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RAPID COMMUNICATION

Evaluation of chemotherapeutics in a three-dimensional breast cancer model

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Abstract
Purpose Utilization of miniaturized three-dimensional (3D) cell culture-based assays enables investigation into the anticancer activity of drug candidates and further elucidation of the anticancer profile of standard-of-care chemotherapeutic agents against tumor cells. Drug discovery assays established using 3D cell culture, which better recapitulate the tumor microenvironment, may more accurately reflect the antitumor activity of compounds.
Methods Several standard-of-care anticancer drugs, epirubicin, paclitaxel and vinorelbine, were evaluated against a panel of breast cancer cell lines grown in a 3D cell culture microenvironment in the presence of extracellular matrix. A comparison of this antitumor activity in 3D conditions was made with that observed in traditional two-dimensional (2D) monolayer conditions.
Results Examination of the above mentioned drugs against breast tumor cells cultured in 3D conditions demonstrated significantly altered potency and efficacy in comparison with cells propagated in a 2D monolayer system. The differences observed were cell line-dependent and drug-specific; the triple-negative cell line MDA-MB-231 and the endocrine receptor-positive cell line MCF-7 consistently displayed resistance to therapeutics with distinct modes of action (i.e., topoisomerase II and microtubules) in 3D cell culture in comparison with ErbB2 receptor-positive BT-474 cells.
Conclusion The data presented herein demonstrates the cellular viability and physical changes observed within the 3D spheroid following exposure to drug, which is not always reflected in 2D cell culture models.

Keywords Tumor microenvironment · 3D cell culture · Drug resistance · Breast cancer

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Introduction

Breast cancer is one of the major cancers affecting the female population, accounting for approximately one quarter of cancer patients diagnosed in 2012 (Ferlay et al. 2013). Breast cancer is a heterogeneous disease, with differences between tumors classified by various factors including morphology (e.g., lobular of ductal) and receptor expression (e.g., endocrine receptor absence or expression). Historically, experimentation performed in vitro generally involves the use of cells cultured as a monolayer; however, these cellular growth conditions are not representative of tumor characteristics in vivo (Kim 2005). Three-dimensional (3D) cell culture methodology closes the gap between in vitro two-dimensional (2D) monolayer cell culture and in vivo models and could provide more accurate predictions of compound activity when investigating novel therapeutics (Breslin and O'Driscoll 2013).

Employing in vitro 3D cultures in association with extracellular matrix (ECM) has made a significant contribution to cancer research [as reviewed in (Lovitt et al. 2014; Weigelt et al. 2014)]. One established approach is the use of Matrigel™, a gelatinous composition of proteins including collagen IV, laminin, perlecan, entacin and growth factors, forming the artificial ECM (Benton et al. 2011). Modeling

cancer in vitro utilizing this 3D anchorage-dependent methodology supports the formation of 3D tissue architecture, which includes diverse morphologies stemming, at least to some extent, from tumor cell characteristics and particular gene/protein expression profiles (Kenny et al. 2007). Undertaking 3D cell culture research utilizing matrices is expensive, which may be prohibitive to large-scale studies (Sodunke et al. 2007). However, miniaturized, semi-automated 3D cell culture models suitable for use in drug discovery programs have been developed (Lovitt et al. 2013). Improvements to fully automate and miniaturize further are ongoing and have the potential to provide a rapid cost-effective means of testing compound activity on cells cultured in 3D conditions. An added value is the ability to recapitulate tumor architecture and its surrounding microenvironment, thus providing more information-rich data for decision making. For instance, utilizing 3D cell cultures for drug evaluations allows parameters such as drug diffusion to be examined, which are unable to be determined in 2D cultures.

Frontline therapies for the treatment of advanced breast cancer include the anthracycline and taxane drug classes, as well as vinorelbine (Nicolini et al. 2006). Anthracyclines prevent cellular growth through inhibition of the topoisomerase II enzyme, whereas taxanes and vinorelbine bind to microtubules, preventing mitosis. We report here the altered sensitivity of these known breast cancer therapeutics observed with breast cancer cell lines grown in 3D culture with exogenous ECM, in comparison with 2D monolayer cell culture. The altered responses of cells cultured as 3D aggregates provide insights into drug activity against breast cancer cells, highlighting specific drug- and cell line-dependency. Collectively, these results demonstrate the decreased activity of chemotherapeutics against cells cultured in a 3D microenvironment, emphasizing the importance of utilizing 3D cell culture in drug discovery practices and mechanistic studies.

Materials and methods

Cell line culture conditions

Breast cancer cell lines MCF-7 (endocrine receptor-positive), BT-474 (ErBb2 receptor overexpression) and MDA-MB-231 (endocrine receptor-negative; seeding stock authenticated and purchased from ATCC) were cultured in phenol red-free DMEM/F12 (Life Technologies) with 10 % heat-inactivated fetal bovine serum (Life Technologies) in a humidified incubator at 37 °C, with 5 % carbon dioxide.

Reagents and drug treatment

Epirubicin, paclitaxel and vinorelbine (Sigma-Aldrich; Tocris Bioscience) were prepared as concentrated stocks (50 mM) in 100 % dimethyl sulfoxide (DMSO) and stored at −20 °C. Drugs were diluted to final concentrations ranging from 0.0002 to 200 μM in a 20-point dose response for 2D monolayer cell culture and a 12-point dose response for 3D cell culture. To perform the 2D and 3D cell culture assays, 384-well optical imaging microtiter plates (PerkinElmer) were utilized. 3D cell culture assays were undertaken utilizing Growth Factor Reduced Matrigel™ (GFR Matrigel; Becton–Dickinson Biosciences) as the biological scaffold. Resazurin sodium salt was used to measure cellular metabolic activity following drug exposure (Sigma-Aldrich).

2D monolayer cell culture assay

Each cell line was seeded at a density of 600 cells (MCF-7, MDA-MB-231) or 1500 cells (BT-474) per well in 45 μl of cell culture media into 384-well microtiter plates, followed by an incubation period of 24 h. Drugs and controls (0.4 % DMSO; negative control and 10 % DMSO; positive control) were subsequently added to wells in an automated fashion utilizing a Bravo liquid handling platform (Agilent Technologies). Media changes and re-addition of drug were undertaken every 48 h for a period of 6 days.

3D cell culture assay

The 3D cell culture assay was performed as described previously by Lovitt et al. (2013). Briefly, 15 μl of 7.6 mg/ml GFR Matrigel was added to each well of the 384-well microtiter plates and allowed to set in standard cell culture conditions at 37 °C. Cells were added at a density of 1000 cells (MDA-MB-231) or 5000 cells (MCF-7 and BT-474) per well in 100 μl of media. After a 72- to 144-h incubation period (to allow spheroid formation to 50–100 μm) the media was changed, and drug along with the appropriate controls were applied to wells. Media changes and re-addition of drug were performed every 48 h for a period of 6 days.

Paclitaxel survival following removal of drug

Four hundred MCF-7 cells per well were seeded into 1536-well microtiter optical imaging plates (PerkinElmer) containing 1.5 μl of 7.6 mg/ml GFR Matrigel in an automated manner using a Bravo. Following a 72-h incubation period, media was changed and drug (between 0.0004 and 40 μM) was applied using a Bravo. Media changes and re-addition of drug were completed every 48 h for a period of 6 days. Following paclitaxel exposure for 6 days, paclitaxel-containing media was removed and replaced with paclitaxel free-media. Three-dimensional cellular structures were cultured for a further 5 days in the absence of paclitaxel (media changed every 48 h).

Measurement of drug activity

At the assay conclusion, a final concentration of 600 μM resazurin was added to wells and incubated in standard cell culture conditions for a period of 2–8 h to measure the metabolic activity of cells following exposure to drug. The total well fluorescence intensity was measured using an EnVision™ multilabel plate reader (PerkinElmer). Raw data values were normalized to between 0 and 100 % and plotted using Graphpad Prism™ software. Statistical analysis was completed using a one-way ANOVA and Bonferroni's post hoc test or the Student's *t* test. Live cell imaging of 3D cell cultures was conducted using an Operetta™ high-content imaging system (PerkinElmer) with the 20× objective. The live cell stain, calcein AM, was added to 3D cell cultures (2 μM, final concentration) and incubated at 37 °C for 2 h prior to live cell imaging.

Results

Several breast cancer standard-of-care therapeutics (paclitaxel, epirubicin and vinorelbine) were evaluated using two alternative cell culture techniques, namely 2D (monolayer) and 3D cell culture. A panel of breast cancer cell lines consisting of a range of phenotypes, from endocrine receptor-positive (MCF-7) to ErBb2 receptor overexpression (BT-474) and to endocrine receptor-negative (MDA-MB-231), was investigated to determine the effect of the aforementioned chemotherapeutics. The cellular metabolic activity was measured for both 2D and 3D cell culture conditions (indirect measure of cellular viability), and imaging was completed for 3D cultures (direct measure of cell viability) to complement the metabolic activity data collected. Three measurement parameters were employed, namely: (1) half-maximal inhibitory concentration (IC_{50} value), the concentration of drug resulting in cell death (potency), (2) area under the curve (AUC), the proportion of cells inhibited over a range of drug doses to be examined (potency and efficacy) and (3) E_{max}, the maximum inhibition (efficacy; Fig. 1; Fallahi-Sichani et al. 2013; Huang and Pang 2012). Evaluating the IC_{50}, AUC and E_{max} allows a range of different features of a dose–response curve to be examined. Specifically, the parameters evaluating efficacy examine the quantity of residual cancer cell populations after drug application. Assessment of potency allows differences in the concentration required to inhibit 50 % of cancer cells between culture conditions and cancer cell types. Following drug exposure, the cellular metabolic activity (2D monolayer and 3D cell cultures) was measured and the morphological profiles (3D cell cultures) were determined.

The anthracycline, epirubicin, was shown to be approximately 12-fold more potent ($p \leq 0.0001$) against MDA-MB-231 cells (2D: 52.2 ± 23.7 nM, 3D: 584.8 ± 176.9 nM) and approximately twofold more potent ($p \leq 0.01$) against MCF-7 cells (2D: 192.4 ± 54.9 nM, 3D: 500 nM) grown in 2D conditions when compared to those cultured in a 3D system (Table 1). Furthermore, there was a significant ($p \leq 0.001$) decrease in efficacy of epirubicin against MDA-MB-231 (2D AUC: 20,181 ± 336 units, 3D AUC: 19,360 ± 30 units) and MCF-7 (2D AUC: 19,737 ± 333 units, 3D AUC: 18,254 ± 303 units) cells cultured in 3D conditions in comparison with those cells cultured in 2D conditions. In contrast, epirubicin activity against BT-474 cells was similar ($p > 0.05$), irrespective of the culturing conditions used.

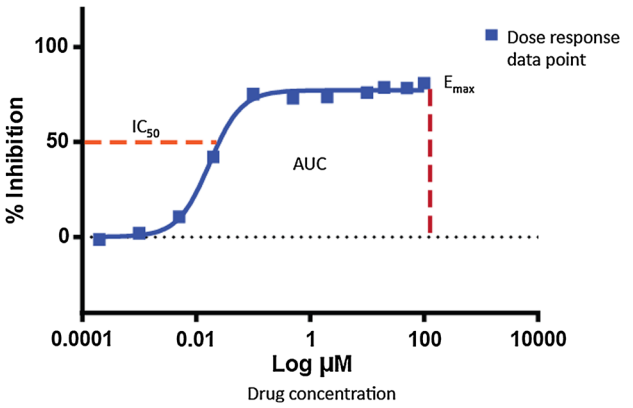


Fig. 1 Diagram illustrating parameters measured in 2D and 3D cell culture assays (Graphpad Prism™)

The activity of vinorelbine, a vinca alkaloid, was also examined in both 2D and 3D cell culture conditions. Vinorelbine did not show differences in potency between 2D and 3D cell cultures. However, vinorelbine demonstrated significantly decreased ($p \leq 0.01$) efficacy against MDA-MB-231 cells (2D AUC: 18,070 ± 477 units, 3D AUC: 12,873 ± 2162 units, 2D E_{max}: 89.2 ± 3.0 %, 3D E_{max}: 63.3 ± 9.0 %) and MCF-7 cells (2D AUC: 19,014 ± 1118 units, 3D AUC: 13,545 ± 1176 units) in 3D cell culture in comparison with 2D cell culture (Table 1). In contrast, similar to epirubicin, vinorelbine demonstrated comparable ($p > 0.05$) potency and efficacy against BT-474 breast cancer cells under all test conditions.

Paclitaxel, belonging to the taxane family, was demonstrated to be significantly ($p \leq 0.0001$) more potent (approximately sevenfold) against MDA-MB-231 cells in 2D (3.6 ± 0.4 nM) than 3D cell culture (29.5 ± 5.5 nM). A significant difference ($p \leq 0.05$) in potency was also detected between 2D and 3D cultures of BT-474 cells (2D: 12.1 ± 1.2 nM, 3D: 18.3 ± 0.1 nM). In addition, the efficacy of paclitaxel against MDA-MB-231 cells (2D AUC: 18,018 ± 893 units, 3D AUC: 14,896 ± 867 units; 2D E_{max}: 93.0 ± 1.3 %, 3D E_{max}: 73.6 ± 4.1 %) and MCF-7 cells (2D

Table 1 Breast cancer cell line response to chemotherapeutic drugs in two-dimensional (2D) and three-dimensional (3D) cell culture. Mean ± standard deviation of three experiments

Drug	MDA-MB-231		MCF-7		BT-474	
	2D	3D	2D	3D	2D	3D
Epirubicin						
Drug IC_{50} (nM)	52.2 ± 23.7	584.8 ± 179.6****	192.4 ± 54.9	500[a], **	171.4 ± 12.8	160.5 ± 3.0
AUC (units)	20,181 ± 336	19,360 ± 30***	19,737 ± 333	18,254 ± 303****	19,206 ± 275	19,276 ± 272
E_{max} (%)	100 ± 0.0	98.7 ± 2.2	96.4 ± 3.5	100[b]	91.3 ± 1.4	94.5 ± 2.7
Vinorelbine						
Drug IC_{50} (nM)	21.4 ± 1.3	104.2 ± 68.9	25.4 ± 6.6	93.3 ± 100.9	25.2 ± 6.0	21.2 ± 4.8
AUC (units)	18,070 ± 477	12,873 ± 2162***	19,014 ± 1118	13,545 ± 1176***	18,305 ± 502	18,050 ± 906
E_{max} (%)	89.2 ± 3.0	63.3 ± 9.0**	89.1 ± 4.4	74.0 ± 6.1	80.0 ± 5.2	88.2 ± 9.0
Paclitaxel						
Drug IC_{50} (nM)	3.6 ± 0.4	29.5 ± 5.5****	3.2 ± 0.7	5.6 ± 0.6	12.1 ± 1.2	18.3 ± 0.1*
AUC (units)	18,018 ± 893	14,896 ± 867**	15,435 ± 277	5793 ± 975****	17,509 ± 600	17,581 ± 99
E_{max} (%)	93.0 ± 1.3	73.6 ± 4.1**	79.8 ± 4.0	30.2 ± 3.7****	84.6 ± 8.3	88.5 ± 1.2

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$

[a] IC_{50} not converged, value approximated based on raw percent inhibition values

[b] value not calculated, estimated based on raw percent inhibition values

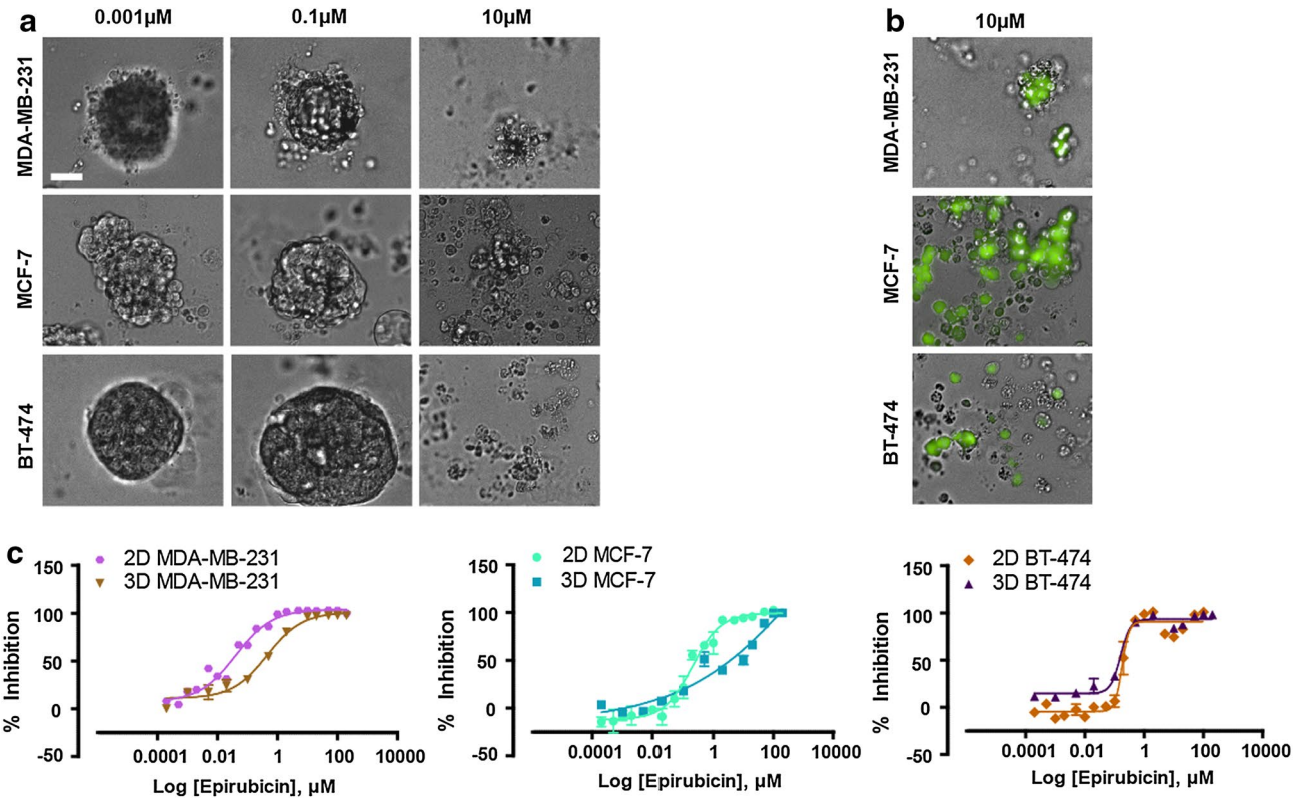


Fig. 2 Morphological response of breast cancer cells to epirubicin. **a** Live cell brightfield imaging of three-dimensional (3D) cultured cells following exposure to epirubicin for 6 days (20× objective, Operetta; PerkinElmer). **b** Live cell staining of cells arranged in 3D aggregates with calcein AM (20× objective, Operetta; PerkinElmer). **c** Dose–response curves illustrating metabolic activity of breast tumor cells following epirubicin application (measured with resazurin). Data represent mean ± standard deviation. *Scale bar* 50 µm

AUC: 15,435 ± 277 units, 3D AUC: 5793 ± 975 units; 2D E_{max}: 79.8 ± 4.0 %, 3D E_{max}: 30.2 ± 3.7 %) was significantly reduced ($p \leq 0.01$) in 3D conditions compared to the corresponding 2D cell cultures (Table 1). Conversely, there were no substantial differences ($p > 0.05$) in paclitaxel efficacy between 2D and 3D cell culture for BT-474 cells.

Cell numbers for both 2D and 3D cell culture assays were evaluated for optimal assay conditions prior to undertaking comparisons of drug activity between 2D and 3D cell culture assays. When utilizing an alternate number of cells than determined optimal for 2D cell culture experiments, the profile (i.e., AUC and E_{max}) of the dose–response curves generated was similar to that of the optimized cell number utilized in 2D experiments (IC_{50} values within threefold).

Collectively, these results demonstrate the differences in potency and efficacy when standard-of-care breast cancer therapeutics were evaluated in 3D cell culture and compared to traditional 2D monolayer cell culture conditions. The data obtained highlight the cell line- and drug-dependent manner in which this occurs.

The morphological response of breast cancer cell lines cultured in 3D conditions following exposure to breast cancer chemotherapeutics was also examined. It was demonstrated that exposure to epirubicin at high concentrations (i.e., 10 μM) resulted in substantial disruption of MDA-MB-231 and BT-474 3D cell cultures, whereas under the same conditions, partially intact cellular aggregates were observed for MCF-7 cells cultured in 3D conditions (Fig. 2a). Live cell staining of 3D breast tumor cultures confirmed that the MCF-7 cells were still viable following treatment with epirubicin at these high concentrations (Fig. 2b). The phenotypic responses observed for the breast cancer cell lines grown as 3D breast cancer aggregates (Fig. 2a) reflect the metabolic activity data obtained following exposure to epirubicin for 6 days (Fig. 2c).

The phenotypic response of MDA-MB-231 and MCF-7 cells exposed to vinorelbine is comparable to the reduced efficacy observed from the metabolic activity studies (Fig. 3a). Following treatment with 10 μM vinorelbine for 6 days, the structural integrity (i.e., the cellular organization within the structure of 3D cell culture) of MDA-MB-231 cell-containing spheroids had deteriorated; however, a 3D cellular configuration was still evident. Furthermore, at 10 μM vinorelbine, MCF-7 3D aggregates were no longer

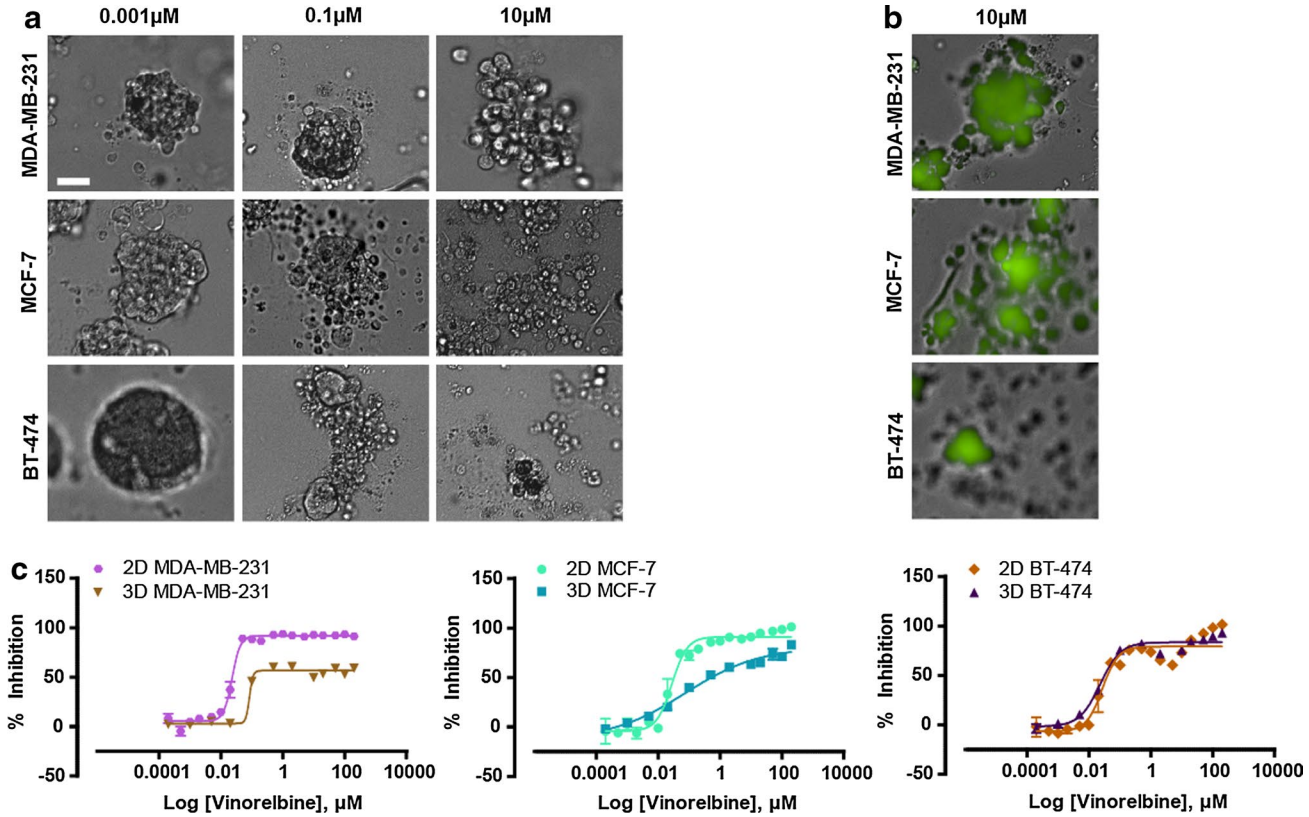


Fig. 3 Morphological response of breast cancer cells to vinorelbine. **a** Live cell brightfield imaging of cells cultured in three-dimensional (3D) conditions following exposure to vinorelbine for 6 days (20× objective, Operetta; PerkinElmer). **b** Live cell staining of cells in 3D aggregates with calcein AM (20× objective, Operetta; PerkinElmer). **c** Dose–response curves illustrating metabolic activity of breast tumor cells following vinorelbine application (measured with resazurin). Data represent mean ± standard deviation. *Scale bar* 50 μm

intact, rather a collection of dispersed single cells. At these high concentrations of vinorelbine, the 3D cellular aggregates were, however, found to be viable (Fig. 3b). The cellular viability data correlate to the dose–response activity data (Fig. 3c).

The morphological response of breast cancer cell lines in 3D cell culture to paclitaxel exposure was also evaluated. At 10 μM concentrations of paclitaxel, MDA-MB-231 and MCF-7 spheroids remained partially structurally intact (Fig. 4a). The MCF-7 cell line was particularly resistant to paclitaxel, with extensive cellular viability of 3D cultures observed at 10 μM paclitaxel (Fig. 4b). This was further exemplified by the reduction in efficacy determined at high concentrations of paclitaxel compared to other breast cancer cell lines evaluated (Fig. 4c). To further examine the extensive paclitaxel resistance observed for MCF-7 cells, paclitaxel was removed from the 3D cell culture microenvironment and the cells were incubated for a further 5 days to evaluate the transient nature of the resistance. At high concentrations of paclitaxel (0.4–40 μM), the metabolic activity of these cultures decreased significantly ($p \leq 0.05$; Fig. 4d), suggesting that either temporary resistance or delayed effects were impacting on the viability of these cells in 3D cell culture. Similar results were obtained for MDA-MB-231 cells (Fig. 4e).

Collectively, we have demonstrated the reduced efficacy of several chemotherapeutic drugs on 3D breast cancer cell cultures. Measurement of drug activity on cell lines can be visualized through evaluation of the morphological structure following drug exposure, which we have demonstrated correlates to the metabolic activity data.

Discussion

Several chemotherapeutic drugs (epirubicin, vinorelbine and paclitaxel) were investigated for their potency and efficacy against a selection of breast cancer cell lines cultured in a 3D scaffold-dependent manner, namely surrounded by exogenous ECM, and the morphological profiles they elicited. Utilizing morphological and metabolic data, we ascertained the activity profile of several representative drugs against three breast cancer cell lines. We were able to demonstrate reduced inhibition of cell lines grown in 3D culture, which may have clinical relevance for tumors with a similar profile.

Differences in drug activity were particularly noticeable in the MCF-7 (endocrine receptor-positive) and MDA-MB-231 (endocrine receptor-negative) cell lines for all of the drugs tested. However, the profile of BT-474 (ErbB2 receptor-positive) cells in response to these chemotherapeutic drugs was similar irrespective of whether the cells were grown as a monolayer (2D) or in 3D culture conditions.

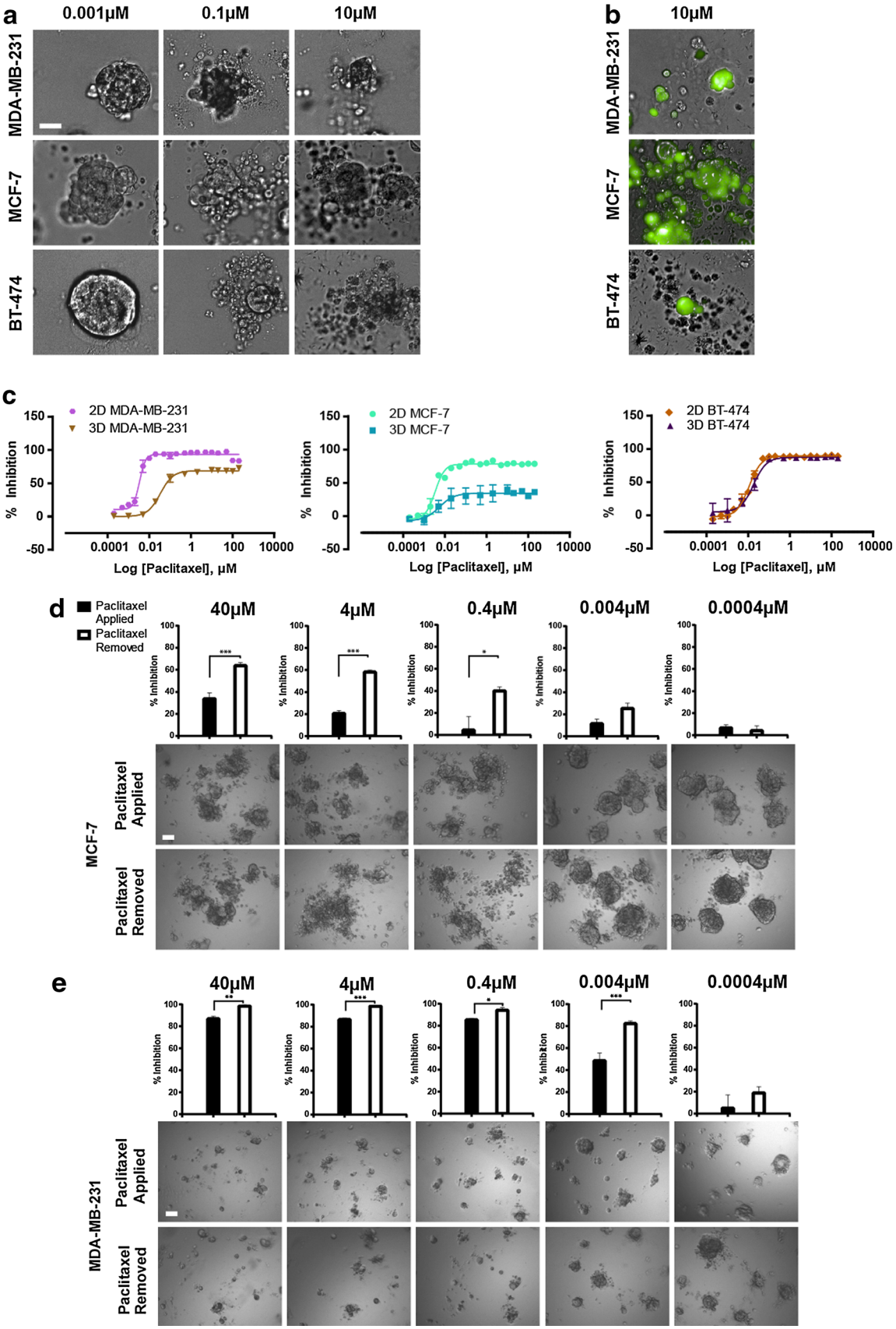
Fig. 4 Response of breast cancer cells to paclitaxel. **a** Breast cancer cell line three-dimensional (3D) cell culture morphological response to paclitaxel; live cell imaging (20× objective, Operetta; PerkinElmer). **b** Live cell staining of cells situated in 3D aggregates with calcein AM (20× objective, Operetta; PerkinElmer). **c** Dose–response curves illustrating metabolic activity of breast tumor cells following paclitaxel application (measured with resazurin). Data represent mean ± standard deviation. 3D breast cancer cell culture of MCF-7 **d** and MDA-MB-231 **e** cells in response to paclitaxel exposure time frame. Paclitaxel was applied to cells in 3D cultures for a period of 6 days (paclitaxel applied ■), followed by removal of paclitaxel from the microenvironment for an extended period of 5 days (paclitaxel removed □). Bright field images were acquired using the Olympus CellR microscope (4× objective). Measurements were completed utilizing resazurin to determine the metabolic activity. *Scale bar* 50 μm. Significance values are: $*p \leq 0.05$; $***p \leq 0.001$. Data represent mean ± standard error

This suggests that the resistance mechanisms involved for these particular drugs are both cell line-dependent, which may be related to cell line receptor status, and drug class-specific, perhaps due to the differing mechanism of action of drugs tested. In addition, the differences in the sensitivity could be attributed to BT-474 cells not acquiring a survival advantage when cells are cultured in a 3D architecture with cell-ECM contacts, unlike MCF-7 and MDA-MB-231 cells.

Previous studies have reported resistance which was cancer cell line- and drug/compound-specific upon exposure to various anticancer agents, including chemotherapeutics, when tumor cells were cultured in 3D cell culture conditions compared to standard 2D cell culture (Nirmalanandhan et al. 2010; Barbone et al. 2008; Vinci et al. 2012). A variety of factors have been implicated in resistance to antitumor agents observed in cells cultured in 3D conditions, including altered signaling pathways (Weigelt et al. 2009), reduced proliferation rates (Burdett et al. 2010) and environment-mediated factors (e.g., ECM-to-integrin signaling) (Huang et al. 2011; Sethi et al. 1999; Muranen et al. 2012).

Preliminary evidence from this study suggests that the altered drug sensitivity may be transient in nature. The potential transient features of paclitaxel resistance are illustrated when MCF-7 cells were cultured in 3D conditions and demonstrated immense resistance against paclitaxel during a 6-day period of exposure in comparison with 2D monolayer cell culture. However, there was a significant decline in cellular viability observed in MCF-7 cells cultured in 3D conditions following removal of drug pressure. The potential transient resistance mechanisms occurring in these 3D cultured MCF-7 cells include signaling derived from cell–ECM adhesions, a factor which may not be present in monolayer cell cultures.

The differences in cellular sensitivity to drugs between the two cell culture conditions were not parameter-specific. For example, there was a significant difference in


a
0.001μM
0.1μM
10μM
MDA-MB-231
MCF-7
BT-474
b
10μM
MDA-MB-231
MCF-7
BT-474
c
2D MDA-MB-231
3D MDA-MB-231
2D MCF-7
3D MCF-7
2D BT-474
3D BT-474
% Inhibition
Log [Paclitaxel], μM
d
Paclitaxel Applied
Paclitaxel Removed
40μM
4μM
0.4μM
0.004μM
0.0004μM
MCF-7
e
MDA-MB-231

the efficacy (E_{max} and AUC) of paclitaxel against MCF-7 cells in 3D cell culture compared to 2D cell culture conditions; however, there was no significant difference detected between the potency results. The data show that a decreased population of cells is affected, but there is no significant difference in cellular sensitivity at the IC_{50} concentration. Chemical entities which demonstrate a high potency may not be effective in inhibiting viability in 100 % of cancer cells examined and vice versa (Nirmalanandhan et al. 2010).

The efficacy (AUC and the E_{max}) is decreased in chemotherapeutic drugs evaluated in 3D cell culture in comparison with 2D culture conditions against MCF-7 and MDA-MB-231 cells. These results indicate that there is increased cellular viability as a result of reduced drug efficacy upon exposure to these drugs in 3D cell culture conditions and may be more indicative of minimal residual disease in vivo than 2D cell culture models. Environment-mediated factors can be a factor in minimal residual disease (Meads et al. 2009), with the more physiologically relevant properties of 3D cell culture, e.g., cell–matrix adhesion and 3D architecture may contribute to the identification of reduced drug efficacy.

Additional characterization of 3D cell culture models for use in drug discovery practices may be valuable for rapidly identifying compounds that demonstrated reduced efficacy in a 3D tumor-like structure. Research focussed on a broad comparative analysis of cellular responses and anticancer drug activity between 3D cell culture models and in vivo tissue would be advantageous (Hickman et al. 2014). Investigation into the predictive nature of 3D cell culture for use in novel compound evaluation may assist in more rapid identification and characterization of active anticancer agents. In addition, expanding 3D cell culture models to study the impact of co-culture on metastatic sites around the body in a format suitable for screening would be highly beneficial. Recent advances in developing selected models have been made, but their use in compound screening has not been evaluated (Marlow et al. 2013).

The results acquired herein demonstrate the increased drug resistance of selected breast cancer cell lines cultured in three-dimensions compared to the same cells in 2D cell culture. Utilizing two distinct approaches for evaluating cellular responses to the exposure of drug, specifically metabolic activity and morphology, provided insights into temporal changes observed within 3D cell cultures. The results obtained also demonstrate the value of evaluating drug candidates in more advanced in vitro cell culture models. Live cell imaging, assessment of phenotypic properties and metabolic activity data evaluation provided a unique perspective on drug activity against well-characterized cell lines in a novel ECM-based cell culture model. These 3D cellular aggregates may be invaluable in the evaluation of new molecular entities as part of drug discovery programs and, in addition, may have an integral role for use as an in vitro model when deconstructing drug resistance mechanisms occurring during therapy in the clinical setting.

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Conflict of interest The authors declare no conflicts of interest.

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