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An in vitro Model for Studying Breast Cancer Invasiveness in Response to Chemotherapy

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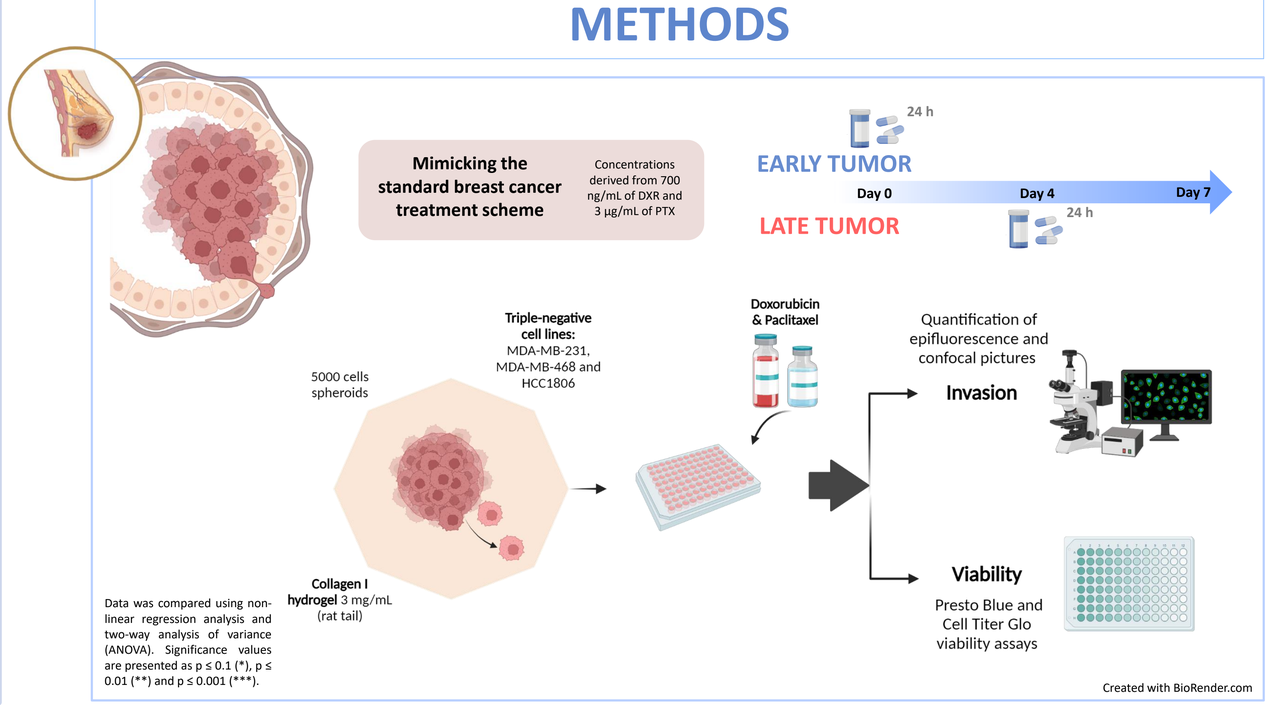
BACKGROUND

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, characterized by its high metastatic potential and lack of targeted therapies [1]. Novel therapeutic strategies for managing TNBC are urgently needed, especially those aimed at prevention during the early stages of metastasis. However, current in vitro models struggle to replicate the complex metastatic cascade, primarily due to the challenge of accurately establishing a tumor microenvironment (TME) [2].

This study aims to evaluate the influence of the TME on TNBC treatment response. Specifically, the effects of the chemotherapeutic agents doxorubicin (DXR) and paclitaxel (PTX) were assessed on three TNBC cell lines (MDA-MB-231, MDA-MB-468, and HCC1806) across different conditions: 2D traditional cell culture, spheroids, and matrix embedded spheroids. Additionally, the impact of these drugs on invasion was analyzed on a 3D invasive model, consisting of MDA-MB-231 spheroids embedded in a collagen I hydrogel simulating the extracellular matrix of cancerous breast tissue.

METHODS



RESULTS

Different response to DXR and PTX in 3D *versus* 2D culture

All the TNBC cell lines studied were less responsive to both DXR y PTX when cultured in three dimensions (spheroids) versus two dimensions.

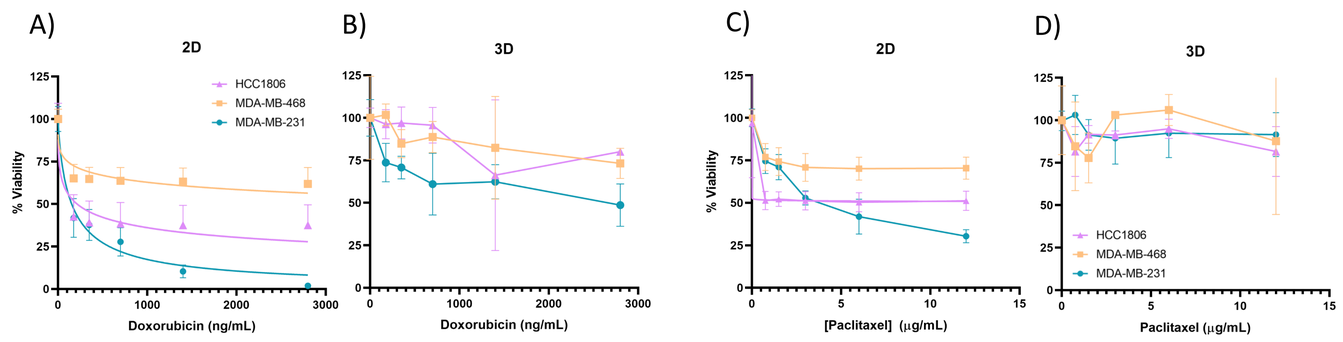


Figure 1. Dose-response studies. Viability of MDA-MB-231, MDA-MB-468 and HCC1806 cell lines cultured in 2D after treatment with A) DXR and C) PTX for 72h (n=3) compared to 3D culture B) and D) (n=3).

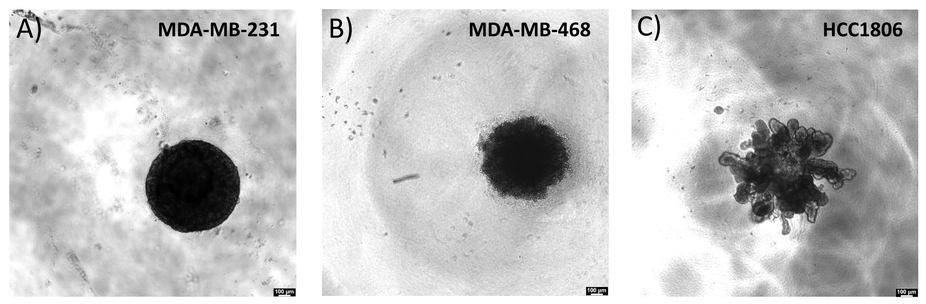


Figure 2. Spheroids of the three triple negative breast cancer cell lines analyzed for viability. A) MDA-MB-231; B) MDA-MB-468; C) HCC1806. All photos were taken at day 8 of growth.

Effect of DXR and PTX on early and late invasion models

Both drugs reduced the invasion and viability of the collagen hydrogel-embedded invasive MDA-MB-231 spheroids (early tumor model). However, in the case of DXR, the drug effect was less pronounced when it was applied after the cells had invaded the extracellular matrix in the so-called late tumor model (day 4 after invasion).

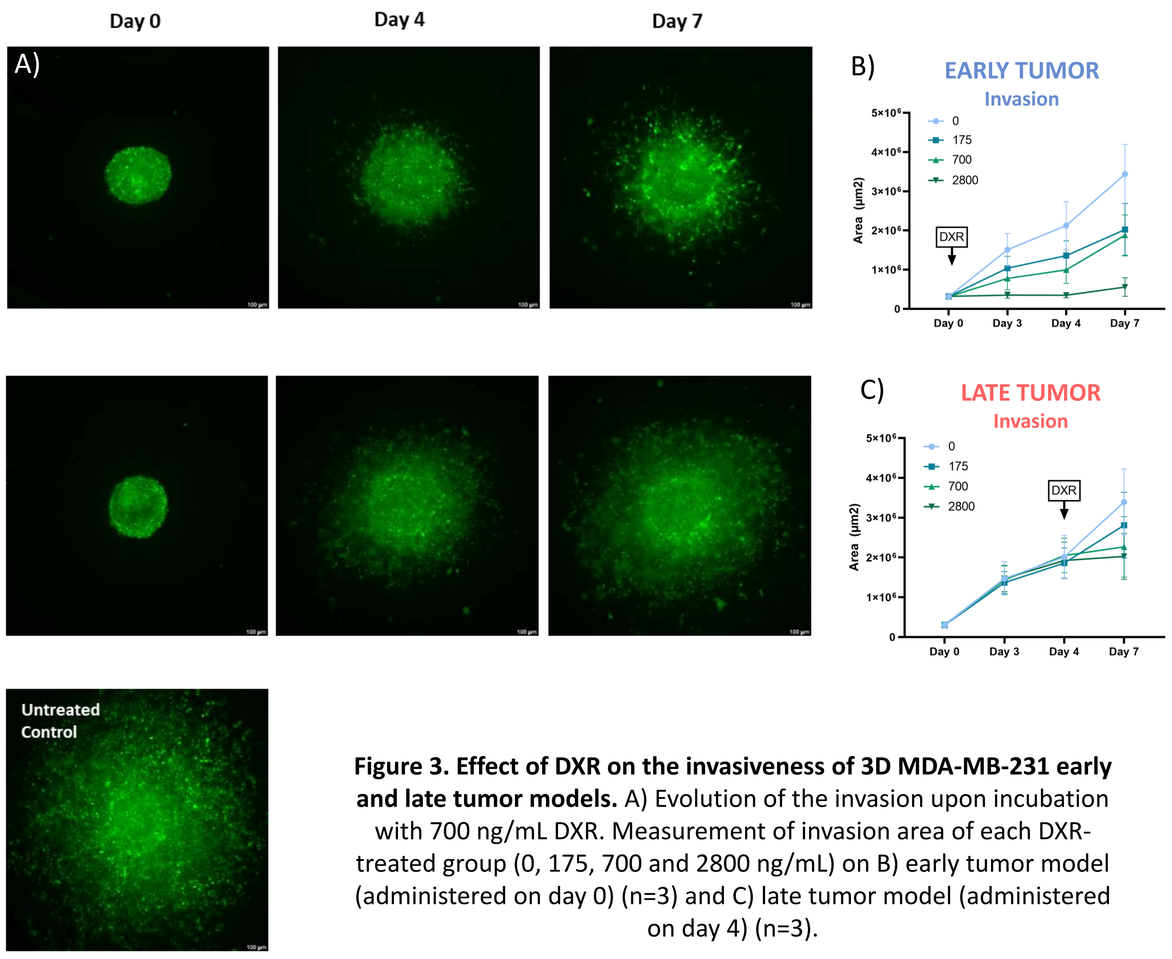


Figure 3. Effect of DXR on the invasiveness of 3D MDA-MB-231 early and late tumor models. A) Evolution of the invasion upon incubation with 700 ng/mL DXR. Measurement of invasion area of each DXR-treated group (0, 175, 700 and 2800 ng/mL) on B) early tumor model (administered on day 0) (n=3) and C) late tumor model (administered on day 4) (n=3).

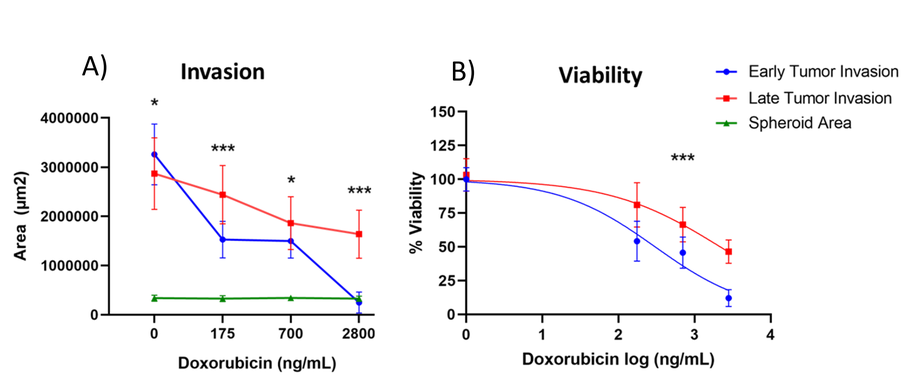


Figure 4. Comparison of the effect of DXR on the early and late 3D MDA-MB-231 tumor models. Analysis of A) invasion and B) viability of both models at day 7 of the experiment (n=3).

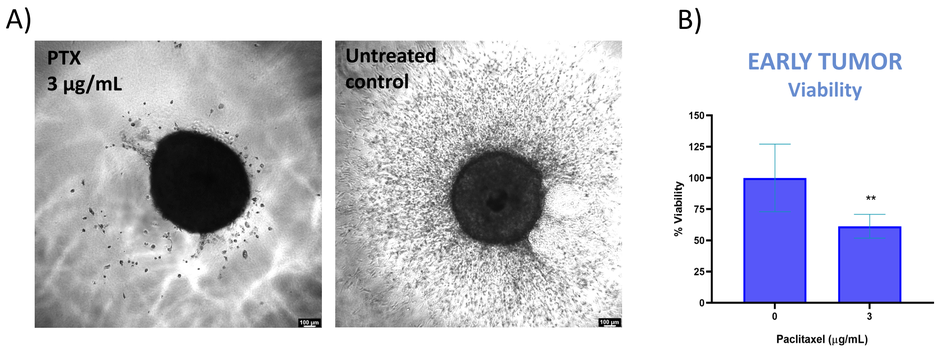


Figure 5. Effect of 3 µg/mL PTX on the invasiveness of 3D MDA-MB-231 early model. A) Invasion of the MDA-MB-231 early model upon incubation with 3 µg/mL PTX, administered on day 0; B) Analysis of cell viability on day 4 after PTX treatment (n=3).

CONCLUSIONS

The fact that all three TNBC cell lines evaluated showed greater resistance to DXR and PTX when cultured on 3D rather than on traditional 2D culture highlights the importance of the TME in determining treatment response. Furthermore, both drugs might reduce the risk of metastasis on TNBC, but once cancer cells have invaded the extracellular matrix, their effect might be compromised.

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