Metastasis on Chip: Modelling Invasive Breast Cancer Cells

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Abstract

The purpose of this work is to employ a three-dimensional model to examine breast cancer metastasis from various perspectives. Using a solid breast tumor model exposed to a standard chemotherapeutic treatment scheme, we aim to replicate the earliest stages of the metastatic cascade, focusing on the drug's effect on invasive cells.

Introduction

Breast cancer (BC) is the greatest cause of mortality for women globally according to the world health organization (WHO) [1], with metastasis serving as the main contributing factor. The effects of metastasis can be attributed to a lack of understanding of the mechanisms at play in the early stages of the metastatic cascade, specifically invasion into the stroma and intravasation into the blood capillaries [stages 1-3], processes where the invasive and subsequently circulating tumor cells (CTCs) go through a number of phenotypic changes with unknown effects on cancer treatment [2]. Although understanding the escape and drug resistance mechanisms occurring at the beginning of metastasis is crucial to reduce BC mortality, there are currently very few in vitro options available to appropriately evaluate this process, partly due to challenges in establishing a precise tumor microenvironment (TME) [3].

Within the framework, the ductal invasive carcinoma cell line MDA-MB-231 is used to study the effect of a three-dimensional (3D) TME on responsiveness to doxorubicin (DXR); an anthracycline chemotherapeutic agent first-line treatment against BC [3]. The aim is to assess the cell proliferation, invasion and sensitivity to doxorubicin on the distinct phenotypes established within the breast cancer-invasive TME.

Additionally, the metastatic TME can be greatly improved by integrating a microfluidic device with the potential of incorporating an endothelium along with physiologically relevant physicochemical stimuli (chemoattractant gradient and sheer stress) to further stimulate invasion. Since pore crossing implies additional genetic modulation for cell deformability, the presence of a permeable membrane allows the analysis of the "intravasation" process and the potential isolation of CTCs.

Materials & Methods

MDA-MB-231 (triple-negative subtype) cell line was used to model invasive breast carcinoma through the formation of spheroid aggregates of 5000 cells. Stable spheroids (600 µm diameter and established necrotic core) were embedded in 3 mg/mL collagen hydrogel matrices and transferred to 96-well plates or microfluidic devices (BE-Transflow chips, BeOnChip). Matrix invasion was assessed from epifluorescence microscope (Leica Microsystems, Thunder) and confocal pictures (Nikon, Eclipse). ImageJ and GraphPad Prism 8 softwares were used for analysis and quantification.

To study DXR effect within several initial metastatic stages, two BC models were established: a primary BC model ("early detected") treated from the beginning of the experiment and an invasive BC model treated after the invasion ("metastatic carcinoma"; Figure 1).

DXR was administered in accordance with the standard BC treatment scheme, consisting of a single application of half and twice a concentration estimated from values obtained in patient blood. Presto Blue and Cell Titer Glo were used to test the viability in 2D and 3D.

Results & Discussion

MDA-MB-231 are more resistant to DXR when cultured in 3D. Viability values were significantly higher in the MDA-MB-231 spheroids than in the cells cultured in 2D.

MDA-MB-231 are more resistant to DXR when already invaded the matrix. The comparison between the models showed reduced invasion and viability in the primary carcinoma model, while the invasive model showed similar values to the untreated control (Figure 2).

MDA-MB-231 invasion can be enhanced on a microfluidic chip. The tests performed on the microfluidic device allowed the successful establishment of a chemoattractant gradient able to trigger cell migration towards the membrane.

Conclusions

This work allows the obtention of a simple BC invasive model for drug evaluation with a closer look at invasive cell response and evasion mechanisms. It has shown promising results while confirming 3D culture and invasive cell resistance to DXR. Considering this, as well as the potential for integrating the model with a microfluidic chip, this model serves to explore the mechanisms of BC progression and invasive cell resistance.

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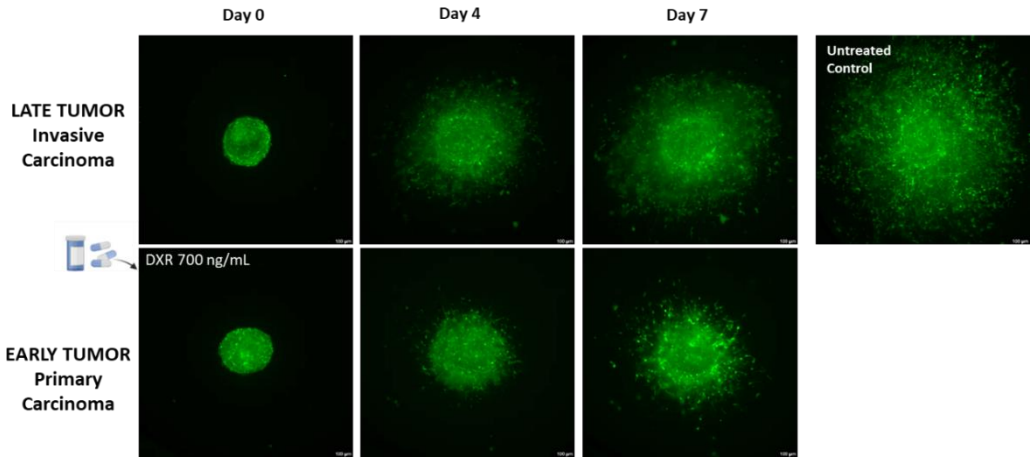

Figura 1. Schematic representation of the two models of breast carcinoma established according to the moment of drug application. An untreated control is presented on the right for visual comparison of invasion.

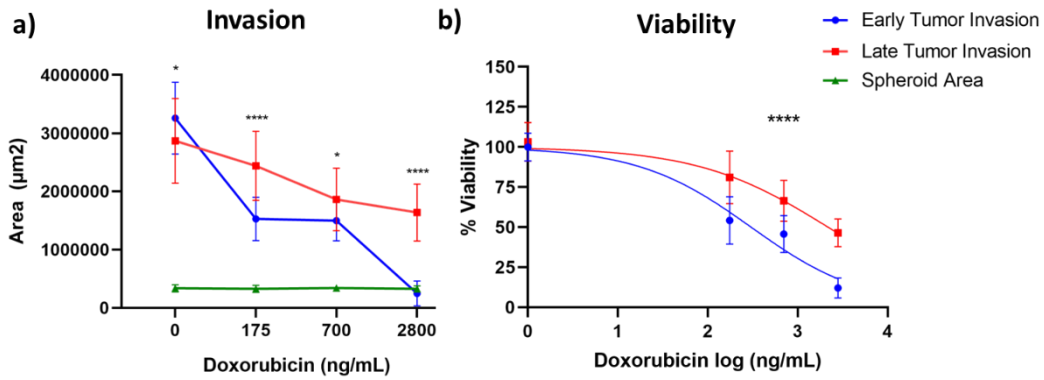

Figura 2. Graphs showing the differences in viability (a) and invasion (b) between the two breast carcinoma models at the endpoint of the experiment (day 7). Significance values are represented as $p \leq 0.1$ (*) and $p \leq 0.0001$ (****).