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

Vira Sharko[1,2], Andrea Pérez-García[1], Inés Mármol[1,2], Estela Solanas[1,2], Ignacio Ochoa[1,2,3]

[1]TME (Tissue Microenvironment) Lab; I3A - Universidad de Zaragoza, Mariano Esquillor s/n, 50018, Zaragoza, Spain
[2]Instituto de Investigación Sanitaria de Aragón (IISA); 50009, Zaragoza, Spain
[3] CIBER BBN Instituto de Salud Carlos III; 50009, Zaragoza, Spain
Tel. +34-976762707, e-mail: 838529@unizar.es

Summary

This work aims to develop an *in vitro* three-dimensional model of triple negative breast cancer to study the influence of tumor microenvironment on treatment response and cell invasion. The model was exposed to chemotherapeutic agents and different responses to treatment were observed when cells were cultured on 2D or 3D.

Introduction

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer. The high metastatic potential of TNBC, together with the lack of targeted therapies, lead to poor prognosis and high mortality rates [1]. The development of novel therapeutic strategies for the management of this breast cancer subtype is urgently needed, especially those focused on the early stages of the metastatic process. However, mainly due to the difficulty in establishing an accurate tumor microenvironment (TME), current *in vitro* models fail to recreate the complex metastatic cascade [2].

Within the framework, the ductal invasive carcinoma cell line MDA-MB-231 was used to develop a three-dimensional (3D) TNBC model to determine the role of the TME on the response to doxorubicin (DXR) and paclitaxel (PTX), both frequently used as first-line treatment in BC [3, 4]. Changes in cell proliferation, invasion and sensitivity to both drugs on the different phenotypes established within the invasive breast cancer TME were analyzed. Furthermore, both drugs were tested on breast metastatic adenocarcinoma MDA-MB-468 and acantholytic variant of squamous cell carcinoma of breast HCC1806 cell lines to further explore their effect on different TNBC *in vitro* models.

Materials and 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 following the liquid overlay method. Further, MDA-MB-231 stable spheroids (600 µm diameter and established necrotic core) were embedded in 3 mg/mL collagen hydrogel matrices and transferred to 96-well plates. MDA-MB-468 and HCC1608 spheroids were developed following the same protocol (Figure 1). Matrix invasion was assessed by epifluorescence microscope (Leica Microsystems, Thunder) and confocal microscope (Nikon, Eclipse). ImageJ and GraphPad Prism 8 softwares were used for analysis and quantification.

DXR and PTX effects were evaluated upon a single application of half and twice a concentration estimated from values obtained in patient blood. Cell Titer Glo was used to test the viability of the 3D model, which was compared with viability of a traditional culture in 2D, measured by Presto Blue, to analyze the influence of TME in response to treatment.

Results

Different response to chemotherapeutic drugs in 3D versus 2D culture. MDA-MB-231 cells were less responsive to DXR and PTX when seeded in spheroids than in 2D. Furthermore, both MDA-MB-468 and HCC1806 cell lines displayed the same behavior when cultured in 2D or 3D dimensions and treated with DXR and PTX.

Effect of chemotherapy on early and late invasion models. Both DXR and PTX reduced the invasion and viability of the collagen hydrogel-embedded MDA-MB-231 spheroid model (Figure 2). However, the above effects were less pronounced when DXR was applied once the cells have exited the spheroid (after 4 days of invasion).

Conclusions

All three breast cancer cell lines tested showed greater resistance to both chemotherapeutic drugs when cultured on 3D rather than on traditional 2D culture. Furthermore, we observed that both DXR and PTX were able to reduce cell invasion when administered prior the metastatic cascade had begun in a 3D MDA-MB-231 cell model. This suggests that both drugs may reduce the risk of metastasis in TNBC. However, since cells once invaded into matrix were less responsive to DXR, the efficacy of this drug may be compromised in advanced stages of this disease.

These results highlight the relevance of considering the effect of TME when evaluating the performance of chemotherapeutic drugs on *in vitro* models. Taken together, these data suggest that this 3D TNBC model, whose development process is easy and user-friendly, offers an in vitro platform suitable for studying cancer cell invasiveness and drug evaluation that more closely resembles physiological responses.

REFERENCES

[1]. ARNOLD, M., et al. Current and future burden of breast cancer: global statistics for 2020 and 2040. *Breast*. 2022. doi.org/10.1016/j.breast.2022.08.010.

[2]. AZIMI, T., LOIZIDOU, M., and DWEK, V. Cancer cells grown in 3D under fluid flow exhibit an aggressive phenotype and reduced responsiveness to the anti-cancer treatment doxorubicin. *Scientific Reports*. 2020. 10 (1), 12020. Available from: doi:10.1038/S41598-020-68999-9.

[3]. LOVITT, C.J., SHELPER, B.T., and AVERY, V.M. Doxorubicin resistance in breast cancer cells is mediated by extracellular matrix proteins. *BMC Cancer*. 2018. 18 (1). doi: 10.1186/S12885-017-3953-6.

[4]. ABU SAMAAN TM, SAMEC M, LISKOVA A, KUBATKA P, BÜSSELBERG D. Paclitaxel's Mechanistic and Clinical Effects on Breast Cancer. Biomolecules. 2019 Nov 27; 9(12): 789. doi: 10.3390/biom9120789
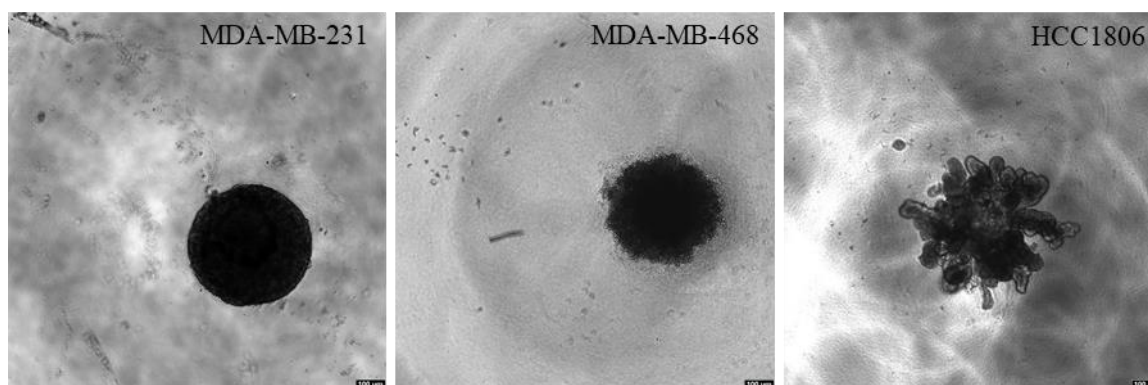
Figure 1. Three-dimensional triple negative breast cancer models (left: MDA-MB-231 cell line); center: MDA-MB-468 cell line; right: HCC1806 cell line).
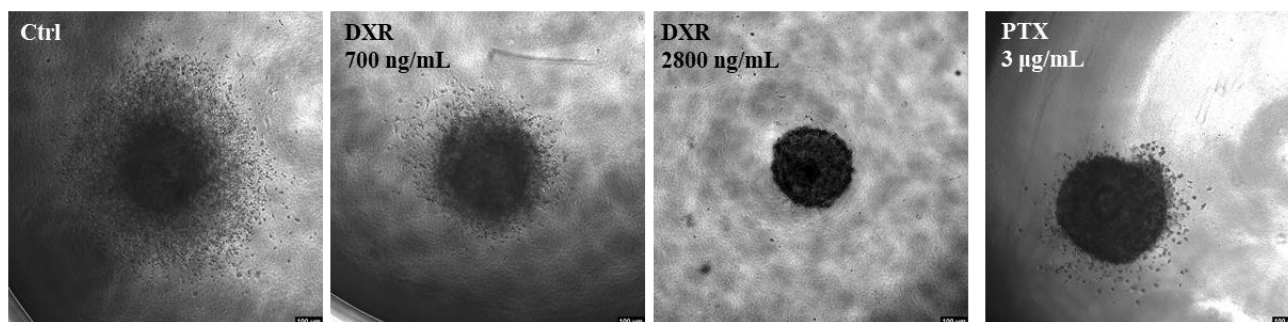

Figure 2. MDA-MB-231 invasion 4 days after treatment with doxorubicin or paclitaxel.