leading to full restoration of saltatory conduction and preventing axonal degeneration by attenuating myelin degradation in MS-like disease states. Authors then sought to shed light on the exact mechanism of macrophage influence on the remyelination process. Macrophage secreted growth factors, including IGF-1 and PDGF-1A, as well as immune-modulatory cytokines, systemic mediators in tissue regeneration, could not be identified as a valid pathway for directly controlling OPC fate. This is further proof that the systemic environment, while capable of modulating and facilitating stem cell differentiation to a certain extent, cannot definitively override the tightly regulated microenvironment within the stem cell niche. Rather than grossly systemic modulation, the decisive role played by macrophages in the remyelination process seems to be honed in on the stem cell microenvironment: fine-tuning of OPC maturation by releasing the inhibitory break of myelin products through CCR2-dependent debris clearance.

Container within the confinements and strict control of their microenvironment, the oligodendroglial niche, OPCs are still susceptible to a certain extent to the modulating effects of external systemic factors. Epigenetic age related attenuation of OPC differentiation and remyelinating capacity seems to be reversible by exposure to a youthful systemic climate thus making the potential of the progenitor response through exogenous factors an appealing future therapeutic strategy in demyelinating disorders. Specific soluble factors, such as leukemia inhibitory factor, capable of crossing from the systemic circulation to the neurovascular niche in order to directly guide stem cell fate and thus bridging the macro and the microenvironment, are still under investigation. However, the role of the vascular network as delivery pipeline for immune cells and the role of the endothelium per se, as the main crosstalk partner in the process of remyelination and tissue regeneration, are becoming increasingly clear. While the full cast of actors involved in this complex process of CNS regeneration is yet to be completely revealed, effective remyelinating therapies might indeed become reality once all the pieces of the puzzle have come together.

**Therapeutic Stem Cells Encapsulated in a Synthetic Extracellular Matrix Selectively Kill Tumor Cells, Delay Tumor Growth, and Increase Survival in a Mouse Resection Model of Malignant Glioma**

One of the main obstacles impeding drug delivery to brain tumors is the blood brain barrier and short systemic half-lives, which prevent drugs from accumulating to therapeutic levels within the tumor cells. Previous research has shown that stem cells selectively migrate to glioblastoma multiforme (GBM) tumors, a property that has been exploited to turn them into a sort of tumor-seeking missile when they are engineered to secrete anti-tumor molecules, such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Moreover, once situated next to the tumor, stem cells offer a continuous and concentrated local delivery of secretable therapeutic molecules, like TRAIL. Thus far, the problem with this approach has been the delivery and retention of these stem cells to the target area. One method that may overcome this challenge is encapsulating the stem cells in a synthetic extracellular matrix (sECM), which provides the cells with a physiological environment promoting their survival while simultaneously permitting their easy transplantation and retention in the target area. In a recent Nature Neuroscience article, Kauer et al show that TRAIL-expressing stem cells encapsulated in a biodegradable sECM were able to home to tumors, delay tumor growth, and increase survival in a mouse resection model of GBM.

While the current standard treatment for GBM involves surgical debulking of the tumor followed by radiation and chemotherapy, most in vivo animal models of GBM focus on targeting the intact solid tumor. In order to more closely mimic the actual clinical scenario of targeting the resected tumor, Kauer et al created a mouse resection model for GBM. The authors transduced U87 GBM cells with mCherry fluorescent protein and firefly luciferase (Fluc), injected these cells into the right hemisphere of athymic mice via a cranial window, and then used in vivo animal models of GBM focus on targeting the intact solid tumor. In order to more closely mimic the actual clinical scenario of targeting the resected tumor, Kauer et al created a mouse resection model for GBM. The authors transduced U87 GBM cells with mCherry fluorescent protein and firefly luciferase (Fluc), injected these cells into the right hemisphere of athymic mice via a cranial window, and then used intravital microscopy (IVM) and bioluminescent imaging (BLI) to monitor tumor growth before resection, the extent of tumor resection, and tumor regrowth following resection. This resection, which removed more than 60 to 80% of the total tumor volume, greatly increased mouse survival.

After establishing a resection model for GBM, Kauer et al set out establishing the efficacy of their stem cell delivery system. They transduced mouse neural stem cells (mNSCs) with green fluorescent protein (GFP) and either Fluc or Fluc and a secretable Remilla luciferase marker (Rluc). These mNSCs were encapsulated in a sECM and intracranially injected into mice. Compared to injections of mNSCs in suspension, the sECM-encapsulated mNSCs showed increased proliferation (as measured by Fluc) and protein secretion (as measured by Rluc) over time. When these encapsulated cells were injected next to the U87 cell xenografts, they migrated out from the sECM and specifically homed in on the tumor over 4 days.

Having established that encapsulation within sECM increases mNSC long-term viability and ultimate migration to the tumor target, the authors tested the therapeutic effect of these stem cells with the cytotoxic TRAIL, would have on GBM. In vitro, mNSCs transduced with TRAIL released high amounts of TRAIL and...
induced apoptosis in GBM cells, which showed increased Caspase-3 and 7 activity. When encapsulated TRAIL-secreting mNSCs were implanted into the resection cavity, they were retained at high concentrations adjacent to the residual tumor and induced a marked increase in tumor cell Caspase 3/7 activity as well as a 80% reduction in residual tumor cells in as early as 3 days. This was a sustained effect as they suppressed the regrowth of residual tumor cells 49 days after resection and all of the mice were alive 42 days after tumor cell injection (compared to a median 14.5 day survival in mice treated with control mNSCs expressing Rluc).

Because primary GBM cell lines often recapitulate clinical GBM tumors better than established GBM cell lines like U87, the authors decided to test the effect their stem cell delivery system had on a primary GBM cell line. They also switched from mNSCs to human bone marrow-derived mesenchymal stem cells (hMSCs), which have been extensively used in past and ongoing clinical trials and allow for autologous transplantation with less chance of immune rejection, to see if this other stem cell type would provide the same therapeutic benefit when transduced with TRAIL. As was the case with the mNSCs and U87 cells, hMSCs migrated out of the sECM and tracked the primary GBM cell lines and induced tumor cell death in both a time- and Caspase 3/7-dependent manner in vitro. Similarly, under in vivo conditions, sECM encapsulation significantly increased the retention time of the hMSCs in the resection cavity, permitting a robust tumor-selective migration of these cells and subsequent directed TRAIL secretion that drastically decreased tumor regrowth and increased mouse survival.

In summary, Kauer et al demonstrated that therapeutic stem cells encapsulated in sECM in a mouse model of human GBM resection showed higher retention in the resection cavity, which facilitated their tumoritropic migration and release of therapeutic protein. In this case, the therapeutic protein used was TRAIL, which eradicated residual tumor cells by inducing caspase-mediated apoptosis, delayed tumor growth, and greatly increased mouse survival.

While other resection models have been previously reported, the author’s combined use of BLI and confocal IVM permits better non-invasive imaging that can characterize multiple events in stem cell therapy in real time and follow tumor growth before and after resection as well as quantitate overall tumor resection. Nevertheless, future studies should begin to incorporate more clinically-relevant imaging modalities, such as MRI, to assist in the translation of this therapy from the bench to the bedside. TRAIL has been found to selectively target tumor cells while remaining harmless to most other normal cells, including the stem cells secreting it; however, many tumor lines have varying sensitivity to TRAIL, with about 50% of established GBM lines resistant to it. While there have been attempts to sensitize tumor cells to TRAIL using methods like irradiation, it will probably be ideal to use stem cells that secrete therapeutic proteins targeting multiple GBM pathways to increase treatment efficacy and minimize resistance.

Figure. A, mice with established U87-Fluc-mCherry GBMs in the cranial window were injected with a blood pool agent, AngioSense-750, before (top) and after (bottom) tumor resection (red, tumor; blue, vasculature). B, Kaplan-Meier survival curves of mice with and without resected U87-Fluc-mCherry tumors. C, IVIM images showing mNSCs (green) and tumor cells (red) on day 1 (top left) and day 4 (top right and bottom) after mNSC implantation (dashed line, encapsulated mNSCs; arrows, mNSC migration). D, Kaplan-Meier survival curves for mice with unresected U87-Fluc-mCherry tumors and resected tumors treated with mNSC-GFP-Rluc cells in sECM, -TRAIL cells in suspension, and –TRAIL cells in sECM. E, hMSCs expressing GFP were encapsulated in sECM and placed in a culture dish containing primary human GBM-Fluc-mCherry cells (left), and they subsequently migrated out of the sECM to the tumor cells (right). F, the same experiment except now the hMSCs express TRAIL, which leads to death of the tumor cells (green, hMSCs; red, GBM cells). G, encapsulated hMSC-TRAIL cells (green) in sECM were implanted intracranially in the tumor resection cavity of mice bearing primary GBM-Fluc-mCherry cells (left). Higher magnification shows hMSC migration toward the residual tumor cells (right). Figures adapted from Kauer et al. Reprinted by permission from Macmillan Publishers Ltd: Nature Neuroscience. Vol 15(2):197-204, copyright 2011.
in the future. At the same time, the long term fate of the stem cells used to treat tumors still needs to be addressed, as they may have tumorigenic potential.\textsuperscript{8,14} The use of induced pluripotent NSCs, such as those that have been produced from skin fibroblasts,\textsuperscript{15} or the incorporation of suicide genes like HSV-TK may help to minimize this risk.

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REFERENCES


Seeing is Believing—Spinal Chamber for Spinal Cord Injury Research

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pineal cord injury remains a vexing problem that has evaded the development of effective treatments despite modern scientific