Mesenchymal stromal cells as a drug delivery system

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Abstract

Mesenchymal stromal cells (MSC) are multipotent cells that can self-renew and at the same time differentiate into multiple lineages with specific surface marker expression. During the past several years, MSCs have generated a great deal of interest in many clinical settings, including regenerative medicine, immune modulation, and tissue engineering. Many studies have demonstrated their remarkable tumor tropic properties. Several pre-clinical and clinical studies have demonstrated the efficacy of genetically modified MSC to express and release therapeutic factors, confirming their ability to serve as an excellent base for cell-mediated gene therapy. This chapter will review the literature on the use of MSCs as a therapeutic drug delivery system.

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1. Introduction

Using viral vectors for gene transfer to tumor tissue is a very effective and promising approach, which allows the achievement of high efficiency (Cavazzana-Calvo et al., 2000), (Davidson et al., 2000) expression in the target (Gao et al., 2007). However, the feasibility of viral vectors for clinical trials has been hindered due to many safety and biological concerns (Cristiano, 1998), (Harrington et al., 2002), (Hacein-Bey-Abina et al., 2003), (Nair, 2008). Though some studies have proposed that viral vectors can be relatively safe (Myers et al., 2008), (Chiocca et al., 2008), even the slightest possibility of increased oncogenesis, introduction of the virus into the germline, or induction of an immune response is unfavorable. Therefore, a better alternative may be to develop a delivery system that would direct therapeutic agents to appropriate sites and express therapeutic genes within or near the tumor. The ideal system for tumor-selective delivery would be cells that possess tumor tropism and at the same time are compatible and non-immunogenic to the host. During the past few years, the ability to transfer genes and express proteins in stem cells has provided the feasibility of using cell-based gene therapy approaches for clinical trials. For clinical applications, the transplanted cells can be used either to repair, replace, or regenerate damaged tissue/organs; express enzyme or gene products to overcome deficiency; or as drug/gene delivery systems.

Aboody et al. (Aboody et al., 2000) demonstrated that neural stem cells (NSCs) administered intracranially possess extensive tropism and homing towards glioma tumors. This behavior of NSCs has been exploited as a tumor targeting strategy for glioma and medulloblastoma gene therapy (Kim et al., 2006), (Shimato et al., 2007). Genetically modified NSCs expressing interleukin (IL) -4 (Benedetti et al., 2000), IL-12 (Ehtesham et al., 2002b), PEX (Kim et al., 2005), carboxyesterase (Danks et al., 2007) or tumor necrosis factor related apoptosis-inducing ligand (TRAIL; Ehtesham et al., 2002a), (Shah et al., 2005) in experimental glioma models exhibited significant antitumor effects following intracranial transplantation. Unfortunately, the preparation of allogenic NSCs for clinical application is a major challenge. There are several ethical and logistical problems in terms of their isolation from neonatal tissue and their immunologic compatibility in allogenic transplantation. Thus, the ideal candidate of cellular therapy for clinical use are cells that can be harvested without difficulty, *ex vivo* processed very efficiently, and later transplanted into patients. Mesenchymal stromal cells (MSCs) indeed possess all these properties and could be a compatible source for use in human clinical trials (Giordano et al., 2007).

2. The potential clinical applications of human MSC

MSCs are non-hematopoietic cells, initially described by Friedenstein (Friedenstein et al., 1970), (Prockop, 1997). These cells are characterized by their plastic adherence in culture, differentiation potential and cell surface marker expression. Based on recent guidelines, MSCs must express CD105, CD73, and CD90, and lack the expression of CD45, CD34, CD14, or CDllb, Cd79a or CD19, and HLA class II surface markers (Dominici et al., 2006). These represent only the minimal requirements for their identification, as MSCs may often express different markers or specific combinations based on their microenvironment. MSCs must also differentiate to osteoblasts, adipocytes, and chondroblasts. Upon commitment to one of these lineages, the morphology of the cells and expression of markers will change to match those of each respective lineage. For example, in the case of osteogenic differentiation, the expression of marker CD106 decreases, and mRNA levels of osteogenic genes, including alkaline phosphatase, bone sialoprotein, osteocalcin, and transcription factors RUNX2 and Osterix increase (Liu et al., 2008). Analogous lineage-specific marker changes occur for differentiation into adipocytes and chondroblasts.

MSCs can be obtained from bone marrow and other tissues, such as peripheral blood, umbilical cord blood, adipose tissue, and placenta (Erices et al., 2000), (Zuk et al., 2002), (Fukuchi et al., 2004), (Kern et al., 2006). Several other sources of MSCs have been reported including liver (Campagnoli et al., 2001), periodontal ligament (Trubiani et al., 2005), hair follicles (Shih et al., 2005), amniotic fluid (De Coppi et al., 2007), and placenta (In ‘t Anker et al., 2004). MSCs reside in the bone marrow in small numbers (0.001–0.01% of nucleated cells (Kawada et al., 2004), but can be easily expanded *in vitro* (*ex vivo*) to yield a sufficient number of cells for clinical issue. Human MSCs exhibit unique characteristics including their ability to differentiate, migrate to sites of tissue injury/inflammation, genetic modifiability, and expression of protein (Chamberlain et al., 2007), (Reiser et al., 2005), (Brooke et al., 2007), (Lazennec and Jorgensen, 2008), (Kumar et al., 2008). MSCs possess strong immunosuppressive properties that can be exploited for successful autologous as well as heterologous therapies (Stagg et al., 2006), (Jones and McTaggart, 2008).

Hoffman and his group have shown that *ex-vivo* expanded allogenic and autologous MSCs transduced with eGFP distributed to a wide range of tissues in baboons, including lung, thymus, bone, skin, cerebellum, and gastrointestinal tract (Devine et al., 2003). The results suggest that MSCs distribute widely to a variety of nonhematopoietic tissues

following systemic infusion and may possess proliferative capacity within these tissues. Intra-arterial and intravenous injections of MSCs in rats, led to early engraftment in the lung and later in the liver and other organs (Gao et al., 2001). In Osteogenesis Imperfecta (OI) transgenic mice, infused wild type MSCs homed to many organs, including the lung, marrow, bone, skin, brain, and spleen (Pereira et al., 1998). In preclinical studies MSCs have been shown to improve myocardial function after myocardial infarction in rodents (Itescu et al., 2003) and pigs (Amado et al., 2005). In experimental autoimmune encephalomyelitis (EAE), Zappia et al. (Zappia et al., 2005) showed that intraperitoneally injected MSCs migrated to the subarachnoidal space in close contact with the immune cells. Houghton et al. (Houghton et al., 2004) was the first to report MSC engraftment into gastric glands in a model of gastric cancer. Khakoo et al (Khakoo et al., 2006) showed that human MSCs injected intravenously (i.v.) home to sites of tumorigenesis in a model of Kaposi's sarcoma. Studies have shown that injection of MSCs in the contralateral hemisphere, the carotid vein or the tail vein, led to MSC homing in the tumor on the other hemisphere (Nakamura et al., 2004), (Nakamizo et al., 2005). Slavin et al (Slavin et al., 2008) have shown in preclinical studies using the animal model of Multiple Sclerosis (MS) and EAE, that bone marrow MSCs migrated to the inflammed central nervous system (CNS) and differentiated into cells expressing neuronal and glial cell markers to regenerate existing defects in the CNS. In summary, MSCs exhibit tropism to sites of wounds, chronic inflammation, and tissue damage as well as to the tumor microenvironment (Spaeth et al., 2008).

The mechanisms underlying the migratory ability of MSCs are not yet fully characterized or known; however, it is likely that signaling factors secreted from the tumor are the cause of this behavior. Studies have shown that differential gene expression of MSCs (Menon et al., 2007) as well as different molecular mechanisms involving chemokine receptors and adhesion molecules (Chamberlain et al., 2007) are associated with MSC migration towards specific tumor microenvironment. Other groups have also shown that there is specific migration of MSCs towards growth factors upregulated in tumors, such as PDGF, EGF, and VEGF (Beckermann et al., 2008), and chemokines (Spaeth et al., 2008). MSCs are likely to have chemotactic properties similar to those of immune cells that allow them to respond to sites of injury or inflammation. This may be demonstrated by the fact that radiation enhances inflammatory signaling in the tumor microenvironment, and this may be used to cause MSC migration. Klopp et al. irradiated murine 4T1 breast carcinomas and monitored subsequent MSC migration within the mice. They found that there was increased MSC migration and engraftment in radiated tumors, and that various cytokines and growth factors, including TGF-β1, VEGF, and PGF-BB, were upregulated in the radiated tumors. Furthermore, the chemokine receptor CCR2 was upregulated in MSCs that migrated towards irradiated tumor cells (Klopp et al., 2007). More recently, Gutova et al. reported that expression of urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR) in cancer cells underlies a novel mechanism of stem cell tropism to malignant solid tumors (Gutova et al. 2008). Thus, signaling factors may likely play a significant role in MSC migration and tumor tropism.

In a clinical trial, breast cancer patients, who received high dose chemotherapy and autologous peripheral blood progenitor cell transfusion, showed facilitated haematopoietic recovery after infusions of autologous MSC (Koc et al., 2000), (Le Blanc et al., 2007). The use of MSCs in patients is rapidly emerging as a new means of graft-versus-host-disease (GVHD) treatment. In one case, MSC DNA was detected in the colon and lymph node of a patient treated with MSCs for steroid resistant GVHD (Le Blanc et al., 2004), (Ringden et al., 2006), (Lazarus et al., 2005). In addition, MSC engraftment was confirmed in the marrow, bone and skin of OI patients after intravenous injection of MSCs (Horwitz et al., 2002; Horwitz et al., 1999). In summary, MSCs possess the potential for a large number of gene therapy applications.

3. Genetically modified MSC for therapy

Cell-based gene therapy represents a promising treatment alternative for patients and has gained momentum in the past several years. Even though several experimental and preclinical studies have paved the way for clinical translation, more studies are needed. Attempts to use MSCs as cellular delivery vehicles have taken advantage of the tumor tropic ability of MSCs, expression of genetically engineered proteins, and utilization for the delivery of therapeutic gene products.

Genetically engineered MSCs have been used for improvement in hematopoietic engraftment following myeloablative transplantation regimens (Nolta et al., 1994), (Dao et al., 1997), (Ringden et al., 2006), and the targeted delivery of antitumor factors by secretion of growth factors and cytokines (Brooke et al., 2007), (Lazennec and Jorgensen, 2008). These studies have led to several clinical trials using MSCs for the treatment of inherited disorders, producing promising results in OI (Horwitz et al., 2002; Horwitz et al., 1999), metachromatic leukodystrophy, and Hurler's syndrome (Koc et al., 2002). Pilot clinical trials to investigate the safety and feasibility of intrathecal treatment with MSCs in conditions of MS and ALS (amyotrophic lateral sclerosis) in patients are underway (Slavin et al., 2008).

Studies have investigated the role of genetically modified MSCs as cellular therapy for diabetes. The introduction of the pancreatic duodenal homeobox-1 (PDX-1) gene into MSCs resulted in their differentiation into functional insulin-producing cells which produced euglycaemia in streptozotocin-induced diabetic mice (Li et al., 2007b), (Karnieli et al., 2007). Meyerrose et al (Meyerrose et al., 2008) examined the utility of human MSCs for treatment of an inherited disorder of enzyme deficiency -mucopolysaccharidosis type VII (MPSVII). MPSVII results from the genetic deficiency of the enzyme β-glucuronidase (GUSB). Transduced human MSCs expressing GUSB retained their normal trafficking ability *in vivo* and mediated relatively high and consistent levels of the protein for an extended time, in an authentic xenotransplantation model of human disease.

4. Genetically modified MSC for cancer

Detailed investigations of MSC migration and the role of factors influencing this tropism have paved the potential for MSC-targeted therapies. Studies have shown that by genetic manipulation of MSCs, either to over express target receptors or by introduction of exogenous genes for expression/secretion of a desired therapeutic factor, the migration efficiency to specific tumor cells can be improved. This specific and directed approach is a very promising step in the field of gene therapy which allows targeted treatment of cancers.

4.1. Bone marrow derived mesenchymal stromal cells

4.1.1. Interferon (IFN) – α and β

IFN-β has a wide range of biological activities including potent antiproliferative (Wong et al., 1989),(Johns et al., 1992) and proapoptotic (Chawla-Sarkar et al., 2001) effects. However, its *in vivo* therapeutic efficacy has been limited due to toxicity associated with systemic administration. Human MSCs, engineered to express interferon β (IFN-β), have been used for targeted delivery of this potent antiproliferative and proapoptotic agent to metastatic breast and melanoma models (Studeny et al., 2004),(Studeny et al., 2002), gliomas (Nakamizo et al., 2005) and lung metastasis (Ren et al., 2008b). Studies reported by Studeny et al (Studeny et al., 2002),(Studeny et al., 2004) showed that subcutaneous tumor growth in a SCID mouse xenograft model was inhibited and prolonged survival of animals was observed only after the coinjection of A375SM melanoma cells with IFN-β-MSCs. Similarly, i.v administration of IFN-β-MSCs significantly prolonged the survival of animals with established metastases of either MDA 231 breast carcinoma or A375SM melanoma tumor in the lung. This effect is presumably due to the local production of IFN-β by MSC IFN-β cells in the tumor microenvironment. Toxicity effects associated with IFN-β were reduced by delivering MSCs expressing IFN-β to tumors.

Nakamizo et al (Nakamizo et al., 2005) have shown that hMSCs have a tropism for human gliomas after intravascular and local delivery of the cells. In this study, this tumor tropism was exploited therapeutically by engineering hMSCs to release IFN-β. hMSC-IFN-β show tropism for human gliomas after either intravascular or local delivery. The results indicate that both intratumoral and regional delivery of hMSC-IFN-β cells into the internal carotid artery by injection significantly extended the survival of animals with established U87 intracranial gliomas.

In a related study, Ren et al (Ren et al., 2008b) evaluated the potential of genetically modified MSCs expressing IFN-β in reducing tumor growth in a model of prostate cancer lung metastasis. Targeted homing of MSCs producing IFN-β was seen at tumor sites in the lungs with established TRAMP-C2 pulmonary metastases, and this resulted in suppression of tumor growth. Cell therapy with MSC-IFN-β cells could be used to increase IFN-β expression in tumors and surrounding tissues and to control the growth of malignant cells. This approach is applicable to any type of malignancy which is sensitive to the antiproliferative or proapoptotic effects of IFN-β.

Studies have also shown the antiproliferative, antitumor, and immunomodulatory effects (Pestka et al., 1987), (Borden, 1992) of IFN-α, a multifunctional regulatory cytokine. IFN-α is one of the most frequently used adjuvant therapies to eradicate micrometastatic deposits in patients with a high risk of systemic recurrence (Grander and Einhorn, 1998), (Lens, 2006). In a similar study, Ren et al (Ren et al., 2008a) evaluated the potential of mouse MSCs transduced with adeno-associated virus expressing murine IFN-α in a mouse B16F10 melanoma lung metastasis model. Preferential homing of MSCs to lung tumor was confirmed after tail vein injection. A significant reduction in lung tumor colonies was observed in the MSC IFN-α treated mice, which resulted in an increase in life span compared to control animals.

4.1.2. Interleukin -2 (IL-2)

Stagg et al (Stagg et al., 2004) investigated the use of MSCs genetically modified with interleukin (IL)-2 to exert an effective immune response against the poorly immunogenic B16 melanoma model. IL-2-producing MSCs, when mixed with B16 cells, significantly delayed tumor growth in a dose-dependent manner. When injected in the vicinity of pre-established B16 tumors, matrix-embedded IL-2-producing MSCs led to an absence of tumor growth in mice. The results demonstrate that tumor-bearing mice treated with IL-2-producing MSCs developed CD8-mediated tumor-specific immunity and significantly delayed tumor growth, by generating effective immune responses against melanoma in mice with normal immune systems.

In a similar study by Nakamura et al (Nakamura et al., 2004), MSCs were genetically modified using an adenoviral vector encoding human IL-2, an immunomodulatory cytokine. To assess the therapeutic efficacy and survival benefit for 9L glioma bearing rats, hMSC IL-2 cells were either coinjected with tumor cells or intratumorally injected 3 days after tumor injection. The results conferred tumor inhibition in both cases when compared to their respective controls. The delay in intracranial tumor growth after MSC injection was confirmed by MRI monitoring *in vivo,* and the results correlated with the prolonged survival of glioma-bearing rats.

4.1.3. Interleukin-12 (IL-12)

MSCs have also been transduced to express interleukin-12 (IL-12), with the rationale of improving the anti-cancer immune surveillance by activating cytotoxic lymphocytes, natural killer cells, and producing IFN-γ (Shrayer et al., 2002). Chen et al. (Chen et al., 2006) transduced MSCs with adenovirus engineered to secrete interleukin-12 (AdIL-12-MSC). In this model, the adIL-12-MSCs were used prophylactically and prevented the development of subcutaneous melanomas (B16), hepatomas (HCC), and lung cancers (LLC Lewis). In the B16 melanoma model, none of the 12 mice in the AdIL-12-MSC group developed tumors, whereas only one out of 12 in the HCC hepatoma model and 2 out of 12 in the LLC lung cancer model receiving AdIL-12-MSC developed tumors. This approach of using AdIL-12-MSC has been shown to have protective anticarcinogenesis on the preneoplastic lesions studied. Another study by the same group (Chen et al., 2008) investigated an integrated immunotherapy method against pre-established metastases in three kinds of advanced cancer models, including B16 melanoma, 4T1 breast tumor, and Hca hepatoma. The progression of metastases into multistep lymph nodes and internal organs was suppressed following intravenous immunotherapy with IL-12-engineered MSCs, without systemic toxic effects. In an attempt to improve the delivery of IL-12 against melanoma, Elzaouk et al. (Elzaouk et al., 2006) evaluated the anti-tumor capacity of human MSCs stably expressing rat IL-12 in the B16F10 mouse melanoma model. The results indicate that treatment with MSCs expressing IL-12 led to a significant reduction of lung metastases in a prophylactic model and an established tumor model.

4.1.4. CX3CL1 (Fractalkine)

A similar approach was used by Xin et al. to reduce the metastastic load caused by the intravenous delivery of melanoma and colon cancer cell lines. In this study, mouse MSCs were transduced with CX3CL1 (fractalkine), an immunostimulatory chemokine, ex vivo using an adenoviral vector with the Arg-Gly-Asp-4C peptide in the fiber knob. CX3CL1 fractalkine is a member of the CX3CL family, and the soluble form of CX3CL1 induces the migration of cells expressing its receptor, CX3CR1, in a manner similar to that of other soluble chemokines (Imai et al., 1997). Systemic administration of CX3CL1-expressing MSCs, to mice bearing lung metastases of C26 and B16F10 cells, strongly inhibited the development of lung metastases and prolonged the survival of tumor-bearing mice (Xin et al., 2007).

4.1.5. Conditionally replicating adenoviruses (CRAds)

Delivery of the CRAds by using cell carriers with endogenous tumor-targeting properties can result in high efficiency. The use of replication-competent oncolytic adenovirus vectors enhances the preferential targeting of tumors and rescues the surrounding normal tissue. These novel vectors replicate and kill tumor cells, and this approach combines cell vehicle therapy and virotherapy with enhanced tumor infectivity and specificity.

The potential of mesenchymal progenitor cells (MPC) as cellular vectors to assist delivery of CRAd to tumors was evaluated (Komarova et al., 2006). The preferential tumor homing of MPCs loaded with oncolytic adenoviruses was confirmed in a pre-established ovarian carcinoma mouse model. The therapeutic efficacy was confirmed in an orthotopic xenograft model of human ovarian cancer in SCID mice. MPC-based viral delivery *in vivo* promoted the oncolytic effect of this treatment and prolonged the survival of tumor bearing animals.

A similar targeting strategy using MSCs as carriers for CRAds was studied in a breast cancer metastasis in a lung model (Stoff-Khalili et al., 2007). Systematically administered CRAd loaded-hMSCs were able to home to and reduce the tumor burden of MDA-MB-231 breast cancer metastases in the lungs of SCID mice. hMSC-based viral delivery enhanced the oncolytic effect and also increased the survival of tumor bearing animals. These results confirm that hMSCs can serve as cellular carriers to deliver CRAds to distant tumors such as ovarian carcinoma /breast cancer metastases in the lungs and mediate oncolysis.

These findings were supported by Sonabend et al. (Sonabend et al., 2008), who studied the feasibility of using hMSCs to deliver CRAds in a model of intracranial malignant glioma. CXCR4-promoter driven CRAds were used to enhance adenoviral glioma targeting. CXCR4 has been shown to be expressed in hMSCs (Ponte et al., 2007), (Honczarenko et al., 2006) and the CXCR4 promoter is highly active in gliomas (Ulasov et al., 2007). The CXCR4 promoter allows replication of the oncolytic virus first in carrier cells and then tumor tissue. Virus-loaded hMSC efficiently migrated *in vitro* and released CRAds which in turn infected U87MG glioma cells. The use of hMSCs to deliver CRAd-CXCR4–5/3 to murine brains led to a higher adenoviral infection of distant glioma cells, and confirms the ability of hMSCs to act as carriers for oncolytic adenoviral vectors for the treatment of malignant glioma.

Hakkarainen et al (Hakkarainen et al., 2007) investigated the efficacy of capsid-modified adenoviruses to infect and replicate in MSCs. The biodistribution and tumor-killing efficacy of virus loaded MSCs were evaluated in orthotopic murine models of lung and breast cancer. *In vivo* intravenously injected MSCs homed primarily to the lungs, and the virus was released into advanced orthotopic breast and lung tumors which resulted in both therapeutic efficacy and increased survival. The liver was the only infected organ when the same dose of virus was injected without a carrier. These results suggest that MSCs loaded with oncolytic adenoviruses could be a potentially powerful tool for improving the bioavailability and delivery of systemically administered oncolytic adenoviruses to tumors.

4.1.6. NK4

NK4 is an antagonist of hepatocyte growth factor (HGF; Matsumoto and Nakamura, 2003). HGF is a strong inducer of tumor growth, angiogenesis and lymphangiogenesis (Bellusci et al., 1994), (Cao et al., 2006). The effect of MSCs expressing adenovirus NK4 on mice with C-26 lung metastases was studied by Kanehira et al (Kanehira et al., 2007). Migrated NK4-expressing MSCs were observed at the sites of lung metastatic tumor and not in normal tissue. Systemically administered MSCs expressing NK4 efficiently inhibited C-26 tumor progression /metastases in the lung and prolonged survival without inducing severe adverse effects. The anti-metastatic effect of NK4-MSCs *in vivo* was due to the inhibition of angiogenesis and lymphangiogenesis within the tumor tissues.

4.1.7. TRAIL – Tumor necrosis factor related apoptosis inducing ligand

TRAIL is a member of the tumor necrosis factor-α family, and induces apoptosis in various tumor cell types (Hao et al., 2001), (Kagawa et al., 2001), while sparing most normal cells. TRAIL triggers apoptosis through interaction with death receptors and by initiating caspase-mediated cell death. Mohr et al (Mohr et al., 2008) reported the ability of an adenoviral vector expressing TRAIL to transduce MSCs and the subsequent therapeutic efficacy of these MSCs in a lung cancer model. MSCs transduced with adenovirus expressing TRAIL induced higher levels of apoptosis in A549 cells. TRAIL-MSCs were efficient in reducing subcutaneous tumor growth in a mouse model, by inducing apoptosis in a TRAIL refractory epithelial lung cancer cell line. Furthermore, TRAIL MSCs can induce apoptosis in A549 lung epithelial cancer cells even in the presence of serum, white bloods cells and erythrocytes which supports the potential of these cells to become an exciting new delivery vector for targeted treatment.

4.2. Adipose tissue derived MSC (AT-MSC)

Adipose tissue, like bone marrow, is a mesodermally derived organ that contains stem cells (Zuk et al., 2001). AT-MSCs share many of the characteristics of their bone marrow counterpart, including cell morphology, extensive proliferation potential, tumor tropism and the ability to undergo multilineage differentiation (Kim et al., 2006), (Strem et al., 2005), (Wagner et al., 2005), (Bieback et al., 2008). AT-MSCs can be obtained by a less invasive method and in larger quantities than bone marrow. The success rate of isolation is 100% and the yield from adipose tissue is 40-fold higher compared with that of bone marrow (Kern et al., 2006). Vilalta et al (Vilalta et al., 2008) have shown that AT-MSCs implanted in immunocompromised mice during a prolonged time period maintained a steady state, did not proliferate rapidly, and did not show any detectable chromosomal abnormalities or formation of tumors in the host's tissues. AT-MSCs genetically modified by bone morphogenic protein-2 produced a significant increase of newly formed bone in a canine bone defect model (Li et al., 2007a). Other studies have reported the use of AT-MSCs in

GVHD (Yanez et al., 2006), as a source of hepatocytes (Banas et al., 2007). A phase I clinical trial involving patients with Crohn's disease, to test the feasibility and safety of autologous stem cells transplantation in the treatment of fistulas, showed no adverse effects and confirmed safety of the therapy (Garcia-Olmo et al., 2005). Another group (Trivedi et al., 2008) reported a clinical trial using insulin-producing human adipose tissue-derived mesenchymal stem cells (h-AD-MSC) transfused with unfractionated cultured bone marrow in Type 1 diabetes mellitus patients. No adverse side effects related to the stem cell infusion, such as infective episodes or GVHD, were observed. Thus, the capability of human adipose tissue derived MSC to serve as vehicles for a cell-based gene therapy is encouraging.

4.2.1. Prodrug therapy – cytosine deaminase

Prodrug gene therapy involves delivery of genes encoding enzymes that convert nontoxic prodrugs into toxic antimetabolites. Three suicide genes that are being evaluated in clinical trials are the Cytosine Deaminase (CD), HSV-1 Thymidine kinase and carboxyesterase genes, which confer sensitivity to 5-fluorocytosine 5-FC, ganciclovir (GCV) and camptothecin-11 (CPT-11), respectively (Freytag et al., 2002; Freytag et al., 2007),(Danks et al., 2007). The CD gene-directed enzyme prodrug therapy approach relies on the ability of bacterial or yeast CD enzyme to convert non toxic substrate antifungal agent 5-FC to antitumor agent 5-fluorouracil (5-FU). 5-FU is capable of nonfacilitated diffusion into and out of cells resulting in a significant bystander effect of CD/5-FC Paillard, 1997. Our group and several others have shown the therapeutic proof-of-concept using neural stem cells (NSC) producing bacterial cytosine deaminase and systemic 5-fluorocytosine (5-FC) prodrug administration in the medulloblastoma (Kim et al., 2006), (Shimato et al., 2007) and melanoma brain metastases (Aboody et al., 2006) models.

In a pilot study, Kucerova et al (Kucerova et al., 2007) showed that AT-MSCs expressing the fusion yeast CD::UPRT gene (CDy-AT-MSC) in combination with the prodrug 5-FC augment potent cytotoxic effects over HT-29 tumor cells *in vitro*. Engineered CD-AT-MSCs combined with 5-FC were significantly effective in suppression of subcutaneous human colon cancer xenograft growth *in vivo*. Kucerova et al (Kucerova et al., 2008) also investigated the therapeutic efficacy of MSCs expressing yeast CD on melanoma. Bystander cytotoxicity was mediated towards MDA-MB-361 breast cancer cells, A375 melanoma cells and HT29 colon cancer cells by CDy-AT-MSC in the presence of prodrug 5-FC in vitro. CD-AT-MSC in combination with 5-FC efficiently inhibited the growth of various human tumor cell lines in coculture experiments. Systemic administration or coinjection of CDy-AT-MSC exerted antitumor effects in the presence of 5-FC in subcutaneous A375 melanoma xenografts. The results confirm the potential clinical utility of these cells and the CD gene as a cell-directed approach for enzyme-mediated prodrug conversion in the field of molecular cancer chemotherapy.

5. Summary

Strategies for the delivery of target genes and chemotherapeutic agents to tumors are compromised by the invasive nature of the tumor or several tumor microenvironment factors. Even though viral and non-viral methods have been employed to target tumors, the poor survival of patients in many cases relates, in part, to the inability to deliver therapeutic agents to the tumor microsatellites that have migrated away from the main tumor mass. It is crucial to develop novel therapeutic approaches which can utilize the chemotherapeutic agents and drugs already in clinical use. Stem cell-based therapies provide a promising approach to the treatment of several diseases in humans. The ability of MSCs to interact with different tissue environments, along with the immune tolerance elicited, and their migratory abilities, present MSCs as an attractive platform for cellular and gene therapy in humans. More studies elucidating the basic biology, trafficking after transplantation, and characterization using *in vivo* disease models are needed to develop MSC-based therapy for application in the fields of stem cell tissue engineering, gene therapy, and cancer biology.

6. References

Aboody, K.S., Brown, A., Rainov, N.G., Bower, K.A., Liu, S., Yang, W., Small, J.E., Herrlinger, U., Ourednik, V., and Black, P.M., et al. (2000). Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci USA *97*, 12846–12851.

Aboody, K.S., Bush, R.A., Garcia, E., Metz, M.Z., Najbauer, J., Justus, K.A., Phelps, D.A., Remack, J.S., Yoon, K.J., and Gillespie, S., et al. (2006). Development of a tumor-selective approach to treat metastatic cancer. PLoS ONE *1*, e23.

Amado, L.C., Saliaris, A.P., Schuleri, K.H., St John, M., Xie, J.S., Cattaneo, S., Durand, D.J., Fitton, T., Kuang, J.Q., and Stewart, G., et al. (2005). Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci USA *102*, 11474–11479.

Banas, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Quinn, G., Okochi, H., and Ochiya, T. (2007). Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology *46*, 219–228.

Beckermann, B.M., Kallifatidis, G., Groth, A., Frommhold, D., Apel, A., Mattern, J., Salnikov, A.V., Moldenhauer, G., Wagner, W., Diehlmann, A., Saffrich, A., Schubert, M., Ho, A.D., Giese, N., Buchler, M.W., Friess, H., Buchler, P., and Herr, I (2008). VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. British Journal of Cancer *99*, 622–631.

Bellusci, S., Moens, G., Gaudino, G., Comoglio, P., Nakamura, T., Thiery, J.P., and Jouanneau, J. (1994). Creation of an hepatocyte growth factor/scatter factor autocrine loop in carcinoma cells induces invasive properties associated with increased tumorigenicity. Oncogene *9*, 1091–1099.

Benedetti, S., Pirola, B., Pollo, B., Magrassi, L., Bruzzone, M.G., Rigamonti, D., Galli, R., Selleri, S., Di Meco, F., and De Fraja, C., et al. (2000). Gene therapy of experimental brain tumors using neural progenitor cells. Nat Med *6*, 447–450.

Bieback, K., Kern, S., Kocaomer, A., Ferlik, K., and Bugert, P. (2008). Comparing mesenchymal stromal cells from different human tissues: bone marrow, adipose tissue and umbilical cord blood. Biomed Mater Eng *18*, S71–76.

Borden, E.C. (1992). Interferons: pleiotropic cellular modulators. Clin Immunol Immunopathol *62*, SI8–24.

Brooke, G., Cook, M., Blair, C., Han, R., Heazlewood, C., Jones, B., Kambouris, M., Kollar, K., McTaggart, S., and Pelekanos, R., et al. (2007). Therapeutic applications of mesenchymal stromal cells. Semin Cell Dev Biol *18*, 846–858.

Campagnoli, C., Roberts, I.A., Kumar, S., Bennett, P.R., Bellantuono, I., and Fisk, N.M. (2001). Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood *98*, 2396–2402.

Cao, R., Bjorndahl, M.A., Gallego, M.I., Chen, S., Religa, P., Hansen, A.J., and Cao, Y. (2006). Hepatocyte growth factor is a lymphangiogenic factor with an indirect mechanism of action. Blood *107*, 3531–3536.

Cavazzana-Calvo, M., Hacein-Bey, S., de Saint Basile, G., Gross, F., Yvon, E., Nusbaum, P., Selz, F., Hue, C., Certain, S., and Casanova, J.L., et al. (2000). Gene therapy of human severe combined immunodeficiency (SCID)-Xl disease. Science *288*, 669–672.

Chamberlain, G., Fox, J., Ashton, B., and Middleton, J. (2007). Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells *25*, 2739–2749.

Chawla-Sarkar, M., Leaman, D.W., and Borden, E.G. (2001). Preferential induction of apoptosis by interferon (IFN)-beta compared with IFN-alpha2: correlation with TRAIL/Apo2L induction in melanoma cell lines. Clin Cancer Res *7*, 1821–1831.

Chen, X., Lin, X., Zhao, J., Shi, W., Zhang, H., Wang, Y., Kan, B., Du, L., Wang, B., and Wei, Y., et al. (2008). A Tumor-selective Biotherapy With Prolonged Impact on Established Metastases Based on Cytokine Gene-engineered MSCs. Mol Ther.

Chen, X.C., Wang, R., Zhao, X., Wei, Y.Q., Hu, M., Wang, Y.S., Zhang, X.W., Zhang, R., Zhang, L., and Yao, B., et al. (2006). Prophylaxis against carcinogenesis in three kinds of unestablished tumor models via IL12-gene-engineered MSCs. Carcinogenesis *27*, 2434–2441.

Chiocca, E.A., Smith, K.M., McKinney, B., Palmer, C.A., Rosenfeld, S., Lillehei, K., Hamilton, A., DeMasters, B.K., Judy, K., and Kirn, D. (2008). A phase I trial of Ad.hlFN-beta gene therapy for glioma. Molecular Therapy *16*, 618–626.

Cristiano, RJ. (1998). Viral and non-viral vectors for cancer gene therapy. Anticancer Res *18*, 3241–3245.

Danks, M.K., Yoon, K.J., Bush, R.A., Remack, J.S., Wierdl, M., Tsurkan, L., Kim, S.U., Garcia, E., Metz, M.Z., and Najbauer, J., et al. (2007). Tumor-targeted enzyme/prodrug therapy mediates long-term disease-free survival of mice bearing disseminated neuroblastoma. Cancer Res *67*, 22–25.

Dao, M.A., Pepper, K.A., and Nolta, J.A. (1997). Long-term cytokine production from engineered primary human stromal cells influences human hematopoiesis in an in vivo xenograft model. Stem Cells *15*, 443–454.

Davidson, B.L., Stein, C.S., Heth, J.A., Martins, I., Kotin, R.M., Derksen, T.A., Zabner, J., Ghodsi, A., and Chiorini, J.A. (2000). Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. Proc Natl Acad Sci USA *97*, 3428–3432.

De Coppi, P., Bartsch, G., Jr., Siddiqui, M.M., Xu, T., Santos, C.C., Perin, L., Mostoslavsky, G., Serre, A.C., Snyder, E.Y., and Yoo, J.J., et al. (2007). Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol *25*, 100–106.

Devine, S.M., Cobbs, C., Jennings, M., Bartholomew, A., and Hoffman, R. (2003). Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood *101*, 2999–3001.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F.C., Krause, D.S., Deans, R.J., Keating, A., Prockop, D.J., and Horwitz, E.M. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy *8*, 315–317.

Ehtesham, M., Kabos, P., Gutierrez, M.A., Chung, N.H., Griffith, T.S., Black, K.L., and Yu, J.S. (2002a). Induction of glioblastoma apoptosis using neural stem cell-mediated delivery of tumor necrosis factor-related apoptosis-inducing ligand. Cancer Res *62*, 7170–7174.

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

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.

Erices, A., Conget, P., and Minguell, J.J. (2000). Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol *109*, 235–242.

Freytag, S.O., Khil, M., Stricker, H., Peabody, J., Menon, M., DePeralta-Venturina, M., Nafziger, D., Pegg, J., Paielli, D., and Brown, S., et al. (2002). Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer. Cancer Res *62*, 4968–4976.

Freytag, S.O., Stricker, H., Peabody, J., Pegg, J., Paielli, D., Movsas, B., Barton, K.N., Brown, S.L., Lu, M., and Kim, J.H. (2007). Five-year follow-up of trial of replication-competent adenovirus-mediated suicide gene therapy for treatment of prostate cancer. Mol Ther *75*, 636–642.

Friedenstein, A.J., Chailakhjan, R.K., and Lalykina, K.S. (1970). The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet *3*, 393–403.

Fukuchi, Y., Nakajima, H., Sugiyama, D., Hirose, I., Kitamura, T., and Tsuji, K. (2004). Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem Cells *22*, 649–658.

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.

Gao, X., Kim, K.S., and Liu, D. (2007). Nonviral gene delivery: what we know and what is next. AAPS J *9*, E92–104.

Garcia-Olmo, D., Garcia-Arranz, M., Herreros, D., Pascual, I., Peiro, C., and Rodriguez-Montes, J.A. (2005). A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum *48*, 1416–1423.

Giordano, A., Galderisi, U., and Marino, I.R. (2007). From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. J Cell Physiol *211*, 27–35.

Grander, D., and Einhorn, S. (1998). Interferon and malignant disease—how does it work and why doesn't it always?. Acta Oncol *37*, 331–338.

Gutova, M., Najbauer, J., Frank, R.T., Kendall, S.E., Gevorgyan, A., Metz, M.Z., Guevorkian, M., Edmiston, M., Zhao, D., Glackin, C.A., Kim, S.U., and Aboody, K.S. (2008). Urokinase plasminogen activator and urokinase plasminogen activator receptor mediate human stem cell tropism to malignant solid tumors. Stem Cells *26*, 1406–1413.

Hacein-Bey-Abina, S., et al. (2003). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science *302*, 415–419.

Hakkarainen, T., Sarkioja, M., Lehenkari, P., Miettinen, S., Ylikomi, T., Suuronen, R., Desmond, R.A., Kanerva, A., and Hemminki, A. (2007). Human mesenchymal stem cells lack tumor tropism but enhance the antitumor activity of oncolytic adenoviruses in orthotopic lung and breast tumors. Hum Gene Ther *18*, 627–641.

Hao, C., Beguinot, F., Condorelli, G., Trencia, A., Van Meir, E.G., Yong, V.W., Parney, I.F., Roa, W.H., and Petruk, K.C. (2001). Induction and intracellular regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apotosis in human malignant glioma cells. Cancer Res *61*, 1162–1170.

Harrington, K., Alvarez-Vallina, L., Crittenden, M., Gough, M., Chong, H., Diaz, R.M., Vassaux, G., Lemoine, N., and Vile, R. (2002). Cells as vehicles for cancer gene therapy: the missing link between targeted vectors and systemic delivery? Hum Gene Ther *13*, 1263–1280.

Honczarenko, M., Le, Y., Swierkowski, M., Ghiran, I., Glodek, A.M., and Silberstein, L.E. (2006). Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. Stem Cells *24*, 1030–1041.

Horwitz, E.M., Gordon, P.L., Koo, W.K., Marx, J.C., Neel, M.D., McNall, R.Y., Muul, L., and Hofmann, T. (2002). Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc Natl Acad Sci USA *99*, 8932–8937.

Horwitz, E.M., Prockop, D.J., Fitzpatrick, L.A., Koo, W.W., Gordon, P.L., Neel, M., Sussman, M., Orchard, P., Marx, J.C., and Pyeritz, R.E., et al. (1999). Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med *5*, 309–313.

Houghton, J., Stoicov, C., Nomura, S., Rogers, A.B., Carlson, J., Li, H., Cai, X., Fox, J.G., Goldenring, J.R., and Wang, T.C. (2004). Gastric cancer originating from bone marrow-derived cells. Science *306*, 1568–1571.

Imai, T., Hieshima, K., Haskell, C., Baba, M., Nagira, M., Nishimura, M., Kakizaki, M., Takagi, S., Nomiyama, H., and Schall, T.J., et al. (1997). Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell *91*, 521–530.

In 't Anker, P.S., Scherjon, S.A., Kleijburg-van der Keur, C., de Groot-Swings, G.M., Claas, F.H., Fibbe, W.E., and Kanhai, H.H. (2004). Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells *22*, 1338–1345.

Itescu, S., Schuster, M.D., and Kocher, A.A. (2003). New directions in strategies using cell therapy for heart disease. J Mol Med *81*, 288–296.

Johns, T.G., Mackay, I.R., Callister, K.A., Hertzog, P.J., Devenish, R.J., and Linnane, A.W. (1992). Antiproliferative potencies of interferons on melanoma cell lines and xenografts: higher efficacy of interferon beta. J Natl Cancer Inst *84*, 1185–1190.

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

Kagawa, S., He, C., Gu, J., Koch, P., Rha, S.J., Roth, J.A., Curley, S.A., Stephens, L.C., and Fang, B. (2001). Antitumor activity and bystander effects of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene. Cancer Res *61*, 3330–3338.

Kanehira, M., Xin, H., Hoshino, K., Maemondo, M., Mizuguchi, H., Hayakawa, T., Matsumoto, K., Nakamura, T., Nukiwa, T., and Saijo, Y. (2007). Targeted delivery of NK4 to multiple lung tumors by bone marrow-derived mesenchymal stem cells. Cancer Gene Ther *14*, 894–903.

Karnieli, O., Izhar-Prato, Y., Bulvik, S., and Efrat, S. (2007). Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. Stem Cells *25*, 2837–2844.

Kawada, H., Fujita, J., Kinjo, K., Matsuzaki, Y., Tsuma, M., Miyatake, H., Muguruma, Y., Tsuboi, K., Itabashi, Y., and Ikeda, Y., et al. (2004). Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood *104*, 3581–3587.

Kern, S., Eichler, H., Stoeve, J., Kluter, H., and Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells *24*, 1294–1301.

Khakoo, A.Y., Pati, S., Anderson, S.A., Reid, W., Elshal, M.F., Rovira, II, Nguyen, A.T., Malide, D., Combs, C.A., and Hall, G., et al. (2006). Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med *203*, 1235–1247.

Kim, S.K., Cargioli, T.G., Machluf, M., Yang, W., Sun, Y., Al-Hashem, R., Kim, S.U., Black, P.M., and Carroll, R.S. (2005). PEX-producing human neural stem cells inhibit tumor growth in a mouse glioma model. Clin Cancer Res *11*, 5965–5970.

Kim, S.K., Kim, S.U., Park, I.H., Bang, J.H., Aboody, K.S., Wang, K.C., Cho, B.K., Kim, M., Menon, L.G., and Black, P.M., et al. (2006). Human neural stem cells target experimental intracranial medulloblastoma and deliver a therapeutic gene leading to tumor regression. Clin Cancer Res *12*, 5550–5556.

Klopp, A.H., Spaeth, E.L., Dembinski, J.L., Woodward, W.A., Munshi, A., Meyn, R.E., Cox, J.D., Andreef, M., and Marini, F.C. (2007). Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. Cancer Research *57*, 11687–11695.

Koc, O.N., Day, J., Nieder, M., Gerson, S.L., Lazarus, H.M., and Krivit, W. (2002). Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). Bone Marrow Transplant *30*, 215–222.

Koc, O.N., Gerson, S.L., Cooper, B.W., Dyhouse, S.M., Haynesworth, S.E., Caplan, A.I., and Lazarus, H.M. (2000). Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol *18*, 307–316.

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.

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.

Kucerova, L., Matuskova, M., Pastorakova, A., Tyciakova, S., Jakubikova, J., Bohovic, R., Altanerova, V., and Altaner, C. (2008). Cytosine deaminase expressing human mesenchymal stem cells mediated tumour regression in melanoma bearing mice J Gene Med..

Kumar, S., Chanda, D., and Ponnazhagan, S. (2008). Therapeutic potential of genetically modified mesenchymal stem cells. Gene Ther *75*, 711–715.

Lazarus, H.M., Koc, O.N., Devine, S.M., Curtin, P., Maziarz, R.T., Holland, H.K., Shpall, E.J., McCarthy, P., Atkinson, K., and Cooper, B.W., et al. (2005). Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant *11*, 389–398.

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

Le Blanc, K., Rasmusson, I., Sundberg, B., Gotherstrom, C., Hassan, M., Uzunel, M., and Ringden, O. (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet *363*, 1439–1441.

Le Blanc, K., Samuelsson, H., Gustafsson, B., Remberger, M., Sundberg, B., Arvidson, J., Ljungman, P., Lonnies, H., Nava, S., and Ringden, O. (2007). Transplantation of mesenchymal stem cells to enhance engraftment of hematopoietic stem cells. Leukemia *21*, 1733–1738.

Lens, M. (2006). Cutaneous melanoma: interferon alpha adjuvant therapy for patients at high risk for recurrent disease. Dermatol Ther *19*, 9–18.

Li, H., Dai, K., Tang, T., Zhang, X., Yan, M., and Lou, J. (2007a). Bone regeneration by implantation of adipose-derived stromal cells expressing BMP-2. Biochem Biophys Res Commun *356*, 836–842.

Li, Y., Zhang, R., Qiao, H., Zhang, H., Wang, Y., Yuan, H., Liu, Q., Liu, D., Chen, L., and Pei, X. (2007b). Generation of insulin-producing cells from PDX-1 gene-modified human mesenchymal stem cells. J Cell Physiol *211*, 36–44.

Liu, F., Akiyama, Y., Tai, S., Maruyama, K., Kawaguchi, Y., Muramatsu, K., and Yamaguchi, K. (2008). Changes in the expression of CD106, osteogenic genes, and transcription factors involved in the osteogenic differentiation of human bone marrow mesenchymal stem cells. Journal of Bone and Mineral Metabolism *26*, 312–320.

Matsumoto, K., and Nakamura, T. (2003). NK4 (HGF-antagonist/angiogenesis inhibitor) in cancer biology and therapeutics. Cancer Sci *94*, 321–327.

Menon, L.G., Picinich, S., Koneru, R., Gao, H., Lin, S.Y., Koneru, M., Mayer-Kuckuk, P., Glod, J., and Banerjee, D. (2007). Differential gene expression associated with migration of mesenchymal stem cells to conditioned medium from tumor cells or bone marrow cells. Stem Cells *25*, 520–528.

Meyerrose, I.E., Roberts, M., Ohlemiller, K.K., Vogler, C.A., Wirthlin, L., Nolta, J.A., and Sands, M.S. (2008). Lentiviral-transduced human mesenchymal stem cells persistently express therapeutic levels of enzyme in a xeno-transplantation model of human disease. Stem Cells *26*, 1713–1722.

Mohr, A., Lyons, M., Deedigan, L., Harte, T., Shaw, G., Howard, L., Barry, F., O'Brien, T., and Zwacka, R. (2008). Mesenchymal Stem Cells expressing TRAIL lead to tumour growth inhibition in an experimental lung cancer model J Cell Mol Med..

Myers, R., Harvey, M., Kaufmann, T.J., Greiner, S.M., Krempski, J.W., Raffel, C., Shelton, S.E., Soeffker, D., Zollman, P., Federspiel, M.J., Blanco, M., and Galanis, E. (2008). Toxicology study of repeat intracerebral administration of a measles virus derivative producing carcinoembryonic antigen in rhesus macaques in support of a phase I/II clinical trial for patients with recurrent gliomas. Hum Gene Ther. *19*, 690–8.

Nair, V. (2008). Retrovirus-induced oncogenesis and safety of retroviral vectors. Current Opinion in Molecular Therapeutics *10*, 431–438.

Nakamizo, A., Marini, F., Amano, T., Khan, A., Studeny, M., Gumin, J., Chen, J., Hentschel, S., Vecil, G., and Dembinski, J., et al. (2005). Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res *65*, 3307–3318.

Nakamura, K., Ito, Y., Kawano, Y., Kurozumi, K., Kobune, M., Tsuda, H., Bizen, A., Honmou, O., Niitsu, Y., and Hamada, H. (2004). Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. Gene Ther *11*, 1155–1164.

Nolta, J.A., Hanley, M.B., and Kohn, D.B. (1994). Sustained human hematopoiesis in immunodeficient mice by cotransplantation of marrow stroma expressing human interleukin-3: analysis of gene transduction of long-lived progenitors. Blood *83*, 3041–3051.

Paillard, F. (1997). Bystander effects in enzyme/prodrug gene therapy. Hum Gene Ther *8*, 1733–1735.

Pereira, R.F., O'Hara, M.D., Laptev, A.V., Halford, K.W., Pollard, M.D., Class, R., Simon, D., Livezey, K., and Prockop, D.J. (1998). Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. Proc Natl Acad Sci USA *95*, 1142–1147.

Pestka, S., Langer, J.A., Zoon, K.C., and Samuel, C.E. (1987). Interferons and their actions. Annu Rev Biochem *56*, 727–777.

Ponte, A.L., Marais, E., Gallay, N., Langonne, A., Delorme, B., Herault, O., Charbord, P., and Domenech, J. (2007). The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells *25*, 1737–1745.

Prockop, D.J. (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. Science *276*, 71–74.

Reiser, J., Zhang, X.Y., Hemenway, C.S., Mondal, D., Pradhan, L., and La Russa, V.F. (2005). Potential of mesenchymal stem cells in gene therapy approaches for inherited and acquired diseases. Expert Opin Biol Ther *5*, 1571–1584.

Ren, C., Kumar, S., Chanda, D., Chen, J., Mountz, J.D., and Ponnazhagan, S. (2008a). Therapeutic potential of mesenchymal stem cells producing IFN-{alpha} in a mouse melanoma lung metastasis model. Stem Cells.

Ren, C., Kumar, S., Chanda, D., Kallman, L., Chen, J., Mountz, J.D., and Ponnazhagan, S. (2008b). Cancer gene therapy using mesenchymal stem cells expressing interferon-beta in a mouse prostate cancer lung metastasis model. Gene Ther.

Ringden, O., Uzunel, M., Rasmusson, I., Remberger, M., Sundberg, B., Lonnies, H., Marschall, H.U., Dlugosz, A., Szakos, A., and Hassan, Z., et al. (2006). Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation *81*, 1390–1397.

Shah, K., Bureau, E., Kim, D.E., Yang, K., Tang, Y., Weissleder, R., and Breakefield, X.O. (2005). Glioma therapy and real-time imaging of neural precursor cell migration and tumor regression. Ann Neurol *57*, 34–41.

Shih, D.T., Lee, D.C., Chen, S.C., Tsai, R.Y., Huang, C.T., Tsai, C.C., Shen, E.Y., and Chiu, W.T. (2005). Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. Stem Cells *23*, 1012–1020.

Shimato, S., Natsume, A., Takeuchi, H., Wakabayashi, T., Fujii, M., Ito, M., Ito, S., Park, I.H., Bang, J.H., and Kim, S.U., et al. (2007). Human neural stem cells target and deliver therapeutic gene to experimental leptomeningeal medulloblastoma. Gene Ther *14*, 1132–1142.

Shrayer, D.P., Bogaars, H., Cole, B., Wolf, S.F., and Wanebo, H.J. (2002). Capacity of murine IL-12 to inhibit the development of primary melanoma tumors and to prevent lung metastases in the melanoma-challenged mice. J Exp Ther Oncol *2*, 93–99.

Slavin, S., Kurkalli, B.G., and Karussis, D. (2008). The potential use of adult stem cells for the treatment of multiple sclerosis and other neurodegenerative disorders. Clin Neurol Neurosurg.

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.

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 *75*, 730–738.

Stagg, J., Lejeune, L., Paquin, A., and Galipeau, J. (2004). Marrow stromal cells for interleukin-2 delivery in cancer immunotherapy. Hum Gene Ther *75*, 597–608.

Stagg, J., Pommey, S., Eliopoulos, N., and Galipeau, J. (2006). Interferon-gamma-stimulated marrow stromal cells: a new type of nonhematopoietic antigen-presenting cell. Blood *107*, 2570–2577.

Stoff-Khalili, M.A., Rivera, A.A., Mathis, J.M., Banerjee, N.S., Moon, A.S., Hess, A., Rocconi, R.P., Numnum, T.M., Everts, M., and Chow, L.T., et al. (2007). Mesenchymal stem cells as a vehicle for targeted delivery of CRAds to lung metastases of breast carcinoma. Breast Cancer Res Treat *105*, 157–167.

Strem, B.M., Hicok, K.C., Zhu, M., Wulur, I., Alfonso, Z., Schreiber, R.E., Fraser, J.K., and Hedrick, M.H. (2005). Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med *54*, 132–141.

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.

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

Trivedi, H.L., Vanikar, A.V., Thakker, U., Firoze, A., Dave, S.D., Patel, C.N., Patel, J.V., Bhargava, A.B., and Shankar, V. (2008). Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin. Transplant Proc *40*, 1135–1139.

Trubiani, O., Di Primio, R., Traini, T., Pizzicannella, J., Scarano, A., Piattelli, A., and Caputi, S. (2005). Morphological and cytofluorimetric analysis of adult mesenchymal stem cells expanded ex vivo from periodontal ligament. Int J Immunopathol Pharmacol *18*, 213–221.

Ulasov, I.V., Rivera, A.A., Sonabend, A.M., Rivera, L.B., Wang, M., Zhu, Z.B., and Lesniak, M.S. (2007). Comparative evaluation of survivin, midkine and CXCR4 promoters for transcriptional targeting of glioma gene therapy. Cancer Biol Ther *6*, 679–685.

Vilalta, M., Degano, I.R., Bago, J., Gould, D., Santos, M., Garcia-Arranz, M., Ayats, R., Fuster, C., Chernajovsky, Y., and Garcia-Olmo, D., et al. (2008). Biodistribution, long-term survival, and safety of human adipose tissue-derived mesenchymal stem cells transplanted in nude mice by high sensitivity non-invasive bioluminescence imaging. Stem Cells Dev.

Wagner, W., Wein, F., Seckinger, A., Frankhauser, M., Wirkner, U., Krause, U., Blake, J., Schwager, C., Eckstein, V., and Ansorge, W., et al. (2005). Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol *33*, 1402–1416.

Wong, V.L., Rieman, D.J., Aronson, L., Dalton, B.J., Greig, R., and Anzano, M.A. (1989). Growth-inhibitory activity of interferon-beta against human colorectal carcinoma cell lines. Int J Cancer *43*, 526–530.

Xin, H., Kanehira, M., Mizuguchi, H., Hayakawa, T., Kikuchi, T., Nukiwa, T., and Saijo, Y. (2007). Targeted delivery of CX3CL1 to multiple lung tumors by mesenchymal stem cells. Stem Cells *25*, 1618–1626.

Yanez, R., Lamana, M.L., Garcia-Castro, J., Colmenero, I., Ramirez, M., and Bueren, J.A. (2006). Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. Stem Cells *24*, 2582–2591.

Zappia, E., Casazza, S., Pedemonte, E., Benvenuto, F., Bonanni, I., Gerdoni, E., Giunti, D., Ceravolo, A., Cazzanti, F., and Frassoni, F., et al. (2005). Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood *106*, 1755–1761.

Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., Fraser, J.K., Benhaim, P., and Hedrick, M.H. (2002). Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell *13*, 4279–4295.

Zuk, P.A., Zhu, M., Mizuno, H., Huang, J., Futrell, J.W., Katz, A.J., Benhaim, P., Lorenz, H.P., and Hedrick, M.H. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng *7*, 211–228.