Approximately 1 in 8 women will develop breast cancer in their lifetime, and many require a combination of debilitating surgery, chemotherapy or radiation for long term survival. Histologic evaluation of biomarkers such as estrogen receptor and Her2 often dictate treatment regimens. However, despite high initial response rates, relapses are common, and choosing the right therapy for each patient remains challenging. In vitro 3D models of breast cancer that maintain biologic features and more closely resemble clinical disease (as compared to 2D models) are a promising option for predicting the best therapies for patients. Utilizing both immortalized cell lines and patient derived xenografts (PDX) lines, we have developed a complex 3D model of breast cancer that combines cancer cells, fibroblasts, and adipocytes in a 3D matrix under perfusion culture (3D microtumors, 3DpMT). We have examined these 3DpMTs for metabolism as measured by redox ratio, biomarker expression, and drug response.

**Methods**

**Perfusion Microtumor Model Development**

**Non-lytic Analysis**

**Redox Ratio = FAD/NAD**

**Perfused Microtumor Secretome**

Static or perfused 3D heterotypic Microtumors were made of adipocytes and fibroblasts/epithelial cells in Matrigel™ and collagen type I and cultured for up to 4 weeks in the 3DKUBE™ microbioreactor, with non-destructive analysis including multiphoton microscopy (MPM) and multiplexed immunoassays (mMAP™) of perfuse. ASC=adipose stem cells; HMF=human mammary fibroblasts, CAF=cancer associated fibroblast, BCE=breast cancer epi.

**Figure 1:** Perfusion culture affects 3DpMT morphology and metabolism. 3DpMT containing MCF7 cells were cultured as shown. (A) Tumor morphology was imaged over time by multiphoton microscopy detecting NADH and FAD autofluorescence. • Arrowheads = MCF7 cells • Arrows = HMF • *Asterisk = Scaffolding (B) Analysis of NADH and FAD autofluorescence at 3 weeks indicated a significant difference in the redox ratio between static and perfusion, with the static condition having a lower redox ratio than perfusion. This may be a result of the presence of far more HMF in the static than perfusion.

**Figure 2: Redox ratios of 3DpMTs.** 2D cell lines and 3DpMT from cell lines and PDX were imaged by multiphoton microscopy. Redox ratios were calculated for each cell within each tissue or for each cell for 2D by measuring the autofluorescence of NADH and FAD. Data represents the combine ratio for >50 cells per tissue.

**Figure 3: Lapatinib Drug Response Curves and Redox ratios of 3DpMTs after 1 week of treatment with drugs.** 3DpMT were treated for 1 week in perfusion and then assayed or imaged by multiphoton microscopy for NADH and FAD autofluorescence in order to obtain the redox ratio. Normalized metabolism (PrestoBlue) and non-linear regression curves of cell lines in response to lapatinib is shown. *Redox ratio detects lapatinib response in SKBR and not MDA-MB-231; resazurin reduction does not.*

**Conclusions**

- Perfusion promotes tumor growth and changes redox ratio
- 3DpMT analyzed with non-destructive methods generate breast cancer subtype-specific metabolic signatures
- 2D cell lines and 3D microtumors differ in their redox ratio unlike normal cells, MCF10A.
- 3DpMT from ER+ and Her2+ PDX have different relative redox ratios than immortalized cell lines
- Redox ratio identifies targeted drug response in 3DpMT whereas standard metabolic assays do not
- 3DpMTs secretomes correlate with redox ratio, 3D perfusion culture, and molecular subtype.

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