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REVIEW

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Stem Cell Based Cancer Gene Therapy

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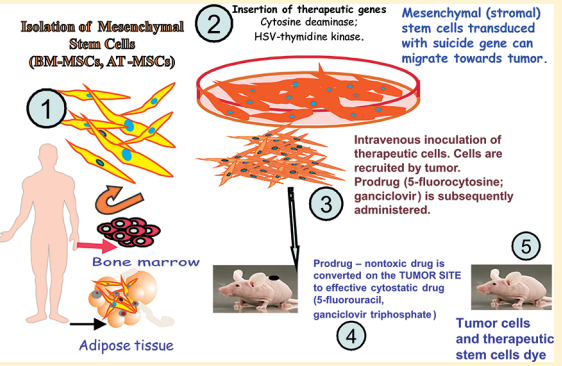
ABSTRACT: The attractiveness of prodrug cancer gene therapy by stem cells targeted to tumors lies in activating the prodrug directly within the tumor mass, thus avoiding systemic toxicity. Suicide gene therapy using genetically engineered mesenchymal stem cells has the advantage of being safe, because prodrug administration not only eliminates tumor cells but consequently kills the more resistant therapeutic stem cells as well. This review provides an explanation of the stem cell-targeted prodrug cancer gene therapy principle, with focus on the choice of prodrug, properties of bone marrow and adipose tissue-derived mesenchymal stem and neural stem cells as well as the mechanisms of their tumor homing ability. Therapeutic achievements of the cytosine deaminase/5-fluorocytosine prodrug system and Herpes simplex virus thymidine kinase/ganciclovir are discussed. In addition, delivery of immunostimulatory cytokines, apoptosis inducing genes, nanoparticles and antiangiogenic proteins by stem cells to tumors and metastases is discussed as a promising approach for antitumor therapy. Combinations of traditional, targeted and stem cell-directed gene therapy could significantly advance the treatment of cancer.



KEYWORDS: mesenchymal stem cells, prodrug enzymes, cytosine deaminase, HSV*tk*, suicide gene therapy, immunostimulation

INTRODUCTION

Tumor stroma is composed of a variety of cells including proliferating tumor cells, cancer stem cells (CSCs), tumor fibroblasts, endothelial cells, lymphocytes and other cells which infiltrate or are incorporated in the tumor mass. It is now generally accepted that cancer is a stem cell disease. CSCs give rise to a hierarchy of progenitor and aberrantly differentiated cells and are therefore entirely responsible for the cellular heterogeneity of the tumor.[1,2] Taking this into account, one of the major goals of cancer therapy should be targeting cancer stem cells. Hypothetically, drugs which would block self-renewal ability of CSCs either by killing them or making them differentiate might lead to cure. Development of therapies targeted at CSCs holds hope for improvement of survival of cancer patients, especially patients with metastatic disease or with aggressive tumors, like glioblastoma, where in addition disseminated tumor cells in brain tissue have to be attacked. Prodrug cancer gene therapy driven by mesenchymal stem cells (MSCs) might be one out of several treatment modalities fulfilling these requirements. It represents an attractive tool for activating the prodrug directly within the tumor mass, thus avoiding systemic toxicity. In addition, MSCs lack major histocompatibility complex MHC-II and show only minimal MHC-I expression.[3−5] Thanks to their immunosuppressive properties, allogeneic MSCs can substitute autologous stem cells in delivering the therapeutic agent in targeted tumor therapy.

SOURCE OF MSCS AND THEIR PROPERTIES

The biological role of MSCs, also known as mesenchymal stromal cells, is to repair damaged and used tissues in the body. Isolation of MSCs has been reported from almost every type of tissue, including bone marrow, adipose tissue, muscles, liver, dental pulp, but also placenta, amniotic fluid, menstrual blood,[6,7] or umbilical cord blood.[8] Mesenchymal stem cells, as we know them today, possess many attributes which support their use as a biologic therapeutic agent. They are easy to isolate, have enormous expansion potential in culture and are able to migrate toward sites of tissue injury. This ability includes tumor-tropic capacities. MSCs reside in the bone marrow (BM-MSCs) in small numbers (about 10 cells per million of mononuclear cells), but can be easily expanded *in vitro* because of their ability to adhere to plastic. Human adipose tissue is about 10 times more abundant for MSCs compared to bone marrow. Adipose-derived mesenchymal stem cells (AT-MSCs) possess similar properties, therefore BM-MSCs and AT-MSCs are used most frequently in stem cell therapies. According to the criteria provided by The International Society for Cellular Therapy, MSCs are defined by their plastic-adherent properties under standard culture conditions, by their ability to differentiate into osteocytes, adipocytes and chondrocytes *in vitro* under specific stimulus and by positive (CD105, CD73 and CD90) or negative (CD45, CD34, CD14 and HLA-DR) expression of specific surface markers.[9] It has

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been demonstrated and well established by many researchers that MSCs possess extraordinary ability to migrate toward and home into sites of injury. The first evidence of homing ability of MSCs was described in primates with severe multiorgan failure by Chapel et al.[10] Tumor microenvironment mimics the environment of an injured tissue and uses many of the same inflammatory mediators to attract the MSCs that are invoked in the wound healing process and may be therefore regarded as wounds that never heal.[11] Recently published data have also revealed an interesting potential of MSCs to suppress bacterial growth. One of the factors responsible for the antimicrobial activity of MSCs may be the human cathelicidin antimicrobial peptide hCAP-18/LL-37.[12]

Neural stem cells (NSCs)—self-renewing, multipotent cells that generate the main phenotypes of the nervous system—have been for their tumor-tropic capacities also exploited in preclinical as well as clinical studies in enzyme prodrug gene therapy approaches. NSCs can be obtained from fetal, neonatal or postnatal tissues.[13] Since it is not easy to isolate NSCs in a sufficient number, immortalized neural progenitor cells lines were prepared and used in several preclinical models of prodrug cancer gene therapy.[14−17] The well-characterized NSC line HB1.F3 was derived from fetal brain at 15 weeks of gestation and is known to be multipotent, migratory and nontumorigenic.

The effect of endometrial regenerative cells (ERCs) isolated from menstrual blood on inhibition of intracranial glioma growth has been also observed. Endometrial regenerative cells are a population of mesenchymal-like cells and are characterized by pluripotent differentiation capacity, expression of unique surface markers and production of a unique set of growth factors. When administered intravenously or intratumorally into rats bearing the aggressive C6 glioblastoma, significant inhibition of the tumor mass has been observed. It is assumed that the effect of ERCs on tumor reduction lies in the inhibition of angiogenesis and reduction of CD133 positive cells (cancer stem cells) in the tumor mass.[18]

■ MECHANISMS OF MSCs MIGRATION AND HOMING TO TUMORS

Stem cell-driven cancer gene therapy is based on the tumor-tropic property of MSCs. Tumor homing ability of MSCs holds therapeutic advantages compared to vehicles such as proteins, antibodies, nanoparticles and in some extent also viruses. Viruses or nonmigratory vector-producing cells have been utilized but display many shortcomings in effective delivery of the therapeutic agents. Virus-mediated gene therapies are limited by the difficulty in tracking cancer cells, which are infiltrating into the surrounding tissue, and by their migratory capacities.[19]

To clarify the mechanisms by which MSCs are able to track and migrate to the injured tissues or tumors, the idea of following the chemokine density gradient has been well established. The increased production of inflammatory mediators found at the sites of a tumor is potentially responsible for recruitment and engraftment of MSCs.[20] The most recent findings by Song suggest that matrix metalloproteinase-2 (MMP-2) and chemokine (C-X-C motif) receptor 4 (CXCR4) are involved in the multistep migration processes of MSCs tropism to tumors. Gene expression profiles of MSCs exposed to conditioned medium of various tumor cell types have been analyzed and revealed that MMP-2 expression was downregulated, while CXCR4 expression was upregulated.[21] The positive role of tumor-secreted chemotactic protein-1 (MCP-1) in stimulating the migration of MSCs to the tumor site was suggested as well.[22] By further investigations of researchers the role of CXCL-12, stem cell-derived factor-1 (SDF-1), CXCL-2, CINC-2, endothelial cell specific molecule-1, fibroblast growth factor-7, nuclear factor-kappaB p105 and thrombomodulin was proposed.[23] Enhanced migration of MSCs also correlated with increased SDF-1 protein production. Knockdown of SDF-1 expression inhibited migration of MSCs, thus confirming the importance of its expression by MSCs in the migration process.[23] The search for finding responsible elements that drive MSCs to home in sites of tissue injury or tumor was pushed forward by the discovery that MSCs express chemokine receptors CXCR1, CXCR2 and CCR2 and migrate upon stimulation with CXCL8.[24] Tumor migratory ability of human AT-MSCs has been confirmed in our laboratory in *in vivo* model of human colon cancer HT-29,[25] both *in vitro* and *in vivo*, on human prostate cancer cells derived from metastases[26] or melanoma *in vivo*.[27] In the model of brain tumors the migratory capacity and tumor tropism of MSCs have been confirmed in rats bearing syngeneic gliomas. MSCs inoculated into the contralateral hemisphere were able to migrate through the corpus callosum toward glioma cells in the opposite hemisphere.[28] Similar observation has been obtained from experiments with immunocompromised mice bearing intracranial xenografts of human glioma. MSCs showed an intrinsic attraction to gliomas and capacity to migrate between hemispheres toward gliomas. They were also able to localize to human gliomas following ipsilateral or contralateral intraarterial delivery. It has been suggested that, at least in part, the tropism of MSCs may be due to specific growth factors and cytokines (e.g., PDGF-BB, EGF, SDF-1α) secreted by the brain tumors.[29] Intratumorally implanted MSCs migrate to invasive glioma and its distant microsatellites in a rat model shown by Bexell et al.[30] The same phenomenon was noticed when implanting NSCs into experimental intracranial gliomas *in vivo* in adult rodents. NSCs distributed extensively throughout the tumor and migrated to aggressively expanding tumor cells. They followed this route when implanted intracranially at distant sites from the tumor or outside the CNS intravasculary. This evidence suggests that migration of NSCs can be extensive.[31] Although it has been well established that systemically administrated MSCs specifically engraft into neoplastic lesions within organism, the role of recruited MSCs in the tumor microenvironment is still not fully elucidated.

■ GENETICALLY MODIFIED MSCs FOR CANCER TREATMENT

Mesenchymal stem cell-targeted cancer gene therapy is a modification of earlier developed gene therapies known under several names such as gene-directed enzyme prodrug therapy, suicide gene therapy and others,[32,33] all describing a two-step treatment process. In the first step, the gene for a foreign enzyme (bacterial, yeast or viral) is delivered and targeted to the tumor by transduced MSCs. In the second step, the enzymatic activity of gene product is able to convert a far less toxic prodrug to its cytotoxic substance at the tumor site (for a review see Altaner[2]). Consequently, the active drug produced by an enzymatic process within transduced MSCs (called therapeutic stem cells) effectively kills neighboring tumor cells and also more resistant cells in which it is formed. This process is called bystander effect or neighboring cell killing effect. Thus the genetically modified

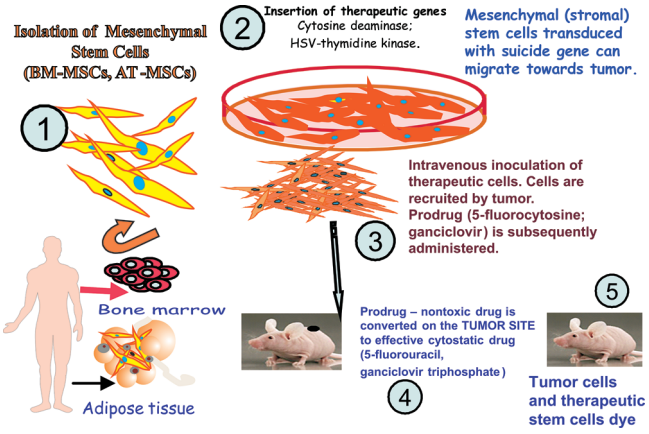

Figure 1. Mesenchymal stem cell targeted cancer gene therapy. AT-MSCs are isolated by collagenase digestion of lipoaspirate material. BM-MSCs are isolated from mononuclear fraction of bone marrow obtained by Percoll density gradient centrifugation. Obtained cells are plated in a plastic dish and expanded. Stem cells are transduced with retrovirus containing suicide gene and gene encoding resistance to antibiotic G418. Transduced cells are exposed to selective medium containing antibiotic G418. The population of selected therapeutic stem cells is expanded and used for systemic or intratumoral injections. Therapeutic cells migrate to the tumor site. Subsequently nontoxic prodrug is administrated, which is at the tumor site converted to toxic drug killing tumor cells and therapeutic cells as well. No adverse systemic toxicities have been observed.

MSCs are eliminated from the organisms. In addition, dying tumor and therapeutic stem cells can induce host immune responses mediated by natural killer (NK) cells and T-cells. This therapeutically beneficial effect is known as the distant bystander effect.[34−36] Prodrug cancer gene therapy driven by MSCs toward brain tumors was recently reviewed by Bexell et al.[37] A schematic picture depicting the steps of mesenchymal stem cell-targeted cancer gene therapy is in Figure 1.

■ STEM CELL DELIVERY OF PRODRUG-CONVERTING ENZYMES

Gene-directed enzyme prodrug therapy (GDEPT) known also as virus-directed enzyme prodrug therapy (VDEPT) or suicide gene therapy (SGT) did not show a clinically significant effect in the past. Besides other factors, the failure was caused by the missing tumor specificity of this approach. On the other hand, stem cell driven prodrug cancer therapy is quite different from the classical prodrug gene cancer therapies. Tumor homing ability of MSCs and *in vitro* preparation of therapeutic stem cells having prodrug converting genes integrated as DNA provirus in the cell genome is the main difference from earlier versions of GDEPT. Stable production of prodrug converting enzyme under strong retroviral promoter in therapeutic stem cells is of great advantage.

Stem cell-based enzyme prodrug therapy represents a more specific, less toxic and tailored approach to treating invasive cancers. Investigation in this area has gained tremendous momentum over the last decades, since it might have the potential to deliberately target and eradicate the tumor initiating stem-like cells known to be the stumbling block to successful treatment. A large number of enzyme prodrug systems have been developed. The most frequently used enzymes are of nonmammalian origin and differ from any circulating endogenous enzymes, thus fulfilling the general requirement to be expressed in such concentrations which enable achievement of sufficient conversion of a prodrug for high therapeutic efficiency. The disadvantage compared to the enzymes of mammalian origin is that they are likely to be immunogenic.

Cytosine Deaminase/5-Fluorocytosine Prodrug System. The basis for the cytosine deaminase/5-FC enzyme prodrug therapy is the ability of bacterial or yeast enzyme cytosine deaminase to convert nontoxic prodrug 5-FC into active substance 5-fluorouracil (5-FU). Although both of them are capable of doing so, yeast cytosine deaminase (yCD) shows much higher efficacy in converting the 5-FC than the bacterial enzyme. It has been shown that yCD produces a 15-fold higher amount of 5-FU compared to bacterial CD.[38] Increased efficiency of 5-FC to 5-FU conversion is achieved when bifunctional yeast fusion gene cytosine deaminase::uracil phosphoribosyltransferase (CD::UPRT) is used. The gene product of a bifunctional chimeric protein shows at least 100-fold higher activity than native yeast CD.[39] We have shown in *in vivo* experiments that human CDy-AT-MSCs administered subcutaneously as a mixture with tumor cells, or intravenously, significantly inhibited the growth of human colon adenocarcinoma cells HT-29.[25] Similar results were obtained when xenografts of human melanoma and glioma in nude mice were treated with CDy-AT-MSCs/5-FC system.[26] Our preclinical study has proven efficacious in the treatment of human tumor cells derived from bone metastases of prostate carcinoma.[26] The observed high efficacy of the yeast CD::UPRT can be assigned to ability of the enzyme to convert formed 5-FU to 5-fluorouridine monophosphate, which directly kills cytosine deaminase expressing cells and surrounding cells via the bystander effect.[40] In addition, we have found that the high efficacy of AT-MSCs transduced with yeast CD::UPRT (CDy-AT-MSC) is caused by induction of proapoptotic genes in tumor cells by their paracrine effect. In experiments where the therapeutic cells CDy-AT-MSC in inserts were seeded into tissue plates with a monolayer of human colorectal carcinoma cells HT29, a strong increase of products of proapoptotic genes Bad, Bax, TRAIL R1/DR4, and FADD was detected. On the other hand, the amount of products of antiapoptotic genes Bcl-2 and Bcl-X decreased. In the same experimental setup AT-MSCs did not influence the expression profiles of human apoptosis-related proteins (Figure 2).

The ability of NSCs, BM-MSCs and AT-MSCs engineered to express suicide gene products to target brain tumors triggered several preclinical studies to test the efficacy of this approach. Strong tropism of NSCs and MSCs toward brain tumors is attributed to receptors for chemokines and growth factors including SDF-1, MCP-1, HGF, IL-8, NT3, TGF-b 0 and VEGF.[29,41−43] BM-MSCs as well as AT-MSCs have been shown to share some characteristics with pericytes.[44] When implanted directly into rat malignant gliomas, MSCs infiltrate the majority of invasive glioma extensions as well as distant tumor microsatellites, where they integrate into tumor vessel walls and express pericyte markers.[37] This property might therefore enhance their migration to highly vascularized tumors. It has been shown that targeting of tumor endothelium and tumor pericytes synergistically affects tumor vascularization and tumor growth.[45]

C6 rat glioma often serves as an experimental model system for glioblastoma. Since the implantation of C6 malignant cells into animal brain resembles *in vivo* tumor growth, it helps to elucidate the mechanism of tumor growth, angiogenesis and invasion and to improve the potential use of gene therapy.[46] It has also been demonstrated that the C6 cell line contains a fraction of cancer

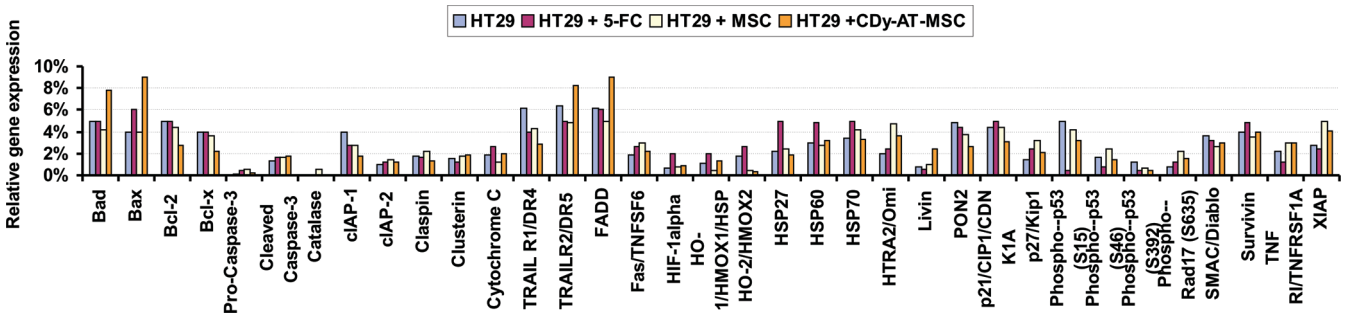

Figure 2. Apoptosis induction by AT-CDy-MSCs in human colorectal cells HT29 detected by Human Apoptosis Array Kit. Inserts containing AT-MSCs or CDy-AT-MSCs were added into the plates with human colorectal cells HT29. As controls served plates with monolayer of human colorectal carcinoma cells HT29 only or the same plates where 5-fluorocytosine (100ug/mL) was added. The plates were incubated for three days, and then cells were lysed. The same amount of cell lysate proteins were analyzed by Human Apoptosis Arrays (R&D Systems). Apoptosis array data on developed X-ray film was quantified by scanning the film on the transmission-mode scanner, and the array image file was analyzed using ImageJ software.

stem cells expressing CD133 and nestin, widely used markers for brain CSCs.[47] Satisfactory evidence of potent anticancer effect of CD/5-FC prodrug enzyme therapy on glioblastoma has been indicated by experiments conducted recently. Rats intracranially inoculated with C6 glioma cells were treated with MSCs engineered to express bacterial cytosine deaminase. Results suggested that the concentration of CD enzyme is crucial because tumor mass was reduced in proportion to the 5-FC dosage. Repeated delivery of CD-MSCs successfully suppressed the tumor growth.[48] Our laboratory has shown that AT-MSCs engineered to express yeast suicide gene CD::UPRT effectively inhibit glioblastoma growth in rats after 5-FC therapy. Likewise, the overall effect on survival of the animals was dependent on the dose of inoculated therapeutic cells. We have also demonstrated that continuous intracerebroventricular delivery of 5-FC using an osmotic pump reduced the dose of prodrug required for the same therapeutic effect when injected intraperitoneally and along with repeated administration of therapeutic stem cells increased the survival time.[49]

The potential of NSCs as an effective delivery system for CD to target medulloblastoma, the most common childhood malignant brain tumor, has been for the first time proposed by research group of Kim.[14] In nude mice with intracranially established medulloblastoma, 76% reduction of tumor mass was observed after injection of cytosine deaminase-modified NSCs and consequent systemic 5-FC treatment. Not only primary brain tumors may be targeted by NSC-based enzyme prodrug gene therapy but also brain metastases. Cytosine deaminase-expressing NSCs and systemic 5-FC prodrug administration showed potential to target disseminated melanoma brain metastases[50] and breast cancer brain metastases.[51] Brain metastases are difficult to treat because of the inability of most drugs to penetrate the blood-brain-barrier (BBB) and because of the dispersed localization of neoplastic cells. The cytotoxic drug 5-FU is effective in the treatment of brain metastases, but unfortunately cannot penetrate the BBB. However, systemically administered 5-FC is able to cross the BBB, and when converted by the CD enzyme at the tumor site, it might then exert a specific cytotoxic effect. In both settings it has been demonstrated that NSCs were attracted by brain metastases equally as by primary brain tumors. The tumor burden in animals with established melanoma brain metastases after intracranial implantation of CD-NSCs has been reduced by 71%.[50] Recently, FDA approved the first human neural stem cell clinical trial in treating recurrent high grade gliomas. Based on the evidence of intrinsic tumor tropism of NSCs, the therapy uses a genetically modified immortalized human neural stem cell line to deliver *Escherichia coli* cytosine deaminase to brain tumor sites in combination with oral 5-FC (http://clinicaltrials.gov/).

Ito et al. applied a combination therapy for the treatment of malignant gliomas using human NSCs expressing CD and IFN-β.[52] The IFN-β cytokine, exerting pleiotropic effects, has shown potent antitumor activity in patients with malignant glioma in previous studies of phase I clinical trial. This combination therapy appeared to exert an additive effect in destroying intracerebral gliomas, intensified the bystander effect and proved to be more effective than CD-based strategy alone.[52]

The antitumor effect of MSCs producing CD followed by 5-FC administration has been demonstrated not only on brain tumor models but also on a mouse gastric cancer xenograft. Tumor volumes of mice injected with CD-MSCs decreased significantly after treatment with 5-FC. 5-Fluorouracil has been widely used as a chemotherapeutic choice for a majority of solid tumors, including colon and gastric cancers. Since its high systemic toxicity, adverse and serious side effects on patients, the results obtained from this study are promising.[53] NSCs expressing 5-FC are also able to target and retard the growth of a tumor induced by stereotactic implantation of human breast cancer cells in an immune-deficient mouse.

Herpes Simplex Virus Thymidine Kinase/Ganciclovir Prodrug System. The first clinical trials for the treatment of malignant glioma were conducted in 1990s with Herpes simplex virus thymidine kinase in combination with ganciclovir.[19]

Ganciclovir (GCV), a nontoxic purine analogue, upon phosphorylation with the enzyme HSV*tk* is further phosphorylated by endogenous kinases to GCV-triphosphate, which inhibits DNA synthesis, thus leading to cell death via apoptosis.[54] It has been demonstrated that AT-MSCs expressing HSV*tk* are able to exert cytotoxic effect on human glioblastoma cells *in vitro* and that formation of gap junctions is crucial for induction of the bystander cytotoxic effect on tumor cells.[55] A preclinical study confirming the feasibility of this approach on glioma *in vivo* level has been demonstrated using NSCs as a HSV*tk*-delivery vehicle.[56] A significant number of experimental animals co-implanted with HSV*tk*-NSCs and C6 cells and treated with ganciclovir (GCV) showed prolonged survival.[57] However, recently published data by Amano et al. suggested that treatment strategy using MSCs transduced with HSV*tk* and GCV is more feasible and practical for clinical application than the method using NSCs.[58] MSCs are much easier to obtain from the adult subjects and exert a sufficient

bystander effect. The efficacy of the HSV*tk*/GCV model of suicide gene therapy exploiting MSCs as delivery vehicles has been confirmed in several studies. Song et al. used lentivirally transduced BM-MSCs expressing HSV*tk* to inhibit the growth of subcutaneous PC3 prostate cancer xenografts as well as metastastic RIF-1 fibrosarcoma tumor in nude mice in the presence of GCV.[59] Mori et al. confirmed the tumor retarding effect of HSV*tk*-expressing BM-MSCs on Fisher rats bearing intracranial murine gliomas.[60] BM-MSCs expressing HSV*tk* combined with overexpression of connexin 43 (Cx43) were shown to enhance the bystander effect of suicide gene therapy by restoration of gap junctions in intercellular communication. In rats bearing C6 gliomas this combined model increased survival time of treated animals and proved to be highly effective.[61]

Bak et al. reported an interesting approach using BM-MSCs transduced with a baculoviral vector harboring the *Herpes simplex virus* thymidine kinase gene. The transduced cells were recruited to tumors after systemic injection. Growth inhibition of mouse xenografts of human U87 glioma cells was observed when transduced cells were inoculated intravenously. Strong expression of transgenes in MSCs transduced by baculovirus vectors indicates that these vectors might serve as an attractive option to other viral vectors for MSC transduction.[62]

Other Prodrug−Enzyme Strategies. Besides well-characterized HSV*tk*/GCV or CD/5-FC systems, other enzyme−prodrug approaches have also been tested. Choi et al. sought to examine the use of human AT-MSCs genetically modified to express rabbit carboxylesterase (rCE) enzyme (AT-MSC.cRE), which can efficiently convert the prodrug CPT-11 into the active drug SN-38. Activation of the prodrug CPT-11 by human esterase is poor, whereas the cRE converts the CPT-11 more effectively into cytotoxic drug SN-38, which acts as a potent topoisomerase I inhibitor. To circumvent the immunological reaction by immune-mediated toxicity of viral vectors, a nonviral transfection of AT-MSCs has been used, with transfection efficacy approximately 90%. The therapeutic potential of AT-MSC.cRE has been tested on brainstem glioma bearing rats. Rats treated with AT-MSC.cRE and CPT-11 survived only 10 days longer than those in control groups. The relatively low success can be assigned to a suboptimal concentration of CPT-11 or ineffectiveness of the drug against gliomas.[63]

Previously described prodrug-activation gene therapy model P450 IFO/CPA has been recently used in combination with NSCs. Mice neural stem cells obtained from two-day-old pups' brains were engineered to express enzyme P450 2B6 (CYP2B6), which catalyzes conversion of cyclophosphamide (CPA) into membrane diffusible DNA-alkylating metabolites. NSCs were then coinjected with glioblastoma cells into mouse brain. Upon CPA administration, the growth of glioblastoma was inhibited in all animals with orthotopic grafting of NSCs-CYP2B6.[64]

Khan et al. designed a novel system, in which tomato thymidine kinase 1 (toTK1) and nucleoside analogue prodrug zidovudine (AZT) is used. The merit of the toTK1 is that it efficiently phosphorylates its substrate AZT not only to AZT monophosphate but also to AZT diphosphate with excellent kinetics, while human thymidilate kinase cannot substantially phosphorylate AZT-MP into the AZT-DP form. When delivered to an intracranial glioblastoma xenograft in nude rats by NSCs, the tumor growth in animals treated with AZT was substantially attenuated. The toTK1/AZT system was found superior and displayed higher efficiency in killing glioblastoma cells than the HSV*tk*/GCV system.[65]

Stem Cell Delivery of Other Therapeutic Agents. One of the strategies in combating brain cancer is to augment the immune response of the afflicted individual. Stem cell-directed therapies started nearly 10 years ago by studies in which human MSCs engineered to express interferon β (IFN-β) were used for targeted delivery of this potent antiproliferative and proapoptotic agent to metastatic breast and melanoma models.[66,67] Studies showed that subcutaneous tumor growth in a SCID mouse xenograft model was inhibited and the survival of animals was prolonged when melanoma cells were coinjected with IFN-β-MSCs. Significantly inhibited intracranial mouse glioma growth was observed when experimental mice were treated with interleukin-12-expressing human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs). UCB-MSCs confirmed their migratory ability toward established mice glioma and exerted antitumor effects through associated increase of local IL-12 levels, T-cell attraction, IFN-γ secretion and inhibition of angiogenesis processes. Interesting to mention, tumor-free mice after treatment with IL-12-modified UCB-MSCs were resistant to repeated establishment of gliomas, which is associated with long-term T cell immunity specifically targeted against tumor.[68] NSCs demonstrated intense tropic traits against glioma cells as well. IL12-producing NSCs targeted disseminated intracranial gliomas and enhanced T-cell immune response and prolonged survival of experimental animals.[69] A similar effect was achieved when C6 glioma bearing rats were presented with MSCs producing interleukin-18 (IL-18) infected by adenoviral vector. The T-cell infiltration was enhanced and long-term immunity established, which led to inhibited glioma growth and prolonged survival of glioma-bearing rats.[70] Gene modification of MSCs by infection with an adenoviral vector encoding human interleukin-2 (IL-2) and its application to 9L glioma-bearing rats showed the same results.[28] MSCs genetically modified with IL-2 induced an effective immune response against melanoma model. Significantly delayed tumor growth was observed when IL-2-producing MSCs in a dose-dependent manner were mixed with melanoma cells.[71] The group of Gunnarsson has observed regression of rat gliomas when intratumoral delivery of interleukin-7 (IL-7) by MSCs was combined with previous immunization using interferon-gamma transduced autologous tumor cells. Combined immunotherapy allowed a higher recruitment of T-cells at the tumor site.[72] Xin et al. demonstrated that MSCs expressing CX3CL1 cytokine administered intratracheally to lung tumor bearing mice resulted in a strong inhibitory effect on lung metastases and prolonged the survival of tumor-bearing mice without apparent adverse effects.[73]

MSCs engineered to express secreted tumor necrosis factor related apoptosis-inducing ligand (TRAIL) induce caspase-mediated apoptosis in established glioma cell lines as well as CD133-positive primary glioma cells *in vitro* and exert profound antitumor effects *in vivo*.[74] UCB-MSCs similarly to BM-MSCs also proved to be capable of delivering secretory trimeric form of TRAIL into mice bearing gliomas and exerting significant reduction in tumor burden.[75,76] When MSCs expressing TRAIL (MSCs-TRAIL) were administrated into a human pancreatic cancer mouse model, they infiltrated both tumor and lymphatic tissues. MSCs-TRAIL triggered limited apoptosis in human pancreatic carcinoma cells that were resistant to soluble recombinant TRAIL. MSCs-TRAIL-mediated cell death was markedly increased by concomitant knockdown of X-linked inhibitor of apoptosis protein XIAP by RNAi in the cancer cells. This interesting report further supports the circumstantial evidence

that MSCs can attack also cancer stem cells. Thus the combined approach using systemic MSC-mediated delivery of TRAIL together with XIAP inhibition suppresses metastatic growth of pancreatic carcinoma.[77]

It has been shown that MSCs can be easily labeled with superparamagnetic iron oxide (SPIO) nanoparticles.[49] They have also been tested as cellular vehicles for delivery of nanoparticles to brain tumors. MSCs carrying two kinds of nanoparticles (poly lactic acid NPs and LNCs) were shown to retain their viability, differentiation and tumor homing capacities.[78]

MSCs can also be utilized for delivery of antiangiogenic proteins. Ghaedi et al. used lentivirus-transduced MSCs for production of functional α1-antitrypsin (AAT) and showed that AAT secreted from transduced cells significantly inhibited human umbilical cord vein endothelial cell (HUVEC) proliferation compared to untransduced MSCs.[79]

CONCLUSION

The success of an enzyme−prodrug gene therapy depends on several factors. The catalytic activity of the enzyme encoded by suicide gene, a suitable prodrug−enzyme combination, ability of the vector to target tumor cells, sufficient transgene expression and very importantly the extent of bystander effect are the main indicators of effective and successful suicide gene therapy. Various combinations of suicide genes, prodrugs and gene transfer technologies have been investigated in order to find the most suitable and effective system in combating the otherwise incurable tumors. The cytosine deaminase/5-fluorocytosine and *Herpes simplex virus* thymidine kinase/ganciclovir suicide gene therapies are considered as the most effective and most promising therapeutic strategies mainly for malignant gliomas, but also for other malignancies. However, the CD/5-FC system is regarded more efficient than the HSV*tk*/GCV system for its stronger bystander effects.[49] 5-FU is a small molecule therefore it is able to enter neighboring cells through simple diffusion, while GCV requires gap junctions to affect surrounding cells. The failures of up to the present suicide gene therapies were mainly caused by the inability of vectors carrying the suicide gene to reach invasive tumor cells distant from the tumor bulk as well as inefficient spread of the vectors within the tumor. Therefore the stem cell-based suicide gene therapy based on the inherent and privileged tumor-tropic nature of mesenchymal or neural stem cells holds great potential of moving suicide gene therapy closer to the patient's bedside.

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