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Drug Delivery using genetically modified Mesenchymal Stem Cells: A promising targeted-delivery method

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Abstract

***Plan**: The review covers the unique role of Mesenchymal stem cells to improve, the poor solubility, premature metabolic inactivation, excretion and bioavailability of different classes of drugs which are insoluble or sparingly soluble in water.*
***Preface**: Mesenchymal stem cells (MSCs) exhibit unique characteristics including their ability to differentiate and migrate to sites of tissue injury/inflammation, genetic modifiability, and expression of protein. Many of the most promising drug targets are intracellular and can only be accessed by drugs capable of traversing the cell membrane. Gene and drug delivery using genetically modified cells offers several unique advantages including ease of modifying, inducible or continuous drug production inside the body, more control on drug target and safety.*
***Outcome**: In this review article, we examine the promising of using MSCs as a drug delivery vehicle for gene therapy, and summarize various challenges and concerns regarding these therapies.*
***Key words**: Mesenchymal stem cell, Drug delivery, Gene delivery*

Introduction

The rapid advances in biomedical research have led to a better understanding of the molecular basis of many diseases. However, many of the most promising targets are intracellular and can only be accessed by drugs capable of traversing the cell membrane. Many of these targets are also either present ubiquitously in many cell types or are present in non-diseased cell types such that therapeutic intervention against these targets could affect non-diseased cells and inflict severe adverse side effects. The bioavailability of many drugs is also often compromised by their poor solubility in aqueous solution, premature metabolic inactivation and excretion. Many of these problems could potentially be resolved by loading the drugs into drug delivery vehicles. Gene therapy is a relatively recent, and still highly experimental, approach to treating human disease.

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While traditional drug therapies involve the administration of chemicals that have been manufactured outside the body, gene therapy takes a very different approach: directing a patient's own cells to produce and deliver a therapeutic agent.

Gene therapy uses genetic engineering to alter or supplement the function of an abnormal gene by providing a copy of a normal gene, to directly repair such a gene, or to provide a gene that adds new functions or regulates the activity of other genes. Gene therapy researchers have employed two major strategies for delivering therapeutic transgenes into human recipients. The first is to "directly" infuse the gene into a person. Viruses that have been altered to prevent them from causing disease are often used as the vehicle for delivering the gene into certain human cell types, in much the same way as ordinary viruses infect cells. This delivery method is fairly imprecise and limited to the specific types of human cells that the viral vehicle can infect. Nonviral vehicles for directly delivering genes into cells are also being explored, including the use of plain DNA and DNA wrapped in a coat of fatty molecules known as liposomes. The second strategy involves the use of living cells to deliver therapeutic transgenes into the body. In this method, the delivery cells—often a type of stem cell, a lymphocyte, or a fibroblast—are removed from the body, and the therapeutic transgene is introduced into them via the same vehicles used in the previously described direct-gene-transfer method. While still in the laboratory, the genetically modified cells are tested and then allowed to grow and multiply and, finally, are infused back into the patient.

Gene therapy using genetically modified cells offers several unique advantages over direct gene transfer into the body and over cell therapy, First, the addition of the therapeutic transgene to the delivery cells takes place outside the patient, which allows researchers an important measure of control because they can select and work only with those cells that both contain the transgene and produce the therapeutic agent in sufficient quantity. Second, investigators can genetically engineer, or "program," the cells' level and rate of production of the therapeutic agent.

Cells can be programmed to steadily churn out a given amount of the therapeutic product. In some cases, it is desirable to program the cells to make large amounts of the therapeutic agent so that the chances that sufficient quantities are secreted and reach the diseased tissue in the patient are high. In other cases, it may be desirable to program the cells to produce the therapeutic agent in a regulated fashion. In this case, the therapeutic transgene would be active only in response to certain signals, such as drugs administered to the patient to turn the therapeutic transgene on and off.

1. Drug delivery vehicles

1.1. Synthetic drug delivery vehicles

Most of the drug delivery vehicles presently in use for clinical or cosmetic applications are chemically synthesized using lipids or lipid-like molecules. Among the synthetic lipid or lipid-like vesicles, the liposome is currently the pharmaceutical vehicle of choice. It has been used for the delivery of anti-cancer drugs [1,2,3,4,5,6,7,8,9,10,11], anti-fungal drugs [12], analgesics [13] etc. Liposomes have many positive attributes that are pivotal in their function as drug delivery vehicles (reviewed in [14]). A major attribute is the ease with which their phospholipid membranes breached the plasma membrane to deliver their drug cargo.

They also have a highly versatile capacity to incorporate both hydrophilic and hydrophobic drugs by simply synthesizing liposomes either as unilamellar vesicles with one lipid bilayer and a large aqueous core to encapsulate water-soluble drugs, or multilamellar vesicles with several concentric lipid bilayers to more efficiently entrap lipophilic drugs. An unexpected perquisite of such drug encapsulation or entrapment is a protective barrier against premature transformation and elimination. Liposomal membranes are also highly amenable to modifications to display ligands or antibody fragments that bind to specific cell types and enhance cell type-specific drug delivery. They can be coated with inert polymers such as PEG to reduce liposome recognition by opsonins and clearance of the liposomes.

1.2. Natural drug delivery vehicles

Despite the remarkable advances and successes in their design and efficacy of synthetic drug vehicles, there is an increasing recognition that nature has particulates with some of the highly desired attributes of drug delivery vehicles. Some of the natural particulate candidates for drug delivery include genetically modified bacteria, viruses, red blood cells, macrophages, lymphocytes and stem cells with vectors.

1.2.1. Gene delivery using Viral vectors

The vector of retrovirus, lentivirus, adenovirus, and adeno-associated virus has been widely used for gene transfer. Viral method is proved to be a very effective approach, which allows the achievement of high transfection efficiency. In a series of studies, fiber-mutant adenovirus vectors were developed and used for cancer gene therapy and induced significant anti-tumor activity when cytokines and/or chemokines were employed as therapeutic genes [14,15] . A variety of studies have been reported to use different viral vector systems to transduce MSCs [16,17]. However, many clinical trials in which viral vectors were used have been terminated since the application of these vectors had induced unexpected adverse effects such as toxicities, immunogenicity and oncogenicity [18,19].

1.2.2. Gene delivery using Non-viral vectors

An increasing number of non-viral vectors are being developed for the purpose of gene delivery nowadays. These vectors have several advantages such as ease of synthesis, cell/tissue targeting, low immune response, and unrestricted plasmid size [20,21]. Till now, a variety of non-viral delivery carriers, including calcium phosphate and the nanoparticles [22], have been under development [23]. More recently, a novel transfection vector, spermine–pullulan, was synthesized by cationization pullulan with the chemical introduction of spermine [24]. It was proved that the transfection efficiency of spermine–pullulan was equal to that of Cyd and was higher than that of Tat-cyd and lipofectamine2000, indicating spermine–pullulan could be used as an effective non-viral gene transfection vector [25].
Although the non-viral vectors hold promise in delivering therapeutic genes to MSCs, most current studies on these vectors are still limited to the in vitro evaluation of their transfection efficiency. As viral vectors can integrate into the host genome, they could result in a stable and long-term gene expression in vivo[26].

However, the gene expression by the non-viral system is transient, so that this system is not suitable to carry out the studies involving the cells for the treatment of diseases for which gene expression is required over long periods of time, as the non-viral vectors have a shorter expression time for the transgenes and a relatively lower transfection efficiency. It was reported that non-viral gene carrier also can prepare cells stably expressed through an antibiotic selection [27]. However, this procedure showed not practical, as it takes a long time to perform and to optimize the conditions. Therefore, to tackle this issue, the controlled release of anticancer agents inside the cells or in the tissue will be promising. Hence, targeting drug delivery system (TDDS) could be practically used to modify and regulate the level and time period of gene expression[28]. In this case, MSCs could be used for the targeted delivery of tumor therapeutic genes transfected by non-viral vectors for their engraftment efficiency to tumor sites.

2. Characterization and utility of Mesenchymal stem cells

MSCs are non-hematopoietic cells, initially described by Friedenstein [29]. 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 [30]. 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 [31]. 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 [32,33]. Several other sources of MSCs have been reported including liver [34], periodontal ligament [35], hair follicles [36], amniotic fluid [37], and placenta ([38]. MSCs reside in the bone marrow in small numbers (0.001–0.01% of nucleated cells [39], 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 [40,41,42]. MSCs possess strong immunosuppressive properties that can be exploited for successful autologous as well as heterologous therapies [43,43].

3. Genetically modified MSC for therapy

Genetically engineered MSCs have been used for improvement in hematopoietic engraftment following myeloablative transplantation regimens [44], and the targeted delivery of antitumor factors by secretion of growth factors and cytokines [45,46]. These studies have led to several clinical trials using MSCs for the treatment of inherited disorders, producing promising results in OI [47], metachromatic leukodystrophy, and Hurler's syndrome [48].

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 [(49)].

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 [(50)]. Meyerrose et al [(51)] 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.

3.1. MSC based cell therapy in brain diseases

Cell replacement and gene transfer to the diseased or injured brain have provided the basis for the development of potentially powerful new therapeutic strategies for a broad spectrum of human neurological diseases including stroke, neurodegenerative diseases such as Parkinson disease, Huntington disease, and Alzheimer disease, and also brain tumors. Recent studies have shown that stem cells can migrate to injured sites and generate new neurons, which has raised hopes for the development of stem cell-based therapy for patients suffering from brain damage [(24)] and [(25)]Stem cell-based cell therapy of brain diseases aims to replace dead or damaged neurons, and provide environmental enrichment to support host neurons by producing neurotrophic factors, thereby beneficial for functional recovery. Besides that, stem cell-based therapy could be improved through the gene transfer technology, that is to say, stem cells could be promising vehicles for therapeutic gene delivery.

Mesenchymal stem cells (MSCs) and gene-engineered MSCs have shown therapeutic benefits in treating brain diseases such as stroke, neurodegenerative diseases and brainstem glioma. Proposed approaches include delivery via intracerebral or intravenous injection, or even infusion via an intranasal route [(37)]. Upon transplantation into the brain, MSCs promote endogenous neuronal growth, decrease apoptosis, reduce levels of free radicals, encourage synaptic connection from damaged neurons and regulate inflammation, primarily through paracrine actions [(36)]. Some chemokines and factors seem to play important roles in homing and therapeutic outcomes of MSCs. Especially, MSCs may encourage repair and new growth of neurons through providing neurotrophic factors, which seems to be a significant aspect among the mechanisms of MSCs for the treatment of brain diseases.

3.2. MSCs for the treatment of ischemic stoke

Acute ischemic stroke causes a disturbance of neuronal circuitry and disruption of the blood–brain barrier that can lead to functional disabilities, which is the most important vascular central nervous system (CNS) disorder that remains a leading cause of death and disability [(38)].

Bone marrow-derived MSCs have great potential as therapeutic agents in stroke management, since they are easily obtained from bone marrow and can be expanded rapidly ex vivo for autologous transplantation [39].

Table 1: MSCs for the treatment of brain diseases

Cells	*Animal model*	*Application*	*Outcomes*
Fibroblast growth factor-2 (FGF-2) gene–transferred MSCs	Aβ-induced mice	Intracerebral transplantation, 1 × 106 cells	Functional recovery[43]
Bone marrow MSCs overexpressed BDNF or NGF	YAC 128 transgenic mice	Stereotaxic injection bilaterally into the striatum, 3 × 105 cells	Reduced clasping, provided behavioral sparing and neuronal sparing [51]
Tyrosine hydroxylase (TH)-gene-engineered Bone marrow MSCs	6-OHDA induced rats	Left striatum injection, 1 × 106 cells	Significant functional recovery [56]
GDNF-transduced MSCs	6-OHDA induced rats	Intrastriatal transplantation, 1 × 105 cells	Induced a pronounced local trophic effect in the striatum [60]
Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) genetically engineered MSCs	F98 tumor-bearing rats	Stereotaxic injection into the brain, 1.6 × 105 cells	Short- and long-term therapeutic efficacy [64]
MSCs engineered to express cytosine deaminase	C6 glioma-bearing rats	Stereotaxic injection into the striatum, 3 × 105 cells	Reduced tumor mass in early stage brain tumors [66]
MSCs transduced with HSVtk	9L glioma-bearing rats	Intracerebral co-inoculation of MSCs/tk and ganciclovir, 5 × 104 cells	Reduced tumor growth, prolonged survival time [67]

Increasing evidences suggest that MSCs can be used to treat stroke with satisfactory results, as MSCs can migrate to the ischemic areas, secrete some trophic factors and even differentiate into neurons and glia cells, which seems to be beneficial for the treatment of ischemia stroke.

After middle cerebral artery occlusion (MCAO), MSCs that are administered by different routes and applied for different time of treatment all show promising therapeutic effects. The three major routes of MSC administration are intrastriatal, intracarotid, and intravenous injection. MSC therapy begins 1, 4, 7 days and even 1 month after MCAO [36].

Transplanting MSCs after MCAO has been found significantly improving neurological functional recovery, as well as promoting endogenous neurogenesis and reducing apoptosis while no significant change of infarct volume was found, and some cells express protein marker phenotypic neural cells, which indicates that a few stem cells differentiate into neural cell lineage [15,24,41,42]. However, gene modification is always a promising way to enhance therapeutic benefits.

Rats with ischemic stroke due to MCAO that received fibroblast growth factor-2 (FGF-2)-modified MSCs or BDNF-modified MSCs had a significantly reduced infarction volume 14 days after MCAO [43,44].

Other combination methods have also been studied and showed great therapeutic benefits, which demonstrates that MSCs have great potential in stroke treatment. Transplanting a composite graft of fresh bone marrow (BM) along with brain-derived neurotrophic factor (BDNF) into the ischemic boundary zone (IBZ) of rat brain can facilitate BM cells to survive and differentiate, and improves functional recovery after middle cerebral artery occlusion (MCAO) [45].

Transplantation of neural progenitor cells (NS-MSCs) in combination with a collagen sponge and bFGF releasing microspheres has been found to significantly improve histological and functional recovery in the rat stroke model [46].Transplanting MSCs increased expression of brain-derived neurotrophic factor (BDNF) [25], nerve growth factor (NGF) [24] and [25], vascular endothelial growth factor (VEGF) [52], basic fibroblast growth factor (bFGF) [15] and so on. These trophic factors function in the processes of induction of angiogenesis, neurogenesis and neuroprotection, and it seems that these factors/cytokines secreted by MSCs play most important role in functional recovery after MCAO.

3.3. MSCs for the treatment of neurodegenerative disease

Neurodegenerative diseases are characterized by the loss of neurons in the brain or spinal cord. In the brain, Alzheimer's disease (AD) and Huntington's disease (HD) result in widespread loss of neurons, whereas Parkinson's disease (PD) involves the specific and localized loss of dopaminergic (DA) neurons in the substantia nigra [35]. The lack of effective therapies for these neurological diseases provokes researchers to find other appropriate ways. MSCs present a promising tool for the treatment of neurodegenerative diseases, as MSCs transplantation has been demonstrated to promote functional recovery in many disease models.

3.3.1. Alzheimer's disease

AD is an age-related progressive neurodegenerative disorder characterized by progressive memory deficits, cognitive impairment, and personality changes associated with the degeneration of multiple neuronal types and pathologically by the presence of neuritic plaques and neurofibrillary tangles [(53)]. Amyloid β-peptide (Aβ) has been suggested to play an etiological, pivotal and likely causal role in the pathogenesis of AD [(54)]. In vitro incubation of primary cultured hippocampal neurons with Aβ, induced apoptosis was ameliorated by MSCs co-culture [55]. In the acute Aβ-induced AD model, MSCs intracerebral transplanted promoted microglial activation, and substantially reduced the Aβ deposits, which suggested that BM-MSCs may be a useful therapeutic agent against AD [56].

Interestingly, in amyloid precursor protein (APP) and presenilin one (PS1) double-transgenic mice, a model of age-dependent AD, MSC treatment promoted microglial activation, rescued cognitive impairment, and reduced Aβ and tau pathology in the brain [(57)]. Huntington's disease (HD) is a fatal inherited neurodegenerative disorder, characterized by a polyglutamine expansion that leads to the production of a mutant huntingtin protein (mHtt), for which no treatment is yet available [(58)]. Mesenchymal stem cell transplantation may help stabilize the striatal environment by producing anti-inflammatory cytokines and neurotrophic factors. BDNF therapy is a leading candidate for use in HD since striatal neurons depend on BDNF levels for function and survival [61].

Therefore, researchers evaluated MSCs engineered to overexpress BDNF or nerve growth factor (NGF) had significant ameliorative effects on disease progression in a mouse model of HD [62]. Collectively, the use of MSCs to deliver factors, confers benefits over other methods because transplanted MSCs have the potential to provide sustainable delivery of trophic factors directly to cells that are at risk of degenerating [13] and [62].

3.3.2. Parkinson's disease

PD is caused by the progressive loss of the dopaminergic neurons in the substantia nigra and is a severe decrease in the dopamine content of the striatum [63]. The goal of cellular therapy to treat Parkinson's disease (PD) is the replacement of lost neurons in the substantia nigra with healthy dopaminergic neurons or the protection of these neurons from further loss. Lusine et al. demonstrated that intranasal (IN) delivery of mesenchymal stem cells (MSCs) to the brains of unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rats led to therapeutic effects on dopaminergic activity, reflected by increases in TH and dopamine levels in the damaged areas of the host tissue, and the neuroprotective features of MSC may prevail over their capacity to replace degenerated neural cells [64]. Furthermore, gene-modified MSCs, MSCs-TH and MSCs-GDNF, also resulted in biologically significant functional recovery. The GDNF-transduced MSCs were capable of inducing a pronounced local trophic effect in the denervated striatum as they induced sprouting from the remaining dopaminergic terminals towards the neurotrophic milieu created, while the TH-modified MSCs significantly protected TH-positive cells from neurotoxicity and increases the DA content in the striatum of the rat brain [65,66,67]. All these indicate MSCs as a potential candidate for treatment of diseases of central
nervous system.

3.4. 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.

3.4.1. Interferon (IFN) – α and β

IFN-β has a wide range of biological activities including potent antiproliferative [81,82] and proapoptotic [83] 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 [84, 85] gliomas [86] and lung metastasis [87].

Ren et al [88] 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. Studies have also shown the antiproliferative, antitumor, and immunomodulatory effects [89,90] 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 [91,92].

In a similar study, Ren et al [93] evaluated the potential of mouse MSCs transduced with adeno-associated virus expressing murine IFN-α in a mouse B16F10 melanoma lung metastasis. 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.

3.4.2. 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 [97]. 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 [98].

3.4.3. Interleukin -2 (IL-2)

In a study by Nakamura et al [94], 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.

3.4.4. NK4

NK4 is an antagonist of hepatocyte growth factor [101]. HGF is a strong inducer of tumor growth, angiogenesis and lymph angiogenesis [102,103]. The effect of MSCs expressing adenovirus NK4 on mice with C-26 lung metastases was studied by Kanehira et al [104]. 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 lymph angiogenesis within the tumor tissues.

3.4.5. 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 [(95)]. Chen et al. [(96)] 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

3.5. 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 [118].

In a pilot study, Kucerova et al [119] 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 [(120)] 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.

3.6. 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 [(105,106)], while sparing most normal cells. TRAIL triggers apoptosis through interaction with death receptors and by initiating caspase-mediated cell death.

Mohr et al [107] 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. 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. Biosafety of genetically engineered human MSC

Several studies have verified that MSC can be efficiently and durably transduced without intensive labor and that this transgene expression is maintained throughout lineage differentiation and without compromising the proliferation rate or quality of progeny [48, 49, 50, 51].

However, a fear for MSC-based tissue therapy is that ectopic bone formation, or even tumor formation, could occur if the cells are not induced into the correct tissue at sites of damage.

This fear is fostered by the fact that human and murine embryonic cells can form teratoma in vivo but should not apply to primary human cells that are subject to proper contact inhibition-mediated cessation of cell division. Tumors and ectopic unwanted tissues are not formed from human bone marrow-derived MSC in immune-deficient mice when conducting careful biosafety studies for retroviral and lentiviral vector trials. Human hematopoietic stem cells and human mesenchymal stem cells carrying two different Moloney-based vectors were co-transplanted together into immune-deficient mice. In a study by Gin [121] A total of 481 mice were monitored for adverse events for 7–18 months post-transplantation.

Following the co-transplantation of the engineered HSC/MSC inoculums, mice were assessed twice a day for signs of ill health, as defined by any of the following indicators: weight loss, hunching, lethargy, rapid breathing, skin discoloration or irregularities, bloating, hemi-paresis, visibly enlarged lymph nodes, or visible solid tumors under the skin.

In addition, 149 mice were transplanted with human hematopoietic progenitor cells transduced with HIV-1-based lentiviral vectors and were followed for 2–6 months. No adverse events caused by the vectors could be observed, and none of the mice had detectable HIV p24 antigen in their serum.

In summary, MSC-based cellular therapy, when combined with genetic engineering, can provide a safe and effective means by which to systemically produce factors that are needed by other cells in the organs of a recipient that has enzymatic or other defects. Numerous MSC-based therapies conducted by Osiris, Athersys, and other companies have demonstrated the safety of systemic infusion in Phase I-III trials [5].

Biosafety data from researchers have further shown that genetic engineering of MSC can provide a safe and effective cell-based therapy for different disorders where a single protein or enzyme is lacking. Genetically engineered MSC should be considered a cell-based therapy for some disorders, especially orphan diseases, when the risk to benefit ratio has been carefully considered.

5. Summary:

It is now clear that the differentiative capacity of MSC is far broader than anyone would have foreseen at the time Friedenstein originally described his bone marrow-derived CFU-F. In addition to this tremendous differentiative potential, the relative ease with which MSC can be isolated, propagated in culture, and modified with a variety of viral and non-viral-based vectors, and their intrinsic ability to seek out sites of injury/inflammation within the body argues that MSC may be ideally suited as cellular therapeutics and gene delivery vehicles for numerous diseases affecting each of the major organ systems of the body.

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.

References

1. Friedenstein AJ. Osteogenic stem cells in the bone marrow. *Bone and Mineral*. **1991**; 7:243–272.
2. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation*. **1999**;17(4):331–340.
3. Caplan AI. Mesenchymal stem cells. *J Orthop Res*. **2007**;9(5):641–650.
4. Galotto M, Berisso G, Delfino L, Podesta M, Ottaggio L, Dallorso S, Dufour C, Ferrara GB, Abbondandolo A, Dini G, Bacigalupo A, Cancedda R, Quarto R. Stromal damage as consequence of high-dose chemo/radiotherapy in bone marrow transplant recipients. *Exp Hematol*. **2010**;27(9):1460–1466.
5. Kassem M. Mesenchymal stem cells: biological characteristics and potential clinical applications. *Cloning Stem Cells*. **2004**;6(4):369–374.
6. Luria EA, Panasyuk AF, Friedenstein AY. Fibroblast colony formation from monolayer cultures of blood cells. *Transfusion*. **2009**;11(6):345–349.
7. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. **1999**;284(5411):143–147.
8. Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood*. **2006**;78(1):55–62.
9. Airey JA, Almeida-Porada G, Colletti EJ, Porada CD, Chamberlain J, Movsesian M, Sutko JL, Zanjani ED. Human mesenchymal stem cells form Purkinje fibers in fetal sheep heart. *Circulation*. **2004**;109(11):1401–1407.
10. Almeida-Porada MG, Porada C, ElShabrawy D, Simmons PJ, Zanjani ED. Human marrow stromal cells (MSC) represent a latent pool of stem cells capable of generating long-term hematopoietic cells. *Blood*. **2001**;98(Part 1):713a.
11. Chamberlain J, Yamagami T, Colletti E, Theise ND, Desai J, Frias A, Pixley J, Zanjani ED, Porada CD, Almeida-Porada G. Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. *Hepatology*. **2007**;46(6):1935–1945.
12. Colletti E, Zanjani ED, Porada CD, Almeida-Porada MG. Tales from the Crypt: Mesenchymal Stem Cells for Replenishing the Intestinal Stem Cell Pool. *Blood*. **2008**;112 Abstract 390.
13. Colletti EJ, AJA, Zanjani ED, Porada CD, Almeida-Porada G. Human Mesenchymal Stem Cells differentiate promptly into tissue-specific cell types without cell fusion, mitochondrial or membrane vesicular transfer in fetal sheep. *Blood*. **2007**;110(11):135a.
14. Colletti EJ, Airey JA, Liu W, Simmons PJ, Zanjani ED, Porada CD, Almeida-Porada G. Generation of tissue-specific cells from MSC does not require fusion or donor-to-host mitochondrial/membrane transfer. *Stem Cell Res*. **2009**;2(2):125–138.
15. Colletti EJ, Almeida-Porada G, Chamberlain J, Zanjani ED, Airey JA. The time course of engraftment of human mesenchymal stem cells in fetal heart demonstrates that Purkinje fiber aggregates derive from a single cell and not multi-cell homing. *Exp Hematol*. **2006**;34(7):926–933.
16. Bruder SP, Ricalton NS, Boynton RE, Connolly TJ, Jaiswal N, Zaia J, Barry FP. Mesenchymal stem cell surface antigen SB-10 corresponds to activated leukocyte cell adhesion molecule and is involved in osteogenic differentiation. *J Bone Miner Res*. **1998**;13(4):655–663.
17. Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone*. **1992**;13(1):69–80.
18. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM. Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells*. **2006**;24(2):376–385.
19. Almeida-Porada G, El Shabrawy D, Porada C, Zanjani ED. Differentiative potential of human metanephric mesenchymal cells. *Exp Hematol*. **2002**;30(12):1454–1462.
20. Fan CG, Tang FW, Zhang QJ, Lu SH, Liu HY, Zhao ZM, Liu B, Han ZB, Han ZC. Characterization and neural differentiation of fetal lung mesenchymal stem cells. *Cell Transplant*. **2005**;14(5):311–321.
21. Gotherstrom C, West A, Liden J, Uzunel M, Lahesmaa R, Le Blanc K. Difference in gene expression between human fetal liver and adult bone marrow mesenchymal stem cells. *Haematologic*a. **2005**;90(8):1017–1026.
22. in 't Anker PS, Noort WA, Scherjon SA, Kleijburg-van der Keur C, Kruisselbrink AB, van Bezooijen RL, Beekhuizen W, Willemze R, Kanhai HH, Fibbe WE. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. *Haematologica*. **2003**;88(8):845–852.
23. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood*. **2004**;103(5):1669–1675.

24. Morizono K, De Ugarte DA, Zhu M, Zuk P, Elbarbary A, Ashjian P, Benhaim P, Chen IS, Hedrick MH. Multilineage cells from adipose tissue as gene delivery vehicles. *Hum Gene Ther*. **2003**;14(1):59–66.
25. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. **2002**;13(12):4279–4295.
26. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. **2001**;7(2):211–228.
27. Garol NJ, Yamagami T, Osborne C, Porada CD, Zanjani ED, Almeida-Porada G. Tissue-specific molecular signature may explain differentiative bias of human MSC from different tissues. *Blood*. **2007**;110(11):570a.
28. Mazhari S, Desai J, Chamberlain J, Porada C, Zanjani ED, Almeida-Porada G. Proteomic Analysis Reveals Intrinsic Differences between Phenotypically Identical Mesenchymal Stem Cells. *Blood*. **2005**;106(11).
29. Mazhari SM, Porada CD, Chamberlain J, Zanjani ED, Almeida-Porada G. Characterization of membrane proteins of mesenchymal stem cells from human liver. *Experimental Hematology*. **2006**;34(9, Suppl. 1):80.
30. Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, Bae YC, Jung JS. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. *Cell Physiol Biochem*. **2004**;14(4–6):311–324.
31. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*. **2006**;24(5):1294–1301.
32. Chamberlain J, Frias A, Porada C, Zanjani ED, Almeida-Porada G. Neural Generation in vivo differs with route of administration and source of mesenchymal stem cells. *Experimental Hematology*. **2005**;33(7):47a.
33. Almeida-Porada MG, Chamberlain J, Frias A, Porada CD, Zanjani ED. Tissue of Origin Influences In Vivo Differentiative Potential of Mesenchymal Stem cells. *Blood*. **2003**;102(11) abstract #1304.
34. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res*. **2007**;67(19):9142–9149.
35. Bochkov NP, Voronina ES, Kosyakova NV, Liehr T, Rzhaninova AA, Katosova LD, Platonova VI, Gol'dshtein DV. Chromosome variability of human multipotent mesenchymal stromal cells. Bull *Exp Biol Med*. **2007**;143(1):122–126.
36. Buyanovskaya OA, Kuleshov NP, Nikitina VA, Voronina ES, Katosova LD, Bochkov NP. Spontaneous aneuploidy and clone formation in adipose tissue stem cells during different periods of culturing. *Bull Exp Biol* Med. **2009**;148(1):109–112.
37. Rubio D, Garcia S, De la Cueva T, Paz MF, Lloyd AC, Bernad A, Garcia-Castro J. Human mesenchymal stem cell transformation is associated with a mesenchymal-epithelial transition. *Exp Cell Res*. **2008**;314(4):691–698.
38. Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A. Spontaneous human adult stem cell transformation. *Cancer Res*. **2005;**65(8):3035–3039.
39. Altanerova V, Horvathova E, Matuskova M, Kucerova L, Altaner C. Genotoxic damage of human adipose-tissue derived mesenchymal stem cells triggers their terminal differentiation. *Neoplasma*. **2009**;56(6):542–547.
40. Crop M, Baan C, Weimar W, Hoogduijn M. Potential of mesenchymal stem cells as immune therapy in solid-organ transplantation. *Transpl Int*. **2009**;22(4):365–376.
41. de Jong DS, Vaes BL, Dechering KJ, Feijen A, Hendriks JM, Wehrens R, Mummery CL, van Zoelen EJ, Olijve W, Steegenga WT. Identification of novel regulators associated with early-phase osteoblast differentiation. J *Bone Miner Res*. **2004**;19(6):947–958.
42. Hishikawa K, Miura S, Marumo T, Yoshioka H, Mori Y, Takato T, Fujita T. Gene expression profile of human mesenchymal stem cells during osteogenesis in three-dimensional thermoreversible gelation polymer. *Biochem Biophys Res Commun*. **2004;**317(4):1103–1107.
43. Nakamura T, Shiojima S, Hirai Y, Iwama T, Tsuruzoe N, Hirasawa A, Katsuma S, Tsujimoto G. Temporal gene expression changes during adipogenesis in human mesenchymal stem cells. *Biochem Biophys Res Commun*. **2003**;303(1):306–312.
44. Ando W, Tateishi K, Hart DA, Katakai D, Tanaka Y, Nakata K, Hashimoto J, Fujie H, Shino K, Yoshikawa H, Nakamura N. Cartilage repair using an in vitro generated scaffold-free tissue-engineered construct derived from porcine synovial mesenchymal stem cells. *Biomaterials*. **2007**;28(36):5462–5470.
45. Cancedda R, Mastrogiacomo M, Bianchi G, Derubeis A, Muraglia A, Quarto R. Bone marrow stromal cells and their use in regenerating bone. *Novartis Found Symp*. **2003**;249:133–143. discussion 143-7, 170-4, 239-41.
46. Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M. Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med*. **2001**;344(5):385–386.
47. Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. *Cell Transplant*. **2004**;13(5):595–600.
48. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T. 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 U S A*. **2002**;99(13):8932–8937.
49. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med*. **1999**;5(3):309–313.
50. Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, McCarville ME, Orchard PJ, Pyeritz RE, Brenner MK. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood*. **2001**;97(5):1227–1231.
51. Le Blanc K, Gotherstrom C, Ringden O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Norden-Lindeberg S, Jansson M, Dalton A, Astrom E, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation*. **2005**;79(11):1607–1614.
52. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve*. **1995**;18(12):1417–1426.
53. Dezawa M, Ishikawa H, Itokazu Y, Yoshihara T, Hoshino M, Takeda S, Ide C, Nabeshima Y. Bone marrow stromal cells generate muscle cells and repair muscle degeneration. *Science*. **2005**;309(5732):314–317.
54. Ichim TE, Alexandrescu DT, Solano F, Lara F, Campion Rde N, Paris E, Woods EJ, Murphy MP, Dasanu CA, Patel AN, Marleau AM, Leal A, Riordan NH. Mesenchymal stem cells as anti-inflammatories: implications for treatment of Duchenne muscular dystrophy. *Cell Immunol*. **2010**;260(2):75–82.
55. Bossolasco P, Cova L, Calzarossa C, Rimoldi SG, Borsotti C, Deliliers GL, Silani V, Soligo D, Polli E. Neuro-glial differentiation of human bone marrow stem cells in vitro. *Exp Neurol*. **2005;**193(2):312–325.
56. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science*. **2000**;290(5497):1775–1779.
57. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci U S A*. **1999**;96(19):10711–10716.
58. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science*. **2000**;290(5497):1779–1782.
59. Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ, Olson L. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci U S A*. **2002**;99(4):2199–2204.
60. Neuhuber B, Timothy Himes B, Shumsky JS, Gallo G, Fischer I. Axon growth and recovery of function supported by human bone marrow stromal cells in the injured spinal cord exhibit donor variations. *Brain Res*. **2005**;1035(1):73–85.
61. Satake K, Lou J, Lenke LG. Migration of mesenchymal stem cells through cerebrospinal fluid into injured spinal cord tissue. *Spine* (Phila Pa 1976) **2004**;29(18):1971–1979.
62. Honma T, Honmou O, Iihoshi S, Harada K, Houkin K, Hamada H, Kocsis JD. Intravenous infusion of immortalized human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. *Exp Neurol*. **2006**;199(1):56–66.

63. Kang SK, Lee DH, Bae YC, Kim HK, Baik SY, Jung JS. Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. *Exp Neurol*. **2003**;183(2):355–366.
64. Nomura T, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. I.V. infusion of brain-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. *Neuroscience*. **2005**;136(1):161–169.
65. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. *J Neurosci Res*. **2003**;73(6):778–786.
66. Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M. Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. *J Neurol Sci*. **2001**;189(1–2):49–57.
67. Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ Res*. **2003**;92(6):692–699.
68. Iihoshi S, Honmou O, Houkin K, Hashi K, Kocsis JD. A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. *Brain Res*. **2004**;1007(1–2):1–9.
69. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. *Mol Ther*. **2005**;11(1):96–104.
70. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H. BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. *Mol Ther*. **2004**;9(2):189–197.
71. Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, Chopp M. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology*. **2002**;59(4):514–523.
72. Zheng W, Honmou O, Miyata K, Harada K, Suzuki J, Liu H, Houkin K, Hamada H, Kocsis JD. Therapeutic benefits of human mesenchymal stem cells derived from bone marrow after global cerebral ischemia. *Brain Res*. **2010**;1310:8–16.
73. Akiyama Y, Radtke C, Kocsis JD. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. *J Neurosci*. **2002**;22(15):6623–6630.
74. Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. *Glia*. **2002**;39(3):229–236.
75. Gerdoni E, Gallo B, Casazza S, Musio S, Bonanni I, Pedemonte E, Mantegazza R, Frassoni F, Mancardi G, Pedotti R, Uccelli A. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. *Ann Neurol*. **2007**;61(3):219–227.
76. Jin HK, Carter JE, Huntley GW, Schuchman EH. Intracerebral transplantation of mesenchymal stem cells into acid sphingomyelinase-deficient mice delays the onset of neurological abnormalities and extends their life span. *J Clin Invest*. **2002**;109(9):1183–1191.
77. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood*. **2005**;106(5):1755–1761.
78. Blandini F, Cova L, Armentero MT, Zennaro E, Levandis G, Bossolasco P, Calzarossa C, Mellone M, Giuseppe B, Deliliers GL, Polli E, Nappi G, Silani V. Transplantation of Undifferentiated Human Mesenchymal Stem Cells Protects against 6-Hydroxydopamine Neurotoxicity in the Rat. *Cell Transplant*. **2009**.
79. Cova L, Armentero MT, Zennaro E, Calzarossa C, Bossolasco P, Busca G, Lambertenghi Deliliers G, Polli E, Nappi G, Silani V, Blandini F. Multiple neurogenic and neurorescue effects of human mesenchymal stem cell after transplantation in an experimental model of Parkinson's disease. *Brain Res*. **2010**;1311:12–27.
80. Li Y, Chen J, Wang L, Zhang L, Lu M, Chopp M. Intracerebral transplantation of bone marrow stromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Neurosci Lett*. **2001**;316(2):67–70.
81. Mahmood A, Lu D, Lu M, Chopp M. Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells. *Neurosurgery*. **2003**;53(3):697–702. discussion 702-3.
82. Phinney DG, Isakova I. Plasticity and therapeutic potential of mesenchymal stem cells in the nervous system. *Curr Pharm Des*. **2005**;11(10):1255–1265.
83. Pisati F, Bossolasco P, Meregalli M, Cova L, Belicchi M, Gavina M, Marchesi C, Calzarossa C, Soligo D, Lambertenghi-Deliliers G, Bresolin N, Silani V, Torrente Y, Polli E. Induction of neurotrophin expression via human adult mesenchymal stem cells: implication for cell therapy in neurodegenerative diseases. *Cell Transplant*. **2007**;16(1):41–55.
84. Prockop DJ, Azizi SA, Phinney DG, Kopen GC, Schwarz EJ. Potential use of marrow stromal cells as therapeutic vectors for diseases of the central nervous system. *Prog Brain Res*. **2000**;128:293–297.
85. Tondreau T, Lagneaux L, Dejeneffe M, Massy M, Mortier C, Delforge A, Bron D. Bone marrow-derived mesenchymal stem cells already express specific neural proteins before any differentiation. *Differentiation*. **2004**;72(7):319–326.
86. Fukuda K. Reprogramming of bone marrow mesenchymal stem cells into cardiomyocytes. *C R Biol*. **2002**;325(10):1027–1038.
87. Shim WS, Jiang S, Wong P, Tan J, Chua YL, Tan YS, Sin YK, Lim CH, Chua T, Teh M, Liu TC, Sim E. Ex vivo differentiation of human adult bone marrow stem cells into cardiomyocyte-like cells. Biochem Biophys *Res Commun*. **2004**;324(2):481–488.
88. Xu W, Zhang X, Qian H, Zhu W, Sun X, Hu J, Zhou H, Chen Y. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro. *Exp Biol Med* (Maywood) **2004**;229(7):623–631.
89. Cao Y, Sun Z, Liao L, Meng Y, Han Q, Zhao RC. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. *Biochem Biophys Res Commun*. **2005**;332(2):370–379.
90. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. **2002**;105(1):93–98.
91. Tomita S, Mickle DA, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y, Liu P, Li RK. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J Thorac Cardiovasc Surg*. **2002**;123(6):1132–1140.
92. Yoon J, Min BG, Kim YH, Shim WJ, Ro YM, Lim DS. Differentiation, engraftment and functional effects of pre-treated mesenchymal stem cells in a rat myocardial infarct model. *Acta Cardiol*. **2005**;60(3):277–284.
93. Huang J, Zhang Z, Guo J, Ni A, Deb A, Zhang L, Mirotsou M, Pratt RE, Dzau VJ. Genetic Modification of Mesenchymal Stem Cells Overexpressing CCR1 Increases Cell Viability, Migration, Engraftment, and Capillary Density in the Injured Myocardium. *Circ Res*. **2010** Apr 8; (Epub ahead of print)
94. Jiang W, Ma A, Wang T, Han K, Liu Y, Zhang Y, Dong A, Du Y, Huang X, Wang J, Lei X, Zheng X. Homing and differentiation of mesenchymal stem cells delivered intravenously to ischemic myocardium in vivo: a time-series study. *Pflugers Arch*. **2006**;453(1):43–52.
95. Jiang W, Ma A, Wang T, Han K, Liu Y, Zhang Y, Zhao X, Dong A, Du Y, Huang X, Wang J, Lei X, Zheng X. Intravenous transplantation of mesenchymal stem cells improves cardiac performance after acute myocardial ischemia in female rats. *Transpl Int*. **2006**;19(7):570–580.
96. Jiang WH, Ma AQ, Zhang YM, Han K, Liu Y, Zhang ZT, Wang TZ, Huang X, Zheng XP. Migration of intravenously grafted mesenchymal stem cells to injured heart in rats. Sheng Li Xue Bao. **2005**;57(5):566–572.
97. Katritsis DG, Sotiropoulou P, Giazitzoglou E, Karvouni E, Papamichail M. Electrophysiological effects of intracoronary transplantation of autologous mesenchymal and endothelial progenitor cells. *Europace*. **2007**;9(3):167–171.
98. Li Q, Turdi S, Thomas DP, Zhou T, Ren J. Intra-myocardial delivery of mesenchymal stem cells ameliorates left ventricular and cardiomyocyte contractile dysfunction following myocardial infarction. *Toxicol Lett*. **2010** March 23; (epub ahead of print).
99. Ohnishi S, Yanagawa B, Tanaka K, Miyahara Y, Obata H, Kataoka M, Kodama M, Ishibashi-Ueda H, Kangawa K, Kitamura S, Nagaya N. Transplantation of mesenchymal stem cells attenuates myocardial injury and dysfunction in a rat model of acute myocarditis. *J Mol Cell Cardiol*. **2007**;42(1):88–97.
100. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A*. **2001**;98(18):10344–10349.
101. Song H, Song BW, Cha MJ, Choi IG, Hwang KC. Modification of mesenchymal stem cells for cardiac regeneration. *Expert Opin Biol Ther*. **2010**;10(3):309–319.

102. Tang J, Wang J, Guo L, Kong X, Yang J, Zheng F, Zhang L, Huang Y. Mesenchymal stem cells modified with stromal cell-derived factor 1 alpha improve cardiac remodeling via paracrine activation of hepatocyte growth factor in a rat model of myocardial infarction. *Mol Cells*. **2010**;29(1):9–19.
103. Westrich J, Yaeger P, He C, Stewart J, Chen R, Seleznik G, Larson S, Wentworth B, O'Callaghan M, Wadsworth S, Akita G, Molnar G. Factors Affecting Residence Time of Mesenchymal Stromal Cells (MSC) Injected into the Myocardium. *Cell Transplant*. **2010** March 31; (epub ahead of print)
104. Xu J, Chen Q, Shi C, Yin Z. Overexpression of CXCR1/CXCR2 on mesenchymal stromal cells may be an effective treatment for acute myocardial infarction. *Cytotherapy*. **2009**;11(8):990–991.
105. Yau TM, Tomita S, Weisel RD, Jia ZQ, Tumiati LC, Mickle DA, Li RK. Beneficial effect of autologous cell transplantation on infarcted heart function: comparison between bone marrow stromal cells and heart cells. *Ann Thorac Surg*. **2003**;75(1):169–176. discussion 176-7.
106. Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D, Pattany PM, Zambrano JP, Hu Q, McNiece I, Heldman AW, Hare JM. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proc Natl Acad Sci U S A.* **2009**;106(33):14022–14027.
107. Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Muller HW, Zanjani E, Wernet P. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med*. **2004**;200(2):123–135.
108. Noort WA, Feye D, Van Den Akker F, Stecher D, Chamuleau SA, Sluijter JP, Doevendans PA. Mesenchymal stromal cells to treat cardiovascular disease: strategies to improve survival and therapeutic results. *Panminerva Med*. **2010**;52(1):27–40.
109. Rameshwar P, Qiu H, Vatner SF. Stem cells in cardiac repair in an inflammatory microenvironment. *Minerva Cardioangiol***. 2010**;58(1):127–146.
110. Kocher AA, Schlechta B, Gasparovicova A, Wolner E, Bonaros N, Laufer G. Stem cells and cardiac regeneration. *Transpl Int*. **2007**;20(9):731–746.
111. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB, Jr, Reisman MA, Schaer GL, Sherman W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol*. **2009**;54(24):2277–2286.
112. Mohyeddin-Bonab M, Mohamad-Hassani MR, Alimoghaddam K, Sanatkar M, Gasemi M, Mirkhani H, Radmehr H, Salehi M, Eslami M, Farhig-Parsa A, Emami-Razavi H, Alemohammad MG, Solimani AA, Ghavamzadeh A, Nikbin B. Autologous in vitro expanded mesenchymal stem cell therapy for human old myocardial infarction. *Arch Iran Med*. **2007**;10(4):467–473.
113. Ripa RS, Haack-Sorensen M, Wang Y, Jorgensen E, Mortensen S, Bindslev L, Friis T, Kastrup J. Bone marrow derived mesenchymal cell mobilization by granulocyte-colony stimulating factor after acute myocardial infarction: results from the Stem Cells in Myocardial Infarction (STEMMI) trial. *Circulation*. **2007**;116(11 Suppl):I24–I30.
114. Hoogduijn MJ, Crop MJ, Peeters AM, Korevaar SS, Eijken M, Drabbels JJ, Roelen DL, Maat AP, Balk AH, Weimar W, Baan CC. Donor-derived mesenchymal stem cells remain present and functional in the transplanted human heart. *Am J Transplant***. 2009**;9(1):222–230.
115. Chen SL, Fang WW, Qian J, Ye F, Liu YH, Shan SJ, Zhang JJ, Lin S, Liao LM, Zhao RC. Improvement of cardiac function after transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. *Chin Med J* (Engl) **2004;**117(10):1443–1448.
116. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, Sun JP. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol*. **2004**;94(1):92–95.
117. Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, Chen JR, Chen YP, Lee OK. In vitro hepatic differentiation of human mesenchymal stem cells. *Hepatology*. **2004**;40(6):1275–1284.
118. Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M, Sato T, Miyanishi K, Takayama T, Takahashi M, Takimoto R, Iyama S, Matsunaga T, Ohtani S, Matsuura A, Hamada H, Niitsu Y. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. Blood. **2005**;106(2):756–763.
119. Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest. **2002**;109(10):1291–1302.
120. Almeida-Porada G, Porada C, Zanjani ED. Adult stem cell plasticity and methods of detection. Rev Clin Exp Hematol. **2001**;5(1):26–41.
121. Almeida-Porada G, Porada C, Zanjani ED. Plasticity of human stem cells in the fetal sheep model of human stem cell transplantation. *Int J Hematol*. **2004**; 79(1):1–6.

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