A screenshot of a computer

Description automatically generated

Generation of GBOs from Resected Patient Glioblastoma Tissue

The remaining tumor pieces not set aside for RNA sequencing, whole exome sequencing, or histology were distributed in ultra-low attachment 6-well culture plates (Corning) with 4 mL of GBO medium containing 50% DMEM:F12 (Thermo Fisher Scientific), 50% Neurobasal (Thermo Fisher Scientific), 1X GlutaMax (Thermo Fisher Scientific), 1X NEAAs (Thermo Fisher Scientific), 1X PenStrep (Thermo Fisher Scientific), 1X N2 supplement (Thermo Fisher Scientific), 1XB27 w/o vitamin A supplement (Thermo Fisher Scientific), 1X 2- mercaptoethanol (Thermo Fisher Scientific), and 2.5 μg/ml human insulin (Sigma) per well and placed on an orbital shaker rotating at 120 rpm within a 37°C, 5% CO2, and 90% humidity sterile incubator. Roughly 75% of the medium was changed every 48 hours by tilting the plates at a 45° angle and aspirating the medium above the sunken GBOs. Within the first week of culture, the tumor pieces often shed cellular and blood debris making the medium slightly cloudy. The shedding soon ceased, and the tumor pieces generally formed rounded organoids within 1-2 weeks, depending on tissue quality and patient-specific tumor growth characteristics. The criteria for successful establishment of GBOs from a given patient’s tumor was that the micro-dissected tumor pieces survived for 2 weeks, developed a spherical morphology, and continuously grew in culture. GBOs cultured for prolonged periods of time (> 1 month) were routinely cut to ~200-500 μm diameter pieces using fine dissection scissors to prevent substantial necrosis within the center due to limited nutrient and oxygen diffusion. GBOs were sampled for RNA sequencing, whole exome sequencing, and histology by the same methods as the corresponding parental tumor pieces.

GBO Growth Analysis—To measure the growth of GBOs over time, similarly sized GBOs (0.5 −1 mm diameter) were placed into individual wells of a 48-well tissue culture plate with 300 μL of

GBO medium per well. Images of individual GBOs were taken every week using a brightfield microscope and Zen software. The 2D projected area of each GBO was quantified in ImageJ by carefully outlining each GBO and measuring the area within the outlined region. The 2D area at each time point was divided by the 2D area at time 0 to calculate a growth ratio for each time point. Ten individual GBOs were measured for each GBO sample. GBOs recovered from the biobank were cultured for 3 days before the start of analysis of GBO growth.