Towards integral mapping of glioblastoma cell invasion in cerebral organoids

T. Van De Looverbosch[1], S. De Beuckeleer[1], S. Peeters[1], J. Van Den Daele[1], J. Sijbers[2], W. De Vos[1,3,4,5]

[1] Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium; [2] Imec-Vision lab, University of Antwerp, Department of Physics, University of Antwerp, Antwerp, Belgium; [3] IMARK, University of Antwerp, Antwerp, Belgium; [4] Antwerp Centre for Advanced Microscopy, University of Antwerp, Antwerp, Belgium; [5] µNeuro Research Centre of Excellence, University of Antwerp, Antwerp, Belgium.

Abstract

Glioblastoma multiforme (GBM) is the most lethal brain tumor in adults. As there is no cure, identification of novel therapeutic entry points is crucial. A significant hurdle in developing effective treatments is the limited understanding and characterization of the spatial distribution and dynamics of the three-dimensional (3D) infiltration of GBM cells in the brain. While recapitulating this complex phenomenon in 2D cell cultures is impossible, it has now become possible to generate human organoids that consist of different cell types with defined 3D organization and patterning using human induced pluripotent stem cell (hiPSC) differentiation protocols. As these models have to potential to better capture local in-vivo conditions, we aim to develop a method to comprehensively quantify GBM cell invasion in human cerebral organoids. Pre-labeled GBM cells were allowed to co-colonize iPSC-derived NPC spheroids and immature cerebral organoids. Thereafter, they were counterstained with a nuclear dye, cleared and visualized using fluorescence microscopy. We imaged the spheroids in-toto, while we used 100 µm thick slices of the much larger organoids (>1000 µm diameter). Even with clearing, the cellular density inside the 3D cultures, the typical anisotropic voxel size of the images, and significant nuclear dysmorphy of GBM cells complicate nuclear segmentation. Therefore, we trained a 3D U-Net based neural network to predict nuclear centroids instead. This showed to be more reliable than existing alternatives, such as Cellpose and StarDist. Using the pre-labeled GBM signal, our approach allowed quantifying the number and distribution of the GBM cells inside the 3D cultures, enabling rapid and integral 3D mapping of GBM infiltration. We are currently using this approach to monitor the impact of selected pharmacological compounds on the infiltration as a function of time. Next, we aim to develop deep learning-based method for label-free detection of GBM cells and integrate our approach into a scalable pipeline to investigate GBM invasion in in-toto cerebral organoids. This way, we aim to expedite drug discovery and pave the way for effective GBM treatments.