**A Patient-Derived Glioblastoma Organoid Model and Biobank Recapitulates Inter- and Intra-tumoral Heterogeneity** (Cell. 2020 January 09; 180(1): 188–204.e22. doi:10.1016/j.cell.2019.11.036.)

Traditional in vitro culture models, both monolayer and tumor sphere cultures, can require a substantial amount of time to establish and use exogenous EGF, bFGF, and/or serum to propagate tumor cells over serial passages with clonal expansion, which are not favorable to maintain various cellular subtypes and key driver gene expression of parental tumors (Ledur et al., 2017; Lee et al., 2006).

In most cases, dissociated tumor cells of epithelial origin are cultured within Matrigel in the presence of exogenous growth factors to form 3D structures, and various cancer organoid biobanks have been established as valuable resources (Bleijs et al., 2019). To study glioblastoma, cerebral organoids have been genetically manipulated to develop oncogenic properties (Bian et al., 2018; Ogawa et al., 2018) or co-cultured with tumor spheres to model tumor cell invasion (da Silva et al., 2018; Linkous et al., 2019).

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**Culture and Banking of Glioblastoma Organoids from Patient Tumors**

To preserve the local cytoarchitecture and native cell-cell interactions of original tumors, and to avoid clonal selection of specific cell populations in culture, we developed a protocol to generate glioblastoma organoids (GBOs) without mechanical or enzymatic dissociation of the resected tumor tissue into single cells (Figure 1A). Furthermore, the optimized medium to establish and maintain GBOs is fully defined, serum-free, and with no added EGF/bFGF or extracellular matrix that may contribute to further selection. We obtained fresh surgically resected glioblastoma tumor tissue from patients after informed consent (Table S1). Optimal GBOs were generated from tissue along the tumor margin with minimal necrosis and little surrounding brain tissue. The resected tissue was cut into ~1 mm diameter pieces using fine dissection scissors (Figure 1A). Debris and red blood cells were removed and tumor pieces were cultured in the GBO medium on an orbital shaker to facilitate organoid formation and increase nutrient and oxygen diffusion. Tumor pieces generally formed round organoids within 1–2 weeks (Figure 1A). GBOs were propagated by cutting them into ~0.5 mm diameter pieces to avoid necrotic cell death in the inner core (Figure 1A). To assess whether GBOs resemble their corresponding parental tumors, we first