Differential response of breast cancer cells cultured in 2D versus 3D to estrogen receptor-targeting therapeutics.

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Introduction

Breast cancer is one of the most commonly diagnosed cancers among American women, and while early detection and intervention have led to a reduction in the associated mortality rate over the past few decades[1], the development of targeted, patient-specific therapies promises greater success in treating the progressed disease. For example, estrogen receptor (ER), which is expressed by tumors in a subset of breast cancer patients, can facilitate expression of genes responsible for growth and proliferation of breast cancer cells[2], and thus represents an attractive therapeutic target.

Given the rapid pace and growing expense of therapeutic development, high-content, in vitro screening approaches represent an attractive approach for assaying target-specific effects of novel drug candidates. However, since cell-extracellular matrix (ECM) interactions, which may not be well represented in traditional 2D cell culture models, are often crucial to the expression of drug targets[3], the application of 2D cell culture models is not always the most effective means to screening targeted therapeutic compounds. Specifically, in the context of breast cancer, several investigations have reported a loss of ER-expression throughout standard 2D culture[4].

In order to explore the effect of 2D versus 3D culture on both ER expression and the consequential response of each culture model to a variety of ER-targeting and non-targeting therapeutics, the commercially available Wood cell model (Cellaria Biosciences), which was derived from an ER (+), PR (weak+), grade 1, stage T2N0M0 invasive ductal and lobular carcinoma of the breast was implemented. Cells were cultured in either 2D or 3D formats, labeled with a viability indicator and antibodies against ER and Ki67 (to determine proliferation), cleared with Visikol® HISTO-M™, and imaged via high-content confocal microscopy. Analysis of viability and proliferation suggested a difference in expression of ER in 2D versus 3D spheroid culture and, consequently, a difference in therapeutic response.

Materials and Methods

Wood cell culture

Wood cells were maintained and passaged according to recommendations by Cellaria Biosciences. For experiments, 1 x 10^3 cells per well were plated in a Corning 384-well Round Bottom Ultra Low Attachment Spheroid Microplate or 2 x 10^3 cells per well were plated in a Nunc 96 well, optical bottom, tissue culture treated plate. After 2 days in culture, cells were exposed to drugs at doses listed in Table 1 and cultures were maintained for an additional 2 days prior to labeling.

Table 1: Dosing scheme

Compound	Assayed concentrations					
Paclitaxel	0.01	0.1	1	10	100	nM
Cisplatin	0.1	1	10	100	500	µM
Carboplatin	1	10	100	1000	10000	µM
Fulvestrant	0.01	0.1	1	10	100	nM
Tamoxifen	0.001	0.01	0.1	1	10	µM
Lapatinib	0.01	0.1	1	10	100	µM

Staining and fixing Wood cells

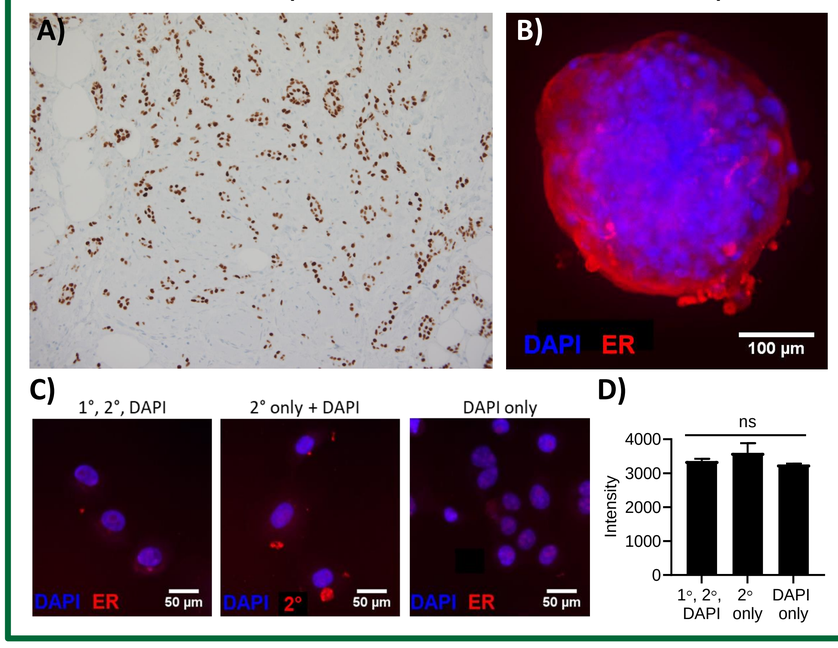
Cells were washed and labeled with 1:1000 Thermo Fisher LIVE/DEAD Fixable Red Dead Cell Stain in PBS with a 1:400 dilution of Molecular Devices Nucview 488. Following 45 minutes of room temperature incubation with the staining solution, cells were washed, fixed with 10% neutral buffered formalin, permeabilized with 0.2% Triton, blocked with Visikol HISTO Blocking Buffer, and labeled with a 1:200 dilution of anti-Ki67 in Visikol HISTO Antibody Buffer. Primary labeled cells were then washed and labeled with a 1:200 dilution of AlexaFluor 647 anti-rabbit secondary antibody plus a 1:5000 dilution of DAPI. Select spheroids and 2D cell cultures were labeled separately with either DAPI only, AlexaFluor 488 anti-mouse secondary antibody only, or anti-ERα plus AlexaFluor 488 anti-mouse secondary and DAPI.

Clearing and high throughput imaging of spheroids and 2D cell cultures

Stained spheroids/cells were washed and gradually dehydrated prior to clearing with Visikol HISTO-M. A CX7 LZR High Content Confocal Imager was used to obtain z-stacks for each spheroid/cell culture (10 µm steps for spheroids, 1 step for 2D cultures), and image analysis was performed using both custom ImageJ macros and CellProfiler.

Results

Wood cells exhibit ER expression in 3D but lack detectable ER expression in traditional 2D cell culture



ER expression, confirmed by IHC of original tumor biopsy (Fig. 1A) was detectable in spheroid culture of the Wood cell line (Fig. 1B). However, in 2D culture, fluorescence intensity of ER-labeled Wood cells was similar to that of non-ER labeled controls (Fig. 1C-D), suggesting that ER is not expressed by Wood cells at detectable levels in 2D culture, despite its expression in 3D cultures.

Figure 1: ER expression in original biopsy (A), 3D (B), and 2D (C) Wood cell cultures. (D) Fluorescence intensity of each labeling condition in C. Data represent mean ± SEM; one-way ANOVA.

Results

Differences in 2D versus 3D drug response implicate ER expression in spheroid culture in inducing sensitivity to ER-targeting drugs

Treated spheroids (Fig. 2, e.g.) or 2D cultures (images not shown) were cleared, imaged, and quantitatively analyzed to elucidate the dose response of Wood cells to various drug compounds in each format.

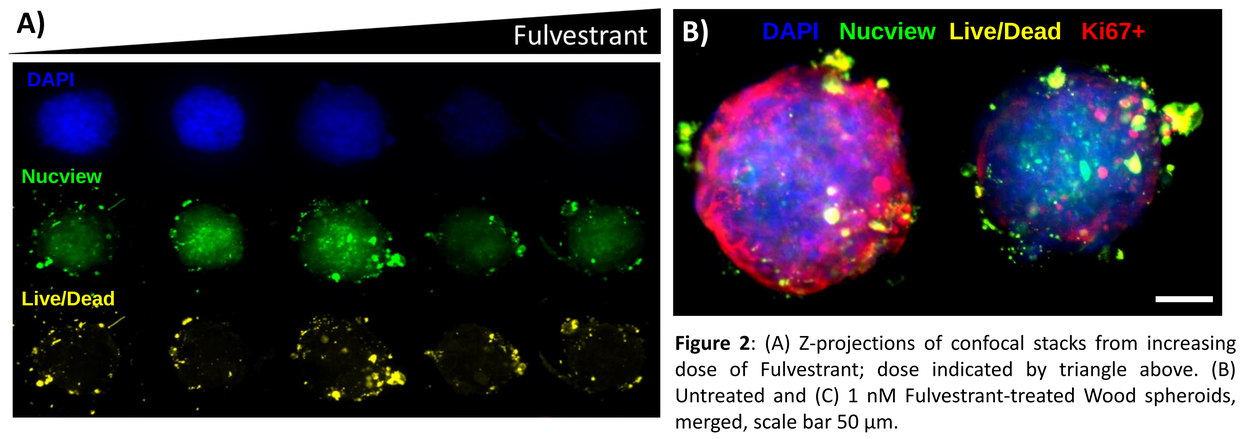
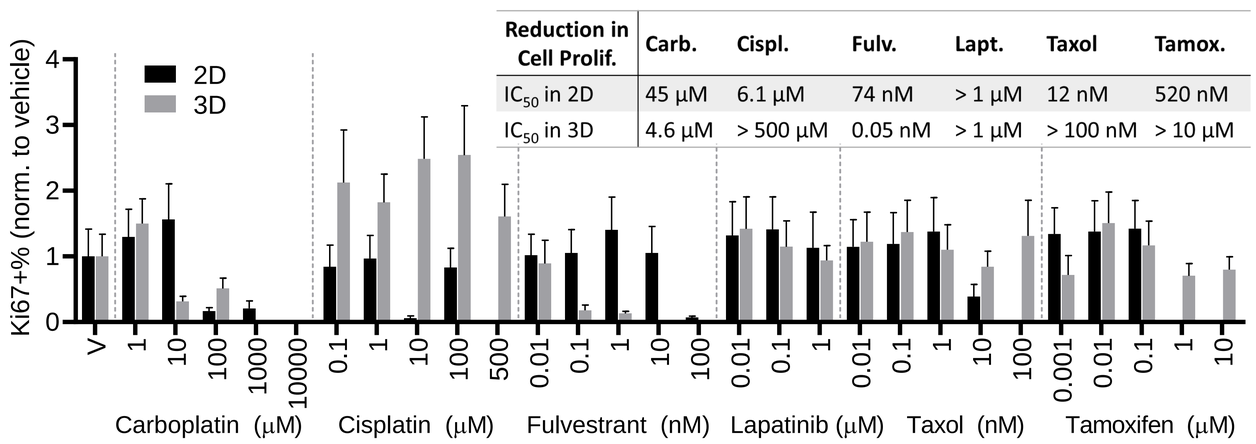


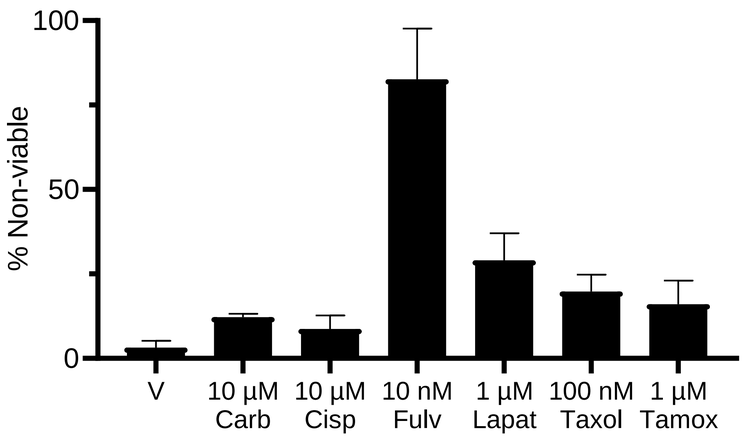
Figure 2: (A) Z-projections of confocal stacks from increasing dose of Fulvestrant; dose indicated by triangle above. (B) Untreated and (C) 1 nM Fulvestrant-treated Wood spheroids, merged, scale bar 50 µm.

Given the higher drug availability in 2D cell culture formats, the dose at which proliferation was reduced by half (denoted as IC_{50}) for several of the assayed drugs was lower in 2D versus 3D Wood cell cultures (i.e. cisplatin, taxol, tamoxifen; Fig. 3, inset table). However, the proliferation of Wood cells cultured in 3D exhibited greater sensitivity to fulvestrant (an ER-targeting drug) relative to 2D cultures (Fig. 3). This is likely attributable to the lack detectable ER expression in 2D cultures (Fig. 1 C-D).



Reduction in Cell Prolif.	Carb.	Cispl.	Fulv.	Lapt.	Taxol	Tamox.
IC_{50} in 2D	45 µM	6.1 µM	74 nM	> 1 µM	12 nM	520 nM
IC_{50} in 3D	4.6 µM	> 500 µM	0.05 nM	> 1 µM	> 100 nM	> 10 µM

Figure 3: Quantification of proliferation (Ki67+, as a percent of total cells, normalized to vehicle controls) of Wood cells, cultured in 2D or 3D formats and treated with a variety of anti-neoplastic agents. IC_{50} from dose-response curves shown in inset table.



Moreover, when Wood cells are cultured in 3D spheroid format and treated with doses of anti-neoplastic agents that correspond to IC_{50} values reported in the literature based on clinical data, the percent of non-viable cells of total was greatest for fulvestrant-treated spheroids, indicating therapeutic utility of this targeted therapeutic.

Figure 3: Quantification of viability (LIVE/DEAD stain+, as a percent of total cells) of Wood cells cultured in 3D and treated with a variety of anti-neoplastic agents at doses similar to literature-reported clinical IC_{50} values.

Conclusions

- Visikol HISTO-M facilitates clear visualization of the interior of multicellular spheroids, thus enabling the detection and quantification of cell viability and proliferation in the context of 3D breast cancer models.
- 3D spheroid and standard 2D cultures of Wood cells exhibit different ER expression and consequently different sensitivity to targeted and non-targeted chemotherapeutic agents, underscoring the importance of model choice (2D versus 3D) in drug screening applications.
- Comparison of in vitro model formats may enable for the generation of mechanistic hypotheses regarding the mechanism of action of targeted and non-targeted therapeutic strategies.

References

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