Imaging and molecular characterizaton of a new 3D bioprinted glioblastoma model for drug screening

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Glioblastoma Multiforme (GBM) is the most aggressive primary brain tumor. Treatment is highly difficult due to its infiltrative nature, high intra- and inter-heterogeneity, and resistance to standard therapies.

Despite significant efforts to develop new strategies against GBM, current treatment relies on the Stupp protocol, which consists of surgical removal followed by radiotherapy and temozolomide chemotherapy.

Conventional 2D models, not taking into account the Extracellular matrix (ECM) signals in Tumour MicroEnvironment (TME), fail to replicate the complex physical, mechanical, and biochemical properties of GBM, limiting their effectiveness for animal-free drug testing.

3D bioprinting allows for the creation of advanced in vitro tumor models that accurately mimic the biochemical and structural properties of the ECM, modulating the functional complexity of the GBM TME and helping to bridge the current gap.

Although the role of glycans is still largely unexplored, it is known that the glycosignature, including sialic acids in post translational modifications of proteins and sulphate glycosaminoglycans (GAGs) in proteoglycans, is involved in the functional modulation of cell fate within the TME. In particular, the disruption of these physiological glycosignatures is strongly linked to the onset of pathological conditions, including the promotion of tumor growth, immune escape, immunosuppression, and invasion by modulating cell signaling, cell-matrix adhesion, and extracellular matrix remodeling.

In this study, the application of hydrogel-based 3D Bioprinted GBM model (3DGlycoGBM) and the use for drug screening applications is presented. Using cutting-edge imaging and molecular techniques such as multiplex immunofluorescence (IF) analysis and high resolution nano-computed tomography (nano CT) analysis we have characterized the 3D bioprinted hydrogel with incorporating patient-derived GBM cells. The 3DGlycoGBM in vitro model, with different glycosignatures, selected using data obtained from ML-guided analytical pipeline GlycoMatrix_GBM demontrates that glycosignatures influences GBM cell growth, matrix deposition and drug response, concomitantly emphasizing the critical necessity of meticulous model validation for the drug preclinical screening.

Keywords: Glioblastoma, 3D bioprinted in vitro model, glycosignature, drug preclinical screening