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Applications of neural and mesenchymal stem cells in the treatment of gliomas

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Abstract

In addition to stem cells providing a better understanding about the biology and origins of gliomas, new therapeutic approaches have been developed based on the use of stem cells as delivery vehicles. The unique ability of stem cells to track down tumor cells makes them a very appealing therapeutic modality. This review introduces neural and mesenchymal stem cells, discusses the advances that have been made in the utilization of these stem cells as therapies and in diagnostic imaging (to track the advancement of the stem cells towards the tumor cells), and concludes by addressing various challenges and concerns regarding these therapies.

Keywords

brain tumor; glioma; mesenchymal stem cell; neural stem cell; stem cell

Despite the large amount of research that has been carried out investigating the biology and treatment of gliomas, the median survival is still 1 year or less for glioblastoma multiforme (GBM) and approximately 3 years for anaplastic astrocytoma [1-5]. High-grade gliomas have the ability to infiltrate local structures and migrate long distances, even to the contralateral hemisphere, leading to disease recurrence despite aggressive resection [6-10]. Even after seemingly curative resection of the tumor, there are often microsatellites of tumor cells scattered throughout normal brain tissue that have the potential to continue proliferating and cause tumor recurrence in other areas of the brain [8,10,11]. Moreover, tumor infiltration of eloquent areas of the brain often limits the extent of tumor resection [5,9,10,12-14]. In the 1930s, Walter Dandy reported recurrence of contralateral gliomas even after hemispherectomy [15]. Radiotherapy [16,17] and chemotherapy [3,18,19] have had limited success in treating gliomas [2,3]. Both are limited by their toxicity to normal brain tissue that could lead to further brain damage and decreased quality of life for patients [20-22]. Moreover, the effect of chemotherapy is often suboptimal because there is limited drug penetration across the blood—brain

barrier [23-25]. Lastly, gliomas evade the immune system by local suppression of immune cells within the tumor microenvironment, thus limiting the ability of the immune system to attack the tumor cells [26,27].

Treatment of gliomas has also been difficult because their origin is still not fully understood (Figure 1) [28]. A significant number of similarities have been found between normal neural stem cells (NSCs) and neoplastic cells of neuroectodermal origin in terms of migratory capacity, self-renewal potential, molecular signature and their ability to integrate themselves into normal tissue [29,30]. Many now believe that the virulence of brain tumors is maintained by a subpopulation of 'stem-like' cells, which are referred to as brain tumor stem cells (BTSCs) [31-34]. There is mounting evidence suggesting that BTSCs are responsible for the highly invasive [35] and resistant [36] potential of many human brain tumors. This unique group of cells has the ability to migrate long distances in the brain parenchyma [37]. Moreover, they exhibit profound resistance to both chemotherapy and radiotherapy [38]. Some studies have even shown that GBMs bordering the lateral ventricle, an area that contains the subventricular zone NSC niche, may carry a worse prognosis for patients [39]. It is still unclear whether the origin of these BTSCs emanates from normal progenitor/stem cells that have become neoplastic or from the dedifferentiation of mature neural cells that have undergone mutations [28]. There is evidence that both etiologies may be possible [40].

The resemblance of the properties and attributes of stem cells to those of BTSCs has initiated interest in how stem cells can be armed to track and eradicate tumors [29,41,42]. In this review, we will discuss the tropism of stem cells for tumors, review the advances in neural and mesenchymal stem cell (MSC) therapies for gliomas, and finally outline the concerns and challenges in making stem cells into a treatment modality in humans.

Stem cell tropism for tumors

One of the remarkable properties of both NSCs and MSCs is their tropism for tumor cells [29,43]. These stem cells have the ability to migrate across the blood—brain barrier into the tumor when administered intra-arterially [29,44]. Stem cells have also been shown to migrate from the contralateral hemisphere into the tumor [29,43]. The tumor tropism of progenitor/stem cells is mediated by several receptor—ligand combinations [45]. The molecular basis of tumor tropism of stem cells *in vivo* is still poorly understood, but multiple *in vitro* studies have provided insight into the cytokines, growth factors and receptors involved in tumor tropism of stem cells [46]. These receptor—ligand interactions include SCF/c-Kit (in NSCs) [47-49], HGF/c-Met (in NSCs) [50], MCP-1/CCR2 (in NSCs) [51] and HMGB1/RAGE (in mesangioblasts) [52]. Kendall *et al.* observed that human HGF elicited the strongest chemotactic response from NSCs and that gliomatropism was critically dependent on c-Met and Ras—PI3K signaling [53]. EGF and its receptor have also been demonstrated to be involved in NSC migration [54], as well as the increased migration and invasion of glioma cells [55].

Malignant gliomas actively recruit mesenchymal progenitor cells by secreting angiogenic cytokines, such as VEGF [56], IL-8, TGF- α 1 and neurotrophin-3 [57]. In addition to its angiogenic properties, VEGF has also been shown to play a role in the tropism of NSCs for glioma cells [58].

Glioma extracellular matrix proteins have also been associated with the tropism of NSCs to gliomas [59] and MSCs to injured tissues [60]. Adhesion molecules, such as b1- and b2-integrins and L-selectin, may play a significant role in the mobilization and homing of MSCs to gliomas [61-63]. Matrix metalloproteases (MMPs) such as MMP2 and membrane type-1 matrix metalloprotease (MT1-MMP), as well as tissue inhibitor of metalloproteases-2 (TIMP-2) have been associated with MSC migration to injured tissues [59,60].

It is noteworthy to discuss the CXCR4—SDF-1 α interaction, which plays an important role in inflammation, tumor tropism of stem cells and the pathology of gliomas [64-66]. The chemokine receptor CXCR4 has been found to be expressed in human and mouse NSCs, while its cognate ligand SDF-1 α is expressed by reactive astrocytes and endothelium within regions of CNS injury and degeneration [64]. SDF-1 α expressed by tumor-derived endothelium attracts NSCs to migrate to the tumor [65,67]. Inhibiting CXCR4—SDF-1 α interactions prevented NSC migration towards gliomas [68]. With regard to its role in glioma pathology, the CXCR4—SDF-1 α interaction increases glioma growth and cell migration as well as tumor angiogenesis [66,69]. Antagonists of CXCR4 have been shown to inhibit the growth of GBMs and medulloblastomas in experimental models [70]. The CXCR4—SDF-1 α interaction may also play a role in metastatic malignancies to the brain [71]. It has been found that the CXCR4 receptor is expressed in breast cancer cells while SDF-1 α is expressed on brain endothelium, thus providing a basis for the migration of circulating metastatic cells through the blood—brain barrier [71]. Inhibiting CXCR4 has been shown to prevent breast cancer metastasis to the brain in animal models [72].

Neural stem cells

The working definition of a stem cell includes the ability to self-renew and differentiate into several cell types [73,74]. Several immunocytochemically detectable markers have been proposed for NSCs, the most important being CD133 [75] and nestin [76,77]; but other less-used markers include mushashi1 [78-80] and Sox1/2 [81-83]. However, there are no markers that are sufficiently specific and sensitive to define a NSC and, thus, many rely on the operational definition of NSCs [84]:

- Multipotency in which the neurospheres have the ability to produce mature cells in all three of the fundamental neural lineages (neurons, astrocytes and oligodendrocytes)
- The ability to populate a developing region of the nervous system and/or an ablated/degenerated area of the nervous system with appropriate cell types
- The ability to be serially transplanted
- Self-renewal properties in which they are able to produce new NSCs

There are several potential sources of human NSCs [85], including the adult brain [86-90], fetal brains [91,92] and embryonic stem cells [93,94]. Within the adult brain, NSCs have been found in the sub ventricular zone (SVZ) [95-97], hippocampus [87-89], subcortical white matter [90], cerebellum [98] and olfactory bulbs [99]. Although adult NSCs and embryonic stem cell-derived neural progenitors exhibit many of the same properties, a few subtle differences in behavior and signaling pathways have been described [100]. For instance, embryonic stem cell-derived neural progenitors have a greater tendency to form neurons and have enhanced MAPK signaling, thus contributing to the high proliferation rate of these cells. Other sources of NSCs that have been explored include MSCs [101], adipose tissue stem cells [102] and skin stem cells [103], all of which have been demonstrated to have the potential to be reprogrammed into NSCs.

The progeny of NSCs are capable of integrating structurally and functionally into the surrounding host nervous system, thus having the potential to serve as treatments for nervous system repair [104-109]. The migratory capability of NSCs, along with their tropism for intracranial pathologies, make them attractive therapeutic agents for several neurological diseases, including stroke, Parkinson's and Alzheimer's disease, as well as brain tumors [79, 92,110-113].

Mesenchymal stem cells

Mesenchymal stem cells are another stem cell population with the ability to self-renew as well as give rise to multiple tissue types [114]. These primitive progenitors exist postnatally and are multipotent, with the ability to generate cartilage, bone, muscle, tendon, ligament and fat [114]. There is a paucity of developmental stage-specific markers for MSCs [115]. However, markers such as STRO-1 have been used in the isolation of MSCs. For isolation of MSCs, our laboratory uses flow cytometry to isolate for a cell population with the following cell marker characteristics: CD31⁻ CD45⁻CD90⁺CD105⁺CD106⁺ [116-118].

Mesenchymal stem cells can be acquired from a variety of sources, including embryonic stem cells [119], bone marrow [120,121], peripheral blood [122], placenta [123], adipose tissue [124], skin [125 and umbilical cord blood [126-128]. Studies are being performed

Box 1 Summary of neural stem cell therapies for gliomas.

Chemotherapeutic vehicles

- Cytosine deaminase [23,110]
- HSV-thymidine kinase [109,112]
- Deoxycytosine kinase (not yet used in NSCs) [113]
- Carboxylesterase (not yet used in NSCs) [114-116]

Viral delivery vehicles

- Replication-conditional HSV-1 vectors [111]
- Conditionally replicating adenovirus [118,119]

Immunotherapeutic vehicles

- IL-4 [120,121]
- IL-12 [122]
- IL-23 [123]
- TRAIL [127-132]
- IFN- β [137]

Inhibition of glioma migration

- PEX [140]

HSV: Herpes simplex virus; NSC: Neural stem cell; PEX: Hemopexin-like domain of MMP-2; TRAIL: TNF-related apoptosis-inducing ligand

to determine the best sources of MSCs. Kern *et al.* observed that bone marrow-derived MSCs had a lower proliferation capacity *in vitro* than MSCs from adipose tissue or umbilical cord blood, making the latter two sources very appealing as sources of MSCs [129]. Moreover, a recent study by Kim *et al.* demonstrated that the IL-8-mediated glioma-tracking behavior of umbilical cord MSCs is much stronger than that of bone marrow-derived MSCs and that this phenomenon is due to overexpression of the IL-8 receptor and CXC chemokine receptors 1 and 2 on umbilical cord MSCs [130]. Due to this difference in receptor expression, umbilical cord-derived MSCs have a much greater capacity to migrate towards glioma cells than bone marrow-derived MSCs [130].

NSCs & MSCs as a therapy for brain tumors

Both NSCs and MSCs have potential use in therapy for brain tumors (see BOXES 1 & 2 for a summary). Studies have suggested that unmodified endogenous NSCs may have a natural ability to suppress tumor growth [131]. It has been demonstrated that endogenous NSCs exhibit significant tropism for gliomas and migrate from the SVZ to surround the tumor graft [131]. The same study found that the endogenous precursor cell accumulation around gliomas decreased with the age of the recipient; this correlated with increased tumor size and decreased survival times in aged mice [131]. Furthermore, when GBM cells were cocultured with unmodified NSCs, the rapid increase in tumor cell number was suppressed and there was increased GBM cell apoptosis [131]. This observation had also been documented in earlier studies by Weinstein *et al.* [132] and Staflin *et al.* [133].

In 2000, Aboody *et al.* demonstrated that modified NSCs migrated great distances to tumor masses when introduced in various locations of the brain, including the ipsilateral and contralateral hemispheres, relative to the glioma [29]. NSCs were

Box 2	
Summary of mesenchymal stem cell therapies for gliomas.	
Chemotherapeutic vehicles	
• HSV-thymidine kinase	[172]
Viral delivery vehicles	
• Conditionally replicating adenovirus	[173]
Immunotherapeutic vehicles	
• IL-2	[34,174,175]
• IL-18	[176]
• IL-23	[124]
• TRAIL	[177]
• IFN- α	[178]
• IFN- β	[36]
HSV: Herpes simplex virus; TRAIL: TNF-related apoptosis-inducing ligand.	

also able to migrate to the intracranial glioma when introduced via the systemic circulation [29]. On histological studies, it was found that NSCs traveled toward and positioned themselves in direct juxtaposition to microsatellites of glioma cells that had migrated away from the tumor bulk and invaded normal brain tissue [29]. Finally, Aboody *et al.* demonstrated that treatment with NSCs genetically modified to deliver a drug-activating enzyme resulted in a reduction in the size of the tumor and increased host survival [29].

The ability of stem cells to efficiently cross the blood—brain barrier, home in on tumor cells and then secrete a therapeutic molecule provides a very enticing approach to treat tumors of the CNS. Compared with some of the other experimental therapies being studied, such as viral therapy, stem cells are showing potential to be much more powerful treatments because, in addition to being able to home in on tumor cells, they are fully capable of transcriptional, translational and post-translational expression of large amounts of genetic information, allowing them to secrete therapeutic substances into the tumor microenvironment [46].

NSCs & MSCs as chemotherapeutic vehicles

There are several ways that stem cells can be used to treat gliomas. One such way is for the stem cells to locally convert a nontoxic prodrug into a toxic drug that will kill tumor cells in close vicinity [29]. This strategy takes advantage of the ability of stem cells to migrate towards areas of tumor mass and distribute themselves evenly throughout the bulk of the tumor to produce high concentrations of prodrug-converting enzyme. Systemically administered prodrug penetrates through the blood—brain barrier into the CNS and is converted into a toxic form only in the close vicinity of tumor cells, thus killing the cancer cells through a ‘bystander effect’ and sparing normal tissue from the toxic effects of the drug.

Aboudy *et al.* demonstrated *in vivo* efficacy of murine NSCs transduced to express cytosine deaminase (CDA), which converts the nontoxic prodrug 5-fluorocytosine (5-FC) into 5-fluoro uracil (5-FU), which is incorporated into the newly created DNA of proliferating tumor cells, causing chain termination and subsequent cell death [29]. There was a significant reduction in tumor burden in mice with intracranial gliomas when the mice were injected intracranially with CDA-expressing NSCs and given systemic 5-FC [29]. In 2003, Baressi *et al.* also demonstrated regression of intracranial gliomas when they used neural progenitors genetically modified to locally convert 5-FC to 5-FU in the tumor microenvironment [134]. If the CDA-expressing NSCs were to be recruited by the tumors to contribute to the tumor bulk, CDA would function as a suicide gene for these NSCs [135]. However, tumor recruitment of these stem cells is not a concern because evidence exists that exogenous NSCs do not contribute to the tumor [136]. Another drug-activating enzyme that has been studied is thymidine kinase [135]. In 2005, Li *et al.* demonstrated increased survival in rats that were treated with NSCs transduced with herpes simplex virus thymidine kinase (HSV-tk) and then given systemic gancyclovir [135]. Uhl *et al.* demonstrated that migratory HSV-tk-transduced NSCs had the ability to eliminate glioma cells purely by means of a gap junction-mediated bystander effect and that the efficacy of the treatment correlated with connexin-43 expression in glioma cells [137]. Deoxycytosine kinase, which activates cytosine arabinoside into a toxic form, is another enzyme that has been used in viral therapies for intracerebral gliomas and has potential for stem cell therapies [138]. Furthermore, carboxylesterase, which converts CPT-11 (irinotecan) to SN-38, may be yet another genetic modification to consider in future NSC therapies [139-141]. Another idea that may be interesting to look into is genetically modifying stem cells to activate radiotherapy-sensitizing agents in the tumor microenvironment.

As with NSCs, MSCs can be engineered to locally convert nontoxic compounds into a toxic drug that will kill glioma cells in close vicinity [142]. In 2007, Miletic *et al.* demonstrated that bone marrow-derived MSCs genetically engineered to express HSV-tk were highly effective in the treatment of gliomas in rodents [142]. Tumor cells were not detected on serial histological sections in the brains of surviving mice who received the tk-MSCs with adjuvant gancyclovir. Only a cavity and scar tissue from the initial tumor mass, which had been successfully treated, was observed in long-term survivors; this tissue was found to be infiltrated by CD45⁺ inflammatory cells [142]. There are several more drug-activating enzymes that can be explored in MSC-based therapies for gliomas.

NSCs & MSCs as viral-delivery vehicles

The delivery of viruses via NSCs that have the ability to home in on areas of pathology and locally produce virus is a significant improvement to the older methods of viral therapies using injection of viral supernatants or immobile virus-producing fibroblasts [143]. NSCs have also been used to deliver oncolytic viruses to kill glioma cells [136]. The modified NSCs home in on tumor cells and release oncolytic viruses, thereby transfecting the tumor cells to express oncolytic genes. An advantage of this form of viral delivery to tumors is that the dispersion of NSCs throughout the tumor bulk allows more extensive delivery of the oncolytic viruses not

only to the tumor bulk but also to glioma cells that have invaded into normal brain parenchyma [136]. The *HSV-tk* system mentioned earlier has also been used in the form of viral therapy in which replication-conditional HSV-tk viruses are delivered by murine NSCs to transfect tumor cells [136]. The systemically administered prodrug, ganciclovir, is then converted into its toxic form in tumor cells that have been transduced to express thymidine kinase [135]. Lastly, NSCs have also been used to deliver conditionally replicating adenovirus to gliomas, and studies have shown that NSC-mediated delivery of the adenovirus enhanced the intratumoral distribution of the oncolytic vector compared with injection of the virus alone [144,145].

Mesenchymal stem cells can also be engineered to deliver oncolytic viruses to glioma cells [146]. Sonabend *et al.* engineered human MSCs to deliver replication-competent oncolytic adenovirus (CRAd) in a model of intracranial malignant glioma in rodents and showed that, when injected away from the tumor site *in vivo*, MSCs migrated to the tumor and delivered 46-fold more viral copies than injection of the virus alone [146]. More studies utilizing MSCs to deliver oncolytic viruses are currently underway.

NSCs & MSCs as immunotherapy

Neural stem cells can also be used as a form of immunotherapy to elicit an immune response against tumor cells by locally secreting cytokines in proximity to the tumor cells [147]. Depending on the cytokine used, a variety of effects can be achieved, including direct cytotoxicity to the tumor cells, arrest of growth and differentiation and host immune system modulation to elicit a stronger anti-tumor response [46]. One such cytokine that has been used is IL-4, which increases T-cell-mediated response to tumor cells [147]. Benedetti *et al.* found that treatment of mice bearing intracranial gliomas with NSCs transduced to express and secrete the IL-4 cytokine increased survival [148]. The group of mice with C6 rat gliomas treated with IL-4-secreting NSCs had a 90-day survival of over 80%, whereas 0% of the untreated mice survived for even 30 days [148]. Likewise, similar treatment with GL261 tumors resulted in 71% of the treatment group surviving compared with 33% of tumor-bearing mice that received control NSCs with no genetic modifications [148]. IL-12 is another cytokine that has therapeutic potential [149]. It is a T-cell-stimulating factor that can stimulate the growth and function of T cells, stimulate the production of IFN- γ and TNF from T cells and natural killer (NK) cells, enhance the cytotoxic effects of NK and CD8⁺ cytotoxic T lymphocytes, and has anti-angiogenic activity. In 2002, Ehtesham *et al.* used IL-12-secreting murine NSCs to treat GL261 tumor-bearing rats [149]. The local expression and secretion of IL-12 in the tumor microenvironment elicited a significant amount of tumor infiltration by T lymphocytes [149]. Another cytokine that has been studied for stem cell therapy for tumors is IL-23, which promotes an inflammatory response by promoting angiogenesis, increasing MMP9 and reducing CD8⁺ T-cell infiltration [150]. There has been some concern that IL-23 may actually promote tumor incidence and growth [150]. However, Yuan *et al.* demonstrated that bone marrow-derived neural stem-like cells engineered to secrete IL-23-inhibited tumor growth in C57BL/6 glioma-bearing mice, and the surviving mice treated with these engineered stem cells, were resistant to tumor rechallenge [151].

TNF-related apoptosis-inducing ligand (TRAIL) is a cell membrane protein that has sparked interest in tumor therapies because it has been shown to specifically induce apoptosis in cancer cells and not in normal tissue [152,153]. Injection of TRAIL-expressing NSCs into nude mice with intracranial GBM xenografts resulted in tumor apoptosis and reduction of the tumor bulk [154]. Similar results have been achieved with human NSCs isolated from the telencephalon of human fetal cadavers (13 weeks' gestation) [155]. Shah *et al.* improved on this treatment by designing a secreted version of TRAIL (S-TRAIL), which increased the delivery of TRAIL to its molecular target [156]. Intracranial injection of NSCs expressing S-TRAIL resulted in a greater than 80% decrease in tumor growth [156]. Furthermore, NSCs have been engineered

to secrete S-TRAIL in combination with small hairpin RNA (shRNA) targeting BCL-2 [157] or with micro-RNA-21 inhibitors [158]. Hingtgen *et al.*, in 2008, demonstrated that combined treatment with NSCs secreting S-TRAIL and temozolomide induced cell killing and markedly upregulated proapoptotic proteins in glioma cells that were least sensitive to TRAIL [159].

Although originally described as antiviral proteins, type I interferons (α/β) also exhibit pleiotropic anti-tumor effects, including direct tumor cytotoxicity [160], immunomodulation [161] and inhibition of angiogenesis [160,162,163]. In a study by Dickson *et al.*, HB1.F3 NSCs engineered to deliver IFN- β were injected into tumor foci with the intent to normalize tumor vasculature before administering cytotoxic agents so that these agents would more extensively penetrate the tumor mass and thus increase the anti-tumor efficacy of the drug [164]. The delivered IFN- β resulted in the maturation of the vasculature inside the tumor, which consequently made it unable to expand to support the tumor growth [164]. The maturation of the tumor vasculature also significantly increased the delivery and anti-tumor effect of adjuvant therapy [164].

Similar to NSCs, MSCs have also been genetically engineered to deliver various immunological cytokines (FIGURE 3) [43]. One of the first studies to explore the effect of genetically altered MSCs for the treatment of gliomas was by Nakamura *et al.* in 2004, where they demonstrated that the MSCs were able to migrate across the corpus callosum to the contralateral hemisphere towards the glioma cells [43]. When MSCs were implanted directly into the tumor, the MSCs localized mainly at the border between the tumor cells and normal brain parenchyma, but they also infiltrated into the tumor bed [43]. Finally, the study demonstrated that genetically modifying the MSCs to secrete IL-2 limited tumor growth and prolonged the survival of tumor-bearing rats [43]. Implantation of IL-2 MSCs induced lymphocyte infiltration into the gliomas, whereas there was minimal inflammatory cell infiltration in the untreated controls [43]. Other studies have also demonstrated that genetically modified MSCs expressing IL-2 slowed tumor development when injected into intracranial gliomas [165,166]. Another cytokine that has been studied in MSC-based therapy is IL-18, which is a proinflammatory cytokine that induces the production of IFN- γ in T cells and NK cells. Xu *et al.* demonstrated that MSCs genetically modified to produce IL-18 inhibited glioma growth and prolonged the survival of glioma-bearing rats [167]. Transplantation of these IL-18-secreting MSCs was associated with increased T-cell infiltration of the gliomas and long-term anti-tumor immunity [167]. Yuan *et al.* showed that IL-23-expressing neural stem-like cells can be generated from the bone marrow of adult mice and, when implanted into the brains of mice with intracranial gliomas, these cells were able to track into the tumor mass as well as to tumor islands that had infiltrated deep into brain tissue [151]. Intratumoral implantation of bone marrow-derived NSCs transduced with IL-23 led to decreased glioma growth and significantly prolonged the survival of treated mice [151].

In 2008, Kim *et al.* demonstrated that human umbilical cord blood-derived MSCs that were genetically modified to secrete TRAIL significantly inhibited tumor growth and prolonged the survival of mice bearing gliomas compared with controls [168]. In 2005, Nakamizo *et al.* demonstrated that human MSCs transduced to express IFN- β significantly increased animal survival compared with controls in an intracranial glioma model [44]. Another study demonstrated that MSCs engineered to secrete IFN- α significantly prolonged the survival of brain tumor-bearing rats [169]. The same study found that transfection of the MSCs with EGF receptor (EGFR) enhanced the migratory responses of the MSCs in glioma-conditioned media and that EGFR MSCs were able to migrate toward GL261 gliomas or B16 melanoma *in vivo*, even after injection into the contralateral hemisphere of mice [169].

It is noteworthy to also mention that activating MSCs with certain cytokines, such as IL-2, IL-15 and GM-CSF, increases the cytotoxicity of genetically modified MSCs [170,171]. In

Kang *et al.*, activation of umbilical cord blood-derived MSCs with cytokines did not increase the number of differentiated immune effector cells in the tumor; however, activated umbilical cord blood-derived MSCs did secrete more immune response-related proteins such as IL-4 and IFN- γ than the MSCs that were not activated [171].

NSCs to inhibit glioma cell migration & invasion

The human metalloprotease-2 (MMP-2) fragment PEX inhibits the proliferation and migration of endothelial and glioma cells, in addition to decreasing angiogenesis [172,173]. In 2005, Kim *et al.* demonstrated that intratumoral injection of the hemopexin-like domain of MMP-2 (PEX)-secreting HB1.F3 NSCs reduced tumor volume by 90% and decreased angiogenesis by 45% and cell proliferation by 24% [174]. Histological analysis showed that these stem cells migrated to the tumor boundary [174]. This is a desirable location for the NSCs to situate themselves considering that the main goal is to inhibit tumor cell migration away from the tumor bulk. Since migration and invasion are such lethal characteristics of glioma cells, additional approaches to inhibit metalloproteases and other proteins conducive to glioma cell invasion should be explored in future therapies. This therapeutic approach should also be explored in MSCs.

Diagnostic imaging using NSCs & MSCs

Since stem cells have a tendency to track down cancer cells in the tumor mass as well as those that have migrated away from the bulk of the tumor into the brain parenchyma, studies have explored the use of stem cells as an imaging modality to determine the extent of tumor infiltration and to determine where the therapeutic stem cells are positioned (FIGURE 2) [175]. Utilizing routine bioluminescence imaging, Tang *et al.* were able to explore the macroscopic migratory abilities of luciferase-expressing murine neural progenitor cells toward brain tumors and demonstrated their persistence in brain tumor tissue 4 weeks after implantation in living mice [176]. Another interesting finding from the study was that intraventricular injection of the NSCs resulted in a greater number of NSCs migrating to the tumor than the intravenous, intracranial or intraperitoneal routes [176]. In a later study, the same group demonstrated that NSCs could be transduced with both S-TRAIL and luciferase to provide for the tracking of *in vivo* migration of the NSCs as they delivered tumoricidal S-TRAIL to glioma cells [177]. Another imaging modality relies on the internalization of tat peptide-derivatized magnetic nanoparticles in hematopoietic and neural progenitor cells and then using MRI for *in vivo* tracking and recovery of the cells [178]. Zhang *et al.* used MRI to demonstrate that neural progenitor cells and MSCs labeled with lipophilic dye-coated superparamagnetic particles migrated toward intra cranial gliomas after injection into the cisterna magna or the rat tail vein [179]. Using MRI, they were able to track the migration of as few as 1000 labeled NSCs *in vivo* [179]. Slotkin *et al.* demonstrated that fluorescent semiconductor quantum dots can be effectively used to label mammalian neural stem and progenitor cells *in vivo* [180]. Lastly, nanoshells are a new class of optically tunable nanoparticles that may provide imaging of NSC migration as well as therapeutic photothermal therapy to treat glioma cells [181]. Loo *et al.* engineered immunotargeted nanoshells that are able to scatter light in the near-infrared region (enabling imaging of the tumor) as well as absorb light (allowing selective destruction of targeted carcinoma cells through photothermal therapy) [181]. This combined imaging and therapeutic modality has great potential in gliomas.

Mesenchymal stem cells can also be labeled so that they can be tracked *in vivo*. In 2005, Anderson *et al.* demonstrated that MRI can detect the incorporation of magnetically labeled bone marrow-derived precursor cells into tumor vasculature as part of ongoing angiogenesis and neovascularization [182]. This labeling of stem cells can be used to directly identify tumor neovasculture *in vivo* and to facilitate gene therapy by noninvasively monitoring these cells

as gene-delivery vectors. In 2008, Wu *et al.* showed on MRI that superparamagnetic iron oxide nanoparticle-labeled MSCs, which were systemically transplanted, migrate toward gliomas with high specificity [183]. Fluorescent quantum dots are another modality for biological imaging [184] and work has already been carried out to label MSCs *in vitro* [185] and *in vivo* [186].

Concerns involving stem cell-based therapies

One of the major concerns involving stem cell therapy is that genetically modified stem cells may generate secondary malignancies [187]. Allogeneic stem cell lines used as therapy for gliomas could theoretically become tumorigenic by three mechanisms [188]:

- Expression of the gene used to immortalize the cell line could transform the cells
- The genetic modification of the stem cells could result in insertion of the gene into a critical locus, dysregulating the normal cell behavior and inducing cancer cell behavior
- The therapeutic stem cell may fuse with endogenous cells, resulting in cells with a transformed phenotype that could potentially be cancerous

With regard to the concern that genetic modifications to immortalize cells can lead to the transformation of the stem cells, there is evidence that several cell lines immortalized with human telomerase reverse transcriptase (*hTERT*) gene and simian virus 40 (*SV40*) large T fragment were observed to be unstable with respect to chromosome number and karyotype [189]. Nevertheless, immortalization of cell lines is important to be able to develop stem cell lines that can proliferate long enough to be well characterized, genetically modified and then amplified to therapeutic quantities [188]. Studies have shown that immortalization of stem cell lines with *v-myc* [188], as well as the use of cells that have only undergone a few passages [190], may decrease the risk for neoplastic transformation of the stem cells. The concern that genetic modification of the stem cells may lead to genetic insertion that will transform the cells into cancer emphasizes the need to establish cloned, extensively characterized stem cell lines in which all the insertion sites can be carefully scrutinized for their safety. Finally, with regard to therapeutic stem cells fusing with endogenous cells, resulting in cells with a transformed phenotype that could potentially be cancerous, there have been no reports of hyperdiploid cells in any of the animals treated with stem cells [188]. Embryonic stem cells do have a greater capacity for fusion than fetal or adult stem cells [191,192], which suggests that nonembryonic stem cells should preferentially be used in stem cell-based glioma therapies.

Another major concern is the recruitment of the stem cells to contribute to the tumor. The capacity of NSCs to self-renew and invade suggests that aberrant NSCs may transform into BTSCs, creating the bulk of the tumor [193-195]. This is an area of heavy research that many laboratories, including ours, are exploring. There is also concern that MSCs may enhance or initiate tumor growth [196,197]. Chen *et al.* suggested that systemic administration of human MSCs can contribute to angiogenesis in the pathological brain [198]. In 2006, Tso *et al.* found that a subset of primary GBM tumors and their derived tumor cell lines express cellular and molecular markers that are associated with MSCs [199]. They also found that GBM cultures can be induced to differentiate into multiple mesenchymal lineage-like cell types [199]. These findings suggest that a subset of primary glioblastomas derive from transformed stem cells containing MSC-like properties or that glioblastomas activate a series of genes resulting in the mesenchymal properties of the cancer cells and providing sustained tumor growth and malignant progression [199]. However, Hall *et al.* pointed out that many of these studies that have shown MSCs to possess tumorigenic properties used extensively passaged and/or genetically altered MSCs [190]. It has been demonstrated in several papers that lower-passage MSCs do not form tumors *in vivo* [200-202]. Whether or not NSCs and MSCs are recruited by

the tumor cells to participate in the expansion of the tumor, it has been demonstrated from numerous animal studies using genetically modified NSCs or MSCs that stem cell therapy actually decreases the tumor burden.

There have also been concerns regarding the immunologic response to therapeutic NSCs or MSCs, especially that stem cells can suppress the immune response to tumors [203,204]. However, we have discussed earlier the many studies that used genetically modified NSCs or MSCs to control tumor growth and stimulate an anti-tumor immune response [44,169]. Another immunologic concern is whether these stem cells will be accepted by the recipient's immune system. As noted by Yip *et al.*, the NSCs used for the treatment of tumors may not necessarily need to be matched to the recipient [46]. If the tumor-infiltrating NSCs were to elicit an immune reaction against themselves, this would attract immune cells into the tumor microenvironment, increasing the immune response to the tumor as well [46]. Thus, it may be feasible to have a universal, readily available stem cell line for use as a therapy.

There are also several practical concerns about the use of these stem cell therapies for the treatment of gliomas before they can be applied to humans. For example, one major concern with NSCs is how autologous NSCs and MSCs would be obtained for patient treatment. There is concern about whether an adequate number of stem cells can be obtained from each patient and the amount of time it would take to make these stem cells ready for treatment. These harvested stem cells then need to be cultured, grown out, genetically modified and then characterized and tested for safety, all of which would take a considerable amount of time. As these stem cells are passaged and prepared for therapy, there is also concern that the original stem cells can significantly change outside their intrinsic *in vivo* environment. Moreover, genetic modifications may actually make these stem cells harmful to the patient rather than beneficial. The most practical way to make stem cells readily available for treatment in patients would be to create universal, immortalized stem cell lines that have been thoroughly characterized for genetic stability, biodistribution, safety and toxicity [188]. These cell lines would be kept in master cell banks and be readily available for clinical use.

Expert commentary

As noted earlier, the current standard of care for many brain tumors, which includes radiation and chemotherapy combined with surgical resection, is limited in its ability to extend survival [2,3,16-18]. Many high-grade gliomas fail to respond to therapy for a variety of reasons, including extensive seeding of microsatellites of tumor cells away from the tumor bulk into normal brain [11] and the resistance of glioma cells to radiation and chemotherapy, in particular BTSCs [36]. Stem cells represent a powerful new way to treat gliomas. Numerous studies have documented the tropism of stem cells for tumor cells and their ability to migrate to areas of pathology [29,43,44]. This behavior of stem cells is exciting in that it provides a novel way to provide therapy and/or elicit an immune response to all glioma cells, even those that have invaded deep into normal brain tissue [205].

In 1995, Brem *et al.* demonstrated the safety and efficacy of controlled drug delivery by biodegradable polymers that were implanted into the tumor resection cavity to release the chemotherapeutic drug carmustine [206]. In the future, we may be able to implant similar biopolymer wafers or gels into the tumor resection cavity to locally release genetically engineered stem cells to track down tumor cells and release tumoricidal substances/viruses as well as elicit a powerful immune response against the tumor cells [207]. Future stem cell therapies will probably be even more effective at tracking down tumor cells, controlling tumor growth, amplifying the anti-tumor immune response and limiting invasion of glioma cells into normal tissue.

Five-year view

Before stem cell therapy can be applied for the treatment of human brain tumors, much more research must be conducted to investigate the biology of stem cells and brain tumors. Thus, in the next 5 years, we believe that significant advances will be made in the understanding of how tumors, stem cells and the immune system interact with one another, and how these interactions can be utilized from a therapeutic standpoint. Further research needs to be carried out in finding new and more powerful genetic modifications to stem cells so that their anti-tumor effects are maximized. As these stem cell therapies advance and become more sophisticated, stem cells will probably be engineered to have multiple genetic modifications and thus be equipped to apply several different therapeutic strategies towards treating the cancer cells. More studies must also be carried out to elucidate the chemotactic factors and signaling pathways involved in the gliomatropic behavior of stem/progenitor cells. Understanding the signals involved in the tropism of stem cells for gliomas will enable better engineering of therapeutic stem cells so that they are more sensitive to signals secreted from the tumor environment and thus better able to track tumor cells. Moreover, there are still many potential applications that have not been explored. For example, there have been no studies that have looked at ways that stem cells can, for instance, increase the radiosensitivity of glioma cells.

Human trials of stem cells for the treatment of gliomas are still a long way off. Before stem cell therapy can be applied to the treatment of gliomas in humans, further *in vivo* human safety and efficacy data must be compiled in terms of cell distribution and duration of survival, and genomic insertion sites for exogenous genes that would minimize the risk of inducing neoplastic mutations in the cell must be identified.

Although there is still much work to be done, the progress in stem cell therapies has provided us with new hope that, in the future, we will be able to better treat aggressive brain tumors such as GBM.

Key issues

- Stem cells have a tropism for glioma cells, enabling them to home in on glioma cells in the tumor bulk as well as in distant tumor microsatellites scattered throughout normal brain tissue.
- Several genetic modifications have been performed on neural and mesenchymal stem cells to increase their tumor cytotoxic effects, their stimulation of an anti-tumor immune response and their glioma-tropic tracking behavior.
- Stem cells can also be used as an imaging modality to track the migration of these stem cells on tumor cells.
- Further research must be performed into the exact role that neural and mesenchymal stem cells play in the tumor environment and whether there is any tumor recruitment of stem cells.

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- of considerable interest

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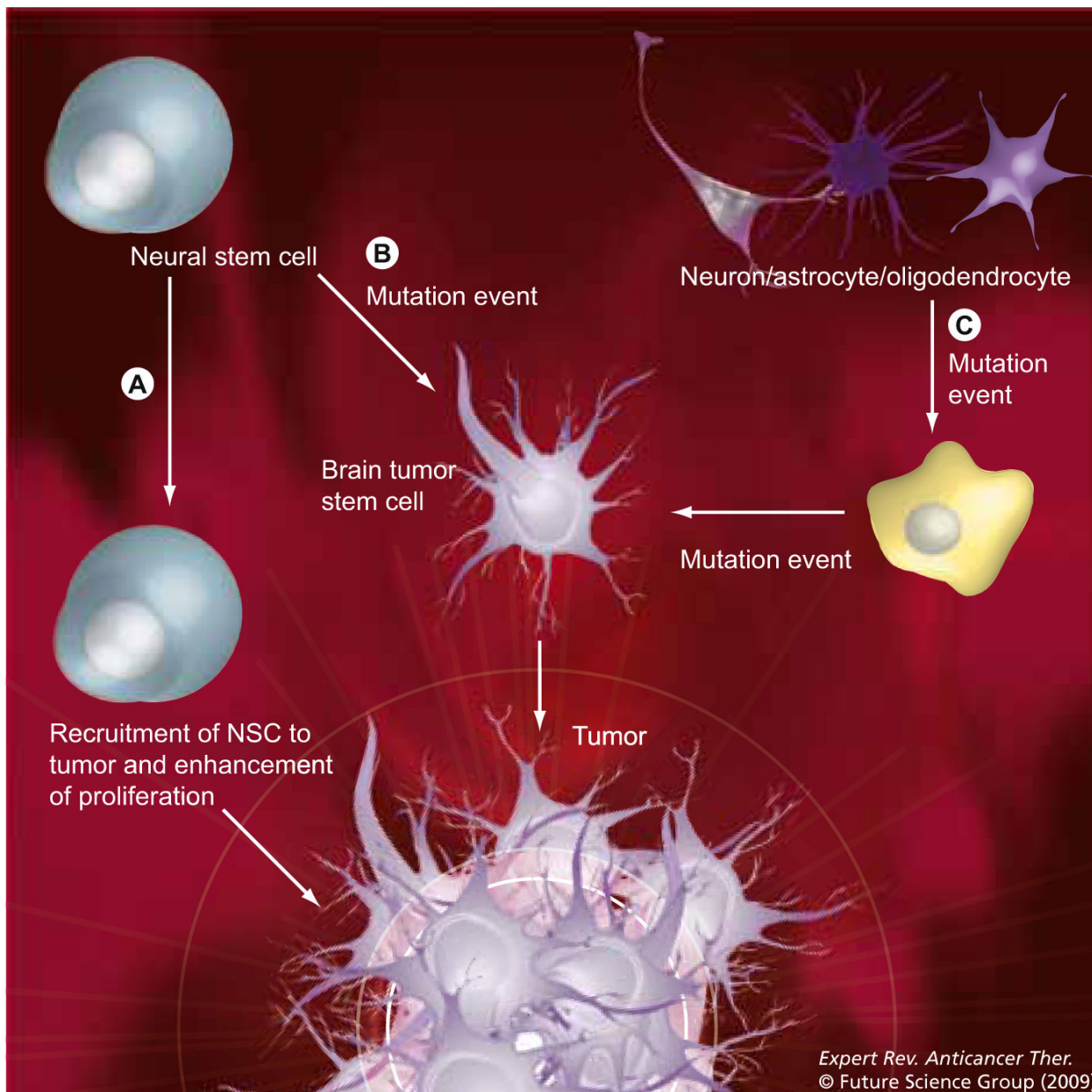


Figure 1. Three models on the origins of CNS malignancies

(A) Normal adult NSCs are recruited to the site of tumor and help to regenerate tumor stromal cells. (B) NSCs undergo malignant transformation into a brain tumor stem cell. (C) Fully committed adult brain cells accumulate mutations over time to transform into brain tumor stem cells.

NSC: Neural stem cell.

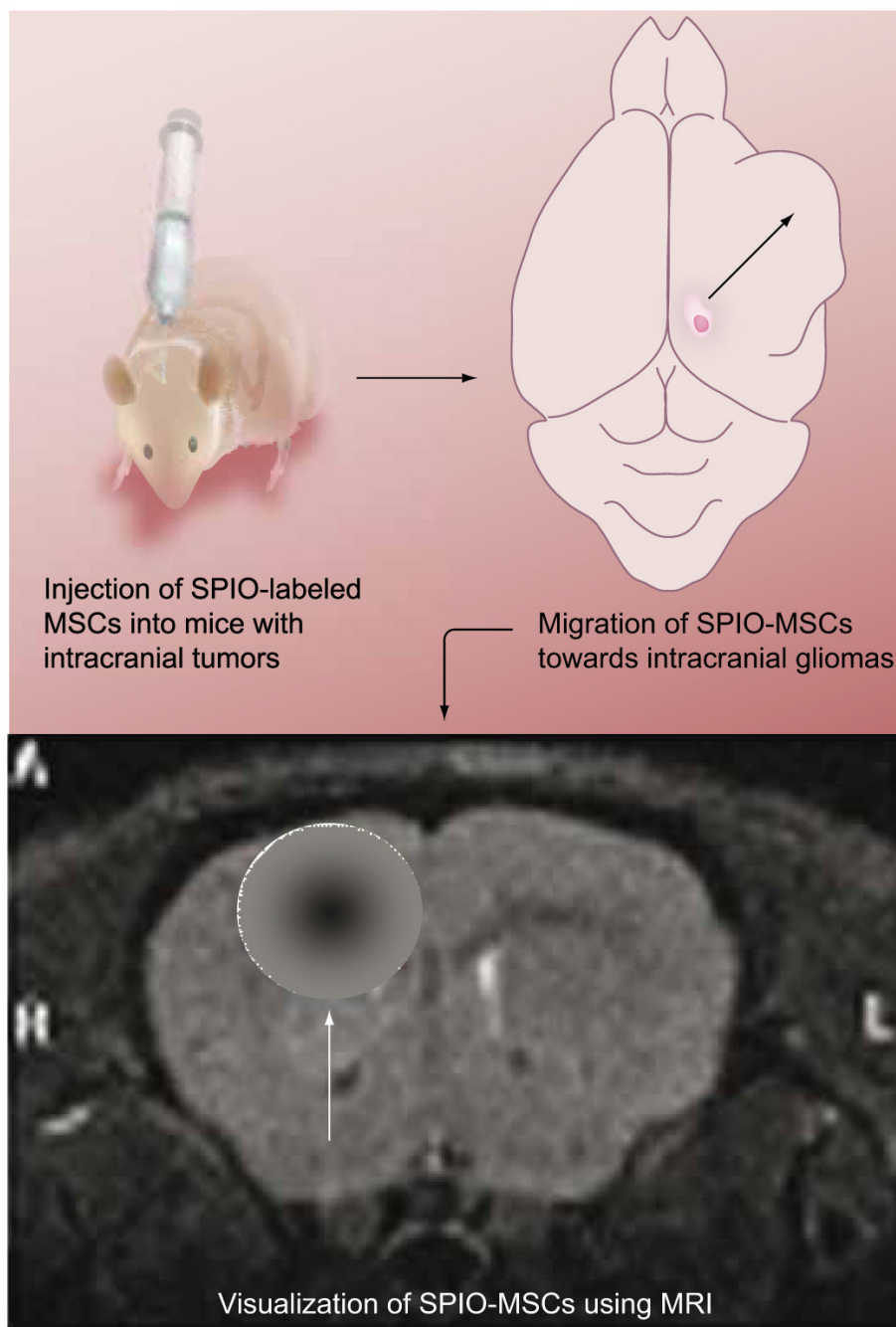


Figure 2. MSCs labeled with SPIO injected into intracranial glioma-bearing mice

The SPIO-labeled MSCs migrate towards the area of the brain containing the tumor. This modification of MSCs and the idea that MSCs home in on tumor cells allow for another way to image brain tumors and the extent of tumor infiltration into normal brain parenchyma. More importantly, it allows for the surveillance of the location of the therapeutic stem cells. Such labeling will be important in future therapies because it will allow clinicians to know whether the therapeutic stem cells have positioned themselves in a location that will be effective for the treatment of the tumor bulk and microsatellites.

MSC: Mesenchymal stem cell; SPIO: Superparamagnetic iron oxide.

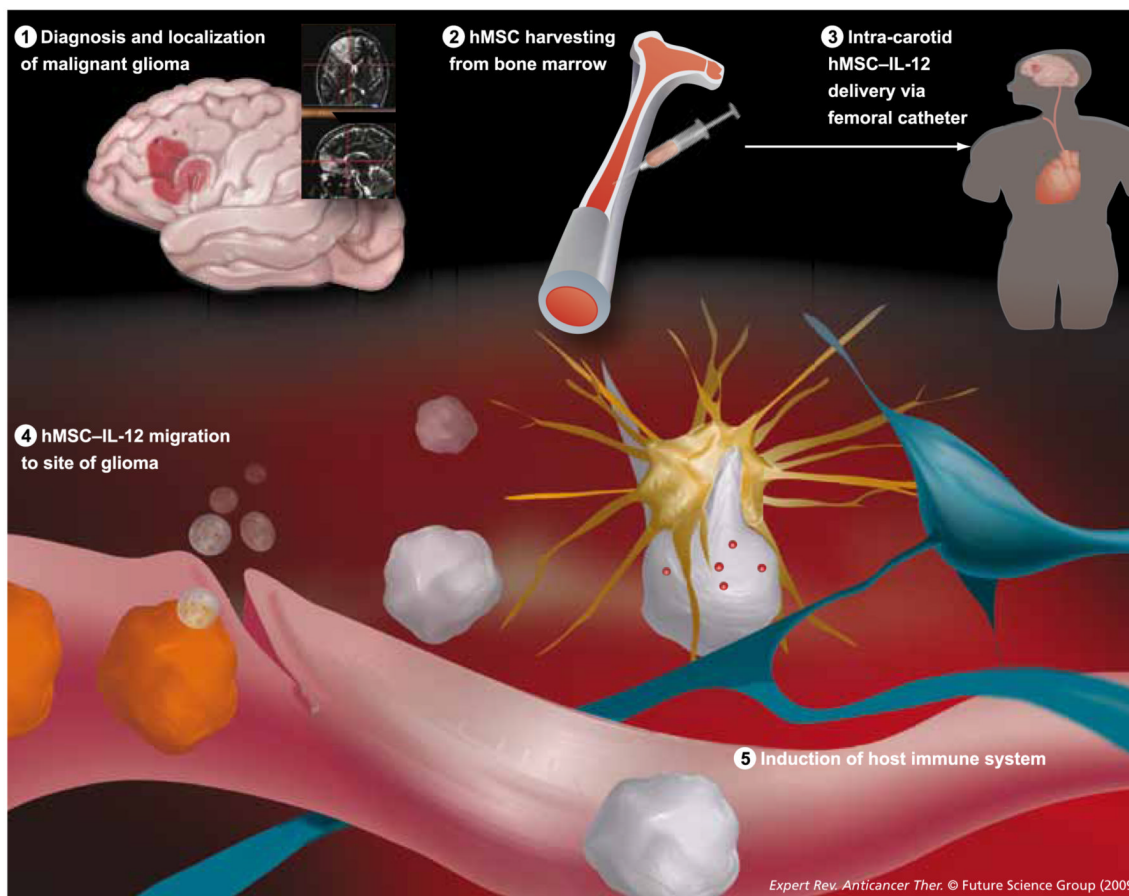


Figure 3. Potential clinical application of genetically engineered IL-12 hMSCs

In our laboratory, we are using hMSCs harvested from bone marrow as well as from adipose tissue. The advantage to adipose-derived MSCs is the ease of harvesting patient-specific MSCs. During surgery for resection of the brain tumor, the surgeon also takes a small fat graft from the patient. These MSCs can then be cultured and transduced to express various proteins, such as IL-12. Ideally, in future therapies, the genetically modified MSCs would be injected intra-arterially through the carotid artery, and the MSCs would migrate to the site of glioma and induce an anti-tumor immune response.

hMSC: Human mesenchymal stem cell; MSC: Mesenchymal stem cell.