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Stem Cells as Vehicles for the Treatment of Brain Cancer

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Malignant gliomas represent a significant challenge in the field of neuro-oncology. Despite dramatic advances in imaging technology, surgical techniques, and adjuvant radio- and chemotherapy, the overall prognosis of this disease remains dismal. The median survival 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 [1,2]. The failure of current therapeutic approaches to treat gliomas effectively centers chiefly on the highly infiltrative nature of these neoplasms. Gliomas invade deep into normal brain parenchyma, thereby making total surgical resection a challenging task [3]. In addition, global treatment strategies, such as external beam radiation and systemic chemotherapy, are unable to effect meaningful therapeutic benefit, given their inability to target disseminated tumor specifically and the fact that these regimens themselves carry significant toxicity and potential for cognitive impairment [4,5].

In light of this, there is an urgent need for the development of more effective therapies that can target residual disseminated tumor burden in the brain. A rational approach to the design of such a treatment would involve the use of a therapeutic delivery system that could "seek out" dispersed tumor cells in the brain and specifically deliver toxic molecules directly to these infiltrative neoplastic foci. One promising method of meeting this challenge is the use of neural stem cells (NSCs) and mesenchymal stem cells (MSCs). Multiple studies have now conclusively demonstrated that stem cells show strong tropism for infiltrating glioma cells within the brain and can be engineered to deliver tumoricidal proteins directly and specifically to tumor microsatellites. In this review, the authors discuss the characteristics of stem cells that make them good candidates for this purpose, explore evidence that supports their use for antitumor therapy, and elucidate different potential tissue sources from which stem cells can be isolated for this use. Finally, the authors present an assessment of future challenges and important questions that need to be addressed before clinical trials using stem cell therapies for glioma can begin.

Relevance of stem cells as delivery vehicles for brain tumors

Tumor-homing capacity of stem cells

Stem cells are defined by two main properties: self-renewal and multipotency [6,7]. Based on their source, they can be divided into embryonic stem cells derived from the blastocyst inner cell mass, fetal stem cells isolated from fetal blood and tissues, and adult stem cells. Adult stem cells are further categorized based on their tissue of origin. To date, the most extensively studied adult stem cells have been hematopoietic stem cells [8], but stem cells from other tissues, including bone

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marrow (BM) [9], central nervous system (CNS) [10], skeletal muscle [11], and adipose tissue [12], have also been isolated and characterized. Numerous reports have demonstrated the ability of adult stem cells to transdifferentiate across lineages, as exemplified by the ability of hematopoietic stem cells to acquire neuronal, hepatic, and endothelial phenotypes, and vice versa [13,14]. Together, these findings demonstrate the substantial plasticity of adult stem cell populations and serve to underscore their tremendous clinical potential.

Several reports have outlined the ability of stem cells to "home in" to areas of tumor throughout the body after local as well as systemic administration [15–18]. In this context, NSCs located within the subventricular zones and dentate gyrus of the hippocampus in the adult CNS have been widely studied for their potential to migrate to areas of intracranial pathologic change [19], neoplastic and nonneoplastic alike. Such observations have suggested that intracranial NSC populations may persist well into adulthood, perhaps serving to facilitate endogenous tissue repair and cell repopulation after injury or insult. To fulfill such a role successfully, NSCs must be responsive to chemotactic signals emanating from diseased or injured areas of the brain, thereby enabling them to migrate toward these areas.

Aboody and colleagues [15] were the first to demonstrate that NSCs migrate expeditiously toward sites of intracranial tumor. Using the immortalized murine NSC line C17.2, originally isolated from the external granule layer of murine neonatal cerebellum, these investigators demonstrated that these cells could track to areas of disseminated intracranial tumor when injected intracerebrally or intravenously into glioma-bearing rodents (Fig. 1). In addition, they showed that NSCs could be engineered to express the bioactive transgene for cytosine deaminase (CD), an enzyme capable of converting the systemically administered nontoxic prodrug 5-fluorocytosine (5-FC) to highly cytotoxic 5-fluorouracil (5-FU), resulting in significant shrinkage of treated tumors compared with controls. The tumor-tropic migratory capacity of NSCs has been subsequently confirmed in extensive studies performed by our group [17,20,21] and others [16]. Specifically, we confirmed that the glioma-directed dissemination of NSCs we observed in vivo was a nonrandom phenomenon, because NSCs injected into non–tumor-bearing brains did not demonstrate any notable migratory activity. These initial studies, although highlighting the clinical implication that NSCs have the potential to target intracranial glioma, did not elucidate the mechanisms by which these cells migrate. Elucidation of the factors mediating the inherent homing potential of NSCs is a necessary prerequisite for developing more effective ways to use these cells for therapeutic applications.

Regulation of glioma-tropic neural stem cell migration

The coordinated migration of neural precursor cells during CNS development is a highly orchestrated and complex phenomenon and critically depends on a plethora of regulatory factors, including soluble proteins, cell adhesion molecules, and extracellular matrix components. In contrast, the molecular mechanisms regulating NSC migration in the adult brain are not yet well understood but are likely to involve similar processes. Elevated concentrations of several soluble growth factors known to regulate NSC migration, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and stem cell factor (SCF), are typical features of malignant gliomas [22,23], and cultured NSCs have been shown to express the corresponding receptors (Table 1), thereby implicating a potential regulatory role for these pathways in glioma-tropic NSC migration. In addition, our group has demonstrated that the cell surface chemokine receptor CXCR4 is a highly potent mediator of NSC trafficking toward brain tumors [21]. The interaction of CXCR4 and its ligand CXCL12 mediates invasiveness in malignant glial cells themselves [24], and we have recently characterized widespread expression of this pathway in high-grade gliomas (manuscript submitted). As such, the CXCR4/CXCL12 chemokine axis is likely to be a significant mechanism that regulates glioma-directed NSC migration, as evidenced by our demonstrated ability to abrogate in vitro glioma-directed NSC trafficking after neutralization of cell surface CXCR4 [21]. What remains unclear is whether the tactic signals responsible for chemoattracting NSCs toward intracranial tumor foci emanate from the tumor cells themselves or are derived from reactive changes in nonneoplastic parenchyma. Evidence in support of the latter possibility includes our recent finding that CXCL12 expression within malignant gliomas seems to be most significant within vascular endothelium and nontumorous perivascular cells that may be neurons or microglia (manuscript submitted).

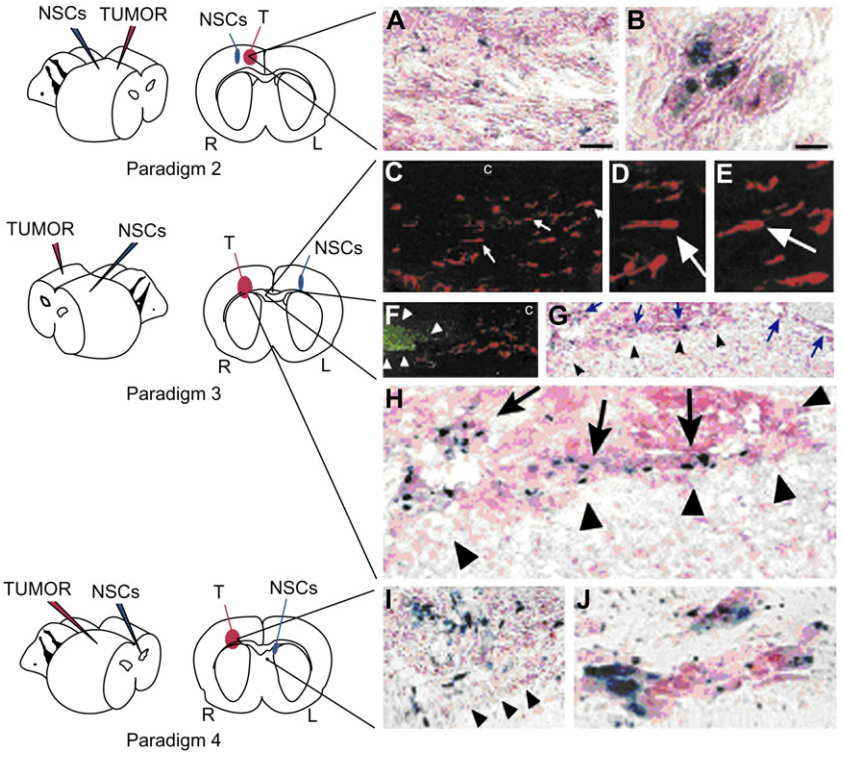

Fig. 1. NSCs display strong tropism for glioma in vivo. (*A, B*) NSCs implanted in the same cerebral hemisphere caudal to the main tumor bed (Paradigm 2) migrate through normal brain parenchyma toward glioma cells. A and B represent sections through intracranial tumor in an adult nude mouse 6 days after NSC implantation. In A, NSCs (stained with X-gal) appear blue and are seen interspersed among hypercellular areas of tumor (counter-stained with neutral red). (*B*) details disseminating NSCs closely following migrating pockets of tumor cells. (*C–H*) Contralateral hemisphere (Paradigm 3). (*C–E*) As indicated on the schematic, these panels are views through the corpus callosum (''c'') where b-gal$^+$ NSCs (*red cells, arrows*) are seen migrating from their site of implantation on one side of the brain toward tumor on the other. Two representative NSCs indicated by arrows in C are viewed at higher magnification in D and E, respectively, to visualize the classic elongated morphology and leading process of a migrating neural progenitor oriented toward its target. In F, b-gal$^+$ NSCs (*red*) are ''homing in'' on the GFP$^+$ tumor (*green, arrowheads*) having migrated from the other hemisphere. In G, and magnified further in H, the X-Gal$^+$ blue NSCs (*arrows*) have now actually entered the neutral red$^+$ tumor (*arrowheads*) from the opposite hemisphere. (*I, J*) Intraventricular (Paradigm 4). Shown here is a section through the brain tumor of an adult nude mouse 6 days following NSC injection into the contralateral cerebral ventricle. In I, as per the schematic, blue X-Gal$^+$ NSCs are distributed within the neutral red$^+$ main tumor bed (*edge delineated by arrowheads*). At higher power in J, the NSCs are in juxtaposition to migrating islands of red glioblastoma cells. Fibroblast control cells never migrated from their injection site in any paradigm. All X-Gal-positivity was corroborated by anti-b-gal immunoreactivity. (Scale bar: A, 20 mm, and applies to C; B, 8 mm, 14 mm in D and E, 30 mm in F and G, 15 mm in H, 20 mm in I, and 15 mm in J.) (*From* Aboody KS, Brown A, Rainov NG, et al. Neural Stem Cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci USA 2000;97:12848; with permission. © Copyright 2000 National Academy of Sciences, USA.)

Drug delivery to disseminated tumor cells

The robust tropism of NSCs for intracranial neoplasms makes them highly attractive as vehicles for the delivery of a wide variety of therapeutic gene products directly to tumor cells. Several different classes of effector molecules have been tested in this context, including immunostimulatory cytokines, prodrug activation enzymes, viral vectors, and proapoptotic proteins. Preliminary results in preclinical rodent models have been encouraging and are summarized here.

Cytokines

Benedetti and colleagues [16] first described the use of cytokine-expressing NSCs in the treatment

Table 1
Factors potentially involved in mediating the neural stem cell tropism for gliomas

Receptor on NSC	Ligand (detected in gliomas)	Suggested role in NSCs	References
CXCR4	CXCL12 (yes)	Proliferation, survival, migration	[21,37]
CCR2	CCL2 or MCP1 (?)	Migration	[38,39]
CCR5	CCL5 or RANTES (yes)	Migration	[40]
CXCR3	I-TAC or CXCL11 (no)	Migration	[41]
VEGFR	VEGF (yes)	Migration	[42]
CX3CR1	Fractalkine	Migration	[40]
c-kit	SCF	Proliferation, migration	[43]

Abbreviations: NSC, neural stem cell; VEGF; vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

of experimental glioma in rodent models. They isolated neural progenitor (npr) cells (NPCs) from the brains of newborn C57/BL6 mice, cultured them as neurospheres, and used retroviral-mediated gene transfer to induce expression of mouse interleukin (IL)-4 (C57.npr.IL-4) or the lysosomal enzyme, galactocerebrosidase (C57.npr.GALC). IL-4 is an immunomodulatory cytokine that stimulates a strong T helper cell type 2 response. These IL-4–producing NPCs were then injected into the striatum of adult mice concurrently with GL261 glioma cells for 5 days after tumor implantation. The animals receiving IL-4–producing NPCs survived longer than those receiving GALC-secreting control cells (Fig. 2A). Benedetti and colleagues [16] were able to recapitulate their findings in a rat model (Fig. 2B), confirming the ability of IL-4–producing NPCs to suppress glioma growth and prolong survival in vivo.

In a murine model of experimental glioblastoma, we have subsequently confirmed that syngeneic NSCs exhibit potent tropism for intracranial tumors, migrating specifically and extensively within the brain, and that they can be used as a tool for delivering tumor-toxic therapy to infiltrated tumor pockets within the brain [17]. In our study, NPCs were harvested from frontoparietal regions of embryonic mice, cultured as neurospheres (Fig. 3A–E), and infected with a replication-defective adenovirus encoding the β-galactosidase gene to facilitate in vivo tracking (Fig. 3F). These NSCs were then stereotactically injected into the brains of animals with established intracranial GL26 gliomas. We were able to detect several different patterns of tumor spread, including thin outgrowths of tumor deep into adjacent normal brain, direct extension of tumor mass into adjacent tissue, migration of glioma cells along established white matter tracts, and dissemination of solitary tumor pockets at a considerable distance from the primary tumor bed. In each case, we detected NSCs tracking these infiltrative glioma cells that had migrated away from the primary tumor mass. In addition, we were able to demonstrate that NSCs inoculated into non–tumor-bearing brains did not randomly dissipate into adjacent tissue. Finally, we confirmed that NSC tropism for glioma could be exploited for therapeutic benefit by engineering NSCs to express and deliver IL-12, a potent immune-enhancing cytokine with demonstrated efficacy against glioma, to established intracranial tumors. To confer IL-12 protein expression to NSCs, we used replication-defective adenovirus encoding the gene for IL-12 to infect NSCs in vitro. Inoculation of IL-12–secreting NSCs into rodent glioblastoma tumors induced robust intratumoral CD4+ and CD8+ T-cell infiltration into disseminated tumor foci and significantly prolonged survival as compared with nonmodified NSCs (Fig. 2C).

Prodrug activation enzymes

Prodrugs are compounds designed to be pharmacologically inactive over a wide range of doses. On chemical modification by specific enzymes, however, they are converted to physiologically active molecules [25]. Aboody and colleagues [15] demonstrated that C17.2 NSCs could be engineered to express the bioactive transgene for CD, an enzyme that converts the nontoxic prodrug 5-FC to 5-FU. 5-FU is a base analogue that blocks DNA replication and causes cell death in actively proliferating cells. Mammalian cells do not have endogenous CD activity, and 5-FC is thus fairly nontoxic. Conversely, when administered systemically, 5-FU causes significant gastrointestinal and hematologic toxicities. Aboody and colleagues [15] demonstrated that when injected intracerebrally or intravenously into glioma-bearing rodents, C17.2 CD cells colocalized with the main tumor mass as well as with infiltrating tumor

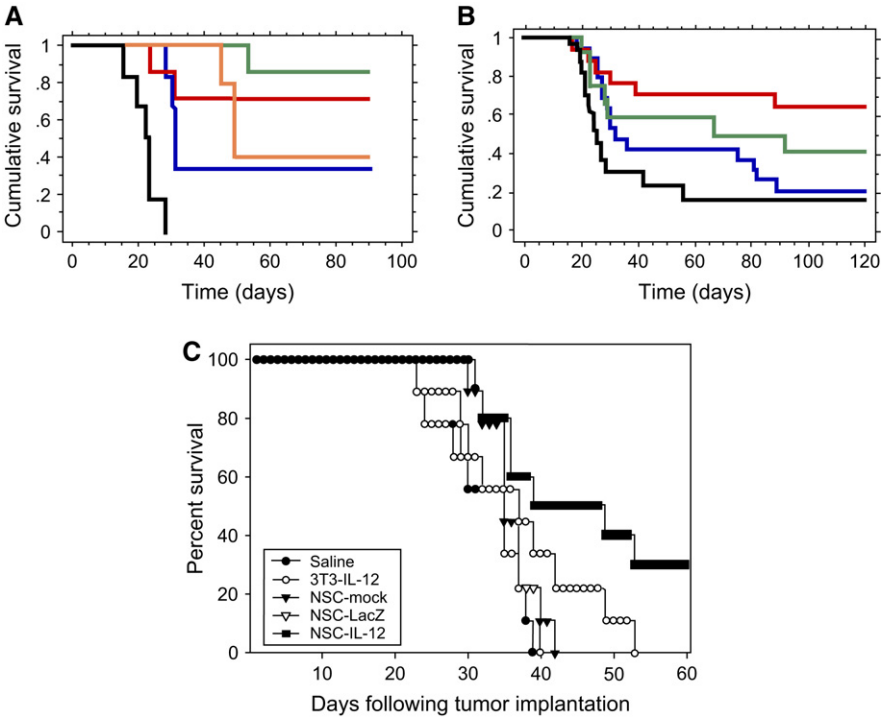

Fig. 2. Effects of IL-4–releasing NPCs on the growth of experimental malignant gliomas. (*A*) Kaplan-Meier survival plots of C57BL6 mice injected concurrently with C57.npr.IL-4 cells and GL261 glioma cells (*green*, n = 6), C57.npr.IL-4 cells injected into established GL261 tumors (*red*, n = 7), C57.npr.GALC control cells mixed with GL261 cells (*yellow*, n = 5), C57.npr.GALC cells injected into established GL261 tumors (*blue*, n = 6), and inoculation with GL261 tumor cells alone (*black*, n = 6). (*B*) Kaplan-Meier survival curves of adult Sprague-Dawley rats injected concurrently with ST14A.IL-4.3 cells and C6 glioma cells (*green*, n = 12), ST14A.IL-4.3 cells injected into animals harboring established C6 tumors (*red*, n = 17), ST14A control cells mixed with C6 cells, ST14A cells injected into established C6 tumors (*blue*, n = 19), and inoculation with C6 cells alone (*black*, n = 33). (*C*) Kaplan-Meier survival curve. Two days after intracranial implantation of 104GL26 tumor cells, mice were inoculated intratumorally with NSC-IL-12, 3T3-IL-12, NSC-LacZ, NSC-mock, or saline and were then followed for survival. (From Benediti S, Pirola B, Pollo B. Gene therapy of experimental brain tumors using neural progenitor cells. Nat Med 2000;6:447–50. Reprinted by permission from Macmillan Publishers Ltd.)

foci. In these animals, after systemic 5-FC therapy, tumor mass was found to be reduced by 80% compared with controls and no significant toxicities were noted. Thus, the highly specific tumor tropism of C17.2 NSCs permitted the delivery of a prodrug-activating enzyme directly to tumor cells, thereby enhancing the tumor-toxic effect of the chemotherapeutic agent while minimizing local toxicity to normal brain parenchyma.

Viral vectors

NSCs have been used as cellular vehicles for the delivery of replication-conditional oncolytic herpes simplex virus (HSV) [26]. In replication-conditional HSV vectors, genes regulating viral DNA replication are deleted or modified, impeding the ability of these vectors to multiply in nondividing cells. These viruses typically retain the ability to replicate in rapidly proliferating cell populations, such as tumor cells and neovascularizing endothelial cells, however. To test a strategy using NSCs to deliver oncolytic HSV to disseminated glioma foci, Herrlinger and colleagues [26] first treated C17.2 NSCs in culture with mimosine to arrest cell proliferation and prevent viral replication within the NSCs. This was followed by infection of these NSCs with replication-conditional HSV-1 (rRP 450). Infected NSCs were then inoculated into the brains of nude mice harboring intracranial gliomas. The NSCs migrated within the main tumor mass as well as to disseminated tumor

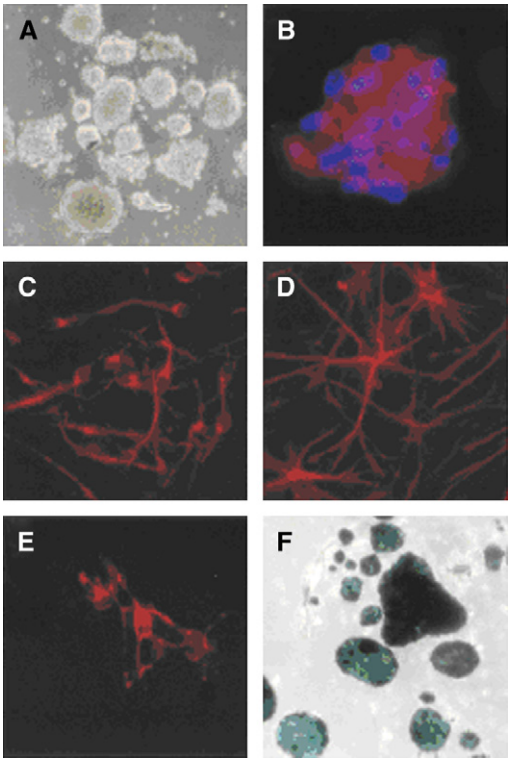

Fig. 3. Generation of NSCs from primary fetal brain culture. (*A*) After harvest and culture in growth factor–supplemented medium (2–3 days), NSCs grew in spherical aggregates. (*B*) Neurospheres are composed of NPCs expressing nestin (*red*). Nuclei are counterstained with 4,6-diamidino-2-phenylindole (DAPI, *blue*). NSCs replated in modified culture conditions were induced to differentiate into β-III tubulin–expressing neurons (*C*), glial fibrillary acidic protein (GFAP)–expressing astrocytes (*D*), and CNPase-expressing oligodendrocytes (*E*). (*F*) Expression of β-galactosidase by NSCs infected in vitro with replication-defective LacZ gene-bearing adenovirus. (Magnification ×400 for *B–E* and ×100 for *A* and *F*). (*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 2002;62:5658; with permission.)

satellites. Eventual loss of intracellular mimosine from the NSCs permitted the replication of HSV-1, resulting in NSC lysis and release of oncolytic virus in the vicinity of dispersed tumor cells. Free virus then infected and lysed the tumor cells. This combination of replication-conditional virus with stem cells capable of homing to intracranial tumor cells may thus provide an important tool for a targeted therapeutic attack against disseminated glioma.

Proapoptotic proteins

A wide range of protein ligands, particularly members of the tumor necrosis factor (TNF) family, have been used as apoptosis-inducing agents in experimental tumor therapies. One such molecule, TNF-related apoptosis-inducing ligand (TRAIL), has been shown to induce apoptosis selectively in malignant glial cells while sparing normal tissue [27]. We have previously shown that NSCs can be engineered to secrete TRAIL [20] and that inoculation of TRAIL-secreting NSCs into athymic mice bearing intracranial human U343 glioblastoma xenografts significantly inhibits glioma growth and can induce marked apoptotic activity not only in the main tumor mass but in tumor microsatellites at a significant distance from the main tumor mass.

Inherent antitumor activity of neural stem cells

Although most recent studies with stem cells have focused on their ability to deliver proteins directly to areas of pathologic change within the brain, some reports have detailed inherent antitumor properties within NSCs themselves. Benedetti and colleagues [16] first presented evidence suggesting that exogenously administered unmodified NSCs may be independently capable of inhibiting glioma proliferation in vivo. In addition, they described that conditioned medium from NSCs suppressed the proliferation of GL261 cells in vitro. More recently, Staflin and coworkers [28] have also showed that select NPC lines have the ability to inhibit glioma growth in vivo. Glass and colleagues [29] recently tested the hypothesis that nontransplanted endogenous neural precursor cells may possess inherent antitumor activity. In their studies, they showed that after the establishment of intracranial gliomas in mice, endogenous neural precursor cells migrated out of the subventricular zone toward growing tumor and that the abundance of neural precursor cells in the vicinity of the tumor was associated with significantly reduced tumor size and increased length of survival. Their report indicated that the pathotropic trafficking of endogenous precursor cells was a specific response to the presence of intracranial tumor, because such migratory activity was not observed in the setting of other nonneoplastic lesions. In addition, they demonstrated that the attraction of precursor cells to glioblastoma declined with increasing age of animals and that glioblastomas implanted into older animals grew more

vigorously, implying a link between levels of neural precursor activity and rate of tumor growth. Despite these important reports, we have not found similar evidence supporting the inherent antitumor capability of unmodified NSCs. The exact mechanisms underlying these observations remain unclear, but it is possible that NSCs may elaborate certain factors, such as transforming growth factor (TGF)-β, capable of inhibiting tumor cell growth. Moreover, it is also possible that this property may vary with the location, differentiation status, and age of NSCs.

Problems with use of neural stem cells and alternate sources of stem cells

The clinical application of NSCs in the treatment of gliomas, although attractive, is limited by fundamental logistic and ethical problems. The use of fetally derived NSCs remains highly controversial. Moreover, the use of allogeneic cells would necessitate immunosuppressive therapy, which would be undesirable in the context of malignant gliomas, because these tumors are already known to exert potent systemic immunosuppressive effects. The potential tumorigenicity of transplanted NSCs also remains a concern. An ideal cellular therapy for use in clinical practice should thus ideally comprise autologous cells that can be harvested without difficulty, processed efficiently in vitro, and reinoculated into the same patient.

In this context, BM represents an alternative and much more clinically accessible pool of stem cells [30]. BM is a major source of adult hematopoietic stem cells that renew circulating blood elements. BM also contains MSCs, which are identified as a subpopulation of BM stromal cells that forms an essential structural and functional component of the BM microenvironment [31]. BM-derived MSCs (BM-MSCs) help to form stroma or connective tissue (bone, fat, muscle, cartilage, and tendons) during development and normally migrate to sites of injury in the body to help facilitate healing. Recent advances in the field of MSC biology have suggested these cells possess several unique properties that make them ideally suited for clinical use, including accessibility, plasticity (ie, the ability to differentiate into different cell types), and robust synthetic activity enabling them to express therapeutic proteins efficiently. Studies have confirmed the ability of BM-MSCs to differentiate into astrocytes and neurons in vitro and in vivo [32,33]. This suggests that BM may contain a master pool of stem cells and that these BM-derived stem cells possess all the properties of organ-specific stem cells (eg, NSCs), such as extensive migratory capacity and tropism for gliomas.

Studeny and colleagues [34] were the first to show that BM-MSCs can be used to deliver toxic proteins directly to tumors. Using a melanoma model, they showed that BM-MSCs (isolated from the BM of normal individuals undergoing BM harvest for allogeneic BM transplantation) become incorporated into the tumor architecture as stromal fibroblasts. They suggested that the microenvironment of solid tumors mimics that of injured organs; therefore, solid tumors secrete factors that mediate this process of recruitment. Furthermore, they demonstrated that MSCs can be transduced to produce interferon (IFN)-β, an antitumor molecule, and that these modified MSCs could inhibit tumor growth in vivo.

Nakamizo and coworkers [35] have recently shown that human BM-MSCs are capable of exhibiting glioma tropic behavior comparable to that demonstrated by fetal-derived NSCs. They isolated MSCs from BM harvested in healthy volunteers and demonstrated selective localization of these cells to human glioma intracranial xenografts in nude mice after intravascular and intracranial injections (Fig. 4). In addition, they showed that conditioned medium from human glioma cell lines (but not from C29 fibroblasts) could stimulate significant MSC migration in vitro. Furthermore, this effect was neutralized by treating the conditioned medium from the glioma cell line with a mixture of antibodies against PDGF, EGF, and CXCL12, suggesting that these factors may be involved in mediating the tropism of MSCs toward glioma. To test the potential of BM-MSCs as therapeutic vehicles for the delivery of antitumor molecules, Nakamizo and colleagues [35] transfected the MSCs with the IFNβ gene using an adenoviral vector and found that the engineered cells were capable of killing human glioma cell lines in vitro as well as significantly extending the length of survival of mice harboring human intracranial gliomas when administered intra-arterially. This effect was not duplicated by the intravenous injection of IFNβ, suggesting that increased survival resulted from local delivery of IFNβ by transfected MSCs to intracranial tumor cells.

Yuan and coworkers [36] have reinforced the concept that BM-derived stem cells can be used in place of fetal-derived NSCs as delivery vehicles

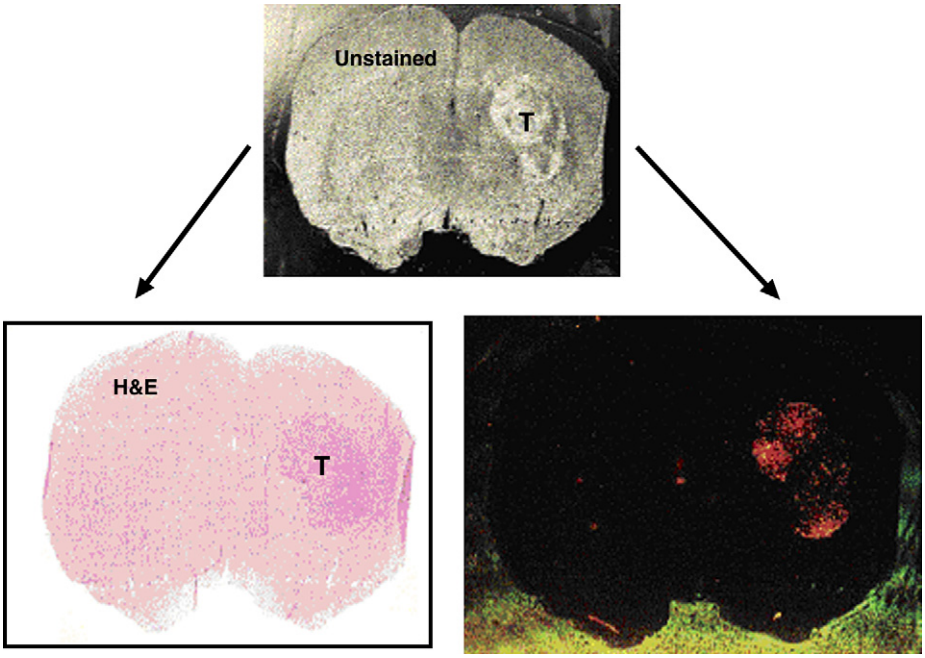

Fig. 4. Adult BM-MSCs demonstrate specific localization to human glioma xenografts after regional intravascular administration. Photomicrograph of a section of the entire brain shows selectivity of human mesenchymal stem cell (hMSC) engraftment for right-hemisphere U87 glioma after left intracarotid injection of SP-DiI–labeled hMSCs (10^6 cells). Light microscopy (*center*) and hematoxylin-eosin (H&E) staining (*bottom left*) reveal tumor (T) in the right hemisphere. Fluorescence microscopy (*bottom right*) shows fluorescently labeled hMSCs (*red*) located within the tumor but not in normal brain. (*From* Nakamizo A, Marini F, Amano T, et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res 2005;65(8):3310; with permission.)

to target brain tumors. Using a mouse model, these investigators showed that neural stem-like cells can be generated from the BM of adult mice (BM-NSCs). These BM-NSCs, when implanted into the brains of mice with intracranial glioma, were able to track not only the main tumor mass but tumor islands that had infiltrated deep into normal neural tissue. Furthermore, these investigators transduced BM-NSCs with IL-23, an antitumor molecule that acts directly on memory T cells and dendritic cells to promote IL-12 and IFNγ production. When implanted into glioma-bearing mice, the engineered BM-NSCs demonstrated antiglioma activity and significantly prolonged the length of survival of mice as compared with controls.

These exciting findings point toward a promising new source of autologous glioma-tropic stem cells for delivery of therapeutic molecules to intracranial tumor. Such a source would eliminate the ethical concerns and logistic issues related to the use of allogeneic fetal-derived NSCs. With their relative ease of accessibility, BM-derived stem cells represent a potentially important step in the clinical implementation of stem cell therapy for brain tumors.

Future perspectives and challenges

Preliminary evidence has established the promise of stem cell therapy for malignant glioma. Significant concerns must be addressed before the true potential of this novel therapy can be fully realized in a clinical setting, however.

Because of the significant ethical and immunologic concerns associated with the use of fetal-derived tissue as a cell source, it is imperative to characterize an easily accessible pool of stem cells before this technology can be used as a clinically viable therapeutic modality. An optimal stem cell candidate must not only be readily accessible but should possess significant migratory capabilities and display robust tumor tropism. Ideally, the chemotropic mechanisms guiding migration of these cells toward intracranial tumor should be well defined, allowing for the selective use of

optimally responsive stem cell subpopulations. Although, to date, most experimental studies have used NSCs, it is critical to determine whether stem cells derived from alternate tissue sources may have additional therapeutic advantages. In this context, BM-derived stem cells represent an attractive option for clinical use, but additional sources must be explored. Furthermore, determining which therapeutic molecules to deliver to foci of intracranial glioma represents another challenge. Although immunomodulatory cytokines, proapoptotic proteins, and prodrug activation enzymes have all been used successfully in animal models, there is no evidence to support the use of one modality over another. Continued research over the next several years should address many of these concepts and should clarify the ultimate clinical viability of stem cell therapy for glioma.

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