Use of genetically engineered stem cells for glioma therapy (Review)

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Abstract. Glioblastoma, the most common and most malignant type of primary brain tumor, is associated with poor prognosis, even when treated using combined therapies, including surgery followed by concomitant radiotherapy with temozolomide-based chemotherapy. The invasive nature of this type of tumor is a major reason underlying treatment failure. The tumor-tropic ability of neural and mesenchymal stem cells offers an alternative therapeutic approach, where these cells may be used as vehicles for the invasion of tumors. Stem cell-based therapy is particularly attractive due to its tumor selectivity, meaning that the stem cells are able to target tumor cells without harming healthy brain tissue, as well as the extensive tumor tropism of stem cells when delivering anti-tumor substances, even to distant tumor microsatellites. Stem cells have previously been used to deliver cytokine genes, suicide genes and oncolytic viruses. The present review will summarize current trends in experimental studies of stem cell-based gene therapy against gliomas, and discuss the potential concerns for translating these promising strategies into clinical use.

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1. Introduction

Gliomas account for ~30% of all brain tumors, and are the most common primary tumors of the central nervous system in Japan (1). Glioblastoma multiforme (GBM), the most common and most malignant type of glioma, has a median survival time of 14.6 months and a 5-year survival rate of <10%, despite various therapeutic strategies, including surgery, radiotherapy and chemotherapy with temozolomide (2,3). Complete surgical removal of GBM is not possible due to the invasive nature of gliomas to the surrounding healthy brain tissue, and the majority of patients die within 1 year of diagnosis as a result of a novel secondary tumor foci forming within 2 cm of the resected area (4,5). Residual tumor cells are typically resistant to standard radiotherapy, and efficient chemotherapy cannot be delivered due to the presence of the blood-brain barrier (BBB) and systemic toxicity (6). The potential effects of radiotherapy on GBM are limited by the associated toxicity to normal tissues. In addition, the efficiency of chemotherapy is also limited, as chemotherapeutic agents are unable to efficiently cross the BBB, while glioma cells also have a high tendency to develop resistance against chemotherapeutic agents. Therefore, novel therapeutic strategies to eliminate invasive tumor cells without damaging the normal brain parenchyma are urgently required (7-9).

Gliomas rarely metastasize outside of the central nervous system, and the majority of recurrence occurs proximal to the resection site, therefore malignant gliomas are recognized as good candidates for local gene therapy (10). One of the first and most widely used local gene therapies is that of the herpes simplex virus-thymidine kinase (HSV-tk)/ganciclovir (GCV) system. However, while clinical studies regarding the retrovirus-mediated HSV-tk/GCV gene therapy have been conducted, only clinical safety has been proven and no therapeutic benefits have been confirmed (10). Although promising results have been observed in studies of viral-mediated gene therapy in animal models of glioma (11), clinical studies have achieved limited success in the attenuation of tumor growth and extension of patient survival (12). These poor results associated with the use of the viral system are associated with, at least in part, the limited distribution of viral vectors throughout the invasive tumor (12). In order to improve the treatment field of local gene therapy, stem cells-based strategies were subsequently introduced.

Recent advances in neural stem cell (NSC) research suggest that the use of genetically engineered NSCs to

produce anti-tumor substances has notable advantages over viral vector-mediated gene delivery of therapeutic genes to gliomas, as NSCs exhibit extensive tropism for intracranial lesions, including gliomas (13). Numerous laboratories have replicated this migratory capacity using various types of stem cell, including multipotent mesenchymal stem cells (MSCs) (14,15), in animal models. In contrast to viral vectors, stem cells are primarily attracted to tumor tissue and not to normal neural cells, and therefore tumor-specific gene delivery is achieved, whilst minimal side effects are exerted on the normal brain tissue (13,16).

Over the past decade, significant attention has been paid to stem cell-based strategies as alternative therapies for the treatment of malignant gliomas. This is a result of the fundamental ability of stem cells to migrate to brain tumors, regardless of the BBB (13). Since then, a wide variety of stem cell-based therapeutics have been evaluated (17). Stem cells are relatively easy to modify to carry therapeutic genes (18) and exert immunosuppressive properties that may abrogate host immunoreaction following implantation (19-22). These cells are also capable of protecting oncolytic viruses from the host immune response, thereby establishing long-term supplies of the therapeutic virus at the tumor site (23).

The present review aims to summarize the current status of genetically engineered stem cell-based gene therapy for the treatment of glioma. The types of cell and therapeutic transgene to be used will be discussed in terms of efficacy and safety for translating experimental findings to a clinical setting.

2. Tumor tropism of stem cells

NSCs and other types of stem cell exhibit tropism for sites of tissue damage, as well as the tumor microenvironment, where a variety of substances are secreted (19), including inflammatory-derived factors and angiogenic factors. Activated astrocytic and microglial cells in the peritumoral edema zone generate an inflammatory tumor microenvironment in glioma (24,25). Interleukin (IL)-8 (26), monocyte chemotactic protein (MCP)-1 (27) and stromal cell-derived factor (SDF)-1α (28) in the peritumoral reactive region attract MSCs. Tumor necrosis factor (TNF)-α contributes to enhancement of the expression of the CXC chemokine receptor (CXCR) 4 on MSCs, which facilitates the chemotactic invasiveness of MSCs towards stroma-derived SDF-1α (29). This effect is also observed in induced pluripotent stem cells (iPSCs) (30,31). MCP-1 expression in gliomas may mediate glioma-tropic migration of NSCs via the CC chemokine receptor 2 (32). Therapeutic irradiation further enhances MSC tropism to glioma, via the inflammatory response (33,34).

Tumor tropism of MSCs is enhanced by tumor angiogenesis and angiogenic signaling molecules including platelet-derived growth factor (PDGF)-BB, PDGF-D (14,16), vascular endothelial growth factor (VEGF)-A, transforming growth factor-β1 and neurotrophin-3 (35,36). NSC migration is also influenced by angiogenic signaling (37). Hypoxia, a condition frequently associated with glioma, upregulates CXCR4, urokinase plasminogen activator receptor and VEGF receptor 2 on NSCs, which enhances their migration towards gliomas (38). The tumor tropism of iPSCs is enhanced by stem cell factor (SCF), PDGF-BB, SDF-1α and VEGF, and the receptors of those factors (c-Kit, intercellular adhesion molecule 1, CXCR4 and VEGFR-2) are upregulated in the iPSCs (30).

The interaction between MSCs and the extracellular matrix (ECM) is significant, particularly in highly migrating MSCs (39). As mentioned previously, multiple factors influence the tumor tropism of stem cells. Further studies are required to integrate these *in vitro* factors into a comprehensive mechanism underlying stem cell migration. Since the *in vivo* tumor microenvironment is markedly more complex, further *in vivo* cell-tracking studies are required for the development of improved clinical protocols using stem cells as therapeutic vehicles for the treatment of gliomas (40).

3. Types of cell vector

NSCs are mainly located in the subependymal zone of the lateral ventricles and the dentate gyrus of the hippocampus, and are capable of differentiating into neurons, astrocytes and oligodendrocytes (41). Since the first study regarding tumor-tropic migration of the immortalized murine NSC line C17.2 (13), numerous *in vivo* studies using NSCs to deliver anti-tumor substances to gliomas have been conducted (17,42-50). Although NSCs may be obtained, even from the adult human brain, it is not easy to quickly expand, modify and characterize these cells in preparation for implantation into GBM patients with a short life expectancy. Therefore, it is possible that immortalized NSC lines that are readily available may be used (https://clinicaltrials.gov/; identifier, NCT01172964). A well-characterized NSC line is able to be cultured and expanded *in vitro* to obtain high numbers of cells ready for transplantation within a short period, as long as the problems associated with immunogenicity and tumorigenicity are solved by, for example, steroid administration. In 2010, a clinical pilot trial using genetically engineered immortalized NSCs was initiated for patients with recurrent high-grade gliomas, where NSCs were applied at the time of surgery (https://clinicaltrials.gov/; identifier, NCT01172964).

MSCs are multipotent stem cells located in the bone marrow, adipose tissue, umbilical cord and placenta, which are able to differentiate into cells of mesenchymal lineage, including osteoblasts, adipocytes, chondrocytes and myocytes (51,52). It is significantly easier to obtain MSCs, for example, via bone marrow aspiration, than NSCs. The relatively easy availability of these cells makes it possible to graft autologous MSCs (isolated from the patient), facilitating the avoidance of graft rejection. However, the expansion, modification and characterization of these MSCs delays the initiation of treatment, compared with that of implantation of readily available, well-characterized, existing cell lines. In addition, there are a variety of concerns regarding the use of MSCs for gene therapy in the treatment of tumors. MSCs may contribute to tumor growth via their immunosuppressive function (53), growth factor production (54) and contributions to pro-tumorigenic stroma (55), as well as through malignant transformation of the recruited MSCs, which may induce tumor growth (53,56,57). The interaction between MSCs and tumor cells, and the potential risk of MSC transformation into malignant cells remain controversial (58-60). MSCs and NSCs have similar tumor tropism and infiltrative potential across the BBB (61). Intracranially implanted MSCs have demonstrated tropism for experimental gliomas, where

MSCs were able to successfully deliver therapeutic substances, thereby contributing to the increased survival of glioma-bearing model animals (14,15).

Hematopoietic progenitor cells are a readily available cell type, which exhibit marked glioma tropism (62,63). Implantation of human skin-derived stem cells, which are able to migrate to experimental gliomas and inhibit tumor angiogenesis, may present an autologous stem cell therapy for the treatment of gliomas (64). Systemically injected endothelial progenitor cells have been demonstrated to be able to target experimental gliomas and assimilate into the tumor vasculature (65,66). Endothelial progenitor cells have been genetically modified to produce oncolytic measles virus and tested as a potential anti-glioma therapy (67), or engineered to express cytotoxic anti-tumor genes (65). Embryonic stem cell-derived astrocytes have demonstrated intracranial migratory potential and therapeutic efficacy following implantation into subcutaneously established gliomas (68). In addition, NSCs derived from iPSCs have also been used as vectors in gene therapy for experimental glioma (69).

4. Cytokine-based therapy

Various types of cytokine have been delivered to gliomas, by NSC or MSC, and have demonstrated therapeutic efficacy (14,43,70,71). Positive therapeutic effects of intratumoral injection of IL-4-producing NSCs on murine glioma growth have been identified (42). NSC-produced IL-4 exerted more powerful anti-tumor effects than that of virus-mediated transfer of IL-4 (42). The capabilities of genetically engineered NSCs and MSCs expressing therapeutic cytokines IL-2 (72), IL-7 (70), IL-12 (43), IL-18 (73) and IL-23 (71) to augment the immune response to the tumor were also evaluated. TNF-related apoptosis-inducing ligand (TRAIL) activates the pro-apoptotic death receptors 4 and 5, which trigger caspase-8-dependent apoptosis (74). TRAIL is able to selectively target tumor cells, whilst sparing the majority of non-malignant cells (75). The tumor-specific therapeutic effect of TRAIL-producing NSCs, MSCs and ESC-derived astrocytes have been shown in several studies of experimental gliomas (76-79). A study, which simulated the clinical scenario of GBM treatment, demonstrated that inoculation of stem cells encapsulated in a biodegradable, synthetic ECM in the resection cavity following surgical debulking of human GBM tumors in mice, effectively inhibited tumor regrowth (80).

5. Enzyme/prodrug-based therapy ('suicide' gene therapy)

Enzyme/prodrug systems, which are also known as 'suicide gene therapies,' have been the most widely used type of gene therapy for glioma treatment. Among these systems, the HSV-tk/GCV system has been the most extensively studied. The HSV-tk gene phosphorylates non-toxic GCV into a toxic GCV-monophosphate in the cells, which is then further phosphorylated by cellular enzymes to GCV-triphosphate. Incorporation of GCV-triphosphate into the DNA results in chain termination of the DNA (10). Activated GCV is toxic not only to the HSV-tk-producing cells, but also to the cells in their vicinity, a phenomenon known as the 'bystander effect'. Since GCV-triphosphate is a relatively large molecule, the bystander effect is hypothesized to be mediated by gap junctions between cells, through which the phosphorylated prodrug is able to be transported (81). In addition, connexin 43 expression is important for the bystander effect (82). Migratory stem cell vectors have been introduced to achieve improved intratumoral distribution of the prodrug-converting enzyme. *In vivo* preclinical studies confirming the feasibility of this approach for the treatment of glioma have been conducted using NSCs (17,44,83) and MSCs (81,84-90) as HSV-tk delivery vehicles. It has also been proven that the 'bystander effect' of HSV-tk/GCV suicide gene therapy does not damage normal brain tissues (91).

Cytosine deaminase (CD) is another well-investigated prodrug-activating enzyme, which converts 5-fluorocytosine (5-FC) to its toxic form, 5-fluorouracil (5-FU), thereby inducing cell death (92). 5-FU is able to diffuse across cell membranes without requiring direct cell-to-cell contact, and exerts a marked bystander effect (93). Since the first report regarding the use of CD-expressing NCSs for the treatment of intracranial rat gliomas (13), multiple *in vivo* preclinical studies have demonstrated the treatment efficacy of the use of NSCs (18,93-95) and MSCs (96-98). As previously mentioned, a clinical pilot trial using immortalized NSCs engineered to produce CD, in combination with oral 5-FC administration, commenced in 2010. This pilot was for patients with recurrent high-grade gliomas, and aimed to examine whether intracerebral NSC implantation and systemic 5-FC administration was safe and feasible.

Rabbit carboxylesterase (CE) is able to convert, more efficiently than human CE (99), the prodrug CPT-11 into the cytotoxic drug 7-ethyl-10-hydroxycamptothecin, which functions as a potent inhibitor of topoisomerase I (100). Intratumoral injection of genetically modified MSCs expressing rabbit CE, in combination with systemic administration of CPT-11, modestly prolonged the survival of brainstem glioma-bearing rats (101). Cytochrome P450 2B6 (CYP2B6) catalyzes the transformation of cyclophosphamide (CPA) into the non-toxic metabolite, 4-hydroxy CPA (102). Co-cultures of CYP2B6-NSC with human CPA-treated U87 Mg glioma cells demonstrated significant bystander effect-mediated cytotoxic effects on tumor cells (102). A further *in vivo* study demonstrated that intracerebral inoculation of CYP2B6-NSCs, prior to intracerebral administration of CPA, effectively inhibited the growth of aggressive high-grade gliomas (46). Previous studies regarding these 'suicide gene therapies' are summarized in Table I.

6. Oncolytic virus-based therapy

Oncolytic virotherapy describes the process where viruses with the capacity to infect tumor cells are delivered to tumors. The viruses are able to replicate within and subsequently lyse the tumor cells. Following cell lysis, the viral particles are released and thus infect the neighboring tumor cells. However, the distribution of locally injected viruses throughout the tumor tissue and to invasive tumor cells is difficult. Furthermore, the viral particles may be attacked and neutralized by the host immune system prior to the exertion of any effects (103). To circumvent these obstacles, tumor-tropic migratory cells may be used to deliver viral particles to the distant parts of tumor and to protect against their attack by the immune system (23). Preclinical experiments using NSCs (49,104,105) and MSCs (106-108) have demonstrated extended delivery of

Table I. Summary of previous preclinical stem cell-based enzyme/prodrug therapies for brain tumors.

Cell line (species)	Tumor type (species)	Experimental animal	Enzyme gene	Prodrug	Ref	Year
NSC (rat)	C6 (rat)	SD rat	HSV-tk	GCV	(17)	2005
NSC (mouse) C17.2	CNS-1 (rat)	Nude mouse	CD	5-FC	(12)	2000
NSC (rat) ST14A	C6 (rat)	SD rat	CD	5-FC	(94)	2003
NSC (human) HB1.F3	Daoy (human)	Nude mouse	CD	5-FC	(95)	2007
NSC (human) HB1.F3	Neuroblastoma (human)	SCID mouse	rCE	CPT-11	(100)	2007
NSC (mouse)	GL261 (mouse)	C57Bl/6 mouse	CYP2B6	CPA	(102)	2008
NSC (mouse)	U87 (human)	Nude mouse	CYP2B6	CPA	(46)	2010
MSC (rat)	9L (rat)	Fischer rat	HSV-tk	GCV	(84)	2007
MSC (rat)	9L (rat)	SD rat	HSV-tk	GCV	(85)	2009
MSC (rat)	C6 (rat)	SD rat	HSV-tk	GCV	(86)	2009
MSC (human) iv	8-MG-BA (human)	Nude mouse	HSV-tk	GCV	(81)	2010
MSC (human) iv	U87 (human)	Nude mouse	HSV-tk	GCV	(89)	2010
MSC (rat)	9L (rat)	Fischer rat	HSV-tk	GCV	(88)	2010
MSC (human)	A172, T98G (human)	Nude mouse	HSV-tk	GCV	(90)	2012
MSC (rat)	9L (rat)	Nude mouse	CD	5-FC	(98)	2012
MSC (human)	C6 (rat)	SD rat	CD:UPRT	5-FC	(97)	2012
MSC (human)	F98 (rat)	Fischer rat	rCE	CPT-11	(101)	2012

NSC, neural stem cell; MSC, mesenchymal stem cell; SD, Sprague-Dawley; HSV-tk, herpes simplex virus-thymidine kinase; CD, cytosine deaminase; rCE, rabbit liver carboxylesterase; CYP2B6, cytochrome P450 2B6; UPRT, uracil phosphoribosyltransferase; GCV, ganciclovir; 5-FC, 5-fluorocytosine ; CPT-11, camptothecin-11; CPA, cyclophosphamide.

oncolytic viruses and prolonged survival of glioma-bearing animals treated with stem cell-mediated oncolytic virotherapy.

7. Conclusion

Neural, mesenchymal and other types of stem cell, engineered to express various therapeutic genes are attractive candidates for use in the treatment of malignant glioma patients. For the evaluation of such novel treatment strategies, cases of glioma which are recurrent following standard therapy, including radiotherapy with temozolomide-based chemotherapy, should be selected for the study cohort. Implanting genetically modified stem cells into any remaining tumor tissue following surgical resection, or stereotactically injecting stem cells into the unresectable tumor are two potential treatment modalities (17). These treatments may prolong the period of re-remission, without inducing the serious side effects associated with more extensive chemotherapeutic strategies (6). In addition, repeated treatment may be possible as a number of the treatment substances employed are essentially non-toxic to humans.

However, despite the abundance of basic findings that support the use of stem cell vectors in tumor therapy, there are also several issues regarding the translation of this strategy into a clinical setting. These issues include the choice of cell vector and therapeutic transgene, as well as the optimal route of administration (for example, intratumoral or intravenous administration). Teratogenicity of the stem cells, particularly that of MSCs, is one of the most significant concerns to be solved prior to the commencement of clinical studies, even when the targets of treatment are fatal diseases, for example GBM. The interaction between MSCs and tumor cells and the potential risk of MSCs transforming into malignant cells remain controversial (58-60). Therefore, the use of readily available well-characterized MSCs, for example the C17.2 NSC line, is suggested. The use of stem cells transduced with suicide genes, for example the HSV-tk gene, to eliminate transplanted therapeutic stem cells may function as an additional 'safety valve'. As a result of the promising preclinical results regarding the use of stem cell-based therapy for glioblastomas, clinical studies should be conducted under careful clinical protocols, including sophisticated imaging techniques for evaluating the fate of the implanted stem cells (40).

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