402-Excellence-8-CRISPRi screening- Executive Summary

CRISPRi screening in cancer spheroids to investigate factors in genome stability

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Background. Non-small cell lung cancer (NSCLC) has a 28% 5-year survival rate and limited treatment options according to the American Cancer Society (2025). A major obstacle in treating NSCLC is the continuous evolution of cancer genomes, which leads to frequent mutations, clonal diversity, and ultimately, resistance to therapeutic treatment. Although 2D cancer cell cultures provide *in vitro* data for BME applications like early cancer drug validation, 3D cell models, known as spheroids, provide a more accurate recapitulation of the *in vivo* environment. Spheroids can be used for studying cancer progression and therapeutic responses *in vitro* without the disadvantages of animal models and with more biological relevance than 2D culture. Increasing the scale of the spheroid model is necessary for analyzing the significance of 2D and 3D differences through RT-qPCR and γH2AX staining to assess DNA damage through double strand breaks (DSBs).

Protocol Optimization. First, the passaging schedule of A549 cells and dependence of doubling time on cell density was established. A549 spheroids were formed using ultra-low attachment plates: an inexpensive and common method. Tissue culture plates were coated with a hydrophilic polyHEMA film that causes cells to self-aggregate into spheroids 2-3 days after seeding. Various conditions, methylcellulose concentration and seeding density, were repeatedly tested to find out the ideal condition. Spheroid count, size, and cell viability were optimized at 0.75% methylcellulose and 75k cells/cm^2. Then, spheroids were dissociated into single cells to determine the doubling time of A549 cells in 3D culture; this protocol was needed for the subsequent RT-qPCR and γH2AX staining protocols. The accutase dissociation protocol, originally written by Honeder et al. (2021), was optimized using available Hess lab equipment to ensure minimal cell loss.

Testing and Results. For spheroid formation, size, and abundance verification testing, the team imaged a 96 well plate with varying methylcellulose concentrations and seeding densities using BioTek Cytation. Next, the CellTiter-Glo® Luminescent Cell Viability Assay was used to compare cell survival in various spheroid conditions. Then, a γH2AX stain was completed to compare prevalence of DSBs in 2D WT A549s, etoposide (common cancer drug) treated 2D WT A549s, spheroids, and etoposide treated spheroid populations. These results confirm the hypothesis that there is an increase in DSBs in etoposide versus control and 3D versus 2D. RT-qPCR provides statistically significant evidence that there are key differences in genetic expression between the 2D and 3D populations.

Future Work. Hess lab will make A549 CRISPR interference (CRISPRi) cell line spheroids and run additional experiments. In addition to RT-qPCR and γH2AX stain data, these protocols will be used for a genome-wide CRISPRi screen to identify tumor-specific factors that regulate genome stability in tumor formation and metastasis.