From Bulk Analysis to Single Cell: Dissecting Proliferative and Metabolic Landscape in Cancer Spheroids

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Abstract:
Cancer is a global spread disease whose complexity demands appropriate and easy-to-handle models able to represent and mimic *in vivo* heterogeneity. Therefore, a good compromise is brought up by *in vitro* three-dimensional models (3D), which, compared to bidimensional cultures (2D) should grant a better understanding of tumoral architecture *(Urzì et al., 2023)*.

In the context of this project, cancer spheroids, one of the simplest 3D models, have been obtained from cellular aggregates, with the aim of comparing 2D and 3D cultures derived from 9 immortalized breast and bladder cancer cell lines, by studying their bulk transcriptome, proliferative rate as well as their metabolic parameters.

Transcriptomics analyses revealed significant alterations emerging from the two models, hinting towards a general metabolic deregulation, the upregulation of hypoxia and a decrease in cell-cycle related processes, the latter being further experimentally validated, showing that within spheroids the percentage S-phase cells is lower compared to 2D cultures. Furthermore, the analysis of metabolic parameters using Seahorse technology has shown that the transition to 3D cellular organization is not necessarily linked to an increase of the glycolytic pathway as could be hypothesized given the physiological formation of a hypoxic core, thus suggesting a more articulated metabolic shift. To effectively investigate such deregulations also accounting for positional aspects, spheroids' cryosections have been stained, by applying IBEX technique *(Radtke et al., 2022)*, with a panel of viability and metabolic markers, focusing on glycolysis and respiration, to shed light upon expression distribution as well as to have a clearer overview on inter-line heterogeneous behaviours.

Further analysis using proper statistical and computational approaches to define eventual patterns or common features among cell lines suggested that the different observed metabolic profiles are not only due to single-cell metabolic landscape but are also linked to spheroids' architecture; Our findings could be further elucidated with the help of spatial transcriptomics as well as using 2-photon microscopy and by amplifying IBEX markers panel to enhance data solidity.