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Seahorse technology applied to 3D cultures: how the workflow optimization impacts on the improvement of data interpretation

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Abstract: Three-dimensional (3D) models of cancer, such as homotypic (obtained from a single cell type) and heterotypic[1] (from two or more different cell types) spheroids, are increasingly used for the study of cancer metabolism because they can closely recapitulate the tumor architecture. Within a spheroid, nutrient and oxygen gradients concur to create a cellular heterogeneity in which proliferative cells stay in the outern layers and quiescent or necrotic cells form the central core of the spheroid[2].
Agilent Seahorse XFe96 is a useful technology for the study of metabolic alterations occurring in cancer cells. However, while the employment of this technology on 2D cultures is very common, its application on 3D cultures is still poorly optimized.
The aim of this work was to develop a reliable and reproducible workflow for the metabolic analysis of three-dimensional cultures by Seahorse XFe96 technology.
We optimized the spheroids' formation protocol, demonstrating that it remarkably improves the reproducibility of Oxygen Consumption Rate (OCR) and ExtraCellular Acidification Rate (ECAR). This protocol, indeed, enables the formation of more regular and similar in size and shape spheroids, reducing the variability among the experimental technical replicates.
Since OCR and ECAR values are strongly influenced by the number of metabolically active cells in each spheroid, normalization is important to compare different types of spheroids and to compare 2D and 3D cell culture Seahorse data. We evaluated different methods of normalization of Seahorse data in 3D cultures (spheroid area, indirectly calculated cell number, protein content and DNA content) and we found that the measurement of spheroids' area using Operetta CLS™ high-content analysis system is the most reliable method for this purpose.
Finally, we validated this workflow on the Seahorse analysis of heterotypic spheroids formed by co-cultures of human normal mammary fibroblast and breast cancer cells.

References:

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