Stem cell therapies for malignant glioma

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The prognosis for patients with malignant glioma, which is the most common primary intracranial neoplasm, remains dismal despite significant progress in neurooncological therapies and technology. This is largely due to the inability of current treatment strategies to address the highly invasive nature of this disease. Malignant glial cells often disseminate throughout the brain, making it exceedingly difficult to target and treat all intracranial neoplastic foci, with the result that tumor recurrence is inevitable despite aggressive surgery and adjuvant radiotherapy and/or chemotherapy. The use of neural stem cells (NSCs) as delivery vehicles for tumor-toxic molecules represents the first experimental strategy aimed specifically at targeting disseminated tumor pockets. Investigators have demonstrated that NSCs possess robust tropism for infiltrating tumor cells, and that they can be used to deliver therapeutic agents directly to tumor satellites, with significant therapeutic benefit. With the aim of developing these findings into a clinically viable technology that would not be hindered by ethical and tissue rejection–related concerns, the use of adult tissue–derived stem cells has recently been explored. These technologies represent important progress in the development of a treatment strategy that can specifically target disseminated neoplastic pockets within the brain. Despite encouraging results in preclinical models, however, there are significant impediments that must be overcome prior to clinical implementation of this strategy. Key among these are an inadequate understanding of the specific tropic mechanisms that govern NSC migration toward invasive tumor, and the need to refine the processes used to generate tumor-tropic stem cells from adult tissues so that this can be accomplished in a clinically practicable fashion. Despite these limitations, the use of stem cell therapies for brain tumors holds significant promise and may emerge as an important therapeutic modality for patients with malignant glioma.

KEY WORDS • brain tumor • glioma • stem cell • cellular therapy • gene therapy

OVERVIEW

Malignant gliomas are the most common primary brain tumors. Despite dramatic advances in imaging, surgical technique, and adjuvant radiotherapy and chemotherapy, the prognosis for patients with malignant glial tumors remains dismal.\(^\text{13,23,24}\) The median survival duration after diagnosis of glioblastoma multiforme, the most common and aggressive subtype of malignant glioma, is less than 1 year, with a 2-year survival rate approaching zero.\(^\text{28}\) The failure of currently available therapeutic approaches, which center on resection followed by adjuvant radio- and/or chemotherapy, is rooted in the disseminated nature of these tumors. Gliomas are highly infiltrative neoplasms, with solitary tumor cells or clusters of neoplastic cells migrating extensively throughout the brain. Despite aggressive surgical intervention and radiation therapy, it is impossible to eliminate successfully all of these tumor foci interspersed within normal brain parenchyma, often at a significant distance from the primary tumor mass. These neoplastic pockets eventually serve as reservoirs for near-universal tumor recurrence, which in turn contributes to the lethality of this disease. Standard adjuvant therapies including radiation and chemotherapy have, despite their modest effects on long-term survival, been unable to effect any meaningful impact on the long-term prognosis.

Numerous experimental treatment modalities for malignant glioma have been tested, including the use of various immunotherapeutic strategies as well as gene therapy and novel approaches for optimization of cytotoxic drug delivery across the blood–brain barrier. Despite encouraging results in the treatment of glioblastomas in animal models, none of these experimental therapies have made a significant impact at the clinical level, largely because of their inability to eradicate disseminated tumor foci within the brain. The development of a successful treatment modality for malignant brain tumors will, therefore, center on the ability to devise a means of eliminating all intracranial neoplastic reservoirs left behind after resection of the primary tumor mass. Only by achieving removal/elimination of more than 99% of the total tumor burden (that is, a greater than 2-log kill) will a treatment strategy be able to effect a meaningful impact on long-term survival.\(^\text{14}\) In the setting of malignant glioma, this remains a daunting task, given the highly disseminated nature of the disease process and our current inability to visualize adequately and target therapeutically every remaining tumor cell.

Abbreviations used in this paper: IFN = interferon; IL = interleukin; MSC = mesenchymal stem cell; NSC = neural stem cell; NSC-IL-12 = IL-12–expressing NSC; TRAIL = tumor necrosis factor–related apoptosis-inducing ligand.
One promising means of targeting treatment to migrating tumor satellites involves the use of NSCs. These cells can be derived from fetal, neonatal, or postnatal tissues and are capable of indefinite propagation as well as terminal differentiation into neurons, astrocytes, and oligodendrocytes. We and others have demonstrated that NSCs exhibit potent tropism for disseminating glioma cells within the brain and can be engineered to deliver tumor-toxic gene products directly to remote neoplastic microsatellites with significant therapeutic efficacy. In this review, we will specifically focus on the relevance and role of NSCs as therapeutic delivery vehicles for targeting disseminated tumor. The ability of NSCs to track migrating tumor cells, as well as evidence supporting their ability to deliver a variety of therapeutic proteins, will be discussed in detail, followed by an analysis of alternative sources for tumor-tropic stem cells and our assessment of the future challenges associated with NSC therapy for malignant brain tumors.

Relevance and Role of NSCs in Treatment of Malignant Glioma

The inability of present therapies to improve outcome for patients with high-grade glioma has spurred the search for alternative treatment strategies. Numerous approaches have been tested, usually in the setting of experimental rodent glioblastomas. Although a multitude of novel therapeutic options have been used, major strategies have generally focused on the delivery of novel tumor-toxic molecules/agents into the tumor bed or across the blood–brain barrier, gene transfer techniques to introduce suicidal or therapeutic gene products into tumor cells, or the use of immunostimulatory therapies to induce endogenous immune responses against the tumor. Of these approaches, none have directly focused on the key issue of specifically targeting tumor satellites. It is these disseminated neoplastic foci that form the main therapy-limiting impediment to current treatment approaches, because they serve as a nidus for tumor recurrence, and therefore predestine standard treatment protocols to failure.

Certain immunotherapeutic approaches, although not intentionally targeted toward the specific eradication of disconnected tumor microsatellites, may have the ability to induce clearance of independent neoplastic pockets by boosting tumoricidal cell-mediated immunity against tumor-specific antigenic targets. This is evidenced by the successful eradication of glioblastomas in experimental rodent models of active immunotherapy and in clinical studies that have demonstrated a highly significant prolongation of median survival duration in patients treated with dendritic cell vaccines. Despite these encouraging results, the clinical impact of these immunostimulatory approaches is limited by a number of significant factors. Chief among these is the well-documented ability of malignant glial tumors to suppress endogenous immune activity, with the result that patients with gliomas frequently present with multiple immune defects, particularly at the level of antigen presentation and cytotoxic T-cell activity. Additionally, given the heterogeneity of all high-grade gliomas, the specific antigenic targets that may elicit tumoricidal immunity remain largely uncharacterized. As a result of the complexity of the immune defects observed in association with malignant gliomas as well as the inability of current immunotherapeutic technologies to specifically target immune responses against glioma-specific antigens in general, and tumor microsatellites in particular, the viability of active immunotherapy protocols in the eradication of all neoplastic foci within the brain remains questionable. As the biological mechanisms governing the interaction between gliomas and the immune system are further investigated, however, and immunotherapeutic protocols are refined accordingly, the use of active immunostimulatory treatments holds significant promise, given the potency of antitumor T-cell immunity and the ability of tumor-specific immune responses to seek out, recognize, and clear tumor antigen–expressing neoplastic cells.

In light of the limitations faced by current clinical as well as experimental treatments in addressing the issue of disseminated glioma pockets, it is apparent that the development of a viable therapeutic approach will center on the ability of a treatment to seek out and specifically target all neoplastic foci within the brain. The possibility of actively identifying all tumor microsatellites with the aim of individually treating each neoplastic pocket remains unrealistic given the potentially staggering number of microsatellites that may be associated with a large intracranial tumor, and the limitations of current imaging technology. An ideal treatment should, therefore, be capable of independently targeting glioma microsatellites without the need for operator involvement. This will require that the therapeutic agent be capable of distinguishing neoplastic cells from normal neurons or glia based on either cell surface or secretory characteristics. Additionally, the treatment will have to be able to locate and subsequently target tumor foci composed of exceedingly small numbers of cells, a level of sensitivity that is beyond the capability of any noninvasive imaging technology currently in use.

The demonstrated ability of NSCs to track to intracerebral glioma microsatellites has brought to light the potential of their use as a tumor-tropic treatment vehicle. Initial treatment options featuring NSCs generally focused on their ability to serve as reconstructive agents following tissue destruction resulting from infarction or neurodegenerative disease. The identification of pluripotent NSCs within the subventricular zones in the adult central nervous system, as well as the ability of these cells to migrate to areas of intracranial disease, has raised the interesting hypothesis that intracranial NSC populations persist well into adulthood and may play a key role in endogenous tissue repair and repopulation following injury or insult. The fulfillment of such a role would necessitate that NSCs be responsive to chemotactic signals that would emanate from zones of pathology within the brain, thereby enabling NSCs to follow chemokine gradients as they migrate toward sites of injury. The first clear demonstration that NSCs could exhibit migratory activity toward the site of intracranial tumor was advanced by Aboody and colleagues. These researchers demonstrated that the immortalized murine NSC line C17.2 could track to areas of disseminated intracranial tumor when injected either intracerebrally or intravenously into glioma-bearing rodents. The authors also demonstrated that their NSCs could be engineered to carry the bioactive transgene for cytokine deaminase, which could convert the systemically administered nontoxic pro-drug 5-fluorocytosine to the cytotoxic 5-fluorouracil, resulting in significant shrinkage of treated tumors compared with controls. These
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results clearly pointed to the potential of NSCs as tumortropic vehicles. Although encouraging as an initial report, the therapeutic modality adopted by Aboody, et al., was limited by certain factors that would restrict its relevance in a clinical setting. The use of an immortalized cell line such as C17.2 is inherently problematic; there remain concerns of potential tumorigenicity given the proliferative potential of cells transformed by means of viral oncogenes. Additionally, the use of an allogeneic transplant carries with it the significant likelihood of immune rejection and would therefore necessitate iatrogenic immune suppression in patients treated in this manner. Given the preexisting immune defects found in patients with high-grade gliomas, and the potential role that such immune deficiencies may play in enhancing the growth of these neoplasms, the use of additional immunosuppressive treatments may be highly undesirable. Importantly, however, the use of an “off-the-shelf” cell line such as C17.2 offers the considerable advantage of an easily usable and uniform therapeutic tool that can be mass produced and made available for clinical testing and/or use without delay. Therefore, the further development of NSC lines for therapeutic purposes deserves continued attention and study.

We and others have hypothesized that the use of a syngeneic, nontransformed NSC population may be more relevant as a tool for delivering tumor-toxic therapy to disseminated tumor pockets within the brain. Benedetti and colleagues first described the use of cytokine-expressing primary NSC in the treatment of experimental gliomas. Nevertheless, they did not characterize the migratory potential of these cells and therefore the underlying survival benefit associated with their therapy was not readily apparent. We have subsequently demonstrated, in a murine model of experimental glioblastoma, that syngeneic NSCs exhibit robust tropism for disseminated tumor and are capable of extensive migration within the brain. In these studies, NSCs were harvested from the frontoparietal regions of Day 15 fetal C57Bl/6 mice and cultured in vitro in combination with basic fibroblast growth factor and epidermal growth factor as previously described. Isolated cells grew primarily as spherical aggregates (Fig. 1A) that were composed mainly of cells that stained strongly for nestin, a marker of neural progenitor cells (Fig. 1B). We confirmed their pluripotency by inducing in vitro differentiation into all three neural lineages, with identifiable populations of neurons, astrocytes, and oligodendrocytes (Fig. 1C–E, respectively). The NSCs were then infected in vitro with replication-defective adenovirus encoding the β-galactosidase gene to facilitate in vivo tracking. After in vitro confirmation of robust β-galactosidase expression by infected cells (Fig. 1F), NSCs were stereotactically inoculated into established intracranial GL26 gliomas. Brains from treated animals were harvested and stained with an X-gal substrate to detect the presence of β-galactosidase–expressing NSCs. The NSCs were readily identifiable dispersed within inoculated tumors and were clearly visible tracking glioma cells as they migrated away from the main tumor mass. We were able to detect several different patterns of tumor spread and found NSC tracking migrating glioma cells in each case. These included thin outgrowths of tumor deep into adjacent normal brain (Fig. 2A and B), direct extension of the tumor mass into adjacent tissue (Fig. 2C), migration of glioma cells along established white matter tracts (Fig. 2D), and dissemination of solitary tumor pockets at a considerable distance from the primary tumor bed (Fig. 2E and F).

To establish that the observed tumor-tracking capacity of NSCs was not random, we inoculated NSCs into the corpus striatum of mice, contralateral to existing gliomas. We found that NSCs did not randomly disperse into adjacent normal tissue (Fig. 2G left), nor could they be seen migrating to any distant nontumorous region of the brain. Nevertheless, some NSCs were visible tracking directly across the
Fig. 2. Photomicrographs of NSCs displaying strong tropism for disseminating glioma in vivo. Tumors from glioma–bearing mice inoculated with NSC-LacZ were stained with X-gal and counterstained with neutral red. The NSCs appear blue as they express β-galactosidase, whereas the tumor appears as hypercellular areas staining intensely with neutral red. Arrows indicate disseminating NSCs closely following migrating pockets of tumor.

Four distinct patterns of tumor spread were detected and NSCs were found tracking migrating glioma in each case. A: Thin outgrowth of tumor cells deep into adjacent normal brain. B: High-power magnification of boxed area in A. C: Direct extension of tumor mass into adjacent tissue. D: Migration of glioma cells away from the primary tumor bed along a white matter tract. E: Tumor microsatellite independent of the main tumor mass. F: High-power photomicrograph of microsatellite (box) in E demonstrating β-galactosidase–positive NSCs interspersed with tumor cells exhibiting visible mitoses. G: Inoculation of NSCs into cerebral hemisphere contralateral to existing tumor. The left panel shows the portion of left cerebral hemisphere where NSCs were inoculated and illustrates that NSCs do not randomly dissipate into adjacent nontumorous tissue. The center panel shows the tumor-bearing portion of right cerebral hemisphere demonstrating specific, nonrandom migration of NSCs across the brain into the vicinity of the tumor (inset). The right panel demonstrates a second tumor-bearing portion of a similarly treated brain. The NSC-LacZ are visible, interspersed within the tumor mass (inset), contralateral to their site of inoculation. H: When administered in a nontumor–bearing brain, the NSC-LacZ remain confined to the needle track (left), and do not randomly disseminate into adjacent tissue or into the contralateral hemisphere (right). N = normal brain tissue; T = tumor mass, outgrowths, and microsatellites. (Reproduced with permission from Ehtesham M, Kabos P, Kabosova A, et al: The use of interleukin 12-secreting neural stem cells for the treatment of intracranial glioma. Cancer Res 62:5657–5663, 2002.)
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brain into the immediate vicinity of the tumor (Fig. 2G center) and into the tumor itself (Fig. 2G right). We also found that NSCs inoculated into nontumor–bearing brains did not randomly dissipate into adjacent tissue or to the contralateral hemisphere (Fig. 2H). These results provide strong evidence that NSCs exhibit potent, specific tropism for intracranial tumor.

Based on the demonstrated ability of syngeneic, primary NSCs to migrate in conjunction with tumor outgrowths and microsatellites, we wished to investigate whether this tropism could be exploited for therapeutic benefit. We and others have previously reported the ability of the immunostimulatory cytokine IL-12 to elicit tumoricidal T-cell responses against experimental gliomas, and we have specifically established the ability of in situ IL-12 gene transfer to promote T-cell infiltration into intracranial tumor tissue. We hypothesized that the delivery of IL-12 by NSCs that have migrated into the proximity of disseminated pockets of tumor cells may induce a T-cell response against these tumor islands and thus elicit immune-mediated clearance of all tumor foci within the brain. We used a replication-defective adenoviral vector encoding the genes for IL-12 to confer IL-12 protein expression in NSCs. These cells, when transplanted into established intracranial murine gliomas, continued to secrete significant amounts of cytokine. To establish whether the migratory capacity of NSCs was of therapeutic relevance in this setting, we inoculated glioma-bearing control animals intratumorally with nonmigratory fibroblasts that secreted similar levels of IL-12 to NSCs in vitro and in vivo. Of significance was our finding that NSC–IL-12 could be found within tumor outgrowths and microsatellites, whereas transplanted fibroblasts were limited to the main tumor mass. Additionally, whereas fibroblast-mediated IL-12 secretion could only induce T-cell infiltration within the main tumor mass, treatment with NSC–IL-12 resulted in robust T-cell aggregation along the tumor/normal tissue boundary as well as within tumor outgrowths and microsatellites (Fig. 3). This was most likely a result of a chemotactic colocalization of T cells with marginating and migrating NSC–IL-12. To determine whether the observed ability of NSC–IL-12 to induce T-cell infiltration within disseminating tumor was therapeutically relevant, we followed glioma-bearing animals treated with intratumoral NSC inoculation for long-term survival. We found that animals treated with tumor-tropic NSC–IL-12 survived longer than animals treated with nonmigratory IL-12–secreting fibroblasts (Fig. 4). These findings clearly illustrate the relevance of delivering therapeutic agents directly to disseminated tumor satellites.

To add further credence to the therapeutic relevance of NSC therapy for high-grade gliomas, we tested the efficiency of NSC as a vehicle for delivery of a nonimmune tumoricidal agent. Using replication-defective adenoviral-mediated gene transfer, we engineered NSC to secrete the tumor-toxic chemokine TRAIL; this substance has been shown to induce apoptosis selectively in transformed cells, with negligible toxicity to normal tissue, and it has been used as an experimental agent in a variety of cancer models including glioma. We demonstrated that in athymic T-cell–incompetent mice bearing intracranial human U343 glioblastoma xenografts, the inoculation of TRAIL-secreting NSCs, also called NSC-TRAIL, significantly inhibited the growth of intracerebral gliomas (Fig. 4). Of significance was our additional finding that NSC-TRAIL could migrate into disconnected tumor pockets at a significant distance from the main tumor mass and induce overwhelming apoptotic activity in these glioma satellites (Fig. 5). These findings clearly establish the superiority (in an experimental setting) of NSC-mediated protein delivery over more conventional methods such as direct gene transfer through viral vectors or direct infusion of recombinant protein into the tumor or postresection tumor cavity. The ability of NSCs to seek out pockets of disseminated tumor and specifically deliver therapeutic agents into the vicinity of these neoplastic foci, without distributing these potentially toxic chemokines throughout the brain, underscores their utility and potential.

The mechanisms that underlie the tumor tracking migratory activity of NSCs are still unclear. It is highly likely, given the reparative role that NSC populations are speculated to have within the adult central nervous system, that migratory NSCs are tracking a chemotactic gradient established by the secretion of inflammatory mediator(s) in disseminating tumor cell pockets or in normal brain parenchyma secondary to injury by infiltrating neoplastic cells. The elucidation of the exact mediators and specific signals governing NSC tropism for migrating tumor will allow further refinement in the use of these cells as, prior to transplantation, NSC populations could be purified in vitro in a phenotypic manner based on the expression of cell surface receptor(s) for the chemokine(s) involved in tumor tracking. Conceivably, the identification of cell surface proteins associated with NSC migratory activity could allow for the enrichment of this expression by in vitro manipulation, thereby increasing the tropic potential of NSCs.

In their report, Benedetti and colleagues presented evidence suggesting that NSCs may be independently capable of inhibiting glioblastoma cell proliferation. In our extensive experiments, we have found no evidence to support this earlier demonstration, because we could only demonstrate a therapeutic benefit when NSCs were engineered to deliver tumor-toxic chemokines. The exact mechanism underlying the observations of Benedetti, et al., remains unclear, although they have reported that NSCs may elaborate a secretory agent that could inhibit tumor cell growth. More recently, Staflin and colleagues have also described the ability of nonengineered rat neural progenitors to inhibit glioma growth in vivo. The ability of NSCs to counter the tumorigenic potential of glioma cells independent of the delivery of therapeutic proteins remains an interesting proposition. If confirmed on further investigation, this phenomenon will add more credence to the utility of NSCs as therapeutic agents for malignant glioma.

Clinically Viable, Nonneural Tissue Sources for Therapeutic Stem Cells

It is evident that the use of NSCs as therapeutic vehicles for glioma holds tremendous potential. Nevertheless, the clinical relevance of this approach in the specific context of NSCs with fetal origins is limited by significant ethical issues. Additionally, the use of allogeneic fetal-derived NSC in a clinical setting would be complicated by concerns of tissue rejection and would therefore necessitate a degree of iatrogenic immunosuppression, which for reasons discussed earlier is undesirable in patients with malignant gliomas. An
Fig. 3. Photomicrographs showing therapeutic response associated with IL-12 delivery by NSCs to glioma in vivo. A: Flow cytometry analysis demonstrating robust intratumoral T-cell infiltration in gliomas inoculated with NSC–IL-12 (left) and 3T3–IL-12 (center). The T-cell content of NSC-LacZ–treated tumors (right) was much lower and comparable to infiltration seen in mock-NSC– and saline-inoculated gliomas (data not shown). B–D: The left panels demonstrate CD4+ and the center and right panels show CD8+ T-cell infiltration. The right panels represent high-power images of the boxed areas in their respective center panels. B: The tumors treated with 3T3–IL-12 demonstrate CD4+ and CD8+ T-cell infiltration, with immunohistochemically positive cells interspersed in tumor tissue. C: The NSC-LacZ–treated tumors display negligible infiltration by CD4+ or CD8+ T-cells. D: The glioma-bearing brains treated with NSC–IL-12 demonstrate robust infiltration of tumors with CD4+ and CD8+ T cells, and numerous aggregates along the tumor/normal tissue boundary (arrows). E: Comparison of T-cell infiltration in comparable outgrowths from primary tumor bed. Tumor microsatellites in tumors treated with NSC–IL-12 demonstrated robust CD8+ T-cell infiltration (left), whereas those in brains inoculated with 3T3–IL-12 did not (right).

ideal cellular therapy for use in clinical practice should, therefore, ideally comprise autologous cells that can be harvested without difficulty, processed efficiently in vitro, and then reinoculated into the same patient.

In this context, Nakamizo and colleagues have recently shown that human bone marrow–derived stem cells are capable of exhibiting tumor-tropic behavior comparable to that demonstrated by fetal NSCs. Using bone marrow–derived MSCs isolated from bone marrow harvested in healthy volunteers, they have been able to demonstrate selective localization of these cells to human glioma intracranial xenografts in nude mice when administered intravascularly (Fig. 6). In addition, these MSCs were observed to migrate effectively toward glioma xenografts after local intracranial delivery into the contralateral cerebral hemisphere. Previous studies have proven the ability of MSCs to differentiate into astrocytes and neurons both in vitro and in vivo, demonstrating that they do in fact represent neural progenitor cells.

As a corollary to these initial studies, Nakamizo, et al., tested the potential of bone marrow–derived MSCs to serve as therapeutic vehicles for the delivery of molecules with antitumoral effects to gliomas, as had been demonstrated previously by Ehtesham and colleagues by using fetal NSC. Using an adenoviral vector, they transfected the IFN-β gene into MSCs and found that the engineered cells released high levels of IFN-β and were capable of killing human glioma cell lines in vitro. Furthermore, intraarterial administration of the transfected MSCs in vivo significantly extended the survival of mice harboring human intracranial gliomas, an effect not duplicated by the intravenous injection of IFN-β or by subcutaneous implantation of IFN-β–transfected MSCs. The investigators thus concluded that the increased survival data reflected the local delivery of IFN-β by transfected MSCs to intracranial tumor tissue, and that this population of adult-derived progenitor cells is capable of replicating not only the tumor-tropic properties of fetal NSCs, but also their ability to be genetically modified to elaborate and deliver tumoricidal substances directly to intracranial tumor cells.

These new findings represent a promising new means of isolating tumor-tropic neural progenitor cells for delivery of therapeutic proteins to disseminated intracranial tumor. Indeed, in conjunction with earlier work demonstrating that multipotent progenitors of various tissue lineages, including neural precursors, can be derived from adult bone marrow, it is likely that bone marrow can serve as a reservoir of pluripotent stem cells that may be readily used for a wide variety of therapeutic applications. With their clinical accessibility, potential for in vitro expansion and modification, and demonstrable migratory activity and tumor tropism, adult-derived neural progenitors represent an important step forward that may prove critical in the clinical implementation of stem cell therapy for malignant glioma.

Preliminary Findings

Preliminary evidence has established that the use of NSCs as tumor-tropic delivery vehicles represents a promising new treatment modality for malignant glioma. It is
important, however, to note that significant impediments remain before the true potential of this novel therapy can be fully realized in a clinical setting. The use of fetal-derived NSCs, although potentially of immense therapeutic relevance, given their robust tropism for intracranial tumor, remains highly controversial because of the reluctance to use embryonic tissue for ethical and legal reasons. Additionally, the necessity of using immunosuppressive therapy in patients who receive transplanted allogeneic cells, whether those constitute fetal NSCs or an “off-the-shelf” immortalized stem cell pool as described by Aboody and colleagues, is undesirable given the known role of endogenous tumor-induced immune defects in supporting the growth of malignant gliomas. The potential tumorigenicity of transplanted NSCs also remains a concern. Even though no evidence has arisen in the extensive studies conducted by our group and Benedetti, et al., indicating that primary NSCs may be tumorigenic, further investigation is necessary to ensure the safety of this therapy. These issues limit the clinical impact that currently validated experimental approaches described by our group and others may have in the immediate future. The ability to translate the promising potential visualized with the use of fetal NSCs into an alternate, clinically viable source of cells with similar tumor-tropic characteristics will, therefore, prove critical to the eventual clinical implementation of this therapeutic approach.

Despite these concerns, significant progress has been made in using adult-derived stem cells for experimental tumor therapy. The implications of this novel process are tremendous; it would allow for the generation of a reservoir of autologous cells, harvested from tissue that is routinely accessible in a clinical setting, and would therefore obviate the ethical and immunological incompatibility-related concerns that preclude the use of fetal NSCs in patients. Before this strategy can be realistically implemented, however, it will be critical to validate the process by demonstrating reproducibility in other laboratories. Furthermore, a greater understanding is needed of the chemotropic mechanisms that mediate stem cell migration toward foci of intracranial neoplasm. In this context, we have recently detailed the important role of the chemokine ligand CXCL12 and its corresponding receptor CXCR4 in governing stem cell dissemination toward glioma. More recently, Schmidt, et al., have also described the contribution of vascular endothelial growth factor to this process. Much work is still necessary in further investigation of the biological pathways underlying stem cell migration toward diseased tissue.

Over the next few years we should see rapid progress toward the development of alternate sources of stem cells that can serve as clinically viable avenues for transplantation. It is likely that tissues such as bone marrow and, potentially, whole blood may eventually serve as routine sources for cell harvest, with the establishment of dedicated core laboratories geared toward the rapid in vitro expansion of autologous neural progenitors. Once such methodology is developed, it is possible that we will see the initiation of clinical trials using stem cell therapy in patients with malignant glioma. Additionally, further investigation of the biological signals that govern stem cell migration toward sites of disseminating tumor will provide greater insight into how stem cells track infiltrating tumor cells and may allow further refinement of this therapeutic modality, because specific populations could be purified or engineered on the basis of enhanced tropic characteristics, thereby improving the efficiency of this treatment strategy for malignant glioma.

**CONCLUSIONS**

Although stem cell technologies are currently in the initial stages of development and preclinical testing, there is tremendous promise in their potential to be of significant therapeutic benefit, particularly in patients with high-grade gliomas.
gliomas. Researchers in the field of neurooncology have been unable to make any satisfactory progress in combating this lethal disease process, largely as a result of the inability of currently available clinical and experimental therapies to target and eliminate residual intracranial neoplastic foci. The use of stem cells represents an important therapeutic attempt that is specifically geared toward addressing this problem. The encouraging results that have been observed to date underscore the potential of this therapy, and despite limitations that may make immediate clinical implementation impractical, further work in this field should be pursued aggressively.

References
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