Stem Cell-Mediated Delivery of Therapies in the Treatment of Glioma

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Abstract: High grade gliomas can be seldom controlled, due to the infiltrative nature of these tumors and the presence of cell populations resistant to radio- and chemotherapy. Current research aims to develop novel therapeutic approaches to track and eliminate the disseminated glioma-driving cells. Selected delivery of therapeutic agents taking advantage of the tropism of normal stem cells for glioma cells might be one.

Keywords: Glioma, stem, gene therapy, cancer, resistance.

INTRODUCTION

High grade gliomas [World Health Organization (WHO) grade III and IV] are seldom-sparing tumors. After initial successful surgical and medical treatments, they almost invariably relapse leading patients to death in a few years (grade III) or months (grade IV) (reviewed in [1]). This is due to the infiltrative nature of these tumors that makes complete resection almost impossible and to the presence of selected populations of cells that survive radio- and chemotherapy and cause tumour recurrence and progression. One possible approach to drive therapies directly to residual brain tumour sites may take advantage of the recently discovered property of normal stem cells to migrate towards glioma cells [2-5].

NEURAL STEM CELLS

Use as Gene Delivery Vehicle

The observation that neural stem cells (NSC) migrate selectively towards brain tumour areas and linger there suggests to use these cells as drug and gene delivery vehicles [6-9]. In order to develop a therapeutic strategy targeting residual disease, human glioblastoma xenografts were inoculated with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-secreting NSC. Apoptotic cells increased in both the bulk of the tumour and in its satellites with significant reduction of tumour growth [10]. NSC tropism was further exploited in the same laboratory to generate an antitumor T-cell response. NSC producing interleukin 12 (IL-12) were inoculated in C57Bl/6 mice bearing orthotopic glioma tumors. Increased survival was observed in these mice in comparison to mice inoculated with non-secretory NSC or saline. Enhanced T-cell infiltration in tumor microsatellites and prolonged antitumor immunity were also observed [11]. Delivery of toxic genes to a malignant glioma *via* NSC has been described by Mercapide and co-workers [12]. NSC showed tropism towards glioma cell-conditioned medium and in adherent cell culture they tracked and fused with the tumour cells (Fig. **1**). Similar fusion hybrids could be formed in suspension cultures as well. NSC were then harnessed with the cyclophosphamide (CPA)-activating enzyme cytochrome p450 2B6 (CYP2B6), which catalyzes CPA transformation into DNA-alkylating metabolites and coinjected into mouse brain with glioblastoma multiforme (GBM) cells. NSC expressing CYP2B6 substantially impaired tumour growth after CPA administration (Fig. **1**). When the cells were injected at a distant site from the tumour, they migrated to the tumour area and could eventually delay tumour growth in the presence of CPA. In both the tumoral and normal tissues the migration track closely followed the pattern of expression of vascular endothelial growth factor (VEGF) which is a motility factor for NSC. Ito and co-workers [13] investigated the antitumoral effect of NSC engineered to express cytosine deaminase (CD) and interferon (IFN)-beta genes. Upon administration of 5-fluorocytosine (5-FC), CD/IFN-beta-expressing NSC exerted a bystander effect on human glioma cells *in vitro*. The bystander effect occurs by two major mechanisms: one involves cell-to-cell transfer of toxic metabolites through intercellular gap junctions; the other involves uptake of toxic metabolites by target cells through apoptotic vesicles produced by bystanding cells [2, 14]. In nude mice bearing orthotopic gliomas, infusion of the CD/IFN-beta-expressing NSC produced partial remission of the tumour upon administration of 5-FC and survival of animals was significantly increased (Fig. **2**). Hence, NSC producing CD and IFN-beta proteins may have a bystander effect on glioma tumors in the presence of 5-FC. The therapeutic potential of genetically engineered human NSC (HB1.F3) encoding CD and IFN-beta (HB1.F3-CD-IFNbeta) against orthotopic rat brainstem gliomas was evaluated by Lee *et al.* as well [15]. A 59% reduction in tumor volume and a 2.3 fold increase of apoptotic cells was observed in the HB1.F3-CD-IFNbeta-treated animals compared with the respective control groups (Fig. **3**), thus confirming that NSC genetically engineered to produce CD and IFN-beta may exert therapeutic efficacy against brainstem gliomas. The ability of NSC transduced with the herpes simplex virus thymidine kinase (HSV-1-tk) gene to migrate to regions

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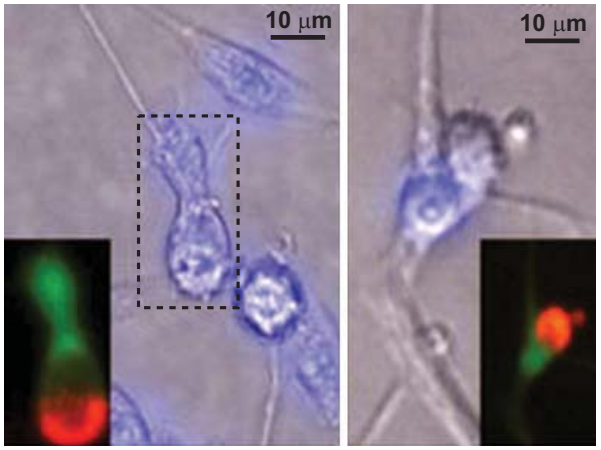
occupied by GBM cells has been further exploited recently to enhance delivery of HSV-1-tk to kill glioma cells [16].



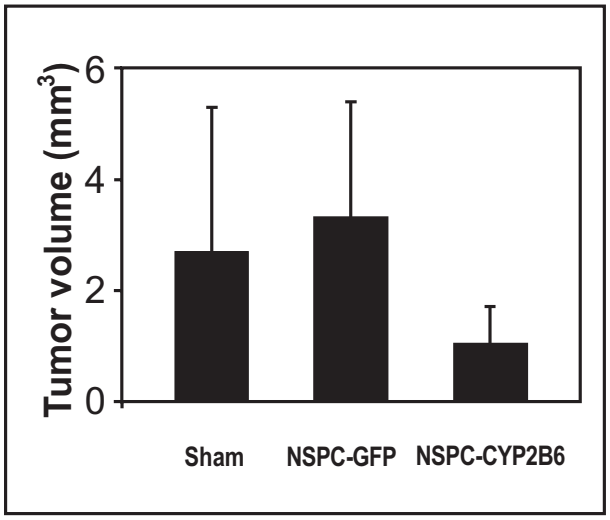
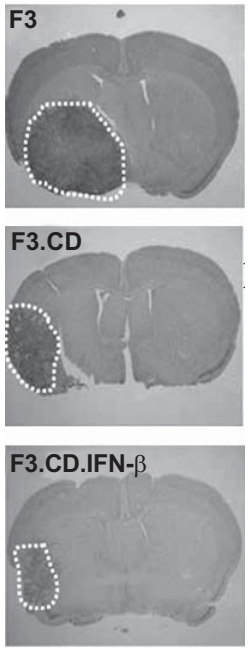


Fig. (1). Glioma-selective migration and antitumor effects of primary gene-engineered neural stem/progenitor cells (NSPC). Top. Membrane fusion in NSPC-GFP/U87 suspension co-culture cells double positive on GFP/CD44 immunofluorescence (insets). Nuclei stained with DAPI. Bottom. NSPC expressing CPA-activating enzyme CYP2B6, which catalyzes CPA prodrug transformation into membrane diffusible DNA-alkylating metabolites specifically target GBM grafts, after travelling through brain parenchyma. Upon CPA administration, NSPC containing CYP2B6 elicit substantial impairment of tumor growth through local activation of CPA (from ref. [12], with permission).

In order to identify human sources of cellular vehicles with glioma tropism other than human brain tissue, the established human NT2 cell line, which shares some features with NSC, was investigated [17]. After treating NT2 cells with retinoic acid, a subpopulation of NT2 cells that migrated towards human U87 GBM cells was isolated using Boyden chambers. In athymic immuno-incompetent nude mice, these cells settled into orthotopic U87 GBM xenografts after systemic administration *via* the tail vein. In order to investigate the potential use of these cells in targeted glioma therapy, the cells were transduced with the HSV-1-tk



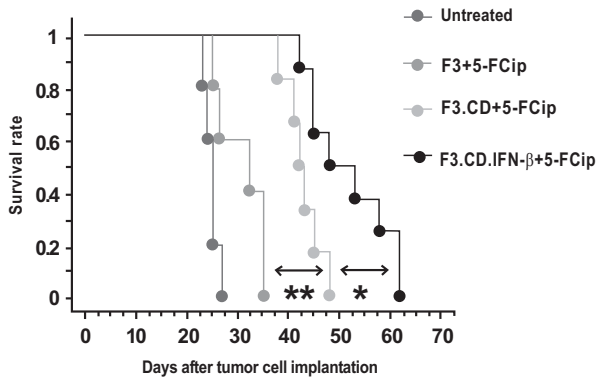


Fig. (2). The growth inhibitory effect of genetically engineered NSC on the glioma cells *in vivo*. Mice with a U251-derived intracerebral tumor were injected with F3 cells, and then i.p. injections of 5-FC were administered for the next 10 days. The volume of the tumour was assessed on day 28 after FC treatment (first picture from top). The residual tumour mass obtained in the group treated with F3.CD.IFN-β cells (third picture from top) was smaller than that obtained in the group treated with F3.CD cells (second picture from top). The survival time of experimental animals (plot). Mice were inoculated with U251 intracranially, and subsequently with each type of NSC (F3.CD.IFN- β, F3.CD and F3), followed by intraperitoneal injection of prodrug 5-FC and compared with untreated animals. Survival time was measured from U251 cell inoculation. The rates of survival of mice treated with F3.CD.IFN-β cells were significantly higher than those of mice treated with F3.CD; mice that received F3.CD therapy had significantly higher survival rates than those that received only F3 cells (from ref. [13], with permission).

gene and injected into the brain side contralateral to a site pre-inoculated with U87 GBM cells. Upon ganciclovir (GCV) injection, tumor growth was inhibited and tumor-inoculated animals survived significantly longer. NT2 cells were stable, easy to cultivate, and amenable to scale-up for

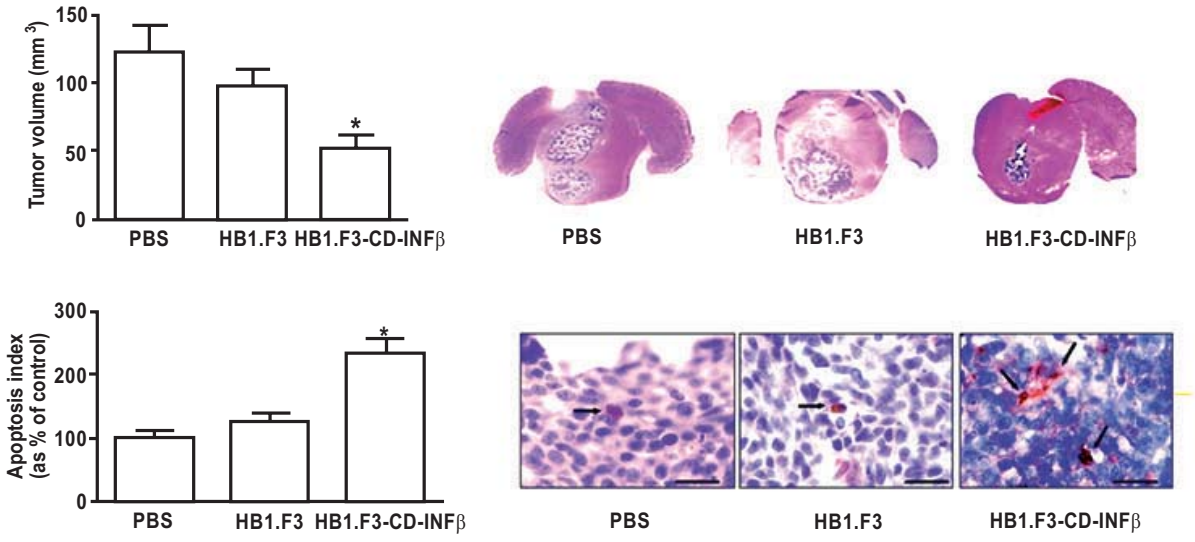


Fig. (3). *In vivo* therapeutic efficacy of genetically engineered human NSC encoding CD and IFN-beta (HB1.F3-CD-IFNβ) and treatment effects assessed by immunohistochemistry. Top left, the tumour volumes were estimated by histologic analysis. The average tumour volume after treatment with 5-FC in the surviving animals in the PBS-treated, HB1.F3-treated, and HB1.F3-CD-IFNβ–treated groups were progressively lower. A significant reduction in tumour volume was observed in the HB1.F3-CD-IFNβ–treated animals relative to that in the PBS-treated animals. Top right, representative histologic images (hematoxylin and eosin staining; magnification, ×1). Bottom left, histologic analysis revealed a significant increase in apoptotic cells in the animals treated with HB1.F3-CD-IFNβ compared with the various control groups. Bottom right, representative histologic images. Dark CASP3–positive cells are shown (arrows). Bar, 20 μm (from ref. [15], with permission).

cell production, thus possibly representing an NSC-like delivery vehicle for clinical cancer therapy [17].

Biological Properties of the Cell Vehicle

The rate and pattern of NSC migration to the tumor mass *in vivo* has been recently described [18]. After NSC injection, about 10% of NSC migrated into the tumour mass by 50 minutes. The number of NSC in the tumor region increased slowly during the first 5 days post-injection and faster during the following 10 days. The rate of NSC migration was approximately 175 microm/min. In the absence of tumor, the injected NSC increased in number approximately 1.7-fold during the first day but their proliferation began to decline at the sixth day after injection [18]. Labeling with ferumoxide-protamine sulfate complex (FE-Pro) has been proposed for real-time tracking of NSC in clinical trials. The Fe-Pro labeled cells retain their proliferative status, tumor tropism, and maintain stemness features, while allowing *in vivo* cellular magnetic resonance imaging (MRI) at 7 Tesla, to monitor their migration and distribution into the tumor mass.

Extracellular matrix (ECM) is subject to wide variations during glioma progression and invasion. Ziu and coworkers [20] have analyzed the effects on NSC migration of the ECM from six glioma tumoural cell lines or normal astrocytes as well as of purified ECM compounds known to be upregulated in the glioma environment. Tumor-produced ECM was highly permissive for NSC migration and tenascin-C was the strongest chemoattractant for NSC. Glioma ECM thus provides a permissive environment for selective migration of NSC to disseminated tumour areas [20].

In the hematopoietic system, monocyte chemoattractant protein-1 (MCP-1) is a chemokine that attracts monocytes, memory T lymphocytes, and natural killer (NK) cells. MCP-1 is often expressed by brain tumour cells while its receptor CCR2 is expressed by NSC. Experiments performed with a Boyden-type chamber assay have shown that NSC migrate towards the tumour expressing MCP-1 [21, 22]. Similar results were obtained using an *in vivo* rat orthotopic glioma model. NSC respond as well to hepatocyte growth factor (HGF) produced by glioma tumors [5]. The NSC response to HGF was mediated by c-Met as downregulation of c-Met by short hairpin RNA expression strongly reduced NSC gliomatropism. Inhibition of phosphoinositide 3-kinase (PI3K) signaling impaired as well the migration of NSC toward HGF and other growth factors. Tropism of NSC toward glioma cells is regulated by a number of chemotactic factors that probably converge on PI3K, causing direct modifications of the cytoskeleton. Those signaling pathways may be partially shared with those promoting tumour invasion [5].

Epidermal growth factor receptor (EGFR) expression and mutation in post-natal NSC may contribute to cellular aggressiveness including enhanced cellular proliferation and migration. Stable subclones of murine NSC were transfected to over-express the wild type EGFR or its most common mutated variant [EGFR variant III (EGFRvIII)]. Activated EGFR signaling in these cells enhanced proliferation,

blocked neuronal differentiation and was associated with a dramatic increase in chemotaxis in the presence of epidermal growth factor (EGF). Activated EGFR signaling may enhance the aggressiveness of NSC promoting their migratory and proliferative capacities [23].

NSC do not naturally reside in bone marrow that is mainly a hematopoietic tissue, but cells that express a few neuronal markers can be induced *in vitro* from bone marrow cells after specific culture conditions. NSC from bone marrow have been successfully isolated and propagated by Kabos and coworkers [24]. Cellular neurospheres able to differentiate to neurons and astrocytes were derived from unfractionated bone marrow cultured *in vitro* with EGF and basic fibroblast growth factor (FGF). The cellular spheres expressed both the neural stem cell marker nestin and the hematopoietic stem cell marker CD90. Bone marrow-derived NSC, which express the chemokine receptor CXC receptor 4 (CXCR4) have been isolated and characterized by Xu and co-workers [8]. When implanted on the brain tumor controlateral side, such bone marrow-derived NSC migrated as early as 24 hours after implantation and this migration was CXCR4-mediated, as indicated by its inhibition in the presence of a blocking anti-CXCR4 antibody. Besides CXCR4, progenitor/stem cell markers were expressed on glioma-tracking NSC. Despite these cells had low/null proliferation capacity, as shown by bromodeoxyuridine incorporation assays and proliferating antigen staining, they survived well in the tumour area with little apoptosis [8].

MESENCHYMAL STEM CELLS

Use as Gene Delivery Vehicle

Likewise NSC, mesenchymal stem cells (MSC) migrate towards glioma lesions and may be taken into consideration as gene delivery vehicles against human malignant gliomas [25]. Human bone marrow-derived MSC (hMSC) have been used as a cellular vehicle for the targeted delivery and local production of TRAIL at the glioma tumour site [26]. Secretable TRAIL (S-TRAIL) and mCherry (red fluorescent protein) were expressed by means of a lentiviral vector in hMSC that retained their migration ability towards the tumour area, as determined by *in vitro* and *in vivo* migration assays. The expression, release and biological activity of S-TRAIL were confirmed by *in vitro* assays. In *in vivo* experiments performed with orthotopic tumors grown in immunodeficient mice, injection of genetically engineered hMSC producing S-TRAIL resulted in inhibition of tumour growth and improved animal survival (Fig. **4**). Tumour cell apoptosis was increased eight fold in the animal group treated with the engineered S-TRAIL hMSC in comparison to controls (Fig. **4**). Hence, hMSC may serve as vehicles of therapeutic proteins to the tumour site and in particular, S-TRAIL-expressing hMSC may have some therapeutic effects against glioma. Similar conclusions were drawn by Yang and co-workers [27]. In this case, MSC were engineered with human TRAIL by a recombinant adeno-associated virus (rAAV). Again, the antiglioma effect of the TRAIL-producing MSC was evaluated on cultured glioma cells and in the *in vivo* human glioma xenograft model in athymic nude mice. There was no increase of apoptosis in TRAIL-producing MSC but their co-culture with U87 human GBM

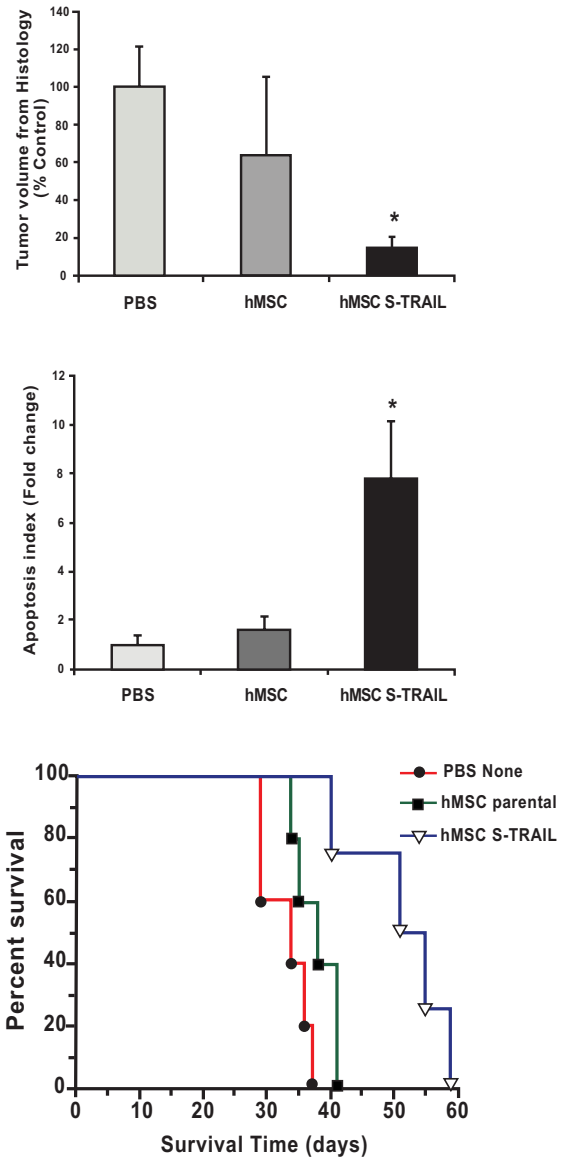


Fig. (4). Tumor volume and survival of U87 glioma-bearing mice treated with hMSC S-TRAIL - *in vivo* study. Top: Tumor volume was determined by quantification of the tumor area from histological sections. PBS alone versus hMSC S-TRAIL difference was significant. Middle. Apoptosis in hMSC S-TRAIL-treated tumors was quantitated by immunohistochemical staining for anticleaved CASP3 in brain sections. The percentage was calculated by counting positively stained cells from five randomly chosen microscopic fields per mouse. Bottom. Mice were injected with U87 cells followed by ipsilateral injection of hMSC S-TRAIL cells or hMSC or PBS on day 5. The survival advantage analyzed by a log-rank test based on the Kaplan–Meier method was statistically significant for PBS versus hMSC S-TRAIL cells and for hMSC versus hMSC S-TRAIL cells (from ref. [26], with permission).

cells caused a selective increase of apoptosis in the tumour cells. Transplanted MSC migrated to a brainstem glioma with high specificity. MSC reached the tumor area through the bloodstream and eventually surrounded the tumour in a chain pattern. Infusion of TRAIL-engineered MSC caused

increased tumoral cell apoptosis and prolonged animal survival as compared to infusion of phosphate-buffered saline (PBS), soluble TRAIL, or hMSC engineered with vector only [27]. The mechanism by which apoptosis is induced in glioma cells after transmembrane expression of TRAIL in MSC still is an open question. The possibilities include transmembrane TRAIL undergoing oligomerization at the cell membrane after binding to its receptor on glioma cells; receptor oligomerization in glioma cells after engagement of membrane-bound TRAIL; suitable ligand/receptor orientation and engagement; and bystander effect. All those possible mechanisms are consistent with the observation that glioma apoptosis seems less efficiently induced by TRAIL transduction of tumoural cells rather than TRAIL presentation by another cell [27].

The migration of human immortalized MSC bearing a human artificial chromosome (HAC) vector containing the HSV-1-tk gene (HAC-tk-hMSC) towards malignant gliomas *in vivo* has been investigated by Kinoshita and co-workers [25]. Labeling with green fluorescent protein (GFP) allowed tracking HAC-tk-hMSC migration by fluorescence microscopy and immunohistochemistry. The therapeutic efficacy *in vivo* was tested by treating HAC-tk-hMSC-injected - immunodeficient mice bearing an orthotopic glioma with GCV or PBS. Mice receiving GCV after HAC-tk-hMSC injection showed a partial reduction of the glioma mass [25]. Similar suicide gene therapy using MSC expressing HSV-1-tk combined with overexpression of connexin 43 (Cx43), which can restore the gap junction of intercellular communication and may enhance the bystander effect of glioma therapy has been described [14]. Co-culture experiments and transwell assays were used to assess the ability of MSC to migrate to C6 glioma tumour cells *in vitro*. HSV-1-tk/GCV treatment mediated by MSC inhibited C6 cell growth and this effect was enhanced after transfection of the tumoral cells with Cx43. The therapeutic gains were confirmed i*n vivo*: BrdU-labeled MSC injected controlateral to the C6 glioma tumor mass in rats could migrate to the tumour xenograft and inhibit its growth [14]. Adult MSC engineered with HSV-1-tk can exert a bystander toxic effect on murine 9L glioma cells as well [28]. Co-culture of 9L glioma cells and HSV-1-tk-transduced MSC [MSC/tk(+)] in the presence of GCV resulted in reduced proliferation and nuclear morphological changes of the glioma cells surrounding the MSC/tk(+). Intracerebral co-inoculation experiments in Fisher rats using 9L glioma cells and either MSC/tk(+) or MSC/tk(-) followed by intraperitoneal injection of GCV showed that the animals co-inoculated with 9L glioma cells and MSC/tk(+) exhibited inhibition of tumor growth and extended survival compared with the animals with 9L glioma cells and MSC/tk(-) [28] .

Replication competent viruses may further been used to lyse glioma cells. However, after injection at the tumour site, oncolytic adenoviruses fail to migrate to distant sites and are fastly eliminated by the immune system. Viral delivery and replication *in vivo* may be enhanced using human bone marrow-derived MSC as delivery vehicles [29, 30]. In particular, delta24-RGD is a conditionally replicative oncolytic adenovirus with ability to target GSC and induce autophagy in gliomas. The hMSC-mediated delivery of delta24-RGD has been explored by Yong and co-workers [31]. hMSC labelled with GFP and transduced with delta24-RGD (hMSC-delta24) were injected into the carotid arteries of immunodeficient mice bearing orthotopic U87 or U251 xenografts and brain sections were analyzed by immunofluorescence for GFP and viral proteins. hMSC-delta24 selectively settled into the tumour releasing the delta24-RGD virus that infected glioma cells. Glioma growth was inhibited and some tumour eradication events were observed in hMSC-delta24-treated animals. There were increased median survival and prolonged survival (beyond 80 days) in hMSC-delta24-treated animals as compared to controls. Hence, the intra-arterially delivered hMSC-delta24 may colonize the tumour mass and release selectively the delta24 therapeutic oncolytic adenovirus with ability to inhibit glioma growth and, in some cases, eradicate the tumour.

Nanotechnologies are currently investigated in glioma therapy in order to protect therapeutic agents and allow their sustained release. As for other therapeutic agents yet, specific targeting and extensive intratumoral distribution of nanoparticles (NP) still are far from our reach [32] . It has been recently observed that poly-lactic acid NP and lipid nanocapsules can be efficiently internalized into MSC with no loss of cell viability and differentiation capacity. These NP-loaded cells are able to migrate towards a human orthotopic glioma developed in athymic nude mice [32] .

Biological Properties of the Cell Vehicle

Likewise NSC (see section "**NEURAL STEM CELLS**"), the mechanisms of MSC mobilization towards the tumour remains poorly defined. The tumor-tropic migratory capacities of NSC and MSC have been compared [15]. Migration capacities were compared to that of human fibroblast cells using an *in vitro* modified transwell migration assay and an *in vivo* assay with stem cell injection into established brainstem gliomas in rats. All the stem cells showed a significant migratory capacity compared with that of the human fibroblasts indicating that there are no major differences in migratory capacities of MSC and NSC. Different MSC lines may display differential migration ability towards human glioma cells and this has prompted Ho and co-workers [33] to compare gene expression profiles of tumor-tropic MSC with those of non-tumor-tropic MSC. Matrix metalloproteinase (MMP) 1 expression and activity were higher in highly migrating MSC compared with poorly migrating MSC. By contrast, there was no change in the expression levels of other MMP. Functional inactivation of MMP1 abrogated tropism of highly-migrating MSC for glioma-conditioned medium and conversely, tumour tropism of non-migratory MSC was increased in the presence of either recombinant MMP1 or conditioned medium from the highly migrating MSC. Expression of MMP1 in these poorly migrating cells also made them responsive to tropic factors released by glioma cells. The migration capability was abrogated by blocking the interaction of MMP1 with its receptor PAR1 thus confirming that MMP1 may be involved in the migration capacity of MSC [33] .

Cultured glioma cells engineered to secrete high levels of platelet derived growth factors (PDGF) effectively attract hMSC as compared to low-secreting glioma cells. Anti-

PDGF or anti-PDGF receptor (PDGFR)-neutralizing antibodies abolished this tropism. *In vivo* experiments with the orthotopic glioma tumour model confirmed increased hMSC attraction by high PDGF-secreting tumors. Incubation of hMSC with neutralizing antibodies against PDGFR-beta decreased the migration towards the xenograft tumour. Hence, attraction of hMSC towards gliomas is mediated by PDGF produced by the tumor that interacts with PDGF-beta receptors present on hMSC [34]. MSC isolated from the umbilical cord blood (hUCB) as well migrate toward glioma cells *in vitro* and *in vivo* and induction of apoptosis of SNB19 glioma cells by hUCB *via* Fas-mediated caspase-8 activation has been observed [35]. Consistent with [34], *in vitro* migration of hUCB was partially dependent on the expression of PDGF from glioma cells and the migration pattern followed the local concentration gradient of PDGF. In animal studies, orthotopically implanted SNB19 cells attracted hUCB cells provided these latter cells were expressing PDGFR. Hence, MSC from various origins are capable of inducing apoptosis in human glioma cells and MSC tropism towards glioma cells is partially dependent on PDGF expression [35].

Likewise NSC, additional tropic factors produced by gliomas that attract MSC may include MCP-1 and stromal cell-derived factor (SDF) -1 alpha. MSC from rat bone marrow express receptors for MCP-1 and SDF-1 alpha such as CXCR4 and *in vitro* migration experiments showed that MCP-1 and SDF-1 alpha induce the migration of MSC towards gliomas [36].

MSC incubated with elevated doses (50 ng/ml) of tumor necrosis factor alpha (TNF-alpha) acquired glial cell morphology and expressed a number of neural genes including LIF (leukemia inhibitory factor), BMP2 (bone morphogenetic protein 2), SOX2 (SRY box 2), and GFAP (glial fibrillary acidic protein), whereas expression of the stem marker nestin was downregulated [37]. The TNF-alpha-mediated upregulation of neural genes in MSC was abolished after inhibition of extracellular signal-regulated kinase 1/2 (ERK1/2) activity. Further, TNF-alpha enhanced expression of the chemokine receptor CXCR4 which facilitated the chemotactic invasiveness of MSC. Increased ability to infiltrate glioma neurospheres by overexpression of MMP and tropism towards intracranial malignant gliomas were observed as well after TNF-alpha treatment of MSC. Hence, TNF-alpha may commit MSC to a neural phenotype that may turn useful as a source of NSC [37].

OTHER STEM CELLS

Adipose-derived stem cells (ADSC) can be infected with wild-type myxoma virus tagged with GFP (vMyxgfp) that replicates efficiently in them resulting in productive infection [38]. Co-culture experiments have shown that myxoma virus-infected ADSC may cross-infect and selectively kill GBM cells. *In vivo* migration experiments in immunodeficient mice in which vMyxgfp-ADSC were injected distantly from an orthotopic tumour, showed that the myxoma virus was delivered efficiently to the tumoural cells, thus improving animal survival. ADSC may be taken into consideration to deliver oncolytic viruses, specifically myxoma virus, to brain tumors. Hematopoietic progenitor cells (HPC) may be further candidates for cell-based delivery of therapies to gliomas. De Palma and coworkers [39] have exploited the tumour tropism of proangiogenic Tie2-expressing monocytes (TEM) for specific delivery of IFN-alpha to the tumour site. Using the orthotopic glioma model system in immunodeficient mice, these authors showed that hematopoietic progenitors transduced with a Tie2 promoter/enhancer-driven IFN-alpha gene could significantly reduce tumour growth and metastasis. Tumour angiogenesis was inhibited as well after TEM-mediated IFN-alpha delivery whereas innate and adaptive immunity were activated. Myelopoiesis and wound healing were not impaired. Delivery of IFN-alpha by TEM may thus contribute, at least in this experimental system, to inhibit glioma growth [39]. The genetic manipulation of HPC with lentiviral vectors and its impact on glioma tropism has been investigated [40]. Transduction of human or murine HPC with a lentivirus did not impair their attraction towards experimental gliomas. Importantly, the injection of lentivirus-transduced HPC into normal brains was not tumorigenic. Likewise, the injection of genetically-modified HPC did not alter survival of animals nor did it reduce the therapeutic effects of radiation treatment. Hence, genetic manipulation of HPC with lentiviral vectors for delivery of therapeutic agents is an apparently safe procedure.

CLINICAL RELEVANCE

Gene therapy may be a worth-investigating approach for some forms of high grade gliomas. This strategy has been hampered by inefficient delivery and expression of the therapeutic genes to the tumour by viral vectors [30]. One possible approach to improve gene therapy of high grade gliomas is delivery of viral vectors to the tumour site by normal stem cells. Using those carrier cells, both delivery and persistence of engineered therapeutic viral vectors are improved, in part as a consequence of the capacity of stem cells from different tissues to home at the tumour site and in part due to shielding of viral particles from the immunological system [41]. Solved one problem yet, one more soon appears: recent evidence indicates that progression of gliomas may be linked to the presence of rare subpopulations of cells [glioma initiating cells (GIC)] that initiate tumour development in limiting dilution assays, resist radio- and chemotherapy and often (but not always) exhibit the ability to differentiate under appropriate conditions to neurons, oligodendrocytes or astrocytes [42, 43]. Specific targeting of GIC might be a *condicio sine qua non* to eradicate the tumour. Unfortunately, whether normal stem cells may specifically target GIC still is an open question. Research on specific delivery of toxic agents to GIC by normal stem cells is required.

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ABBREVIATIONS

ADSC = adipose-derived stem cell(s)

BMP = bone morphogenetic protein

CD = cytosine deaminase

CPA = cyclophosphamide

Cx43 = connexin 43

CXCR4 = chemokine CXC receptor 4

CYP2B6 = cytochrome p450 2B6

ECM = extracellular matrix

EGF = epidermal growth factor

EGFR = EGF receptor

EGFRvIII = EGFR variant III

ERK½ = extracellular signal-regulated kinase ½

5-FC = 5-fluorocytosine

Fe-Pro = ferumoxide-protamine sulphate

FGF = fibroblast growth factor

GBM = glioblastoma multiforme

GCV = ganciclovir

GFAP = glial fibrillary acidic protein

GFP = green fluorescent protein

GIC = glioma initiating cell(s)

HAC = human artificial chromosome

HAC-tk-hMSC = human MSC bearing a HAC vector containing the HSV-1-tk gene

HGF = hepatocyte growth factor

hMSC = human MSC

hMSC-delta 24 = hMSC carrying the oncolytic adenovirus delta 24

hMSC S-TRAIL = human MSC producing TRAIL

HPC = hematopoietic progenitor cells

HSV-1-tk = herpes simplex virus thymidine kinase

hUCB = human umbilical cord blood

IFN = interferon

IL = interleukin

LIF = leukemia inhibitory factor

MCP-1 = monocyte chemoattractant protein-1

MMP = matrix metalloproteinase

MRI = magnetic resonance imaging

MSC = mesenchymal stem cell(s)

NK = natural killer

NP = nanoparticles

NSC = neural stem cell(s)

NSPC = neural stem/progenitor cell(s)

PBS = phosphate-buffered saline

PDGF = platelet-derived growth factor

PDGFR = PDGF receptor

PI3K = phosphoinositide 3-kinase

rAAV = recombinant adeno-associated virus

SDF = stromal cell derived factor

SOX = SRY box

S-TRAIL = secretable TRAIL

TEM = Tie2-expressing monocytes

TNF = tumour necrosis factor

TRAIL = tumour necrosis factor-related apoptosis-inducing ligand

VEGF = vascular endothelial growth factor

vMyxgfp = wild-type myxoma virus tagged with gfp

WHO = world health organization.

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