3D immunochemistry

Starting with fresh high grade astrocytoma tumors dissociated into single cells, we use the neurosphere assay as an enrichment method for cells presenting cancer stem cell phenotype, including expression of neural stem cell markers, long term self-renewal in vitro, and the ability to form orthotopic xenograft tumors. This method has been previously proposed, and is now in use by several investigators. Based on our experience of dissociating and culturing 125 glioblastoma specimens, we arrived at the detailed protocol we present here, suitable for routine neurosphere culturing of high grade astrocytomas and large scale expansion of tumorigenic cells for preclinical studies. We report on the efficiency of successful long term cultures using this protocol and suggest affordable alternatives for culturing dissociated glioblastoma cells that fail to grow as neurospheres. We also describe in detail a protocol for preserving the neurospheres 3D architecture for immunohistochemistry. Cell cultures enriched in CSCs, capable of generating orthotopic xenograft models that preserve the molecular signatures and heterogeneity of Glioblastomas, are becoming increasingly popular for the study of the biology of Glioblastomas and for the improved design of preclinical testing of potential therapies [1)].

1)
Hasselbach LA, Irtenkauf SM, Lemke NW, Nelson KK, Berezovsky AD, Carlton ET, Transou AD, Mikkelsen T, deCarvalho AC. Optimization of high-grade glioma cell culture from surgical specimens for use in clinically relevant animal models and 3D immunochemistry. J Vis Exp. 2014 Jan 7;(83):e51088. doi: 10.3791/51088. PubMed PMID: 24429465; PubMed Central PMCID: PMC4089397.