Contractile response of alveolar epithelial cells to biochemical or mechanical stimulation probed by traction microscopy

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Chapter 6. Conclusions of the thesis

Implementation of a traction microscopy setup specifically adapted to measure the contractile forces of human alveolar epithelial cells in culture

1. A traction microscopy setup to study the contractile properties of human alveolar epithelial cells in culture has been implemented. The setup developed allows determination of the total force magnitude exerted with ~ 1μm spatial resolution and 5 nN force resolution (aims 1.1 and 1.2).
2. A software to determine the contour of an adhered cell from a brightfield or phase contrast image of the cell has been developed. Unlike most of the traction microscopy studies reported to date, this software enables objective determination of the cell contour (aim 1.3)

Contractile response of human alveolar epithelial cells to a biochemical stimulus (thrombin)

3. A protocol for constructing suitable polyacrilamide gels to perform traction microscopy experiments on human alveolar epithelial cells has been adjusted. In addition, the elastic properties of the gels have been determined using atomic force microscopy (aims 2.1 and 2.2).
4. The time-course of the contractile response of alveolar epithelial cells to thrombin challenge has been measured. Thrombin (1 U/ml) challenge induced a fast 2.5-fold increase in total force magnitude. Thrombin-induced contraction reached a plateau 8 min after challenge (aim 2.3).

5. The distribution of contractile forces exerted by adhered cells before and after thrombin stimulation was studied. Contractile forces were mainly exerted at the cell periphery and pointed towards the nucleus. Thrombin addition did not change force distribution but increased the centripetal direction of forces (aim 2.4).
6. Actin polymerization and reorganization induced by thrombin challenge was observed. Thrombin induced actin polymerization and formation of a peripheral actin rim (aim 2.5).
7. The role of the actin cytoskeleton on the thrombin-induced cell contraction was studied. Depolymerization of the actin cytoskeleton with cytochalasin D inhibited thrombin-induced cell contraction and actin polymerization (aim 2.6).
8. The role of pathways signalling MLC phosphorilation in the contractile response to thrombin were studied. Inhibition of MLCK with ML7 inhibited the contractile response induced by thrombin but not actin polymerization. Inhibition of Rho-kinase with Y-27632 inhibited thrombin-induced contraction and reduced actin polymerization (aim 2.7).

Contractile response of human alveolar epithelial cells subjected to stretch.

9. A protocol for constructing suitable collagen gels which firmly attached to silastic membranes and supported ~14% lineal deformation was adjusted. In addition, the elastic properties of the gels at different strain levels were determined using atomic force microscopy (aims 3.1 and 3.2).
10. A stretching device to apply controlled biaxial and uniform strains to cultured cells and simultaneously measure contractile forces by deforming the gel substrate to which they are adhered has been designed, implemented and validated (aim 3.3).
11. Contractile forces of human alveolar epithelial cells before, during and after being subjected to a stepwise deformation were measured. Stretch application induced increase in contractile forces up to 2-fold for 11% linear strain. This increase depended on the amount of applied stretch (aim 3.4).
12. Stretch release resulted in cell relaxation, reaching 33% of baseline value for 11% linear strain. Cell relaxation also depended on the amount of previously applied stretch (aim 3.4).
13. The role of actin polymerization in the contractile response to stretch was studied. No significant changes were found on the amount of polymerized actin before,

during or after stretch release (aim 3.5).

14. The role of actomyosin crossbridges detachment in the contractile response to stretch was studied. When contractile force generation was reduced by inhibiting MLCK, the relative increase in traction forces due to stretch application was larger (140% vs baseline) than in control conditions (45% vs baseline) (aim 3.6).
15. The relative decrease in contractile forces after stretch release in MLCK inhibited cells was smaller than in control conditions (aim 3.6).
16. Together, conclusions number 14 and 15 suggest that stretch induces actomyosin crossbridges detachment (aim 3.6).
17. The temporal changes in cell contractility after stretch release were measured. After stretch release, contractile forces progressively recovered, reaching the baseline values 8 minutes later (aim 3.7).

General conclusions

18. Thrombin and stretch, which are stimuli characteristic of ALI/VILI, induce contraction of alveolar epithelial cells.
19. The observed cell contraction can impair the force balance of the alveolar epithelial barrier. Potentially resulting paracellular gaps could potentially initiate *the novo* injury in healthy lungs or exacerbating pre-existing lung injury.