



Department of  
Biomedical Engineering  
UNIVERSITY OF WISCONSIN-MADISON

# CRISPRi Screening in Cancer Spheroids

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Clients: Ms. Carley Schwartz and Dr. Gaelen Hess

Advisor: Dr. Paul Campagnola

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# Client Description

- Dr. Gaelen Hess
  - Hess Laboratory within the Wisconsin Institutes for Medical Research (WIMR)
  - Assistant professor in the Department of Biomolecular Chemistry and Center for Human Genomics and Precision Medicine [1].
  - High-throughput genomics to investigate DNA repair and pathogenic effectors [1].
- Ms. Carley Schwartz
  - Lab manager and research intern at Hess Lab



**Figure 1:** a) Dr. Gaelen Hess [1], b) Ms. Carley Schwartz [2].

# Problem Statement

- **Current Limitations:**

Existing CRISPRi screening in 2D monolayers fail to identify factors that regulate genome stability and DNA damage in the 3D environment of tumors

- **3D Models:**

Using a 3D spheroid model the tumor microenvironment can be semi-replicated to better assess gene function and cellular responses

- **Project Objective:**

The project aims to identify tumor-specific factors that regulate genome stability using CRISPRi screening in optimized 3D model to assess DNA damage

# Motivation & Impact

## Motivation

- 2.2 million new cancer cases and 736,790 deaths reported in the United States in 2024 [3]
- 2D monolayer fails to accurately represent the 3D tumor microenvironment [4]
  - Increased cell-cell and cell-matrix interactions
  - Better recapitulation oxygen and nutrient diffusion compared to 2D
- 3D CRISPRi screening can help identify sources of DNA damage and regulatory genes not found with 2D cultures

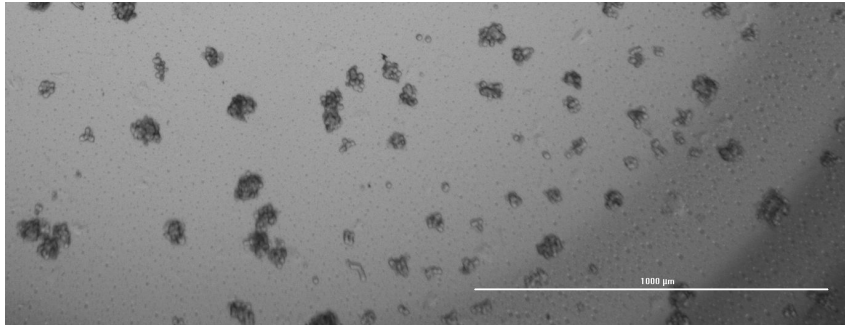
## Impact

- Early cancer detection
- 2D and 3D genetic differences on a cellular level
- Efficacy of cancer treatment

# Previous Work

## Cell Line Selection: A549 [5]

- Non-small cell lung cancer (NSCLC)
- Adenocarcinoma
- Adherent
- 50  $\mu\text{m}$  cell diameter
- Doubling time: 22 hours



**Figure 2:** Brightfield image of spheroids 3 days after seeding at density of 75k cells/cm<sup>2</sup>

## Spheroid Formation Protocol:

### PolyHEMA Plates:

- Stock: 1.3 g poly-HEMA + 33 mL 99% ethanol
- 50  $\mu\text{L}$  of PolyHEMA stock per well in 96-well flat-bottom plate

### Spheroid Seeding:

- Seed cells in 320  $\mu\text{L}$  of serum-free DMEM with 0.75% methylcellulose/cm<sup>2</sup>
- Ideal density: 50k -75k cells/cm<sup>2</sup>

### Spheroid Imaging:

- Dissociated cells with Accutase (150  $\mu\text{L}$ /well) after 3-4 days [6]
- BioTek and Cytation to image cells at 5 z-stack levels per well



# Project Design Specifications

1

## Optimization:

Perform a cell viability assay to analyze additional testing to determine optimal cell seeding density for best spheroid formation results

2

## Optimization 2:

Run testing on differing methylcellulose concentrations to optimize spheroid formation.

3

## BSL-2 Safety:

Handling infectious agents must be conducted in BSCs by trained personnel in a restricted laboratory

4

## Staining:

Develop a protocol to stain for  $\gamma$ H2AX: an indicator of DNA damage by DSB [7]

5

## Screen:

Perform a high-throughput genome-wide CRISPR screen.

6

## Budget:

\$1000



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# Timeline

- Spheroid repeat (2/14)
  - Cell density 50k cells/cm<sup>2</sup>: 0.75%, 1%, and 1.25% Methylcellulose/cm<sup>2</sup>
  - Cell density 70k cells/cm<sup>2</sup>: 0.75%, 1%, and 1.25% Methylcellulose/cm<sup>2</sup>
- qPCR for SOX2 on spheroids with ideal methylcellulose and ideal density (2/21)
  - SOX2 contributes to spheroid formation of A549 [8]
  - Choose and order primer
- Hess lab Gamma-H2AX stain #1 (2/28)
  - 2D without and with drug (etoposide) treatment of A549 Line
  - 3D without and with drug treatment of A549 Line
- Hess lab Gamma-H2AX stain #2 (TBD)
  - Tweak protocol after stain #1
  - Same experimental groups

# Budget

- Spheroid cost estimate for one trial was \$92.62
- Team will be doing this three times: \$277.86
- Many of the materials used are provided by the Hess Lab: Flasks, A549 cell line, DMEM, FBS, Trypsin, etc
- Hess Lab is providing Etoposide for treating spheroids to prepare for  $\gamma$ H2AX stain
- Primer for qPCR estimated at \$109.00 for 0.5 mL GAPDH [5]



# Final Prototype

1. Spheroid formation protocol: seeding density and methylcellulose concentration optimized
2.  $\gamma$ -H2AX staining protocol: optimized for 3D
3. Scale up spheroid formation (50 million cells) for client to proceed with CRISPRi screening on A549 spheroids for tumor specific factors

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# References

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