Investigating the Role of the Perivascular Niche on Glioma Stem Cell Invasion in a Three-Dimensional Microfluidic Tumor Microenvironment Model

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Introduction: Glioblastoma Multiforme is a Grade IV astrocytoma and the most aggressive form of brain cancer. Despite significant advances in treatment options, it still has very low survival rate due to the wide intratumoral heterogeneity of the tumor. A significant contribution to this heterogeneity is the tumor microenvironment (TME) in which it resides which comprises of various factors and niches, including the perivascular niche, which allows for the persistence of a glioma stem cell (GSC) population. It has therefore become imperative to understand the role of the perivascular niche on GSCs through *in vitro* modelling in order to improve the efficacy of therapeutic treatment. In our previous studies, a 3D microfluidic platform was used to establish the role of the CXCL12 signaling pathway on GSC invasion in the perivascular niche, however, there still remains a need to understand the contributions of other factors present in the perivascular niche. To this end, a unique 3D microfluidic platform that permitted the study of intercellular reactions between three different cell types in the perivascular niche was developed and utilized, and their role on GSC invasion was studied.

Materials and Methods: The 3D microfluidic platform is made of three different cell culture regions evenly separated by hexagonal microposts to allow spatial organization of cells and diffusion of nutrients **(Fig 1A)**. To form a vascularized region, human umbilical vein endothelial cells (HUVECs) were embedded in a fibrin matrix and introduced into the vascular region of the device **(Fig 1B)**. Patient-derived GSCs, and human astrocytes were also embedded in Matrigel® hydrogel and introduced into respective regions of the device consecutively. Invasion of GSCs within the device was monitored, and the proliferative and phenotypic properties of invading GSCs were investigated.

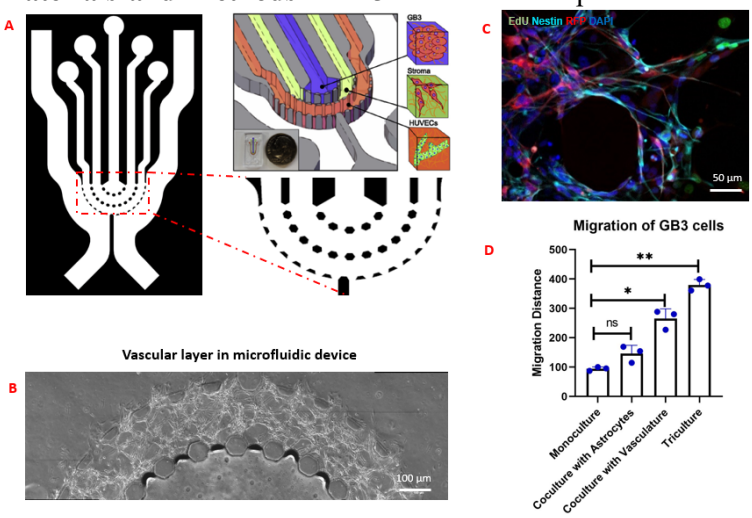

Figure 1. A.) Schematic of microfluidic device with cell culture regions and hexagonal microposts. B.)Stemness and proliferative markers observed through immunoflourescence C.) Phase contrast of vascular layer within device D.) Recorded migration distance of GSCs at Day 6 of experiment.

Results and Discussion: The presence of stroma cells increased the invasion of the GSCs while maintaining the phenotype of the GSCs, similar to previous *in vivo* studies. To confidently make these deductions, four experimental conditions were set-up comprising of different factors present in the perivascular niche. Phenotypically, stemness markers like Nestin, were expressed **(Fig 1C)**. These findings indicate that astrocytes in the perivascular niche significantly increase the migratory **(Fig 1D)** and proliferative properties of GSCs in the tumor microenvironment.

Conclusion: The micro-engineered model developed herein confirms results obtain through *in vivo* experiments and hence, could be used as a platform for further in-depth cellular and molecular level studies to dissect the influence of individual factors within the tumor microenvironment, while serving as a model for developing targeted therapies.

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