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The role of Prolactin (PRL) in activating the signaling pathways of the Jak STAT complex as a potential candidate in radioresistant MDAMB-231 cells in 3D cultures

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1. Introduction

Breast cancer is an aggressive disease whose incidence and mortality has increased in recent years. The studies have advanced significantly in recent decades, early diagnosis and adequate therapy, as the results favor cancer treatment continue to be very challenging.[1,2] Considering the failure of some treatments for tumors resistant to some drugs and also to radiotherapy, it becomes relevant to investigate biomarkers and signaling pathways involved in highly radioresistant cells, such as the case of MDAMB-231, which may be involved, investigation of potential candidates for prognostic monitoring of breast cancer that present high affinity and specificity as potential candidates. Within this context, Prolactin (PRL), a polypeptide composed of 199 amino acids, appears to be involved in the activation of co-factors and tumor clonogenic signaling pathways. [3,4] PRL seems to establish a relationship between the development of breast cancer in triple-negative tumors, its role being still obscure. [5,6,7] One of the questions still not clarified is the extension of the expression of the non-tumoral PRLR receptor, including the activation of the clonogenic signaling pathways of the JAK-STAT complex. In particular, STAT-3 has emerged as a potential molecular candidate in cancer therapy. [8, 9,10,11]

The cultures and tests were carried out in two-dimensional models, where the cell-cell interaction occurs laterally, not allowing the formation of an environment that simulates the natural spatial distribution of the cells in our tissues. However, scientific evidence will show that these models do not allow cells to express their characteristics in a manner similar to what occurs in an organism. However, an alternative is to use three-dimensional models so that the tests can be assembled more years later in vivo, as the physiological behavior of a group of cells is influenced not only by the cell type, but also by the spatial organization of the cells and the zones. of communication on its surfaces. Furthermore, the mechanical stress resulting from cell grouping tends to confer similar physiological functions to both tissues *in vivo*. [5,6]

There are different methods to form three-dimensional models, such as the formation of spheroids from the formation of porous structures (scaffolds) made of biocompatible polymers and various types of hydrogen. These models can also be formed by magnetic levitation of cells in culture, using biocompatible paramagnetic microparticles, or by addition of nanoparticles to the cell surface and also by treatment of culture plates that prevent cell adhesion, or use of Pluronic® F-127 for coating plates or culture flasks that impetus to cellular adhesion, enabling the formation of free spheroids in the field of formation. This last method presents advantages in relation to previous years, because the formation of two spheroids is not controlled by any material, since the cell group is formed naturally, causing a cellular interaction similar to the organism that can stimulate the cells to produce their own extracellular matrix. .

2. Methodology

Cell culture: MDAMB-231 (human breast adenocarcinoma) cells cultured in DMEM (Eagle modified by Dulbecco) supplemented with 10% (v/v) bovine fetal sorus (Cultlab), and 3% (v/v)) of antibiotic solution (10,000 IU/mL of penicillin, 10 mg/mL of streptomycin, Gibco) and the HF002-J cells (Human fibroblasts derived from primary cultures) isolated from humans at the Radiation Technology Center, CETER – IPEN , as quais cultured in 25 cm2 cell culture bottles containing RPMI 1640 (Sigma-Aldrich) supplemented with 10% (v/v) bovine fetal sorum (Cultlab), and 1% (v/v) solution of antibiotics (10,000 IU/mL penicillin, 10 mg/mL streptomycin, Gibco), kept in incubator at 37°C, 5% CO2 with controlled humidity. Ao reach confluência between 60-70%, the cells form highlighted with trypsin solution (0.05%).
Production of Spheroids: The NIH/3T3 cells after trypsinization and contagion will be suspended in the solutions in the concentrations 1 x 10^5 cells per well in a 24-well plate pre-treated with Pluronic® F-127 solution (0.5g/mL in 2 -propanol) and the spheroids are formed after 3 days of application of the cells in the plate.
Pre-treatment of plates: Foram used cell culture plates with 24 wells, with foram pre-treated with Pluronic® F-127 solution (0.5 g/mL in 2-propanol). Each well received 1000 (24 wells) microliters/well and will remain at room temperature for 24 hours within laminar flow. After this period, the maximum amount of liquid was removed by suction and the plates. Format posts for drying in a sterile laminar flux cape under UV irradiation to eliminate or contamination risk for 30 minutes. With this procedure, the copolymer molecules are Start in "pancake" configuration with its hydrophobic portion directed towards the center of the little, thus preventing non-plastic cellular adhesion of culture.
Lines established and maintenance of *in vitro* culture Peripheral blood mononuclear cells (PBMCs): were obtained from healthy volunteers in a sterile manner and placed in culture with RPMI-1640 meio, supplemented with 10% bovine fetal serum and antibiotic gentamicin (50 μg.μL-1) and streptomycin (500 mg. mL-1) in T-25 culture flasks, in a humid atmosphere containing 5% CO2 at 37°C. Separation of PBMC. The separation of the PBMCs was carried out according to the procedure described by Falcão et al., 2015. The heparinized blood was applied in 15 mL, silicone tubes, containing a mixture of Ficoll-diatrozate (OrganonTeknika Corporation; Durham, NC), in a proportion of a part Ficoll-diatrozato in two parts of blood. The solution was centrifuged for 30min at 1,400 RPM at room temperature. At the end of the spin cycle the panel of mononuclear cells at the interface between the Ficoll. The panel of PBMCs was carefully removed by Pasteur pipette and transferred to 15mL tubes and centrifuged at 3X no supplement for 10min at 1,200 RPM. The cell concentration was corrected to suspension with 1.0 × 10^6 cells/ml in supplemented RPMI-1640, being manipulated under stereo conditions inside a laminar flow cabinet model Biological Cabinet BBL 60474.
Iradiation: To prepare the plate before irradiation, add 300 ng/ml of prolactin (PRL) soon after forming two spheroids. After 10 minutes, the plaques irradiated with a dose of 2 Gy using the gamma radiation source ^{60}Co (GammaCell) from the Radiation Technology Center (CETER – IPEN), at a dose rate of 130 kGy/h. After irradiation, the cultures 3D placed in the incubator at 37ºC and 5% CO2 for 24 hours and analyze using the INCell Analyzer 2500 HS optical microscope (Cytiva Lifesciences) located on the grounds of CEBIO, IPEN – CNEN.
Cell viability: The contained cells are coated with fluorescent dyes. For isso, The half of culture was removed and new half of culture was added with Hoescht 33342 (10 mg/mL) and SYTOX™ Green (5 mM) in 195 μL of RPMI 1640, adding 100 μL per well to each solution. The structures verified by INCell Analyzer 2500 HS microscope (Cytiva Biosciences), which after excited in the violet light spectrum, the cells marked in blue (Hoescht) are shown as cells cut with SYTOX™ Green marked in green invisible cells.

3. Results and Discussion

The results derived from the analysis carried out with the INCell Analyzer 2500 HS microscope (Cytiva Biosciences) indicate that the approach used for the three-dimensional model reproduces cellular interaction in a more efficient way, given that it allows interactions between different cell types similar to those found in living tissues, also maintain a free three-dimensional conformation. In this context, it was possible to confirm that prolactin (PRL) plays a relevant role in 3D co-cultures involving MDAMB-231 cells, influencing their differentiation, proliferation and cloning over a culture period of 24, 48 and 72 hours. A clear difference was observed between the addition of prolactin and its absence, when compared to cultures containing only cells from the MDAMB-231 tumor line. This response was also accentuated by the presence of HF002-J (human fibroblasts derived from primary cultures) and PBMC cells (peripheral blood mononuclear cells), resulting in an increase in cell viability in the few contained in these cells. Even cells exposed to 2 Gy of gamma radiation maintain a response similar to control cells, showing tumor proliferation instead of mortality. Furthermore, it was observed that fibroblasts, as observed in previous in *vivo* studies, envelop the tumor as it forms, being highly reconstituted by PBMC cells, especially in the presence of prolactin. [12, 13, 14] The PBMC cells migrate into the spheroid after the first 4 hours of incubation, concentrating especially in the tumor region, which suggests a preferential recognition of the environment. [15,16]

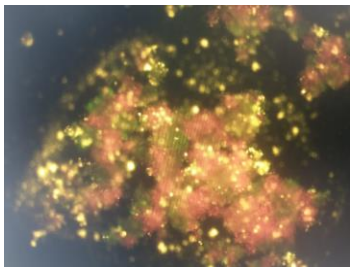

Figure 1 - MDA MB-231 tumor cells in co-culture
of fibroblasts and PBMC + Prolactin (3D - the INCell Analyzer)

4. Conclusions

In summary, research addresses the crucial need to explore new methods and three-dimensional models to better understand the interaction between tumor cells and their microenvironment, aiming to advance the diagnosis and treatment of breast cancer. The complexity and diversity of cellular interactions, particularly in a three-dimensional context, offers a closer perspective on the real physiology of the tumor tissue, potentially revealing some relevant therapeutics and biomarkers. The use of 3D cultures, as demonstrated in this study, allows a more precise evaluation of the effects of molecules such as prolactin and the influence of different cell types, such as fibroblasts and peripheral blood mononuclear cells, on the dynamics of breast cancer. The results obtained highlight the importance of three-dimensional models in oncological research, providing valuable insights that can be translated into more effective and individualized therapeutic strategies for patients with breast cancer. For future work, higher doses of radiation will be performed in parallel with prolactin antagonists to try to prevent tumor proliferation and differentiation.

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