# Modulation of EMT Gene Expression Using CRISPRa/i to Investigate Lung Cancer Mechanosensation

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Cellular mechanosensation is an important biological function that enables cells to sense the mechanical cues of its extracellular environment and to change phenotypes and cell lineage accordingly. Most growing primary tumors originate from stationary epithelial cells and undergo epithelial-mesenchymal transition (EMT) to trans-differentiate and transform into mobile and invasive mesenchymal cancer cells, allowing cancer to metastasize. This study aims to establish and utilize a CRISPRa/i (activation/interference) system to modulate the gene expression of EMT Markers in A549 lung cancer cells to study phenotypic changes. To establish the CRISPRa/i system, I used a 2nd generation lentiviral transduction system as a delivery method to stably integrate desired plasmids into the host cell genome for efficient and long-term expression. The delivery process is separated into two stages. I created lentivirus containing the components of CRISPRa/i system. Then I transduced the lentivirus into A549 cells and separated A549 CRISPRa and CRISPRi cell lines. I then modulated LMNA expression and used Western Blots to measure Lamin A/C protein level. I observed successful over/under expression of Lamin A/C comparative to the control, verifying the CRISPRa/i system. I then utilized the successful CRISPRa/i system to modulate the expression levels of specific EMT markers in A549 mechanosensitive cells to find the factors that drive mechanosensation. I then cultured these transduced A549 CRISPRa/i cells in soft/stiff gels and found phenotypic changes. Certain modulated A549 CRISPRa/i cells acted abnormally in soft/stiff gels, suggesting reduced or altered mechanosensing activity. This information can be used to further study cancer invasitivity.

**Awards Won:**