Stem cell-based therapy for malignant glioma.

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Published in:
Cancer Treatment Reviews

DOI:
10.1016/j.ctrv.2012.06.006

2013

REVIEW:

Title: Stem Cell-Based Therapy for Malignant Glioma

Running title: Stem Cells for Malignant Glioma

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ABSTRACT

Stem cells have been extensively investigated as tumour-tropic vectors for gene delivery to solid tumours. In this review, we discuss the potential for using stem cells as cellular vector systems in gene therapy for malignant gliomas, with a focus on neural stem cells, and multipotent mesenchymal stromal cells. Tumour cell-derived substances and factors associated with tumour-induced inflammation and tumour neovascularisation can specifically attract stem cells to invasive gliomas. Injected stem cells engineered to produce anti-tumour substances have shown strong therapeutic effects in experimental glioma models. However, the potential caveats include the immunosuppressive functions of multipotent mesenchymal stromal cells, the contribution of stem cells to the pro-tumorigenic stroma, and the malignant transformation of implanted stem cells. In addition, it is not yet known which stem cell types and therapeutic genes will be most effective for the treatment of glioma patients. Here, we highlight the possibilities and problems for translating promising experimental findings in glioma models into the clinic.

KEY WORDS: STEM CELLS, MESENCHYMAL STEM CELLS, NEURAL STEM CELLS, TUMOUR, GLIOBLASTOMA, GLIOMA, GENE THERAPY

INTRODUCTION

Glioblastoma (GBM) is the most common and severe form of malignant glioma. Despite enormous efforts, the prognosis for GBM patients is still poor. The median survival of GBM patients is 14,6 months, and around 10% of GBM patients survive more than 5 years, despite receiving surgery, radiotherapy, and chemotherapy with temozolomide (TMZ)[1, 2]. A major portion of treatment failure is due to the invasive growth of GBM. Microscopic tumour extensions and distant tumour microsatellites grow along white matter fibre tracts and normal brain tissue blood vessels[3]. Therefore, complete surgical resection is rarely achieved. Other GBM therapy challenges include an increased interstitial fluid pressure within the tumour, resulting in low concentrations of systemically delivered drugs, an intrinsic and acquired drug resistance of tumour cells, and treatment neurotoxicity[4]. Furthermore, although GBM presents with a dysfunctional and leaky blood-brain barrier (BBB), single infiltrative GBM cells reside deep within the normal brain parenchyma with an intact BBB; therefore, they are protected from many blood-borne drugs[5]. A successful GBM treatment requires several criteria to be fulfilled, including the targeting of invasive tumour cells, the targeting of tumour cells characterised by different genetic aberrations (including putative cancer stem cells), and the selective elimination of tumour cells while sparing normal neural cells[6].

In this review, we present the concept of using genetically engineered stem cells in gene therapy for brain tumours. We discuss different stem cell types that are used for glioma gene therapy, mechanisms by which stem cells are attracted to tumours, and the major principles of their therapeutic functions. We also highlight critical issues for translating the experimental findings to the clinic.

SCIENTIFIC RATIONALE FOR STEM CELL-BASED THERAPY FOR GBM

Gene therapy using viral vectors has been explored in several clinical GBM treatment trials[7]. Proteins aimed at inhibiting tumour angiogenesis, enhancing anti-tumour immune responses, and correcting tumour-specific genetic defects have been expressed in GBM by using locally or systemically administered viral gene vectors. Viral-mediated GBM gene therapy has shown promising results in animal models[8]. However, clinical studies have had modest success at limiting tumour growth and extending patient survival[7]. Failure has been attributed mainly to difficulties in achieving the distribution of viral vectors throughout the invasive tumour. In addition, the viral transduction efficiency of GBM cells has been low[7].

Implanting or injecting stem or progenitor cells that have been genetically modified to produce anti-tumour substances has several advantages over viral-vector mediated gene delivery. Initially described by Aboody et al., implanted neural stem cells (NSCs) possess the capacity to migrate to and within intracranial tumours in which the NSCs deliver a cytotoxic substance that can reduce tumour growth[9]. The tumour-tropic homing and migratory capacity has been replicated by many groups using different types of stem/progenitor cells, including multipotent mesenchymal stromal cells (MSCs)[10, 11], and hematopoietic progenitor cells (HPCs)[12] in animal models. For example, a single MSC implantation into an invasive rat glioma results in MSC migration to the majority of the infiltrative tumour extensions and a fraction of distant tumour microsatellites[13]. Differentiated cells, such as fibroblasts, do not exhibit a similar tumour tropism[9-12]. In contrast to viral vectors, stem cells are attracted primarily to tumour tissue, whereas they show minimal tropism for normal neural cells. Therefore, tumour-specific gene delivery is feasible, and cerebral side effects can potentially be avoided[9, 13]. Numerous studies have demonstrated the potential of stem cell vectors in the treatment of brain tumours and many other invasive solid tumour types[14]. Thus, although each

tumour type may require tailor-made cellular vehicles and transgenes, the results from work with non-neural tumours may contribute to the development of a successful stem cell therapy for GBM and vice versa.

DIFFERENT TYPES OF CELL VECTORS

Neural stem cells

NSCs can give rise to neurons, astrocytes, and oligodendrocytes. In the adult rodent brain, NSCs are located mainly within two neurogenic zones: the subependymal zone lining the lateral ventricles and the dentate gyrus of the hippocampus[15]. *In vitro*, NSCs are cultured and expanded as floating cellular aggregates called neurospheres (**Fig. 1A**). Initial findings demonstrated a tumour-tropic migration of the immortalised murine NSC line C17.2, following implantation into, or at a distance from, the experimental gliomas[9]. Subsequently, immortalised murine or human NSC lines and primary NSCs have been widely used for their tumour-tropic capacity and potential to deliver anti-tumour substances to gliomas[16-23] (**figure 1B**). An advantage of immortalised NSC lines (**figure 1A**) is that they are readily available. A well-characterised NSC line can be cultured and expanded *in vitro* to obtain high numbers of cells ready for transplantation within a short period. In contrast, although it is possible to harvest autologous neural precursor cells from the adult human brain[24], it could take too long to expand, modify, and characterise these cells to prepare them for implantation into GBM patients. Furthermore, grafting immortalised NSC lines into the brain is associated with two main problems: immunogenicity and tumorigenicity. Immunogenicity implies that the immune system may attack and neutralise the grafted non-autologous cells. This could impair NSC survival and migration to the infiltrative tumour parts, which is crucial for therapeutic function. Immortalised NSCs carrying a proto-oncogene, such as v-*myc*, may transform and

develop into secondary malignancies following implantation into the tumour tissue. It has been reported that a fraction of the NSCs continues to proliferate after implantation or injection to gliomas[9, 23]. In addition, when subjected to growth factors and a tumour microenvironment, normal neural progenitors in the brain can be driven towards malignant masses and potentially contribute to glioma progression[25, 26]. It will be important to determine whether implanted and *in vivo* proliferating NSCs can form tumorigenic masses after long-term growth *in vivo*. One way to avoid such an occurrence would be to administer NSCs that are genetically modified to carry a "suicide" gene, such as herpes simplex virus thymidine kinase (HSV-tk). With this approach, it would be possible to eliminate the administered NSCs within the tumours at any given time.

Multipotent mesenchymal stromal cells

Multipotent mesenchymal stromal cells, sometimes called mesenchymal stem cells, are non-hematopoietic stem cells. MSCs are thought to be the precursors of the bone marrow stroma; at a minimum, they can differentiate into chondrocytes, adipocytes, and osteoblasts[27]. MSCs are usually isolated by their adherent growth in culture (**figure 1C)**, differentiation capacity, expression of surface markers (including CD73, CD90, CD105, CD146, CD271, and STRO-1) and lack of expression of the hematopoietic markers CD34 and CD45[27].

Intracranially implanted or injected mouse, rat, and human MSCs have shown tropism for experimental gliomas in which MSCs can deliver a therapeutic substance leading to an increased survival of the glioma-bearing animals[10, 11]. However, rat MSCs lack the long-distance migratory capacity through normal brain tissue towards the rat glioma when implanted at a distance (i.e., a few millimetres) from the tumour[28]. In contrast, intratumoral implantation of rat MSCs directly into rat gliomas results in an MSC migration to the majority

of invasive tumour extensions and a fraction of distant tumour microsatellites[13] (**figure 1D**). Importantly, the tumour-specific distribution of intratumorally implanted MSC makes the cells well suited for transporting toxic substances specifically into tumours while potentially sparing the normal brain tissue[13].

MSCs are promising cell therapy candidates because it is easy to obtain MSCs through a bone marrow puncture and, subsequently, to culture and expand the cells *in vitro*. In principle, this makes it possible to graft autologous MSCs (isolated from the patient). Autologous MSC implantation would avoid graft rejection immunograft issues, but expansion, modification, and characterisation of the MSCs would delay the onset of treatment compared to implantation of a readily available, well-characterised cell line. In addition, there are a number of concerns about the use of MSCs for tumour gene therapy. The findings from other solid tumour types have shown that MSCs may contribute to tumour growth through their immunosuppressive properties, growth factor production, and contribution to the pro-tumorigenic stroma, as well as by the malignant progression of the recruited MSCs, which can drive tumour growth[29-33]. As part of the tumour microenvironment, MSCs can promote experimental ovarian cancer growth[34] and increase the metastatic capacity of breast cancer cells[35]. In contrast, non-modified MSCs can reduce tumour vascularisation and suppress tumour growth in a malignant melanoma model[36]. Importantly, independent groups have reported that implanted non-modified human or rat MSCs display no glioma-promoting effects[13, 37]. Other findings suggest a glioma-suppressing effect upon implanting human adipose tissue-derived MSCs[38] or bone marrow-derived rat MSCs[11] into gliomas. These divergent results may depend on the differences in the MSC sub-populations (e.g., heterogeneity of MSCs within and between the cell cultures leading to functionally different MSCs in different experiments)[39, 40] and MSC species differences (human MSCs may not

necessarily yield the same results as mouse and/or rat MSCs). The conflicting findings of the interaction between MSCs and tumour cells are further reviewed in the literature[41]. The potential risk that human MSCs can undergo transformation into malignant cells under *in vitro* culture conditions is controversial and reviewed by Prockop et al.[42].

Alternative types of cellular vectors

Other types of stem and progenitor cells may also serve as migratory vectors to gliomas. The hematopoietic progenitor cell (HPC) is an easily accessible cell type with glioma tropism[12]. Systemically injected human peripheral blood-derived and murine bone marrow-derived HPCs are attracted specifically to experimental gliomas through transforming growth factor (TGF)-β and stromal cell-derived factor-1α (SDF-1α)/CXC chemokine ligand 12-dependent migration and homing[12]. Tumour endothelial cells express E-selectin (CD62E), which is critical for HPC homing to gliomas by mediating adhesion of circulating HPCs to glioma endothelium[43]. Hypoxia, cerebral irradiation, and chemotherapy (TMZ) further enhance HPC attraction to tumour cells, suggesting that a combined treatment approach by HPCs and irradiation and/or chemotherapy may be advantageous[44].

Implanted human skin-derived stem cells can migrate to experimental gliomas in which the cells reduce tumour angiogenesis and adopt a pericytic phenotype. The effects on glioma growth and prolonged animal survival indicate that skin-derived cells may be an autologous alternative for stem cell therapy of gliomas[45].

Furthermore, systemically injected endothelial progenitor cells can home to experimental gliomas and integrate into the tumour vasculature[46, 47]. Therapeutic anti-glioma effects have

been achieved by injecting endothelial progenitor cells that have been modified to produce oncolytic measles virus[48] or engineered to express cytotoxic antitumor genes[46].

Embryonic stem cell-derived astrocytes have shown intracranial migratory capacity and therapeutic efficacy following implantation into subcutaneously established gliomas[49]. NSCs generated from induced pluripotent stem (iPS) cells have also been used as vectors in glioma gene therapy[50]; however, compared to the other cell vectors described above, the clear advantages of using iPS cells for glioma gene therapy have not yet been demonstrated.

Thus, different types of stem and progenitor cells have been utilised for glioma gene therapy, and each cell type has advantages and disadvantages. There are at least three critical requirements that cell carriers should meet: 1) neoplasm-specific extensive migration within the glioma and to infiltrative GBM cells; 2) stable production and delivery of an oncolytic substance; and 3) implanted cells should not cause any substantial harm to the surrounding brain parenchyma. However, studies comparing migratory potential, proliferative capacity, cell survival, and delivery efficacy of different cellular vectors implanted into intracranial gliomas are lacking and highly warranted.

MECHANISMS OF MIGRATION

Neural stem cells and MSCs have been delivered to orthotopic glioma in preclinical studies by various routes of administration. The different ways to administer therapeutic stem cells to gliomas include direct implantation into tumours, intracerebral injection at a site located at a distance from the tumour, in the contralateral hemisphere, intracerebroventricular deposition, intravenous and intra-arterial injections[9, 10, 19, 21, 51, 52]. Although intratumoral MSC grafting

can yield effective and tumour-selective distribution[13], in terms of efficacy and safety, it is not clear which is the preferred route of administration.

A schematic overview of the tumour components regulating stem cell migration is given in **figure 2**.

Inflammatory-derived factors can attract NSCs and MSCs to glioma. The peritumoral oedema zone in glioma is characterised by a high number of activated astrocytic and microglial cells and constitutes an inflammatory tumour microenvironment[53]. Several factors, notably interleukin (IL)-8[54], monocyte chemotactic protein (MCP)-1[55], and stromal derived factor-1 alpha (SDF-1α)[56], are present within glioma or in the peritumoral reactive region and have been implicated in MSC migration to tumours. For instance, tumour necrosis factor-α can enhance the expression of CXC chemokine receptor (CXCR) 4 on MSCs, which results in increased MSC migration towards stroma-derived SDF-1α[57]. MCP-1 expression in gliomas can mediate glioma-tropic migration of NSCs through the CC chemokine receptor 2[58]. Therapeutic irradiation produces an inflammatory response, and MSC tropism to the glioma is increased following brain irradiation[59, 60]. The irradiation-enhanced MSC tumour tropism is mediated in part by an increased IL-8 production from irradiated gliomas and CXCR1 upregulation on migratory MSCs[59]. Further information describing MSC migration to the inflammatory components of tumours is detailed by Spaeth et al.[61].

In addition to inflammation, tumour angiogenesis and angiogenic signalling molecules influence MSC tropism to neoplasms. Findings from *in vitro* assays suggest that platelet-derived growth factor (PDGF)-BB, PDGF-D, vascular endothelial growth factor (VEGF)-A, TGF-β1, and neurotrophin-3, all of which are involved in tumour angiogenesis, mediate MSC

recruitment to gliomas[10, 62, 63]. Intratumorally grafted MSCs exhibit a marked tropism to tumour vasculature following intratumoral grafting, integrate into tumour vessel walls, and display a pericyte-like phenotype[13]. Tumour angiogenic signalling factors may also regulate MSC migration intratumorally *in vivo*[13]. Glioma angiogenesis inhibition, by the anti-angiogenic drug sunitinib, substantially decreased the migration of grafted MSCs to tumours, indicating that tumour angiogenesis is critical for MSC intratumoral migration[13]. Angiogenic signalling has also been demonstrated to be important for NSC tracking of glioma cells[64]. The dependence on ongoing angiogenic signalling may confer a powerful glioma specificity to grafted, migratory stem cells because there is no active angiogenesis in the surrounding normal brain. Hypoxia upregulates CXCR4, urokinase plasminogen activator (uPA) receptor, and VEGF receptor 2 on NSCs, which enhances their migration towards gliomas[65]. These findings, in combination with the observed localisation of injected NSCs close to hypoxic areas within experimental gliomas, suggest that hypoxia is a critical factor for NSC tropism for gliomas[65].

The migrating MSCs interact with and remodel the extracellular matrix (ECM) during migration. Matrix metalloproteinase (MMP)-1, a matrix-degrading enzyme, is upregulated specifically in MSCs, displaying a high propensity for glioma-directed migration. Conversely, overexpression of MMP-1 on relatively immobile subpopulations of MSCs increase their migration towards gliomas[66]. The composition of the glioma-derived ECM also influences NSC migration towards infiltrating glioma cells[67].

Interestingly, molecules involved in chemotaxis during normal development are upregulated in malignant brain tumours and augment NSC and MSC tropism. Human NSCs are attracted to glioma cell-derived factors (hepatocyte growth factor, epidermal growth factor (EGF) and

VEGF, uPA, and uPA receptor). It has been suggested that NSCs and migratory glioma cells use the same or similar pathways to enable their motility[68].

Most mechanistic findings underlying NSC and MSC glioma tropism have been derived from *in vitro* experiments. These results have to be interpreted with caution because the *in vivo* tumour microenvironment is much more complex compared to the artificial *in vitro* microenvironment. Nevertheless, *in vitro* mechanistic knowledge responsible for NSC and MSC tumour tropism has been exploited to increase the efficacy of brain tumour homing for therapeutic purposes. EGF receptor overexpression on MSCs increases their infiltration into EGF-expressing gliomas[69]. Similarly, CXCR3 overexpressing HiB5 neural progenitor cells (NPCs) exhibit enhanced *in vivo* migration towards rat gliomas compared to non-transduced HiB5 cells[70].

ANTI-TUMOR SUBSTANCE DELIVERY

Pro-inflammatory cytokines

A variety of cytokines have been delivered by NSCs or MSCs to gliomas and have demonstrated therapeutic efficacy alone or in combination with other treatment modalities[10, 17, 71, 72]. Early work demonstrated the therapeutic effects of implanting IL-4-producing NSCs on murine glioma growth[16]. Notably, the effects of NSC-produced IL-4 were more powerful than the virus-mediated transfer of IL-4[16]. Interleukin-mediated effects are related to a pro-inflammatory reaction, including an increased infiltration of anti-tumour immune cells (e.g., CD4+ and CD8+ cytotoxic T-cells and natural killer cells) into tumours[71, 72]. The anti-tumour immune response can be further enhanced by combining the intratumoral implantation of cytokine-producing MSCs with systemic immunotherapy[72].

Tumour necrosis factor-related apoptosis-inducing ligand

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) activates the pro-apoptotic death receptors (DRs) 4 and 5, which induce caspase-8-dependent apoptosis[73]. TRAIL can selectively target tumour cells while sparing most non-malignant cells[74]. TRAIL-producing NSC implantation into human gliomas in mice leads to increased tumour apoptosis and decreased tumour growth. However, the normal neural cells do not undergo apoptosis[75]. Several studies have shown the therapeutic and tumour-specific effects by intracranially implanting or intravenously injecting TRAIL-producing MSCs[37, 76, 77, 78] , NPCs[79], or ESC-derived astrocytes[49] into experimental gliomas.

PEX

PEX is a human MMP-2 fragment that exerts anti-tumour effects by inhibiting glioma angiogenesis and tumour cell proliferation[80]. The intracranial implantation of human immortalised NSCs that are modified to produce PEX leads to decreased tumour vascularisation and tumour cell proliferation, resulting in an inhibited glioma growth[18].

Pro-drug converting enzymes ("suicide" gene therapy)

Enzymes capable of converting inactive pro-drugs into toxic substances have been widely used in clinical glioma gene therapy. The intratumoral transfer of herpes simplex virus type 1 thymidine kinase (HSV-tk) in combination with the pro-drug ganciclovir (GCV) is the most extensively studied system. HSV-tk phosphorylates the guanosine analogue GCV, which is then incorporated into the DNA strand, leading to disturbed DNA synthesis and cell death. The activated GCV is toxic to the HSV-tk-producing cells and the cells in their vicinity. This is due to a bystander effect, exerted by gap junctions between the cells through which the

phosphorylated pro-drug is transported[81]. In clinical trials, HSV-tk has been transferred to tumours mainly using viral gene vectors. A phase III multicenter randomized clinical trial including 248 GBM patients showed no significant difference in survival between the HSV-tk gene therapy group and control group[82]. HSV-tk clinical gene therapy studies are reviewed by Pulkkanen et al.[7]. It was shown that the therapeutic effects were poor due to a low transduction efficiency of tumour cells *in vivo* and an ineffective vector distribution within the tumours[7]. Consequently, migratory stem cell vectors have been introduced in preclinical studies to achieve a better intratumoral distribution of the pro-drug converting enzyme. Indeed, HSV-tk transfer using bone marrow-derived progenitor cells[83], NSCs[84, 85], and MSCs[52], can lead to therapeutic effects through bystander-mediated glioma cell killing[83, 85, 86].

Another well-investigated pro-drug activating enzyme is cytosine deaminase (CD), which converts 5-fluorocytosine (5-FC) to its toxic form, 5-fluorouracil, causing cell death[87]. CD-expressing NSCs have been used to treat intracranial rat gliomas[9] or human medulloblastomas in immunocompromised mice[88]. In 2010, a clinical pilot trial using immortalised NSCs engineered to produce CD in combination with oral 5-FC was initiated for patients undergoing surgery for recurrent high-grade gliomas. The aim of the study is to clarify the safety and feasibility of intracerebral NSC implantation and systemic 5-FC administration in glioma patients (ClinicalTrials.gov; Identifier: NCT01172964). Obviously, two critical issues for achieving therapeutic effects are the distribution of the pro-drug converting enzyme (i.e., administered stem cells) to the invasive glioma and the magnitude of the bystander effect *in vivo*.

Oncolytic viruses

In oncolytic virotherapy, viruses with the capacity to infect tumour cells are delivered systemically or locally to tumours. The viruses replicate within and lyse the tumour cells, whereby they are released for subsequent uptake by the neighbouring tumour cells. Although oncolytic virotherapy has delivered promising preclinical results, there are several obstacles for a successful clinical translation. First, viral particle delivery throughout the tumour tissue and to invasive tumour cells has been difficult. Second, the host immune system can attack and neutralise the viral particles before they can exert any effect[89]. It is possible to deliver viral particles to distant parts of the tumour using tumour-tropic migratory cells as oncolytic virus carriers. Furthermore, the viral particles within cells may be protected from the immune system[90]. NSCs, MSCs, and adipose-derived stem cells have been used for delivering oncolytic viruses (e.g., a conditionally replicating adenovirus) to experimental gliomas. These results suggest that stem cell-mediated oncolytic virus delivery is superior to viral delivery alone for the survival of glioma-bearing animals[22, 91-94].

Antibodies

The administration of engineered stem cells may be an effective way to sustain local anti-tumour antibody delivery. Furthermore, stem-cell-based delivery of antibodies has the potential to reduce the toxic side-effects caused by intravenous antibody administration[95]. Co-injection of human glioma xenografts and human MSCs transfected to express a cell surface-bound single-chain antibody (scFv) against the EGF receptor variant III results in reduced tumour vascularisation and increased survival of glioma-bearing mice[96].

Table 1 summarises selected stem cell-based glioma therapy studies.

TOWARDS AN EFFECTIVE STEM CELL-BASED THERAPY FOR MALIGNANT GLIOMA

The tumour-tropic migration of human, rat, and mouse NSCs and MSCs is associated with general GBM features (e.g., neovascularisation, inflammation, and growth factor production) and, importantly, are not animal model-specific. Therefore, it is conceivable that implanted human stem and precursor cells will migrate within the vascularised tumours of patients. A prospect for stem cell-based therapy for treating glioma patients includes implanting genetically modified stem cells into the remaining tumour after a surgical resection. One alternative could be to inject stem cells at multiple sites to target as many distant tumour satellites as possible. Subsequent stem cell implantations could be performed at later times using image-guided stereotaxic techniques. Irradiation can enhance tumour-tropic stem cell migration; therefore, a combined treatment with stem cells and radiotherapy may be effective[59, 60].

However, even though the basic findings support the use of stem cell vectors in tumour therapy, numerous hurdles should be overcome. Here, we outline the future advancements in four areas, which will be important for clinical cell therapy development for glioma treatment.

Stem cell biology

The divergent results obtained from studying the interaction between stem cells, in particular MSCs, and tumour cells (discussed previously and reviewed by Klopp et al.[41]) highlights the need for further studies on stem cell biology and the interplay between stem cells and gliomas *in vivo*. It is crucial to characterise the different MSC populations and to elucidate how they function with GBM tumor and stromal cells. Similarly, a deeper understanding of the

interaction between NSCs and GBM tissue can lead to a safer and more effective stem cell-based therapy against gliomas.

Animal models

Preclinical results have been obtained from the use of glioma animal models that have been established only a few days prior to the initiation of treatment. The results derived from such studies can be valuable; currently, however, the experimental glioma features do not necessarily resemble the complex GBM characteristics in a patient. To show the clinical potential, experimental studies need to be designed in a "clinical time frame" manner. In particular, it is important to develop effective experimental stem cell-based therapies against glioma animal models that are highly infiltrative, vascularised and cellularly and genetically heterogeneous at the onset of treatment.

Choice of cell type and transgene

It is difficult to compare the results of many of the preclinical studies because there are important differences in the experimental settings. Thus, it would be valuable to systematically compare the migratory capacity and the long-term fate (i.e., proliferation and survival) of the different tumour-tropic cell types in glioma models. Similarly, the choice of stem cell-delivered therapeutic transgenes should be evaluated in a more systematic and comparative way. The results from such studies would provide valuable information when planning clinical trials.

Imaging

Non-invasive imaging will be important for following the migration and survival of implanted/injected stem cells into gliomas. Bioluminescence imaging, magnetic resonance

imaging and positron emission tomography have been used to detect the administered stem cells in glioma animal models[21, 23, 83]. The different imaging modalities will provide information concerning the long-term fate of the implanted stem cells. Further technological improvements can provide a higher spatial resolution with the potential to trace stem cells at the single-cell level within gliomas.

CONCLUSIONS

Stem cell vectors may, by their capacity to target infiltrative tumour cells, provide a powerful treatment modality for GBM. However, many issues, including the choice of cell vector, choice of therapeutic transgene, the optimal route of administration and biosafety, need to be addressed. In light of the previous difficulties in translating experimental glioma therapy into successful clinical therapy, researchers should make a systematic and concerted effort to further examine the problems and the possibilities associated with stem cell vectors in order to clarify their clinical potential.

FIGURE LEGENDS

Figure 1. A) Phase-contrast micrograph of immortalised human NSCs after being subjected to cell culture conditions supporting neuronal differentiation. Insert in A) Human NSCs grown as free-floating spheres in cell culture medium stimulating proliferation. B) Immunofluorescent photomicrograph of human NSCs (red) infiltrating a human glioma (green) implanted into the immunocompromised mouse brain. C) Phase-contrast photomicrograph of human MSCs growing adherently on a plastic surface *in vitro*. D) Rat MSCs (red) infiltrating an invasively growing rat glioma (green) *in vivo*. Scale bar: 60 μm in

A (250 µm in inflicted neurosphere photograph), 150 µm in B, 100 µm in C, and 120 µm in D. Figure 1B is reproduced by permission of the publisher and the author[23].

Figure 2. Schematic illustration of the major mechanisms contributing to tumour-selective tropism of grafted stem cells in glioblastoma.

ACKNOWLEDGEMENTS

We thank Olle Lindvall and Arne Lindgren for providing valuable comments on the manuscript. Khalid Shah, Gesine Paul and Zaal Kokaia are acknowledged for providing photographs. This work was supported by the Swedish Cancer Society; the Swedish Childhood Cancer Foundation; the Crafoord Foundation; the Gunnar Nilsson Cancer Foundation; the Royal Physiographic Society in Lund; Magnus Bergvall Foundation; ALF (Government Public Health Grant); and the Skåne University Hospital in Lund.

CONFLICTS OF INTEREST STATEMENT

There are no conflicts of interest.

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Table 1. Selected preclinical stem cell-based glioma therapy studies

Therapeutic modification of stem cells	Substance	Cell type	Route of administration	Ref.
Pro-inflammatory cytokines	IL-4	NPC	co-injection/i.t.	16
	IL-7[a]	MSC	i.t.	72
	IL-12	NSC	i.t./i.c.	17
	IL-23	BM-NSLC	i.t.	71
	IFN-β	MSC	i.t.	10
Tumour apoptosis-inducing substances	TRAIL	NSC	i.t.	75
	S-TRAIL[b]	NSC	i.t.	79
	S-TRAIL	MSC	i.c.	77
	S-TRAIL	MSC	co-injection/i.t.	37
Pro-drug converting enzymes	CD[c]	NSC	co-injection	9
	HSV-tk[d]	NSC	*in vitro* study	85
	HSV-tk[d]	BM-TIC	i.t.	83
Oncolytic viruses	HSV-1	NSC	i.t.	91
	CRAd	MSC	i.c.	93
	CRAd	MSC	i.a.	94
Antibodies	scFv anti-EGFRvIII	MSC	co-injection/i.t.	96

Abbreviations: BM-NSLC, bone marrow-derived neural stem-like cell; CD, cytosine deaminase; CRAd, conditionally replicating adenovirus; EGFR, epidermal growth factor receptor; HSV-1, herpes simplex virus type 1; HSV-tk, herpes simplex virus type 1 thymidine kinase; i.a., intra-arterial; i.c., intracerebral (at a distance from the tumour); i.t., intratumoral; IL, interleukin; MSC, multipotent mesenchymal stromal cell; NPC, neural progenitor cell; NSC, neural stem cell; scFv, single-chain antibody fragment; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand. [a]Combined with peripheral immunotherapy. [b]Combined with PI-103. [c]Combined with 5-fluorocytosine. [d]Combined with ganciclovir.

Figure 1

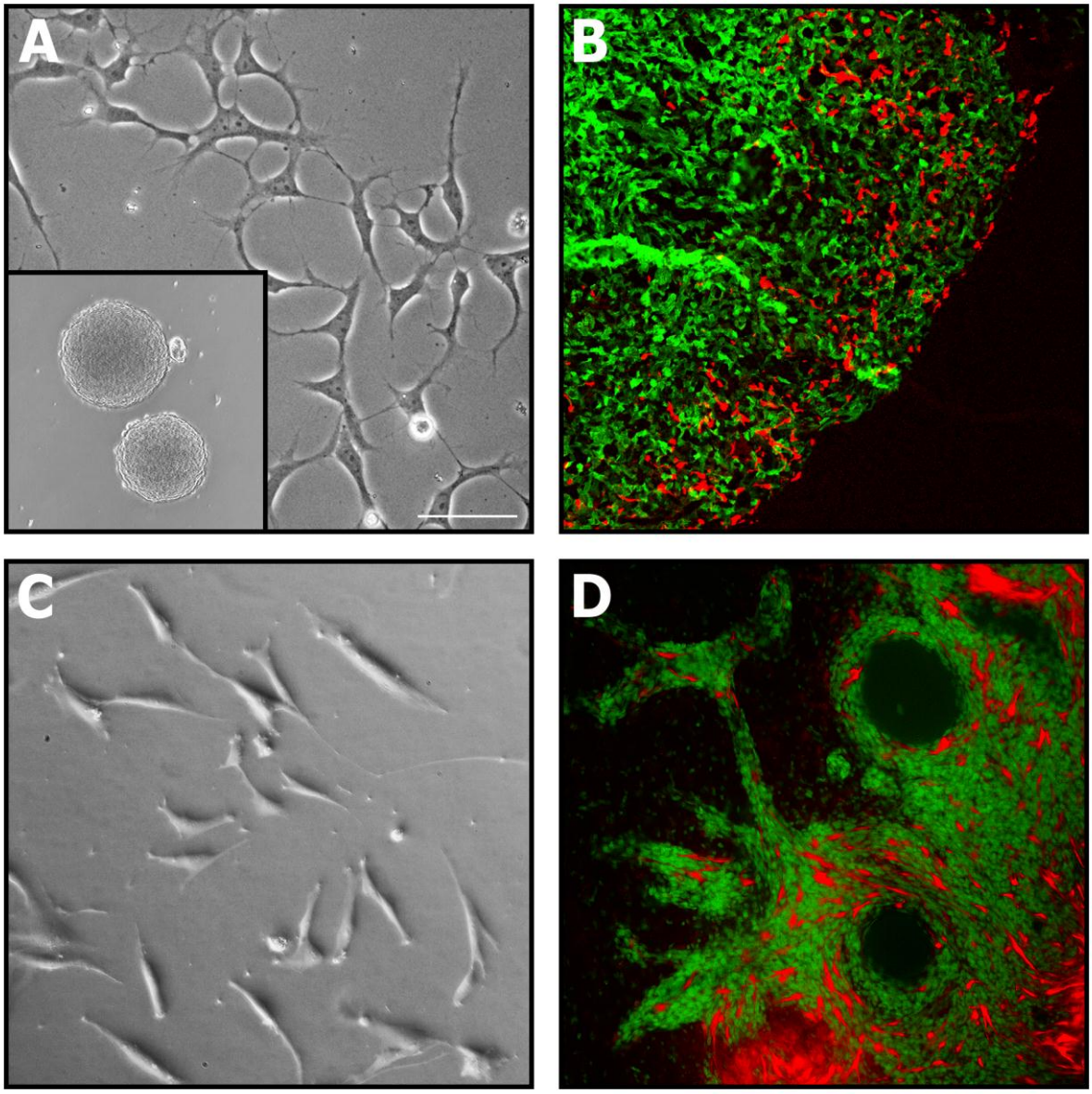
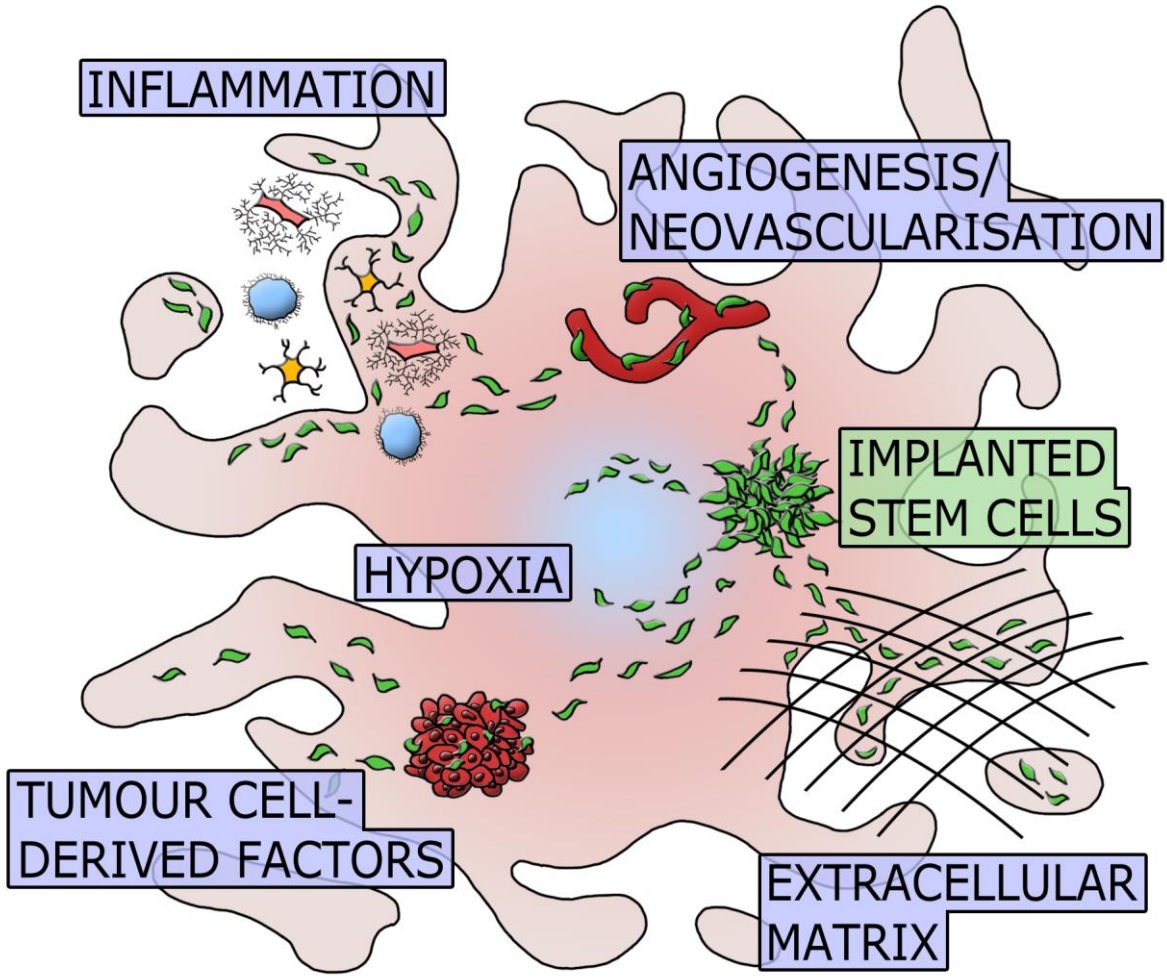


Figure 2


INFLAMMATION
ANGIOGENESIS/
NEOVASCULARISATION
IMPLANTED
STEM CELLS
HYPOXIA
TUMOUR CELL-
DERIVED FACTORS
EXTRACELLULAR
MATRIX