3D Modeling of Immune Cell Interactions in Breast Cancer and Prediction of Immunotherapy Response

#4834

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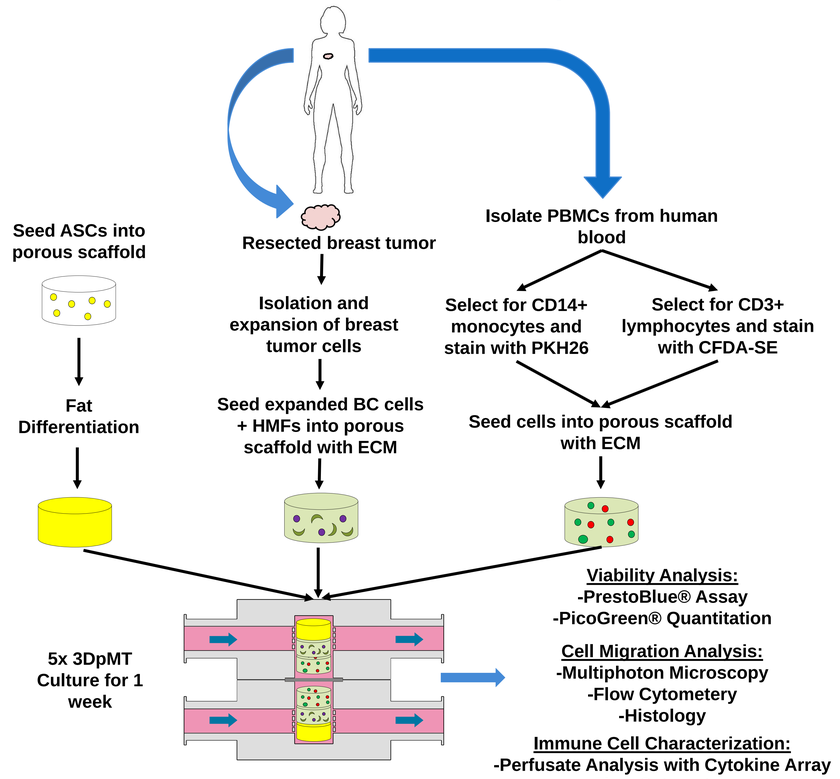
Background

While breast cancer has an overall 5-year survival rate of 89%, the rate for patients with stage 4 metastatic disease is only 26%. Immunotherapies have the potential to improve the prognosis for these patients while also providing better treatment options for all breast cancer patients since they have fewer side effects enabling longer treatment times and the use of combination therapies and reduced chances of developing resistance. Currently these treatments are tested in standard 2D cell cultures that are inaccurate in mimicking in vivo drug response or animal models where the immune system differs from humans in numerous ways including T-cell subsets, cytokine receptors, and costimulatory molecule expression. We have developed 3D models of human breast cancer that span the subtypes, ER+, HER2+, and triple negative, are composed of numerous stromal cell types including fibroblasts and adipocytes, and incorporate immune cell types including macrophages and T-cells under either static or perfusion culture systems.

Methods

Microtumor Model Development



Primary Breast Cancer Cell Expansion

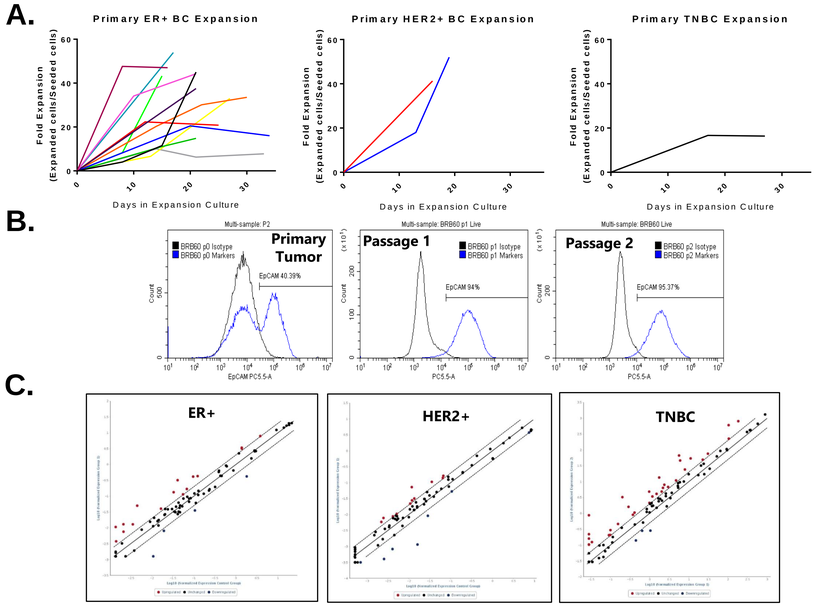


Figure 1: Expansion and Characterization of Expanded Primary Breast Cancer Cells. Cells were isolated from primary breast tumor samples and cultured with our expansion protocol. **(A)** Successful expansion of primary breast cancer cells across all subtypes as shown by fold increase in cell number. **(B)** Representative flow analysis of expanded cells indicate expansion and maintenance of EpCAM+ cell population. **(C)** Analysis of gene expression of passage 1 vs passage 3 expanded cells with RT^2 Breast Cancer PCR Arrays reveal stable gene expression.

Patient Derived 5x 3D Perfusion Microtumor

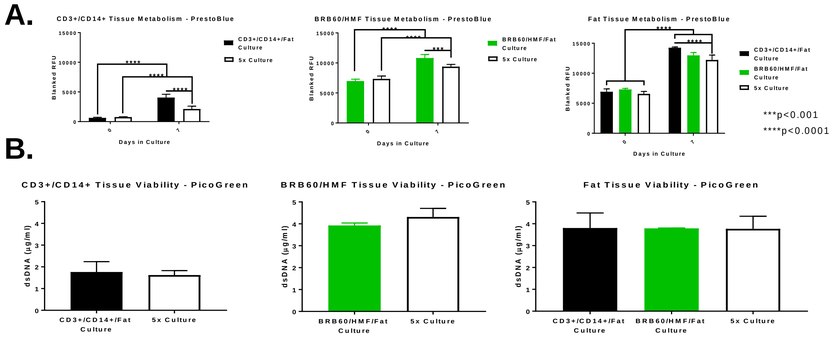


Figure 2: BRB60 5x 3D Perfusion Microtumor. BRB60/HMF/Fat/CD3+/CD14+ 5x microtumors were compared to BRB60/HMF/Fat and CD3+/CD14+/Fat microtumors for cell viability. **(A)** The metabolism of each tissue compartment was assessed. Perfusion culture led to increased metabolism of all tissue compartments in 1 week. 5x microtumors had decreased metabolism in all compartments **(B)** Viability of these tissues were further assessed by DNA content quantification. No significant differences in DNA quantification were observed.

Immune Characterization in 5x 3DpMT

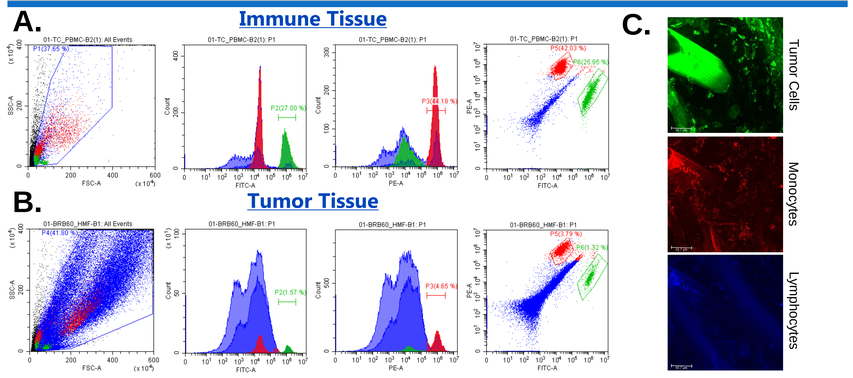


Figure 3: CD3+ and CD14+ Cells Invade into Tumor Tissue in 5x 3D Perfusion Microtumors. Cells were isolated from CD3+/CD14+ tissue **(A)** and BRB60/HMF tissue **(B)** after 1 week of 5x culture and analyzed by flow cytometry. Presence of CFDA-SE+ and PKH26+ cells in BRB60/HMF tissue indicate migration and invasion of CD3+ lymphocytes/CD14+ monocytes into the tumor tissue. **(C)** Immune cell infiltration into the BRB60/HMF tissue compartment was also observed by multiphoton microscopy. Green = tumor, red = monocytes, blue = T-cells.

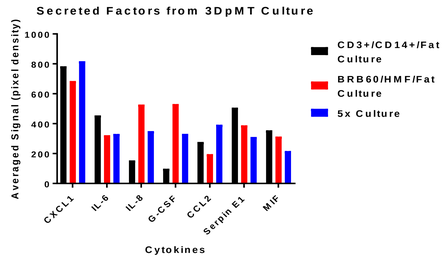


Figure 4: Perfusate from 5X 3D Perfusion Microtumors Analyzed with a Human Cytokine Array. A panel of human cytokines were examined in the supernatant from 5x 3D perfusion microtumor after 1 week in culture. Increased CXCL1 and CCL2 in 5x culture indicate a pro-inflammatory phenotype.

Conclusions

- **Expansion of primary breast cancer cells was successful across all subtypes with an 80% success rate (16/20) on primary samples; Cells isolated from breast core biopsies have also been successfully expanded (data not shown)**
- Expanded primary breast cancer cells demonstrated high EpCAM positivity and stable gene expression throughout expansion culture
- Our 5x 3D perfusion microtumor culture models lymphocyte and monocyte migration and invasion in the breast cancer microenvironment.
- Incorporation of CD3+ lymphocytes and CD14+ monocytes into our 5x 3D perfusion microtumor culture led to a pro-inflammatory phenotype.
- The design and use of the 3DKUBE® is covered by patents 8,865,460 and 9,575,055
- This work is supported by NCI SBIR Contract #: HHSN261201400019C

With Special Thanks to the Biorepository

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