Functional characterization and metabolic studies of breast cancer three-dimensional model by flow cytometry

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BACKGROUND: Breast cancer is the most common cancer and the first cause of cancer-related death worldwide in women. Despite the improvement in targeted therapy, breast cancer relapses and metastasis treatments represent still a massive clinical need. The generation and analysis of metabolomics and transcriptomics data could represent a real chance to find new clinical approaches; however, the analysis has been mainly limited to blood and tissue samples, without the possibility to evaluate dynamic changes. To perform these analysis new models resembling the tumor and suitable for high throughput screening are needed.
AIM: to develop three-dimensional in vitro models able to recapitulate the architectural complexity and heterogeneity of breast cancer, to be used for metabolic characterization and suitable for omics studies.

METHODS: Immortalized cell lines, derived from normal and tumoral breast tissue (triple negative breast cancer: SUM159PT, MDAMB231; estrogen receptor positive breast cancer: MCF7; immortalized normal mammary tissue: MCF10A), are used to test different protocols for 3D culture and to evaluate spheroid morphology and dimensions (phase-contrast microscopy), and architecture (confocal microscopy imaging) to define the faster and more reproducible method. Flow cytometry was used to study cell differentiation and structural markers, as well as mitochondrial activity to characterize the model. Next, spheroid generation and growth were studied under metabolic perturbation obtained through drug treatment targeting metabolic pathways and nutrient limitation and deprivation.

RESULTS AND CONCLUSIONS: We were able to set up successful protocols for spheroid culture and characterization. SUM159PT, derived from a primary tumor, showed the highest propensity to form tight spheroids, while MDAMB231, derived from pleural metastasis, showed the lowest, with loose and jagged structure. MCF7, as expected, was easily able to form spheroids both with suspension and drip culture methods. The non-tumoral epithelium cell line MCF-10A did not form spheroids able to sustain a stable culture and suitable for the metabolic analyses in any condition tested. The results of spheroid formation assay suggest an important role of glutamine and glucose in promoting spheroid formation in triple negative breast cancer cell lines, while mitochondrial respiration is probably only partially involved in this process. Hormone sensitive cell line MCF7 capability of spheroid generation was not inhibited by drugs targeting metabolism but cell viability was strongly affected.
MDAMB231 and SUM159PT cell lines showed at baseline opposite differentiation marker distribution. While nutrient limitation seems not to affect mitochondrial function and cell differentiation, culture time affects cell phenotype while grown as spheroids, depicting a more dynamic model compared to monolayer culture.