Macrophage Incorporation into a 3D Perfusion Tri-Culture Model of Human Breast Cancer

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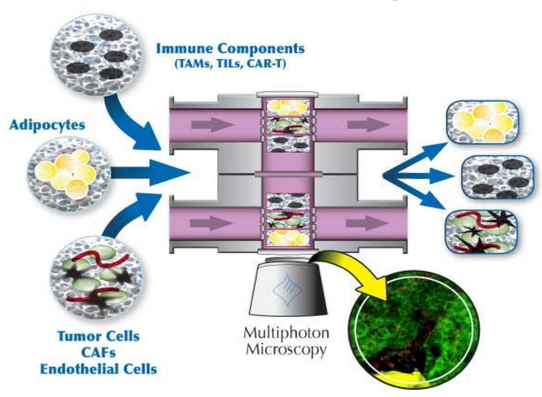
Background

Immunotherapy has recently shown promising clinical activity in multiple tumor types but standard *in vitro* immuno-oncology models for preclinical and clinical predictivity are lacking. Macrophages have been shown to have both tumor promoting and tumor preventing properties dependent upon their differentiation state with M2 macrophages thought to promote tumor growth via enhanced angiogenesis, metastasis and immune evasion. 3D tissue models have been shown to better represent the growth and function of breast tumors and the incorporation of other cell types, such as fibroblasts and adipocytes, and perfusion flow has have been shown by us and others to produce more relevant drug responses. An extension of this work, we have incorporated patient-derived macrophages into our current 3D perfusion tri-culture breast tumor model to form a tetra-culture model consisting of cancer cells, fibroblasts, adipocytes, and macrophages.

Microtumor Development



Perfused 3D heterotypic Microtumors (**3D pMT**) were made of adipocytes, immune cells, and tumor cells/fibroblasts in Matrigel™ and collagen type I and cultured in the 3DKUBE™ perfusion microbioreactor, which supports non-destructive analysis including multiphoton microscopy (MPM) and multiplexed immunoassays (xMAP™) of perfusate. De-identified, primary tissue samples were obtained under IRB-approved prospective research study.

CD14+ Immune Cell Incorporation

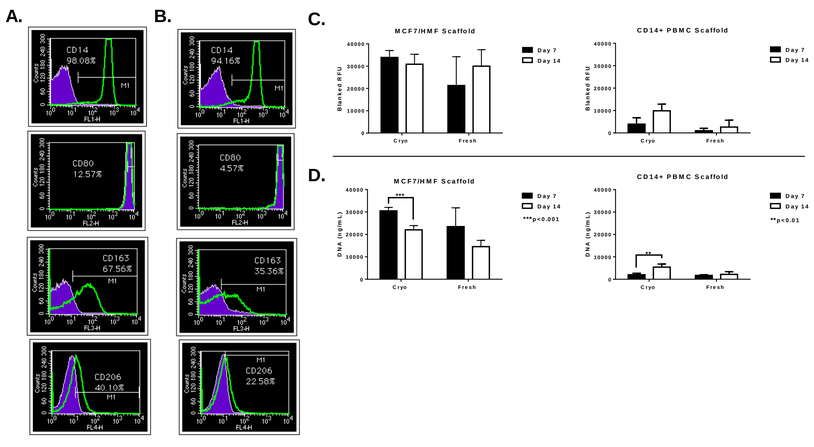


Figure 1: Co-culture with peripheral blood mononuclear cells (PBMCs) effects tumor cell proliferation. 3DpMT containing MCF7 cells, fibroblasts, and either cryopreserved or freshly isolated CD14+ PBMCs were co-cultured in perfusion. No difference in immunophenotype of cryopreserved (**B**) or fresh (**A**) PBMCs was observed. (**C**) Tumor and PBMC metabolic activity did not significantly change over time. (**D**) However, the DNA content of the tumor module co-cultured with cryopreserved PBMCs did decrease, whereas the cryopreserved PBMC module increased. ***These data demonstrate the feasibility of using banked CD14+ PBMCs to develop tumor-macrophage co-culture models.***

M1/M2 Macrophage Incorporation

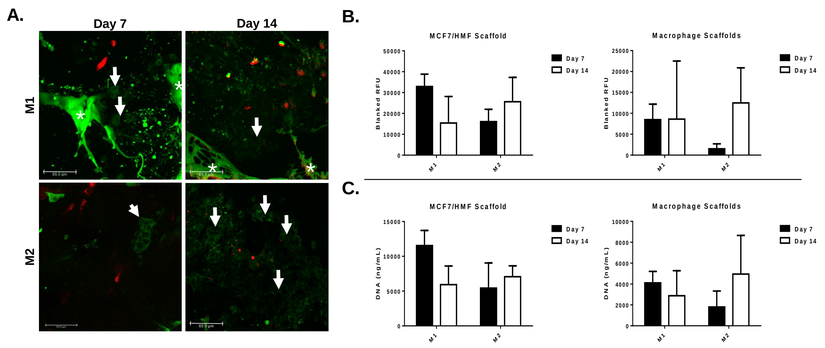


Figure 2: Co-culture with macrophages effects tumor cell proliferation dependent upon macrophage polarity. 3D pMT containing MCF7 cells, fibroblasts, and either pre-labelled (PKH-26) M1 or M2 polarized macrophages were grown under perfusion flow for 2 weeks. (**A**) MCF7/HMF scaffolds were imaged by MPM. Macrophages in the MCF7/HMF scaffolds are evident by red fluorescent cells. Scale bars = 50 µm, solid white arrows-MCF7 cells, white asterisk-scaffolding material. (**B**) Tumor metabolic activity changed over time dependent upon the presence of M1 or M2 macrophages. No significant change in the metabolic activity of the M1 macrophage scaffold, but metabolic activity of the M1 co-cultured MCF7/HMFs trended down. The inverse was true when the M2 co-cultured MCF7/HMFs. The M2 macrophage scaffold increased in metabolic activity over time. (**C**) DNA content supported the metabolic activity data with a loss of DNA in the MCF7/HMF scaffold co-cultured with M1 macrophages and a gain in DNA content in the M2 macrophage scaffold over time possibly indicating de-differentiation. ***These data suggest that macrophage polarity may change in p3DMT while supporting tumor growth over time.***

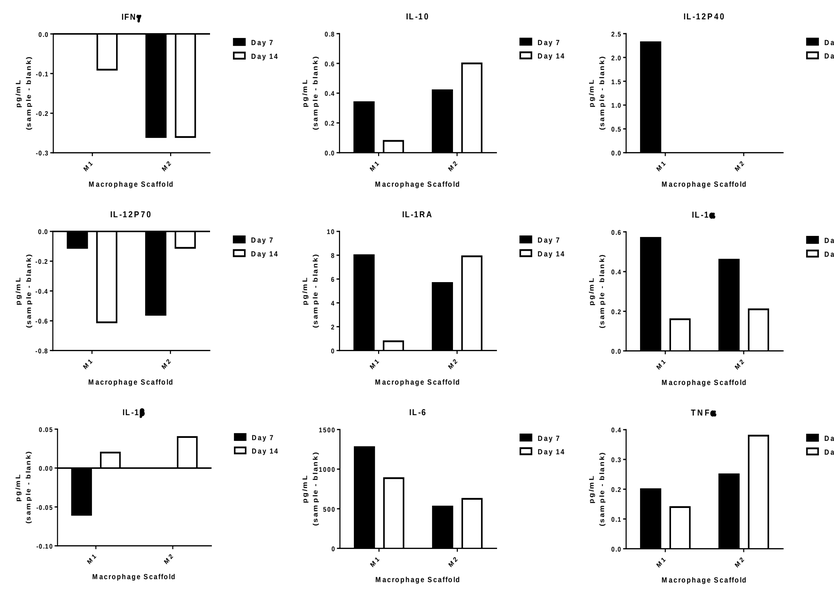


Figure 3: xMAP™ analysis of 3DpMT perfusate containing either M1 or M2 macrophages. 3DpMTs containing MCF7/HMF and either M1 or M2 macrophages were co-cultured for 2 weeks and the perfusate was analyzed for a number of analytes. Mean (n=4) values are shown, blanked to media. ***Suggestive of a "immune protective" phenotype, 3DpMTs co-cultured with M2 macrophages have decreased IFNγ and an increase in IL-10 and TNFα.***

Patient-Derived Immune Microtumors

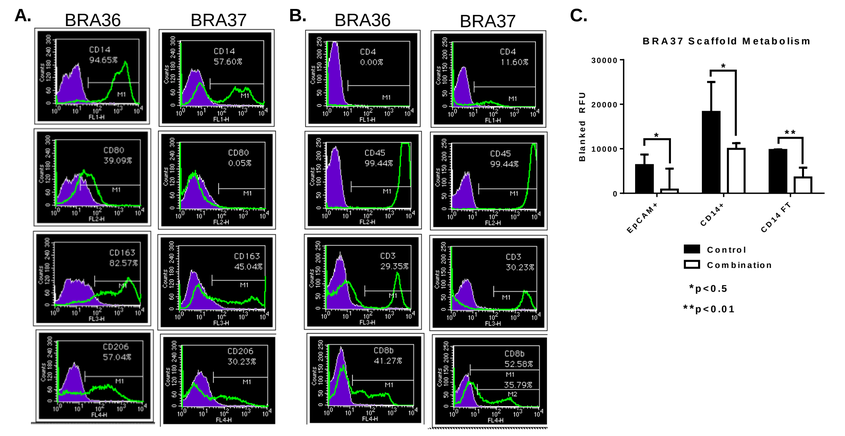


Figure 4: Immune modulation over time from the same patient and 3DpMT formation. Ascitic fluid collected from the same patient at different timepoints was analyzed for immune markers in both the CD14+ selected cell populations (**A**) and the flow through populations (**B**). In both cases, the CD14+ selected cells have a M2-like phenotype and the negatively selected cells are primarily CD45+ with a large population of CD3+ and CD8b positive cells. *CD4+ cell population increases with the second ascitic fluid sample.* (**C**) The cells in the 2nd ascetic fluid sample, BRA37, were separated into EpCAM+, CD14+, and the CD14 flow through and formed into separate modules for 3DpMT development. After 1 week of various culture conditions, metabolism was assessed by PrestoBlue. **In this patient-specific 3DpMT**, ***there was a significant <u>decrease</u> in cellular metabolism for all three cell types when cultured in combination compared to separately***.

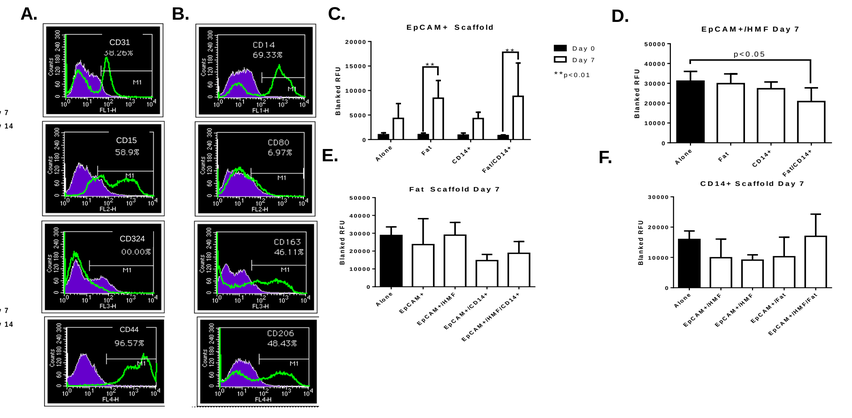


Figure 5: Patient-derived 3DpMT, BRA50. Cells isolated from ascitic fluid of a metastatic ER+ breast cancer patient were selected for EpCAM+ cells and CD14+ cells. (**A**) Flow cytometry analysis of the EpCAM+ cell portion indicated a lack of E-cadherin consistent with a metastatic phenotype; these cells were also CD15+. (**B**) Flow cytometry analysis of the CD14+ cell portion indicated a predominantly M2 macrophage polarity. A 3DpMT was formed with these cells and then cultured either with other modules or alone. Changes in cellular metabolism were measured with PrestoBlue (**C**) The metabolism of EpCAM+ cells without HMFs increased significantly in both fat and CD14+ cells, whereas EpCAM+ cells with HMFs decreased (**D**). There was no significant impact upon the cellular metabolism of either the fat module (**E**) or the CD14+ module (**F**). **In this patient-specific 3DpMT**, ***there was a significant <u>increase</u> in cellular metabolism for tumor cells co-cultured with fat and immne cells, and normal mammary fibroblasts decreased viability over time.***

Conclusions

- **3D perfused microtumors (3DpMT) were developed from CD14+ PBMCs and M1 and M2 macrophages**
- **Use of cryopreserved PBMCs is feasible, but may impact 3DpMT metabolism**
- **M2 macrophages support 3DpMT metabolism whereas M1 macrophages do not**
- **3DpMTs with M2 macrophages have a "immunoprotective" secretome**
- **Patient-specific 3DpMTs can be developed as immuno-oncology models for precision medicine applications**

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