2024 International Nuclear Atlantic Conference – INAC 2024
Escola de Guerra Naval, Rio de Janeiro, RJ, May 6 - 10, 2024

High-throughput Production of Malignant Melanoma Spheroids (sk-mel-37) Using Simple Plate Treatment and Automated Fluorescence Microscopy Analysis

S. R. Prudente[1], E. C. dos Santos[1], G. D. da Silva[1], M. M. P. Lima[1], P. L. Falcão[1], T. M. da Silva[1], D. P. Vieira[1]

[1] *Instituto de Pesquisa Energéticas e Nucleares (IPEN) – São Paulo, Brazil*

suleyna@gmail.com

1. Introduction

Cancer is one of the leading causes of death worldwide and represents a significant challenge to increasing life expectancy in many countries. Aging and behavioral and environmental changes, such as mobility, recreation, diet, and exposure to environmental pollutants, contribute to the rising incidence and mortality rates of the disease. In 2020, according to estimates from the Global Cancer Observatory (Globocan), compiled by the International Agency for Research on Cancer (IARC), there were 19.3 million new cases of cancer worldwide. In the case of Brazil, estimates for the triennium 2023 to 2025 suggest that 704 thousand new cases will occur. [1]

Malignant cutaneous melanoma represents about 5% of malignant skin tumors and shows increasing incidence and high lethality, being responsible for a large portion of skin cancer deaths. In Brazil, estimates for the year 2015 indicated incidence rates of 3.47 new cases per 100,000 inhabitants for men and 3.07 for women. The mortality rates for the same year were 1.22 deaths per 100,000 inhabitants for men and 0.86 for women. [2]

For the triennium from 2023 to 2025, the estimated number of new cases is 8,980, corresponding to a risk of 4.13 per 100,000 inhabitants. In the Southern Region, melanoma skin cancer is more incident when compared to other regions. [1]

Regarding risk factors, professional activities requiring sun exposure such as tourism, fishing, agriculture, and construction are notable. [2]

Currently, preclinical studies in oncology are conducted through in vivo and in vitro assays. With the progress of research in this field, there is a need to develop new in vitro models aiming to reduce the use of animal methods, replace them with equally or more effective alternatives, and improve existing methodologies to minimize the impact of research on animal use, in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement) proposed by Russel and Burch in 1953. [3]

However, traditional cell culture-based in vitro assays are not always suitable. In the context of cancer research, one of the main challenges in understanding the involved biological processes and evaluating the efficacy of anticancer drugs is the lack of appropriate in vitro models. This is because two-dimensional cell culture assays fail to faithfully replicate the complexity of tumor physiology, including aspects such as hypoxia and drug penetration. In this regard, three-dimensional models provide cellular aggregates with

characteristics more like in vivo conditions, featuring more organized structures and greater cellular heterogeneity, allowing for interaction among cells, the extracellular matrix (ECM), and the tumor microenvironment in a more realistic manner. [4]

This study aims to standardize the production of human melanoma spheroids using Pluronic F®-127 pre-treated plates and their response to cobalt therapy. Additionally, it aims to assess their compatibility with high throughput screening (HTS) techniques for production optimization.

2. Methodology

Human melanoma cell lines (SK-MEL-37) were cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% (v/v) fetal bovine serum (Cultilab) and 2% (v/v) antibiotic solution (10,000 U/mL penicillin, 10 mg/mL streptomycin, and 1 mg/mL amphotericin B, Gibco) and maintained in an incubator at 37°C, 5% CO2, and controlled humidity. 24-well cell culture plates were pre-treated with Pluronic® F-127 solution (0.5 g/mL in 2-propanol), 1 mL per well, and left at room temperature for 48 hours. After this period, the liquid was removed by suction, and the plates were dried in a sterile laminar flow hood and under UV irradiation for 30 minutes. Upon reaching 60-70% confluence, cells were washed with phosphate-buffered saline (PBS) and detached using trypsin solution. Afterwards, they were seeded at a density of 1×10^5 and 2×10^5 cells in 24-well plates and returned to the incubator for 72 hours for spheroid formation.

The spheroids produced in the 24-well plates were irradiated with gamma radiation from a 60-Co GammaCell 220 source (Atomic Energy Canada Co.) at 2 Gy. The control group consisted of non-irradiated samples (0 Gy) but subjected to the same conditions. After 24 hours, cell viability was analyzed.

For the cell viability assay by fluorescence microscopy, the cells in the plates were stained with Hoescht 33342 (10µg/mL) and 400µM de SYTOX® Green (Thermo-Fisher Scientific, S7020) and incubated for 60 minutes. The material was viewed and photographed on 4x on imaging platform HCS/HTS (High Content-Screening / High Throughput Screening) INCell Analyzer 2500HS – Cytiva. Cell nuclei were highlighted in blue, marked by Hoescht 33342 and non-viable cells were highlighted in green for Sytox®Green.

The images were evaluated using INCarta 1.17 software (Cytiva), and the numbers of viable and non-viable cell nuclei, as well as morphological parameters such as area and sphericity, were analyzed.

3. Results and Discussion

It was possible to observe that, using Pluronic® F-127, the spheroids form freely, without external constraints such as gravity or magnetic interaction.

Properly cohesive spheroids were obtained, with 20-30 spheroids per well. At the end of the experiment, only a small fraction of cells (about 5%) were considered non-viable by SYTOX® staining. There was no significant difference between the control groups (0 Gy) and the irradiated groups (2 Gy) regarding the volume of cellular aggregates, volume of cellular nuclei, and volume of non-viable nuclei.

Melanoma has a high potential for metastatic spread, making it a challenging tumor to eradicate, and is traditionally known as a radioresistant tumor, which may explain the limited role of radiotherapy in melanoma treatment compared to other tumors. Studies demonstrated that an improved response to low radiation (up to 8 Gy) only after radiosensitization using RO4929097 to inhibit Notch signaling, which is

consistent with the small variation in results found at control group and irradiated group in this study. [5]

Principal Component Analysis (PCA) using 13 variables, and further Principal Component Regression (PCR), revealed that nuclei mean and maximum intensities (Hoechst), as well as nuclei volume, are the most relevant variables correlated to the number of plated cells.

4. Conclusions

It was concluded that the methodology to produce spheroids for melanoma cell lines presented is simple, fast and cheap, in which, in 72 hours, the spheroids form freely, without restriction of shape and size and presenting low cell death, being also compatible with the high throughput screening technique (HTS). Nuclei volume and intensity proved to be good analysis parameters and can be used in future studies to assess cell global viability in spheroids in large-scale.

Acknowledgements

The author thanks IPEN/CNEN-SP for funding, infrastructure and equipment used for the development of the project.

References

[1] Instituto Nacional de Câncer José Alencar Gomes da Silva. *Estimativa 2023: incidência de câncer no Brasil*, Inca, Rio de Janeiro, Brasil (2022). Available from: https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2023.pdf

[2] C. A dos Santos, D. L. B. Souza, “Melanoma mortality in Brazil: Trends and projections (1998-2032)”, *Ciencia e Saude Coletiva*, vol. 24, n. 4, pp. 1551–61 (2019).

[3] “Ministério da Ciência tecnologia e inovações”, https://antigo.mctic.gov.br/mctic/opencms/ciencia/SEPED/Saude/renama/renama.html (2023).

[4] P. Q. S. Passos, “Modelo tridimensional in vitro de adenocarcinoma prostático humano produzido por levitação magnética”. *Instituto de Pesquisas Energéticas e Nucleares*; São Paulo, 2020. Available from: https://www.teses.usp.br/teses/disponiveis/85/85131/tde-01032021-114311/publico/2020PassosModelo.pdf

[5] K. T. Jayaprakash, M. Hussein, R. Shaffer, A. Michael, A. Nisbet, M. Ajaz, “In Vitro Evaluation of Notch Inhibition to Enhance Efficacy of Radiation Therapy in Melanoma”. *Adv Radiat Oncol*, vol. 6, n. 2 (2021).