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N. A. Hasaneen, S. Zucker, R. Z. Lin, G. G. Vaday, R. A. Panettieri and H. D. Foda
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Mechanical force-induced signal transduction in lung cells

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Liu, Mingyao, A. Keith Tanswell, and Martin Post. Mechanical force-induced signal transduction in lung cells. *Am. J. Physiol.* 277 (*Lung Cell. Mol. Physiol.* 21): L667–L683, 1999.—The lung is a unique organ in that it is exposed to physical forces derived from breathing, blood flow, and surface tension throughout life. Over the past decade, significant progress has been made at the cellular and molecular levels regarding the mechanisms by which physical forces affect lung morphogenesis, function, and metabolism. With the use of newly developed devices, mechanical forces have been applied to a variety of lung cells including fetal lung cells, adult alveolar epithelial cells, fibroblasts, airway epithelial and smooth muscle cells, pulmonary endothelial and smooth muscle cells, and mesothelial cells. These studies have led to new insights into how cells sense mechanical stimulation, transmit signals intra- and intercellularly, and regulate gene expression at the transcriptional and posttranscriptional levels. These advances have significantly increased our understanding of the process of mechanotransduction in lung cells. Further investigation in this exciting research field will facilitate our understanding of pulmonary physiology and pathophysiology at the cellular and molecular levels.

physical force; mechanical stimulation; cell proliferation; cell differentiation

PHYSICAL FORCES play an important role in regulating the structure, function, and metabolism of the lung (97). Abnormal physical forces exerted on lung tissues contribute to many pathological situations. A better understanding of how physical forces act on lung cells may help us design strategies in the treatment and prevention of physical force-related disorders such as pulmonary hypoplasia, barotrauma, pulmonary hypertension, asthma, and chronic obstructive pulmonary diseases. Ten years ago, the Division of Lung Diseases of the National Heart, Lung, and Blood Institute (National Institutes of Health, Bethesda, MD) sponsored a workshop that stressed the need to study the effects of physical forces on the lung at the cellular and molecular levels (97). It is our intention to summarize the subsequent progress and current knowledge of mechanotransduction and mechanoreception in lung cells.

Physical forces are regulators of cell proliferation and differentiation in a variety of mammalian cells and have been the subject of several excellent reviews (21, 43, 100). Most of these review articles focused on a particular organ system, e.g., cellular and molecular responses of cardiac myocytes to mechanical stretch

(100), hemodynamic forces as regulators for endothelial gene expression (94), and mechanotransduction in vascular smooth muscle (83), bone (135), and endothelial cells (20, 21). In this review, we discuss investigations in other organ systems only as they illustrate potential approaches for future studies on lung cells.

The lung is a unique organ in terms of its anatomic structure and physiological role. The lung is subjected to several complex physical forces including breathing, pulmonary blood flow, and surface tension. Breathing is a unique feature of the lung. "Breathing movements" can be observed by sonography in the human fetus as early as 10 wk of gestation. After birth, the pulmonary vascular system has a very low resistance. The lung receives the same blood flow as the whole systemic circulation. Blood flow comes from both pulmonary and bronchial arteries. These blood vessels are subjected to both shear stress and pressure-generated stretch from the blood flow. The pressure in the pulmonary arteries is normally much lower than that in the arteries of other organs. Furthermore, the sum of the stresses experienced by the pulmonary vascular system is heavily influenced by changes in lung volume during

respiration. Another unique feature of the physical forces in the lung is the shear stress and pressure of the airflow exerted on the airway. Surface tension formed at the air-liquid interface also affects compliance of the lung (80) and resistance of the small airways (65). The lung shares with the heart the thoracic space, and the physical forces derived from the beating heart may influence the function and metabolism of the lung, especially during fetal development.

From clinical observations and physiological studies, it has been recognized that the structure, function, and metabolism of lung cells are influenced by physical forces (97). For instance, it is well documented that fetal lung growth and maturation are regulated by physical factors (34, 36, 47, 48). Physical forces also influence lung growth after birth (156) and cell proliferation and differentiation during adulthood. For instance, lung inflation initiates growth of the lung after pneumonectomy (89). The pattern of breathing and mechanical ventilation affects pulmonary surfactant synthesis and secretion (13). Physical forces may also contribute to the vasculogenesis and angiogenesis of pulmonary blood vessels (96) and branching of the airways during lung development.

Inappropriate physical forces are associated with many pathophysiological conditions. Excessive mechanical ventilation may induce lung injury at the cellular level (23, 72). Physical factors such as the inflation status during lung preservation (22), the initial reperfusion rate, and the ventilation pattern affect the function of transplanted lung (86). Chronic airway obstruction contributes to airway smooth muscle hyperplasia and hypertrophy. Increased physical forces are also involved in the development of pulmonary hypertension (74, 138). Herein, we review the literature pertaining to studies carried out at the cellular and molecular levels. We first briefly review the models that have been used to manipulate lung cells *in vitro* and the major effects of physical forces on lung cells. We then review the possible mechanisms by which cells can sense mechanical stimulation, the pathways that trans-

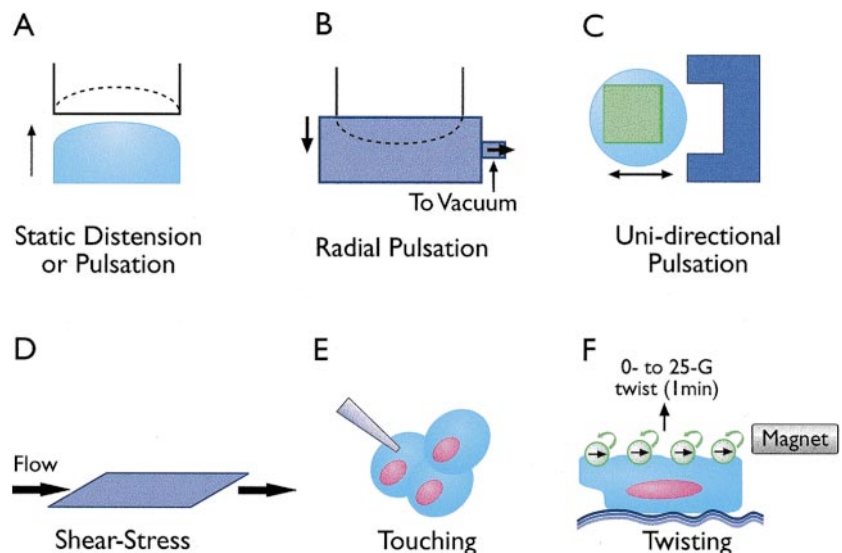
mit the signals initiated by physical forces, and the regulatory effects that physical force-initiated signals have at various levels of cellular metabolism and mechanical stress-initiated intercellular communication.

EXPERIMENTAL METHODS FOR APPLYING PHYSICAL FORCES TO LUNG CELLS

Although it is evident that physical forces are important regulators of lung formation, function, and metabolism, studies at the cellular and molecular levels have been limited by the lack of suitable tools to simulate various physical forces on cells *in vitro*. Over the past decade, several devices have been developed to apply various types of forces to lung cells, some of which have become commercially available.

In principle, the physical forces applied to cells should be quantified in specific physical terms such as stress, strain, and shear stress. However, because of the relatively small size of an individual cell and the complexity of cellular morphology, in most circumstances, the forces applied to individual cells cannot be precisely classified. For example, one-dimensional strain can be defined as the change in length divided by the initial length. However, when cells are strained in one direction, they are also subjected to compression in the direction perpendicular to the pulling force. Therefore, other terms such as stretch and deformation have also been used. These terms may better reflect the nature of the physical forces applied to cells in living systems. Techniques for applying mechanical stimulation to cells and definitions and mathematical analyses of physical forces generated from many devices have been described in detail (131). We discuss those apparatuses that have been specially developed or that have been commonly used for lung cells (Fig. 1) such that the reader may appreciate the strengths and/or limitations of individual approaches.

Fig. 1. A schematic representation of devices used to subject cells to mechanical stimulation. *A*: cells are cultured on an elastic membrane that is pushed upward (arrow) statically or by pulsation. *B*: cells cultured on an elastic membrane are pulled downward (down arrow) by negative pressure. *C*: cells are cultured on a Gelfoam sponge, one end of which is glued to bottom of a petri dish while the other end is attached to a metal bar. A magnetic force generated through a computer-controlled solenoid (arrow) acts on the metal bar to apply strain to organotypic cell cultures on the sponge. *D*: a parallel-plate flow channel applies shear stress to cells cultured as a monolayer. *E*: a micropipette touches apical membrane of a single cell and distorts cellular membrane. *F*: after ligand-coated microbeads attached to surface receptors are magnetized in 1 direction, a second, weaker magnetic field oriented at 90° "twists" the beads with a controlled shear stress.



Mechanical Stretch

Lung expansion and relaxation during breathing movements apply deformation to lung cells. In addition to shear stress, bulk air and blood flow also generate pressures that stretch the walls of the airways and blood vessels. Therefore, several devices have been designed to simulate this type of mechanical stimulation on lung cells. These can be divided into different groupings according to their major physical features, for example, static versus cyclic stretch, uniaxial versus biaxial stretch, and two-dimensional versus three-dimensional stretch.

Static stretch. Although the lung is subjected to cyclic stretch from pulsatile airflow and blood flow while breathing, single static stretch devices have relatively simple designs and have the advantage that changes in cellular morphology, intracellular Ca^{2+} , and other signals can be examined directly under the microscope. For example, one static cell strain device applies biaxial strain to cells cultured on a flexible membrane by pushing the substratum up under positive pressure (146) (Fig. 1A). Another static uniaxial cell stretch device pulls the elastic substratum horizontally (7).

Cyclic stretch. A commercially available device, the Flexercell strain unit, has been used to generate cyclic strain on cells. This equipment uses a vacuum pump to drag a cell-covered flexible membrane downward (Fig. 1B). The membrane can be precoated with various chemicals or extracellular matrix (ECM) components (4). The predicted strain distributions within the flexible membrane in this apparatus has been analyzed (27), which showed inhomogeneous strain of the membrane. A uniaxial circular well device and a linear stretching tub were used to study cyclic stretch on pulmonary arterial smooth muscle cells (51). Several biaxial cyclic cell strain devices have been designed to apply strain on lung cells (8, 133, 144).

Deformation of cells and their substrata has been examined. The strain applied to a cell is not always equivalent to that applied to the substratum. With fluorescent-labeled beads as cell surface markers, Winston et al. (144) found that cell elongation was ~60% of that in the substratum. The strain-induced distension of fetal lung cells, muscle, and bone cells has been measured with the Flexercell unit. Differences between cell strain and membrane strain were also noted (2). Wirtz and Dobbs (146) observed that the change in cell surface area was smaller than that of the membrane surface area with a static cell strain device. They speculated that cell attachment sites to the substratum might be broken when cells are stretched. Alternatively, cells or matrix may slide on the membrane, resulting in a lesser distension of cells than of the membrane (146). With an equibiaxial strain system, however, it was found that the strain for rat cardiac fibroblasts and membrane strain were highly correlated (54). With a similar system, adult rat lung alveolar epithelial cells were studied, and it was reported that the cell deformation was indistinguishable from the deformation of the underlying membrane

(133). The discrepancy between these observations will require further study.

Stretching cells in three-dimensional culture. It is well known that maintaining the phenotype of lung cells in vitro, especially alveolar epithelial cells, is very difficult. When cells are cultured at low density, type II cells quickly flatten and lose their ability to produce surfactant. Culturing fetal lung cells in a three-dimensional environment with sponges has been shown to improve the maintenance of cell morphology and phenotype (114). In such cultures, a denatured collagen sponge is used as the substratum for cell adhesion. Dispersed fetal lung cells reaggregate to form highly organized epithelial "alveolar-like structures" in sponges, which are surrounded by mesenchymal cells (63, 67, 114). Mesenchymal-epithelial interactions, a controlling factor in fetal lung growth and differentiation (117), as well as cell boundaries and tight junctions, are preserved in these cultures (114). Several other cell types are also able to attach to sponges and develop organized structures (61). Based on these observations, a unique three-dimensional cell-stretching device originally designed by Skinner (115), was further developed into a computerized Bio-Stretch System (61) (Fig. 1C).

Shear Stress

Shear stress is defined as the frictional force per unit surface area in the direction of flow exerted at the fluid-solid interface. A parallel-plate flow channel is the most widely used instrument to apply shear stress to cells cultured as a monolayer (131) (Fig. 1D). A cone-in-plate viscometer has also been used to study the interaction between neutrophils and pulmonary endothelial cells (147). Shear stress has also been generated by perfusing a column of beads covered with endothelial or mesothelial cells (140, 142). To simulate shear stress in the microcirculation, a perfusion system was constructed with micropipette glass and Teflon tubing that allowed cells to be cultured in 20- to 50- μm branching tubes. Rabbit lung microvascular endothelial cells have been cultured and perfused with this system (26).

Shear stress and mechanical stretch. Airways, pulmonary blood vessels, and the pleural space are influenced not only by shear stress but also by mechanical stretch. The postnatal lung receives a blood flow equivalent to the whole systemic circulation but with a much lower vascular resistance. Therefore, the relative contribution of fluid shear stress and mechanical strain on pulmonary arterial endothelial cells must be different from that experienced in other organs. To determine the relative contribution of these two components, an apparatus that can selectively control shear stress and mechanical stretch has been described (73). A perfused transcapillary coculture system has been developed that permits the chronic exposure of endothelial cells and smooth muscle cells to a physiological range of shear stress and pressure (90).

“Touching” and “Twisting” Devices

A simple and unique model that can selectively apply pressure to single cells has been used to study the physical forces on pulmonary airway epithelial cells (105). A glass pipette $\sim 1 \mu\text{m}$ in tip diameter was positioned near the apical membrane of a single cell by a micromanipulator. The pipette was deflected downward by a computer-controlled piezoelectric device to transiently distort the cellular membrane (Fig. 1E).

Another interesting device allows cells to bind spherical ferromagnetic microbeads coated with specific receptor ligands. By magnetizing these surface-bound beads in one direction and then applying a second, weaker magnetic field oriented at 90° , one can twist the beads in place and therefore apply a controlled shear stress on bound cell surface receptors (Fig. 1F) (139). This device has been used to study the contractile activity of human airway smooth muscle cells (40).

Although many devices have been developed, most are not standardized equipment. Furthermore, because of the variety of physical forces, new devices are needed to simulate the specific physical forces to which cells are subjected under various physiological or pathophysiological conditions.

EFFECTS OF PHYSICAL FORCES ON LUNG CELLS

Mechanical forces have profound effects on cell proliferation and differentiation related to lung development, physiological functions, and pathological processes of the lung. In this section, we briefly summarize studies related to cell proliferation, surfactant metabolism, ECM turnover, inflammatory mediator production, and cell permeability.

Cell Proliferation

Fetal lung cells. To determine whether intermittent stretch derived from fetal breathing movements can influence proliferation of fetal lung cells, organotypic cell cultures have been subjected to a uniaxial intermittent strain (5% elongation of sponges, 60 cycles/min, 15 min/h), which increased DNA synthesis and cell division of fetal rat lung cells on *day 19* of gestation (canalicular stage of lung development; term 22 days) (63). This strain regimen is similar to the reported frequency, amplitude, and periodicity of normal human fetal breathing movements *in vivo* (34). The growth of an embryonic human lung fibroblast cell line (IMR-90) was also stimulated by cyclic strain (8). These results are consistent with the cyclic stretch imposed by fetal breathing movements being an important stimulator for fetal lung growth. To define the responding cell types and the role of mesenchymal-epithelial interactions, isolated fetal rat lung epithelial cells and fibroblasts were subjected to mechanical strain either alone or combined at various ratios. Strain increased DNA synthesis by both cell types (67, 151). Mechanical strain-induced proliferative activity was observed in a gestation-dependent manner, and the responsiveness of mixed cells was mainly determined by that of the mesenchymal cell component (151).

Adult alveolar epithelial cells. Alveolar epithelial cell proliferation plays an important role in maintaining the integrity of the epithelium, especially during the repair process after lung injury. *In vitro*, adult type II cells proliferate when cultured at low density in a well-defined culture medium (57, 126). Appropriate conditions for the growth of adult type II cells on elastic membrane for mechanical stretch have not been described. When sparsely cultured adult type II epithelial cells (85), as well as fetal lung epithelial cells (67, 111) and fibroblasts (67), were stretched with the Flexercell strain unit, cell detachment occurred even with a very low stretch amplitude. When primary cells were cultured at high density, contact inhibition limits cell proliferation. A cell line (H441) derived from a human lung epithelial carcinoma has been used to study mechanical strain-induced proliferation (16). However, because the differentiated characteristics of cell lines differ from primary cultured cells, such data must be interpreted with caution when used as models for lung alveolar epithelial cells. Adult type II cells subjected to cyclic mechanical stress with the Flexercell unit showed increased DNA synthesis after exposure to conditioned medium from lung fibroblasts compared with static cultured type II cells (85). In agreement with this observation, mechanical strain of fetal rat lung cells increased gene and protein expression of the platelet-derived growth factor (PDGF) β -receptor (PDGFR). Blockade of the receptor with a protein tyrosine kinase (PTK) inhibitor or antisense oligonucleotides abolished mechanical strain-induced cell proliferation (60). These results suggest that mechanical forces not only stimulate growth factor production (as discussed in *Soluble Factors*) but also regulate the responsiveness of cells to mitogenic activity.

Airway smooth muscle cells. Hyperplasia and hypertrophy of airway smooth muscle cells are common findings in asthma, chronic obstructive pulmonary diseases, and other chronic diseases with increased airway resistance. When airway smooth muscle cells isolated from canine tracheae and bronchi were subjected to cyclic strain with the Flexercell strain unit, increases in both cell number and DNA synthesis were observed after 5 or 14 days in culture (120). The content of total cellular protein, especially contractile proteins including myosin, myosin light chain kinase, and desmin, was increased compared with that in static cultured cells (121). The activity of contractile enzymes such as myosin light chain kinase and catomyosin ATPase in airway smooth muscle cells was also increased in the strained cells compared with that in the static cultured control cells (122). These approaches provide model systems for further investigation of mechanisms regulating mechanical stimulation-induced cell proliferation.

Surfactant Metabolism

Synthesis and secretion of pulmonary surfactant are major functions of type II alveolar epithelial cells. The expansion and contraction of the lung during respiration stretch the alveolar epithelium. To test whether

mechanical distension of the alveolus causes surfactant secretion, Wirtz and Dobbs (146) applied a static stretch for 30 min on primary cultured type II alveolar epithelial cells. The static stretch caused a rapid increase in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and subsequently an increase in surfactant phospholipid release (146). During development, fetal lung epithelial cells undergo continuous differentiation. Pulmonary surfactant production is a marker of lung maturation. Applying cyclic strain to primary cultures of fetal rabbit epithelial cells in monolayer culture increased the synthesis and secretion of surfactant phospholipids (111). Intermittent strain of fetal rat lung cells in organotypic culture caused a selective increase in steady-state mRNA levels of surfactant protein (SP) C but not of SP-A (79).

ECM Turnover

Mechanical strain differentially regulated gene expression of the major ECM components in fetal lung cells. An intermittent stretch induced an increase in steady-state mRNA levels of type IV collagen and tropoelastin but inhibited the accumulation of $\alpha_1(\text{I})$ -procollagen, biglycan, and fibronectin mRNAs (79, 150) in organotypic cultured fetal lung cells. The gene expression and activity of matrix metalloproteinases and their inhibitor (tissue inhibitor of metalloproteinase-1) were not affected by mechanical stimulation (150). The release of glycosaminoglycans and proteoglycans was increased by mechanical strain through both constitutive and regulated secretion pathways (149). The strain-induced glycosaminoglycan production was associated with increased antithrombin activity due to an increase in the concentration of active chondroitin sulfate. Strain also downregulated secretion of tissue factor procoagulant activity, which may lead to decreased thrombin generation on the surface of fetal lung cells (12). Strain also increased fibronectin production by upregulating fibronectin protein synthesis and secretion (77).

When primary cultured bovine pulmonary arterial endothelial cells were stretched with a biaxial cell strain apparatus, cyclic strain initially reduced the release of fibronectin into the culture medium (29). With an extended period of stretch, fibronectin in the medium began to approach nonstrained control values, whereas the cell layer-associated fibronectin was significantly increased (28). The distribution of stress fibers visualized by fluorescent staining was also altered in strained cultures. These results suggest that pulmonary arterial endothelial cells respond to cyclic biaxial strain by enhancing structural components associated with cell adhesion (28).

Inflammatory Mediator Production and Cell Injury

Ventilation-induced cytokine release. Mechanical ventilation, an indispensable therapeutic modality for the treatment of respiratory failure, can lead to a number of serious complications, including initiation or exacerbation of underlying lung injury. Injurious ventilation

strategies increased cytokines, including tumor necrosis factor (TNF)- α , interleukins (ILs) such as IL-1 β , IL-6, and IL-10, macrophage inflammatory protein-2, and interferon- γ , in the lavage fluid collected from an isolated rat lung model (132). Inflammatory cytokines produced in the lung during ventilation may contribute to acute lung injury and the development of multiple organ dysfunction syndrome (64). Mechanical stretch with the Flexercell unit increased secretion of IL-8 from A549 cells, a human alveolar epithelial cell line (137). Increased amplitude (10% elongation) or continuous stretch induced injury of fetal lung cells in organotypic culture (63, 127), which was associated with increased expression of proinflammatory cytokines and chemokines (76).

With a cyclic equibiaxial strain apparatus, it was found that primary cultured alveolar epithelial cells on *day 1* of culture can be significantly injured by increasing the magnitude of mechanical deformation. Interestingly, when cell stretch was performed after 5 days in culture, the percentage of injured cells was significantly decreased (133). During prolonged culture, the morphology of type II cells usually changes toward a type I-like appearance. It is plausible that type II cells are more vulnerable to mechanical deformation-induced injury. When human and cat airway epithelial cells were cultured on flexible membranes and wounded by scraping with a metal spatula, both cyclic strain and compression inhibited the repair of the wound (108). These interesting observations provide useful model systems to further study the effect of mechanical ventilation-induced cytokine production, cell injury, and cell repair mechanisms.

Prostaglandin synthesis. Synthesis of biologically active eicosanoids by airway epithelial cells contributes to the regulation of airway smooth muscle tone and inflammatory responses. During ventilation, the airways undergo cyclic stretching. With the Flexercell strain unit, it has been shown that cyclic stretch downregulated the synthesis of prostaglandins (PGs) including PGE_2 , PGI_2 , and thromboxane A_2 of cat and human airway epithelial cells (107). This inhibitory effect seems to be due to an inactivation of cyclooxygenase. In contrast, shear stress increased the production of prostacyclin by lung endothelial cells (91) and mechanical strain induced a rapid release of prostacyclin by fetal rat lung cells (116). These results are consistent with an alteration of physical forces being an important factor in inflammatory diseases in the lung.

Permeability of Cells

Pulmonary endothelial cells. The permeability of pulmonary endothelial cells is a highly regulated process. The effect of shear stress on the permeability of bovine pulmonary arterial endothelial cells was tested by culturing cells on permeable microcarrier beads placed in a chromatography column with variable perfusion rates. When the flow rate was increased from 0.9 to 3.2 ml/min (corresponding to average shear stresses of 4.7 and 16.8 dyn/cm^2), permeability in-

creased within minutes of the flow increase and was reversed by decreasing the flow rate (140).

Mesothelial cells. The mesothelial cells of the visceral pleural surface function as an interface between the lung surface and the pleural space, providing a barrier between the lung and the surrounding fluid and tissues. These cells are exposed to a high level of mechanical stimulation due to the respiratory motion of the lung. When the lung expands and contracts, mesothelial cells are cyclically stretched. During lung expansion, some regions of the visceral pleura slide relative to the parietal pleura of the chest wall and are thus subjected to fluid shear stress. The permeability of these cells could be reversibly altered by fluid shear stress in a model using a perfused column of cell-covered beads, which suggests that the barrier function of mesothelial cells is responsive to changes in fluid shear stress (142).

MECHANORECEPTION

How mechanical forces can be sensed by cells and converted into biochemical signals for intracellular signal transduction is still unknown. Because of the complexity of organ structures, the variety of cell types, and the variety of physical forces to which cells are exposed in the body, mechanisms by which mechanical stimulation is perceived may vary. Among several proposed mechanisms (5, 41), stretch-activated ion channels and the ECM-integrin-cytoskeleton pathway have received the most attention.

Mechanoreceptors

It has long been recognized that specialized nerve endings are localized to the muscle spindles of the chest wall. These nerve endings transmit information to the respiratory neurons about forces exerted by respiratory muscles and about thoracic movement (106). Stretch receptors have been found within the smooth muscle layer of the extrapulmonary airways and are classified as rapidly and slowly adapting receptors based on their electrical activities and the distribution of their terminals (18). It has been speculated that the function of this type of mechanoreceptor is to minimize ventilatory work output for a given minute volume and to be involved in neural regulation of the airway smooth muscle and bronchial blood flow (17). The nature of these specialized mechanosensory receptors, however, is unknown. There is evidence to suggest that stretch-activated ion channels (see *Stretch-Activated Ion Channels*) may be one component (32).

Stretch-Activated Ion Channels

Because physical forces are generally applied directly or indirectly to the plasma membrane of cells, it has been noted that mechanical forces can affect the permeability of the cellular membrane to various ions. Both stretch-activated and -inactivated ion channels have been characterized in many cell types (99).

Stretch-activated ion-channel activities have been found in lung cells. Ca^{2+} is one of the most common

molecules that mediate the intracellular signaling initiated by mechanical stress. A 20% single static stretch of rat pulmonary arterial smooth muscle cells increased both Ca^{2+} influx and efflux (7). Stretch-stimulated Ca^{2+} influx does not need Na^{+} influx and is mediated in part by a pathway sensitive to both gadolinium and verapamil. Stretch-stimulated Ca^{2+} efflux requires both Ca^{2+} influx via a gadolinium-sensitive pathway and the mobilization of intracellular calcium stores (7). Biaxial strain of bovine pulmonary arterial endothelial cells also resulted in an increase in cell Ca^{2+} content from increased entry and release from intracellular stores (145). Cyclic intermittent stretch induced a rapid Ca^{2+} influx via gadolinium-sensitive stretch-activated ion channels in fetal rat lung cells (68). Spritzing bath solution onto cells as a mechanical stimulus induced a transient increase in $[\text{Ca}^{2+}]_i$ of a human lung epithelial cell line (REPF-LC-AI), which could be inhibited by gadolinium or by removing extracellular Ca^{2+} and was sensitized by lysophosphatidic acid (82).

Shear stress did not change $[\text{Ca}^{2+}]_i$ of pulmonary arterial endothelial cells (110) but induced a transient increase in membrane K^{+} permeability (1, 110). Boitano et al. (10) found that Ca^{2+} -conducting channels in airway epithelial cells were opened when mechanical stimulation was applied to the cellular membrane via a micropipette. However, they further demonstrated that mechanical stimulation caused a rapid depolarization of the stimulated cell. This, in turn, activated voltage-sensitive channels that could be blocked by Ni^{2+} or nifedipine (11). Therefore, although mechanical stimulation can induce Ca^{2+} influx and/or efflux, the types of ion channels affected may vary.

Gadolinium, an inhibitor of stretch-activated ion channels, blocked baroreceptor discharge in carotid receptors (31) as well as mechanotransduction in the nodose sensory neurons that project to the carotid (112, 125). Specialized mechanoreceptors in the lung have a similar structure and function. Stretch-activated ion channels may therefore also be present in these nerve endings (see *Mechanoreceptors*).

A mechanosensitive ion channel has been cloned and identified from bacteria (124). Recently, the structure of the mechanosensitive ion channel homolog from *Mycobacterium tuberculosis* was determined by X-ray crystallography (14). Based on genetic and molecular studies of touch avoidance in the nematode *Caenorhabditis elegans*, a molecular model for a mechanotransducing complex has been proposed (128). In this model, the *mec-4* and *mec-10* genes encode proteins that are hypothesized to be subunits of a mechanically gated ion channel that are related to subunits of the vertebrate amiloride-sensitive epithelial Na^{+} channel (128). Molecular characterization of stretch-activated ion channels from mammalian species will provide us with fundamental information for the understanding of mechanoreception mechanisms.

ECM-Integrin-Cytoskeleton Pathway

Cells are attached to the ECM via integrins that are linked to the cytoskeleton. This complex provides a

structural connection to transmit signal from ECM to cells (45). Ingber (42) proposed a tensegrity model, which considers the transmembrane ECM receptors, cytoskeleton filaments, and nuclear scaffolds as a “hard-wired” network. As in any architectural structure, mechanical loads are transmitted across the cell surface and into the cell by these physically interconnected structures. When integrins were pulled by micropipettes, cytoskeletal filaments reoriented, nuclei were distorted, and nucleoli were redistributed along the axis of the applied tension field (71). Mechanical stress-induced changes in cytoskeletal stiffness and deformation may alter the function of the structural molecules that comprise the cytoskeleton and nucleus, including some critical regulatory proteins (41). Changing structural arrangements within the cytoskeleton and nuclear matrix may expose or obscure internal molecular binding sites, release mechanical constraints for molecular remodeling, or change the porosity of the lattice (41, 42). This model has been further developed and supported with additional evidence (43). For example, with a magnetic twisting device, mechanical stresses applied directly to cell surface integrins induced focal adhesion formation and a force-dependent stiffening response of the cytoskeleton (139). With this device, it has been found that cytoskeletal stiffness of human airway smooth muscle cells was modulated by agents that can change the contractile activity of smooth muscle (40). Using electron and fluorescence microscopy, Smith et al. (119) demonstrated that the orientation of airway smooth muscle cells and actin stress fibers within the cells were reorganized after mechanical strain. They found marked increases in the number and length of focal adhesions between the cell membrane and the substratum of cultured cells (119). Therefore, the cytoskeleton elements can be reorganized to increase the efficiency of transmitting signals from the ECM into the cell interior (119). This autofeedback mechanism could be very important for cells adapting to changes in their external environment.

INTRACELLULAR SIGNAL TRANSDUCTION

During evolution, mechanical stimulation, as well as other physical factors such as temperature, pH, and light, has been a critical environmental signal sensed by cells. It is plausible that the intracellular signal transduction pathways originally developed by cells to respond to those basic physical stimuli have been preserved during evolution and further developed to respond to other external stimuli. Thus the biochemical reactions involved in mechanotransduction may be shared by other stimuli. The signaling events initiated by mechanical stimulation include generation of second messengers, change of phosphorylation status of proteins, amplification through enzymatic cascades, and transmission via a complicated network of signaling molecules.

The phosphorylation status of proteins is one of the main determinants of the activity of many enzymes in the cells. It is also involved in cell signaling mechanisms and is of profound importance for cell prolifera-

tion and differentiation (70). The effects of physical forces on protein kinase (PK) activities have been the focus of many mechanotransduction studies. As for signals initiated by other biological stimuli, mechanical stimulation causes activation of multiple second messengers to transmit its signals within the cell (100). The activity of PKs is usually regulated through such second messengers.

PKA

PKA is also named cAMP-dependent PK because it uses cAMP as its second messenger. Increased cAMP concentration and PKA activity were observed in lung tissues after partial pneumonectomy or mechanical ventilation (98). A mechanical strain-induced increase in cAMP content has been reported in fetal rabbit epithelial cells after a continuous, high-amplitude strain for 24 h with the Flexercell unit (111). When organotypic cultured fetal rat lung cells were exposed to intermittent strain at an 18% elongation, an increase in cAMP production was observed (116). With a 5% elongation, however, neither the cAMP content nor the activity of PKA was affected by strain (66). The stimulatory effect of strain on DNA synthesis was also not influenced by inhibition of the cAMP-PKA pathway in fetal lung cells (66). Thus activation of the cAMP pathway may be dependent on the pattern and amplitude of mechanical strain.

PKC

PKC is a family of proteins with an increasingly recognized number of isozymes. The activity of several major isozymes of PKC is regulated by the intracellular concentrations of free Ca^{2+} and diacylglycerol (DAG) (55). The intracellular storage of Ca^{2+} can be mobilized from an inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$]-dependent compartment or from the entry of Ca^{2+} via ion channels (6). $\text{Ins}(1,4,5)\text{P}_3$ and DAG can be generated from the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipases (PLs) (6, 55).

Pulmonary arterial smooth muscle cells respond to a single static stretch with a transient increase in D-myoinositol 1,4,5-trisphosphate and D-myoinositol 1,3,4,5-tetrakisphosphate (52) that may be partially attributed to mechanical stretch-induced Ca^{2+} elevation in these cells. Static biaxial stretch-induced secretion of pulmonary surfactant phospholipid from alveolar type II epithelial cells is mediated through the intracellular mobilization of Ca^{2+} (146). Here, the role of PKC in mediating strain-induced proliferation of fetal rat lung cells is presented as an example to illustrate the cascade of mechanical strain-induced signaling.

PLC- γ -PKC pathway mediates strain-induced fetal lung cell proliferation. In fetal lung cells, both intracellular and extracellular Ca^{2+} are involved in the modulation of strain-induced proliferative activity. Mechanical strain increased Ca^{2+} influx through a gadolinium-sensitive stretch-activated ion channel, which contributed to strain-induced PKC activation and DNA synthesis (Fig. 2) (68). Intracellular concentrations of

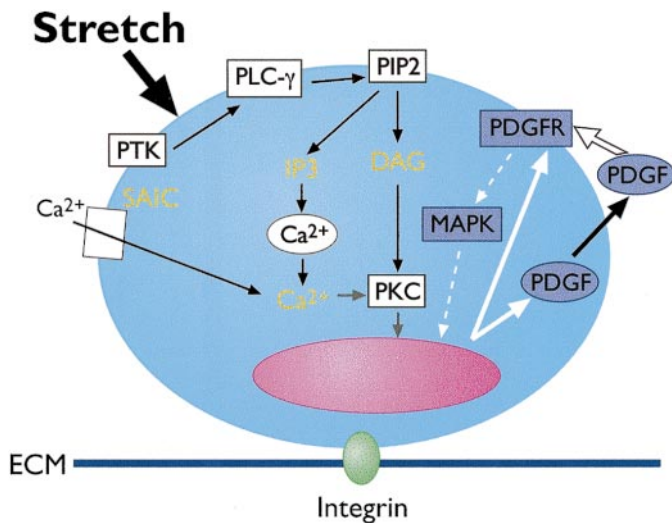


Fig. 2. Mechanotransduction of strain-induced fetal lung cell proliferation. Mechanical stretch induces Ca^{2+} influx and activates protein tyrosine kinases (PTK) such as pp60^{src}. Activation of phospholipase C- γ (PLC- γ) via its tyrosine phosphorylation mediates hydrolysis of phosphatidylinositol 4,5-diphosphate (PIP₂) to generate inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ mobilizes intracellular Ca^{2+} . DAG in presence of Ca^{2+} activates protein kinase C (PKC) and downstream events. Gene expression and protein synthesis of platelet-derived growth factor (PDGF)-B and PDGF β -receptor (PDGFR) are increased by mechanical strain. They then stimulate cell proliferation through autocrine/paracrine mechanisms. MAPK, mitogen-activated protein kinase; ECM, extracellular matrix; SAIC, stretch-activated ion channel.

Ins(1,4,5) P_3 and DAG were dramatically increased after a short period of strain, which may be due to the activation of PLC- γ 1 by an increased tyrosine phosphorylation (Fig. 2) (66). PKC was rapidly activated and translocated from the cytosolic fraction to the membrane-bound fraction and remained activated throughout a 48-h period of intermittent strain. Strain-induced PKC activation and DNA synthesis were blocked by PKC inhibitors as well as by a PLC inhibitor (66). Mechanical strain also activated PLD, which may contribute to the prolonged activation of PKC (66). The activation of PKC has been reported for mechanical force-induced signal transduction in other cell types such as in stretch-induced *c-fos* gene expression in cardiac myocytes (101) and in shear-induced PDGF-B gene expression in human endothelial cells (39). It is a common feature for several cell types in response to mechanical stimulation.

PTK

Cellular phosphotyrosyl levels are regulated by the activities of PTKs and protein tyrosine phosphatases. Several members of the cytoplasmic PTKs belong to a so-called *Src* family. Some newly characterized pp60^{src} (*Src*) substrates are either cytoskeletal proteins or proteins associated with the cytoskeleton. These proteins include pp125^{FAK}, a focal adhesion PTK (109); p120, a tyrosine-phosphorylated protein that shares sequence similarity with cadherin-binding factors (95); an actin filament-associated protein of 110 kDa (AFAP-110) (25); cortactin, an 80/85-kDa filamentous actin-

binding protein that is enriched in the cell cortex (148); and paxillin, a focal adhesion protein associated with vinculin (134).

PTK activation is an upstream event of the PLC- γ -PKC pathway. The mechanical strain-induced rapid increase in tyrosine phosphorylation of PLC- γ 1 in fetal rat lung cells suggested that PTK activation is an upstream event of the PLC- γ 1-PKC pathway. Indeed, it was found that total PTK activity in cell lysates increased within 15 min of strain (62). Tyrosine phosphorylation of proteins was observed within 5 min from the onset of strain, especially in a band corresponding to 110–130 kDa, which is a common phenomenon in ECM-integrin-cytoskeleton-mediated signal transduction. Several of these proteins were identified as pp60^{src} substrates, including p120, AFAP-110, and cortactin (62). *Src*-related PTK activity significantly increased in the cytoskeleton fraction after 5 min of mechanical strain. The amount of pp60^{src} in the cytoskeleton fractions also increased rapidly. Strain-induced pp60^{src} translocation to the cytoskeletal matrix may be mediated through AFAP-110. Strain-induced PKC activation and translocation from the cytosol to the membrane, as well as DNA synthesis, were blocked by the PTK inhibitor herbimycin A (62). Taken together, these studies suggest that the PTK-PLC- γ -PKC axis is a major pathway for strain-induced fetal lung cell growth (Fig. 2). In agreement with these observations, it has been reported that pressure-induced rat vascular smooth muscle cell proliferation can be inhibited by PTK, PLC, or PKC inhibitors (38).

pp125^{FAK} pathway. pp125^{FAK} was first identified as a substrate of pp60^{src} (109). It was subsequently found to function as a PTK that is localized at the focal adhesion plaque. pp125^{FAK} is associated with the cytoskeleton via paxillin, and it has been shown to bind to other signal transduction proteins. These features make it and its substrates very attractive candidates for mechanotransduction. The tyrosine phosphorylation of pp125^{FAK} and paxillin was not influenced by mechanical strain in fetal rat lung cells (62) or in a human pulmonary epithelial H441 cell line (16). In contrast, mechanical strain rapidly increased tyrosine phosphorylation of pp125^{FAK} and paxillin in airway smooth muscle cells cultured on type I collagen. Tyrosine phosphorylation of pp125^{FAK} decreased within 4 h but remained elevated in paxillin at 24 h. In addition, tyrosine kinase inhibitors (genistein and herbimycin A) inhibited strain-induced reorientation and elongation of airway smooth muscle cells (118). Therefore, the role of this pathway in transmitting mechanical stimulation-induced signals appears to be cell-type dependent.

Mitogen-Activated Protein Kinase

Many mitogenic activities stimulated by growth factors and other mitogens are mediated through mitogen-activated protein (MAP) kinases, or so-called extracellular signal-regulated kinases (ERKs). Static stretch of rat cardiac myocytes activated a 42-kDa MAP kinase (155) and its upstream activator MAP kinase kinase (154). Stretch-induced activation of MAP kinase was

partially inhibited by PTK or PKC inhibitors or by blocking Ca^{2+} influx (155). Activation of MAP kinase by stretch was thought to be partially mediated through the enhanced secretion of angiotensin II (153) and endothelin-1 (152). However, mechanical stretch resulted in significantly greater activation of MAP kinases in cardiomyocytes isolated from angiotensinogen-deficient mice than from wild-type mice, suggesting that the presence of angiotensin II is not obligatory (81). Shear stress induced a transient activation of ERKs in bovine aortic endothelial cells. Its upstream regulators were found to be $\text{G}_{i,2}$ protein, Ras, and PTK (44).

Mechanical strain rapidly activated ERKs in a human pulmonary epithelial (H441) cell line (16). Because mechanical stretch induced an increase in PDGF-BB production from fetal lung cells (60) and PDGF-BB stimulated MAP kinase activation in a concentration- and time-dependent manner in fetal lung fibroblasts (59), it is possible that mechanical stretch-induced MAP kinase activation is indirectly mediated via the increase in PDGF-BB production (Fig. 2).

Parallel to the ERK pathway, there are two newly defined pathways related to cellular stress, namely, the p38 MAP kinase and c-Jun NH_2 -terminal (JNK) kinase pathways. JNK is also called stress-activated protein kinase (SAPK). These two pathways are activated by many cellular stress stimuli, including heat shock, ultraviolet radiation, endotoxin, and cytokines such as $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ (64). It has been shown that mechanical stretch increased SAPK activity rapidly in rat cardiac myocytes. This activation was not dependent on secreted angiotensin II, PKC, or Ca^{2+} (50). Shear stress also activated JNK in bovine aortic endothelial cells but with a much slower and prolonged time course. This activation was regulated by mechanisms involving $\text{G}\beta/\gamma$, Ras, and PTK (44). Mechanical stretch (5% strain at 6 cycles/min for 2 h) activated SAPK in human lung epithelial (A549) cells, which may be involved in the stretch-induced release of cytokines (87).

REGULATION OF MECHANICAL FORCES AT TRANSCRIPTION, TRANSLATION, AND POSTTRANSLATIONAL LEVELS

Similar to signals activated by other mechanisms, mechanical-initiated biochemical reactions influence cellular functions at multiple levels. As elucidated in Fig. 3, mechanical stimulation-initiated signals may regulate gene expression, protein synthesis, and post-translational modification and secretion of proteins and other cellular products. Each cellular response is controlled by different pathways at various levels. Each pathway may be involved in many different cellular responses.

Transcriptional Regulation

In the cell, many pathways transmit signals from the plasma membrane toward the nucleus to alter the expression of targeted genes. Physical forces can exert

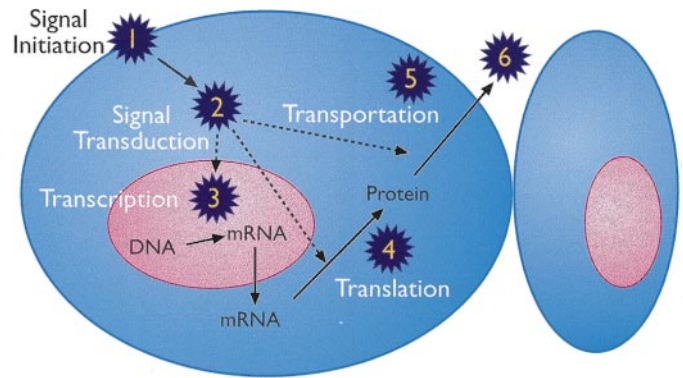


Fig. 3. Mechanical force-induced signals regulate cell functions at multiple levels. Mechanical stimulation applied to a cellular surface may initiate mechanotransduction by activating stretch-activated ion channels or ECM-integrin-cytoskeleton pathway (1). Intracellular signals (2) can be transmitted toward the nucleus to regulate gene expression (3) or can affect translation (4) and secretory process (5). Secreted soluble factors or ECM molecules (6) may further regulate cell proliferation and differentiation.

their effects on cells by influencing expression of so-called immediate early response genes (such as *c-fos*, *c-jun*, *c-myc*, *JE*, and *Egr-1*), which encode proteins related to transcriptional factors and signal transduction (49). Physical forces can also affect the expression of genes encoding secreted proteins such as growth factors, cytokines, and ECM molecules, as well as genes encoding structural proteins of the cell.

Mechanical strain of fetal lung cells increased the mRNA levels of PDGF-B and PDGFR- β within 5 min of the onset of strain. The rapidity of this increase implies transcriptional activation (60). Mechanical strain of fetal lung cells also induced increases in steady-state mRNA levels of tropoelastin and SP-C. By examining the heterogeneous nuclear RNA levels of these two genes, it was further confirmed that the upregulation of these genes is mainly due to increased transcriptional activity (79).

One of the mechanisms to activate transcription is through the binding of activated transcriptional factor(s) to the regulatory element(s) of the promoter on the target gene. With the use of cardiac myocytes, it was found that the "stretch response element" of the *c-fos* gene is present within 356 bp of the 5'-flanking region. In contrast, the stretch response elements of the atrial natriuretic factor and the β -myosin heavy chain genes are probably located outside of 3,412 and 628 bp of the 5'-flanking region (102).

A *cis*-acting fluid shear stress-responsive element was identified in the PDGF-B chain promoter. This putative transcription factor binding site is also present in the promoters of other genes that are induced by shear stress, including tissue plasminogen activator, intercellular adhesion molecule-1, and transforming growth factor- β 1 (92). An increased intercellular adhesion molecule-1 gene transcript was observed as early as 2 h after the onset of shear stress. Expression of two cell adhesion molecules that do not have a shear stress response element in their promoter regions, E-selectin and vascular cell adhesion molecule-1, was not affected

by shear stress (78). With a gel mobility shift assay and *in vitro* DNase I footprinting, it was found that nuclear factor- κ B p50/p65 heterodimers accumulate in the nuclei of vascular endothelial cells exposed to fluid shear stress and bind to the PDGF-B chain shear stress response element in a specific manner (46). A new shear stress response element was found in the PDGF-A promoter that contained a binding site for the transcription factors Egr1/Sp1 (94).

The *cis*-acting phorbol ester "12-*O*-tetradecanoylphorbol 13-acetate" response element is involved in shear stress-induced gene expression of a chemokine, monocyte chemoattractant protein-1 (113). Sp1 was recently identified as a shear stress-responsive element in the tissue factor promoter (58). Further characterization of these positive and other negative genetic regulatory elements and their *trans*-activating factors will enhance our understanding of physical forces as a regulator of cellular functions (93).

Posttranscriptional Regulation

Mechanical-induced signals can also affect cellular functions at the posttranscriptional level. There are several examples that show that mechanical stimulation-induced signals differentially regulate the expression of a particular protein between the transcriptional and posttranscriptional levels.

The mRNA levels of fibronectin in fetal lung cells increased gradually in static culture. However, when the cells were subjected to mechanical strain, the accumulation of fibronectin mRNA was inhibited. Interestingly, despite relatively lower mRNA levels, fibronectin was significantly increased in the culture medium. Pulse labeling with [³⁵S]methionine revealed that the synthesis of fibronectin was stimulated by mechanical strain even in the presence of actinomycin D, an inhibitor of transcription. These data suggest that strain-induced fibronectin synthesis is mainly regulated at the posttranscriptional level. With the use of cycloheximide to block protein synthesis, strain increased the secretion of prelabeled fibronectin, suggest-

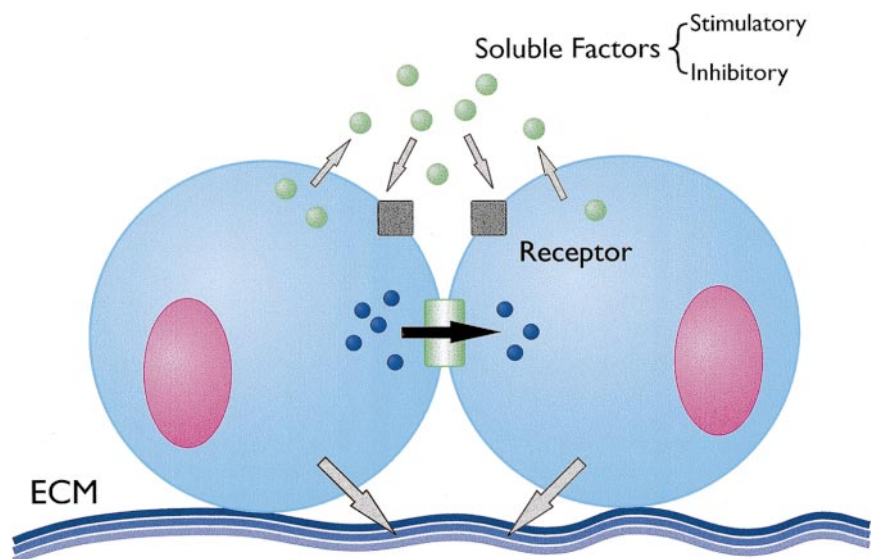
ing that strain also stimulated the secretion of fibronectin (77).

Mechanical strain also increased the sulfation and secretion of glycosaminoglycans (115, 149) and proteoglycans from fetal lung cells (149). The steady-state levels of mRNA encoding proteoglycan core proteins were either not altered or decreased on mechanical strain. With the use of [³⁵S]sulfate to label newly synthesized glycosaminoglycan, it was found that mechanical strain stimulated the constitutive secretion of glycosaminoglycan, which was abrogated by inhibitors for cytoskeletal dynamics (149). Mechanical strain also stimulated the regulatory secretion of these molecules by increasing [Ca²⁺]_i, which was blocked by gadolinium, an inhibitor of the stretch-activated ion channel (149). These studies provided evidence that mechanical stretch-initiated signals can affect posttranslational modification and intracellular trafficking of proteins.

INTERCELLULAR COMMUNICATION

Mechanical force-initiated signals need to be transmitted through intercellular communication. Cell-cell and cell-matrix interactions are essential to coordinate the response of various cell types to internal and external stimuli. For example, mesenchymal-epithelial interactions are a controlling factor for lung growth and maturation (117). Pressure-induced connective tissue synthesis in pulmonary arterial segments is dependent on an intact endothelium (130). Endothelial or airway epithelial cells control the smooth muscle tone of pulmonary blood vessels and airways, respectively. Alveolar macrophages and inflammatory cells interact with epithelial and endothelial cells. Physical forces could regulate these biological processes. There are several ways in which cells may communicate with each other. Cells commonly use cytokines, growth factors, and other small soluble factors for communication. Cells can also transmit messages directly from one to another through cellular junctions or by their reciprocal interaction with the ECM (Fig. 4).

Fig. 4. Mechanical force-induced intercellular signaling may involve autocrine, paracrine, juxtacrine, and cell-matrix interaction. Mechanical stimulation may induce synthesis and release of soluble factors such as growth factors and cytokines, which can further affect cellular functions via autocrine and/or paracrine mechanisms. Cells may also transmit signaling molecules, such as Ca²⁺ and IP₃, through cellular junctions. ECM structure may also determine responsiveness of cells to mechanical stimulation. Conversely, on mechanical stimulation, altered ECM molecule production and degradation may influence functions of surrounding cells.



Soluble Factors

Mechanical stimulation usually induces very transient signals yet can trigger events leading to a long-lasting effect on cell proliferation or differentiation. The strain-induced growth-promoting effect on fetal lung cells appears to be mediated by an increased production of endogenous growth factors. The growth-stimulating activity of conditioned medium from strained fetal rat lung cells is considerably greater than that from control cells (69). Similar results were obtained with an embryonic human lung fibroblast cell line (8). One of these growth factors has been identified as PDGF-BB (60). Both PDGF-B gene expression and PDGF-BB proteins were increased by mechanical strain. The strain-induced stimulatory effect on fetal lung cell proliferation was blocked by antisense PDGF-B oligonucleotides, neutralizing PDGF-BB antibodies, or a PDGFR-associated tyrosine kinase inhibitor (60). A study by Harding et al. (37) has shown that the abolition of fetal breathing movements by spinal cord transection leads to a reduction in DNA synthesis and insulin-like growth factor-II gene expression. Changes in lung volume due to either tracheal obstruction or lung liquid drainage also alter insulin-like growth factor-II expression in fetal sheep (35). These findings are compatible with fetal breathing movements controlling fetal lung growth via activation of growth factor expression. Furthermore, these results reveal that mechanotransduction of fetal lung cell proliferation is a multiple-step process. First, mechanical strain has to be sensed by cells to initiate biochemical reactions for intracellular signaling. Second, these signals have to be transmitted across the cytoplasm to the nucleus. Third, strain-induced signals specifically turn on or off some genes encoding growth-related proteins such as growth factors. Moreover, strain-induced signals may regulate the translational and posttranslational modification of these proteins. Strain-induced growth factors may also initiate intracellular signals such as the MAP kinase pathway to regulate cell proliferation (Fig. 2).

Both the parietal and visceral pleura are involved in growth factor synthesis, ECM production, and secretion of inflammatory mediators (141). The effects of shear stress and mechanical stretch on growth factor production by mesothelial cells were examined both by perfusing columns of cell-covered beads and with the Flexercell unit. Both manipulations increased the release of endothelin-1 but had no effect on the release of PDGF (141). The mitogenic activity from culture medium conditioned by cells subjected to shear stress was significantly decreased, suggesting that mechanical stimulation may also reduce some stimulators or induce some inhibitors of cell growth (141).

Intercellular Junctions

The ciliated airway epithelium plays an important role in host defense by transporting ions and water to control the tonicity of the periciliary layer and maintain efficient mucociliary clearance. A coordinated increase in ciliary beating frequency of multiple airway

epithelial cells has been noted after mechanical stimulation of a single airway epithelial cell (103). Further studies revealed that touching a single airway epithelial cell with a micropipette induced a rapid increase in $[Ca^{2+}]_i$ in the stimulated cell. After a brief delay, the increase in $[Ca^{2+}]_i$ was communicated to adjacent cells and propagated through five to seven cells in all directions to form an intercellular Ca^{2+} wave. The Ca^{2+} wave may coordinate cellular activity, including ciliary beating of airway epithelial cells (10, 24). The Ca^{2+} wave can be initiated by the microinjection of $Ins(1,4,5)P_3$ (104) and is blocked by agents that limit $Ins(1,4,5)P_3$ -dependent Ca^{2+} release from intracellular stores (9). The Ca^{2+} wave can be propagated in the absence of extracellular Ca^{2+} and blocked by agents that inhibit gap junctions (104). The Ca^{2+} wave is not dependent on ATP or other compounds released by the stimulated cell acting in a paracrine fashion (33). These observations indicate that the movement of $Ins(1,4,5)P_3$ through gap junctions mediates the propagation of the Ca^{2+} wave initiated by mechanical stimulation of a single cell. The concentration of $Ins(1,4,5)P_3$ required for each cell to propagate the Ca^{2+} wave may not be achieved by passive diffusion of $Ins(1,4,5)P_3$. A Ca^{2+} -independent regenerative production of $Ins(1,4,5)P_3$ may be necessary (123).

A gap junction is constructed from transmembrane proteins that form structures called connexons, which are composed of six subunits called connexins. In addition to airway epithelial cells, mRNAs for the gap junctional connexins 26, 32, and 43 were found in freshly isolated type II alveolar epithelial cells. The expression of connexin 43 increased significantly during cell culture. Membrane deformation in single type II cells resulted in an increase in $[Ca^{2+}]_i$, which spread rapidly to neighboring cells by octanol-sensitive mechanisms (56).

Mechanical stimulation can regulate not only the gap junction function but also the amount of gap junction proteins. A recent study (84) demonstrated that the expression of connexin 43, but not of connexin 26, in the rat myometrium during pregnancy and labor is regulated by mechanical stretch. It is possible that connexin expression is a tissue-specific response to mechanical strain. Hypertension increased expression of connexin 43 in the rat aorta but not in cardiac muscle (30).

In addition to gap junctions, which are specialized for intercellular communication, cells have two other types of junctions: occluding and anchoring (53). Occluding junctions or tight junctions can seal cells together in an epithelial sheet. Anchoring junctions mechanically attach cells and their cytoskeleton to their neighbors or to the ECM. Cadherins, a group of transmembrane linker proteins, play an important role in cell-cell anchoring junctions by holding the interacting plasma membranes together. These junctions cannot transmit chemical signals but can transmit physical forces directly from one cell to another. For example, the alveolar epithelial surface is mainly covered by type I cells that are stretched during respiration or mechanical ventilation, whereas type II cells are localized at the corners of

alveolar spaces. To affect functions of type II cells, mechanical forces need to be transmitted from type I cells to type II cells. The role of tight junctions and anchoring junctions in intercellular mechanotransduction should be investigated.

Cell-Matrix Interaction

Many cell types in an integrated organ such as the lung share the ECM. Most cell types in lung tissue contribute to the dynamic changes in the ECM. By changing the components and structure of the ECM, cells are continuously remodeling their microenvironment. A change in ECM components and structure influences the proliferation and differentiation state of the cells. Cells can be switched between growth and differentiation states or between survival and apoptosis by modulating cell shape via changes in cell-ECM interactions (15). As discussed in previous sections, the ECM is involved in mechanoreception via the ECM-integrin-cytoskeleton pathway, and mechanical stimulation affects the turnover of ECM molecules. This reciprocal interaction between cells and their matrix adds to the complexity of intercellular communication.

In fetal rat lung cells, the growth-promoting effect of strain was observed only when cells were cultured in a three-dimensional environment (67). Intracellular signal transduction and the morphology of fetal lung cells also varied according to the two- or three-dimensional culture environment (67). These data imply that cell shape or cytoskeletal architecture determined by the spatial environment of cells plays a crucial role in mechanical deformation-induced cellular activity (67). In addition to the spatial structure of the ECM, the responses of cells to mechanical stimulation are also influenced by ECM components. The mitogenic response of rat vascular smooth muscle cells to mechanical strain was matrix dependent. Strain increased DNA synthesis in cells on collagen, fibronectin, or vitronectin but not in cells on elastin or laminin (143). Many growth factors are present in the ECM in their latent form. Cleavage of growth factors from the ECM or cleavage of growth factors from their latent precursor may also be an important regulatory mechanism for intercellular signaling.

FUTURE STUDIES

Studies of the effects of mechanical forces have, to date, been initiated and conducted on the major cell types in the lung. Undoubtedly, such studies will increase as new devices and techniques are developed and with our improved understanding of lung cell and molecular biology. Although it is impossible to predict future directions for this proliferating research field with any degree of certainty, we would like to draw attention to several specific issues.

Mechanical Force-Induced Specific Effects in the Lung

To explore specific effects of mechanical stimulation on lung cells, the unique structure and physiological characteristics of the lung have to be considered. For

example, the role of endogenous nitric oxide production by the pulmonary endothelium in maintaining the relatively lower vascular resistance in the lung is very important. It has been shown that gene expression of endothelial nitric oxide synthase is regulated by shear stress (88, 136) and cyclic strain (3). It will be interesting to know how pulmonary endothelial cells respond to shear stress and/or mechanical strain under reduced pressure and higher flow rate. Genes specifically sensitive to shear stress have been identified by differential display. With this technique, one can first select genes that are differently expressed between control and treated conditions, i.e., shear stress, and then determine the role of these genes and their products in mechanotransduction. With this strategy, it has been found that cyclooxygenase-2, manganese superoxide dismutase, and endothelial nitric oxide synthase are selectively increased by steady laminar shear stress (129). The effect of shear and strain on gene expression in lung cells can be investigated with this and other advanced molecular techniques.

Physical Force Disorders and Pulmonary Diseases

Many studies have focused on the effects of mechanical forces on physiological functions of lung cells. There are few reports about the effects of mechanical factors on cell injury in the development of lung diseases such as congenital diaphragmatic hernia, asthma, pulmonary hypertension, fibrosis, barotrauma, and ischemia-reperfusion injury of lung transplants. There are two strategies that can be used to study the contribution of physical factors in these diseases. One can collect cells from animal models of these diseases or from human patients and then study their responsiveness to mechanical stimulation *in vitro*. Alternatively, if the properties of abnormal physical factors are well defined, one can simulate these conditions with the various devices previously discussed. For example, one can compare the effects of overstretch versus normal stretch; can stretch cells with different amplitudes, frequencies, and stretch-relaxation ratios; and can apply mechanical stimulation in the presence of other injurious physical factors such as hyperoxia, hypoxia, and ischemia-reperfusion.

Interaction Between Physical Factors and Other Factors

In most mechanotransduction studies, identifying the effect of mechanical stimulation on cells requires that the role of other factors be excluded during the initial studies. With established cell culture models, the interaction between mechanical stimulation and other confounding factors can be studied with various combinations. For example, fetal lung maturation is controlled by mechanical and hormonal factors. The contribution and interactions between these factors can be elucidated under better-controlled culture conditions. Similarly, both physical force disorders and other confounding factors influence the pathogenesis of lung diseases. The interactions between physical forces and

bacterial products, cytokines, chemokines, and other injurious factors can be simultaneously tested on cultured cells.

The signal transduction pathways initiated by mechanical stimulation can be altered significantly in the presence of other stimuli. The expression of a particular gene is usually controlled by several transcriptional factors in its promoter and enhancers. Physical forces and other factors may selectively influence their activities. Furthermore, the multiple steps from gene transcription and translation to posttranslational modification can also be regulated differently by physical forces and other signals.

Combining In Vitro and In Vivo Studies

In this article, we have focused on information collected from studies conducted at the cellular level. Cell culture has the advantage of studying the effects of physical forces on one particular cell type under well-controlled conditions. However, the complexity of cell-cell and cell-matrix interactions and the complicated intercellular communications between different cell types cannot be completely simulated in cell cultures. Cellular behaviors and phenotypes are influenced by culture conditions including nutrients, growth factors, the culture substratum on which the cells are attached, and the geometric structure of the culture substratum. Therefore, studies with isolated organs or tissue strips are useful approaches to bridge the cell culture studies to those conducted at organ or whole animal levels. Many newly developed cellular and molecular techniques can be applied in vivo (19). For example, transgenic animal models can be used to study the effect of physical forces at a particular stage of lung growth or a lung disease by switching on the transgene expression at that particular time (75). Furthermore, lung tissues collected from surgical operations are invaluable for confirmation of in vitro mechanotransduction studies. It is likely that combined investigations both in vitro and in vivo will increase our understanding for the role of physical forces in normal lung cell function and in the development of pulmonary disease.

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