HHS Public Access

Author manuscript

Methods. Author manuscript; available in PMC 2017 May 18.

Published in final edited form as:

Methods. 2016 April 15; 99: 37–43. doi:10.1016/j.ymeth.2015.08.013.

Neural stem cell therapy for cancer

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Abstract

Cancers of the brain remain one of the greatest medical challenges. Traditional surgery and chemoradiation therapy are unable to eradicate diffuse cancer cells and tumor recurrence is nearly inevitable. In contrast to traditional regenerative medicine applications, engineered neural stem cells (NSCs) are emerging as a promising new therapeutic strategy for cancer therapy. The tumor-homing properties allow NSCs to access both primary and invasive tumor foci, creating a novel delivery platform. NSCs engineered with a wide array of cytotoxic agents have been found to significantly reduce tumor volumes and markedly extend survival in preclinical models. With the recent launch of new clinical trials, the potential to successfully manage cancer in human patients with cytotoxic NSC therapy is moving closer to becoming a reality.

Keywords

Neural stem cell; Cancer therapy; Drug delivery; Glioblastoma; Gene therapy

1. Introduction

Over the past two decades, the dogma in the central nervous system has shifted from one of a static organ incapable of change to the current understanding of adult neurogenesis [1]. The key to much of this change was the discovery of neural stem cells (NSCs). NSCs are generated by the differentiation of embryonic tissue and can serve as a source for replenishing neurons and glial cells in the adult brain throughout life. NSCs are defined by the expression of classic markers, including Nestin and Sox2, as well as their expansion in growth factor rich media that contains fibroblast growth factor and epidermal growth factor. NSCs display the hallmarks of stem cells, both self-renewing as well as differentiating into neurons, astrocytes, and oligodendrocytes. This differentiation capacity has led to significant investigation into the use of NSCs for regenerative medicine applications to correct damage

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to the brain and central nervous system caused by physical trauma or disease states. These studies have shown that NSC transplants survive in the diseased or damaged brain, and have therapeutic benefits in certain disease models.

In contrast to these traditional NSC therapies, the use of NSCs as tumor-homing drug carriers is an emerging area of interest that holds promise for treating malignant brain cancer [2–5]. Pioneering studies by Aboody et al. and Benedetti et al. first revealed the unique ability of NSCs to home to brain cancer [6,7]. These studies showed that NSCs transplanted at different sites throughout the brain migrated through the non-diseased parenchyma to localize selectively with cancer foci. When the NSCs were engineered with anticancer gene products, cytotoxic NSC therapy significantly inhibited the progression of cancer xenografts. These studies opened the door to the possibility of harnessing drug-loaded NSCs as a tumorhoming therapy. Ensuing studies over the past 15 years have further developed this concept, exploring novel cytotoxic agents, different routes of administration, and numerous molecular assays to define the mechanisms of migration. This exciting work has rapidly moved cytotoxic NSC therapy from preclinical mouse studies to a recent first-in-human clinical trial.

2. Glioblastoma

Glioblastoma (GBM) is the most common primary brain tumor, yet effectively treating this aggressive form of cancer remains a daunting challenge. GBM is classified as a grade IV glioma by the World Health Organization [8,9]. The current clinical standard of care for GBM is surgical resection followed by chemo- and radiation therapy. Yet, median survival for GBM remains only 12–15 months and only 5% of patients survive 5 years [10–12]. GBM survival has not significantly improved in several decades despite the advent of numerous therapeutic agents. This is due in large part to the aggressive and infiltrative nature of the cancer, as well as the heterogeneity of the disease. GBM is a highly infiltrative cancer, and surgical resection is unable to remove all of the invasive GBM foci. Chemotherapeutic regimens are complicated by the blood-brain barrier (BBB) that prevents more than 98% of all drugs from accessing the brain [13]. Those agents capable of crossing the BBB are typically unable to accumulate at therapeutically relevant concentrations.

As a result of these therapeutic challenges, numerous studies have explored new strategies to improve drug delivery to GBM. One of the earliest examples is the development of the Gliadel wafer. Gliadel is a biodegradable wafer consisting of a biocompatible polymer impregnated with the anti-cancer drug carmustine [14,15]. Gliadel was approved for use as an adjunct to surgery in 1997. Yet, Gliadel is often only minimally effective, particularly because the passively diffused drug is unable to reach distant invasive foci [16]. Additionally, Gliadel can cause numerous adverse side effects, ranging from impaired neurosurgical wound healing to seizures. More recently, the vascular endothelial growth factor (VEGF) inhibitor Bevacizumab has entered clinical use in GBM patients [17]. Blood vessels are essential for the growth of tumors, and inhibition of VEGF-mediated vessel formation could impair tumor progression. Studies have shown that Bevacizumab treatment leads to significant radiographic responses. However, these responses were only temporary and the ability of Bevacizumab to prolong overall survival of GBM patients remains in

question [18]. New therapies capable of seeking out delivering cytotoxic agents to both the primary and infiltrative tumor foci are needed to improve therapy for GBM. Engineered cytotoxic NSCs are one of the most promising strategies for GBM therapy.

3. Sources of NSCs

3.1. Endogenous NSCs

NSCs possess the capacity to both self-replicate as well as differentiate into the primary cell types found in the CNS: neurons, astrocytes, and oligodendrocytes. While NSCs are ubiquitous in the developing brain, small populations of dormant NSCs that respond to injury can also be harvested from the subventricular zone (SVZ) or the subgranular zone (SGZ) of the dentate gyrus (DG) in adults [1,19]. Isolation procedures vary yet typically involve microdissection and enzymatic digestion of tissue slices [20]. For the highest yield, cells taken from the SVZ or SGZ are cultured as neurospheres or monolayers, respectively, whereupon neural stemness is confirmed with Nestin, Sox2, or Msi1 markers [21,22]. Prolamin (CD133) can be used to distinguish NSCs from native astrocytes, but due to its expression on a range of stem cell types (including embryonic and cancerous) should be used in conjunction with other markers to identify neural-specific stem cell populations [23,24]. Stemness can be prolonged with the addition of fibroblast growth factor 2 (FGF2) and endothelial growth factor (EGF) in media, enabling thorough characterization of the cultured cells [25].

NSCs can be harvested from endogenous sources, expanded or established into stable lines *in vitro*, and subsequently transplanted to sites of injury for regenerative therapies with exciting clinical ramifications. One such example is partially-committed glial-restricted progenitors, which differentiate into oligodendrocytes and re-sheath demyelinated axons [26,27]. Cells isolated from non-neurogenic regions such as the spinal cord in adult mice [28] or the retina and cortex in humans [29] are also being advanced towards the clinic. The leader in the neural stem cell drug delivery field, however, has been the HB1 cell line established from human fetal donor CNS tissue at 8–18 weeks of gestation [30,31]. In addition to possessing proliferative and regenerative capacity, these cells have been found to natively exhibit tumor tropism, making them ideal drug delivery vehicles in cancer applications. The clonal population HB1.F3 has recently entered clinical trials in which they are being used to deliver chemotherapeutic payloads to recurrent GBM foci [32].

3.2. Induced pluripotent stem cells

In 2006, Takahashi and Yamanaka reported a ground-breaking discovery. By transducing mouse fibroblasts with Oct 3/4, Sox2, Klf4, and c-Myc, they were able to reprogram somatic cells into a pluripotent state where the cells had properties similar to embryonic stem cells [33]. The new cells were termed induced pluripotent stem cells (iPSCs). The discovery of iPSCs showed the fate of a cell could be changed. It also suggested a new approach to cell therapy where a large number of therapeutic cells could be created from a patient’s skin for autologous transplant. The iPSC strategy also eliminated ethical concerns and government restrictions surrounding the use of embryonic stem cells. This enormous promise has driven rapid progress in iPSC technology. Takahashi and Yu reported the generation of human

iPSCs simultaneously in 2007 [34,35]. Subsequently, iPSCs have been generated from a variety of different cell types, showing the majority of somatic cells can be reprogrammed to pluripotency. iPSCs were initially generated using integrating viral vectors, but this strategy risked insertional mutagenesis. New methods have now been reported that allow iPSC generation from non-integrating vectors, small molecule compounds, as well as RNA.

iPSCs can be converted into NSCs using strategies that mimic the conversion of embryonic stem cells into NSCs [36]. The resulting iPSC-derived NSC can be differentiated into oligodendrocytes, astrocytes, or neuronal cell types though culturing with specific factors including retinoic acid, fibroblast growth factor, or Wnts. An iPSC-based strategy to generate patient-specific NSCs could be a valuable clinical tool for future GBM therapies, but the field is still in its early stages. Lee et al. showed that iPSC-derived NSCs expressing thymidine kinase reduced the progression of intracranial U87 xenografts following injection of the ganciclovir (GCV) pro-drug [37]. Yamazoe et al. recently revealed that iPS-derived NSCs home to human and mouse GBM cells in culture, as well as syngeneic GL261 GBM xenografts *in vivo* [38]. These studies have begun to suggest that NSCs created by the conversion of iPSCs are tumor-homing drug carriers with potential to deliver anticancer agents to treat GBM.

3.3. Direct reprogramming

One of the newest areas in cell reprogramming is direct reprogramming. In this approach, somatic cells are directly converted into a distinct lineage without passing through a pluripotent intermediate. This is accomplished using lineage-specific reprogramming factors that are distinct from the Yamanaka factors that produce iPSCs. In contrast to iPSC technology, direct reprogramming was first reported *in vivo* followed by several *in vitro* studies. In this study, three transcription factors (Ngn3, Pdx1, and Mafa) were used to directly convert exocrine cells into endocrine cells within the pancreas of mice with the resulting cells exhibiting numerous properties of endogenous islet beta-cells [39]. In 2011, Marius Wernig and colleagues demonstrated that lineage-specific factors could be used to directly convert mouse and human fibroblasts into neuronal cells, referred to as iNs [40]. The field of direct reprogramming moved into NSCs with four studies published in 2012 [41–44]. These studies showed that different gene cocktails could be used to convert mouse and human fibroblasts into multi-potent expandable NSCs, referred to as induced NSCs (iNSCs). The iNSC strategy provided advantages over iPSC generation in terms of speed and reprogramming efficiency, and has been accomplished by two general strategies. In the first approach, a lineage-specific gene cocktail is used, similar to the iN strategy. This employs reprogramming cocktails such as Brn2, FoxG1, and Sox2. iNSC generation has also been accomplished using Sox2 alone but required extensive culturing on feeder cells. Alternatively, restricting the expression of the Yamanaka factors (Oct4, Sox2, Klf4, c-Myc) can also be used to create iNSCs. Regardless of the approach, the forced expression of these “master regulators” controls numerous downstream pathways that are critical for initiating NSC lineage-specific differentiation, thus bypassing the iPSC stage to directly converting fibroblasts into iNSCs. iNSCs have been shown to differentiate into astrocytes, neurons, and oligodendrocytes in *vitro* and *in vivo*. Unlike iPSCs, iNSCs have been shown to not form cancerous teratomas when transplanted *in vivo*. This is an important advancement in safety

for cell transplant therapy. Similar to iPSC-derived NSCs, iNSCs hold significant potential for creating personalized NSC therapies without the need for invasive intracranial biopsy. Extensive future studies will be needed to fully uncover the potential of iNSCs as a valid source of drug carriers for treatment of GBM.

4. Homing

The main function of NSCs in the brain is to replace lost or injured neurons and glia by differentiation after migration to the injured zone. This migration to injured tissue is triggered by hypoxia through the associated up-regulation of the transcription factor hypoxia-inducible factor-1α (HIF-1α), which in turn activates the expression of NSC chemoattractants. These include chemokines and pro-angiogenic growth factors such as stromal cell-derived factor 1 (SDF-1), monocyte chemotactic protein 1 (MCP1), FGF2, insulin-like growth factor 1 (IGF1) and vascular endothelial growth factor (VEGF) [45]. NSC migration not only occurs towards areas of injury, but also occurs towards tumor foci. The tumoritropic homing of NSCs is driven by chemoattractants produced by cells in the normal brain that are injured due to tumor growth or directly released from the GBM cells themselves [46,47]. The tumoritropic migration of NSCs occurs across different NSC lines, GBMs lines, and routes of NSC administration. The NSC cell lines studied include C17.2 (murine) [48,49], HB1. F3 (human) [50,51], bone marrow-derived neural progenitor stem cells (murine) [52], primary murine NSC [53], and HNT2RA2 (human) [54]. The number of GBM cell lines used for these studies are large and include murine cell lines such as GL261 [53], RG2 [52], C6 [55], and CNS-1 [6] as well as the human cell lines U87 [54], U-373 [50] and U251 [56]. Additionally, new invasive patient-derived GBM lines are allowing investigations into the ability of NSCs to track GBM cells as they invade the brain parenchyma [57,58]. The ability of the NSCs to migrate towards the tumor has been described by different groups and is not restricted to tumors of glial origin [30], as NSCs are known to migrate to metastatic breast cancer and melanoma foci in the brain [59,60]. This is important considering that metastatic brain tumors are estimated to be about ten times more common than primary brain cancers.

NSC tropism is typically studied *in vivo* by implanting GBMs and NSCs in different hemispheres of the rodent brain [6,32,61]. The most common route of administration *in vivo* has been contralateral injection of NSCs and GBM. Tissue sections are then collected to visualize the dynamics of NSC co-localization with GBM foci over time. However, other administration routes have been investigated, including tail vein [6,56,62], ipsilateral [32,55], intracarotid [63], and intraventricular [6,64]. Despite extensive studies of cell migration, no approach has considered the more clinically-relevant case of NSC administration after resection of the tumor. As a consequence of the environment created after resection, both survival and migration patterns can differ considerably from those observed to date [65]. Due to the large number of different conditions applied, it is difficult to definitively compare NSC homing to GBM among cell types. However, in general, the kinetics observed describes a migratory movement that starts as soon as 50 min after implantation [50] and persists for up to 2 weeks [51]. These models are allowing unique insights into the kinetics of NSC migration, but the small size of the rodent models have left many translational questions unanswered. Investigating NSC tumoritropic migration in

large-scale models will be essential for disentangling questions of generation and therapeutic efficacy related to size and species differences that could dramatically impact clinical testing of NSC therapies in GBM patients.

The majority of *in vitro* studies still rely on 2-dimensional (2-D) cell culture models, such as Boyden chamber assays [62,66]. Advances in three-dimensional (3-D) cell culture have raised the possibility of exploring NSC migration in models that better reproduce physiologic conditions [67]. Culturing cells in 2-D or 3-D affects cellular behaviors in many ways such as attachment, growth, spreading, morphology, polarity, motility, protein expression, proliferation, viability, and response to stimuli. The main challenge in 3-D migration assays is to choose matrices with close characteristics to *in vivo* conditions [38]. For this purpose, different natural and synthetic scaffolds have been used that include hydrogel or solid state polymers. However, reproducing all the components present in physiological conditions is complicated. Organotypic slice cultures are a convenient approach to mimicking in vivo conditions [68]. In this approach, NSC migration is assayed over brain slices from rodents previously cut into slices 250 μm thick.

The mechanism guiding the homing to GBM is still not completely understood. Nevertheless, it seems clear that as in ischemia, hypoxia is a key factor that triggers homing to GBM. In hypoxic conditions, GBM cells upregulate the expression of numerous pro-angiogenic factors and chemoattractants. To prove the importance of hypoxia in tumoritropic migration of NSC towards glioblastoma cells, different siRNA-mediated knockdowns have been used. For example, knockdown of HIF-1α in GBM cells reduces the expression of SDF-1, uPA and VEGF, resulting in no tumor tropism of NSC [46]. Other cytokines, growth factors, and receptors have been reported to mediate tumoritropic migration, including (SCF)/c-Kit [69], monocyte chemotactic protein-1 (MCP-1)/CCL2 [48], annexin A2 [70], hepatocyte growth factor (HGF)/c-Met [71] and HMGB1/RAGE [72].

The presence of functional tumoritropic receptors on NSCs dictates the migration pattern as is proven by function-inhibiting antibodies to these receptors that reduce NSC migration towards GBMs. Future research focusing on increasing the number of tumoritropic receptors on NSCs could considerably improve NSC homing to GBMs. One of these approaches could include hypoxic preconditioning of the NSCs. Tumoritropic receptors have been reported to be upregulated on NSCs under hypoxic conditions [46]. Additionally, chemotherapy and radiation have both been shown to promote hypoxia-induced secretion of chemokines by tumor cells [73]. This suggests that hypoxia synergistically upregulates the chemotactic signal strength emanating from the tumor as well as the sensitivity of the NSCs to that signal, potentiating enhanced NSC tropism in a clinical setting where these treatment regimens are frequently combined. Another approach could involve genetically engineering NSCs to overexpress tumoritropic proteins for enhanced GBM homing [49]. This type of approach is currently widely used in tissue regeneration applications to increase stem cell homing to sites of injury [74].

5. Tumoricidal agents

5.1. Enzyme/prodrug

Enzyme/prodrug therapy was the first approach used for engineered NSC therapy and the first strategy to enter human patient testing [6,32]. In this approach, the NSCs are engineered to express an enzyme that converts a non-toxic prodrug into a cytotoxic product. This allows more precise control of the timing, levels, and location of drug release. This approach also adds an additional layer of safety as the prodrug typically kills the NSC drug carrier [75]. Cytosine deaminase (CD) was used in the first pioneering studies by Aboody et al. [6]. This enzyme converts the 5-fluorocytosine (5-FC) prodrug into the toxic 5-fluorouracil (5-FU) variant. Initial studies showed mouse NSCs bearing CD significantly inhibited growth of GBMs following 5-FC administration. The human HB1.F3 cell line was engineered with CD (HB1.F3.CD) and has been one of the most widely used cytotoxic human NSC therapies [2]. Extensive pre-clinical data showing the efficacy and safety of the HB1.F3.CD/5-FC therapy recently advanced the field of cytotoxic NSC therapy for GBM into the first-in-human clinical trial (ClinicalTrials.gov identifier NCT01172964). In this trial, HB1.F3.CD cells are injected into the walls of the GBM resection cavity and patients are administered oral 5-FC [32]. A new clinical trial has recently been launch around a second enzyme/prodrug approach (ClinicalTrials.gov identifier NCT02192359), exploring the efficacy of allogeneic human NSCs expressing carboxylesterase (CE) in combination with intravenous irinotecan also for recurrent GBM patients. CE converts irinotecan into the toxic agent SN-38 and was expressed in the HB1.F3.CD cells using adenoviral transduction [76].

Thymidine kinase from the herpes simplex virus (HSV-TK) was used in multiple proof-of-concept suicide gene therapy studies [77–80]. Variations of this system and HSV-TK-based combination therapies remain some of the most widely used approaches in both clinical and experimental gene therapy applications. Mechanistically, HSV-TK phosphorylates the prodrug monophosphorylate ganciclovir (GCV) into cytotoxic triphosphate ganciclovir (GCV-TP). However, since it reacts with HSV-TK at a 1000-fold higher efficiency compared to endogenous TK, GCV can be administered systemically while only producing local GCV-TP in targeted regions. GCV-TP is then integrated into nearby cells' DNA during division, causing inhibition of DNA polymerase, rapid chain termination, and the formation of single strand breaks, leading to cell death [81,82]. This process primarily affects highly proliferative cancer cells, further minimizing damage to healthy tissue.

Cells transduced with HSV-TK in the presence of GCV exert potent bystander effects by transfer of GCV-TP across gap junctions [78]. In a phase III clinical trial, fibroblasts transduced with HSV-TK were injected into the walls of the brain cavity after tumor resection [83]. Unfortunately, no significant differences were observed between treated and control groups. The possible causes can be the use of murine-derived cells, non-migratory fibroblasts, or a combination of various technical factors. Since this study, several preclinical models have been developed which use tumorhoming NSCs in place of non-migratory fibroblasts to improve upon therapeutic efficacy. Li et al. showed that intratumoral injection of NSCs transduced with HSV-TK (NSCs-TK) followed by daily intraperitoneal injections of GCV for 10 days (two 15 mg/kg doses per day) effectively treated C6 gliomas in rats,

with six of nine animals surviving 100 days post injection with no remaining signs of tumor presence [79]. In a more clinically-relevant approach, Zhao et al. injected U87 cells into the right hemisphere of mouse brains followed by NSC-TK injection in the contralateral hemisphere 7 days later. Despite having to migrate to the opposite hemisphere to co-localize with tumor cells, GCV-treated mice survived nearly twice as long as control groups [84]. These preclinical studies establish that problems associated with failure of the phase III clinical trial may be circumvented in future clinical studies, and that TK remains a promising option for glioma therapy. Additionally, new HSV-TK mutants which potentiate the bystander effect with increased substrate specificity have been discovered and characterized. The most extensively evaluated of these is the mutant SR39, which is 43-fold more sensitive to GCV than the parental HSV-TK [85,86].

5.2. Secreted agents

NSCs can function as *in situ* drug factories, secreting anti-cancer agents for long durations. Selecting agents that induce killing in GBM cells without killing the NSC is a challenge. One of the most commonly used secreted therapeutic agents is TNFα-related apoptosis-inducing ligand (TRAIL) [87]. The interest in TRAIL as a prime candidate for NSC-based cancer therapy is due to its ability to induce apoptosis in a tumor-specific manner. TRAIL-induced cell death begins with the molecule binding as a trimer to death receptors which are present on the surface of cancer cells but absent on most normal tissue [88]. Engagement of the death receptor triggers the assembly of the death-inducing signaling complex (DISC), Fas-associated protein with death domain recruitment of caspases, and autocatalytic caspase activation that further activates downstream signaling molecules to stimulate the apoptotic cascade. Numerous variants of TRAIL have been generated [87]. One of the most potent variants designed specifically for secretion from NSCs was created by fusing the extracellular domain of Flt3 ligand to the amino-terminus of the TRAIL extracellular domain via a leucine-zipper motif (S-TRAIL) [61]. This new molecule was shown to be robustly released by NSCs and induce potent bystander effects in various preclinical models of GBM. Variations in the expression levels of death receptors on GBM cells can result in heterogeneous TRAIL killing. Pre-treatment of GBM cells with the clinically utilized chemotherapy temozolomide increases death receptor expression and improves killing of TRAIL-resistant tumor cells [89]. The histone deacetylase inhibitor MS-275 was also found to upregulate death receptors in numerous GBM cell lines and sensitize tumor cells to TRAIL-induced apoptosis [90]. In a new approach to TRAIL therapy, a fusion between an anti-epidermal growth factor receptor nanobody and TRAIL showed significant cytotoxicity against a panel of GBM cells in culture and reduced the volume of both solid and invasive GBM xenografts in mice [58].

A variety of studies have explored the delivery of immune-modulating agents from NSCs. Interleukin (IL)-4 [7], IL-12 [91], IL-23 [92], and IL-24 [93] have all been delivered by genetically modified NSCs. This therapeutic strategy has been shown to slow GBM growth in preclinical models and significantly extend the survival of tumor bearing mice. Similarly, NSCs transduced with interferon-β significantly inhibited growth of human GBM xenografts and demonstrated a substantial bystander effect [94].

Targeted immunotoxins are a new cytotoxic agent that can be delivered from stem cells. These fusion proteins are comprised of a cytokine fused to a toxin [95,96]. The cytokine targets the fusion to receptors selectively expressed on cancer cells, while the toxin induces tumor cell death. Immunotoxins, such as interleukin 13-pseudomonas exotoxin fusion (IL13-PE) have shown promise in early stage clinical trials of GBM therapy [97], but the efficacy of this strategy was limited by inefficient delivery [98]. We recently developed a new strategy to engineer NSCs with IL13-PE or other immunotoxins and found this approach significantly inhibited GBM recurrence while prolonging survival of tumor-bearing mice [99]. An interesting advancement in this study was that the NSCs had to first be engineered to be resistant to the toxin. In previous studies, the interleukins, TRAIL, and other secreted factors had minimal effects on the viability of the engineered NSCs. Yet, PE in the immunotoxin induced death of NSCs until they were modified with a single-stranded DNA oligonucleotide. This suggests similar strategies could be used to engineer NSCs that are resistant to numerous cytotoxic agents and create a multitude of new cytotoxic NSC therapies. This could allow therapeutic NSCs to kill a broader range of GBM cells with more potent efficacy.

Determining the duration of delivery, bio-distribution, and overall dynamics of NSC-based drug delivery is essential for maximizing tumor kill while protecting non-diseased tissue from damage. Several novel fusion proteins have been developed that incorporate optical reporters conjugated to cytotoxic protein domains. These include a modified Renilla luciferase variant fused to TRAIL as well as a Gaussia luciferase fused to interleukin-24 [100]. Both fusion variants could be detected *in vitro* and *in vivo* by bioluminescence imaging while retaining the capacity to kill GBM cells. Simultaneous optical tracking of NSCs and secreted fusion variants revealed differences in the levels and duration of drug release exist between NSCs and mesenchymal stem cells that significantly impacts tumor killing. *In vivo* tracking showed NSC-based delivery significantly prolongs the duration of drug delivery while reducing drug accumulation on non-tumor tissue compared to traditional intravenous infusion (I.V.) or intratumoral injection (I.T.). Additionally, the improved drug delivery translated to improved tumor killing from a single infusion of cytotoxic NSCs compared to I.V. or I.T. delivery of free protein therapies.

5.3. Viral therapy

Oncolytic viral (OV) therapy is a promising strategy for GBM [101]. Seven different strains of OVs have been tested in over 20 different clinical trials for GBM therapy. Unlike traditional attenuated viruses, OVs have the potential to conditionally replicate in cancer cells. This replication ultimately causes the tumor cells to lyse and release the newly produced virus that infects neighboring tumor cells. In this way, OV therapy spreads through the tumor leading to killing. NSCs can be loaded with OV to improve the spread and hide the virus from the immune system [66,102]. NSCs transduced with OVs retain the ability to home to GBM. *In vivo*, NSC delivery of OVs showed significantly greater GBM killing than the virus alone demonstrating the promise of this approach. As NSC-based OV therapy for GBM moves towards clinical trials, the timing of administration may be critical. A recent study found NSC-based OV therapy delivered with radiation and temozolomide treatment

increased survival, but this was dependent on delivering the NSC/OV after radiation and temozolomide treatment was administered [103].

6. Routes of administration

Determining the most effective route to administer cytotoxic NSC therapies represents an important step for eventual human use. Direct injection into the established GBM has been the mainstay of cytotoxic NSC delivery, and as numerous studies have found, this method leads to efficient NSC transplant and robust tumor killing [2,93,100]. However, directly injecting NSCs into the immunosuppressive tumor niche improves the survival of human NSC transplants and neglects their defining ability to scavenge distant tumor foci [104]. This behavior can instead be mimicked by implanting therapeutic NSCs outside the established GBM [32,61]. As expected, NSC therapies implanted in the proximity of GBMs are still able to slow tumor progression but the effects are not as pronounced as when therapeutic NSCs are entirely co-localized with the GBM cells. Interestingly, NSCs delivered by intravenous infusion were found to cross the blood-brain barrier and co-localize with GBM by immunohistochemical analysis [56,64]. Quantitative optical tracking suggested approximately 1.4% of the cells co-localized with the GBM. Of note, this same study reported over 4% of NSCs reached the GBM when delivered by intraventricular infusion [64].

Surgical resection is part of the clinical standard of care for human GBM patients [105], making it important to define strategies capable of efficiently transplanting cytotoxic NSCs into postoperative GBM patients. Using a mouse model of GBM resection/recurrence [106], we found that NSCs directly injected into the walls of the resection cavity were cleared in 7 days and the remaining cells only extended survival of tumor-bearing mice by 1 week [65]. In contrast, transplanting NSCs encapsulated in hydrogel scaffolds increased the intracavity persistence of therapeutic NSCs to over 28 days, improved tumor killing, and extended the survival of tumor-bearing mice to over 63 days post-transplant. The mechanisms driving the loss of directly injected NSCs from the resection cavity are unclear, yet these findings further emphasize the importance of new strategies to effectively transplant cytotoxic NSCs into human GBM patients undergoing surgical debulking.

7. Conclusions

Tumoricidal NSC therapy is opening new doors for cancer therapy. The tumor-homing capacity of these cells creates a powerful drug delivery platform that provides access to invasive cancer foci which traditional surgery, chemotherapy, and radio-therapy cannot typically access. NSCs have been engineered with a wide range of therapeutic agents, and typically achieve tumor reductions of 70–90% in preclinical models. Despite the success of these studies, many challenges still remain. The treatment durability of NSC therapies needs to be improved, as tumor recurrence typically occurs despite the initial robust tumor killing. As with most chemotherapies, it is unlikely that a single-agent NSC therapy will eradicate the tumor. The optimal rationally selected drug combination needs to be determined. The recent initiation of two clinical trials will provide exciting new clinical feedback on the performance of NSC therapies in human GBM patients. Undoubtedly, new challenges will

arise from these trials, and will provide new opportunities to further improve the performance of this promising therapy. In this way, a cytotoxic NSC therapy can be developed that is capable of providing a true therapeutic benefit to brain cancer patients in a clinical setting.

Acknowledgments

This work was supported by the UNC Lineberger Comprehensive Cancer Center's University Cancer Research Fund and the UNC Translational and Clinical Sciences Institute (KL2TR001109, UL1TR001111).

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