Breast and lung cancer spheroids as tools to investigate novel radiotherapeutic strategies

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Breast cancer (BC) and lung cancer (LC) remain two of the most prevalent malignancies worldwide.[1] Cancer 3D models, such as spheroids and tumoroids, have gained relevancy during the past years due to their better ability to mimic the tumor microenvironment, a key factor in tumor drug response.[2] Radiotherapy (RT) is one of the main types of treatment used on cancer patients, especially in combination with chemotherapeutic drugs.[3, 4] Photon and proton RT effect on tumoral cells may differ, possibly resulting in distinct outcomes for molecular (DNA damage and repair) and cellular (cell invasion) processes.[4-6]

Here, we report on the establishment and characterization of spheroids from BC (MCF7, MDA-MB 231, and SKBR3) and LC (A549 and H69) cell lines and the assessment of their radiosensitivity to gamma radiation. The establishment of spheroids was optimized for all cell lines using an exogenous extracellular matrix (Matrigel® GFR) to obtain spheroids with an appropriate diameter (300-500 μm) on the 3rd day of culture. Such range allows the generation of spheroids with an appropriate size to have a hypoxic core without necrosis, ensuring the optimal gradient of oxygen and nutrients, which affects tumor resistance.[7] The characterization of the 3D spheroids included daily growth monitoring of the diameter, area, and circularity. The expression of relevant markers such as HIF-1**α** (hypoxia marker) and PARP (DNA damage and proliferation marker) was assessed using Western Blot. Initial optimization studies of fluorescence microscopy to analyze spheroid apoptosis and necrosis were conducted after incubation with a chemotherapeutic drug (cisplatin), which could be used as a control in future assays. The radiosensitivity of spheroids was assessed through viability (CellTiterGlo 3D) and survival assays after irradiation with γ photons.

These initial studies demonstrated that spheroids from different cell lines respond differently to the γ-radiation treatment. However, further optimization and studies are required to fully assess their radiosensitivity, as well as the treatment effects on DNA damage and ROS production.

Reproducible establishment of spheroids and their characterization are important factors to properly assess novel therapeutic strategies, thus, the present work presents an initial study on the optimization and use of BC and LC spheroids for the preclinical evaluation of RT approaches in combination with chemotherapeutic drugs. In the future, the treatment's effect will also be tested on tumoroids, as a more complex model of the *in vivo* tumors.

Acknowledgment: This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal, through the grant UID/Multi/04349/2020 to C2TN and the PhD Fellowship to DME. FMendes acknowledges FCT and Campus France for PESSOA grant 2021.09137.CBM, and FCT and UTAustin Portugal Program for project 2022.15449.UTA

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