Toward Brain Tumor Gene Therapy Using Multipotent Mesenchymal Stromal Cell Vectors.

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REVIEW:

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Short title: MSCs as vectors in glioma therapy

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Abstract:

Gene therapy of solid cancers has been severely restricted by the limited distribution of vectors within tumors. However, cellular vectors have emerged as an effective migratory system for gene delivery to invasive cancers. Implanted and injected multipotent mesenchymal stromal cells (MSCs) have shown tropism for several types of primary tumors and metastases. This capacity of MSCs forms the basis for their use as a gene vector system in neoplasms. Here, we review the tumor-directed migratory potential of MSCs, mechanisms of the migration and the choice of therapeutic transgenes, with a focus on malignant gliomas as a model system for invasive and highly vascularized tumors. We examine recent findings demonstrating that MSCs share many characteristics with pericytes and that implanted MSCs localize primarily to perivascular niches within tumors, which might have therapeutic implications. The use of MSC vectors in cancer gene therapy raises concerns, however, including a possible MSC contribution to tumor stroma and vasculature, MSC-mediated anti-tumor immune suppression and the potential malignant transformation of cultured MSCs. Nonetheless, we highlight the novel prospects of MSC-based tumor therapy, which appears to be a promising approach.

Introduction

Tumor invasiveness and metastasis are the main causes of death in cancer patients and present challenging scientific and clinical problems. Glioblastoma multiforme (GBM) is an aggressive and invasive neoplasm characterized by extensive neovascularization. GBM cells grow in a highly invasive pattern along blood vessels and white matter tracts in the brain. The median survival time for GBM patients undergoing conventional treatment (i.e., surgery, radiotherapy and chemotherapy) is only 14.6 months[1]. The shortcomings of conventional GBM therapy can be attributed, at least in part, to a failure to target the invasive tumor cells. It is therefore obvious that effective treatment against GBM and other highly invasive tumors must include the killing not only of cancer cells in the main tumor mass but also of tumor cells that have dispersed deeply into surrounding normal tissue. Experimental evidence suggests that the growth of GBM is maintained by cancer stem cells (CSCs) residing within a perivascular niche[2, 3]. CSCs are a subpopulation of cancer cells that maintain and propagate tumor growth[4]. However, whether CSCs are present in GBM is still a matter of controversy[4].

This article reviews evidence that multipotent mesenchymal stromal cells can act as a gene therapy vector system with the potential to migrate to and within invasive solid cancers. We focus on GBM as a model for invasive tumors, but we also include findings from other experimental tumor models.

Multipotent mesenchymal stromal cells

Bone marrow mononuclear cells contain a rare population of non-hematopoietic mesenchymal progenitor cells that, in *in vitro* culture, adhere and give rise to fibroblastoid colonies (fibroblastoid colony-forming units (CFU-Fs)). Upon further culture, these cells are referred to as mesenchymal stromal cells or marrow stromal cells (MSCs). Cultured MSCs have also been

commonly (and incorrectly) called mesenchymal stem cells; however, cultured MSCs do not fulfill stringent stem cell criteria, in contrast to their uncultured *in vivo* precursors. Nevertheless, cultured MSCs possess a number of intriguing properties (such as proliferation and differentiation capacities, stroma function and immunomodulatory properties) that make them suitable candidates for cell therapy applications.

MSCs display adipogenic, chondrogenic, osteogenic and myogenic differentiation capacities **(Figure 1 a-c)** and possibly others [5, 6]. For a review on MSC differentiation capacities, see Caplan[5]. At present, no single surface marker is available that specifically identifies MSCs. Therefore, MSCs are defined by the expression of combinations of certain surface markers, including CD73+, CD90+, CD105+, CD146+, CD271+ and STRO-1+, and by the lack of expression of hematopoietic markers, such as CD34 and CD45 [7, 8]. Additional properties of MSCs include the capacity to form a hematopoietic microenvironment that is capable of supporting the long-term maintenance and differentiation of hematopoietic stem cells[9]. MSCs play an important role in tissue regeneration and have been used to experimentally repair tissue damage in various disease conditions[10]. MSCs also possess immunosuppressive properties through the modulation of cytotoxic T-cells, antigen-presenting cells, natural killer cells and B-cells[11], and several ongoing promising clinical studies are investigating the potent immunomodulatory effect of MSCs (e.g., in patients who have developed severe acute graft-versus-host disease after allogeneic stem cell transplantation)[12].

Most of the available information concerns human MSCs, especially for bone marrow-derived MSCs, because human MSCs are relatively easy to culture (in contrast to other species, such as mice). In addition to bone marrow, culture of MSCs has been reported from most other organs, including adipose tissue, skeletal muscle, pancreas, placenta, dental pulp and umbilical cord blood[6, 8]. In this review, we focus on bone marrow-derived MSCs except where

otherwise stated. Recent findings indicate that primary MSCs are localized perivascularly and that MSCs share many properties with pericytes. However, not all primary MSCs are found adjacent to vessels, and not all pericytes are MSCs[6, 13, 14].

MSCs display tumor-tropic capacities

Gene therapy utilizing viral vectors to deliver anti-tumor substances has been successful in experimental cancer studies, but most clinical studies have had only limited success[15]. The inefficient spread of vectors within the tumor and the inability to reach invasive tumor cells distant from the tumor bulk can, at least in part, explain these shortcomings. In light of this, the discovery that implanted neural stem cells (NSCs) are able to migrate throughout normal brain tissue to experimental gliomas, where NSCs can deliver a cytotoxic substance, is promising[16]. Notably, the implantation of interleukin-4-producing NSCs into gliomas shows considerably better therapeutic efficiency than the retrovirus-mediated *in vivo* transfer of interleukin-4[17]. Subsequently, NSCs, MSCs, endothelial, hematopoietic, skin-derived and endometrial precursor cells have been utilized as migratory cellular vectors to tumors[18-23].

The first evidence of the tropism of MSCs to gliomas was demonstrated by implantation of rat MSCs into rats bearing syngeneic gliomas[20]. Intracranially implanted MSCs were found to migrate to and disperse throughout the tumor mass. MSCs are also able to migrate along the corpus callosum toward established gliomas in the contralateral hemisphere[20, 24]. The tumor-tropic migratory capacity of MSCs is further strengthened by findings that human MSCs specifically home to human gliomas of immunocompromised mice following injections into the ipsilateral and contralateral carotid arteries[24]. Another research group showed that rat bone marrow-derived multipotent adult progenitor cells (MAPCs), a population of progenitor cells distinct from MSCs, implanted directly into rat gliomas or in the vicinity spread extensively

within gliomas, whereas implanted rat fibroblasts do not migrate but remain at the injection site adjacent to the tumors[25]. Our laboratory has shown that intratumorally implanted rat MSCs possess the ability to migrate to invasive rat glioma extensions and distant tumor microsatellites. MSCs, however, largely avoid normal brain gray matter **(Figure 1 d-e)**[26]. The attraction of MSCs to tumors is not limited to gliomas but has been reported for several experimental tumor models, including malignant melanoma[27], Kaposi's sarcoma[28], colon cancer[29], ovarian cancer[30], pancreatic cancer[31], Ewing sarcoma[32], fibrosarcoma[33], breast cancer[34] and renal cell carcinoma[35]. The tumor-specific migratory pattern makes it possible to utilize intratumorally administered MSCs for delivery of toxic substances to the main tumor mass, as well as to invasive parts of the tumor, without adverse effects on normal brain tissue. Indeed, the tumor-specific migratory capacity of implanted/injected MSCs is fundamental for the development of MSCs as vehicles in cancer therapy. In this respect, MSCs show clear therapeutic advantages compared to vehicles with a more limited distribution potential, such as viruses, antibodies, nanoparticles and liposomes.

MSCs have been delivered to a variety of different tumor models using intravenous (i.v.)[27], intra-arterial[24] and intraperitoneal injections[30] and intracerebral[24], intracerebroventricular[36] and intratumoral implantation[20], as well as intratracheal administration[37]. Both the route of MSC delivery and tumor localization have a decisive influence on the extent of MSC engraftment into tumors. Intratumoral implantation of rat MSCs results in a much more efficient distribution of MSCs within rat gliomas compared to i.v. administration[26]. However, i.v.-injected human MSCs were shown to engraft into a mouse model of pulmonary metastasis produced by i.v. injection of human melanoma cells[27]**.** The i.v.-injected MSCs are initially trapped within both normal lung and tumor tissue, but over time, MSCs persist within tumors, while their numbers gradually decrease in normal lung tissue[27]. It

is likely that the tumor microenvironment promotes a permissive niche for MSC survival as compared to normal lung tissue. Although easy to administer, systemically injected MSCs might cause adverse effects if they locate to normal organs. In fact, reports have shown that the majority (>90%) of i.v.-injected human MSCs are trapped within the lungs of mice and do not reach the arterial system[10, 38, 39]. Experimental evidence thus indicates that intratumoral administration, in addition to producing a superior tumor infiltration, is a safer mode of delivery.

The important role of the tumor microenvironment for MSC survival is further strengthened by findings of absent or minimal MSC survival upon grafting into non-tumoral tissue (see, e.g., Koponen et al.[40]). Factors that might promote MSC survival within tumors are discussed below.

Mechanisms of MSC migration and homing to tumors

One early report found that epidermal growth factor (EGF), platelet-derived growth factor (PDGF)-BB and stromal-derived factor-1 (SDF-1), but not vascular endothelial growth factor (VEGF), are key factors mediating the tropism of human MSCs to human glioma cells *in vitro*[24]. Mouse MSCs transduced with the EGF receptor exhibit enhanced *in vitro* and *in vivo* migration toward mouse gliomas as compared to non-transduced MSCs[41]. Other investigators have reported that glioma-produced angiogenic cytokines, such as VEGF-A, transforming growth factor-β1, interleukin-8 and neurotrophin-3, are mediators of human MSC migration to tumors *in vitro*[42, 43]. Angiogenic cytokines are involved in tumor angiogenesis, indicating that similar pathways are used for tumor angiogenesis and MSC migration within tumors[42]. Furthermore, *in vivo* inhibition of angiogenic signaling factors using sunitinib (a broad-spectrum receptor tyrosine kinase inhibitor) is associated with decreased vessel formation and decreased MSC migration[26]. The tropism of grafted MSCs to neoplastic vessels should be considered from the

perspective of findings that endogenous bone marrow-derived periendothelial vascular mural cells (pericytes) contribute to tumor stroma and neovascularization[44-46]. Thus, it seems that an inherent MSC dependence on angiogenic signaling factors confers, at least in part, the migratory specificity of grafted and endogenous MSCs. This “neoangiotropism” of grafted MSCs might preferentially direct these cells toward cancerous tissue characterized by active angiogenesis. This feature could possibly be exploited to gain maximum therapeutic benefit where it is most needed, namely, in the most rapidly growing parts of the tumor.

Recent data suggest that MSCs share important characteristics with pericytes[6, 13]. Pericytes are important regulators of microvessel blood flow and interact with and support endothelial cells. Within tumors, pericytes are thought to regulate vessel integrity, maintenance and function[47] (for a review on the role of pericytes in health and disease, see Bergers et al.[48]). Similar to the way that endogenous MSCs localize mainly to a perivascular niche within the bone marrow, implanted rat MSCs track and localize to a tumor perivascular niche following implantation into rat gliomas[26]. Within gliomas, most implanted MSCs continue to express pericyte markers, such as neuron-glia 2 (NG2), PDGF-receptor-B and alpha-smooth muscle actin, but not endothelial markers **(Figure 2)**[26].

In addition to the “neoangiotropism” of MSCs, inflammatory mediators released from the tumor cells or tumor stroma or from surrounding peritumoral reactive cells help to attract MSCs to neoplasms; MSCs home to sites of nonspecific tissue damage and inflammation, where MSCs contribute to wound healing[49, 50]. Tumors and their immediate surroundings can be considered “wounds that never heal” [51] and contain high numbers of inflammatory cells and cytokines that attract MSCs, e.g., via monocyte chemotactic protein-1[52]. Hence, it is possible that the extensive and tumor-specific migration capacity of implanted and injected MSCs

represents a common response to injury-induced inflammation. For a detailed review of MSC migration and inflammation, see Spaeth et al.[53].

Owing to the lack of specific MSC subpopulation markers, most studies have isolated, propagated and characterized MSCs by their adherent growth in selected fetal calf serum (FCS)-containing medium, differentiation assays and surface marker expression. This procedure, however, does not take into account the wide heterogeneity of MSCs in culture. Given differences in isolation and culture protocols, it might be difficult to compare studies on MSC migration mechanisms between different research groups. MSC heterogeneity is beginning to be elucidated[54], but there are still large knowledge gaps. Recently, it was shown that a subpopulation of human MSCs that display high matrix metalloproteinase 1 (MMP1) expression migrate toward glioma cells to a much greater extent compared with a subset of low-MMP1-expressing MSCs[55]. In addition to clarifying mechanisms of MSC migration, this study stresses the importance of identifying and characterizing subpopulations in cultured MSC preparations. Obviously, we need to gain further detailed information about the molecular mechanisms determining MSC migratory behavior before we can exploit the full potential of these cells as vectors in cancer therapy.

Effects of MSCs on tumor growth

The grafting of MSCs not modified to produce a therapeutic transgene but often carrying a marker gene to allow for visualization, e.g., enhanced green fluorescent protein (eGFP), has produced conflicting results with respect to tumor growth. Native MSCs have been shown to suppress tumor growth in models of glioma[20], Kaposi's sarcoma[28], malignant melanoma[56, 57], Lewis lung carcinoma[56], colon carcinoma[58] and other tumor models[59]. Suppression of Kaposi's sarcoma growth is associated with MSC inhibition of the Akt protein[28]. Otsu et al.

found that implantation of mouse MSCs into established subcutaneous mouse melanomas results in lower vascular density and inhibition of tumor growth[57]. In contrast, we and others have found no apparent effect on tumor growth following implantation/injection of MSCs[26, 30, 60]. On the other hand, several studies have reported that MSCs can augment tumor growth[61-64]. Promotion of tumor growth is possibly mediated by MSC production of immunosuppressive factors and by the contribution of MSCs to tumor stroma and tumor vascularization[65]. Beckermann et al. reported that the injection of human MSCs into nude mice carrying xenografts of human pancreatic tumors results in an increase in tumor vessel density[66]. The different effects of MSCs on tumor growth are striking and illustrate the complexity of the role of MSCs in cancer. It is possible that the heterogeneity of cultured MSCs (i.e., multiple MSC subpopulations with different properties), differences in tumor cells and models, the use of MSCs from different species and species-specific interactions between tumor cells, MSCs and hosts account for some of the differences observed.

MSC delivery of prodrug-converting enzymes

Clinical gene therapy in malignant glioma was pioneered utilizing gene transfer of the herpes simplex virus thymidine kinase (HSV-tk) gene in combination with the systemic administration of ganciclovir[15]. HSV-tk transfer was mediated by stereotactic or intraoperative intratumoral injections of, e.g., adenoviral vectors. Within tumors, HSV-tk converts (phosphorylates) the prodrug ganciclovir into its toxic form, which inhibits DNA synthesis, leading to cell death. In addition, there is a substantial bystander effect that leads to cell death of neighboring cells. However, as discussed previously, there is a need for a more efficient and specific vector system to achieve a substantial therapeutic effect[15]. The migratory capacity of transduced MAPCs/MSCs permits more efficient distribution of the HSV-tk gene within tumors as compared

to injections of viral vectors. Accordingly, HSV-tk has been successfully transferred via MAPCs to experimental gliomas[25] and via MSCs to pancreatic cancer[67] and gliomas[36]. Significant anti-tumor effects were demonstrated by the administration of MAPCs/MSCs expressing the HSV-tk gene in combination with systemically administered ganciclovir through bystander-mediated tumor cell killing[25, 67]. The migratory capacity of the transduced MSCs/MAPCs is crucial for therapeutic efficiency because administration of transduced non-migratory fibroblasts does not lead to similar therapeutic effects[25, 36].

Adipose tissue-derived MSCs have been used to deliver another prodrug-converting enzyme, cytosine deaminase, followed by systemic administration of the prodrug 5-fluorocytosine, which is converted to the active toxic drug 5-fluorouracil in tumors. This therapeutic regimen has proven efficacious in the treatment of experimental colon carcinoma[60], prostate tumor growth[68] and melanoma growth[69].

Cytokine delivery by MSCs

MSCs can also be utilized to deliver immunomodulatory cytokines in order to augment the host's anti-tumor immune response. Tumor regression by cytokine transfer using MSCs has been demonstrated in several tumor models using a variety of immunostimulatory substances, including interleukin-2[20], interleukin-12[70, 71], interleukin-18[72], interferon-alpha[41],interferon-beta[27, 73] and CXC3CL1 (fractalkine)[74]. The therapeutic effects are often attributed to the increased tumor infiltration of anti-tumor immune cells, e.g., CD4+ and CD8+ T-cells and natural killer cells[20, 74, 75]. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) has gained attention in cancer gene therapy because of its capacity to induce apoptosis specifically in tumor cells. Recent studies demonstrate the ability of umbilical cord blood-derived or bone marrow-derived TRAIL-producing MSCs to effectively inhibit the

growth of gliomas[76-78] and breast cancer-derived lung metastases[79]. Work from our group has demonstrated regression of rat gliomas as a result of combining peripheral immunization using interferon-gamma-transduced autologous tumor cells with the intratumoral delivery of interleukin-7 by rat MSCs[75]

MSC delivery of oncolytic viruses

A major obstacle to the use of oncolytic viruses in the treatment of experimental tumors using direct viral transfer has been the high immunogenicity of the viral particles. Oncolytic viruses are often neutralized by an immunological reaction before they can exert substantial anti-tumor effects. Furthermore, limited vector spread within tumors makes the efficient treatment of invasive tumors difficult[80]. To overcome this problem, cellular vectors for oncolytic viruses are being explored[81]. By using cells as vehicles, viruses are protected from the host immune system while being delivered to the tumor site. Human MSCs loaded to deliver a conditionally replicating oncolytic adenovirus (CRAd) can migrate to gliomas and release CRAds that infect human glioma cells[82]. MSC-based delivery of oncolytic adenoviruses has demonstrated therapeutic effects in mice bearing human ovarian cancer and human gliomas[30, 83].

Pericyte-like MSCs as therapeutic vehicles

The finding that MSCs localize to tumor vasculature upon intratumoral implantation offers opportunities for therapeutic targeting, especially of vascularized tumors[26]. The tumor neovasculature is critical for tumor growth[84], and implanted MSCs may be utilized as vehicles for the delivery of anti-angiogenic substances to vascularized tumors. Combinatorial targeting of both tumor endothelium and tumor pericytes has been shown to synergistically affect tumor vascularization and tumor growth[85]. The association of implanted MSCs with tumor vessels

might thus offer an opportunity to locally and specifically target both tumor endothelial cells and tumor pericytes. In addition, implanted perivascular MSCs are strategically located to deliver substances to target putative CSCs/tumor-initiating cells known to reside within a perivascular niche[2, 86].

Recent findings demonstrating that MSCs can be considered members of the pericyte family and that intratumorally grafted MSCs display pericyte markers allow one to speculate that implanted MSCs could possibly function as tumor pericytes. Tumor pericytes contribute to vascular normalization, a process in which otherwise leaky and nonfunctional tumor blood vessels become "normalized," which allows for more regular blood flow[87]. This in turn can be exploited for therapeutic benefit because vascular normalization leads to an increased influx of anti-tumor immune effector cells[87]. Accordingly, it would be interesting to address the question of whether implanted MSCs contribute to normalization of tumor vasculature and thus enhance the anti-tumor immune response. On the other hand, the addition of pericytes to tumors could possibly also lead to negative effects on blood flow and tumor growth, and certainly more research is required to address these questions. **Table 1** lists selected studies using native or genetically modified MSCs in various tumor models.

In vivo imaging of MSCs

Clinical application of cellular vectors for cancer therapy will require sensitive and specific methods for *in vivo* imaging of vector distribution and transgene expression. Imaging must also allow for sensitive and early detection of treatment effects. Non-invasive *in vivo* imaging in gene therapy applications has previously been reviewed in detail[88]. Today, methods to investigate stem cell homing and migration to tumors include *in vivo* bioluminescence imaging of firefly luciferase-expressing MSCs, magnetic resonance imaging (MRI) of cells containing

biocompatible superparamagnetic iron oxide nanoparticles and positron emission tomography (PET) imaging. These imaging modalities allow for the non-invasive serial detection of implanted/injected cells.

MRI requires a costly imaging device but offers several advantages over the other techniques: no radioactive labeling is needed, more than one physiological parameter can be studied using different pulse sequences and the spatial resolution is superior to that of other imaging modalities[88]. MRI has been utilized to detect iron-labeled MSCs[28, 89-91], Sca1+ bone marrow-derived cells[92] and NSCs[93] upon local or systemic administration of cells to tumors. As few as 1000 labeled human MSCs were detectable by MRI 1 month following co-injection with breast cancer cells[89]. Furthermore, MRI allows for the detection of i.v.-injected human MSCs homing to multiple lung metastases of breast cancer[89]. Moreover, iron-labeled Sca1+ bone marrow-derived cells that incorporate into newly formed vessels have been utilized to detect ongoing tumor angiogenesis using MRI[92]. It is possible that pericyte-like MSCs might be used not only to deliver anti-angiogenic substances to tumors but also to detect treatment effects of anti-angiogenic therapy.

Bioluminescence imaging has been used to track the tropism of luciferase gene-transduced MSCs to breast and ovarian tumor models[34] and lung metastases of breast cancer[94] as well as to track NSC migration to experimental brain tumors [95]. Although this is a very sensitive technique, it is hampered by poor spatial resolution compared to MRI and poor light penetration through tissue, essentially limiting this mode of imaging to studies in small animals.

Tumor targeting using human MSCs has also been studied using PET[29], which is very sensitive but requires radiolabeling of cells prior to grafting. Again, spatial resolution is

poor, and the short decay times of the radioligands make it difficult for sequential imaging of cell vector distribution over time.

Concerns for the development of MSC-based tumor therapy

To translate MSC-based anticancer strategies into clinical therapy, it is essential to identify and minimize treatment-associated risks. Indeed, potential hazards linked to the use of MSC gene therapy vectors have emerged.

Currently, MSCs are usually cultured in FCS-containing medium, even for clinical use. Unquestionably, this is a concern because FCS is a very complex supplement containing an undefined mixture of proteins, growth factors, hormones, amino acids, etc., and possibly also infectious agents such as prions. FCS is therefore considered a high-risk ancillary material when used for the manufacture of cell, gene and tissue-engineered products. Thus, FCS alternatives, such as platelet lysate, have been investigated for MSC culturing[96-98]. Moreover, several companies have developed serum-free MSC culture systems, which might be used in a clinical setting, provided that media production is performed under Good Manufacturing Practice conditions. On the other hand, the safety of allogeneic MSC therapy with regard to bacterial and viral transmission is not a major concern because MSC donors and products can be screened with an efficiency similar to that used for volunteer blood donors and stem cell donors. Moreover, using off-the-shelf third-party MSCs allows for repetitive donor testing before cells are released for transplantation.

Another possible risk for clinical application is that MSC culturing, which is necessary to obtain sufficient numbers for therapeutic use, might result in malignant transformation. It has been reported that human MSCs can be cultured safely for standard expansion periods (6-12 weeks[99, 100]). However, conflicting data exist on the risk of the

malignant transformation of murine and human MSCs following long-term *ex vivo* culturing[99, 101]. Clearly, for clinical use, well-defined *in vitro* procedures avoiding long-term passage of MSC cultures will be required, along with thorough investigation of possible chromosomal abnormalities.

MSCs can also contribute to the tumor neovascular network and to tumor stroma formation[65, 73]. Human MSCs can switch phenotypes into tumor-associated fibroblast-like cells and provide structural support that stimulates tumor growth, e.g., by the production of interleukin-6[65, 102]. The role of MSCs is ambiguous with regard to tumor metastasis. It has been shown that tumor pericytes can limit tumor cell metastasis[103]. The loss of mouse tumor vessel pericytes dramatically increases blood-borne tumor cell dissemination by destabilizing tumor microvessels[103]. In contrast, when mixed with breast cancer cells, human MSCs were shown to increase the metastatic potential [63]. This effect is dependent on MSC secretion of the chemokine CCL5, which acts in a paracrine fashion on the cancer cells and enhances their invasive capacity[63].

Immunomodulatory properties of MSCs can suppress the anti-tumor immune response[62]. MSCs exert immunosuppressive effects via several mechanisms, including suppression of T-cell proliferation and cytokine production[104]. However, the effects of MSCs on immune effector cells are diverse and complex. MSCs can both suppress and promote T-cell proliferation[105],and recent results show that MSCs require a sufficiently strong, ongoing immune response to exert their immunosuppressive potential[106]. In addition, the magnitude and mechanisms of MSC-mediated immune suppression vary among different species[107].

Finally, bone marrow-derived cells can undergo fusion with tumor cells, which can potentially result in increased growth, drug resistance and metastatic ability of tumor cells[108].

Obviously, this phenomenon needs to be clarified before MSCs can be utilized as vectors in clinical therapy.

The functional role of MSCs/pericytes in cancer growth, invasion and metastasis is currently a subject of controversy and intense research[109] **(Table 2)**. Hopefully, the identification of MSC subpopulations and the development of standardized culture protocols will help to solve some of these controversies. However, even when using standardized MSC culture protocols and defined MSC subpopulations, it is likely that the effect of MSCs/pericytes on tumors will vary depending on the tumor type and localization. Nevertheless, the above-mentioned concerns should not halt the ongoing development of MSC-based tumor gene therapy but rather justify the need for further studies.

Prospects for clinical MSC-based tumor therapy

A clinical scenario in which genetically modified MSCs (or alternative cells) are implanted into inoperable tumors or into parts of partially resected invasive tumors (e.g., GBM) can be envisaged. MSCs could be harvested quickly from third-party allogeneic donors, characterized, subtyped and then transduced with therapeutic and diagnostic transgenes prior to implantation. The operative procedure would likely include injections at multiple sites within the tumor, possibly using stereotactic technique. The MSC intratumoral distribution pattern within experimental invasive tumors suggests that human MSCs implanted into a patient's tumors will migrate and deliver their therapeutic substance to the parts of the tumor that are inaccessible to surgery. Interestingly, a recent study showed that tumor irradiation enhances the migration of injected mouse MSCs toward tumors in mice[110]. This effect might be due to irradiation-induced increased inflammatory components that attract MSCs to tumors. Thus, synergy between

treatment modalities might be possible, and treatment effects could possibly be followed by serial MRI of labeled MSCs.

It is clear that the successful translation of experimental findings into the clinic is challenging. Issues such as MSC biosafety, culturing protocols, choice of therapeutic transgenes and efficient and minimally invasive delivery protocols, as well as cost-effectiveness, need to be resolved before MSC-based therapy can be brought into routine clinical use **(Table 2)**. Still, MSCs might, by virtue of their capacity to target tumor extensions and distant metastases, constitute a powerful treatment modality in our future repertoire of therapy options for otherwise incurable invasive cancers. Exciting prospects include the potential use of pericyte-like perivascular MSCs in antiangiogenic tumor therapy and the eradication of microvascular niche-residing cancer stem cells in highly malignant gliomas.

Figure Legends

Fig. 1

(a) Spindle-shaped morphology of rat bone marrow-derived multipotent mesenchymal stromal cells (MSCs) *in vitro*. MSCs possess the capacity to differentiate into **(b)** osteoblasts and **(c)** adipocytes upon induction of differentiation. **(d)** Implantation of enhanced green fluorescent protein (eGFP)-expressing rat MSCs (green) into orthotopic rat gliomas (Hoechst blue). MSCs spread extensively within the glioma but largely avoid adjacent normal brain parenchyma. **(e)** eGFP-MSCs migrate specifically along invasive DsRed-labeled glioma cells (red). The scale bar is 50 µm in a, b and c, 200 µm in d and 100 µm in e. Fig.1 a-c and e are reproduced from Bexell et al. [26] with permission from the publisher.

Fig. 2

(a) Implanted rat eGFP+ MSCs (green) are attracted to perivascular niches within gliomas. Tumor blood vessels are delineated by the endothelial cell marker rat endothelial cell antigen (RECA, red). MSC expression of the pericyte markers **(b)** platelet-derived growth factor receptor-B and **(c)** desmin *in vitro*. **(d)** Implanted eGFP-MSCs (green) express pericyte marker alpha-smooth muscle actin (red) within tumors. The scale bar is 100 µm in a, 50 µm in b and c and 20 µm in d.

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References

1. Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., et al. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352: 987-996.

2. Calabrese, C., Poppleton, H., Kocak, M., Hogg, T. L., Fuller, C., Hamner, B., et al. (2007). A perivascular niche for brain tumor stem cells. *Cancer Cell* 11: 69-82.

3. Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., et al. (2004). Identification of human brain tumour initiating cells. *Nature* 432: 396-401.

4. Stiles, C. D., and Rowitch, D. H. (2008). Glioma stem cells: a midterm exam. *Neuron* 58: 832-846.

5. Caplan, A. I. (2009). Why are MSCs therapeutic? New data: new insight. *J Pathol* 217: 318-324.

6. Crisan, M., Yap, S., Casteilla, L., Chen, C. W., Corselli, M., Park, T. S., et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3: 301-313.

7. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315-317.

8. Baksh, D., Song, L., and Tuan, R. S. (2004). Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *J Cell Mol Med* 8: 301-316.

9. Friedenstein, A. J., Chailakhyan, R. K., Latsinik, N. V., Panasyuk, A. F., and Keiliss-Borok, I. V. (1974). Stromal cells responsible for transferring the microenvironment of

the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* 17: 331-340.

10. Prockop, D. J. (2009). Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. *Mol Ther* 17: 939-946.

11. Jones, B. J., and McTaggart, S. J. (2008). Immunosuppression by mesenchymal stromal cells: from culture to clinic. *Exp Hematol* 36: 733-741.

12. Le Blanc, K., Frassoni, F., Ball, L., Locatelli, F., Roelofs, H., Lewis, I., et al. (2008). Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 371: 1579-1586.

13. Au, P., Tam, J., Fukumura, D., and Jain, R. K. (2008). Bone marrow-derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. *Blood* 111: 4551-4558.

14. Sacchetti, B., Funari, A., Michienzi, S., Di Cesare, S., Piersanti, S., Saggio, I., et al. (2007). Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 131: 324-336.

15. Pulkkanen, K. J., and Yla-Herttuala, S. (2005). Gene therapy for malignant glioma: current clinical status. *Mol Ther* 12: 585-598.

16. Aboody, K. S., Brown, A., Rainov, N. G., Bower, K. A., Liu, S., Yang, W., et al. (2000). Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc Natl Acad Sci U S A* 97: 12846-12851.

17. Benedetti, S., Pirola, B., Pollo, B., Magrassi, L., Bruzzone, M. G., Rigamonti, D., et al. (2000). Gene therapy of experimental brain tumors using neural progenitor cells. *Nat Med* 6: 447-450.

18. Ehtesham, M., Kabos, P., Kabosova, A., Neuman, T., Black, K. L., and Yu, J. S. (2002). The use of interleukin 12-secreting neural stem cells for the treatment of intracranial glioma. *Cancer Res* 62: 5657-5663.

19. Moore, X. L., Lu, J., Sun, L., Zhu, C. J., Tan, P., and Wong, M. C. (2004). Endothelial progenitor cells' "homing" specificity to brain tumors. *Gene Ther* 11: 811-818.

20. Nakamura, K., Ito, Y., Kawano, Y., Kurozumi, K., Kobune, M., Tsuda, H., et al. (2004). Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. *Gene Ther* 11: 1155-1164.

21. Pisati, F., Belicchi, M., Acerbi, F., Marchesi, C., Giussani, C., Gavina, M., et al. (2007). Effect of human skin-derived stem cells on vessel architecture, tumor growth, and tumor invasion in brain tumor animal models. *Cancer Res* 67: 3054-3063.

22. Tabatabai, G., Bahr, O., Mohle, R., Eyupoglu, I. Y., Boehmler, A. M., Wischhusen, J., et al. (2005). Lessons from the bone marrow: how malignant glioma cells attract adult haematopoietic progenitor cells. *Brain* 128: 2200-2211.

23. Han, X., Meng, X., Yin, Z., Rogers, A., Zhong, J., Rillema, P., et al. (2009). Inhibition of intracranial glioma growth by endometrial regenerative cells. *Cell Cycle* 8: 606-610.

24. Nakamizo, A., Marini, F., Amano, T., Khan, A., Studeny, M., Gumin, J., et al. (2005). Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res* 65: 3307-3318.

25. Miletic, H., Fischer, Y., Litwak, S., Giroglou, T., Waerzeggers, Y., Winkeler, A., et al. (2007). Bystander killing of malignant glioma by bone marrow-derived tumor-infiltrating progenitor cells expressing a suicide gene. *Mol Ther* 15: 1373-1381.

26. Bexell, D., Gunnarsson, S., Tormin, A., Darabi, A., Gisselsson, D., Roybon, L., et al. (2009). Bone marrow multipotent mesenchymal stroma cells act as pericyte-like migratory vehicles in experimental gliomas. *Mol Ther* 17: 183-190.

27. Studeny, M., Marini, F. C., Champlin, R. E., Zompetta, C., Fidler, I. J., and Andreeff, M. (2002). Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. *Cancer Res* 62: 3603-3608.

28. Khakoo, A. Y., Pati, S., Anderson, S. A., Reid, W., Elshal, M. F., Rovira, II, et al. (2006). Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* 203: 1235-1247.

29. Hung, S. C., Deng, W. P., Yang, W. K., Liu, R. S., Lee, C. C., Su, T. C., et al. (2005). Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. *Clin Cancer Res* 11: 7749-7756.

30. Komarova, S., Kawakami, Y., Stoff-Khalili, M. A., Curiel, D. T., and Pereboeva, L. (2006). Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. *Mol Cancer Ther* 5: 755-766.

31. Kallifatidis, G., Beckermann, B. M., Groth, A., Schubert, M., Apel, A., Khamidjanov, A., et al. (2008). Improved lentiviral transduction of human mesenchymal stem cells for therapeutic intervention in pancreatic cancer. *Cancer Gene Ther* 15: 231-240.

32. Duan, X., Guan, H., Cao, Y., and Kleinerman, E. S. (2009). Murine bone marrow-derived mesenchymal stem cells as vehicles for interleukin-12 gene delivery into Ewing sarcoma tumors. *Cancer* 115: 13-22.

33. Xiang, J., Tang, J., Song, C., Yang, Z., Hirst, D. G., Zheng, Q. J., et al. (2009). Mesenchymal stem cells as a gene therapy carrier for treatment of fibrosarcoma. *Cytotherapy* 11: 516-526.

34. Kidd, S., Spaeth, E., Dembinski, J. L., Dietrich, M., Watson, K., Klopp, A., et al. (2009). Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging. *Stem Cells* 27: 2614-2623.

35. Gao, P., Ding, Q., Wu, Z., Jiang, H., and Fang, Z. (2009). Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma. *Cancer Lett*.

36. Uchibori, R., Okada, T., Ito, T., Urabe, M., Mizukami, H., Kume, A., et al. (2009). Retroviral vector-producing mesenchymal stem cells for targeted suicide cancer gene therapy. *J Gene Med* 11: 373-381.

37. Xin, H., Sun, R., Kanehira, M., Takahata, T., Itoh, J., Mizuguchi, H., et al. (2009). Intratracheal delivery of CX3CL1-expressing mesenchymal stem cells to multiple lung tumors. *Mol Med* 15: 321-327.

38. Gao, J., Dennis, J. E., Muzic, R. F., Lundberg, M., and Caplan, A. I. (2001). The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 169: 12-20.

39. Harting, M. T., Jimenez, F., Xue, H., Fischer, U. M., Baumgartner, J., Dash, P. K., et al. (2009). Intravenous mesenchymal stem cell therapy for traumatic brain injury. *J Neurosurg* 110: 1189-1197.

40. Koponen, J. K., Kekarainen, T., S, E. H., Laitinen, A., Nystedt, J., Laine, J., et al. (2007). Umbilical cord blood-derived progenitor cells enhance muscle regeneration in mouse hindlimb ischemia model. *Mol Ther* 15: 2172-2177.

41. Sato, H., Kuwashima, N., Sakaida, T., Hatano, M., Dusak, J. E., Fellows-Mayle, W. K., et al. (2005). Epidermal growth factor receptor-transfected bone marrow stromal cells exhibit enhanced migratory response and therapeutic potential against murine brain tumors. *Cancer Gene Ther* 12: 757-768.

42. Birnbaum, T., Roider, J., Schankin, C. J., Padovan, C. S., Schichor, C., Goldbrunner, R., et al. (2007). Malignant gliomas actively recruit bone marrow stromal cells by secreting angiogenic cytokines. *J Neurooncol* 83: 241-247.

43. Schichor, C., Birnbaum, T., Etminan, N., Schnell, O., Grau, S., Miebach, S., et al. (2006). Vascular endothelial growth factor A contributes to glioma-induced migration of human marrow stromal cells (hMSC). *Exp Neurol* 199: 301-310.

44. Aghi, M., and Chiocca, E. A. (2005). Contribution of bone marrow-derived cells to blood vessels in ischemic tissues and tumors. *Mol Ther* 12: 994-1005.

45. Bababeygy, S. R., Cheshier, S. H., Hou, L. C., Higgins, D. M., Weissman, I. L., and Tse, V. C. (2008). Hematopoietic Stem Cell-Derived Pericytic Cells in Brain Tumor Angio-Architecture. *Stem Cells Dev* 17: 11-18.

46. Rajantie, I., Ilmonen, M., Alminaite, A., Ozerdem, U., Alitalo, K., and Salven, P. (2004). Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. *Blood* 104: 2084-2086.

47. Bergers, G., and Benjamin, L. E. (2003). Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3: 401-410.

48. Bergers, G., and Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol* 7: 452-464.

49. Sasaki, M., Abe, R., Fujita, Y., Ando, S., Inokuma, D., and Shimizu, H. (2008). Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol* 180: 2581-2587.

50. Wu, Y., Chen, L., Scott, P. G., and Tredget, E. E. (2007). Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 25: 2648-2659.

51. Dvorak, H. F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 315: 1650-1659.

52. Dwyer, R. M., Potter-Beirne, S. M., Harrington, K. A., Lowery, A. J., Hennessy, E., Murphy, J. M., et al. (2007). Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. *Clin Cancer Res* 13: 5020-5027.

53. Spaeth, E., Klopp, A., Dembinski, J., Andreeff, M., and Marini, F. (2008). Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. *Gene Ther* 15: 730-738.

54. Tormin, A., Brune, J. C., Olsson, E., Valcich, J., Neuman, U., Olofsson, T., et al. (2009). Characterization of bone marrow-derived mesenchymal stromal cells (MSC) based on gene expression profiling of functionally defined MSC subsets. *Cytotherapy* 11: 114-128.

55. Ho, I. A., Chan, K. Y., Ng, W. H., Guo, C. M., Hui, K. M., Cheang, P., et al. (2009). Matrix metalloproteinase 1 is necessary for the migration of human bone marrow-derived mesenchymal stem cells toward human glioma. *Stem Cells* 27: 1366-1375.

56. Maestroni, G. J., Hertens, E., and Galli, P. (1999). Factor(s) from nonmacrophage bone marrow stromal cells inhibit Lewis lung carcinoma and B16 melanoma growth in mice. *Cell Mol Life Sci* 55: 663-667.

57. Otsu, K., Das, S., Houser, S. D., Quadri, S. K., Bhattacharya, S., and Bhattacharya, J. (2009). Concentration-dependent inhibition of angiogenesis by mesenchymal stem cells. *Blood* 113: 4197-4205.

58. Ohlsson, L. B., Varas, L., Kjellman, C., Edvardsen, K., and Lindvall, M. (2003). Mesenchymal progenitor cell-mediated inhibition of tumor growth in vivo and in vitro in gelatin matrix. *Exp Mol Pathol* 75: 248-255.

59. Lu, Y. R., Yuan, Y., Wang, X. J., Wei, L. L., Chen, Y. N., Cong, C., et al. (2008). The growth inhibitory effect of mesenchymal stem cells on tumor cells in vitro and in vivo. *Cancer Biol Ther* 7: 245-251.

60. Kucerova, L., Altanerova, V., Matuskova, M., Tyciakova, S., and Altaner, C. (2007). Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. *Cancer Res* 67: 6304-6313.

61. Coffelt, S. B., Marini, F. C., Watson, K., Zwezdaryk, K. J., Dembinski, J. L., LaMarca, H. L., et al. (2009). The pro-inflammatory peptide LL-37 promotes ovarian tumor progression through recruitment of multipotent mesenchymal stromal cells. *Proc Natl Acad Sci U S A* 106: 3806-3811.

62. Djouad, F., Plence, P., Bony, C., Tropel, P., Apparailly, F., Sany, J., et al. (2003). Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood* 102: 3837-3844.

63. Karnoub, A. E., Dash, A. B., Vo, A. P., Sullivan, A., Brooks, M. W., Bell, G. W., et al. (2007). Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 449: 557-563.

64. Zhu, W., Xu, W., Jiang, R., Qian, H., Chen, M., Hu, J., et al. (2006). Mesenchymal stem cells derived from bone marrow favor tumor cell growth in vivo. *Exp Mol Pathol* 80: 267-274.

65. Spaeth, E. L., Dembinski, J. L., Sasser, A. K., Watson, K., Klopp, A., Hall, B., et al. (2009). Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. *PLoS One* 4: e4992.

66. Beckermann, B. M., Kallifatidis, G., Groth, A., Frommhold, D., Apel, A., Mattern, J., et al. (2008). VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. *Br J Cancer* 99: 622-631.

67. Zischek, C., Niess, H., Ischenko, I., Conrad, C., Huss, R., Jauch, K. W., et al. (2009). Targeting Tumor Stroma Using Engineered Mesenchymal Stem Cells Reduces the Growth of Pancreatic Carcinoma. *Ann Surg* 250: 747-753.

68. Cavarretta, I. T., Altanerova, V., Matuskova, M., Kucerova, L., Culig, Z., and Altaner, C. Adipose tissue-derived mesenchymal stem cells expressing prodrug-converting enzyme inhibit human prostate tumor growth. *Mol Ther* 18: 223-231.

69. Kucerova, L., Matuskova, M., Pastorakova, A., Tyciakova, S., Jakubikova, J., Bohovic, R., et al. (2008). Cytosine deaminase expressing human mesenchymal stem cells mediated tumour regression in melanoma bearing mice. *J Gene Med* 10: 1071-1082.

70. Elzaouk, L., Moelling, K., and Pavlovic, J. (2006). Anti-tumor activity of mesenchymal stem cells producing IL-12 in a mouse melanoma model. *Exp Dermatol* 15: 865-874.

71. Hong, X., Miller, C., Savant-Bhonsale, S., and Kalkanis, S. N. (2009). Antitumor treatment using interleukin- 12-secreting marrow stromal cells in an invasive glioma model. *Neurosurgery* 64: 1139-1146; discussion 1146-1137.

72. Xu, G., Jiang, X. D., Xu, Y., Zhang, J., Huang, F. H., Chen, Z. Z., et al. (2009). Adenoviral-mediated interleukin-18 expression in mesenchymal stem cells effectively suppresses the growth of glioma in rats. *Cell Biol Int* 33: 466-474.

73. Studeny, M., Marini, F. C., Dembinski, J. L., Zompetta, C., Cabreira-Hansen, M., Bekele, B. N., et al. (2004). Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *J Natl Cancer Inst* 96: 1593-1603.

74. Xin, H., Kanehira, M., Mizuguchi, H., Hayakawa, T., Kikuchi, T., Nukiwa, T., et al. (2007). Targeted delivery of CX3CL1 to multiple lung tumors by mesenchymal stem cells. *Stem Cells* 25: 1618-1626.

75. Gunnarsson, S., Bexell, D., Svensson, A., Siesjo, P., Darabi, A., and Bengzon, J. Intratumoral IL-7 delivery by mesenchymal stromal cells potentiates IFNgamma-transduced tumor cell immunotherapy of experimental glioma. *J Neuroimmunol* 218: 140-144.

76. Kim, S. M., Lim, J. Y., Park, S. I., Jeong, C. H., Oh, J. H., Jeong, M., et al. (2008). Gene therapy using TRAIL-secreting human umbilical cord blood-derived mesenchymal stem cells against intracranial glioma. *Cancer Res* 68: 9614-9623.

77. Menon, L. G., Kelly, K., Yang, H. W., Kim, S. K., Black, P. M., and Carroll, R. S. (2009). Human bone marrow-derived mesenchymal stromal cells expressing S-TRAIL as a cellular delivery vehicle for human glioma therapy. *Stem Cells* 27: 2320-2330.

78. Sasportas, L. S., Kasmieh, R., Wakimoto, H., Hingtgen, S., van de Water, J. A., Mohapatra, G., et al. (2009). Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. *Proc Natl Acad Sci U S A* 106: 4822-4827.

79. Loebinger, M. R., Eddaoudi, A., Davies, D., and Janes, S. M. (2009). Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. *Cancer Res* 69: 4134-4142.

80. Yamamoto, M., and Curiel, D. T. (2009). Current Issues and Future Directions of Oncolytic Adenoviruses. *Mol Ther*.

81. Power, A. T., and Bell, J. C. (2007). Cell-based delivery of oncolytic viruses: a new strategic alliance for a biological strike against cancer. *Mol Ther* 15: 660-665.

82. Sonabend, A. M., Ulasov, I. V., Tyler, M. A., Rivera, A. A., Mathis, J. M., and Lesniak, M. S. (2008). Mesenchymal stem cells effectively deliver an oncolytic adenovirus to intracranial glioma. *Stem Cells* 26: 831-841.

83. Yong, R. L., Shinojima, N., Fueyo, J., Gumin, J., Vecil, G. G., Marini, F. C., et al. (2009). Human bone marrow-derived mesenchymal stem cells for intravascular delivery of oncolytic adenovirus Delta24-RGD to human gliomas. *Cancer Res* 69: 8932-8940.

84. Hanahan, D., and Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86: 353-364.

85. Bergers, G., Song, S., Meyer-Morse, N., Bergsland, E., and Hanahan, D. (2003). Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 111: 1287-1295.

86. Pietras, A., Gisselsson, D., Ora, I., Noguera, R., Beckman, S., Navarro, S., et al. (2008). High levels of HIF-2alpha highlight an immature neural crest-like neuroblastoma cell cohort located in a perivascular niche. *J Pathol* 214: 482-488.

87. Hamzah, J., Jugold, M., Kiessling, F., Rigby, P., Manzur, M., Marti, H. H., et al. (2008). Vascular normalization in Rgs5-deficient tumours promotes immune destruction. *Nature* 453: 410-414.

88. Raty, J. K., Liimatainen, T., Unelma Kaikkonen, M., Grohn, O., Airenne, K. J., and Yla-Herttuala, S. (2007). Non-invasive Imaging in Gene Therapy. *Mol Ther* 15: 1579-1586.

89. Loebinger, M. R., Kyrtatos, P. G., Turmaine, M., Price, A. N., Pankhurst, Q., Lythgoe, M. F., et al. (2009). Magnetic resonance imaging of mesenchymal stem cells homing to pulmonary metastases using biocompatible magnetic nanoparticles. *Cancer Res* 69: 8862-8867.

90. Wu, X., Hu, J., Zhou, L., Mao, Y., Yang, B., Gao, L., et al. (2008). In vivo tracking of superparamagnetic iron oxide nanoparticle-labeled mesenchymal stem cell tropism to malignant gliomas using magnetic resonance imaging. Laboratory investigation. *J Neurosurg* 108: 320-329.

91. Zhang, Z., Jiang, Q., Jiang, F., Ding, G., Zhang, R., Wang, L., et al. (2004). In vivo magnetic resonance imaging tracks adult neural progenitor cell targeting of brain tumor. *Neuroimage* 23: 281-287.

92. Anderson, S. A., Glod, J., Arbab, A. S., Noel, M., Ashari, P., Fine, H. A., et al. (2005). Noninvasive MR imaging of magnetically labeled stem cells to directly identify neovasculature in a glioma model. *Blood* 105: 420-425.

93. Thu, M. S., Najbauer, J., Kendall, S. E., Harutyunyan, I., Sangalang, N., Gutova, M., et al. (2009). Iron labeling and pre-clinical MRI visualization of therapeutic human neural stem cells in a murine glioma model. *PLoS One* 4: e7218.

94. Wang, H., Cao, F., De, A., Cao, Y., Contag, C., Gambhir, S. S., et al. (2009). Trafficking mesenchymal stem cell engraftment and differentiation in tumor-bearing mice by bioluminescence imaging. *Stem Cells* 27: 1548-1558.

95. Tang, Y., Shah, K., Messerli, S. M., Snyder, E., Breakefield, X., and Weissleder, R. (2003). In vivo tracking of neural progenitor cell migration to glioblastomas. *Hum Gene Ther* 14: 1247-1254.

96. Bieback, K., Hecker, A., Kocaomer, A., Lannert, H., Schallmoser, K., Strunk, D., et al. (2009). Human alternatives to fetal bovine serum for the expansion of mesenchymal stromal cells from bone marrow. *Stem Cells* 27: 2331-2341.

97. Muller, I., Kordowich, S., Holzwarth, C., Spano, C., Isensee, G., Staiber, A., et al. (2006). Animal serum-free culture conditions for isolation and expansion of multipotent mesenchymal stromal cells from human BM. *Cytotherapy* 8: 437-444.

98. Schallmoser, K., Rohde, E., Reinisch, A., Bartmann, C., Thaler, D., Drexler, C., et al. (2008). Rapid large-scale expansion of functional mesenchymal stem cells from unmanipulated bone marrow without animal serum. *Tissue Eng Part C Methods* 14: 185-196.

99. Bernardo, M. E., Zaffaroni, N., Novara, F., Cometa, A. M., Avanzini, M. A., Moretta, A., et al. (2007). Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* 67: 9142-9149.

100. Rubio, D., Garcia-Castro, J., Martin, M. C., de la Fuente, R., Cigudosa, J. C., Lloyd, A. C., et al. (2005). Spontaneous human adult stem cell transformation. *Cancer Res* 65: 3035-3039.

101. Rosland, G. V., Svendsen, A., Torsvik, A., Sobala, E., McCormack, E., Immervoll, H., et al. (2009). Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res* 69: 5331-5339.

102. Mishra, P. J., Mishra, P. J., Humeniuk, R., Medina, D. J., Alexe, G., Mesirov, J. P., et al. (2008). Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. *Cancer Res* 68: 4331-4339.

103. Xian, X., Hakansson, J., Stahlberg, A., Lindblom, P., Betsholtz, C., Gerhardt, H., et al. (2006). Pericytes limit tumor cell metastasis. *J Clin Invest* 116: 642-651.

104. Uccelli, A., Moretta, L., and Pistoia, V. (2008). Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 8: 726-736.

105. Najar, M., Rouas, R., Raicevic, G., Boufker, H. I., Lewalle, P., Meuleman, N., et al. (2009). Mesenchymal stromal cells promote or suppress the proliferation of T lymphocytes from cord blood and peripheral blood: the importance of low cell ratio and role of interleukin-6. *Cytotherapy* 11: 570-583.

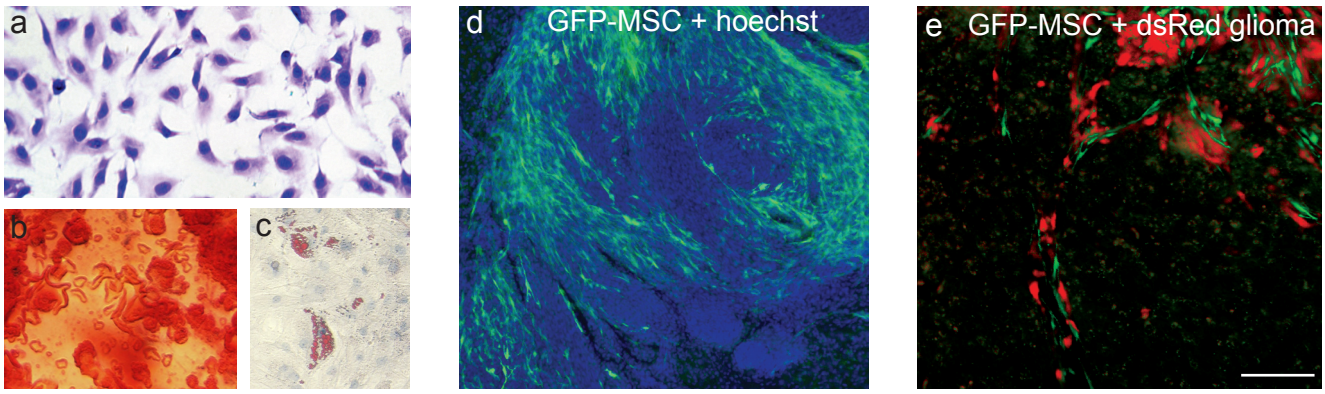
106. Renner, P., Eggenhofer, E., Rosenauer, A., Popp, F. C., Steinmann, J. F., Slowik, P., et al. (2009). Mesenchymal stem cells require a sufficient, ongoing immune response to exert their immunosuppressive function. *Transplant Proc* 41: 2607-2611.

107. Ren, G., Su, J., Zhang, L., Zhao, X., Ling, W., L'Huillie, A., et al. (2009). Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. *Stem Cells* 27: 1954-1962.

108. Pawelek, J. M., and Chakraborty, A. K. (2008). Fusion of tumour cells with bone marrow-derived cells: a unifying explanation for metastasis. *Nat Rev Cancer* 8: 377-386.

109. Lazennec, G., and Jorgensen, C. (2008). Concise review: adult multipotent stromal cells and cancer: risk or benefit? *Stem Cells* 26: 1387-1394.

110. Klopp, A. H., Spaeth, E. L., Dembinski, J. L., Woodward, W. A., Munshi, A., Meyn, R. E., et al. (2007). Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. *Cancer Res* 67: 11687-11695.


a
b
c
d
GFP-MSC + hoechst
e
GFP-MSC + dsRed glioma

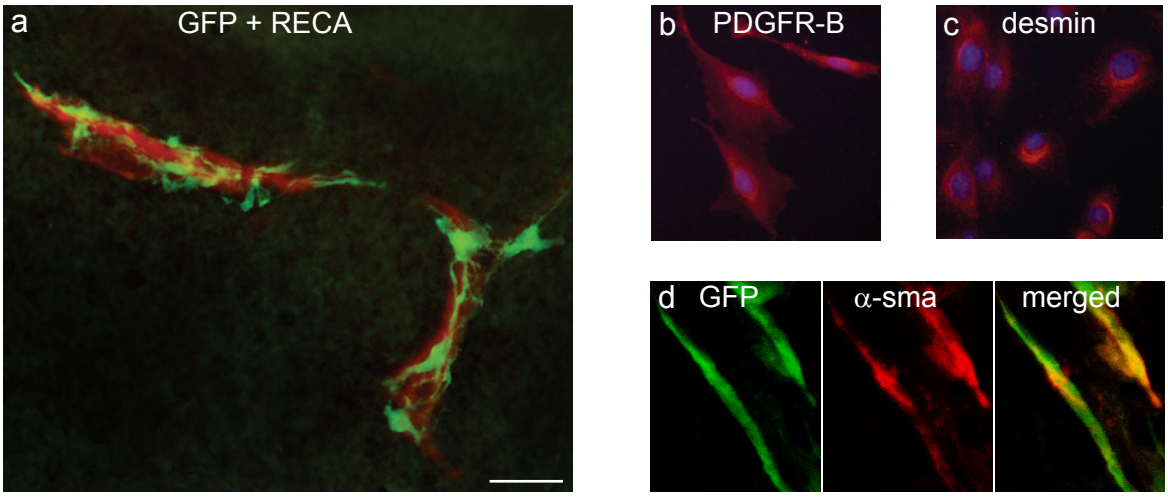

a
GFP + RECA
b
PDGFR-B
c
desmin
d
GFP
α-sma
merged

Table 1 Selected examples of different effects of MSCs in cancer models

Principal use of MSCs	Therapeutic modification	Tumor model	Route of MSC administration	Species: MSC/tumor/host	Refs.
Inhibition of tumor growth by delivery of pro-drug converting enzymes	HSV-tk[a]	glioma	i.t.	rat/rat/rat	25
	CD[b]	colon carcinoma	s.c./i.v.	hu/hu/mo	60
	CD[b]	prostate cancer	s.c./i.v.	hu/hu/mo	68
Inhibition of tumor growth by delivery of immunostimulatory cytokines	IL-2	glioma	i.t./i.c.	rat/rat/rat	20
	IL-7[c]	glioma	i.t.	rat/rat/rat	75
	IL-12	malignant melanoma	i.t./i.p.	hu/mo/mo	70
	IL-18	glioma	i.t.	rat/rat/rat	72
	IFNα	glioma	i.t./i.c.	mo/mo/mo	41
	IFNβ	breast cancer	s.c./i.v.	hu/hu/mo	27
	TRAIL	glioma	i.t.	hu/hu/mo	78
	TRAIL	glioma	i.c.	hu/hu/mo	77
	TRAIL	lung metastases	i.v.	hu/hu/mo	79
	CX3CL1	lung metastases	i.v.	mo/mo/mo	74
Delivery of oncolytic viruses	CrAD	ovarian cancer	i.p.	hu/hu/mo	30
	CrAD	glioma	i.c.	hu/hu/mo	82
	CrAD	glioma	i.a.	hu/hu/mo	83
Inhibition of tumor growth by un-modified MSCs	none	glioma	i.t./i.c.	rat/rat/rat	20
		Kaposi´s sarcoma	i.v.	hu/hu/mo	28
		malignant melanoma	i.t.	mo/mo/mo	57
		colon carcinoma	s.c.	rat/rat/rat	58
Promotion of tumor growth by un-modified MSCs	none	ovarian carcinoma	i.p.	hu/hu/mo	61
		malignant melanoma	s.c./i.v.	mo/mo/mo	62
		breast cancer	s.c.	hu/hu/mo	63
		colon carcinoma	s.c.	hu/hu/mo	64

Abbreviations: CD, cytosine deaminase; CrAD, conditionally replicating oncolytic adenovirus; HSV-tk, herpres simplex virus -thymidine kinase; hu, human; i.a., intra-arterial; i.c., intracerebral (at a distance to tumor); i.p., intraperitoneal; i.t., intra-tumoral; i.v., intravenous; IFN, interferon; IL, interleukin; mo, mouse; MAPC, multipotent adult progenitor cell; MSC, multipotent mesenchymal stromal cell; s.c., subcutaneous; TRAIL, tumor necrosis factor-related apoptosis inducing ligand

[a] MAPCs were delivered in combination with systemic administration of ganciclovir

[b] in combination with systemic administration of 5-fluorocytosine

[c] in combination with peripheral immunotherapy

Table 2 Critical issues for development of MSC-based cancer therapy

Issue	Status	Selected key refs.
Specific MSC tumor tropism	Demonstrated in numerous model systems	20, 24-36, 41-43, 52, 55
Therapeutic effect *in vivo* through transgene delivery	Demonstrated in numerous model systems	see table 1
Contribution to tumor stroma, vascularization and metastasis	Conflicting data	20, 29, 57-59, 63, 65, 66
Promotion of tumor growth by MSC suppression of host anti-tumor immune response	Conflicting data	26, 62, 78
MSC transformation *in vitro*	Conflicting data	99-101
Most efficient route of administration	Conflicting data	20, 24, 26, 27