Establish Biomimetic 3D Immune-Spheroids as a Model to Address Triple-Negative Breast Cancer Radioresistance

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Introduction

Triple-negative breast cancer (TNBC):
• aggressive subtype of cancer that lacks the expression of therapeutic targets (ER PR HER2) [1];
• more radioresistant than the other breast cancer subtypes [2];
• associated with a higher risk of locoregional recurrence after radiotherapy [2].

Figure 1- Triple-negative breast cancer (TNBC) microenvironment. After radiotherapy, immune cells, namely macrophages, are highly recruited to the tumor microenvironment and may dictate radiotherapy responsiveness [3, 4]. Figure created with Biorender.

Aim

To develop a biomimetic 3D model that gathers radioresistant or radiosensitive TNBC cells, and human macrophages, to mimic the tumor microenvironment.

Specific Aims

1. Establish immune-spheroids with radioresistant or radiosensitive TNBC in culture with human macrophages;
2. Immune-spheroids characterization regarding to cancer cells immunogenicity and macrophages inflammatory profile;
3. Evaluate the impact of radiotherapy on macrophage and cancer cells interaction.

I. Methodology and Results

MDA-MB-231 cells (TNBC cell line) were seeded in 96-well plate coated with 1.5% (w/v) agarose (Figure 2) at three different densities: 500, 1000, and 2500 cells/well and at two different mediums: DMEM (Gibco) and DMEM high glucose (ATCC) (Figure 3).

Figure 2- MDA-MB-231 cells seeded in 96-well plates coated with 1.5% (w/v) agarose in dh, O. Figure created with Biorender.

After five days, the breast cancer spheroids were observed in all conditions. However, these spheroids are very heterogeneous, and after five days some cell dispersion is visible.

II. Methodology and Results

To overcome the heterogeneity observed in spheroids formed with the previous methodology, the TNBC-spheroids were formed using agarose micro-molds. This methodology allows us to create in the single micro-mold 81 homogenous spheroids (Figure 4).

Figure 4- Agarose micro-molds formation. A) 3D Petri Dishes micro-molds. B) Fill micro-mold with 2% (w/v) agarose in 3-D Petri Dishes micro-mold. C) Separate the gelled agarose from 3D Petri Dishes micro-mold. D) Agarose micro-mold. Figure created with Adobe Illustrator.

II. Methodology and Results

MDA-MB-231 cells were seeded in 2% (w/v) agarose molds (Figure 5) at three different densities: 1000, 2500, and 5000 cells/spheroid and two different mediums: DMEM/F12 (Gibco) and DMEM high glucose (ATCC) (Figure 6).

Figure 5- MDA-MB-231 cells seeded in agarose micro-molds. Figure created with Biorender.

After seven days, the spheroids were collected and dissociated for cell viability analysis by flow cytometry (Figure 7).

Figure 6- MDA-MB-231 cell viability assessed by flow cytometry analyses. A) Gating strategy used to select live cells in 2500 cells/well DMEM high glucose condition. B) Percentage of live cells in different conditions.

These results lead us to select the condition of 5000 cells/spheroid seeded in DMEM high glucose for further experiments.

Future Work

Figure 7- Radiosensitive Immune-spheroid

References