Characterization of Lung Cancer Mechanosensation

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This study is a proof-of-concept to validate if CRISPR a/i (activation/interference) system can modulate LMNA gene expression in lung cancer cells. To establish the CRISPRa/i system, I used a 2nd generation lentiviral transduction system as an effective delivery method of stably integrating desired plasmids into the host cell genome for efficient and long-term expression. The delivery process is separated into two stages: lentiviral particle production by transfecting HEK293T cells and transduction of the lentiviral particles into target A549 cells. Three plasmids {packaging plasmid, envelope plasmid, transfer plasmid} were transfected into HEK293T cells to utilize the host cell to produce lentiviruses. Following an optimized lentiviral production protocol, 3 lentiviruses for 3 different transfer plasmids of interest {Plasmid #1 VP64-GFP, Plasmid #2 dCas9-10XGCN4-BFP, Plasmid #3 dCas9-KRAB-BFP} were produced and transduced into A549 cells. Then, I used fluorescent cell sorting to select for GFP/BFP expressing cells and create A549 CRISPRa and CRISPRi cell lines. After transducing both cell lines with another lentivirus containing the gRNA for LMNA gene, I performed Western Blots to measure Lamin A/C protein level to determine if LMNA expression was modulated. I observed successful over/under expression of Lamin A/C comparative to the controlled expression of B-actin. This evidence verifies the CRISPRa/i system can modulate the expression of any targeted gene in A549 cells. Since this is a continuation project, next year, I plan to utilize the CRISPRa/i system to modulate the expression levels of specific EMT markers in A549 mechanosensitive cells to find the factors that drive mechanosensation.

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