A 3D Breast Tumor Model to Identify Treatments Against Cancer Cell Invasion

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Statement of Purpose: Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype that often metastasizes and is very difficult to treat. This is due to a lack of estrogen and progesterone receptors and non-amplified HER2 that make targeted therapies not feasible [1]. Thus, chemotherapy is the mainstay treatment for TNBC. However, chemotherapy drugs mainly prevent cell growth and do not necessarily affect mechanisms of cell invasion that is a critical process leading to metastasis. This study aims to develop a 3D model of TNBC cell invasion and examine the anti-invasive efficacy of common chemotherapeutics as well as molecular inhibitors.

Methods: Two TNBC cell lines were used, MDA-MB-231 and SUM159. Cells were transfected to express a green fluorescent protein. Tumor spheroids were made from each cell line by confining cells in an aqueous polyethylene glycol (PEG) phase drop immersed in an aqueous dextran phase, resulting in self-assembly of cells into a spheroid [2]. The spheroids were encapsulated in human-derived type I collagen, as the dominant fibrillar extracellular matrix protein in breast tumors (Figure 1). The spheroids were treated does-dependently with chemotherapeutics (5-FU, carboplatin, doxorubicin, gemcitabine, and paclitaxel) or molecular inhibitors (dactolisib and trametinib). After four days for the MDA-MB-231 model or six days for the SUM159 model, the models were imaged with a confocal microscope in 20 µM slices, which were collapsed to create a single image (Figure 2). Invasion was quantified by measuring the total pixel area of the invading cells from the collapsed z-stack image for each spheroid. The invasion areas were used to create a dose response curve and to find the IC_{50} concentration for each pair of drug and cell line.

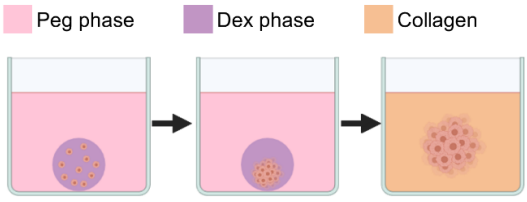

Figure 1: Process of forming tumor models using aqueous two-phase system technology.

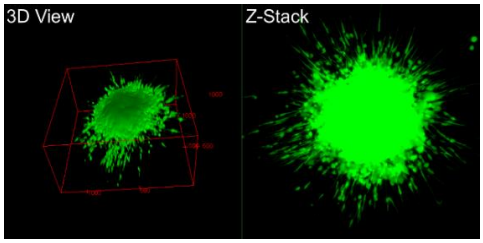

Figure 2: 3D and 2D collapsed z-stack of SUM159 spheroid.

Results: We found that three of the drug compounds effectively inhibited collagen invasion of TNBC cells. Doxorubicin gave a maximum invasion inhibition of MDA-MB-231 cells by 94% (IC_{50}= 1364 nM) and of SUM159 cells by 98% (IC_{50}=227.9 nM). Paclitaxel inhibited MDA-MB-231 cell invasion by 98% (IC50= 0.0064 nM) and SUM159 cell invasion by 98% (IC50= 0.30 nM). Trametinib showed the highest inhibition of invasion of MDA-MB-231 cells by 100% (IC_{50}=1.35 nM) and of SUM159 cells invasion by 57% (IC_{50}=92.7 nM) (Figure 3). Other compounds did not significantly inhibit invasion of cancer cells.

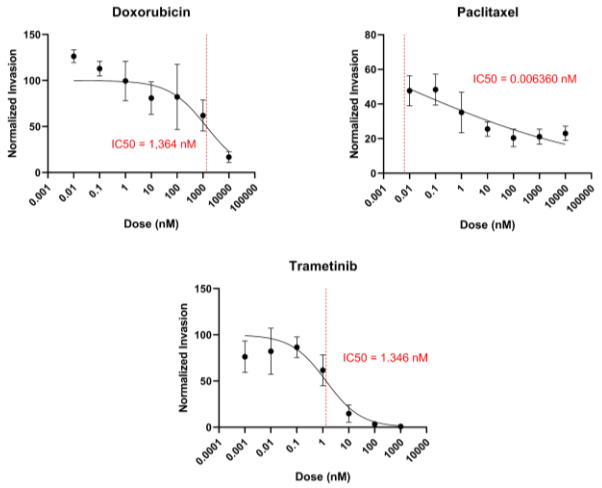

Figure 3: Dose response curves of MDA-MB-231 spheroids treated with doxorubicin, paclitaxel, and trametinib. The IC_{50} value have been shown in red.

Conclusions: The 3D tumor models enabled us to determine which chemotherapeutics or molecular inhibitors are effective at preventing invasion of TNBC cells. While cytotoxic chemotherapy drugs doxorubicin and paclitaxel had inhibitory effects against cell invaison, inhibitors of MAPK pathway that is active in TNBC cells produced the greatest benefit. Therefore, future studies should evaluate a broader panel of such inhibitors not only against tumor growth, but also for their potency against invasiveness of cancer cells. Additionally, the efficacy of these treatments in more complete 3D tumor models containing soluble proteins and stromal cells may help guide treatments selections against invasion and metastasis of TNBC.

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References:

1. Gluz, O., et al., *Triple-negative breast cancer—current status and future directions.* Annals of Oncology, 2009. **20**(12): p. 1913-1927.
2. Atefi, E., et al., *High Throughput, Polymeric Aqueous Two-Phase Printing of Tumor Spheroids.* Advanced Functional Materials, 2014. **24**(41): p. 6509-6515.