Evaluating Tumor-Homing Neural Stem Cell Therapy Using Bio-Inspired 3D Models of Brain Cancer

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Engineered neural stem cells (NSCs) have recently emerged as a promising therapy for the highly aggressive and lethal brain cancer Glioblastoma (GBM). Acting as a tumor-homing drug-delivery system, NSCs are known to migrate through brain tissue and seek out primary and invasive tumor foci. NSCs can deliver therapeutic agents, such as TNFα-related apoptosis-inducing ligand (TRAIL), directly to the tumor and have been shown to suppress both solid and post-surgical GBM in mouse models of cancer. Despite this promise, mouse models have been the mainstay for evaluating NSC migration and efficacy where the low-throughput, small scale, and challenges with implanting and tracking cells in the murine brain have left key translational questions unexplored. To circumvent these challenges, we developed a 3D, bio-inspired culture system composed of PLA microfibers suspended in an agarose hydrogel and a custom bioreactor apparatus. We then sought to determine the potential of this model to assess tumor growth, stem cell migration, and stem cell-based treatment efficacy at a mid- and human-scale. First, we investigated tumor growth in the models by implanting a panel of 5×10^5 human GBM and metastatic cancer cells into small scale microfiber/agar models approximately 6 cm in diameter. Real-time serial imaging showed over one week GBM tumor cells proliferated nearly 50-fold, while metastatic-breast cancer cells proliferated more than 3-fold. To evaluate NSC migration in the models, we created another set of microfiber/agar models in which NSCs were implanted 3mm laterally from the GBM foci. Fluorescence imaging showed directional migration of the NSCs towards the tumor over the course of one week, whereas NSCs implanted laterally from non-cancerous fibroblasts showed no directional migration. To investigate the efficacy of NSC therapy in this model, we first explored the impact of NSC distance from the tumo. NSCs were implanted 0, 2, 5, or 10 mm laterally from a GBM foci and imaging was to track GBM growth. We found that NSCs at all distances were efficacious, with NSCs co-injected with tumor inducing 99% reduction in less than 10 days, while NSCs implanted 10 mm laterally from the tumor reached a similar level but not until 30 days post-treatment. At distances of 2 and 5 mm, a similar response was observed at 20 and 30 days post-treatment, respectively. Next, we studied NSC migration and efficacy in a multifocal tumor model. Tumor cells were injected into each hemisphere of the brain model, and NSCs were implanted directly adjacent to the tumor in the right hemisphere. Interestingly, imaging showed 54% of NSCs migrated to the

tumor in the left hemisphere while only 29% of NSCs migrated directionally to the tumor in the right hemisphere. In conclusion, our results suggest the microfiber/agar system is a useful tool for investigating multiple parameters around cell therapy for cancer, including migration and tumor response. The precise control over cell implant, compatibility with kinetic imaging, and high-throughput nature of the system could allow this system to be a useful surrogate for investigating key questions that are beyond the reach of traditional animal or cell cultures as new cell therapies are advanced towards human patients.