The effects of cyclic stretch on the cytoskeleton and its role in exogenous gene expression in the alveolar epithelium

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ABSTRACT

Cells in the lung undergo cyclic deformation under both normal breathing processes as well as mechanical ventilation. While large deformations due to disease or mechanical ventilation can cause cellular damage such as that induced by cyclic stretch, our lab has found that smaller, physiologically relevant deformations have a positive effect on gene transfection in an in vitro pulmonary epithelial model, increasing reporter gene products up to 12-fold in A549 cells. This result is not surprising considering exogenous mechanical forces have been shown to alter the same cellular barriers efficient gene transfer faces; namely changes in the cytoskeleton and extracellular matrix, activation of cell signaling pathways and changes in transcription factor activation. Previous studies from our lab have shown that this stretch-induced increase in exogenous gene expression is not at the level of transcription or translation, nor is it at the level of cellular entry, suggesting increased trafficking of the plasmid DNA through the cytoskeleton toward the nucleus. To understand what happens to these cells under stretch conditions, we grew A549 cells on deformable elastic membranes to 85% confluency and found that imposing a 10% change in membranes surface area in an equibiaxial manner at 30 cycles per minute with a duty cycle of 50% caused large cytoskeletal rearrangements within 30 minutes of the applied stretch. Stabilization of either the actin or the microtubule cytoskeleton prevented the stretch-induced increase in gene expression; however depolymerization of either cytoskeleton was insufficient to increase gene expression. Due to this, our current investigations focus on determining what stretch-induced events, beyond cytoskeletal depolymerization, are critical to enable cells to undergo stretch-enhanced gene transfer.

INTRODUCTION

Gene therapy is a promising medical advancement with the potential to revolutionize disease treatment protocols. However, current gene delivery techniques are limited by their inability to deliver therapeutic levels of exogenous genes to patients, thus driving research to find better gene delivery modalities. The goal of our research is to understand and develop new approaches for gene delivery under conditions of mechanical stretch that mimics those found during lung injury and its management (i.e., ventilation). A significant number of studies over the past two decades have examined how cells sense mechanical signals, as well as how they respond to these signals both in vivo and in vitro. These responses can be broadly classified into four distinct areas: alterations in the cytoskeleton and extracellular matrix, activation of cell signaling pathways, alteration of transcription factor function and gene regulation, and changes in cell proliferation. Intriguingly, these responses also directly influence the process of gene delivery to cells and tissues.

Exogenous DNA, either viral or non-viral, must cross the plasma membrane and enter the cell, travel through the cytoplasm, enter the nucleus, and be transcribed in order for gene transfection to be successful. Due to the limited diffusion capabilities of plasmid DNA, movement through the cytoplasm is likely to be achieved via an active process. This process may utilize cytoskeletal elements as a key component for transport. Once the DNA reaches the nucleus, we have previously shown that plasmid DNA requires specific transcription factors to confer nuclear import. With mechanical stress altering both the cytoskeletal levels and subcellular localization of many transcription factors, it is likely that mechanical strain will play a large role in gene delivery.

CONCLUSIONS

• Physiologic cyclic stretch (10% change in basement surface area) causes a 4.4-fold increase in exogenous gene expression in pulmonary epithelial cells.

• Cyclic stretch causes significant (50%) depolymerization and rearrangement of the microtubule network within 30 minutes of stretch. Furthermore, these changes are not reversible as determined by static growth out to 24 hours.

• Cyclic stretch does not significantly change the actin network, with a less than 20% change identified by biochemical assays. However, fluorescent microscopy suggests a noticeable rearrangement of the network.

• Cytoskeletal depolymerization is necessary, but not necessarily sufficient for enhanced exogenous gene expression.

• MAPK activation, specifically p44/p42 activation, resulting from stretch appears to play an important role in exogenous gene expression.

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