Three-dimensional models to investigate breast cancer morpho-functional features

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Abstract: Breast cancer (BC) is the most diagnosed type of cancer and the leading cause of cancer-related death in women worldwide[1]. It is a heterogeneous disease that needs adequate models to simulate its complexity. To this end, three-dimensional (3D) models provide a good compromise between biological complexity and feasibility of handling as compared to animal models and bi-dimensional (2D) monolayer cell cultures[2]. Since metabolic reprogramming is recognized as one of the hallmarks of cancer[3], this aspect can be studied in BC to identify new potential targets to be exploited for novel therapies. In this work we have studied the proliferative, differentiative, metabolic and morpho-functional features of three BC cell lines (MCF7, MDA-MB-231 and SUM159PT), comparing 2D cultures and spheroids (3D). The results show that within the spheroids the number of vital cells does not increase over time and the percentage of cells in S-phase of cell cycle is reduced compared to 2D cultures. This aspect can be partially explained in MDA-MB-231 and SUM159PT by a differentiative rearrangement observed in the cell populations expressing markers of basal and luminal progenitors. Surprisingly, the analysis of metabolic parameters by Seahorse technology demonstrates that the transition to the 3D architecture is not necessarily linked to an increase of the glycolytic pathway as could be supposed considering the physiological formation of a hypoxic *core*. However, all these observations refer to average values among all cells of the spheroid, not addressing the relevant problem of spatial diversification of cells within it due to the different exposure to nutrients, oxygen, and waste products among the external and internal layers. Therefore, we are developing a cycling immunolabeling method for high-content imaging on spheroids' sections[4] to investigate spatial resolution of metabolic enzymes within the spheroids.

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