Background

- Immunotherapy
- Successes against certain blood cancers with CAR T cell therapy highlights the potential of immunotherapy. However, CAR T cell therapy has little effect against solid tumors.
- NK cell advantage: it can kill without antigens. Especially with dense tumors, cancer cells often downregulate their major histocompatibility (MHC) class I antigens.
- Drug development
- Large gap between 2D models and human body means that most in vitro drugs fail to work past clinical trials (Huang, Guo, 2018).
- 3D tumor spheroids exist as complex in vitro models with a nutrient gradient, a hypoxic / quiescent tumor core, and tumor density.
- Cheaper, more ethical, and more efficient than human tests and much more accurate
- 4T1 cancer cell line
- Adherent murine breast cancer cell line widely used as a model for stage IV human breast cancer. (Yoo et al., 2017)
- Easy to maintain and grow and forms spheroids on its own.

Objectives

- Short-Term: Set a control to establish effect of PBMCs on 4T1 cells to as a baseline for drug treatments.
- Long-Term: Determine specific treatments for cancer cells or immune cells to facilitate NK cell killing of dense tumors. Increase immune cell penetration of solid tumors to decrease the chance of cancer relapse.

Method

1. Growing and Maintaining Cells
   - Add medium into flask with cells
   - Place in incubator at 37°C and 5% CO₂
2. Seeding Cells
   - Add 1750 4T1 cells in 75 microtiter per well of Ultra-Low Attachment 384 well plate with BioTek MultiFlo automated microplate dispenser
3. Drug Treatment
   - Prepare solutions of Dactinomycin, Mycophenolic acid, Daunorubicin, Piplartine, Tylosin
   - Add to well plate with BioTek MultiFlo and Microlab STAR
   - Incubate for 15-20 min in incubator at 37°C and 5% CO₂
6. Imaging with operetta
   - Set plate and plate map
   - Select dye channels
   - Run

Result

- Spheroid Formation
  - Day 0: Spherical Area [um²] – Mean per well (10^3)
  - Day 3: Spherical Area [um²] – Mean per well (10^3)

- Spheroid Timeline: spheroid formation, drug addition, PBMCs

- PBMC effect on tumor spheroids

References


