with fibronectin in Dulbecco’s modified Eagle’s medium (GIBCO BRL, Gaithersburg, MD) containing 5% fetal bovine serum, 100 U penicillin/ml, and 10 µg gentamicin sulfate/ml (all from Univ. of California, San Francisco, Cell Culture Facility). Membranes were placed in stretching devices that provided space for two groups of three membranes per device. Each group of three membranes can be stretched independently, allowing three stretched membranes to be compared with three unstretched membranes under equivalent conditions in the same stretching device. Membranes were placed onto a...
porous base overlying a fluid-filled chamber. Membranes were held in place by an acrylic top plate with round borings forming the wells. One-half of the membranes in each chamber were distended by applying hydrostatic pressure beneath the membranes (Fig. 1). The amount of distension was controlled by the volume of fluid added. Changes in two-dimensional cellular surface area previously had been correlated with volume of fluid added to the same system (38). In the current experiment, we used a distending volume that resulted in a 21% increase in cellular surface area. Membranes were maintained in the distended state for 18 h. Maintenance of distension was assessed by evaluating the devices for leaks as well as the amount of fluid removal required to return the membranes to their relaxed state. After the 18-h experimental period, tissue culture medium was removed and membranes were washed twice with sterile phosphate-buffered saline at 4°C. Cells were harvested and samples were processed as described below.

Preparation of RNA, RT-PCR, Southern blotting, and hybridization. Total cellular RNA was extracted and isolated using RNA-STAT (Tel-Test, Friendswood, TX). To obtain sufficient amounts of RNA for accurate quantitation and analysis of four genes by RT-PCR, in each experiment we pooled cells from three membranes. The amount of total recovered RNA varied <15% from sample to sample and between groups. The concentration of each sample was adjusted to 0.125 μg RNA/μl with diethyl pyrocarbonate-treated water, 0.5 μg of RNA was loaded onto a 1% agarose gel, and the RNA was fractionated by electrophoresis. Ethidium bromide staining showed that lanes contained recovered RNA varied.

Fig. 1. Stretch device. Type II cells were cultured on circular silicone membranes. Membranes were placed in stretch devices onto a porous base overlying a fluid-filled chamber. Membranes were held in place by an acrylic top with round borings forming wells. Membranes were distended by applying hydrostatic pressure beneath membranes. Amount of distension was controlled by volume of fluid added.

Fig. 2. Evaluation of total RNA. Total cellular RNA was extracted as described in Methods. RNA (0.5 μg) from each sample was size fractionated by electrophoresis through 1% agarose gels and stained with ethidium bromide, and the 18S RNA was visualized using ultraviolet light (A). RNA was transferred to Nytran filters. Filters were probed with [32P]dCTP-labeled cDNA for 18S RNA before undergoing autoradiography (B). In each, first 2 lanes represent control samples and second 2 lanes represent stretch samples.

blastosis virus (Boehringer Mannheim) in the presence of 10X PCR buffer (Perkin-Elmer, Branchburg, NJ) and all four deoxynucleotides to convert mRNA to cDNA. The cDNA was then amplified using Taq polymerase (Perkin-Elmer) in the presence of the above reaction mixture. The following specific oligonucleotide PCR primers were used to amplify the genes of interest (Biomedical Resource Center, San Francisco, CA): RTI40:5'-GCCATC GGT GCG CTA GAA GAT GAT-3' (identical to bases 53–80), 5'-GTG ATC GTG GTC GGA GGT TCC TGA GGT-3' (complementary to bases 201–257); SP-A: 5'-TTT CCA GCT TAC CTG GAT GAG G-3' (identical to bases 13–25), 5'-GGG TCA TGG TCT TCA ATC ATG C-3' (complementary to bases 301–323); SP-B: 5'-AAT GAC CTG TGC CAA GAG TGT G-3' (identical to bases 196–218), 5'-AGG ACC AGG TTC CCT GGA GCA GCT G-3' (identical to bases 509–531); SP-C: 5'-GTG GTT GTG GTG GTA GTG CTT G-3' (identical to bases 127–149), 5'-TAG CAG TAG GTT CCT GGA GCA GCT G-3' (complementary to bases 380–402); GAPDH: 5'-GAG AAG ATG GTG AAG GTC GG-3' (identical to bases 25–44), 5'-CAT GGA CTG TGG TCA TGA GC-3' (complementary to bases 543–562). The number of amplification cycles was determined by evaluating samples subjected to serial amplifications to determine the linear range for each target cDNA and then choosing an amplification number within the linear range (12 for RTI40; 24 for SP-A, SP-B, and GAPDH; and 30 for SP-C).

PCR products were separated by electrophoresis through 2% agarose gels, stained with ethidium bromide, and visualized with ultraviolet light. The cDNA was then transferred to Nytran filters by capillary action. Filters were probed with the corresponding full-length cDNAs for rat RTI40, SP-A, SP-B, SP-C, and GAPDH. All cDNA inserts were excised intact from their vectors with an appropriate restriction enzyme (Boehringer Mannheim), purified by electrophoresis through an agarose gel, and then labeled with [α-32P]dCTP (NEN Research Products, Boston, MA) by random-primer second-strand synthesis using Random Primer Labeling Kit (GIBCO BRL). Unincorporated nucleotides were removed using a NucTrap Probe Purification column (Stratagene). Filters were prehybridized for 10 min in QuikHybe hybridization solution (Stratagene) at 68°C. Hybridization was performed in 10 ml of QuikHybe solution containing 1.25 × 106 disintegrations·min⁻¹·dpm⁻¹·ml⁻¹ for 18 h at 68°C. Hybridized filters were washed three times with a solution of 2× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0)–0.1% sodium dodecyl sulfate (SDS) at 25°C for 15 min and once with a solution of 0.1× SSC–0.1% SDS at 60°C for 30
Isolation of nuclei and run-on transcription assay. Cells were removed from membranes by scraping membranes twice in phosphate-buffered saline; pelleted by centrifugation in a tabletop centrifuge at 500 rpm. Nuclei were pelleted by centrifugation at 500 g for 5 min. RNA was extracted a second time with phenol-chloroform-isooamyl alcohol. Unincorporated nucleotides were removed from membranes by scraping membranes twice in 5% tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 10 mM NaCl, 3 mM MgCl\(_2\), and 0.1% Nonidet P-40 (Pharmacia). Nuclei were pelleted by centrifugation at 500 g for 5 min at 4°C. Pellets containing nuclei were suspended in a solution of 50 mM Tris-HCl, 40% glycerol, 5 mM MgCl\(_2\), and 0.1 mM EDTA (all purchased from Sigma). Nuclei were centrifuged again at 500 g at 4°C and resuspended in 100 μl of the same buffer. Nuclei were used immediately. Extracts from twelve cultured membranes yielding a total of 10–12 × 10\(^6\) nuclei were processed for each condition in the run-on transcription assays.

Nuclei were added to 100 μl of a reaction buffer containing 10 mM Tris-HCl, 5 mM MgCl\(_2\), 300 mM KCl (all from Sigma), 10 mM ATP, 10 mM CTP, 10 mM GTP, 0.1 mM cold UTP (all from Boehringer Mannheim), 10 mM dithiothreitol (Pro-mega), 10 U RNasin (GIBCO BRL), and 200 U of [α-32P]UTP (NEN). After incubation at 25°C for 20 min, nuclei were placed at 4°C, and 5 units of ribonuclease-free deoxyribonuclease I (GIBCO BRL) and 10 μl of 20 mM CaCl\(_2\) were added to the mixture. Samples were mixed by gentle shaking and incubated at 37°C for 30 min. Forty units of protease K (Boehringer Mannheim) were added, and nuclei were lysed in 5% SDS, 50 mM EDTA, and 100 mM Tris-HCl. Fifty micrograms of yeast tRNA (GIBCO BRL) were added to the lysate as a carrier, and samples were mixed and allowed to incubate at 37°C for 30 min. RNA was extracted by incubating samples in 5% β-mercaptoethanol, 0.2% sarcosyl, 2M sodium acetate, and acidic phenol and chloroform-isoamyl alcohol (24:1) on ice for 15 min before centrifugation at 14,000 revolutions/min for 5 min. RNA was extracted a second time with phenol-chloroform-isoamyl alcohol. Unincorporated nucleotides were removed using a Centricon 100 concentrator (Amicon, Beverly, MA).

Equal amounts of radioactive elongated RNA (2–5 × 10\(^6\) dpm) were hybridized to 5 μg of linearized, denatured plasmids containing cDNA inserts for rTI40, SP-A, SP-B, or SP-C or control plasmid DNA with no insert that had been spotted onto Nytran filters (Schleicher and Schuell), using a vacuum slot blower (Schleicher and Schuell). Filters were prehybridized for 2 h at 65°C in a 15-ml centrifuge tube (Corning) containing 5 ml of a solution of 10× Denhardt’s solution (1× Denhardt’s is 0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), 6× SSPE (1× SSPE is 0.15 M NaCl, 0.01 M NaHPO\(_4\), and 0.001 M EDTA, pH 7.4), 1% SDS, and 10 μg salmon sperm DNA/ml. Hybridization was performed using the same solution with the addition of 5% dextran sulfate containing labeled RNA for 20 h at 65°C. Hybridized filters were washed once with 0.1% SSPE-1% SDS for 15 min at 25°C and then twice with the same solution at 65°C. Filters were then subjected to autoradiography as described above; quantitation of radioactivity was performed by volume integration, using phosphorimage analysis (Imagequant, Molecular Dynamics).

Statistical analysis. Results are expressed as the percent change from unstretched controls, means ± SD of 3 experiments, each with a different preparation of cells. The effects of mechanical distension on type II cells were compared with those of undistended controls by the Wilcoxon signed rank test. A value of P < 0.05 was considered statistically significant. Controls varied from experiment to experiment by <25%.

RESULTS

Distension of type II cells causes an increase in the mRNA content of rTI40. RNA isolated from cells undergoing mechanical distension and subjected to RT-PCR for rTI40 yielded a 68 ± 25% (n = 3, P < 0.05) increase in mRNA for rTI40 compared with mRNA obtained from control cells cultured in parallel in the same device but not subjected to mechanical distension (Fig. 3). The content of mRNA was quantitated by Southern blot and phosphorimage analyses of RT-PCR products. These findings support the hypothesis that mechanical distension favors expression of the type I phenotype.

Distension of type II cells causes a decrease in the mRNA content of SP-B and SP-C. In contrast to the effects of mechanical distension on rTI40 mRNA, mechanical distension caused a reduction in the expression of mRNA for two of the markers of the type II phenotype. Southern blot and phosphorimage analyses of mRNA subjected to 24 cycles of amplification by RT-PCR for SP-B showed that mechanical distension resulted in a decrease in mRNA content for SP-B to 35 ± 19% (n = 3, P < 0.005) of control (undistended) cells cultured in parallel chambers of the same device. Similar analysis of RT-PCR products after 30 cycles of amplification for SP-C revealed a decrease in mRNA content for SP-C to 20 ± 6.7% (n = 3, P < 0.005) of control (undistended) cells (Fig. 4).

Distension of type II cells has no effect on the mRNA content of SP-A and GAPDH. A third marker of the type II phenotype, SP-A, was unaffected by mechanical
Mechanical distension modulates lung phenotypic expression

The lung is a dynamic organ in which volume changes regularly, both with normal tidal breathing and with periodic larger breaths (sighs). Although it remains controversial whether individual alveolar cells are stretched or unfolded in vivo, it has been suggested that mechanical factors may be important in modulating alveolar epithelial phenotypic expression. Information regarding the relationship between mechanical factors and alveolar epithelial phenotypic expression has been largely derived from studies with fetal model systems (1). These experiments support the concept that mechanical forces influence expression of alveolar cell phenotype during lung development. In studies performed on fetal sheep lungs in utero, maintaining the fetal lung in an overstretched state by tracheal ligation subjectively favored the expression of the type I phenotype while inhibiting the expression of the type II phenotype; underdistension of fetal lung in utero by chronic tracheal drainage has the opposite effect (1). However, none of these reports provided quantitative data regarding numbers of type I and type II cells or biochemical data supporting these subjective conclusions. The effects of mechanical forces on alveolar phenotypic expression in adult lung remain unexplored.

The content of SP-A mRNA after 24 cycles of RT-PCR was not significantly different when RNA prepared from stretched cells was used in comparison with RNA from unstretched control cells (Fig. 4). Although all conditions of RT-PCR were selected to be in the linear range, to be sure that differences between groups were not obscured by overamplification, we amplified samples for 6, 12, 18, 24, and 30 cycles; there were no differences in SP-A mRNA content between distended or unstretched controls under any of these RT-PCR conditions (data not shown).

Aliquots of RNA were also amplified for GAPDH. Mechanical distension did not result in a significant change in the mRNA content of GAPDH, and it appears that GAPDH is constitutively expressed under both stretched and control conditions. GAPDH was therefore used as a control to ensure quantitative recovery of cDNA following reverse transcription.

Nuclear run-on transcription assays. To determine whether the effects of mechanical distension on the content of specific mRNAs were due to transcriptional or posttranscriptional changes, we performed nuclear run-on assays. The results are shown in Fig. 5, which summarizes the results and shows the amount of radioactivity for each condition as quantitated by phosphorimaging analysis. Mechanical distension caused a very small (3.3%) difference in the level of transcription for rTI40; this difference is not statistically significantly different from that of unstretched control cells. In contrast, there was a marked decrease in the transcription of SP-B and SP-C. The level of transcription of SP-B was decreased to 47 ± 6% (n = 3, P < 0.05) of that of control unstretched cells, and the level of transcription of SP-C was decreased to 25 ± 13% (n = 3, P < 0.05) of that of controls. There were no observed differences in the levels of transcription of SP-A between nuclei obtained from stretched cells subjected to distension relative to nuclei obtained from control cells (data not shown).

Together, these data show that the decrease in mRNA content of SP-B and SP-C may be due to changes at the transcriptional level. It is likely that at least some of the changes in the mRNA content of rTI40 are due to posttranscriptional events.

Discussion

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In the present study, cultures of type II cells were subjected to 18 h of tonic mechanical distension. Cells were distended after only 20–24 h in culture to allow adherence to the membranes while minimizing the loss of differentiated function known to occur when type II cells are cultured on a substratum that promotes spreading. The amount of distension chosen for this study results in an increase in cell surface area of 21% and is known to stimulate calcium mobilization and surfactant secretion in type II cells in vitro (38). We used rTI40, a gene that encodes a protein that is localized by immunochemical methods to the apical plasma membrane of type I cells (11), as a marker for the differentiated type I cell phenotype. This gene is identical to OTS-8, a sequence isolated from transformed mouse osteoblastic cells (26), and has also been called T1α after its cloning and characterization from rat lung (32). Although the function of the protein encoded by this gene remains unknown, rTI40 has proven to be a useful marker of the extent of injury to the alveolar epithelium (23). SP-A, -B, and -C were used as markers of the differentiated type II cell phenotype. Mechanical distension of type II cells for 18 h resulted in a 68% increase in the mRNA content of a marker of the type I phenotype and a decrease in the mRNA content of two of the markers of the type II phenotype to 20% (SP-C) and 35% (SP-B) of control. Interestingly, there was no observed effect on mRNA content of SP-A, another, although less specific than SP-C, marker of the type II phenotype. This apparent coregulation of SP-B and SP-C expression, both different from SP-A expression, has been observed previously. For example, in the developing lung, agents that increase intracellular cAMP concentration cause an increase in SP-A mRNA but have only a modest effect on mRNA content of SP-B and SP-C (18, 27). In human fetal lung tissue in vitro, glucocorticoids exert a marked stimulatory effect on the levels of SP-B and SP-C mRNAs (28, 37) while exerting both stimulatory (at low concentrations) and inhibitory (at high concentrations) effects on the levels of SP-A mRNA (2). In our system, mechanical distension favors the type I phenotype while inhibiting two of the markers of the type II phenotype. These findings are consistent with studies with other model systems (34, 35) in which there is inverse coregulation of the expression of the type I and type II phenotypes (3). We do not know whether the observed changes reflect alterations in cells already expressing the mRNAs of interest and/or reflect a change in the percentage of cells expressing these mRNAs.

The observed differences in mRNAs appear to be due to changes at both the transcriptional and posttranscriptional levels. Mechanical distension resulted in a decrease in newly transcribed mRNA for SP-B to 45% of control and a decrease for SP-C to 25% of control values by nuclear run-on transcription assays. Although we have not directly measured mRNA stability, the differences in transcriptional rates of SP-B and SP-C suggest that the observed differences are regulated by transcriptional events. There was a small increase observed in the transcriptional rate of rTI40. The differences in mRNA content for rTI40 may therefore occur primarily at the posttranscriptional level, although larger initial changes in transcription, as well as in mRNA content, may have been missed by assessing only transcriptional rates at the end of the study period.

These data demonstrate that mechanical distension influences alveolar epithelial phenotypic expression in vitro. Previously, the expression of markers of alveolar epithelial phenotype was felt to be influenced primarily by cell shape (34, 35), extracellular matrix (35), and hormones (2, 29). Type II cells cultured on plastic and allowed to flatten and spread in some aspects morphologically resemble type I cells. We have previously reported that type II cells cultured on tissue culture plastic for 4 days express increasing amounts of rTI40 (11). In contrast, these cells contain decreased content of mRNAs for SPs (35) and have a diminished capability to synthesize phospholipids (20). Type II cells cultured on an Engelbreth-Holm-Swarm tumor basement membrane and floating collagen gels remain cuboidal and retain more characteristics of differentiated type II cells (34). In a previous study, we demonstrated that both single and multiple mechanical stretches-relaxations applied to cultured type II cells first stimulate calcium mobilization and then surfactant secretion (38), providing direct evidence that type II cells in culture respond to mechanical stimuli. In the present study, we have provided evidence of a direct link between mechanical forces and regulation of the genes for the markers of differentiated alveolar cell phenotype.

These findings provide another example of the important role mechanical forces play in modulating gene expression in various biological systems. One of the best studied systems of physical forces and cell phenotypic expression is that of skeletal muscle (14). Both gene expression and muscle fiber type appear to be markedly affected by stretch and force generation. Mechanical loads on cultured cells can have dramatic effects on gene regulation. Shear stress stimulates the expression of mRNAs for platelet-derived growth factor A and platelet-derived growth factor B (30, 17), tissue plasminogen activator (5), and intracellular adhesion molecule-1 in human vascular endothelial cells (25). Mechanical stretching also causes an increase in the amount of mRNA for atrial natriuretic factor in neonatal rat cardiac myocytes (13). The recent identification of stress-responsive elements in the promoter regions of some genes has provided a possible direct link between physical force and gene expression (31). In endothelial cells, one pathway by which shear stress regulates gene expression is the binding of transcription factors to a specific 6- to 12-base pair "shear-stress response element" (SSRE) found upstream from the start site of shear-stress-sensitive genes (30). The 6-base pair SSRE occurs four times in the 5' flanking region of rTI40 and once in the 5' flanking region of SP-C, suggesting that good candidates for mechanosensitive response elements exist in both of these genes. The SSRE is not, however, involved in the regulation of
some mechanically sensitive genes (16), suggesting that other mechanisms also may exist.

The objective of the present study was to determine the effects of mechanical distension on the phenotypic expression of markers of pulmonary alveolar cell differentiation. Although prior studies of fetal lung development in vivo support the concept that mechanical factors affect both growth and phenotypic expression of alveolar epithelium, the relevance of our observations in vitro to lung development is uncertain because of the limitations of our model system. For the current studies, we used type II cells in primary culture, an accepted model for many studies of type II cell functions such as surfactant synthesis, secretion, and rep-take (39) and ion transport (21, 15). We used type II cells cultured for 40 h in these studies. Type II cells in primary culture gradually (over days) cease expressing markers of the type II cell phenotype (6, 19) and express markers of the type I phenotype (4, 10). It is not currently known whether these cells in transition are phenotypically more similar to type II cells, type I cells, an intermediate II-I cell type, or a putative alveolar epithelial stem cell. Although the type II phenotype can be better preserved by culturing type II cells on different matrices or with different apical surface conditions (9, 34), we have found that we cannot apply mechanical forces to cells cultured in this fashion. Despite these considerations, the data presented in this report support the hypothesis that mechanical distension influences alveolar epithelial phenotypic expression in vitro. Both transcriptional and posttranscriptional mechanisms appear to be involved. These findings provide the first biochemical support linking mechanical forces and the regulation of alveolar epithelial phenotypic expression.

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