We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 8

Mesenchymal Stem Cells as Gene Delivery Vehicles

Christopher D. Porada and Graça Almeida-Porada

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53240

1. Introduction

Mesenchymal stem cells (MSCs) possess a battery of unique properties which make them ideally suited not only for cellular therapies/regenerative medicine, but also as vehicles for gene delivery in a wide array of clinical settings. These include: 1) widespread distribution throughout the body; 2) ease of isolation and ability to be extensively expanded in culture without loss of potential; 3) the ability to differentiate into a wide array of functional cell types in vitro and in vivo; 4) they exert pronounced anti-inflammatory and immunomodulatory effects upon transplantation; and 5) the ability to home to damaged tissues, solid tumors, and metastases following in vivo administration.

In this Chapter, we will summarize the latest research in the use of MSC in regenerative medicine, focusing predominantly on their use as vehicles for transferring exogenous genes. To highlight the immense potential these cells possess for gene therapy applications, we will attempt to paint as broad a canvas as possible, starting with a discussion about the basic biology of MSC, and their unique properties which combine to make MSC one of the most promising stem cell populations for use in gene therapy studies and trials. We will reveal the versatility of MSC as gene delivery vehicles by summarizing some of the most recent studies showing the ease with which MSC can be modified with a wide range of both viral and non-viral vector systems, and highlighting some of the advantages to delivering transgenes within a cellular vehicle, as opposed to administering vectors directly into the body. We will then discuss the engineering of MSC to enhance their natural abilities to mediate repair within various tissues; one of the most popular uses of MSC to-date in the gene therapy arena. We will discuss our recent work, and that of others, using MSC to deliver coagulation factors to treat the hemophilias, with hemophilia A serving as a paradigm for how MSC could be used to deliver a therapeutic transgene, and thereby correct essentially any inherited disease. The Chapter will conclude with a discussion of MSC's ability to selectively migrate to forming solid tumors following intravenous administration, and to actively seek out

INTECH
open science | open minds

© 2013 Porada and Almeida-Porada; licensee InTech. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

metastases at sites far removed from the primary site of the tumor. We will summarize exciting recent work showing that it is possible to exploit this property to achieve sustained, high-level expression of pro-apoptotic gene products within the tumor, obtaining greatly improved anti-tumor effects, while essentially eliminating the systemic toxicities that plague current radiation/chemotherapy-based treatments.

2. Isolation and characterization of MSC

More than 30 years ago, Friedenstein pioneered the concept that the marrow microenvironment resided within the so-called stromal cells of the marrow, by demonstrating that fibroblastoid cells obtained from the bone marrow were capable of transferring the hematopoietic microenvironment to ectopic sites [1, 2]. Years later, scientists began to explore the full potential of these microenvironmental cells, and results of these studies led to the realization that this population harbored cells with properties of true stem cells. These cells were officially termed mesenchymal stem cells (MSC) [3]. MSC are now recognized to be a key part of the microenvironment/niche that supports the hematopoietic stem cell and drives the process of hematopoiesis, yet despite serving this vital function, MSC only comprise ~0.001-0.01% of cells within the marrow [4], making methods for isolating/enriching and for expanding these cells essential to their study and their ultimate clinical use. The most straightforward method for obtaining MSC is to exploit their propensity to adhere to plastic and their ability to be passaged with trypsin (contaminating hematopoietic cells do not passage with trypsin) to rapidly obtain a relatively morphologically homogeneous population of fibroblastic cells from a bulk mononuclear cell preparation [5-7]. Unfortunately, true MSC (defined by function) account for only a small percentage of the highly heterogeneous resultant population, making results obtained with cells prepared in this fashion difficult to interpret, and inconsistent from experiment-to-experiment and from group-to-group. The identification of antigens that are unique to MSC would eliminate this problem. Human MSC do not express markers which have been associated with other stem cell populations (like hematopoietic stem cells) such as CD34, CD133, or c-kit, nor hematopoietic markers such as CD45, CD14, and CD19. Moreover, no marker has been identified to date that specifically identifies MSC. Nevertheless, several surface antigens have proven useful for obtaining highly enriched MSC populations. The first of these markers to be identified was Stro-1, an antibody that reacts with non-hematopoietic bone marrow stromal precursor cells [8]. Although the antigen recognized by this antibody has not yet been identified, we and others have found that by tri-labeling bone marrow cells with Stro-1, anti-CD45, and anti-GlyA, and selecting the Stro-1+CD45-GlyA- cells, it is possible to consistently obtain a homogeneous population that is highly enriched for MSC [9-15]. In addition to Stro-1, antibodies such as SB-10, SH2, SH3, and SH4 have been developed over the years and numerous surface antigens such CD13, CD29, CD44, CD63, CD73, CD90, CD105, and CD166 have been used to attempt to identify and isolate MSC [16-18]. Unfortunately, all of these antigens appear to be expressed on a wide range of cell types within the body in addition to MSC. This lack of a unique marker suggests that to obtain a pure population of MSC that are functionally homo-

http://dx.doi.org/10.5772/53240

geneous, investigators will likely either have to await the development of novel antibodies that recognize as yet unidentified antigens that are unique to primitive MSC, or employ strategies in which multiple antibodies are combined to allow for positive selection of MSC and depletion of cells of other lineages that share expression of the antigens recognized by the MSC antibody in question, as we have done with Stro-1, CD45, and GlyA.

3. Sources of MSC

Although much of the work to date has focused on MSC isolated from adult bone marrow, we and others have isolated cells that appear phenotypically and functionally to be MSC, from numerous tissues including brain, liver, lung, fetal blood, umbilical cord blood, kidney, and even liposuction material [19-26]. The broad distribution of MSC throughout the body leads one to postulate that MSC may play a critical role in organ homeostasis by providing supportive factors and/or mediating maintenance/repair within their respective tissue. Importantly, although MSC from each of these tissues appear similar with respect to phenotype and overall differentiative potential, studies at the RNA and protein level have now revealed that subtle differences exist between MSC from these various tissues, with MSC from each tissue possessing a molecular fingerprint indicative of their tissue of origin [21, 22, 27-31]. Using a non-injury fetal sheep transplantation model, we showed that these differences result in a bias for human MSC to home to and give rise to cells of their tissue of origin in vivo [32, 33]. This suggests that, to use MSC as therapeutics or as gene delivery vehicles, the ideal source of MSC may differ depending on the specific disease to be treated and the desired target organ.

Despite the apparent presence of MSC within many of the major organs of the body, the relatively non-invasive fashion with which adipose tissue or bone marrow can be obtained, and the fact that both these tissues could readily be obtained autologously, combine to suggest that these two tissues will likely be the predominant source of MSC employed in clinical applications in the foreseeable future. However, additional experiments will need to be performed to rigorously assess the inherent safety of adipose tissue-derived MSC before these cells will see widespread clinical use, since several recent studies have suggested that they may be inherently less genetically stable than MSC isolated from bone marrow [34], exhibiting aneuploidy [35, 36] and undergoing transformation [37, 38] upon prolonged propagation in vitro. However, another recent study provided evidence that, even if genomic instability is intentionally induced with genotoxic agents, adipose tissue-derived MSC respond to this insult by undergoing terminal adipogenic differentiation rather than transformation [39]. The dramatically conflicting nature of the results from these different studies could, perhaps, be due to differing methods employed for isolating and culturing MSC, differing levels of contaminating non-MSC cells in the cultures, as well as the duration of the culture (i.e., the number of times the cells have been passaged). Bearing this possible instability in mind, the recommendation has been put forward to only make clinical use of cells that have been passaged fewer than 25 times in culture, regardless of the source of MSC [40].

4. MSC as vehicles for delivering therapeutic genes

While MSC possess tremendous therapeutic potential by virtue of their ability to lodge/engraft within multiple tissues in the body and both give rise to tissue-specific cells and release trophic factors that trigger the tissue's own endogenous repair pathways [41-59], gene therapists have realized that these properties are just the beginning of the therapeutic applications for MSC [24, 60, 61]. By using gene therapy to engineer MSC to either augment their own natural production of specific desired proteins or to enable them to express proteins outside of their native repertoire, it is possible to greatly broaden the spectrum of diseases for which MSC could provide therapeutic benefit. Unlike hematopoietic stem cells which are notoriously difficult to modify with most viral vectors while preserving their in vivo potential, MSC can be readily transduced with all of the major clinically prevalent viral vector systems including those based upon adenovirus [62-64], the murine retroviruses [64-68], lentiviruses [69-74], and AAV [75, 76], and efficiently produce a wide range of cytoplasmic, membrane-bound, and secreted protein products. This ease of transduction coupled with the ability to subsequently select and expand only the gene-modified cells in vitro to generate adequate numbers for transplantation combine to make MSC one of the most promising stem cell populations for use in gene therapy studies and trials.

To date, the majority of studies using gene-modified MSC have been undertaken with the purpose of enhancing the natural abilities of MSC to mediate repair within various tissues. Using the heart as an example, once investigators discovered the identity of some of the key trophic factors responsible for MSC's beneficial effect on the injured myocardium, they undertook studies using MSC engineered to overexpress a number of these factors [69, 77-86]. As anticipated, the "gene-enhanced" MSC were substantially more effective than their unmodified counterparts, producing greatly enhanced therapeutic effects. Similar studies have also been performed to repair the damaged/diseased CNS using MSC engineered to produce neurotrophic factors [87-94], to repair the injured liver using MSC expressing proteins involved in hepatocyte differentiation and/or proliferation [95, 96], to repair ischemia/reperfusion injury [97-102], and to repair the kidney [103-105]. In each case thus far, MSC engineered to express higher levels of proteins known to be beneficial for the tissue in question and/or to promote survival have produced markedly better results than unmodified MSC.

Despite the many advantages of using MSC as gene delivery vehicles, however, relatively few studies have thus far explored this potential for the treatment of genetic diseases. One disease for which we and others are actively investigating MSC for delivery of a therapeutic gene is hemophilia A [106-112].

5. Hemophilia A as a paradigm for the use of gene-modified msc to correct genetic diseases

Hemophilia A represents the most common inheritable deficiency of the coagulation proteins [113]. The severity of hemophilia A is traditionally based on plasma levels of FVIII,

with persons exhibiting less than 1% normal factor (<0.01 IU/mL) being considered to have severe hemophilia, persons with 1-5% normal factor moderately severe, and persons with 5%-40% of the normal FVIII levels mild [114-116]. Up to 70% of hemophilia A patients present with the severe form of the disease, and suffer from frequent hemorrhaging, leading to chronic debilitating arthropathy, hematomas of subcutaneous connective tissue/muscle, and internal bleeding. Over time, the collective complications of recurrent hemorrhaging result in chronic pain, absences from school and work, and permanent disability [114]. Current state-of-the-art treatment consists of frequent prophylactic infusions of plasma-derived or recombinant FVIII protein to maintain hemostasis, and has greatly increased life expectancy and quality of life for many hemophilia A patients.

This treatment approach is, however, far from ideal, due to the need for lifelong intravenous infusions and the high treatment cost. Moreover, this treatment is unavailable to a large percentage of the world's hemophiliacs, placing these patients at great risk of severe, permanent disabilities and life-threatening bleeds. Furthermore, even among the patients who are fortunate enough to have access to, and the financial means to afford, prophylactic FVIII infusions, approximately 30% will form FVIII inhibitors [117]. The formation of these inhibitors greatly reduces the efficacy of subsequent FVIII infusions, and can ultimately lead to treatment failure, placing the patient at risk of life-threatening hemorrhage. There is thus a significant need to develop novel, longer-lasting hemophilia A therapies.

In the past three decades, the remarkable progress in the understanding of the molecular basis of the disease, the identification and characterization of FVIII gene, structure, and biology has heightened the interest and feasibility of treating hemophilia A with gene therapy. In contrast to current protein-based therapeutics, lifelong improvement or permanent cure of hemophilia A is theoretically possible after only a single gene therapy treatment; indeed, several aspects of hemophilia A make it ideally suited for correction by gene therapy [118-126]. First, in contrast to many other genetic diseases, the missing protein (coagulation FVIII) does not need to be expressed in either a cell- or tissue-specific fashion to mediate correction. Although the liver is thought to be the primary natural site of synthesis of FVIII, expression of this factor in other tissues exerts no deleterious effects. As long as the protein is expressed in cells which have ready access to the circulation, it can be secreted into the bloodstream and exert its appropriate clotting activity. Second, even modest levels (3-5%) of FVIII-expressing cells would be expected to convert severe hemophilia A to a moderate/mild phenotype, reducing or eliminating episodes of spontaneous bleeding and greatly improving quality of life. Thus, even with the low levels of transduction that are routinely obtained with many of the current viral-based gene delivery systems, a marked clinical improvement would be anticipated in patients with hemophilia A. Conversely, even supraphysiologic levels of FVIII as high as 150% of normal are predicted to be well tolerated, making the therapeutic window extremely wide [116]. Based on this knowledge, the American Society of Gene and Cell Therapy (www.ASGCT.org) recently provided NIH director, Dr. Francis Collins, with a roadmap of disease indications that it feels will be viable gene therapy products within the next 5-7 years. The hemophilias were identified as belonging to the most promising, "Target 10", group of diseases.

6. Mesenchymal Stem Cells (MSC) as hemophilia A therapeutics

As discussed in the preceding section, the liver is thought to be the primary site of FVIII synthesis within the body. We and others have devoted a great deal of energy to demonstrating the ability of MSC from various sources to serve as therapeutics for liver disease [11, 13, 14, 33, 96, 127-152]. It is now clear that, not only do MSC have the ability to generate, in vitro and in vivo, cells which are indistinguishable from native hepatocytes, but transplantation of MSC in a range of model systems can result in fairly robust formation of hepatocytes which repair a variety of inborn genetic defects, toxin-induced injuries, and even fibrosis. The fetal sheep model provides a unique system in which to explore the full differentiative potential of various stem cell populations, since the continuous need for new cells within all of the organs during fetal development provides a permissive milieu in which gene-modified donor cells can engraft, proliferate, and differentiate. Furthermore, by performing the transplant at a stage in gestation when the fetus is considered to be largely immuno-naïve, it is possible to engraft human cells at significant levels, which persist for the lifespan of the animal due to induction of donor-specific tolerance [130-132]. Indeed, in ongoing studies, we have found that, after transplantation into fetal sheep, human MSC engraft at levels of up to 12% within the recipient liver [11, 131, 132, 153-158], and contribute to both the parenchyma and the perivascular zones of the engrafted organs, placing them ideally for delivering FVIII into the circulation. Since FVIII levels of 3-5% of normal would convert a patient with severe hemophilia A to a moderate or mild phenotype, these levels of engraftment should be highly therapeutic. These collective results thus suggest that MSC may represent an ideal cell type for treating hemophilia A.

However, although MSC engrafted (following transplantation in utero) at significant levels within organs that are natural sites of FVIII synthesis, only a small percent expressed endogenous FVIII, suggesting that simply transplanting "healthy" MSC will not likely provide an effective means of treating/curing hemophilia A. By using gene therapy to engineer MSC to express FVIII, however, it is highly probable that the levels of engrafted MSC we have thus far achieved in utero would provide marked therapeutic benefit in hemophilia A. By transducing the MSC in vitro, rather than performing gene therapy by injecting the vector directly, as is the current practice in clinical gene therapy trials, there is no risk of off-target transduction, and the vector being employed simply needs a strong constitutively active promoter to ensure that all cells derived from the transplanted MSC continue to express FVIII and mediate a therapeutic effect. Importantly, the only documented cases of retroviral-induced insertional mutagenesis have been observed following genetic modification of hematopoietic stem cells [159-161]; there is no evidence that MSC transform or progress to clonal dominance following transduction, suggesting they represent safe cellular vehicles for delivering FVIII (or other transgenes).

Importantly, critical proof-of-principle studies have already shown that MSC can be transduced with FVIII-expressing viral vectors and secrete high levels of FVIII protein in vitro and following transplantation in vivo [106-109]. FVIII purified from the conditioned medium of the transduced MSC was proven to have a specific activity, relative electrophoretic

http://dx.doi.org/10.5772/53240

mobility, and proteolytic activation pattern that was virtually identical to that of FVIII produced by other commercial cell lines [109]. Given the widespread distribution and engraftment of MSC following their systemic infusion, the ability of MSC to give rise, in vivo, to cells of numerous tissue types, and their ability to efficiently process and secrete high amounts of biologically active FVIII, they are, not surprisingly, being viewed as ideal vehicles for delivering a FVIII transgene throughout the body and thus providing long-term/permanent correction of hemophilia A [106-109, 162].

In addition to their widespread engraftment and their ability to serve as delivery vehicles for the FVIII gene, the rather unique immunological properties of MSC may further increase their utility for treating hemophilia A. MSC do not normally express MHC class II or the co-stimulatory molecules CD80 and CD82, unless they are stimulated with IFN-γ, and are thus viewed as being relatively hypo-immunogenic. As such, they do not provoke the proliferation of allogeneic lymphocytes or serve as very effective targets for lysis by cytotoxic T cells or NK cells. In fact, a large body of evidence is now accumulating that MSC can be readily transplanted across allogeneic barriers without eliciting an immune response [163, 164]. Thus, if one wished to use MSC to treat hemophilia A, off-the-shelf MSC from an unrelated donor could theoretically be used, greatly increasing the feasibility of obtaining and using these stem cells for therapy.

Perhaps even more important from the standpoint of their potential use as hemophilia A therapeutics, more recent studies have provided evidence that MSC also appear to have the ability to exert both immunosuppressive and anti-inflammatory properties both in vitro and in vivo. These properties appear to result from MSC's ability to intervene, at multiple levels, with the generation and propagation of an immune response. To name just a few examples, MSC have been demonstrated to interfere with the generation and maturation of cytotoxic and helper T cells [165-174], dendritic cells [175-178], and B cells [179]. In addition to actively shutting down the generation of immune effector cells, MSC also have the ability to induce the formation of potent Tregs, although the mechanism by which this comes about is still the subject of active research [40, 180-182]. MSC are also known to express a battery of factors [40, 168-170, 180, 183-187] that reduce local inflammation, blunt immune response, and counteract the chemotactic signals responsible for recruiting immune cells to sites of injury/inflammation. One could thus envision these immune-dampening properties enabling the delivery of FVIII without eliciting an immune response and subsequent inhibitor formation, thus overcoming one of the major hurdles that plague current treatment/management of hemophilia A. As will be discussed in the next section, however, our postnatal studies in the hemophilic sheep suggest that further work will be required to discover how to obtain these potential immune benefits in the context of the ongoing injury/inflammation present in animals/patients with clinically advanced hemophilia A.

In addition to the aforementioned properties, preclinical animal studies examining the potential of MSC isolated from adult tissues have also highlighted another interesting and clinically valuable characteristic of MSC; their ability to selectively navigate to sites of injury and/or inflammation within the body. Once reaching these specific sites, the MSC then mediate repair both by engrafting and generating tissue-specific cells within the injured tissue

[188-190], and by releasing trophic factors that blunt the inflammatory response and often promote healing by activating the tissue's own endogenous repair mechanisms. While the mechanisms responsible for this trafficking to sites of injury are still being elucidated [191-193], this observation raises the exciting possibility that, following systemic infusion, FVIII-expressing MSC could efficiently migrate to sites of active bleeding/injury, thereby releasing FVIII locally and focusing the therapy where it is most needed. As will be discussed in the following section, over the past 2-3 years, we have begun exploring whether it is possible to exploit these many advantages of MSC as a cellular vehicle for delivering a FVIII gene by testing the ability of FVIII-expressing MSC to correct hemophilia A in a new large animal model; sheep.

7. Establishment of a new preclinical model of hemophilia A and success with MSC-based treatment

A number of animal models have been developed to evaluate new methods of not only treatment of coagulation disorders, but also the prevention and treatment of inhibitor formation. Transient hemophilic rabbit models induced by infusion of plasma containing inhibitors have been used to evaluate the effect of different bypass products to factor VIII [194], but these models, while valuable for inhibitor studies, do not accurately recapitulate the human disease, precluding their use for gene therapy studies. Fortunately, dog models of hemophilia A with congenital deficiency [195, 196] and mouse models obtained by gene targeting and knockout technology [197] are available to study FVIII function and gene therapy approaches for treating hemophilia A. Therapeutic benefit has been obtained in numerous studies using a variety of vector systems in the murine model [121, 122, 198-204], and phenotypic correction of hemophilia A in the dog has been achieved, but has proven to be far more difficult than in mice [205, 206]. Despite promising results in both canine and murine models, however, no clinical gene therapy trial has shown phenotypic/clinical improvement of hemophilia A in human patients. This is in marked contrast to the recent clinical successes with gene therapy for hemophilia B [207]. The reasons for the disparity in the efficacy of gene therapy for treating hemophilia A versus B is not presently clear. Nonetheless, based on the disappointing results to-date, there are currently no active hemophilia A clinical gene therapy trials, even though hemophilia A accounts for roughly 80% of all cases of hemophilia.

The difficulties seen thus far translating success in animal models into therapeutic benefit in human patients underscores the importance of preclinical animal models that both precisely mimic the disease process of hemophilia A, and closely parallel normal human immunology and physiology. To this end, between 1979 and 1982, a number of male offspring of a single white alpine ewe at the Swiss Federal Institute of Technology all died several hours postpartum due to severe bleeding from the umbilical cord [208-210]. Daughters and granddaughters of this ewe also gave birth to lambs exhibiting the same pathology. Investigation of the affected animals showed extensive subcutaneous and intramuscular hematomas. Spontaneous hemarthroses were also frequent, leading to reduced locomotion and symp-

http://dx.doi.org/10.5772/53240

toms of pain in standing up, restricting nursing activity. Stronger injuries that arose when animals were not placed in carefully controlled isolation resulted in heavy bleeding and intensive pain. Laboratory tests showed increased PTT, and FVIII levels (as assessed by aPTT) of about 1% of control animals. Replacement therapy with human FVIII (hFVIII) concentrate or fresh sheep plasma resulted in remission of disease and rapid clinical improvement.

Unfortunately, due to the expense and effort of maintaining these sheep, the Swiss investigators allowed the line to die out, saving only a few straws of semen prior to allowing this valuable resource to pass into extinction. We recently used a variety of reproductive technologies to successfully re-establish this line of hemophilia A sheep, and fully characterized both the clinical parameters and the precise molecular basis for their disease [211-216]. In similarity to mutations seen in many human patients [217], these animals possess a premature stop codon with a frameshift mutation. This is the only animal model of hemophilia A with this clinically relevant mutation-type, providing a unique opportunity to study therapies in this context. All ten animals to-date have exhibited bleeding from the umbilical cord, prolonged tail and "cuticle" bleeding time, and multiple episodes of severe spontaneous bleeding including hemarthroses, muscle hematomas, and hematuria, all of which have responded to human FVIII. Just like human patients with severe hemophilia A, a hallmark symptom in these sheep is repeated spontaneous joint bleeds, which lead to chronic, debilitating arthropathies and reduced mobility. Importantly, chromogenic assays performed independently at the BloodCenter of Wisconsin and Emory University revealed undetectable FVIII activity in the circulation of these sheep, explaining their severe, life-threatening phenotype.

In addition to the value of another large animal model of hemophilia A and the uniqueness of the mutation, sheep possess many characteristics that make them an ideal preclinical model for gene therapy. The first of these is the size. Sheep are fairly close in size to humans, weighing roughly 8lbs at birth and 150-200lbs as adults, likely obviating the need for scale-up of cell/vector dose to move from experiments in sheep to trials in humans. This is in marked contrast to mice which are ~2800 times smaller than a typical human patient [218]. Secondly, sheep share many important physiological and developmental characteristics with humans; for example, the pattern of fetal to adult hemoglobin switching, and the naturally occurring changes in the primary sites of hematopoiesis from yolk sac to fetal liver and finally to the bone marrow near the end of gestation. Thirdly, sheep are outbred, and thus represent a wide spectrum of genetic determinants of the immune response, as do humans. As the immune response to both the vector and the vector–encoded FVIII are likely to play a key role in FVIII inhibitor formation (or lack thereof), this represents an advantage not found in most other models, with the possible exception of the dog, which could conceivably be outbred as well to achieve a broader genetic spectrum. In addition, the development of the sheep immune system has been investigated in detail [219-225], making sheep well suited for studying the immunological aspects of gene-based therapies for hemophilia A. Importantly, the large size of the sheep, their long life span (9-12 years), and their relative ease of maintenance and breeding make it possible to conduct long-term studies in relatively large numbers of animals to fully evaluate the efficacy and safety issues related to gene

therapy. For these reasons, we feel that the sheep are a particularly relevant model in which to examine gene and cell-based therapies for hemophilia A. An additional unique advantage to using sheep to study hemophilia A treatment is that in sheep, like human, a large percentage of the vWF is found within platelets rather than free in plasma. This is in contrast to dog (in which vWF circulates free in plasma [226, 227]), and makes the sheep an ideal large animal model in which to explore the use of platelet-targeted gene therapy for hemophilia A [126, 228-230].

To experimentally test the ability of MSC to serve as FVIII delivery vehicles and thus treat hemophilia A, we recently tested a novel, non-ablative transplant-based gene therapy in 2 pediatric hemophilia A lambs [110-112]. During the first 3-5 months of life, both these animals had received frequent, on-demand infusions of human FVIII for multiple hematomas and chronic, progressive, debilitating hemarthroses of the leg joints which had resulted in severe defects in posture and gait, rendering them nearly immobile. In an ideal situation, one would use autologous cells to deliver a FVIII transgene, and thus avoid any complications due to MHC-mismatching. Unfortunately, the severe life-threatening phenotype of the hemophilia A sheep prevented us from collecting bone marrow aspirates to isolate autologous cells. We therefore elected to utilize cells from the ram that had sired the two hemophiliac lambs, hoping that, by using paternal (haploidentical) MSC, immunologic incompatibility between the donor and recipient should be minimized sufficiently to allow engraftment, especially given the large body of evidence now accumulating that MSC can be transplanted across allogeneic barriers without eliciting an immune response [163, 164].

Based on our prior work in the fetal sheep model, we knew that the intraperitoneal (IP) transplantation of MSC results in widespread engraftment throughout all of the major organs [11, 131, 157, 231-233] and durable expression of vector-encoded genes [232-234]. We further reasoned that using the IP route would also have the advantage of enabling the cells to enter the circulation in an almost time-release fashion, after being engulfed by the omentum and absorbed through the peritoneal lymphatics. Importantly, we also felt that the use of the IP route would enable us to avoid the lung-trapping which hinders the efficient trafficking of MSC to desired target organs following IV administration, and also poses clinical risks due to emboli formation [235, 236].

Following isolation, MSC were simultaneously transduced with 2 HIV-based lentivectors, the first of which encoded an expression/secretion optimized porcine FVIII (pFVIII) transgene [112]. We selected a pFVIII transgene for two reasons. First, we had not yet cloned the ovine FVIII cDNA and constructed a B domain-deleted cassette that would fit in a lentivector. Secondly, the pFVIII transgene had previously been shown, in human cells, to be expressed/secreted at 10-100 times higher levels than human FVIII [120, 121, 237]. We thus felt that these very high levels of expression/secretion might enable us to achieve a therapeutic benefit, even in the event we obtained very low levels of engraftment of the transduced paternal MSC. The second lentivector encoded eGFP to facilitate tracking and identification of donor cells in vivo. Combining the 2 vectors unexpectedly resulted in preferential transduction with the eGFP vector, such that only about 15% of the MSC were transduced with the pFVIII-encoding vector, as assessed by qPCR. Once the transduced MSC had been suffi-

ciently expanded, the first animal to be transplanted was treated with a dose of hFVIII calculated to correct the levels to 200%, to ensure no procedure-related bleeding occurred. The animal was then sedated, and 30x10^6 transduced MSC were transplanted into the peritoneal cavity under ultrasound guidance in the absence of any preconditioning.

Following transplantation, FVIII activity (assessed by chromogenic assay) was undetectable in the circulation, but this animal's clinical picture improved dramatically. All spontaneous bleeding events ceased, and he enjoyed an event-free clinical course, devoid of spontaneous bleeds, enabling us to cease hFVIII infusions. Existing hemarthroses resolved, the animal's joints recovered fully and resumed normal appearance, and he regained normal posture and gait, resuming a normal activity level. To our knowledge, this represents the first report of phenotypic correction of severe hemophilia A in a large animal model following transplantation of cells engineered to produce FVIII, and the first time that reversal of chronic debilitating hemarthroses has been achieved.

Based on the remarkable clinical improvement we had achieved in this first animal, we transplanted a second animal with 120x10^6 paternal MSC, >95% of which were transduced and expressing pFVIII. We anticipated that by transplanting 4x's the number of cells with roughly 6x's the transduction efficiency, we would achieve pronounced improvement and therapeutic levels of FVIII in the circulation of this animal. In similarity to the first animal, hemarthroses present in this second animal at the time of transplant resolved, and he resumed normal activity shortly after transplantation. This second animal also became factor-independent following the transplant. These results thus confirm the ability of this MSC-based approach to provide phenotypic correction in this large animal model of hemophilia A. However, just as we had observed in the first animal, no FVIII was detectable in the circulation of this animal, making the mechanism by which this procedure mediated such pronounced clinical improvement uncertain.

Despite the pronounced clinical improvement we observed in the first animal, he mounted a rapid and fairly robust immune response to FVIII, in similarity to prior studies performed with hemophilia A mice [237]. Before transplant, this first animal had Bethesda titers against hFVIII of only ~3, yet this lifesaving procedure resulted in a rise in Bethesda titer to ~800 against the vector-encoded pFVIII and nearly 700 to hFVIII. The formation of such high titer inhibitors with cross-reactivity to the human protein was surprising, given the well established ability to successfully use porcine FVIII products in human patients to bypass existing anti-hFVIII inhibitors [238-241]. Similarly, despite having no detectable inhibitors prior to transplant, the second animal receiving the higher FVIII-expressing cell dose developed titers of ~150 Bethesda units against the vector-encoded pFVIII following transplantation which also exhibited cross-reactivity to the human protein.

Following euthanasia of these animals, we performed a detailed tissue analysis to begin deciphering the mechanism whereby this novel MSC-based gene delivery produced its pronounced therapeutic effect at a systemic level. PCR analysis demonstrated readily detectable levels of MSC engraftment in nearly all tissues analyzed, including liver, lymph nodes, intestine, lung, kidney, omentum, and thymus. These molecular analyses thereby proved that it is possible to achieve widespread durable engraftment of MSC following transplantation

in a postnatal setting in a large animal model without the need for preconditioning/ablation, and in the absence of any selective advantage for the donor cells.

Confocal immunofluorescence analysis revealed large numbers of FVIII-expressing MSC within the synovium of the joints which exhibited hemarthrosis at the time of transplant, demonstrating (just as we had hoped/predicted) that the transplanted MSC possessed the intrinsic ability to home to and persist within sites of ongoing injury/inflammation, releasing FVIII locally within the joint, providing an explanation for the dramatic improvement we observed in the animal's joints. This finding is in agreement with prior studies [242], showing that local delivery of FIX-AAV to the joints of mice with injury-induced hemarthroses led to resolution of the hemarthroses in the absence of any detectable FIX in the circulation. While this finding provides an explanation for the reversal of the joint pathology present in these animals at transplant, it cannot explain the observed systemic benefits such as the cessation of spontaneous bleeding events.

Confocal analysis also revealed engrafted cells within the small intestine, demonstrating that MSC can still engraft within the intestine following postnatal transplantation, just as we had observed in prior studies in fetal recipients [232]. Given the ease with which proteins secreted from cells within the intestine can enter the circulation, future studies aimed at improving the levels of engraftment within the intestine have the potential to greatly improve the systemic release of FVIII. In addition to the intestine and hemarthrotic joints, significant levels of engraftment were also seen within the thymus of this animal. While the ability of the transplanted MSC to traffic to the thymus could clearly have important implications for the likelihood of long-term correction with this approach to hemophilia A treatment, additional studies are required to determine with which cells within the thymus these MSC are interacting to ascertain the immunologic ramifications of thymic engraftment.

The marked phenotypic improvement and improvement in quality of life we have observed in our studies, to date, in the sheep model thus support the further development of therapeutic strategies for hemophilia A and, perhaps, other coagulation disorders, employing MSC as cellular vehicles to deliver the required transgene.

8. MSC as anti-cancer gene delivery vehicles

As alluded to earlier, a large number of preclinical animal studies examining the differentiative potential of MSC isolated from a variety of adult tissues have also highlighted another interesting and clinically valuable characteristic of MSC; their ability to selectively navigate to sites of injury and/or inflammation within the body [192, 193, 243-247]. Once reaching these specific sites, the MSC then mediate repair both by engrafting and generating tissue-specific cells within the injured tissue (but contributing very little if at all to other tissues that are functionally normal [188-190]), and by releasing trophic factors that blunt the inflammatory response and often promote healing by activating the tissue's own endogenous repair mechanisms. While the mechanisms responsible for this trafficking to sites of injury

are currently not well understood, this observation has raised the exciting prospect of using MSC to treat a wide array of diseases in which inflammation plays a key role such as stroke [87, 88, 92, 248-255], rheumatoid arthritis [256], asthma [257-259] and allergic rhinitis [260], and both acute and chronic lung injury [261].

Cancer represents another condition in which there is a selective need for new cells created by the forming tumor, and a chronic state of insult/inflammation within the surrounding tumor microenvironment. Studies over the last several years have now revealed that MSC have the ability to "sense" this need for cells and the perceived injury to the tissue surrounding the tumor. As a result, both endogenous bone marrow- and adipose-resident MSC, as well as intravenously infused MSC, all appear to have the ability to efficiently migrate to the forming tumor, and contribute to the newly forming tumor "stroma" [191, 262-266]. Clearly, this may not seem ideal, since the MSC could, in fact, provide support to the growing tumor, potentially worsening the prognosis. Indeed, unraveling the role played by MSC within the tumor microenvironment is currently an area of active research [191, 192, 262-265, 267-269]. Irrespective of their role in the tumor's health/biology, however, the ability of MSC to selectively traffic to and integrate into the tumor microenvironment can be viewed as a double-edged sword, since this ability has now been recognized to present a very powerful and unique means of selectively delivering anti-cancer gene products to tumor cells in vivo [270-274]. Four of the gene products which have thus far received the most attention are IL-2 [275, 276], IL-12 [277-284], IFN-β [270, 271, 285, 286], and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [287-298]. Unfortunately, the utility of these and many other biological agents that could be used for cancer therapy is often limited by both their short half-life in vivo and their pronounced toxicity due to effects on normal, non-malignant cells within the body. Using MSC to deliver these therapeutics promises to solve both of these problems, since the MSC can selectively migrate to the tumor site and release their therapeutic payload locally. This would be predicted to greatly increase the agent's concentration within the tumor and significantly lower its systemic toxicity. In addition, by genetically modifying the MSC with viral vectors, the engrafted MSC will steadily release the therapeutic agent, allowing a single administration to result in long-lasting effects. Other studies have now provided evidence that MSC have the ability to not only selectively home to solid tumors [270, 271, 287, 299], but also to actively seek out metastases at sites far removed from the primary site of the tumor [271, 288, 290, 299, 300]. This ability has recently been proven to be of great therapeutic value in the treatment of lung metastases arising from both breast cancer and melanoma in a murine xenograft model [271, 299]. Given the difficulty and frequent lack of success using traditional approaches such as surgery, radiotherapy, and chemotherapeutic agents to treat tumors which are either highly invasive or prone to metastasis, this property of MSC will likely prove to be of great clinical value in the near future.

One form of cancer for which the use of MSC is receiving a great deal of attention is glioblastoma multiforme (GBM). GBM represents the most common form of malignant glioma. Despite decades of research and many advances in the treatment of this disease with con-

ventional surgery, radiotherapy, and chemotherapy, there is no cure, and the current prognosis is abysmal, with a median survival of only 6-18 months. The failure of current therapies to cure this disease arises predominantly from the highly invasive nature of this cancer and the inability of these agents to effectively target tumor cells which have disseminated into the normal parenchyma of the brain, at sites distant from the main tumor mass. Given the ability of MSC to home to tumors and their ability to track to metastases throughout the body, gene-modified MSC are receiving a great deal of attention as a possible therapy for GBM. Studies have now shown that MSC migrate through the normal brain parenchyma towards gliomas and appear to possess the uncanny ability to track microscopic tumor deposits and individual tumor cells which have infiltrated the normal brain parenchyma [276, 282, 289, 290, 301-310]. While these migratory properties are certainly interesting, even more exciting are the dramatic therapeutic benefits these same studies have shown, with reduction in tumor size, and pronounced improvements in survival. It is important to note that, in most of these studies, MSC were used as the sole therapy, and definite benefits were observed. In the clinical setting, the current plan is to use gene-modified MSC as an adjunct after surgical resection. In this scenario, the vast majority of the tumor mass would be surgically removed, and the MSC would then be transplanted, in the hopes that they would then remove the residual malignant cells at the site of the tumor, and then hunt down and eliminate any invasive tumor cells that have migrated away from the site of the primary tumor. In this context, one would imagine that the therapeutic benefit of the MSC will likely be even more pronounced, since their anti-tumor effects could be focused only on the small number of residual tumor cells that evaded removal during surgery. The remarkable success seen in studies aimed at treating GBM, one of the most devastating forms of cancer, thus serve to highlight the tremendous potential MSC harbor as gene delivery vehicles for the treatment of many forms of cancer for which current therapeutic strategies are ineffective.

9. Conclusions

Numerous investigators around the globe have now provided compelling evidence that MSC from a variety of tissues possess a far broader differentiative capacity than anyone would have foreseen at the time Friedenstein originally described his bone marrow-derived stromal cells. Extrapolating the work thus far on using MSC to deliver FVIII to treat hemophilia A, and the rapidly growing number of studies showing the tremendous potential of MSC as anti-cancer gene delivery vehicles, and combining this with the relative ease with which MSC can be isolated, propagated in culture, and modified with a variety of viral-based vectors, and their intrinsic ability to seek out sites of injury/inflammation within the body, one can readily see why MSC are widely viewed as being ideally suited not only as cellular therapeutics, but as vehicles to deliver gene therapy vectors to numerous tissues in the body, thus promising to provide a permanent cure for a diverse range of diseases.

Author details

Christopher D. Porada and Graça Almeida-Porada

Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA

References

[1] Friedenstein, A.J., *Osteogenic stem cells in the bone marrow.* Bone and Mineral, 1991. 7: p. 243-72.

[2] Friedenstein, A.J., et al., *Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo.* Transplantation, 1974. 17(4): p. 331-40.

[3] Caplan, A.I., *Mesenchymal stem cells.* J Orthop Res, 1991. 9(5): p. 641-50.

[4] Galotto, M., et al., *Stromal damage as consequence of high-dose chemo/radiotherapy in bone marrow transplant recipients.* Exp Hematol, 1999. 27(9): p. 1460-6.

[5] Kassem, M., *Mesenchymal stem cells: biological characteristics and potential clinical applications.* Cloning Stem Cells, 2004. 6(4): p. 369-74.

[6] Luria, E.A., A.F. Panasyuk, and A.Y. Friedenstein, *Fibroblast colony formation from monolayer cultures of blood cells.* Transfusion, 1971. 11(6): p. 345-9.

[7] Pittenger, M.F., et al., *Multilineage potential of adult human mesenchymal stem cells.* Science, 1999. 284(5411): p. 143-7.

[8] Simmons, P.J. and B. Torok-Storb, *Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1.* Blood, 1991. 78(1): p. 55-62.

[9] Airey, J.A., et al., *Human mesenchymal stem cells form Purkinje fibers in fetal sheep heart.* Circulation, 2004. 109(11): p. 1401-7.

[10] Almeida-Porada, M.G., Porada, C., ElShabrawy, D., Simmons, P.J., Zanjani, E.D., *Human marrow stromal cells (MSC) represent a latent pool of stem cells capable of generating long-term hematopoietic cells..* Blood, 2001. 98((Part 1)): p. 713a.

[11] Chamberlain, J., et al., *Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep.* Hepatology, 2007. 46(6): p. 1935-45.

[12] Colletti, E., Zanjani, E. D., Porada, C. D., Almeida-Porada, M. G., *Tales from the Crypt: Mesenchymal Stem Cells for Replenishing the Intestinal Stem Cell Pool..* Blood 2008. 112:. p. Abstract 390.

[13] Colletti E.J., A.J.A., Zanjani E.D., Porada C.D., 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): p. 135a.

[14] Colletti, E.J., et al., *Generation of tissue-specific cells from MSC does not require fusion or donor-to-host mitochondrial/membrane transfer.* Stem Cell Res, 2009. 2(2): p. 125-38.

[15] Colletti, E.J., et al., *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): p. 926-33.

[16] Bruder, S.P., et al., *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): p. 655-63.

[17] Haynesworth, S.E., M.A. Baber, and A.I. Caplan, *Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies.* Bone, 1992. 13(1): p. 69-80.

[18] Mitchell, J.B., et al., *Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers.* Stem Cells, 2006. 24(2): p. 376-85.

[19] Almeida-Porada, G., et al., *Differentiative potential of human metanephric mesenchymal cells.* Exp Hematol, 2002. 30(12): p. 1454-62.

[20] Fan, C.G., et al., *Characterization and neural differentiation of fetal lung mesenchymal stem cells.* Cell Transplant, 2005. 14(5): p. 311-21.

[21] Gotherstrom, C., et al., *Difference in gene expression between human fetal liver and adult bone marrow mesenchymal stem cells.* Haematologica, 2005. 90(8): p. 1017-26.

[22] in 't Anker, P.S., et al., *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): p. 845-52.

[23] Lee, O.K., et al., *Isolation of multipotent mesenchymal stem cells from umbilical cord blood.* Blood, 2004. 103(5): p. 1669-75.

[24] Morizono, K., et al., *Multilineage cells from adipose tissue as gene delivery vehicles.* Hum Gene Ther, 2003. 14(1): p. 59-66.

[25] Zuk, P.A., et al., *Human adipose tissue is a source of multipotent stem cells.* Mol Biol Cell, 2002. 13(12): p. 4279-95.

[26] Zuk, P.A., et al., *Multilineage cells from human adipose tissue: implications for cell-based therapies.* Tissue Eng, 2001. 7(2): p. 211-28.

[27] Garol, N.J., Yamagami, T., Osborne C., Porada, C.D., Zanjani, E.D., Almeida-Porada, G., *Tissue-specific molecular signature may explain differentiative bias of human MSC from different tissues..* Blood, 2007. 110(11): p. 570a.

[28] Mazhari, S., Desai, J., Chamberlain, J., Porada, C., Zanjani, E.D., Almeida-Porada, G., *Proteomic Analysis Reveals Intrinsic Differences between Phenotypically Identical Mesenchymal Stem Cells.* Blood, 2005. 106(11).

[29] Mazhari, S.M., Porada, C.D., Chamberlain, J., Zanjani, E.D., Almeida-Porada, G., *Characterization of membrane proteins of mesenchymal stem cells from human liver* Experimental Hematology, 2006. 34(9, Suppl. 1): p. 80.

[30] Lee, R.H., et al., *Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue.* Cell Physiol Biochem, 2004. 14(4-6): p. 311-24.

[31] Kern, S., et al., *Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue.* Stem Cells, 2006. 24(5): p. 1294-301.

[32] Chamberlain, J., Frias, A., Porada, C., Zanjani, E. D., Almeida-Porada, G., *Neural Generation in vivo differs with route of administration and source of mesenchymal stem cells.* Experimental Hematology, 2005. 33(7): p. 47a.

[33] Almeida-Porada, M.G., Chamberlain, J., Frias, A., Porada, C.D., Zanjani, E.D., *Tissue of Origin Influences In Vivo Differentiative Potential of Mesenchymal Stem Cells..* Blood 2003. 102(11): p. abstract #1304.

[34] Bernardo, M.E., et al., *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): p. 9142-9.

[35] Bochkov, N.P., et al., *Chromosome variability of human multipotent mesenchymal stromal cells.* Bull Exp Biol Med, 2007. 143(1): p. 122-6.

[36] Buyanovskaya, O.A., et al., *Spontaneous aneuploidy and clone formation in adipose tissue stem cells during different periods of culturing.* Bull Exp Biol Med, 2009. 148(1): p. 109-12.

[37] Rubio, D., et al., *Human mesenchymal stem cell transformation is associated with a mesenchymal-epithelial transition.* Exp Cell Res, 2008. 314(4): p. 691-8.

[38] Rubio, D., et al., *Spontaneous human adult stem cell transformation.* Cancer Res, 2005. 65(8): p. 3035-9.

[39] Altanerova, V., et al., *Genotoxic damage of human adipose-tissue derived mesenchymal stem cells triggers their terminal differentiation.* Neoplasma, 2009. 56(6): p. 542-7.

[40] Crop, M., et al., *Potential of mesenchymal stem cells as immune therapy in solid-organ transplantation.* Transpl Int, 2009. 22(4): p. 365-76.

[41] Caplan, A.I. and J.E. Dennis, *Mesenchymal stem cells as trophic mediators.* J Cell Biochem, 2006. 98(5): p. 1076-84.

[42] Chen, T.S., et al., *Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs.* Nucleic Acids Res, 2010. 38(1): p. 215-24.

[43] Dai, W., S.L. Hale, and R.A. Kloner, *Role of a paracrine action of mesenchymal stem cells in the improvement of left ventricular function after coronary artery occlusion in rats.* Regen Med, 2007. 2(1): p. 63-8.

[44] Gnecchi, M., et al., *Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells.* Nat Med, 2005. 11(4): p. 367-8.

[45] Haynesworth, S.E., M.A. Baber, and A.I. Caplan, *Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha.* J Cell Physiol, 1996. 166(3): p. 585-92.

[46] Huang, N.F., et al., *Bone marrow-derived mesenchymal stem cells in fibrin augment angiogenesis in the chronically infarcted myocardium.* Regen Med, 2009. 4(4): p. 527-38.

[47] Kuo, T.K., et al., *Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells.* Gastroenterology, 2008. 134(7): p. 2111-21, 2121 e1-3.

[48] Ladage, D., et al., *Mesenchymal stem cells induce endothelial activation via paracine mechanisms.* Endothelium, 2007. 14(2): p. 53-63.

[49] Lai, R.C., et al., *Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury.* Stem Cell Res, 2010. 4(3): p. 214-22.

[50] Li, Z., et al., *Paracrine role for mesenchymal stem cells in acute myocardial infarction.* Biol Pharm Bull, 2009. 32(8): p. 1343-6.

[51] Mias, C., et al., *Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction.* Stem Cells, 2009. 27(11): p. 2734-43.

[52] Ohnishi, S., et al., *Mesenchymal stem cells attenuate cardiac fibroblast proliferation and collagen synthesis through paracrine actions.* FEBS Lett, 2007. 581(21): p. 3961-6.

[53] Parekkadan, B., et al., *Immunomodulation of activated hepatic stellate cells by mesenchymal stem cells.* Biochem Biophys Res Commun, 2007. 363(2): p. 247-52.

[54] Parekkadan, B., et al., *Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure.* PLoS One, 2007. 2(9): p. e941.

[55] Shabbir, A., et al., *Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen.* Am J Physiol Heart Circ Physiol, 2009. 296(6): p. H1888-97.

[56] Timmers, L., et al., *Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium.* Stem Cell Res, 2007. 1(2): p. 129-37.

[57] van Poll, D., et al., *Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo.* Hepatology, 2008. 47(5): p. 1634-43.

[58] Xiang, M.X., et al., *Protective paracrine effect of mesenchymal stem cells on cardiomyocytes.* J Zhejiang Univ Sci B, 2009. 10(8): p. 619-24.

[59] Yu, X.Y., et al., *The effects of mesenchymal stem cells on c-kit up-regulation and cell-cycle re-entry of neonatal cardiomyocytes are mediated by activation of insulin-like growth factor 1 receptor.* Mol Cell Biochem, 2009. 332(1-2): p. 25-32.

[60] Ozawa, K., et al., *Cell and gene therapy using mesenchymal stem cells (MSCs).* J Autoimmun, 2008. 30(3): p. 121-7.

[61] Reiser, J., et al., *Potential of mesenchymal stem cells in gene therapy approaches for inherited and acquired diseases.* Expert Opin Biol Ther, 2005. 5(12): p. 1571-84.

[62] Bosch, P., et al., *Efficient adenoviral-mediated gene delivery into porcine mesenchymal stem cells.* Mol Reprod Dev, 2006. 73(11): p. 1393-403.

[63] Bosch, P. and S.L. Stice, *Adenoviral transduction of mesenchymal stem cells.* Methods Mol Biol, 2007. 407: p. 265-74.

[64] Roelants, V., et al., *Comparison between adenoviral and retroviral vectors for the transduction of the thymidine kinase PET reporter gene in rat mesenchymal stem cells.* J Nucl Med, 2008. 49(11): p. 1836-44.

[65] Gnecchi, M. and L.G. Melo, *Bone marrow-derived mesenchymal stem cells: isolation, expansion, characterization, viral transduction, and production of conditioned medium.* Methods Mol Biol, 2009. 482: p. 281-94.

[66] Meyerrose, T.E., et al., *In vivo distribution of human adipose-derived mesenchymal stem cells in novel xenotransplantation models.* Stem Cells, 2007. 25(1): p. 220-7.

[67] Piccoli, C., et al., *Transformation by retroviral vectors of bone marrow-derived mesenchymal cells induces mitochondria-dependent cAMP-sensitive reactive oxygen species production.* Stem Cells, 2008. 26(11): p. 2843-54.

[68] Sales, V.L., et al., *Endothelial progenitor and mesenchymal stem cell-derived cells persist in tissue-engineered patch in vivo: application of green and red fluorescent protein-expressing retroviral vector.* Tissue Eng, 2007. 13(3): p. 525-35.

[69] Fan, L., et al., *Transplantation with survivin-engineered mesenchymal stem cells results in better prognosis in a rat model of myocardial infarction.* Eur J Heart Fail, 2009. 11(11): p. 1023-30.

[70] Meyerrose, T.E., et al., *Lentiviral-transduced human mesenchymal stem cells persistently express therapeutic levels of enzyme in a xenotransplantation model of human disease.* Stem Cells, 2008. 26(7): p. 1713-22.

[71] Wang, F., et al., *Transcriptional profiling of human mesenchymal stem cells transduced with reporter genes for imaging.* Physiol Genomics, 2009. 37(1): p. 23-34.

[72] Xiang, J., et al., *Mesenchymal stem cells as a gene therapy carrier for treatment of fibrosarcoma.* Cytotherapy, 2009. 11(5): p. 516-26.

[73] Zhang, X.Y., V.F. La Russa, and J. Reiser, *Transduction of bone-marrow-derived mesenchymal stem cells by using lentivirus vectors pseudotyped with modified RD114 envelope glycoproteins.* J Virol, 2004. 78(3): p. 1219-29.

[74] Zhang, X.Y., et al., *Lentiviral vectors for sustained transgene expression in human bone marrow-derived stromal cells.* Mol Ther, 2002. 5(5 Pt 1): p. 555-65.

[75] Kumar, S., et al., *Osteogenic differentiation of recombinant adeno-associated virus 2-transduced murine mesenchymal stem cells and development of an immunocompetent mouse model for ex vivo osteoporosis gene therapy.* Hum Gene Ther, 2004. 15(12): p. 1197-206.

[76] Stender, S., et al., *Adeno-associated viral vector transduction of human mesenchymal stem cells.* Eur Cell Mater, 2007. 13: p. 93-9; discussion 99.

[77] Deuse, T., et al., *Hepatocyte growth factor or vascular endothelial growth factor gene transfer maximizes mesenchymal stem cell-based myocardial salvage after acute myocardial infarction.* Circulation, 2009. 120(11 Suppl): p. S247-54.

[78] Guo, Y.H., et al., *Hepatocyte growth factor and granulocyte colony-stimulating factor form a combined neovasculogenic therapy for ischemic cardiomyopathy.* Cytotherapy, 2008. 10(8): p. 857-67.

[79] Liu, X.H., et al., *Therapeutic potential of angiogenin modified mesenchymal stem cells: angiogenin improves mesenchymal stem cells survival under hypoxia and enhances vasculogenesis in myocardial infarction.* Microvasc Res, 2008. 76(1): p. 23-30.

[80] Tang, J., et al., *Mesenchymal stem cells over-expressing SDF-1 promote angiogenesis and improve heart function in experimental myocardial infarction in rats.* Eur J Cardiothorac Surg, 2009. 36(4): p. 644-50.

[81] Wang, Y., et al., *Adenovirus-mediated hypoxia-inducible factor 1alpha double-mutant promotes differentiation of bone marrow stem cells to cardiomyocytes.* J Physiol Sci, 2009. 59(6): p. 413-20.

[82] Wang, Y., et al., *Bone marrow derived stromal cells modified by adenovirus-mediated HIF-1alpha double mutant protect cardiac myocytes against CoCl2-induced apoptosis.* Toxicol In Vitro, 2009. 23(6): p. 1069-75.

[83] Huang, J., et al., *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].

[84] Zhu, K., et al., *Novel vascular endothelial growth factor gene delivery system-manipulated mesenchymal stem cells repair infarcted myocardium.* Exp Biol Med (Maywood), 2012. 237(6): p. 678-87.

[85] Cho, Y.H., et al., *Enhancement of MSC adhesion and therapeutic efficiency in ischemic heart using lentivirus delivery with periostin.* Biomaterials, 2012. 33(5): p. 1376-85.

[86] Holladay, C.A., et al., *Recovery of cardiac function mediated by MSC and interleukin-10 plasmid functionalised scaffold.* Biomaterials, 2012. 33(5): p. 1303-14.

[87] Kurozumi, K., et al., *Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model.* Mol Ther, 2005. 11(1): p. 96-104.

[88] Kurozumi, K., et al., *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): p. 189-97.

[89] Lu, Z., et al., *Overexpression of CNTF in Mesenchymal Stem Cells reduces demyelination and induces clinical recovery in experimental autoimmune encephalomyelitis mice.* J Neuroimmunol, 2009. 206(1-2): p. 58-69.

[90] Lu, Z.Q., et al., *[Bone marrow stromal cells transfected with ciliary neurotrophic factor gene ameliorates the symptoms and inflammation in C57BL/6 mice with experimental allergic encephalomyelitis].* Nan Fang Yi Ke Da Xue Xue Bao, 2009. 29(12): p. 2355-61.

[91] Zhao, M.Z., et al., *Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF gene transfer with HSV-1 vector.* J Cereb Blood Flow Metab, 2006. 26(9): p. 1176-88.

[92] Nomura, T., et al., *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): p. 161-9.

[93] Glavaski-Joksimovic, A., et al., *Glial cell line-derived neurotrophic factor-secreting genetically modified human bone marrow-derived mesenchymal stem cells promote recovery in a rat model of Parkinson's disease.* J Neurosci Res, 2010. 88(12): p. 2669-81.

[94] Olson, S.D., et al., *Examination of mesenchymal stem cell-mediated RNAi transfer to Huntington's disease affected neuronal cells for reduction of huntingtin.* Mol Cell Neurosci, 2012. 49(3): p. 271-81.

[95] Aquino, J.B., et al., *Mesenchymal stem cells as therapeutic tools and gene carriers in liver fibrosis and hepatocellular carcinoma.* Gene Ther, 2010. March 12. [epub ahead of print].

[96] Ishikawa, T., et al., *Fibroblast growth factor 2 facilitates the differentiation of transplanted bone marrow cells into hepatocytes.* Cell Tissue Res, 2006. 323(2): p. 221-31.

[97] McGinley, L., et al., *Lentiviral vector mediated modification of mesenchymal stem cells & enhanced survival in an in vitro model of ischaemia.* Stem Cell Res Ther, 2011. 2(2): p. 12.

[98] Li, Y.L., et al., *[Effect of mesenchymal stem cells transfected with human vegf-165 gene carried by adenovirus on revascularization for hind limb ischemic necrosis in rat model].* Zhongguo Shi Yan Xue Ye Xue Za Zhi, 2011. 18(6): p. 1568-73.

[99] Hagiwara, M., et al., *Kallikrein-modified mesenchymal stem cell implantation provides enhanced protection against acute ischemic kidney injury by inhibiting apoptosis and inflammation.* Hum Gene Ther, 2008. 19(8): p. 807-19.

[100] Jiang, Y.B., et al., *[Effects of autologous mesenchymal stem cells transfected with heme oxygenase-1 gene transplantation on ischemic Swine hearts].* Zhonghua Xin Xue Guan Bing Za Zhi, 2009. 37(8): p. 692-5.

[101] Manning, E., et al., *Interleukin-10 delivery via mesenchymal stem cells: a novel gene therapy approach to prevent lung ischemia-reperfusion injury.* Hum Gene Ther, 2010. 21(6): p. 713-27.

[102] Cao, H., et al., *Mesenchymal stem cells derived from human umbilical cord ameliorate ischemia/reperfusion-induced acute renal failure in rats.* Biotechnol Lett, 2010. 32(5): p. 725-32.

[103] Huang, Z.Y., et al., *Infusion of mesenchymal stem cells overexpressing GDNF ameliorates renal function in nephrotoxic serum nephritis.* Cell Biochem Funct, 2012. 30(2): p. 139-44.

[104] Zhen-Qiang, F., et al., *Localized expression of human BMP-7 by BM-MSCs enhances renal repair in an in vivo model of ischemia-reperfusion injury.* Genes Cells, 2012. 17(1): p. 53-64.

[105] Yuan, L., et al., *VEGF-modified human embryonic mesenchymal stem cell implantation enhances protection against cisplatin-induced acute kidney injury.* Am J Physiol Renal Physiol, 2011. 300(1): p. F207-18.

[106] Van Damme, A., et al., *Bone marrow mesenchymal cells for haemophilia A gene therapy using retroviral vectors with modified long-terminal repeats.* Haemophilia, 2003. 9(1): p. 94-103.

[107] Chuah, M.K., et al., *Long-term persistence of human bone marrow stromal cells transduced with factor VIII-retroviral vectors and transient production of therapeutic levels of human factor VIII in nonmyeloablated immunodeficient mice.* Hum Gene Ther, 2000. 11(5): p. 729-38.

[108] Chuah, M.K., et al., *Bone marrow stromal cells as targets for gene therapy of hemophilia A.* Hum Gene Ther, 1998. 9(3): p. 353-65.

[109] Doering, C.B., *Retroviral modification of mesenchymal stem cells for gene therapy of hemophilia.* Methods Mol Biol, 2008. 433: p. 203-12.

[110] Porada, C.D., Sanada, C., Kuo, E., Colletti, E.J., Moot, R., Doering, C., Spencer, H.T., Almeida-Porada, G., *Phenotypic Correction of Hemophilia A by Postnatal Intraperitoneal Transplantation of FVIII-Expressing MSC.* Blood, 2010. 116(21): p. 249a.

[111] Porada, C.D., Sanada, C., Kuo, E., Colletti, E.J., Moot, R., Doering, C., Spencer, H.T., Almeida-Porada, G., *Phenotypic Correction of Hemophilia A by Postnatal Intraperitoneal Transplantation of FVIII-Expressing MSC..* Molecular Therapy, 2011. 19(Suppl 1): p. 873a.

[112] Porada, C.D., et al., *Phenotypic correction of hemophilia A in sheep by postnatal intraperitoneal transplantation of FVIII-expressing MSC.* Exp Hematol, 2011. 39(12): p. 1124-1135.

[113] Mannucci, P.M. and E.G. Tuddenham, *The hemophilias--from royal genes to gene therapy.* N Engl J Med, 2001. 344(23): p. 1773-9.

[114] Agaliotis, D., *Hemophilia, Overview*. 2006.

[115] High, K.A., *Gene transfer as an approach to treating hemophilia.* Semin Thromb Hemost, 2003. 29(1): p. 107-20.

[116] Kay, M.A. and K. High, *Gene therapy for the hemophilias.* Proc Natl Acad Sci U S A, 1999. 96(18): p. 9973-5.

[117] Kaveri, S.V., et al., *Factor VIII inhibitors: role of von Willebrand factor on the uptake of factor VIII by dendritic cells.* Haemophilia, 2007. 13 Suppl 5: p. 61-4.

[118] High, K.A., *Gene therapy for haemophilia: a long and winding road.* J Thromb Haemost, 2011. 9 Suppl 1: p. 2-11.

[119] Arruda, V.R., *Toward gene therapy for hemophilia A with novel adenoviral vectors: successes and limitations in canine models.* J Thromb Haemost, 2006. 4(6): p. 1215-7.

[120] Doering, C.B., et al., *Directed Engineering of a High-expression Chimeric Transgene as a Strategy for Gene Therapy of Hemophilia A.* Mol Ther, 2009.

[121] Doering, C.B., et al., *Hematopoietic stem cells encoding porcine factor VIII induce pro-coagulant activity in hemophilia A mice with pre-existing factor VIII immunity.* Mol Ther, 2007. 15(6): p. 1093-9.

[122] Ide, L.M., et al., *Hematopoietic stem-cell gene therapy of hemophilia A incorporating a porcine factor VIII transgene and nonmyeloablative conditioning regimens.* Blood, 2007. 110(8): p. 2855-63.

[123] Lipshutz, G.S., et al., *Short-term correction of factor VIII deficiency in a murine model of hemophilia A after delivery of adenovirus murine factor VIII in utero.* Proc Natl Acad Sci U S A, 1999. 96(23): p. 13324-9.

[124] Nichols, T.C., et al., *Protein replacement therapy and gene transfer in canine models of hemophilia A, hemophilia B, von willebrand disease, and factor VII deficiency.* ILAR J, 2009. 50(2): p. 144-67.

[125] Ponder, K.P., *Gene therapy for hemophilia.* Curr Opin Hematol, 2006. 13(5): p. 301-7.

[126] Shi, Q., et al., *Syngeneic transplantation of hematopoietic stem cells that are genetically modified to express factor VIII in platelets restores hemostasis to hemophilia A mice with preexisting FVIII immunity.* Blood, 2008. 112(7): p. 2713-21.

[127] Lee, K.D., et al., *In vitro hepatic differentiation of human mesenchymal stem cells.* Hepatology, 2004. 40(6): p. 1275-84.

[128] Sato, Y., et al., *Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion.* Blood, 2005. 106(2): p. 756-63.

[129] Schwartz, R.E., et al., *Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells.* J Clin Invest, 2002. 109(10): p. 1291-302.

[130] Almeida-Porada, G., C. Porada, and E.D. Zanjani, *Adult stem cell plasticity and methods of detection.* Rev Clin Exp Hematol, 2001. 5(1): p. 26-41.

[131] Almeida-Porada, G., C. Porada, and E.D. Zanjani, *Plasticity of human stem cells in the fetal sheep model of human stem cell transplantation.* Int J Hematol, 2004. 79(1): p. 1-6.

[132] Almeida-Porada, G. and E.D. Zanjani, *A large animal noninjury model for study of human stem cell plasticity.* Blood Cells Mol Dis, 2004. 32(1): p. 77-81.

[133] Aurich, H., et al., *Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo.* Gut, 2008.

[134] Aurich, I., et al., *Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers.* Gut, 2007. 56(3): p. 405-15.

[135] Banas, A., et al., *Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure.* J Gastroenterol Hepatol, 2008.

[136] Banas, A., et al., *IFATS collection: in vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury.* Stem Cells, 2008. 26(10): p. 2705-12.

[137] Banas, A., et al., *Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes.* Hepatology, 2007. 46(1): p. 219-28.

[138] di Bonzo, L.V., et al., *Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential.* Gut, 2008. 57(2): p. 223-31.

[139] Enns, G.M. and M.T. Millan, *Cell-based therapies for metabolic liver disease.* Mol Genet Metab, 2008. 95(1-2): p. 3-10.

[140] Fang, B., et al., *Systemic infusion of FLK1(+) mesenchymal stem cells ameliorate carbon tetrachloride-induced liver fibrosis in mice.* Transplantation, 2004. 78(1): p. 83-8.

[141] Higashiyama, R., et al., *Bone marrow-derived cells express matrix metalloproteinases and contribute to regression of liver fibrosis in mice.* Hepatology, 2007. 45(1): p. 213-22.

[142] Luk, J.M., et al., *Hepatic potential of bone marrow stromal cells: development of in vitro co-culture and intra-portal transplantation models.* J Immunol Methods, 2005. 305(1): p. 39-47.

[143] Lysy, P.A., et al., *Persistence of a chimerical phenotype after hepatocyte differentiation of human bone marrow mesenchymal stem cells.* Cell Prolif, 2008. 41(1): p. 36-58.

[144] Muraca, M., et al., *Liver repopulation with bone marrow derived cells improves the metabolic disorder in the Gunn rat.* Gut, 2007. 56(12): p. 1725-35.

[145] Oyagi, S., et al., *Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCl4-injured rats.* J Hepatol, 2006. 44(4): p. 742-8.

[146] Popp, F.C., et al., *Therapeutic potential of bone marrow stem cells for liver diseases.* Curr Stem Cell Res Ther, 2006. 1(3): p. 411-8.

[147] Sakaida, I., et al., *Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice.* Hepatology, 2004. 40(6): p. 1304-11.

[148] Sgodda, M., et al., *Hepatocyte differentiation of mesenchymal stem cells from rat peritoneal adipose tissue in vitro and in vivo.* Exp Cell Res, 2007. 313(13): p. 2875-86.

[149] Talens-Visconti, R., et al., *Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells.* World J Gastroenterol, 2006. 12(36): p. 5834-45.

[150] Theise, N.D. and D.S. Krause, *Bone marrow to liver: the blood of Prometheus.* Semin Cell Dev Biol, 2002. 13(6): p. 411-7.

[151] Zhao, D.C., et al., *Bone marrow-derived mesenchymal stem cells protect against experimental liver fibrosis in rats.* World J Gastroenterol, 2005. 11(22): p. 3431-40.

[152] Zheng, J.F. and L.J. Liang, *Intra-portal transplantation of bone marrow stromal cells ameliorates liver fibrosis in mice.* Hepatobiliary Pancreat Dis Int, 2008. 7(3): p. 264-70.

[153] Almeida-Porada G. ElShabrawy D, P.C., Ascensao JL,, Zanjani ED., *Clonally Derived MSCs Populations are able to Differentiate into Blood Liver and Skin Cells..* Blood, 2001. 98: p. abstract.

[154] Almeida-Porada G., P.C.D., Brouard N., Simmons P.J., Ascensao J.L., Zanjani E.D., *Generation of hematopoietic and hepatic cells by human bone marrow stromal cells in vivo..* Blood, 2000. 96(1): p. 570a.

[155] Almeida-Porada, M., et al., *Intra-Hepatic Injection of Clonally Derived Mesenchymal Stem Cell (MSC) Populations Results in the Successful and Efficient Generation of Liver Cells.* Blood, 2003. 102(11): p. abstract #1229.

[156] Chamberlain, J., Frias, A., Porada, C., Zanjani, E. D., Almeida-Porada, G., *Clonally derived mesenchymal stem cell (MSC) populations generate liver cells by intra-hepatic injection without the need for a hematopoietic intermediate..* Exp. Hematol., 2004. 32(7): p. 48.

[157] Porada, C.D. and G. Almeida-Porada, *Mesenchymal stem cells as therapeutics and vehicles for gene and drug delivery.* Adv Drug Deliv Rev, 2010.

[158] Porada CD, Z.E., Almeida-Porada G, *Adult mesenchymal stem cells: a pluripotent population with multiple applications..* Current Stem Cell Research and Therapy, 2006. 1(1): p. 231-238.

[159] Aiuti, A. and M.G. Roncarolo, *Ten years of gene therapy for primary immune deficiencies.* Hematology Am Soc Hematol Educ Program, 2009: p. 682-9.

[160] Nienhuis, A.W., C.E. Dunbar, and B.P. Sorrentino, *Genotoxicity of retroviral integration in hematopoietic cells.* Mol Ther, 2006. 13(6): p. 1031-49.

[161] Persons, D.A., *Lentiviral vector gene therapy: effective and safe?* Mol Ther, 2010. 18(5): p. 861-2.

[162] Pipe, S.W., et al., *Progress in the molecular biology of inherited bleeding disorders.* Haemophilia, 2008. 14 Suppl 3: p. 130-7.

[163] Bartholomew, A., et al., *Baboon mesenchymal stem cells can be genetically modified to secrete human erythropoietin in vivo.* Hum Gene Ther, 2001. 12(12): p. 1527-41.

[164] Devine, S.M., et al., *Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion.* Exp Hematol, 2001. 29(2): p. 244-55.

[165] Le Blanc, K. and O. Ringden, *Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation.* Biol Blood Marrow Transplant, 2005. 11(5): p. 321-34.

[166] Le Blanc, K., et al., *Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex.* Scand J Immunol, 2003. 57(1): p. 11-20.

[167] Puissant, B., et al., *Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells.* Br J Haematol, 2005. 129(1): p. 118-29.

[168] Batten, P., et al., *Human mesenchymal stem cells induce T cell anergy and downregulate T cell allo-responses via the TH2 pathway: relevance to tissue engineering human heart valves.* Tissue Eng, 2006. 12(8): p. 2263-73.

[169] Di Nicola, M., et al., *Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli.* Blood, 2002. 99(10): p. 3838-43.

[170] Groh, M.E., et al., *Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells.* Exp Hematol, 2005. 33(8): p. 928-34.

[171] Krampera, M., et al., *Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide.* Blood, 2003. 101(9): p. 3722-9.

[172] Jones, S., et al., *The antiproliferative effect of mesenchymal stem cells is a fundamental property shared by all stromal cells.* J Immunol, 2007. 179(5): p. 2824-31.

[173] Klyushnenkova, E., et al., *T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression.* J Biomed Sci, 2005. 12(1): p. 47-57.

[174] Tse, W.T., et al., *Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation.* Transplantation, 2003. 75(3): p. 389-97.

[175] Djouad, F., et al., *Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism.* Stem Cells, 2007. 25(8): p. 2025-32.

[176] Jiang, X.X., et al., *Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells.* Blood, 2005. 105(10): p. 4120-6.

[177] Nauta, A.J., et al., *Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells.* J Immunol, 2006. 177(4): p. 2080-7.

[178] Zhang, W., et al., *Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells.* Stem Cells Dev, 2004. 13(3): p. 263-71.

[179] Corcione, A., et al., *Human mesenchymal stem cells modulate B-cell functions.* Blood, 2006. 107(1): p. 367-72.

[180] Aggarwal, S. and M.F. Pittenger, *Human mesenchymal stem cells modulate allogeneic immune cell responses.* Blood, 2005. 105(4): p. 1815-22.

[181] Maccario, R., et al., *Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype.* Haematologica, 2005. 90(4): p. 516-25.

[182] Prevosto, C., et al., *Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction.* Haematologica, 2007. 92(7): p. 881-8.

[183] Sato, K., et al., *Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells.* Blood, 2007. 109(1): p. 228-34.

[184] Nasef, A., et al., *Identification of IL-10 and TGF-beta transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells.* Gene Expr, 2007. 13(4-5): p. 217-26.

[185] Nasef, A., et al., *Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G.* Transplantation, 2007. 84(2): p. 231-7.

[186] Hainz, U., B. Jurgens, and A. Heitger, *The role of indoleamine 2,3-dioxygenase in transplantation.* Transpl Int, 2007. 20(2): p. 118-27.

[187] Meisel, R., et al., *Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation.* Blood, 2004. 103(12): p. 4619-21.

[188] Jiang, W., et al., *Homing and differentiation of mesenchymal stem cells delivered intravenously to ischemic myocardium in vivo: a time-series study.* Pflugers Arch, 2006. 453(1): p. 43-52.

[189] Jiang, W., et al., *Intravenous transplantation of mesenchymal stem cells improves cardiac performance after acute myocardial ischemia in female rats.* Transpl Int, 2006. 19(7): p. 570-80.

[190] Jiang, W.H., et al., *Migration of intravenously grafted mesenchymal stem cells to injured heart in rats.* Sheng Li Xue Bao, 2005. 57(5): p. 566-72.

[191] Kidd, S., et al., *Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging.* Stem Cells, 2009. 27(10): p. 2614-23.

[192] Spaeth, E.L., S. Kidd, and F.C. Marini, *Tracking inflammation-induced mobilization of mesenchymal stem cells.* Methods Mol Biol, 2012. 904: p. 173-90.

[193] Spaeth, E.L. and F.C. Marini, *Dissecting mesenchymal stem cell movement: migration assays for tracing and deducing cell migration.* Methods Mol Biol, 2011. 750: p. 241-59.

[194] Turecek, P.L., et al., *Assessment of bleeding for the evaluation of therapeutic preparations in small animal models of antibody-induced hemophilia and von Willebrand disease.* Thromb Haemost, 1997. 77(3): p. 591-9.

[195] Hough, C., et al., *Aberrant splicing and premature termination of transcription of the FVIII gene as a cause of severe canine hemophilia A: similarities with the intron 22 inversion mutation in human hemophilia.* Thromb Haemost, 2002. 87(4): p. 659-65.

[196] Lozier, J.N., et al., *The Chapel Hill hemophilia A dog colony exhibits a factor VIII gene inversion.* Proc Natl Acad Sci U S A, 2002. 99(20): p. 12991-6.

[197] Bi, L., et al., *Targeted disruption of the mouse factor VIII gene produces a model of haemophilia A.* Nat Genet, 1995. 10(1): p. 119-21.

[198] Gallo-Penn, A.M., et al., *In vivo evaluation of an adenoviral vector encoding canine factor VIII: high-level, sustained expression in hemophiliac mice.* Hum Gene Ther, 1999. 10(11): p. 1791-802.

[199] Garcia-Martin, C., et al., *Therapeutic levels of human factor VIII in mice implanted with encapsulated cells: potential for gene therapy of haemophilia A.* J Gene Med, 2002. 4(2): p. 215-23.

[200] Moayeri, M., T.S. Hawley, and R.G. Hawley, *Correction of murine hemophilia A by hematopoietic stem cell gene therapy.* Mol Ther, 2005. 12(6): p. 1034-42.

[201] Moayeri, M., et al., *Sustained phenotypic correction of hemophilia a mice following oncoretroviral-mediated expression of a bioengineered human factor VIII gene in long-term hematopoietic repopulating cells.* Mol Ther, 2004. 10(5): p. 892-902.

[202] Reddy, P.S., et al., *Sustained human factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector.* Mol Ther, 2002. 5(1): p. 63-73.

[203] Sarkar, R., et al., *Total correction of hemophilia A mice with canine FVIII using an AAV 8 serotype.* Blood, 2004. 103(4): p. 1253-60.

[204] Ide, L.M., et al., *Functional aspects of factor VIII expression after transplantation of genetically-modified hematopoietic stem cells for hemophilia A.* J Gene Med, 2010. 12(4): p. 333-44.

[205] Gallo-Penn, A.M., et al., *Systemic delivery of an adenoviral vector encoding canine factor VIII results in short-term phenotypic correction, inhibitor development, and biphasic liver toxicity in hemophilia A dogs.* Blood, 2001. 97(1): p. 107-13.

[206] Scallan, C.D., et al., *Sustained phenotypic correction of canine hemophilia A using an adeno-associated viral vector.* Blood, 2003. 102(6): p. 2031-7.

[207] Nathwani, A.C., et al., *Adenovirus-associated virus vector-mediated gene transfer in hemophilia B.* N Engl J Med, 2011. 365(25): p. 2357-65.

[208] Neuenschwander, S., et al., *Inherited defect of blood clotting factor VIII (haemophilia A) in sheep.* Thromb Haemost, 1992. 68(5): p. 618-20.

[209] Backfisch, W., et al., *Carrier detection of ovine hemophilia A using an RFLP marker, and mapping of the factor VIII gene on the ovine X-chromosome.* J Hered, 1994. 85(6): p. 474-8.

[210] Neuenschwander, S. and V. Pliska, *Factor VIII in blood plasma of haemophilic sheep: analysis of clotting time-plasma dilution curves.* Haemostasis, 1994. 24(1): p. 27-35.

[211] Bormann C., L.C., Menges S., Hanna C., Foxworth G., Shin T., Westhusin M., Pliska V., Stranzinger G., Joerg H., Glimp H., Millsap L., Porada C., Almeida-Porada G., Kraemer D., *Reestablishment of an Extinct Strain of Sheep From a Limited Supply of Frozen Semen.* Reproduction, Fertility and Development 2005. 18(2): p. 201-202.

[212] Almeida-Porada, G., Desai, J., Long, C., Westhusin, M., Pliska, V., Stranzinger, G., Joerg, H., Thain, D., Glimp, H., Kraemer, D., Porada, C.D., *Re-establishment and characterization of an extinct line of sheep with a spontaneous bleeding disorder that closely recapitulates human hemophilia A.* Blood, 2007. 110(11): p. 347a.

[213] Sanada, C., Wood, J.A., Liu, W., Lozier, J.N., Almeida-Porada, G., Porada, C.D., *A Frame Shift-Induced Stop Codon Causes Hemophilia A in Sheep..* Blood, 2008. 112: p. Abstract #3378.

[214] Porada, C.D., et al., *Clinical and molecular characterization of a re-established line of sheep exhibiting hemophilia A.* J Thromb Haemost, 2010. 8(2): p. 276-85.

[215] Bormann, C., Long, C., Menges, S., Hanna, C., Foxworth, G., Westhusin, M., Pliska, V., Stranzinger, G., Glimp, H., Millsap, L., Porada, C., Almeida-Porada, G., Kraemer, D., *Reestablishment of an extinct strain of sheep utilizing assisted reproductive technologies..* Reproduction Fertility and Development 2007. 21(1): p. 153.

[216] Bormann C., L.C., Menges S., Hanna C., Foxworth G., Shin T., Westhusin M., Pliska V., Stranzinger G., Joerg H., Glimp H., Millsap L., Porada C., Almeida-Porada G., Kraemer D., *Reestablishment of an Extinct Strain of Sheep From a Limited Supply of Frozen Semen.* Reproduction, Fertility and Development 2006. 18(1): p. 201.

[217] Vidal, F., et al., *A novel mutation (2409delT) in exon 14 of the factor VIII gene causes severe haemophilia A.* Hum Hered, 2000. 50(4): p. 266-7.

[218] High, K., *Gene transfer for hemophilia: can therapeutic efficacy in large animals be safely translated to patients?* J Thromb Haemost, 2005. 3(8): p. 1682-91.

[219] Maddox, J.F., C.R. Mackay, and M.R. Brandon, *Ontogeny of ovine lymphocytes. I. An immunohistological study on the development of T lymphocytes in the sheep embryo and fetal thymus.* Immunology, 1987. 62(1): p. 97-105.

[220] Maddox, J.F., C.R. Mackay, and M.R. Brandon, *Ontogeny of ovine lymphocytes. III. An immunohistological study on the development of T lymphocytes in sheep fetal lymph nodes.* Immunology, 1987. 62(1): p. 113-8.

[221] Maddox, J.F., C.R. Mackay, and M.R. Brandon, *Ontogeny of ovine lymphocytes. II. An immunohistological study on the development of T lymphocytes in the sheep fetal spleen.* Immunology, 1987. 62(1): p. 107-12.

[222] Osburn, B.I., *The ontogeny of the ruminant immune system and its significance in the understanding of maternal-fetal-neonatal relationships.* Adv Exp Med Biol, 1981. 137: p. 91-103.

[223] Sawyer, M., J. Moe, and B.I. Osburn, *Ontogeny of immunity and leukocytes in the ovine fetus and elevation of immunoglobulins related to congenital infection.* Am J Vet Res, 1978. 39(4): p. 643-8.

[224] Silverstein, A.M., C.J. Parshall, Jr., and J.W. Uhr, *Immunologic maturation in utero: kinetics of the primary antibody response in the fetal lamb.* Science, 1966. 154(757): p. 1675-7.

[225] Tuboly, S., R. Glavits, and M. Bucsek, *Stages in the development of the ovine immune system.* Zentralbl Veterinarmed B, 1984. 31(2): p. 81-95.

[226] McCarroll, D.R., et al., *Canine platelet von Willebrand factor: quantification and multimeric analysis.* Exp Hematol, 1988. 16(11): p. 929-37.

[227] Parker, M.T., M.A. Turrentine, and G.S. Johnson, *von Willebrand factor in lysates of washed canine platelets.* Am J Vet Res, 1991. 52(1): p. 119-25.

[228] Montgomery, R.R. and Q. Shi, *Platelet and endothelial expression of clotting factors for the treatment of hemophilia.* Thromb Res, 2012.

[229] Shi, Q. and R.R. Montgomery, *Platelets as delivery systems for disease treatments.* Adv Drug Deliv Rev, 2010. 62(12): p. 1196-203.

[230] Shi, Q., et al., *Factor VIII ectopically targeted to platelets is therapeutic in hemophilia A with high-titer inhibitory antibodies.* J Clin Invest, 2006. 116(7): p. 1974-82.

[231] Almeida-Porada, G., E.D. Zanjani, and C.D. Porada, *Bone marrow stem cells and liver regeneration.* Exp Hematol, 2010. 38(7): p. 574-80.

[232] Colletti, E., Zanjani, E. D., Porada, C. D., Almeida-Porada, M. G., *Tales from the Crypt: Mesenchymal Stem Cells for Replenishing the Intestinal Stem Cell Pool.* Blood 2008. 112: p. abstract #390.

[233] Colletti, E., Airey, J.A., Liu, W., Simmons, P.J., Zanjani, E.D., Porada, C.D., Almeida-Porada, G., *Generation of tissue-specific cells by MSC does not require fusion or donor to host mitochondrial/membrane transfer.* Stem Cell Research, 2009. In Press.

[234] Yamagami, T., Colletti E, Wiendl, H, Zanjani, E, Porada, C, and Almeida-Porada, G, *Expression of Molecules Involved in Fetal-Maternal Tolerance Allows Human Mesenchymal*

Stem Cells to Engraft at High Levels across Immunologic Barriers Blood, 2008. 112(11): p. 3480.

[235] Fischer, U.M., et al., *Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect.* Stem Cells Dev, 2009. 18(5): p. 683-92.

[236] Schrepfer, S., et al., *Stem cell transplantation: the lung barrier.* Transplant Proc, 2007. 39(2): p. 573-6.

[237] Gangadharan, B., et al., *High-level expression of porcine factor VIII from genetically modified bone marrow-derived stem cells.* Blood, 2006. 107(10): p. 3859-64.

[238] Barrow, R.T., et al., *Reduction of the antigenicity of factor VIII toward complex inhibitory antibody plasmas using multiply-substituted hybrid human/porcine factor VIII molecules.* Blood, 2000. 95(2): p. 564-8.

[239] Garvey, M.B., *Porcine factor VIII in the treatment of high-titre inhibitor patients.* Haemophilia, 2002. 8 Suppl 1: p. 5-8; discussion 28-32.

[240] Hermans, C., et al., *Single-dose pharmacokinetics of porcine factor VIII (Hyate C).* Haemophilia, 2002. 8 Suppl 1: p. 33-8.

[241] Kulkarni, R., et al., *Therapeutic choices for patients with hemophilia and high-titer inhibitors.* Am J Hematol, 2001. 67(4): p. 240-6.

[242] Sun, J., et al., *Intraarticular factor IX protein or gene replacement protects against development of hemophilic synovitis in the absence of circulating factor IX.* Blood, 2008. 112(12): p. 4532-41.

[243] Auletta, J.J., R.J. Deans, and A.M. Bartholomew, *Emerging roles for multipotent, bone marrow-derived stromal cells in host defense.* Blood, 2012. 119(8): p. 1801-9.

[244] Nurmenniemi, S., et al., *Toll-like receptor 9 ligands enhance mesenchymal stem cell invasion and expression of matrix metalloprotease-13.* Exp Cell Res, 2010. 316(16): p. 2676-82.

[245] Qiu, Y., L.A. Marquez-Curtis, and A. Janowska-Wieczorek, *Mesenchymal stromal cells derived from umbilical cord blood migrate in response to complement C1q.* Cytotherapy, 2012. 14(3): p. 285-95.

[246] Raheja, L.F., et al., *Hypoxic regulation of mesenchymal stem cell migration: the role of RhoA and HIF-1alpha.* Cell Biol Int, 2011. 35(10): p. 981-9.

[247] Teo, G.S., et al., *Mesenchymal Stem Cells Transmigrate Between and Directly Through TNF-alpha-activated Endothelial Cells.* Stem Cells, 2012.

[248] Honma, T., et al., *Intravenous infusion of immortalized human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat.* Exp Neurol, 2006. 199(1): p. 56-66.

[249] Kang, S.K., et al., *Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats.* Exp Neurol, 2003. 183(2): p. 355-66.

[250] Chen, J., et al., *Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat.* J Neurosci Res, 2003. 73(6): p. 778-86.

[251] Chen, J., et al., *Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats.* J Neurol Sci, 2001. 189(1-2): p. 49-57.

[252] Chen, J., et al., *Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats.* Circ Res, 2003. 92(6): p. 692-9.

[253] Iihoshi, S., et al., *A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats.* Brain Res, 2004. 1007(1-2): p. 1-9.

[254] Li, Y., et al., *Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery.* Neurology, 2002. 59(4): p. 514-23.

[255] Zheng, W., et al., *Therapeutic benefits of human mesenchymal stem cells derived from bone marrow after global cerebral ischemia.* Brain Res, 2010. 1310: p. 8-16.

[256] Augello, A., et al., *Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis.* Arthritis Rheum, 2007. 56(4): p. 1175-86.

[257] Park, H.K., et al., *Adipose-derived stromal cells inhibit allergic airway inflammation in mice.* Stem Cells Dev, 2010. March 17. [epub ahead of print].

[258] Weiss, D.J., et al., *Stem cells and cell therapies in lung biology and lung diseases.* Proc Am Thorac Soc, 2008. 5(5): p. 637-67.

[259] Nemeth, K., et al., *Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma.* Proc Natl Acad Sci U S A, 2010. 107(12): p. 5652-7.

[260] Cho, K.S., et al., *IFATS collection: Immunomodulatory effects of adipose tissue-derived stem cells in an allergic rhinitis mouse model.* Stem Cells, 2009. 27(1): p. 259-65.

[261] Iyer, S.S., C. Co, and M. Rojas, *Mesenchymal stem cells and inflammatory lung diseases.* Panminerva Med, 2009. 51(1): p. 5-16.

[262] Kidd, S., et al., *Origins of the tumor microenvironment: quantitative assessment of adipose-derived and bone marrow-derived stroma.* PLoS One, 2012. 7(2): p. e30563.

[263] Klopp, A.H., et al., *Concise review: Dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth?* Stem Cells, 2011. 29(1): p. 11-9.

[264] Marini, F.C., *The complex love-hate relationship between mesenchymal stromal cells and tumors.* Cytotherapy, 2009. 11(4): p. 375-6.

[265] Martin, F.T., et al., *Potential role of mesenchymal stem cells (MSCs) in the breast tumour microenvironment: stimulation of epithelial to mesenchymal transition (EMT).* Breast Cancer Res Treat, 2010. 124(2): p. 317-26.

[266] Spaeth, E., et al., *Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells.* Gene Ther, 2008. 15(10): p. 730-8.

[267] Subramanian, A., et al., *Human umbilical cord Wharton's jelly mesenchymal stem cells do not transform to tumor-associated fibroblasts in the presence of breast and ovarian cancer cells unlike bone marrow mesenchymal stem cells.* J Cell Biochem, 2012. 113(6): p. 1886-95.

[268] Wong, R.S., *Mesenchymal stem cells: angels or demons?* J Biomed Biotechnol, 2011. 2011: p. 459510.

[269] Bergfeld, S.A. and Y.A. DeClerck, *Bone marrow-derived mesenchymal stem cells and the tumor microenvironment.* Cancer Metastasis Rev, 2010. 29(2): p. 249-61.

[270] Studeny, M., et al., *Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors.* Cancer Res, 2002. 62(13): p. 3603-8.

[271] Studeny, M., et al., *Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents.* J Natl Cancer Inst, 2004. 96(21): p. 1593-603.

[272] Hall, B., et al., *Mesenchymal stem cells in cancer: tumor-associated fibroblasts and cell-based delivery vehicles.* Int J Hematol, 2007. 86(1): p. 8-16.

[273] Hall, B., M. Andreeff, and F. Marini, *The participation of mesenchymal stem cells in tumor stroma formation and their application as targeted-gene delivery vehicles.* Handb Exp Pharmacol, 2007(180): p. 263-83.

[274] Shah, K., *Mesenchymal stem cells engineered for cancer therapy.* Adv Drug Deliv Rev, 2012. 64(8): p. 739-48.

[275] Aboody, K.S., J. Najbauer, and M.K. Danks, *Stem and progenitor cell-mediated tumor selective gene therapy.* Gene Ther, 2008. 15(10): p. 739-52.

[276] Nakamura, K., et al., *Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model.* Gene Ther, 2004. 11(14): p. 1155-64.

[277] Chen, X.C., et al., *Prophylaxis against carcinogenesis in three kinds of unestablished tumor models via IL12-gene-engineered MSCs.* Carcinogenesis, 2006. 27(12): p. 2434-41.

[278] Eliopoulos, N., et al., *Neo-organoid of marrow mesenchymal stromal cells secreting interleukin-12 for breast cancer therapy.* Cancer Res, 2008. 68(12): p. 4810-8.

[279] Elzaouk, L., K. Moelling, and J. Pavlovic, *Anti-tumor activity of mesenchymal stem cells producing IL-12 in a mouse melanoma model.* Exp Dermatol, 2006. 15(11): p. 865-74.

[280] Gao, P., et al., *Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma.* Cancer Lett, 2010. 290(2): p. 157-66.

[281] Hong, X., et al., *Antitumor treatment using interleukin- 12-secreting marrow stromal cells in an invasive glioma model.* Neurosurgery, 2009. 64(6): p. 1139-46; discussion 1146-7.

[282] Ryu, C.H., et al., *Gene therapy of intracranial glioma using interleukin 12-secreting human umbilical cord blood-derived mesenchymal stem cells.* Hum Gene Ther, 2011. 22(6): p. 733-43.

[283] Seo, S.H., et al., *The effects of mesenchymal stem cells injected via different routes on modified IL-12-mediated antitumor activity.* Gene Ther, 2011. 18(5): p. 488-95.

[284] Zhao, W.H., et al., *[Human umbilical cord mesenchymal stem cells with adenovirus-mediated interleukin 12 gene transduction inhibits the growth of ovarian carcinoma cells both in vitro and in vivo].* Nan Fang Yi Ke Da Xue Xue Bao, 2011. 31(5): p. 903-7.

[285] Kidd, S., et al., *Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors in vivo, an effect countered by anti-inflammatory treatment.* Cytotherapy, 2010. 12(5): p. 615-25.

[286] Ling, X., et al., *Mesenchymal Stem Cells Overexpressing IFN-beta Inhibit Breast Cancer Growth and Metastases through Stat3 Signaling in a Syngeneic Tumor Model.* Cancer Microenviron, 2010. 3(1): p. 83-95.

[287] Grisendi, G., et al., *Adipose-Derived Mesenchymal Stem Cells as Stable Source of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Delivery for Cancer Therapy.* Cancer Res, 2010. Apr 13. [Epub ahead of print].

[288] Loebinger, M.R., et al., *Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer.* Cancer Res, 2009. 69(10): p. 4134-42.

[289] Menon, L.G., et al., *Human bone marrow-derived mesenchymal stromal cells expressing S-TRAIL as a cellular delivery vehicle for human glioma therapy.* Stem Cells, 2009. 27(9): p. 2320-30.

[290] Sasportas, L.S., et al., *Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy.* Proc Natl Acad Sci U S A, 2009. 106(12): p. 4822-7.

[291] Ciavarella, S., et al., *In vitro anti-myeloma activity of TRAIL-expressing adipose-derived mesenchymal stem cells.* Br J Haematol, 2012. 157(5): p. 586-98.

[292] Grisendi, G., et al., *Adipose-derived mesenchymal stem cells as stable source of tumor necrosis factor-related apoptosis-inducing ligand delivery for cancer therapy.* Cancer Res, 2010. 70(9): p. 3718-29.

[293] Kim, S.M., et al., *Irradiation enhances the tumor tropism and therapeutic potential of tumor necrosis factor-related apoptosis-inducing ligand-secreting human umbilical cord blood-derived mesenchymal stem cells in glioma therapy.* Stem Cells, 2010. 28(12): p. 2217-28.

[294] Luetzkendorf, J., et al., *Growth inhibition of colorectal carcinoma by lentiviral TRAIL-transgenic human mesenchymal stem cells requires their substantial intratumoral presence.* J Cell Mol Med, 2010. 14(9): p. 2292-304.

[295] Moniri, M.R., et al., *TRAIL-engineered pancreas-derived mesenchymal stem cells: characterization and cytotoxic effects on pancreatic cancer cells.* Cancer Gene Ther, 2012. 19(9): p. 652-8.

[296] Mueller, L.P., et al., *TRAIL-transduced multipotent mesenchymal stromal cells (TRAIL-MSC) overcome TRAIL resistance in selected CRC cell lines in vitro and in vivo.* Cancer Gene Ther, 2011. 18(4): p. 229-39.

[297] Sun, X.Y., et al., *MSC(TRAIL)-mediated HepG2 cell death in direct and indirect co-cultures.* Anticancer Res, 2011. 31(11): p. 3705-12.

[298] Yang, B., et al., *Dual-targeted antitumor effects against brainstem glioma by intravenous delivery of tumor necrosis factor-related, apoptosis-inducing, ligand-engineered human mesenchymal stem cells.* Neurosurgery, 2009. 65(3): p. 610-24; discussion 624.

[299] Chen, X., et al., *A tumor-selective biotherapy with prolonged impact on established metastases based on cytokine gene-engineered MSCs.* Mol Ther, 2008. 16(4): p. 749-56.

[300] Hamada, H., et al., *Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy.* Cancer Sci, 2005. 96(3): p. 149-56.

[301] Nakamizo, A., et al., *Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas.* Cancer Res, 2005. 65(8): p. 3307-18.

[302] Ahmed, A.U., et al., *A comparative study of neural and mesenchymal stem cell-based carriers for oncolytic adenovirus in a model of malignant glioma.* Mol Pharm, 2011. 8(5): p. 1559-72.

[303] Doucette, T., et al., *Mesenchymal stem cells display tumor-specific tropism in an RCAS/Ntv-a glioma model.* Neoplasia, 2011. 13(8): p. 716-25.

[304] Fei, S., et al., *The antitumor effect of mesenchymal stem cells transduced with a lentiviral vector expressing cytosine deaminase in a rat glioma model.* J Cancer Res Clin Oncol, 2012. 138(2): p. 347-57.

[305] Choi, S.A., et al., *Human adipose tissue-derived mesenchymal stem cells: characteristics and therapeutic potential as cellular vehicles for prodrug gene therapy against brainstem gliomas.* Eur J Cancer, 2012. 48(1): p. 129-37.

[306] Kim, S.M., et al., *CXC chemokine receptor 1 enhances the ability of human umbilical cord blood-derived mesenchymal stem cells to migrate toward gliomas.* Biochem Biophys Res Commun, 2012. 407(4): p. 741-6.

[307] Kosaka, H., et al., *Therapeutic effect of suicide gene-transferred mesenchymal stem cells in a rat model of glioma.* Cancer Gene Ther, 2012. 19(8): p. 572-8.

[308] Park, S.A., et al., *CXCR4-transfected human umbilical cord blood-derived mesenchymal stem cells exhibit enhanced migratory capacity toward gliomas.* Int J Oncol, 2011. 38(1): p. 97-103.

[309] Wang, Q., et al., *Mesenchymal stem cells over-expressing PEDF decreased the angiogenesis of gliomas.* Biosci Rep, 2012.

[310] Yin, J., et al., *hMSC-mediated concurrent delivery of endostatin and carboxylesterase to mouse xenografts suppresses glioma initiation and recurrence.* Mol Ther, 2011. 19(6): p. 1161-9.

IntechOpen

IntechOpen