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BREAST CANCER SPHEROID MODELS AS A SCREENING PLATFORM FOR COMBINATION THERAPIES

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INTRODUCTION

3D culture as spheroids better recapitulates the tumor micro-environment[1] when compared to conventional adherent 2D cultures. Therefore, in vitro spheroid models will represent an important tool in cancer research, allowing optimized drug selection and improved drug tumor distribution. In breast cancer (BC), 3D models will contribute to a reduction in the number of experimental animals employed and associated costs related to drug screening[2]. Spheroidal growth originates from self-renewing breast cancer stem cells (CSCs - $CD44^{+}CD24^{low}$)[3], whose presence correlates well to long-term resistance to chemotherapy in patients.

The molecular complexity of BC requires the application of drug combinations to inhibit tumorigenic growth and metastasis efficiently. We are currently working to identify drugs that preferentially affect 3D models and then incorporate them into rationally-designed combination therapies via the conjugation to polymeric scaffolds as polymer therapeutics[4-7].

OBJECTIVES

1. Production and characterization of spheroid models representative of the four clinical BC subtypes: MDA-MB-231 (ER-PR-HER-, triple negative), MDA-MB-453 (ER-PR-HER2+, Her2+), ZR75.1 (ER+PR+HER2+, Luminal B), MCF7 (ER+ PR+ HER2-, Luminal A)
2. Comparison of high throughput screening (HTS) of a chemical library in mammospheres vs. adherent models in order to identify differential candidates
3. Design and synthesis of polymers conjugates with the drug candidates (single or in combination) to improve therapeutic outcomes towards metastatic phenotypes

METHODOLOGY

We cultured MDA-MB-231, MDA-MB-453, MCF7, and ZR75.1 cell lines in suspension as mammospheres in the presence of EGF2 and B27 using low adherence plates. We employed markers of CSCs (CD44, CD24), the undifferentiated state (SOX2), and proliferation (Ki67) in a flow cytometry characterization step to evaluate differential cell behavior when grown as mammospheres. With the challenge of finding drugs with better efficacy in spheroid models (3D) than in adherent models (2D), we performed HTS with a commercial chemical library using the MTS assay. To understand the effects of the positive anti-tumor drugs screened in mammosphere model, we measured the number and diameter of treated mammospheres.

RESULTS

Our preliminary results demonstrate that the triple negative MDA-MB-231 spheroid model presents a large number of quiescent cells and an enrichment of CSCs when compared to the higher proliferation rate observed in MDA-MB-453 and ZR75 spheroid models. These findings could be an explanation for therapeutic drug resistance characteristic in this type of tumor.

We performed an HTS with a commercial chemical library to study the effect of drugs in 3D models compared to adherent cell culture (2D models). HTS revealed that certain compounds functioned more efficiently in spheroids derived from the MDA-MB-231 model than in 2D cultures and provided evidence that some drugs may function in 3D but not in 2D culture, perhaps as the molecular target is expressed only in the 3D environment. Overall, drug treatment significantly reduced the number and size of spheroids. Currently, we are

synthesizing polymer-based anti-tumor combination therapeutics for assessment in spheroid models following the previous experience that our laboratory has in polymerization techniques to further enhance the effectiveness of selected drugs.[4-7]

CONCLUSION

We have established mammospheres of four BC cell lines representative of breast cancer clinical subtypes as an improved approach to study tumorigenesis. Flow cytometry for SOX2 and Ki67 markers demonstrates that cells grown as 3D mammospheres are present in a more differentiated and less proliferative state, a phenotype more marked in the triple negative model, which also exhibits an enrichment of CSCs. This combination may explain characteristics such as elevated aggressivity and drug resistance. Drug HTS has allowed us to identify drugs with greater anti-tumorigenic capabilities in mammospheres than in adherent models. We now aim to design polymer conjugates of these drugs to create enhanced therapies for BC treatment.

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