Modeling the Metastatic Niche Interactions Between Patient Tumor and Mesenchymal Cells to Identify Drivers of Chemotherapy Drug Resistance.

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ABSTRACT

Cancer research relies on a plethora of disease-relevant tumor models, but patient specific models are still being developed. In this study, we show the utility of patient-specific pancreatic, ovarian and breast cancer models in the context of the metastatic niche, composed of co-cultured mesenchymal stem cells (MSC) in three-dimensional tumor spheroids. Additionally, different ratios of MSC's were seeded with the cancer spheroids to interrogate the effect of MSC's on chemotherapeutic drug response in defined media conditions. The patient-specific and treatment-naive cancer models represent a diverse range of disease type, progression grade, and genetic profiles. These models were co-cultured with MSC's sourced from adipose tissue. These co-culture models were tested with a panel of common, broad-range chemotherapeutic agents as well as several gene-specific drugs.

The resulting drug responses show that the presence of MSC's within the context of the metastatic niche causes attenuation towards a higher chemotherapeutic drug resistance. The tumor models exhibit a varied chemotherapeutic response when MSC's are seeded in 50:1 or 1:1 ratios with cancer cells, suggesting that even a small presence of MSC's may have a significant effect on the drug resistance of metastatic tumor spheroids. While this MSC-induced drug response attenuation is observed across the broad spectrum of patient profiles and disease types, the strength of the effect is not homogenous. The observed variability in the strength of the effect was impacted by the disease type of the cancer cells, the source of the MSC's, and the specific drug used in the metastatic tumor model.

We propose that using these reproducible and easily scaled models of metastatic tumors to find effective chemotherapeutic drugs could increase our understanding of the tumor microenvironment, the onset of metastasis, and the process of cancer grafting into other tissues. Discovering effective and targeted therapies utilizing a more advanced model of the metastatic tumor niche could increase the success rate of a subsequent clinical trial.

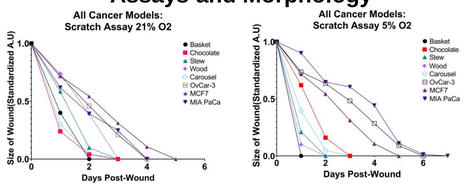
METHODS

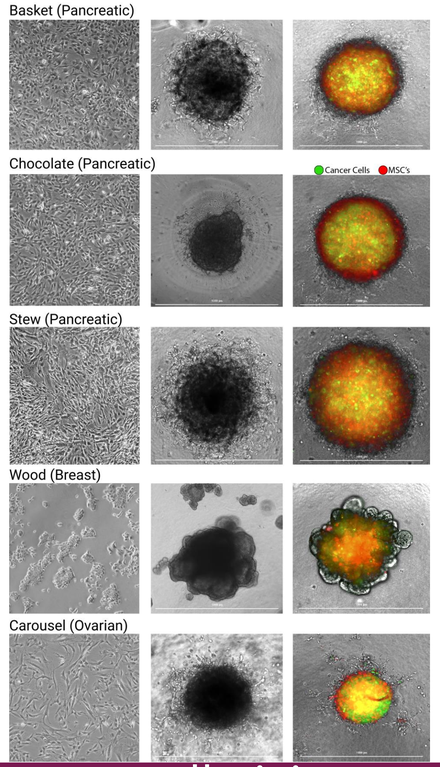
1. All cell models were grown in Cellaria™ **Renaissance Essential tumor medium (RETM**) with 5% FBS.
2. Culture conditions of the cells were either normoxic (21% O2) or hypoxic (5% O2) at 37° C.
3. 3D Spheroids composed of 5K cancer cells and 100/5000 Adipose-Derived MSC's were established in **Corning™ U-Bottom ultra-low-attachment plates.**
4. 3D scaffolds were formed using **Corning Matrigel™**
5. Drug response assays were performed utilizing **ThermoFisher XTT**.
6. Gene expression analysis was performed using the **Nanostring™** platform's Pan-Cancer pathway analysis.
7. Logarithmic curves were visualized using the Cellaria drug response database's visualization toolkit.

A. Cell Model Clinical Profiles

Cell Line	AJCC/UICC Stage	Mutations	Co-Indications	Clinical Info
Basket (Pancreatic)	T3N1Mx	No Known Mutations	Jaundice, Osteoporosis, Anxiety, Arthritis	70-75 Caucasian Female, Naive
Chocolate (Pancreatic)	T3N0Mx	CDKN2A – R58X on exon 2 - Pathogenic TP53 – E326X on exon 9 - Pathogenic KRAS – G12D on exon 2 – Pathogenic	Diabetes Mellitus	50-55, Caucasian Female, Naive
Stew (Pancreatic)	T3N1Mx	KRAS – G12V on exon 2 - Pathogenic TP53 – R337L on exon 10 - Pathogenic WT1 – R434H on exon 8 – Pathogenic	Diabetes Mellitus, Congestive Heart Failure, COPD, Emphysema, Arthritis	65-70, Caucasian Male, Naive
Carousel (Ovarian)	T1cN0M1	No Known Mutations	N/A	65-69, Caucasian Female, Naive
Wood (Breast)	T2N0M0	EGFR – E424Q on exon 11 – Variant of Unknown Significance	N/A	60-65, Caucasian Female, Naive

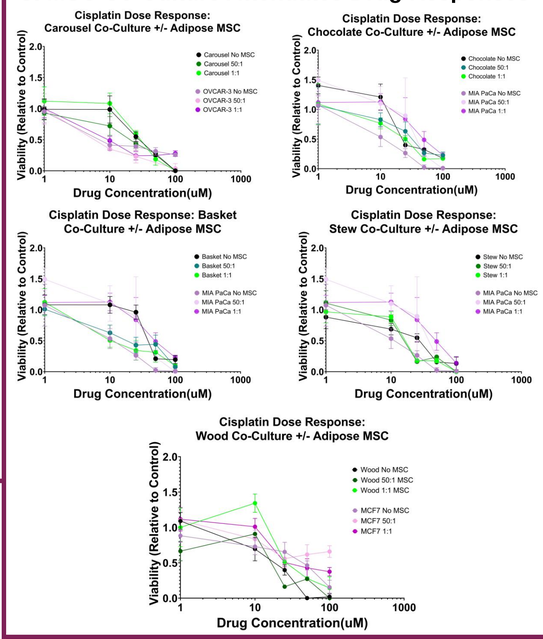
B. Cell Model Characterization: Scratch Assays and Morphology



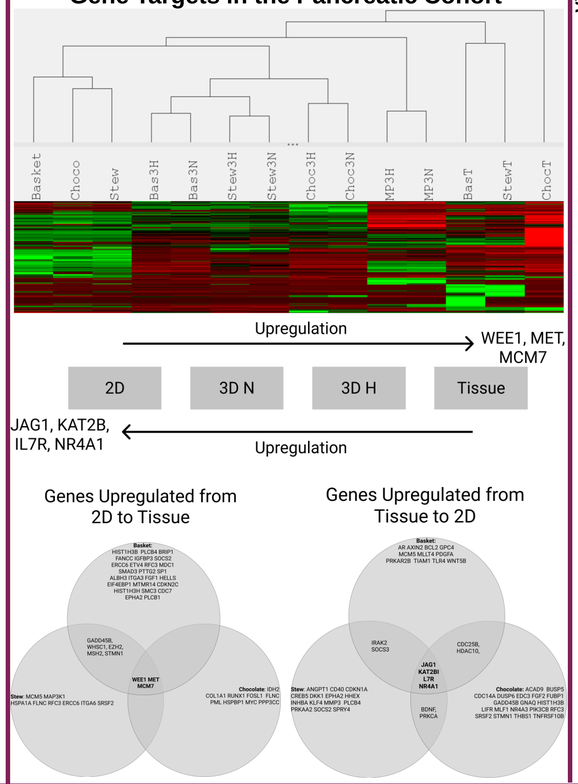


RESULTS

C. MSC Co-Culture Attenuates Drug Responses



D. Altering Cell Culture Format Helps Identify Gene Targets in the Pancreatic Cohort

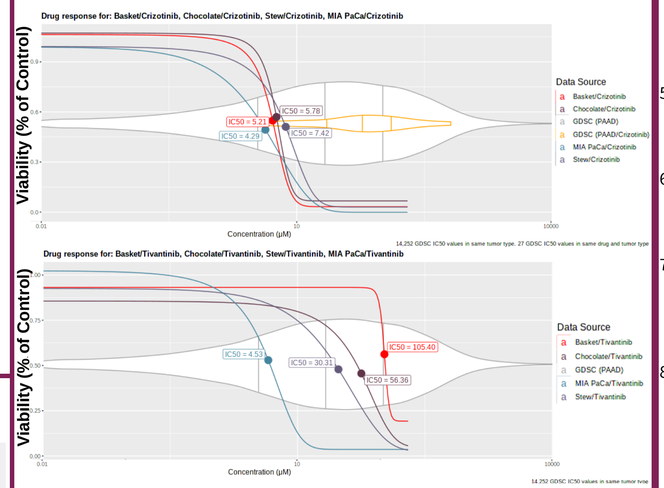


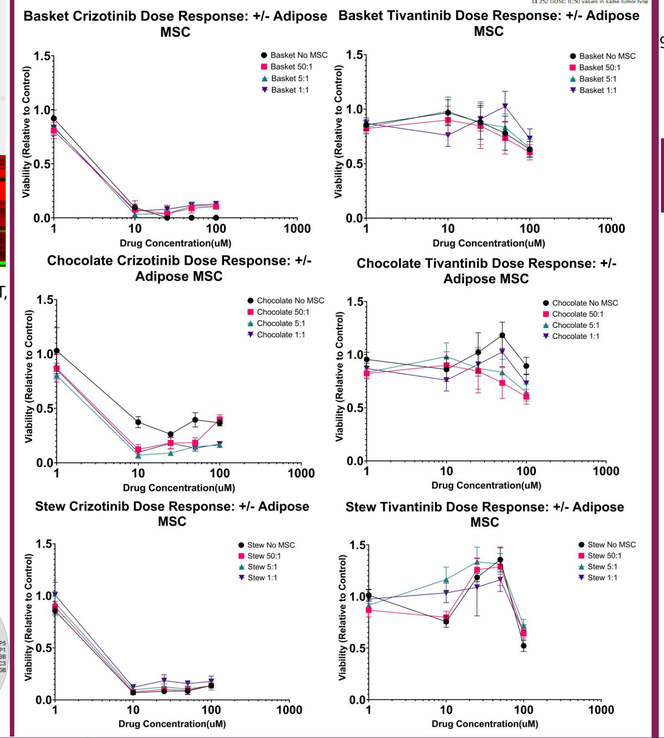
Basket (Pancreatic) MET Expression			
Format Transition	2D to 3D N	3D N to 3D H	3D H to Tissue
Log2 Change	0.38	0.71	3.77

Stew (Pancreatic) MET Expression			
Format Transition	2D to 3D N	3D N to 3D H	3D H to Tissue
Log2 Change	0.09	0.12	1.6

Chocolate (Pancreatic) MET Expression			
Format Transition	2D to 3D N	3D N to 3D H	3D H to Tissue
Log2 Change	0.1	0.74	3.55

E. MET-Targeting Drugs are Effective Across Growth Format





KEY FINDINGS

1. Spheroid models of patient-specific cancers have specific gene expression patterns that are present in the initial biopsy and not in the planar (2D) tissue culture format.
2. A hypoxic (5% O2) tissue culture condition changes gene and cell motility patterns in patient-specific cancer models[1].
3. In Cellaria models of the metastatic tumor niche, all patient-specific cell types readily formed spheroids with mesenchymal stem cells when grown in RETM media [3 4 5].
4. Assessing the gene expression changes that occur between planar, three-dimensional growth formats, and the original patient biopsies produced several gene targets for drug screening.
5. MET gene expression gradually increases from planar to biopsy levels and was observed among all three models of the Pancreatic patient cohort [2].
6. Despite maintaining a similar trend, expression levels of MET varied significantly within the cohort of pancreatic models.
7. C-MET Targeting drugs are not equally effective due to possible differences in their mode of action, and 3D culture shows an increased resistance to MET-targeting drug efficacy observed in planar cultures[8].
8. Adipose-derived MSC's (adMSCs) attenuate Cisplatin drug responses in different ways. A 46% *decrease* in Cisplatin resistance was seen in Basket 1:1 co-culture (Figure C.) Additionally, a 52% *increased* resistance to Cisplatin was seen in the Wood 1:1 co-culture model of breast cancer (Figure C).
9. Metastatic niche modeling using adMSCs did not affect the pancreatic cancer models' response to MET-Targeting drugs[8], contrary to the Cisplatin responses as noted above.

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