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STEM-01. DEVELOPMENT OF A HUMAN-SPECIFIC 3D IN VITRO PAEDIATRIC CEREBELLAR TUMOUR MODEL USING DECELLULARISED BRAIN AUTOPSY TISSUE
Phoebe McCrone, Harry Porter, Mohammed Diksin, Cara Moloney, Richard Grundy, Ruman Rahman; University of Nottingham, Nottingham, United Kingdom

BACKGROUND: Medulloblastoma (MB) and Atypical Teratoid/ Rhabdoid Tumours (ATRT) represent paediatric cerebellar tumours with a dismal prognosis. For ATRT in particular, 5-year survival remains at less than 32%. We have developed a 3D in vitro model termed 'tumoursphere matrix' which recapitulates in vivo crosstalk between tumour and reactive brain. METHODS AND RESULTS: We co-cultured D283 (Group 3-MYC amplified MB) and BT12 (ATRT-MYC) cells with primary human cerebellar astrocytes within a PEGDA hydrogel containing decellularised cerebellar extracellular matrix (ECM) derived from non-disease human autopsy brain. Cerebellar ECM was extensively characterised, showing a significant reduction in cellular material whilst retaining ECM ligands. Reduction of DNA content to 174 ng/mg was corroborated by an absence of nuclei in immunohistochemical staining. Colorimetric assays indicated collagen and glycosaminoglycan retention in decellularised ECM, and Immunofluorescence confirmed retention of fibronectin, laminin and hyaluronic acid. Detection of all ECM ligands was cross-validated by Orbitrap-Secondary Ion Mass Spectrometry. A solubilised ECM/PEGDA hydrogel was used to coat an AggreWell™ plate. Primary human cerebellar astrocytes, D283 and BT12 cells were cultured as spheroids within the ECM/PEGDA hydrogel for 7 days with no significant decrease in cellular viability. Cytokine and Human Matrix Metalloproteinase (MMP) Antibody Arrays showed cytokine and MMP expression in all three cell lines was retained in the presence of ECM material. Furthermore, D283 and BT12 cells were co-cultured with primary astrocytes for 7 days prior to separation using fluorescence activated cell sorting to generate individual cell populations. CONCLUSIONS: We have developed a 3D in vitro model which can co-culture cells for over 7 days, whilst recapitulating the in vivo tumour environment. RNA sequencing is currently in progress to identify differentially expressed genes in cerebellar tumour cells upon astrocytic and brain ECM crosstalk. We hypothesise that biochemical signalling underlying this communication will reveal putative therapeutic vulnerabilities.