Chapter 15
Genetically Engineered Mesenchymal Stem Cells for Cell and Gene Therapy

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Abstract Stem cells have enormous potential for regenerative medicine to treat fatal diseases and injuries that cannot otherwise be healed. In particular, adult stem cell-based therapies have been studied for several decades. Mesenchymal stem cells/marrow stromal cells (MSCs) have shown safety and therapeutic efficacy in preclinical models of various diseases such as cardiovascular disease, cancer, bone defects, renal failure, and neurodegenerative disorders. In spite of the great potential, several factors including low survival rate, low efficiency of MSC homing to injured sites, and poor levels of engraftment and retention have been major

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technical challenges to be overcome before MSC-based therapy can be applied to clinical applications in a consistently therapeutic manner. Genetically modified MSCs can be one option to overcome some of these problems and to deliver therapeutic agents. MSCs are powerful delivery vehicles and potent protein synthesis factories, and therefore the use of gene-modified MSCs to provide growth factors and other signals to improve the repair of damaged or diseased tissues holds much promise. Here we review the basic biology of human MSCs and the current status of preclinical and clinical trials using genetically engineered MSCs.

Keywords Mesenchymal stem cells • Gene therapy • Cellular therapy • Preclinical models • Growth factor production • Revascularization • Cancer • Renal disease • Bone disease • Neurodegenerative disorders • Clinical trials • Regenerative medicine

15.1 Human Diseases and Stem Cells

15.1.1 Degenerative Diseases

The human body is made up of millions of cells. When cells are injured, we may experience disease or disability. Those with type I diabetes have damaged or decreased numbers of islet cells, which are unable to produce sufficient amounts of insulin. Such individuals must substitute what is missing by taking frequent, daily insulin injections. Those with spinal cord injuries have damaged nerve cells which are no longer able to conduct messages from limbs to the brain and back and as a result have lost the ability to move some part of their body. Some organs like the liver and skin are excellent at repairing and regenerating themselves, while other organs or tissues have far less capacity to do so.

Islet or whole organ transplantation is one of the methods to cure these types of diseases but immune rejection is a major problem and the patients must often remain immunosuppressed, increasing risk of infections and cancers. Organ transplantation is also hampered by severe donor shortages. In spinal cord injury, transplantation is not even an option to consider. One promising new way to treat some diseases and disabilities is to regenerate injured or missing cells with stem cells, either by replacing those that have been damaged in the tissue or organ (e.g., pluripotent cell-derived differentiated cell types), or to provide cells that deliver factors that can encourage endogenous recovery (e.g., mesenchymal stem cells).

15.1.2 Pluripotent Stem Cells and Their Therapeutic Potential

Both embryonic stem cells (ESCs) and adult stem cells (e.g., hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs)) are under examination in clinical trials of cell therapy. Bone marrow (BM) transplantation, which has provided great success in transplantation for more than 50 years, has shown a great therapeutic benefit for the blood-forming system and this may extend to promoting healing of other tissues [1].

Recently, researchers' attention has been focused on human (h) ESCs, which theoretically have the potential to differentiate into all types of adult human tissues (pluripotency) and can grow indefinitely (self-renewal) [2]. Since their initial derivation, hESCs have become a promising tool for developmental biology and regenerative medicine. However, concerns related to ethical objections regarding the use of human embryos for hESC derivation have dramatically restricted research using these cells and therefore have set back the development of hESCs for clinical trials, although recent first-in-human phase I clinical trials from the company Geron were initiated in 2011 [3]. Later, Geron announced that they pulled out their entire program due to the financial reasons, but to date, safety has been demonstrated [4].

Due to their allogeneic nature, immune rejection of transplanted cells or tissues derived from hESCs is another potential drawback to therapeutic applications. The immune system of the patient recognizes transplanted "foreign" cells or tissues and escalates a rapid response, attacking the graft. This attack can result in the loss of the graft, which can lead to the death of patients if it was an organ on which the patient was reliant (e.g., heart). Immunosuppressive drug regimens, similar to those used for current human tissue and organ transplant procedures, might lessen the severity of the anticipated immune rejection, but at the same time may put the tissue recipient at an increased risk for infections. This risk can be lessened by application of human leukocyte antigen (HLA)-matched tissue, as is currently being practiced in organ transplantation, or could be completely eliminated by the use of the patient's own tissue. The latter possibility might, in the future, be achieved by reprogramming the patient's own somatic cells to induced pluripotent stem cells (iPSCs) [5].

Takahashi and Yamanaka pioneered methods to generate iPSCs by virally transducing four transcription factors into human somatic cells and showed that the resultant iPSCs have similar characteristics to ESCs [5]. Recent reports, however, have indicated that iPSCs are not exactly the same as ESCs and may be more prone to genetic mutations during the reprogramming and expansion phases [6]. Also, abnormal gene expression in some differentiated cells from iPSCs can induce T-cell-dependent immune responses in autologous transplantations done in mice [7]. Differentiated progenies from iPSCs and hESCs still have an immature status similar to early human fetal tissues (<6 weeks), and it is too early to tell whether it will be appropriate to apply these cells for transplantation therapies in humans [8].

15.1.3 Mesenchymal Stem Cells and Clinical Trials

Over the last half century, adult stem cell therapies in the form of bone marrow, mobilized peripheral blood, and umbilical cord blood transplantations have rescued thousands of patients from induced or genetic disorders [9]. After the first human hematopoietic stem cell transplantation in 1956, the technique gradually evolved to become a standard clinical procedure (reviewed in [10]). Mesenchymal stem cells (MSCs) were first described as adherent "marrow stromal cells" and were studied for their role in supporting hematopoiesis and were then engineered to provide

factors for other cells (reviewed in [11]). Later, these cells were found to differentiate into cartilage, bone, fat, tendon, and fibroblasts [9]. Over the past three decades of study, MSCs have become a tool for regenerative applications either through direct differentiation into specific tissues (e.g., bone), or indirectly through protein or cytokine secretion and immune suppression [9, 11–14]. MSCs have become a promising cell-based therapeutic because they are easily accessible from various tissues (e.g., bone marrow, fat and umbilical cord tissue) and are easily grown in culture. MSCs can be expanded in vitro to a clinical scale and can be cryopreserved without the loss of their integrity.

MSCs have demonstrated systemic migration after intravenous injection, in particular to areas of hypoxia or tissue damage [15]. The systemic administration of allogeneic MSCs has not been observed to cause any adverse effects in numerous treated patients, in part due to immunomodulatory effects [16, 17]. Also, MSCs have been considered safe as they do not show tumor formation after transplantation [18] and have been widely tested and shown efficacy in preclinical and clinical studies for cardiovascular, neurodegenerative, graft-versus-host (GvHD), and autoimmune diseases [9, 11]. Due to the MSC's osteoblast differentiation potential, Caplan and colleagues applied allogeneic MSCs to osteogenesis imperfecta patients [19]. In addition, LeBlanc et al. investigated the immunomodulatory effects of transplanted MSC for steroid-resistant GvHD [16], and similar methods were applied to other diseases [9]. These early studies have established a good clinical record of safety for systemic MSC administration.

15.2 Genetic Modification of Mesenchymal Stem Cells

Even though there have been remarkable advances in demonstrating safety in MSC clinical trials, therapeutic efficacy is still debated [9]. Unsolved problems such as low cell survival and engraftment efficiency after MSC transplantation still remain to be resolved [13, 14]. Genetic engineering of MSCs is a potential means to improve their therapeutic potential. MSCs can be modified to express therapeutic agents, to improve cell survival or to possess an enhanced ability to home to a disease site. In the following section, we will briefly discuss the pros and cons of several gene modification methods.

15.2.1 Choice of Vector Systems

Genetic modification of MSCs can be achieved by permanent integration or episomal expression of target genes via viral vector transduction or by transient expression of specific genes using nonviral delivery [20]. Viral delivery of desired genes to MSCs is one of the most utilized methods in preclinical studies due to the ability to achieve high infectivity with broad tropism. However, the transduction efficiency

Table 15.1 Summary of viral vectors

Characteristics	Retroviral vector	Lentiviral vector	Adenoviral vector	AAV vector
Cloning capacity (kb)	8	9	8-10	4.9
Chromosomal integration	Yes	Yes	No	No (yes if rep is included)
Transgene activity	Long-term	Long-term	Weeks	>1 year
Infect nondividing cells	No	Yes	Yes	Yes
Oncolytic activity	No	No	Yes	No

Abbreviation: *kb* kilobase

depends on the target cells (Table 15.1). Clonal analysis of transduced MSCs has shown that these cells often contain several thousand copies of transgene RNA per cell and can maintain transgene expression for 6 months or longer [11, 21–23]. In MSC studies, retroviral, lentiviral, adenoviral, and adeno-associated virus (AAV) vectors are generally used [20]. Adenoviral and AAV vectors do not integrate into the host genome but can express transgenes in an episomal manner. In non-dividing cells, these viral particles can sustain long-term transgene expression but would be diminished as cells proliferate in dividing cells due to the dilution of viruses. However, it is well documented that the capsids of adenoviral vector can be recognized by the patient's innate immune system and can cause adverse events [24]. Even though success has been shown with the delivery of factor IX with AAV into the hepatocytes of human hemophilia patients, transduced hepatocytes were cleared due to an immune reaction to capsids from the AAV [25]. To achieve short-term transgene expression in MSCs for applications such as angiogenic growth factor expression after myocardial infarction or surface antigen modification for increase cell survival, it may be beneficial to choose AAV vectors.

MoMuLV-based retroviral and HIV-based lentiviral vectors can offer long-term expression of transgenes in target cells due to their permanent integration into the host genome. While lentiviral vectors can transduce both dividing and non-dividing cells, retroviral vectors can only transduce dividing cells (Table 15.1). In terms of chromosomal integration sites, there are differences in the integration hot spots between these two viral vectors, but most integration is into the active regions of chromosomes. Retroviral vectors tend to favor integration into transcriptional start sites, promoter regions, or CpG islands, while lentiviral vectors do not appear to have preferential integration regions [26, 27]. Numerous papers, including work from our own lab, have demonstrated that MSCs can be transduced with retroviral or lentiviral vectors and can retain transgene expression for many passages. In addition, transduced cells retain in vitro lineage-specific differentiation and in vivo engraftment, with no detectable complications caused by viral integration [11, 18, 22, 23, 28–31].

Although a promising method, nonviral gene transfer must overcome low efficiency (discussed in [32]). To test gene delivery methods, McMahon et al. tested GFP expression via adenoviral, AAV, lentiviral, plasmid transfection, and electroporation in rat MSCs [33]. Lentiviral delivery showed the highest GFP expression with minimal cell death. Adenoviral vectors provided effective GFP expression but with

a reduced transduction efficiency and an increased cell toxicity compared to lentiviral vectors. In that study, AAV vectors could not effectively deliver transgenes into rat MSCs, although another study showed that AAV vectors could infect human MSCs [34]. To improve the transduction efficiency for AAV vectors, specific serotypes of AAV must be chosen as only serotype 2 AAV vectors were shown to have a high MSC transduction efficiency [35]. Transfection and electroporation of plasmids were not as effective as viral delivery of GFP and were harmful to MSCs due to the associated cytotoxicity. Furthermore, transfected plasmids can randomly integrate as concatamers into host chromosomes at a frequency of 1/3000 to 1/5000 [36].

15.2.2 Safety Considerations for Genetic Modifications

Even though long-term gene expression can be achieved by retroviral and lentiviral vectors, the risk of insertional mutagenesis remains a concern when considering genetically engineered MSC for clinical trials. In the hematopoietic stem cell (HSC) clinical trial that used retroviral vector for X-linked severe combined immunodeficiency disease (X-SCID) in France, four out of eleven children developed leukemia [37, 38]. One out of ten patients treated by hematopoietic stem cell gene therapy for Wiskott–Aldrich syndrome also developed acute lymphocytic leukemia [39]. Later, it was discovered that the leukemia in both cases was due to the long-terminal repeat (LTR) of the retroviral cassette having integrated in the proximity of the *LMO2* proto-oncogene promoter. As a result, the integrated LTR acted as a promoter to drive LMO2 expression and lead to leukemia, in cells that were greatly expanded already due to the selection process in both trials, and delivery of a growth factor receptor in the case of XSCID.

Naldini and colleagues compared the in vivo tumor induction capacity by both retroviral and lentiviral vectors [40]. Using the tumor-prone p16 knockout mouse strain, this group found that retroviral vectors triggered a dose-dependent induction of tumor onset, while lentiviral vectors showed low genotoxicity upon integration. Later, the same group also showed that the retroviral LTR is the major component capable of generating unregulated cell growth in this tumor-prone mouse model, by swapping between retroviral vector LTR and self-inactivating (SIN)-LTR in lentiviral plasmids [41]. Indeed, additional gene-modified stem cell clinical trials using SIN-LTR lentiviral vectors have avoided this outcome [42]. The possibility of adverse events can be monitored by serial transplantation experiments in vivo [18, 40].

In contrast to hematopoietic stem cells which are capable of long-term self-renewal and differentiation in vivo, gene-modified MSCs have not been reported to cause tumors, using in vivo assays. We have completed a comprehensive decade-long study of the biosafety of MSCs stably transduced by retro- and lentiviral vectors and did not observe adverse events arising from the human cells, in sensitive xenograft assays [18]. However, it should be noted that we did not perform serial transplantations and the follow-up of the human MSCs in immune-deficient mice was limited to 18 months, due to the natural lifespan of the mice.

A way to potentially avoid risks from random gene insertion would be to use human embryonic stem cell (ESC) or human induced pluripotent stem cell (hiPSC)-derived MSCs [43] which have safe harbor integrations of the transgenes and have been subsequently expanded in vitro. The lifespan of primary MSCs are limited up to 40 doublings during in vitro expansion [44]. Aging significantly reduces the in vitro and in vivo survival and differentiation potential of primary MSCs [45]. This could be potentially overcome in the future by the use of hESC or hiPSC-derived MSCs, since these cell types could theoretically be expanded indefinitely after transduction and prior to differentiation to the MSC lineage (discussed in [46]).

15.3 Gene-Modified MSCs in Pre-clinical Models

MSCs genetically modified to secrete cytokines and other growth factors have been successfully used in animal models for various diseases, and are therefore poised to be tested in human clinical trials [11]. Accumulating evidence indicates that genetically modified MSCs have therapeutic potential in various disease models and that genetic engineering of the cells can improve cell survival, homing to the disease sites, secretion of therapeutic agents and differentiation into different cell types. Here we will describe the current status of gene-modified MSCs in various preclinical models.

15.3.1 MSC Survival After Transplantation

The majority of intravenously transplanted MSCs appear to die within several hours or lodge nonspecifically in the lung, spleen, liver, or kidney [11, 14, 22, 47]. To improve MSC survival after transplantation, several approaches have been examined including the overexpression of proliferation-related or anti-apoptotic-related genes or preconditioning using hypoxia or other approaches prior to transplantation (Table 15.2).

Several laboratories have focused on the overexpression of Akt, a protein which inhibits apoptosis. MSCs engineered to overexpress Akt survived longer than unmodified MSCs after transplantation in a variety of animal models. Dzau and colleagues documented that Akt-overexpressing MSCs had a higher survival rate after transplantation into an ischemic heart model and showed that the improvement of cardiac function following transplantation was due to the paracrine factors secreted from the surviving MSCs [48]. Another paper from the same group further demonstrated that transplanted Akt-modified MSCs balanced the metabolism and pH of the myocardium [49]. Recently, a swine myocardial infarction model also added to the evidence showing that Akt transduced MSCs survived longer and showed greater efficacy than unmodified MSCs [50]. Our group has shown that, rather than performing gene modification, hypoxic preconditioning of human MSCs at 3% oxygen

Table 15.2 Summary of survival-related gene expressing MSCs in preclinical models

Transgene	Model	Route of administration	Effect	Ref
Akt	Rat infarcted myocardium	Intra-myocardial	Improved cardiac function	[48]
Akt	Rat infarcted myocardium	Intra-myocardial	Improved cardiac function	[49]
Akt	Swine infarcted myocardium	Intra-myocardial	Improved cardiac function	[50]
Connexin43	Rat infarcted myocardium	Intra-myocardial	Improved cardiac function	[51]
Hsp20	Rat infarcted myocardium	Intra-myocardial	Improved cardiac function	[52]
Bcl-2	Rat infarcted myocardium	Intra-myocardial	Improved cardiac function	[53]
Bcl-xL	Rabbit cartilage defect	Intra-articular	Improved cartilage healing	[54]
HO-1	Rat infarcted myocardium	Intra-myocardial	Improved cardiac function	[55–58]
HO-1	Mouse infarcted myocardium	Intra-myocardial	Improved cardiac function	[59]
HO-1	Swine infarcted myocardium	Intra-myocardial	Improved cardiac function	[60]

Abbreviation: *Hsp20* heat shock protein 20, *Bcl-2* B-cell lymphoma 2, *Bcl-xL* B-cell lymphoma-extra large, *HO-1* Heme oxygenase 1

for 24 h prior to transplantation will upregulate AKT activity, enhance cell homing and survival, and enhance their in vivo capacity to promote revascularization in a xenograft model of hindlimb ischemia [61].

Overexpression of connexin43, a gap junction protein, also showed a higher MSC survival rate and improvement of cardiac function. This result was shown to be due to the fact that these cells expressed more Bcl-2, one of the negative regulators in the apoptotic pathway, along with phosphorylated Akt. These cells also expressed less Bax, a pro-apoptotic protein [51]. In the same fashion, Hsp20 overexpression in MSCs has been shown to increase the cell survival rate along with reduced fibrosis. Hsp20 can protect other proteins against heat-induced cellular stress. The beneficial effects of these gene-modified MSCs were associated with enhanced Akt activation and increased secretion of growth factors such as VEGF, FGF-2, and IGF-1 [52].

MSCs engineered to express Bcl-2, which is one of the key anti-apoptotic proteins, have shown better survival and improved cardiac function due to reduced apoptotic events and increased VEGF secretion [53]. Recent studies using Bcl-xL-(one of the Bcl-2 family members) modified MSCs also supports this concept [54]. Direct intra-articular injection of Bcl-xL overexpressing MSCs into a rabbit articular cartilage defect model improved MSC survival and increased cartilage healing. Several studies mediated Heme oxygenase (HO-1), which is known for protection against apoptosis in rat [55–58], mouse [59], and swine cardiac ischemic models [60]. It is well known that HO-1 itself has therapeutic potential in the treatment of cardiac disease [62].

Although overexpression of pro-proliferative and anti-apoptotic genes in MSCs improved cell survival after transplantation, there must be extreme caution for any consideration of application to human clinical trials as these genes are related to

Table 15.3 Summary of homing-related gene expressing MSCs in preclinical models

Transgene	Model	Route of administration	Effect	Ref
CXCR4	Rat infarcted myocardium	Intra-myocardial	Improved MSC homing and cardiac function	[69]
CXCR4	Mouse infarcted myocardium	Intra-myocardial	Improved MSC homing and cardiac function	[70]
CCR1	Mouse infarcted myocardium	Intra-myocardial	Improved MSC survival, homing and cardiac function	[71]

Abbreviation: *CXCR4* C–X–C chemokine receptor type 4; *CCR1* C–C chemokine receptor type 1

cancer activity and overexpression may lead to cell transformation. Therefore it is most prudent to learn from the overexpression data in these instances and apply this knowledge to developing novel ways to precondition cells prior to transplantation to achieve similar effects. For example, our group others have explored the use of hypoxic prestimulation prior to transplantation [61, 63, 64].

15.3.2 MSC Homing

Efficient MSC homing to the tissue of interest is one of the most important aspects of effective MSC therapy. Extensive studies have shown that MSC migration is mediated by growth factor or chemokine/receptor pairs such as SDF-1/CXCR4, HGF/c-MET, VEGF/VEGFR, MCP-1/CCR2, and others (detailed review in [14]). The well-known homing receptor CXCR4 is a chemotactic receptor for stromal cell-derived factor-1α (SDF-1). CXCR4 is absent in populations of in vitro expanded MSCs, but freshly isolated MSCs have a small positive population [65–67]. It has been shown that hypoxic (tissue normoxic) preculture can induce CXCR4 expression [64]. Surface antigen modification of CD44 by the FUT VI enzyme improved homing efficiency of BM-MSCs into the bone in NOD/SCID mice without the expression of CXCR4 [67]; ex vivo engineered E-selectin, which is not expressed naturally in MSCs, was also shown to be sufficient to home MSCs into bone[67]. Also, our group has also shown that modification of MSCs with bone-homing ligands tethered to bisphosphonate has also resulted can result in homing of MSCs back to the bone [68].

In a rat myocardial infarction model, MSCs engineered to overexpress CXCR4 showed greater numbers of cells that had homed to ischemic sites and improved left ventricular function, as compared to unmodified MSCs [69] (Table 15.3). Huang et al. further analyzed that CXCR4-overexpressing mouse MSCs migrated to the infarction site and released the collagen degrading enzyme, matrix metalloproteinase-9 (MMP-9), which lead to a reduction of the remodeling of infarcted myocardium [70]. Dzau and colleagues turned their focus on a different chemokine receptor, CCR1 [71]. This receptor is one of the G protein-coupled receptors known to bind to CCL7 and is usually expressed by MSCs at low levels.. This group noticed that

infarcted hearts have higher expression levels of CCL7. To better guide MSCs to ischemic sites, they overexpressed CCR1 in murine MSCs. These cells had better survival, reduced cardiac remodeling and increased cardiac functions in comparison with non-engineered MSCs.

Since permanent expression of E-selectin is not required to home MSC to bone, transient expression of these homing proteins can be considered [67]. Nonviral methods such as plasmid transfection, cytokine treatment, hypoxia, and others that can increase levels of homing receptors can be an alternative method to improve MSC localization to bone, to the perivascular space, and to damaged tissues in general.

15.3.3 Cardiovascular Diseases

Cardiovascular diseases are the leading cause of death in the USA. An estimated 79 million American adults (1 in 3) have one or more types of cardiovascular diseases [72]. Ischemia and hypertensive heart failure cause irreversible loss of cardiomyocytes. Potent pharmacological treatments have significantly improved morbidity and mortality [73]. These methods along with the development of implantable cardioverter-defibrillators [74] and left ventricular assist devices have all significantly increased survival rates [75]. Despite all these improvements in clinical management, the prevalence of heart failure remains. The current best therapy for cardiac failure, heart transplantation, is hampered by the shortage of organ donors. Stem/progenitor cell transplantation for curing cardiac diseases remains an attractive concept that is studied in numerous preclinical and clinical trials.

So far, most gene-modified MSC studies using anti-apoptotic and proliferative genes showed improvement of cardiac function in acute cardiac infarction cases due to better survival of transplanted MSCs and secretion of various growth factors (Table 15.4). Among the growth factors, the most heavily studied is vascular endothelial growth factor (VEGF). VEGF-overexpressing MSCs administered to treat acutely infarcted heart in mouse [76] and rat [77, 78] significantly increased vascular density, reduced the infarcted area and improved cardiac function. Human MSCs genetically modified to secrete VEGF were also found to significantly enhance blood flow recovery in an immune-deficient mouse model of hindlimb ischemia [83]. Hepatocyte growth factor (HGF) is also one of the promising options to improve cardiac ischemia. Ectopic expression of HGF in MSCs improved cardiac function, reduced ventricular remodeling, and enhanced vascular density in rat models [79–81]. Another study confirmed that HGF or VEGF-expressing MSCs also improved cardiac function [82].

In coronary or peripheral artery diseases, bypass surgery or angioplasty is popular solution. Our laboratory examined cell fates, proliferation of growth factor overexpressing MSCs and angiogenesis using VEGF-overexpressing human MSCs in an immune-deficient mouse ischemic hind limb injury model [30]. Other studies have focused on the therapeutic potential of factor releasing MSCs, but cell fate decisions and the proliferation potential of vector containing MSCs are less well illustrated.

Table 15.4 Summary of growth factor expressing MSCs in cardiovascular preclinical models

Transgene	Model	Route of administration	Effect	Ref
VEGF	Mouse infarcted myocardium	Intra-myocardial	Increased vascular density and cardiac function	[76]
VEGF	Rat infarcted myocardium	Intra-myocardial	Improved MSC homing and cardiac function	[77, 78]
HGF	Rat infarcted myocardium	Intra-myocardial	Improved MSC homing, reduced remodeling, increased cardiac function	[79–81]
HGF/VEGF	Mouse infarcted myocardium	Intra-myocardial	Increased vascular density and cardiac function	[82]
VEGF	Mouse ischemic hind limb model	Intravenous injection	Enhanced blood flow restoration in ischemic hind limb model	[30]

Abbreviation: *VEGF* Vascular endothelial growth factor, *HGF* Hepatocyte growth factor

We showed that bFGF or PDGF-B overexpression in MSCs increased proliferation. When cultured in differentiation conditions, both bFGF and PDGF-B overexpressing MSCs showed enhanced osteogenesis, but strong inhibition was shown for adipogenesis in MSC overexpressing PDGF-B and only mildly affected in the MSCs overexpressing bFGF. Overexpression of TGF-β_1 blocked both osteogenic and adipogenic differentiation but VEGF overexpression did not vary in any of these differentiation assays, most probably due to the lack of VEGF receptor expression on MSCs. Therefore, due to the lack of autocrine effects on the MSCs that would produce it, we further examined the role of MSCs engineered to produce VEGF165a in vivo. VEGF overexpressing MSCs were demonstrated to significantly enhance blood flow restoration in a xenograft model of hind limb ischemia, without adverse events [30].

15.3.4 Cancers

Cancer is the second leading cause of death in the USA, accounting for 1 in every 4 deaths over all ages in 2010 [84]. It is estimated that approximately 1 in 2 men (44%) and 1 in 3 women (38%) have a lifetime probability of being diagnosed with an invasive cancer. Since the declaration of "War on Cancer" in 1971, there have been tremendous advancements in cancer biology and successful drug treatments. In consensus, metastatic cancer is the major cause of deaths, not the primary cancer. Metastatic cells spread to the bones, lung, kidney, liver, brain, and other organs, and it is very difficult to locate these metastases by established diagnostics. The short half-life of some drugs limits their delivery to some metastatic tumor sites and side effects on non-tumor cells is one of the major impediments to curing cancers.

MSCs have been proposed as one of the several treatment modalities for cancer therapy due to supposed antitumor effects, but this is still highly controversial. Some

papers claimed that MSCs had antitumor properties such as inhibiting the proliferation of glioma, melanoma, lung cancer, hepatoma, and breast cancer [85]. Others showed that MSCs secreted IL-6 and this increased proliferation [86] or production of CCL5 from MSCs and increased metastasis of breast cancer cells [87]. One thing that both sides agreed on is that MSCs migrate into cancer sites with not fully understood mechanisms [85]. With this notion in mind, many groups modified MSCs as delivery vehicles for therapeutic reagents; categorized as immunostimulatory agents, cytotoxic agents, prodrug activators, and viral vector delivery (detailed review in [85]).

15.3.4.1 Immunostimulatory Agents

Cancers have an ability to modulate their environments to hide their identity [88]. Stimulating endogenous immune systems by cytokines is one of the interesting options to treat cancers. Interleukins are known to regulate inflammatory and immune responses [89]. IL-12 and IL-18 are known to kill tumors directly and to recruit T cells and natural killer cells and those cells can eradicate tumors [90]. Administration of MSCs expressing IL-12 compared to adenoviral delivery of IL-12 every 5 days for 4 times showed reduction in the spread of metastatic melanoma, breast cancer, and hepatoma [91] (Table 15.5). IL-12 delivered by adenoviral vector showed toxicity and the levels of IL-12 were only elevated in the serum, but not the intratumoral environment. However, MSC overexpressing IL-12 showed increased apoptosis of tumor cells and higher levels of IL-12 in the intratumoral samples.

The same concept to eradicate renal carcinoma was applied by Gao et al. [92]. They injected MSCs bearing IL-12 once in xenografted nude mouse models and showed reduction of tumor growth and prolonged survival compared to systemic administration of adenoviral delivery of IL-12. Other teams reported that IL-12 expressing MSCs showed therapeutic efficacy on melanoma and cervical cancers [93] as well as intracranial glioma [94]. Similarly, IL-18 modified MSCs also have been investigated to treat glioma in a rat model [95]. IL-18 expressing MSCs were systemically administered and showed inhibition of glioma growth and prolonged survival of rats bearing glioma. IL-2 expressing MSCs also showed similar efficacy in a rat glioma model [96].

Interferons (IFNs) are cytokines released from the host cells and have functions to activate natural killer cells or macrophages and to increase antigen presentation to be recognized by T cells [90]. IFNα and IFNβ were pursued to treat various tumors using MSCs as the vehicle to deliver them, because systemic administrations of IFNs cause toxicity in vivo. As described earlier, MSCs are prone to migrate into tumor sites and IFN expressing MSCs recruit cells of the host immune system. IFNα overexpressing MSCs were evaluated in mouse melanoma lung metastasis models [97] and a mouse plasmacytoma model [98]. Both studies showed that intravenously [97] and subcutaneously [98] injected MSCs producing IFNα increased tumor apoptosis and decreased cancer proliferation along with prolonged survival of mice bearing tumors. Several laboratories utilized IFNβ expressing MSCs to treat

Table 15.5 Summary of immune-stimulatory gene expressing MSCs in cancer preclinical models

Transgene	Model	Route of administration	Effect	Ref
IL-12	Metastatic mouse model	Subcutaneous injection every 5 days for 4 times	Prevention of metastatic melanoma, breast cancer, and hepatoma	[91]
IL-12	Renal carcinoma in xenograft nude mouse model	One time tail vein injection	Reduction of tumor growth and prolonged survival of mice	[92]
IL-12	Melanoma and cervical cancer mouse model	Intravenous injection	Anti-metastatic effects	[93]
IL-12	Intracranial glioma mouse model	Ipsilateral hemisphere injection	T-cell infiltration in intracranial gliomas, and anti-angiogenesis	[94]
IL-18	Rat glioma model		Inhibition of glioma growth and prolonged survival of glioma-bearing rats	[95]
IL-2	Rat glioma model	Injection to contralateral hemisphere	Inhibition of glioma growth and prolonged survival of glioma-bearing rats	[96]
IFNα	Mouse melanoma metastasis model	Intravenous injection	Increase in tumor apoptosis and a decrease in proliferation and blood vasculature and prolonged survival of mouse bearing tumors	[97]
IFNα	Mouse plasmacytoma model	Subcutaneous injection	Increase in tumor apoptosis and a decrease in proliferation and prolonged survival of mouse bearing tumors	[98]
IFNβ	Melanoma nude mouse model	Intravenous injection	Inhibition of the growth of malignant cells *in vivo*	[99]
IFNβ	Melanoma and breast cancer mouse model	Intravenous injection	Inhibition of tumor cells growth and prolonged survival of mice bearing tumors	[100, 101]
IFNβ	Prostate cancer lung metastasis model	Tail vein injection	Increased tumor cell apoptosis and natural killer cell activity, decreased tumor cell proliferation and blood vessel counts	[102]
IFNβ	Metastatic pancreatic cancer model	Intraperitoneal injection	Increased tumor growth inhibition. anti-inflammatory drug reverses the MSC-mediated effects	[103]

Abbreviation: *IL-12* Interleukin-12, *IL-18* Interleukin-18, *IL-2* Interleukin-2, *INFα* Interferon α, *IFNβ* Interferon β

various tumors in rodent models [99, 100, 102, 103]. Studeny et al. showed that IFNβ-modified MSCs inhibited melanoma growth in vivo [99]. Interestingly, therapeutic efficacy was only shown when MSCs had migrated to tumor sites but systemically delivered IFNβ or that produced by MSCs at a site distant from the tumors did not. Similar approaches were applied to a breast cancer model [100, 101], prostate cancers [102], and pancreatic cancers [103].

15.3.4.2 Cytotoxic Agents

Tumor necrosis factor-related apoptosis-induced ligand (TRAIL) is a pro-apoptotic protein that will enter cancer cells but normal cells are not affected [104, 105]; TRAIL-induced apoptosis occurs via the caspase pathway. A major drawback of systemic administration of TRAIL is a large amount of TRAIL needed to kill cancers due to the fast clearance of TRAIL by the kidney [106]. Several groups investigated MSCs expressing TRAIL as a vehicle to deliver locally to tumor sites and to sustain the TRAIL expression enough to kill the cancer (Table 15.6). Szegezdi et al. showed that MSCs were not sensitive to TRAIL-induced apoptosis because TRAIL receptors in MSCs were inactive and downstream genes of the TRAIL pathway were rarely expressed [119]. Another report also confirmed that TRAIL did not affect the MSC characteristics such as cell proliferation and differentiation into osteogenic and adipogenic lineages, and that it enhanced the migration of MSCs [120]. With these characteristics, the therapeutic efficacy of TRAIL-secreting MSCs was evaluated in various cancer models. MSC-mediated TRAIL delivery in a human/mouse xenograft model was performed by Mohr et al. [107]. They showed that TRAIL-expressing MSCs could reduce the growth of human lung carcinoma xenografted into immune-deficient mice. TRAIL-expressing MSCs were evaluated in different cancer types including glioma [108, 109, 111, 112, 116, 117], lung cancer [110], breast cancer [110, 113], squamous cancer [110], cervical cancer [110, 113], pancreatic cancer [113, 115], and colon cancer [113, 114, 118].

Several papers have shown that some cancers have a subset that are resistant to TRAIL-mediated apoptosis due to low levels of TRAIL receptors. To address this problem, a variety of methods to sensitize the cancer cells to TRAIL were applied; several laboratories conducted combination studies using TRAIL secreting MSCs in conjunction with drugs [113], RNAi [114], irradiation to the cancers [115], or 5-fluorouracil (5-FU) [118]. Grisendi et al. found that BT549 breast cancer cell lines survived in the high concentration of TRAIL due to the lack of the expression of TRAIL receptor, DR4 and DR5 [113]. They treated BT549 with the proteosome inhibitor PS-341, also known as Bortezomib, which upregulates expression of the DR5 receptor. With the combination of Bortezomib and TRAIL-producing MSCs, tumor apoptosis was increased. Mohr et al. investigated the use of RNAi in combination with TRAIL secreting MSCs to treat metastatic pancreatic carcinoma to treat TRAIL-resistant cells [115].

X-linked inhibitor of apoptosis protein (XIAP), which prevents apoptosis by inhibition of caspase-3 and caspase-9 activation, leading to the resistance to TRAIL treatment, was silenced by the shRNA technique and in combination with TRAIL-

Table 15.6 Summary of TRAIL releasing MSCs in cancer preclinical models

Transgene	Model	Route of administration	Effect	Ref
TRAIL	lung carcinoma xenograft	Direct injection into tumor site	Reduction of tumor growth	[107]
TRAIL	Human glioma nude mouse model	Direct injection into tumor site or opposite hemisphere injection	Reduction of tumor mass and prolonger mice survival	[108]
TRAIL	Glioblastoma multiforme mouse xenograft model	Stereotactic injection	Antitumor effects	[109]
TRAIL	Lung (A549), breast (MDAMB231), squamous (H357), and cervical (Hela) cancer mouse xenograft model	Subcutaneous injection	Reduced the metastatic lung cancer	[110]
TRAIL	Glioma mouse xenograft model	Intravenous injection	Prolonged survival of xenograft mice bearing tumors	[111]
TRAIL	Glioma mouse xenograft model	Ipsilateral injection	Inhibition of tumor growth in vivo	[112]
TRAIL	Human cervical carcinoma, pancreatic cancer, colon cancer, and, with bortezomib to TRAIL-resistant breast cancer xenograft model	Flank injection	Increased apoptosis of cancers in vivo	[113]
TRAIL	Colorectal carcinoma xenograft model	Flank injection	Reduction of tumor growth in vivo	[114]
sTRAIL RNAi toXIAP	Pancreatic carcinoma xenograft model	Intravenous injection	Growth retardation on treatment with sTRAIL-MSCs Remission by sTRAIL-MSCs with RNAi to XIAP	[115]
TRAIL Irradiation	Glioma xenograft model	Stereotactic injection	Sequential treatment of irradiation and TRAIL-MSCs enhanced therapeutic efficacy to kill TRAIL-resistant glioma	[116]
TRAIL	Brainstem glioma rat model	Stereotactic injection	Short- and long-term prolonged survival of rats bearing tumor	[117]
TRAIL	Colorectal carcinoma xenograft model	Flank injection	Showed colon cancers were resistant to TRAIL-MSCs Intraperitoneally injected 5-FU with TRAIL-MSCs overcome the resistance	[118]

Abbreviation: *TRAIL* Tumor necrosis factor-related apoptosis induced ligand, *RNAi* RNA interference, *XIAP* X-linked inhibitor of apoptosis protein, *5-FU* 5-fluorouracil

expressing MSCs and RNAi, metastatic pancreatic cancer in human to mouse xenograft models went into remission. TRAIL-secreting MSCs alone showed reduced growth of tumor but could not block the tumor growth enough. Sequential treatments of irradiation and TRAIL expressing MSCs showed killing of TRAIL-resistant glioma cells [116]. Mueller et al. found that a subset of colon carcinoma cells were resistant to TRAIL-induced apoptosis [118]. They injected 5-FU, which is an active form of prodrug to kill cancers, with TRAIL-expressing MSCs, and showed improved efficacy.

15.3.4.3 Prodrug Activators

Prodrug systems, which convert nontoxic prodrugs into cytotoxic materials, are also utilized to treat various types of cancer (detailed review in [121]). Currently, several types of prodrug activation systems are available. Cytosine deaminase (CD) converts nontoxic 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU) and herpes simplex virus (HSV) thymidine kinase (TK) is sensitive to ganciclovir. Activated prodrugs are known to kill cancers through the bystander effect [121]. Several papers have exploited the CD-mediated prodrug activation approach (Table 15.7). MSCs producing CD can convert 5-FC to 5-FU, then 5-FU diffuses out from MSCs to kill rapidly dividing cells. Several cancer models such as colon cancer [122], melanoma [123], gastric cancer [124], prostate cancer [125], glioma [126], and a rat glioblastoma model [127] have been evaluated with intravenously injected CD-producing MSCs and have shown inhibition of tumor growth. In the prostate cancer [125] and rat glioblastoma model [127], cytosine deaminase::uracil phosphoribosyltransferase (CD::UPRT), which is a better converter than CD alone, has been exploited.

For TK-mediated cancer treatment, TK-expressing MSCs were able to deliver cytotoxic effects to human glioblastoma cells, but delivery to HeLa cells and MCF7 breast cancer cells was not achieved with the same efficacy [128]. It turned out that cytotoxic effects were transferred into adjacent cells by gap junctions, and HeLa and MCF7 cells did not form gap junctions with MSCs, making TK-mediated induction of apoptosis less effective. Intravenously injected TK-expressing MSCs were effective in reducing tumor volume in the nude mouse model. The same approach was applied to prostate cancer [129] and glioma [130, 131]. Huang et al. reconstructed the gap junction connection by overexpressing Connexin43 in combination with TK [131]. Using this approach, they showed enhanced inhibition of tumor growth as compared to MSC therapy with TK-alone.

15.3.4.4 Viral Vector Delivery

Oncolytic viruses such as adenovirus are able to replicate and selectively kill cancer cells, sparing normal cells [132] (Table 15.1). Direct injection of oncolytic viruses to intratumoral sites showed efficacy and tumor regression in clinical trials, but intravenous injection did not [132]. However, it is well known that adenoviral

Table 15.7 Summary of prodrug converter gene expressing MSCs in cancer preclinical models

Transgene	Model	Route of administration	Effect	Ref
CD	Colon cancer xenograft model	Intravenous injection	MSCs migrated into tumor sites and reduced tumor growth	[122]
CD	Melanoma xenograft model	Intravenous or intraperitoneal injection	MSCs migrated into tumor sites and reduced tumor growth	[123]
CD	Gastric cancer xenograft model	Intravenous injection	MSCs migrated into tumor sites and reduced tumor growth	[124]
CD::UPRT	Prostate cancer xenograft model	Intravenous injection	MSCs migrated into tumor sites and reduced tumor growth	[125]
CD	Glioma xenograft model	Stereotactic injection	Multiple injections of MSCs reduce the preexisting tumor size	[126]
CD::UPRT	Rat glioblastoma model	Intracerebral injection	Complete tumor regression	[127]
TK	Human glioblastoma nude mouse model	Intravenous injection	Reduction of tumor volume	[128]
TK	Prostate cancer nude mouse model	Intravenous injection	Reduction of tumor volume and prolonged survival of tumor-inoculated mice	[129]
TK	Glioma xenograft model	Intravenous injection	Inhibition of tumor growth and prolonged survival of tumor-inoculated mice	[130]
TK/Connexin43	Glioma rat model	intracerebral injection	MSCs migrate into tumor sites and enhanced inhibition of tumor growth compared with TK-MSCs	[131]

Abbreviation: *CD* Cytosine deaminase, *UPRT* uracil phosphoribosyltransferase, *TK* thymidine kinase

vectors cause a host immune reaction. High concentrations of adenovirus can cause a transient elevation in liver enzymes, a sign of an immune reaction, and has been associated with severe adverse events.

Since MSCs are used as a vehicle to deliver to tumor sites and as a reservoir for the adenovirus, it is feasible to apply this approach for cancer treatment (Table 15.8). Komarova et al. first utilized MSCs to deliver oncolytic adenovirus [133]. Several papers also showed efficacy with adenovirus-loaded MSCs using a breast cancer lung metastasis xenograft model [134], intracranial glioma [135–137], ovarian cancer [133], and metastatic neuroblastoma [138]. Instead of adenovirus, Mader et al. utilized oncolytic measles virus-loaded MSCs to treat ovarian cancer in a xenograft model [139]. The measles virus is known to induce cytopathic effects on cancers, but native viruses are neutralized by preexisting antiviral antibodies. MSCs bearing

Table 15.8 Summary of oncolytic virus containing MSCs in cancer preclinical models

Transgene	Model	Route of administration	Effect	Ref
Adenovirus	Ovarian cancer mouse xenograft model	Intraperitoneal injection	Reduction of tumor size and prolonged survival mice bearing tumor	[133]
Adenovirus	Breast cancer metastatic lung cancer xenograft model	Intravenous injection	MSCs migrated to tumor site and reduced the tumor growth in vivo.	[134]
Adenovirus	Intracranial glioma	Stereotactic injection	MSCs migrated to tumor site and release viruses	[135]
Adenovirus	Intracranial glioma	Intra-arterial injection	MSCs migrated to tumor site and reduced the tumor growth in vivo and prolonged survival of mice bearing tumor	[136]
Adenovirus	Malignant glioma	Stereotactic injection	Prolonged survival of mice bearing tumor	[137]
Adenovirus	Metastatic neuroblastoma in human patients	Intravenous infusion to patients	One out of four children had complete remission	[138]
Measles virus	Ovarian cancer xenograft model	Intraperitoneal injection	Prolonged survival of mice bearing tumor	[139]

measles viruses formed syncytia in the presence of antiviral antibodies and enhanced the survival of mice bearing tumors.

In Spain, there has been progress towards the clinical application of adenovirus-loaded MSCs to treat neuroblastoma [138]. In this study, 4 children from ages 2 to 5 with metastatic refractory stage IV neuroblastoma were infused at least twice with MSCs bearing oncolytic adenovirus. The clinical team followed the patient's renal and liver functions, white and red blood cell and platelets counts, and they checked the adenovirus concentration in serum and urine every 2 weeks. One of the four patients showed that metastatic tumors had disappeared and is now in complete remission for 36 months after the first treatment.

15.3.5 Bone-Related Diseases

It is not surprising that one of the first human clinical trials using MSCs was to treat osteogenesis imperfecta, a genetic bone disease, because MSCs can form bone [9]. Several laboratories tried to enhance osteogenesis by overexpressing bone morphogenetic protein 2 (BMP2), insulin-like growth factor 1 (IGF1), VEGF, and human telomerase reverse transcriptase (hTERT) (Table 15.9). Shi et al. examined whether hTERT overexpression can maintain MSC proliferation and osteogenic differentiation potential in ex vivo culture [147]. An in vitro osteogenic differentiation assay in hTERT overexpressing MSCs showed more mineralized bone structure than

Table 15.9 Summary of pro-osteogenesis gene expressing MSCs in bone defect preclinical models

Transgene	Model	Route of administration	Effect	Ref
hTERT	Beige mouse	Subcutaneous injection	More osteogenic cells and osteogenic potential	[140]
Wnt-4	• Nude rat periodontal defect model • Craniofacial Defect Model	Embedded with Polylactic co-glycolide polymer scaffolds (periodontal defect model)	Enhanced osteogenesis and showed extensive periodontal bone regeneration and improved the repair of craniofacial defects in vivo	[141]
BMP2	Ulnar bone defects in the canine model	MSCs mixed with β-tricalcium phosphate ceramic granules and placed onto the defect area	Significant increase of newly formed bone area and healed or partly healed all of the bone defects	[142]
BMP2	Ovariectomized, female C57BL/6 mice	Intravenous injection	Significant increase in bone mineral density and bone mineral content and more trabecular bone following MSC-BMP2 therapy	[143]
BMP2	Periodontal defects rabbit model	Not available	Regenerated cementum with Sharpey's fiber insertion and bone formation	[144]
BMP2 Runx2	Nude mouse model	Subcutaneous injection with PLGA	Enhanced bone formation compared to BMP2 expressing MSCs	[145]
IGF1	Insulin-receptor-substrate knock-out mice	Intravenous injection	Restored the fracture by new bone formation and promoted the occurrence of a well-organized callus	[146]

Abbreviation: *hTERT* human Telomerase reverse transcriptase, *Wnt-4* wingless-type MMTV integration site family, member 4, *BMP2* Bone morphogenetic protein 2, *Runx2* runt-related transcription factor 2

unmodified MSCs. In their following paper, hTERT overexpressing MSCs were subcutaneously injected into beige mice and showed more osteogenic cells than MSCs alone, along with an increased osteogenic potential due to the upregulation of CBFA1, osterix, and osteocalcin [140].

Chang et al. evaluated the possibility of whether non-canonical Wnt-4 regulates the osteogenic pathway [141]. They tested two different bone defect models, a periodontal bone defect model and a craniofacial defect model with Wnt-4 expressing MSCs embedded in polylactic co-glycolide polymer scaffolds. Wnt-4 overexpressing MSCs increased osteogenesis and showed extensive periodontal bone regeneration and improved the repair of craniofacial defects in vivo. Several papers used BMP2 expressing MSCs to enhance bone formation. Li et al. evaluated BMP2-expressing canine MSCs on ulnar bone defects in the canine model [142]. Sixteen weeks after

the transplantation, BMP2 overexpressing MSCs increased the area of newly formed bone and healed or partially healed all of the bone defects.

Ponnazhagan and colleagues looked at bone regeneration in an osteopenic mouse model with BMP2 expressing MSCs [143]. Intravenously injected BMP2 secreting MSCs enhanced bone mineral deposits and more trabecular bone formation than MSCs alone. Chen et al. engineered MSCs to express BMP2 and implanted them into a periodontal defect rabbit model [144]. BMP2 overexpressing MSCs regenerated cementum with Sharpey's fiber and enhanced bone formation where it attached to periodontal fibers. To enhance bone formation compared to BMP2 expressing MSCs, Runx2, one of the master regulators of osteogenesis, was co-expressed [145]. Runx2/BMP2 co-expressing MSCs were embedded within a PLGA scaffold and implanted subcutaneously into athymic nude mice. BMP2/Runx2 expressing MSCs showed enhanced bone formation compared to MSC only and BMP2 expressing MSCs. Instead of BMP2, efficacy using IGF1 overexpressing MSCs was recently evaluated in a tibia fracture model [146]. IGF1 secreting MSCs were intravenously injected into the tail of insulin-receptor-substrate knock-out (Irs(−/−)) mice, which lack the ability to repair fractures. Authors claimed that IGF1 expressing MSCs improved new bone formation and restored the tibia fracture in Irs(−/−) mice. From the in vitro and in vivo assays, they showed that IGF1 induced osteogenesis via the Irs1-PI3K signaling pathway, with autocrine and paracrine effects.

15.3.6 Renal Failure

Most kidney diseases are related to the characteristics of ischemic, inflammatory and immunologic injury. MSC-mediated treatments were pursued as cellular therapy to improve these problems. It is known that erythropoietin (EPO) is downregulated at the end stage of renal failures. Eliopoulos et al. transduced EPO into murine MSCs and injected them subcutaneously into syngeneic mice with chronic renal failure [148] (Table 15.10). Among various doses, higher doses showed increased hematocrit levels to normal compared to controls and better survival of the mice. In a follow-up study, the same group co-introduced IGF-1 and EPO secreting mouse MSCs subcutaneously to the renal failure mouse model. An enhanced hematocrit level was achieved and cardiac function was improved [149]. Our group has also overexpressed EPO from human MSCs, in the late 1990s, and found a significant increase in hematocrit and differentiation of co-transplanted human hematopoietic stem cells to the red cell lineage [31]. However, toxicity occurred from the very high RBC counts resulting from the high unregulated dosages of EPO that MSCs can produce if the transgene is not under the control of a regulated inducible promoter (reviewed in [11]). These studies confirmed that MSCs can be powerful in vivo delivery vehicles, but suggest that, with growth factor expression, it will be important to regulate the amounts of protein produced.

For the acute renal failure model, Hagiwara et al. examined the production of kallikrein, which makes cells resistance to oxidative stress-induced apoptosis, from

Table 15.10 Summary of MSCs expressing kidney-related genes in renal failure preclinical models

Transgene	Model	Route of administration	Effect	Ref
EPO	Mouse chronic renal failure	Subcutaneous	Increased hematocrit and activity of mice	[148]
IGF1/EPO	Mouse chronic renal failure	Subcutaneous	Increased hematocrit and activity of mice	[149]
Kallikrein	Rat acute renal failure	Carotid artery injection	Protection against apoptosis in acute renal failure model	[150]
VEGF	Nude mouse renal failure	Intravenous tail injection	Protection against apoptosis in acute renal failure model and better renal function	[151]

Abbreviation: *EPO* Erythropoietin, *IGF1* Insulin-like growth factor 1

gene-modified modified MSCs. Kallikrein engineered rat MSCs showed significant reduction of apoptosis induced by H_2O_2 and inhibition of neutrophil and monocyte infiltration [150]. A recent paper showed VEGF-mediated protection and improvement of acute renal failure in a nude mouse model [151]. In that report, VEGF-engineered human fetal MSCs also showed better survival of renal epithelium by increased cell proliferation and reduced apoptosis, better renal function, and increased peritubular capillary density [151].

15.3.7 Neurological Diseases

MSC therapies have shown efficacy in preclinical models of various neurological diseases such as Parkinson's disease, Huntington's disease, multiple sclerosis, and stroke (detailed review in [152]). Here we review genetically engineered MSCs in preclinical models of Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (Table 15.11). Stroke models are reviewed in [157].

15.3.7.1 Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease, which is caused by the excessive expression of cytotoxic polyglutamine (poly-Q) in the mutant huntingtin protein HTT and death of medium spiny neurons due to HTT toxicity and the lower expression of brain-derived neurotrophic factor (BDNF) [158]. Unfortunately, there is currently no cure for HD [159]. Canals et al. showed that disease onset is dependent on BDNF expression levels in the R6/2 HD mouse model and BDNF can improve symptoms and extend life span [160]. With this notion in mind, efficacy of MSCs expressing BDNF and nerve growth factor (NGF) transplantation in the YAC128 HD mouse model were evaluated [153]. For the YAC128 HD model, hyperkinesis starts at 3 months of age with progressive motor

Table 15.11 Summary of neuroprotection-related gene expressing MSCs in neurodegenerative disease preclinical models

Transgene	Model	Route of administration	Effect	Ref
BDNF	YAC128 Huntington's mouse model	Intrastriatal injection	Ameliorated symptoms of HD	[153]
GDNF	SOD1 ALS rat model	Intramuscular injection	Increased the number of neuromuscular connections and motor neuron cell bodies in the spinal cord and prolonged survival	[154]
GDNF	6-OHDA rat Parkinson's disease model	Intrastriatal injection	Decreased amphetamine-induced rotation and rejuvenated tyrosine hydroxylase-immunoreactive fibers in short-term treatment	[155]
BDNF	6-OHDA Parkinson's disease model	Intrastriatal injection	Decreased clinical symptoms and rejuvenated DA neurons	[156]

Abbreviation: *BDNF* Brain-derived neurotrophic factor, *GDNF* Glial cell-derived neurotrophic factor, *HD* Huntington's disease, *YAC128* yeast artificial chromosome containing 128 CAG repeats, *SOD1* superoxide dismutase 1, *ALS* Amyotrophic lateral sclerosis, *6-OHDA* 6-hydroxydopamine, *DA* dopaminergic

neuron impairment at 6 months of age and neurodegenerative features showing at 9 months of age. In the report by Dey et al., they transplanted BDNF secreting MSCs in a preventive manner at 4 months of age, which is ahead of the onset of motor neuron impairment. As expected, BDNF expressing MSCs transplanted into the striatum of HD mice showed significant improvement in motor function, as measured by performance on the rotarod, and significant reduction in levels of hindlimb clasping, a hallmark phenotype of affected HD mice. The least amount of neuronal loss within the striatum of the YAC128 mice at 13 months of age was observed in those transplanted with the growth factor-producing MSCs.

The underlying mechanisms for the beneficial effects from BDNF overexpressing MSCs are not completely known. These effects may represent a combination of the anti-apoptotic and axon-extending properties of MSCs, combined with the effects of the naturally produced neurotrophins in conjunction with the additional BDNF expression. BDNF therapies for HD have been extensively reviewed by Zuccato and Cattaneo [161–163]. It will be interesting to see if the effects of BDNF expressing MSCs can help to prevent the worsening of the symptoms or reverse the course of disease progression in this currently untreatable severe neurodegenerative disorder.

15.3.7.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a neurodegenerative disorder caused by the loss of motor neurons connected to muscle, and failure of this neuromuscular junction leads to paralysis of patients [152]. Most ALS cases are sporadic events and only 10% of them are familial cases. Currently,

the underlying disease etiology of sporadic ALS is unknown but in familial cases, it is linked to point mutations of cytosolic Cu^{2+}/Zn^{2+} superoxide dismutase 1 (SOD1). Currently, there are no treatments available that prevent neuromuscular decline to significantly delay the progression of ALS.

Glial cell-derived growth factor (GDNF) has been shown to have neuroprotective function in motor neurons of the SOD1 ALS mouse [164]. Following intramuscular transplantation of MSCs engineered to express GDNF in a rat model of familial ALS, Svendsen and colleagues showed an increased number of neuromuscular connections and motor neuron cell bodies in the spinal cord at mid-stages of the disease [154]. Furthermore, they showed that GDNF secreting MSCs could delay the disease progression and significantly extended lifespan in the SOD1 rat model [154].

15.3.7.3 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disease caused by the progressive degeneration of dopaminergic (DA) neurons in the midbrain [165]. Currently, there is no cure for PD, although implantation of deep brain stimulation devices and pharmacological agents can ameliorate clinical symptoms [166]. Different growth factors have been evaluated in an attempt to recover the damaged DA neurons, or to delay the rate of decline. GDNF secreting MSCs promoted rejuvenation of host striatal DA fibers and improvement in DA-dependent behavioral function in a rat model of PD [155]. Similar results were confirmed using BDNF secreting MSCs, where intrastriatally injected BDNF overexpressing MSCs showed improved clinical symptoms and rejuvenated striatal DA fibers in a rat model of Parkinson's disease [156].

15.4 Beyond the Preclinical Models: Future Directions for Genetically Engineered MSC Therapy in Working Toward Human Clinical Trials

In human clinical trials, safety is one of the major concerns and it is critical to identify and minimize risks associated with treatment [46]. In current MSC culture protocols, MSCs are cultured in fetal bovine serum-containing media. To generate safe and clinically acceptable MSC expansion protocols, xeno-free cell culture media should be better developed to allow optimized growth and subsequent in vivo function, while minimizing the risk of transmitting pathogens or causing human immune reactions [167]. In the case of hESC culture, Martin et al. found that xenogeneic serum replacement is the source of nonhuman sialic acid Neu5Gc, which causes immunological reactions involving human antibodies [168]. Therefore, human clinical applications of MSCs would best employ chemically defined media.

Karyotypic stability is a highly important criterion for any cell type expanded in culture, prior to consideration of clinical trials. In contrast to murine MSCs which

can easily undergo transformation in culture, there has been no documented evidence that human MSCs can be transformed during short-term in vitro expansion due to the development of chromosomal abnormalities. There has been one report that was retracted because it was found that the MSC cultures were contaminated with HeLa cells [169–171]. This type of contamination can occur readily in laboratories that use aspiration flasks to remove media from culture flasks, since all cell cultures are eventually mixed by regurgitation from the hose line, in spite of new sterile pipettes for each culture. Aspiration flaks must never be used in Good Laboratory Practices or in Good Manufacturing Practice Facilities, to ensure the identity of the culture. A second group, de la Fuente et al. retracted their paper because they could not reproduce the transformation data [172]. Nevertheless, well characterized in vitro MSC culture protocols and carefully adhered to standard operating procedures must be followed, along with the establishment of sensitive techniques to investigate chromosomal abnormalities.

Even though genetically engineered MSCs showed enhanced efficacy in the various preclinical disease models discussed here (including cardiovascular, cancer, bone formation defects, renal damage, neurological diseases, and others), there are so far no studies approved to move forward with human clinical trials. Currently, there are 123 human clinical trials registered using MSCs worldwide and all of the studies are utilizing unmodified MSCs [173].

There are barriers toward human clinical trials using genetically modified MSCs as there is no safe standard protocol to engineer MSCs to express transgenes. Each vector system has its own advantages and limitations with regard to efficacy and safety for the planned human clinical trials. As discussed earlier, most preclinical studies have utilized permanently integrating viral vectors as a delivery method for the gene of interest in order to continually express the transgene. Transient expression of genes of interest are not effective in most cases, but can be used to modify surface antigens of MSCs in order to increase MSC homing capacity and survival after transplantation. The risk of insertional mutagenesis caused by viral cassette integration into the host genome must be considered prior to the planned clinical trial. The potential risk to benefit ratio for that disorder or disease must be carefully evaluated, as is currently done for hematopoietic stem cell gene therapy applications [20].

Site-specific integration can be one of the options to eliminate insertional mutagenesis. It is well known that AAV vectors including rep protein integrate into chromosome 19 [174]. With rep, however, the cloning capacity of AAV vectors is reduced significantly. Annealing two inverted repeats (ITRs) can extend its cloning capacity to double the size of the insert [175]. These modified AAV vectors can be one of the options to avoid insertional mutagenesis with sustained expression of the genes of interest. Zinc-finger nuclease (ZFN)-mediated homologous recombination (HR) could be another option to modify MSCs safely, if success rates can be improved [176–179]. Zinc fingers have specific binding sites to DNA and engineered zinc-fingers with nucleases can cut the specific genomic regions of DNA. After the cleavage of specific DNA by ZFNs, the therapeutic cassette can be inserted by Homologous Recombination to create safe site-specific integration. Even though there are tremendous efforts to optimize ZFNs, nonspecific cleavages by ZFNs are still problematic [180].

Benabdallah et al. investigated whether ZFN-mediated targeted gene addition to safe harbor sites are possible [181]. They inserted Epo into the C–C chemokine receptor 5 (CCR5) gene loci, a putative safe harbor site, in MSCs by ZFN-mediated HR. Up to 40% of MSCs were successfully modified with EPO in CCR5 loci. Then, they injected these modified MSCs into NOD/SCID interleukin-2Rγ null (NSG) mice and these mice showed higher hemocrit levels in comparison with unmodified MSCs.

In the practical setting, identification of genetically engineered MSCs, which have safe harbor integrations of the transgenes, is restricted due to the limited lifespan of primary MSCs during in vitro expansion. Aging, moreover, significantly reduces the survival and differentiation potential of BM-MSCs [45]. hESC or hiPSC-derived MSCs can be considered in this case. With human pluripotent stem cells (hESC or hiPSC), a vector integration site could be mapped and cells with safe harbor integrations could potentially be expanded nearly indefinitely to generate differentiated MSCs with safe harbor integrations. Our group and others are working toward this future goal [46]. We have shown that pluripotent stem cell-derived MSCs can perform in vivo in a manner analogous to adult MSCs, by homing into areas of hypoxic injury [43].

With current techniques, it is difficult to track where the transplanted MSCs go in humans and to evaluate their long-term survival and function [14, 46]. Gene marking studies, using non-therapeutic genes such as eGFP or luciferase to track transplanted cells, are prohibited in clinical trials. Therefore, mysteries remain and can only be deciphered from large animal models. To ensure integrity and safety of the transplanted MSCs, suicide genes can be utilized to eliminate gene-modified MSCs if they are found to cause problems in patients [182, 183]. Schuldiner et al. showed that HSV-TK expressing hESCs had self-renewal and pluripotency and were sensitive to ganciclovir to kill cells [182]. They, moreover, could ablate teratoma that had arisen from the subcutaneous injection of undifferentiated TK-hESCs by intraperitoneal injection of ganciclovir. However, caution must be used when considering this strategy for MSCs, since TK has bystander effects on nearby cells and MSCs are known to effectively transfer the protein.

15.5 Conclusions

MSCs have been shown to be safe and have early evidence of efficacy in various clinical trials for heart attack, stroke, graft-vs.-host disease, and multiple sclerosis, among others [184–188], but some problems still need to be solved and efficiency and reproducibility need to be improved. Genetically modified MSCs can potentially overcome these barriers to increase the efficiency of therapy for many disorders. Given the possibility of immune reaction or insertional mutagenesis for vector transduced MSCs, long-term observation of modified MSCs must be followed carefully to meet safety regulations. The field looks to future applications of gene delivery to safe harbor sites to improve biosafety. Since they are powerful delivery

vehicles and potent protein synthesis factories, the use of gene-modified MSCs to provide missing enzymes or growth factors and other signals to improve the repair of damaged or diseased tissues holds almost unlimited potential.

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