Abstract: Glioblastomas are aggressive intrinsic brain tumors. The median overall survival does not exceed 15 months despite surgical resection, radiotherapy, and chemotherapy even in selected clinical trial populations. One reason for this poor outcome is the characteristic infiltrative growth pattern of glioblastomas with tumor cells deeply infiltrating into the normal brain parenchyma and thereby escaping surgical debulking and involved-field radiation therapy. Novel therapeutic strategies are urgently needed including those that target disseminated tumor cells, too. In this regard, the application of adult stem cells as cellular vehicles for the delivery of therapeutic molecules has emerged during the last decade as an experimental approach. Adult stem cells with a tropism for gliomas include neural stem and progenitor cells, mesenchymal stem cells, hematopoietic progenitor cells, and endothelial progenitor cells. Importantly, these candidate cellular carriers also localize to sites of hypoxia and invasive tumor borders which are usually not targeted by currently available therapeutic approaches. Stem cell-based therapeutic approaches could therefore help to overcome some of the current limitations of radio- and chemotherapy and may circumvent toxicity to normal resident cells of the central nervous system. The development of neural stem- and progenitor-based therapies is advanced with a currently ongoing phase I clinical study. We review rationale, achievements, and future challenges in this field.

Introduction

Tumors in the brain are either primary brain tumors, i.e., neoplasms that arise from resident cells of the central nervous system (CNS), or metastases, i.e., lesions that are derived from tumor cells originating from outside the CNS. Primary brain tumors comprise a heterogeneous group of neoplasms and are classified into four ascending grades of malignancy according to the World Health Organization (Louis et al., 2007). Gliomas, i.e., tumors thought to be of glial origin, are the most frequent cluster and include astrocytomas, mixed gliomas, oligodendrogliomas, and ependymomas. Grade I gliomas occur almost exclusively in childhood. Grade III-IV gliomas are referred to as high-grade or malignant gliomas. Glioblastomas are grade IV glial tumors arising either from lower grade gliomas, so called secondary glioblastomas, or apparently de novo. Of note, the precise cellular origin of primary brain tumors has remained elusive. The stem cell hypothesis was generated in the last decade, proposing that brain tumors are hierarchically organized and maintained by a small subpopulation of glioma stem-like cells. The cell of origin might be a normal neural stem or progenitor cell that has undergone malignant transformation or a mature resident cell of the central nervous system that dedifferentiated (reviewed in Tabatabai and Weller, 2011).
Stem Cell-mediated Gene Therapies for Malignant Gliomas: A Promising Approach

Placenta. Intraarterially injected bone marrow-derived MSC migrate towards intracerebral U87MG, U251, or L229 gliomas. MSC were genetically engineered with retroviral vectors to express therapeutic genes and were used as cellular carriers that display a tropism for glioma cells. The formation of neurospheres in culture was observed (Gritti et al., 1996). In addition to primary cultures, immortalized NPC cell lines have been generated, e.g., the murine cell line C17.2 (Snyder et al., 1992) or the human HBI.F3 line (Kim et al., 2002).

Primary NPC from C57BL/6 mice were transduced with interleukin-4 (IL-4). IL-4-producing primary NPC were co-injected with syngeneic GL-261 glioma cells at a ratio of 1:1 into the brain of C57BL/6 mice. Six of seven mice injected with GL-261 cells injected with IL-4-releasing NPC were still alive at day 90 whereas all animals of the respective control group died within 30 days. Moreover, the treatment of established GL-261 gliomas or C6 gliomas was more effective with NPC-based delivery of IL-4 than retrovirus-mediated delivery of IL-4 (Benedetti et al., 2000). The advantage of using NPC as cellular carriers was underscored by a study demonstrating that LacZ-expressing NPC could be exploited as carriers for a ribonucleotide reductase-deficient HSV-1 mutant virus that only replicates in dividing cells. In this study, an immortalized neural stem cell line, C17.2, was used. To circumvent death of these cells by the virus load, cells were pretreated with ramosine and thereby arrested in G1 phase. HSV-1 virus-infected C17.2 cells were injected into intracerebral C57BL/6 mice in nude mice with an estimated C17.2/tumor cell ratio of 1:5 to 1:10. Three days later, the brains were harvested for histological analysis. Galactosidase staining showed that C17.2 cells had migrated extensively throughout the tumor and even into the surrounding parenchyma. The tumor mass and disseminated tumor metastasis was significantly reduced in MSC-transplanted EAE mice indicating an immune-suppressive effect (Karnoub et al., 2007). Further, MSC have immunosuppressive effects as demonstrated in models of experimental autoimmune encephalitis (EAE). The evaluation of biopsies and autopsies from patients who were treated with this cell-based gene therapeutic approach showed very low rejection by the immune system. Encapsulated cells were injected in C6 gliomas. Intravital video microscopy revealed an anti-angiogenic effect and reduced tumor sizes (Bjerkgv et al., 2003).

Despite these promising experimental findings, fibroblasts and HEK293 cells are most likely not the appropriate vehicle since they are not motile and therefore the delivery of therapeutic load to invading tumor cells is probably precluded. Consequently, cellular carriers with migratory ability and a tropism for glioma cells would be more attractive candidates to overcome this problem. In this regard, adult stem cells have gained attention in the last decade. Studies using neural, mesenchymal, hematopoietic, or epithelial stem and progenitor cells are discussed in the following paragraphs.

**Neural Stem Cells**

Primary neural stem and progenitor cells (NPC) can be isolated from the cortex of mice or rats, e.g., on postnatal days 1-4. The cortex-derived cell suspension can be plated on untreated tissue culture plates in the presence of recombinant epidermal growth factor and fibroblast growth factor in serum-free medium. The formation of neurospheres in culture was rapidly observed (Gritti et al., 1996). In addition to primary cultures, immortalized NPC cell lines have been generated, e.g., the murine cell line C17.2 (Snyder et al., 1992) or the human HBI.F3 line (Kim et al., 2002).

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MSC-based therapeutic approaches are summarized in Table 1.

Mesenchymal stem cells (MSC) are isolated from various sources including autologous, e.g., bone marrow or peripheral blood, and allogeneic sources, e.g., umbilical cord blood and placenta. Intraarterially injected bone marrow-derived MSC migrate towards intracerebral U87MG, U251, or L229 gliomas. MSC were genetically engineered with retroviral vectors to express therapeutic genes and were used as cellular carriers that display a tropism for glioma cells. The formation of neurospheres in culture was observed (Gritti et al., 1996). In addition to primary cultures, immortalized NPC cell lines have been generated, e.g., the murine cell line C17.2 (Snyder et al., 1992) or the human HBI.F3 line (Kim et al., 2002).

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### Table 1: Summary of MSC-based Therapeutic Approaches

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<th>Approach</th>
<th>Summary</th>
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<tr>
<td>Retroviral vectors</td>
<td>Co-injected with 9L glioma cells. After systemic application of ganciclovir the tumor volumes were significantly reduced. However, vector-transduced dividing cells. Replication deficiency of MLV was achieved by removing parts of the viral genome encoding for reverse transcriptase, envelope, and capsid.</td>
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<tr>
<td>TRAIL releasing human MSC</td>
<td>Injected into intracerebral CNS-1 rat gliomas in nude mice with an estimated C17.2:tumor cell ratio of 1:5 to 1:10. Three days later, the brains were harvested for histological analysis. Galactosidase staining showed that C17.2 cells had migrated extensively throughout the tumor and even into the surrounding parenchyma. The tumor mass and disseminated tumor metastasis was significantly reduced in MSC-transplanted EAE mice indicating an immune-suppressive effect (Karnoub et al., 2007). Further, MSC have immunosuppressive effects as demonstrated in models of experimental autoimmune encephalitis (EAE). The evaluation of biopsies and autopsies from patients who were treated with this cell-based gene therapeutic approach showed very low rejection by the immune system. Encapsulated cells were injected in C6 gliomas. Intravital video microscopy revealed an anti-angiogenic effect and reduced tumor sizes (Bjerkgv et al., 2003).</td>
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<td>Interleukin-4</td>
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Endogenous bone marrow-derived cells including hematopoietic and endothelial progenitor cells may contribute to glioma vasculogenesis (Koo et al., 2010). Both progenitor cell types can be isolated from the bone marrow and from peripheral blood. The human CD34 antigen is a reliable marker for enrichment of a small fraction of human bone marrow and periphery residing hematopoietic stem cells (Andrews et al., 1994). Marine HPC can be isolated from the bone marrow, e.g., by cryoablation, depletion of lineage-committed progenitor cells, and further positive selection for cells that express stem cell antigen 1 (Vajkoczy et al., 2005). De Palma et al. (2008) transplanted HPC that were ex vivo with a Ti2c promoter-driven interferon (IFN) alpha gene into myeloablative irradiated mice. Ti2c-IFN-alpha expressing cells were attracted by orthotopic intracerebral gliomas and tumor angiogenesis was inhibited (De Palma et al., 2008). Intravenously injected marine or human HPC displayed a tropism for intracerebral experimental gliomas and tumor angiogenesis was inhibited (De Palma et al., 2008).

Another approach to overcome the limitations of bone marrow-derived stem cells is ex vivo gene therapy. HPC transplantation has been successfully performed in a mouse model for Huntington’s disease (Amariglio et al., 2009). The first clinical trial of human mesenchymal stem cells was performed in a patient with Huntington’s disease (Amariglio et al., 2009). A patient with Huntington’s disease received intrastriatal fetal neural stem cell transplantation. Four years after the first treatment multifocal brain tumors occurred. The biopsied tumor was diagnosed as a glioneuronal neoplasm. Molecular and cytogenetic studies revealed that the tumor was derived from the transplanted NSC (Amariglio et al., 2009). A young male patient with ataxia telangiectasia received intracerebral neural transplantation. At the age of 13, he developed multifocal brain tumors that were diagnosed as gliomas with features of adult stem cell origin (Kuwamura et al., 2008). This supports the hypothesis that NSC might be used for gene therapy also in human diseases with NSC contribution.

Discussions and Outlook

The success of these approaches critically depends on the availability of the stem cell population for clinical use and on the ability of the cellular carrier to deliver sufficient amounts of therapeutic molecules to the tumor. The development of NSC-based therapies is hindered by several current limitations of the procedures and cell properties. One of the most serious current limitations is the lack of effective tools for NSC delivery and the inability to monitor the fate of transplanted NSC. The development of imaging strategies for detection of transplanted NSC opens new perspectives for clinical application of NSC therapies. The development of effective imaging strategies is an essential requirement for successful clinical application of NSC therapies.

The authors do not have any conflicts of interests to declare.

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