Mapping the cell state plasticity of patient-derived glioma stem-like cells

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Glioblastoma is the predominant form of brain cancer in adults. Due to its aggressive nature, less than 10% of patients survive longer than 5 years post-diagnosis. To date, no treatment is available that can eradicate all tumor cells and avoid relapse. This is in part due to the presence of stem-like glioma cells (GSCs), which can self-renew, invade and communicate with each other and the tumor microenvironment. Recent single cell transcriptomics analyses have revealed a continuum of four distinct transcriptional signatures within the GSC population. However, significant cell state plasticity within and between patients complicates our understanding of their individual contribution to GBM aggressiveness. To address this gap, we are developing an microscopy strategy to map individual cell states in relation to the local tumor microenvironment. First, we have established and validated a multiplex marker panel that allows unequivocal documentation of the four major cell states. Using this panel, we found significant heterogeneity in cell state composition in a panel of patient-derived GSCs. When growing the GSCs as 3D tumoroids, we could observe a phenotypic switch over time which differs between patients. Next, to fully recapitulate the different zones of the tumor microenvironment, we established an assembloid model by fusing a cerebral organoid with a GSC-derived tumoroid. Using an-house developed pipeline for in-flow, high-throughput light-sheet microscopy, we are currently screening assembloids with different patient cells to correlate GSC subtype diversity with infiltrative behavior. This will allow us to untangle the connection between GSC plasticity and tumor aggressiveness. Stratifying patient- and cell-specific migration patterns will offer an unprecedented view on in situ GSC behavior and pave the way to more personalized treatment in the future.