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REVIEW

Growth Factor Gene-Modified Mesenchymal Stem Cells in Tissue Regeneration

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Abstract: There have been marked changes in the field of stem cell therapeutics in recent years, with many clinical trials having been conducted to date in an effort to treat myriad diseases. Mesenchymal stem cells (MSCs) are the cell type most frequently utilized in stem cell therapeutic and tissue regenerative strategies, and have been used with excellent safety to date. Unfortunately, these MSCs have limited ability to engraft and survive, reducing their clinical utility. MSCs are able to secrete growth factors that can support the regeneration of tissues, and engineering MSCs to express such growth factors can improve their survival, proliferation, differentiation, and tissue reconstructing abilities. As such, it is likely that such genetically modified MSCs may represent the next stage of regenerative therapy. Indeed, increasing volumes of preclinical research suggests that such modified MSCs expressing growth factors can effectively treat many forms of tissue damage. In the present review, we survey recent approaches to producing and utilizing growth factor gene-modified MSCs in the context of tissue repair and discuss its prospects for clinical application.

Keywords: growth factor, mesenchymal stem cell, tissue regeneration, genetic engineering

Background

In settings where the human body is unable to partially or fully heal a given tissue injury, the use of stem-cell based regenerative therapies offers great promise as a means of improving patient outcomes.[1] Indeed, such therapies can support heart or kidney transplants, bone reconstruction, or the repair of skin, cartilage, and neurons. In patients suffering from pathological conditions, such therapies can also potentially restore compromised tissue function.[2–4] Mesenchymal stem cells (MSCs) are a form of multipotent stem cell capable of differentiating into a subset of distinct cell types such as myocytes, adipocytes, chondrocytes, and osteoblasts. As they are capable of differentiating into several cell types, homing to target tissues, and secreting growth factors and immunomodulatory compounds, MSCs represent an ideal cell type to use for treating a range of disease types. Importantly, these cells can also be easily obtained and amplified in vitro without engendering substantial ethical concerns, allowing them to be safely and readily used in patients.

Most organs in human adults are limited in their ability to undergo tissue regeneration, instead undergoing scarring that can disrupt organ function. As such, the utilization of MSCs to facilitate true tissue reconstruction rather than scarring represents an ideal means of maintaining normal tissue function in the context of injury. Many studies to date have explored the ability of MSCs to support bone development, restoration of ventricular functional, and improved renal tubular

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function in vivo and in clinical settings.[4–6] Unfortunately, however, these cells are limited in their therapeutic efficacy, particularly in contexts where injuries or the associated ischemic damage are severe and irreversible. Indeed, preclinical animal models suggest that MSCs have a poor ability to engraft, and they are also hampered by limited homing and survival in vivo owing to factors including inflammation, ischemia, and anoikis.[7] One strategy proposed to overcome such limitations centers on the use of MSCs engineered to express specific genes.

Growth factors (GFs) are well known to be key mediators that can support MSC survival and proliferation, in addition to being key drivers of tissue regenerative processes. Many recent studies have utilized MSCs in order to deliver specific GFs to a target site of tissue regeneration either via utilizing cells naturally secreting these factors, or by engineering these cells to overexpress GFs of interest. Indeed, many recent studies have explored the therapeutic potential of MSCs engineered to express particular GFs in a therapeutic context. In the present review, we offer an overview of recent studies exploring the application of GF gene-modified MSCs in the field of tissue repair and reconstruction.

The Relationship Between MSC Biology and GF Secretion

MSCs are a readily isolated cell type that expand rapidly in culture without losing the ability to undergo self-renewal, permitting their use for reconstructing damaged tissues and organs via extensive amplification.[8] In addition to their multipotent ability to differentiate into a range of cell types, MSCs can orchestrate and enhance proximal or distal cell functionality via paracrine signaling and endocrine mechanisms. Studies have shown MSCs to be capable of promoting tissue regeneration via secreting exosomes and GFs including hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF).[9] Additionally, these cells express high levels of factors known to regulate hematopoietic cell function such as CXCL12, vascular cell adhesion molecule 1, interleukin-7, angiopoietin-1 (Ang-1), and osteopontin.[10] Consistent with these findings, in vivo studies also support the fact that the paracrine secretion of GFs by MSCs is a key mechanism whereby they support target tissue healing, as while these cells can migrate to sites of injury, the cells derived therefrom contribute only to a limited degree to therapeutic efficacy. Many recent studies have suggested that the secretion of GFs and other bioactive molecules may be one of the primary mechanisms whereby MSCs mediate their therapeutic efficacy. These secreted compounds can inhibit a range of processes such as apoptotic cell death and fibrosis,[11] in addition to being able to drive angiogenesis,[12,13] and to regulate the immune response.[14,15]

Without any exogenous manipulation, MSCs achieve limited therapeutic efficacy due to their poor survival and limited GF secretion upon transplantation. The therapeutic efficacy of MSCs ultimately depend upon the number of cells implanted, the function of these cells, when they are administered, and what condition they are being used to treat.[9,16-18] Poor MSC engraftment can be attributable to limited cell survival as a consequence of ischemia, anoikis, loss of trophic factors, or localized inflammation.[19] It is thus vital that MSC survival and differentiation be improved following transplantation in order to enhance therapeutic outcomes in treated patients. To that end, studies have explored the use of MSCs modified to express certain exogenous genes that can enhance their ability to promote angiogenesis and target tissue homing.[13,20] These genetically engineered MSCs can thereby both improve MSC engraftment and functionality, while also allowing for the targeted delivery of therapeutic gene products that can enhance local tissue healing.[21] Indeed, MSCs can secret a broad profile of active molecules including hematopoietic growth factors, angiogenic growth factors, trophic molecules, immunomodulatory cytokines, and chemokines. The best-characterized GFs and cytokines produced by these cells are compiled in Table 1. Based on these previous findings, it is clear that engineering MSCs to overexpress GFs may be an optimal means of improving the therapeutic efficacy of these cells.

Vectors Used for GF Overexpression in MSCs

Both non-viral vectors such as lipids or polymers, as well as viral vectors (including retroviruses, adenoviruses, lentiviruses and adeno-associated viruses) have been used to mediate GF overexpression in MSCs. The most common vectors used for such approaches are compiled in Table 2.[31–39] Using viral vectors to insert genes into MSCs is a high transduction efficiency approach that has the potential to induce off-target effects owing to insertional mutagenesis.[32,35,40,41] Viral systems are also limited by relatively small transgene cargo capacity, high production cost, difficulties in production and scale-up, and adverse

Table 1 Secretome of Mesenchymal Stem Cells

Type of Secreted Factors	Active Molecules	Ref
Hematopoietic growth factors	SCF, FLT3LG, Thrombopoietin, IL-3, IL-6, GM-CSF, M-CSF	[22–24]
Angiogenic growth factors	HGF, VEGF, Angiopoietin, PDGF, IGF-1, FGF-2, FGF-4, FGF-7	[22,23,25,26]
Trophic molecules	Adiponectin, Adrenomedullin, Osteoprotegerin, MMP10, MMP13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, Leptin, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, BDNF, GDNF, NGF, PIGF	[22,23,27]
Immunomodulatory cytokines	IL-1α, IL-1β, IL-2, TSG-6, OSM, IL-7, IL-10, IL-11, IL-12, IL-13, IL-16, IFN-γ, TNF-α, LIF, TGF-β, MIF	[23,24,28]
Chemokines	CCL1, CCL2, CCL5, CCL8, CCL11, CCL16, CCL18, CCL22, CCL23, CCL24, CCL26, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCL11, CXCL12, CXCL13, CX3CL1, XCL1	[22,23,29,30]

Abbreviations: SCF, stem cell factor; FLT3LG, Fms-related tyrosine kinase 3 ligand; IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factors; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; FGF, fibroblast growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; IGFBP, insulin-like growth factor-binding protein; BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor; NGF, nerve growth factor; PIGF, placenta growth factor; TSG, tumor necrosis factor-stimulated gene; OSM, oncostatin; IFN, interferon; TNF, tumor necrosis factor; LIF, leukemia inhibitory factor; TGF, transforming growth factor; MIF, macrophage migration inhibitory factor; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; CX3CL, C-X3-C motif chemokine ligand; XCL, X-C motif chemokine ligand.

immune reactions. There are advantages and disadvantages to all known viral vectors, with the selection of an appropriate vector being dependent upon transduction rates and the desired duration of treatment and target gene expression. It is also essential that such modified MSCs be extensively screened for safety reasons, thus potentially reducing the cost-effectiveness of such approaches in a clinical context.

To avoid the limitations of viral vectors, non-viral vectors such as nanoparticles (NPs) or cationic liposomes have been utilized to deliver vectors into MSCs. These alternative delivery strategies are more scalable and flexible, easier to synthesize and target to tissues, less likely to drive immune stimulation, and more amenable to scale-up manufacturing.[37] However, the disadvantages of non-viral vectors can include their transient expression with low efficiencies, and their potential for associated toxicity. He et al[42] utilized the cationic polymer pullulan-spermine to overexpress HGF encoded in the pMEX vector in MSCs, resulting in high in vitro HGF expression.[43] In contrast to such success, however, Tan et al[44] found that such plasmid-containing liposomes were only able to mediate FGF expression in BMSCs at a relatively low transfection efficiency, although they were able to achieve expression at levels sufficient to support periodontal regeneration. Still other authors have utilized lipid-based NPs to achieve target gene expression in MSCs for a sustained period of time.[45,46] These vectors, however, have recently been suggested to have the potential to induce genotoxicity, thus potentially mediating oncogenesis.[47,48] It is thus important to weight the relative costs and benefits of these different strategies to MSC engineering in order to produce a safe, effective, and sustainable approach for clinical use.

The Impact of GF Overexpression on MSCs in Tissue Regeneration

The Primary Impact of GF Gene-Modified MSCs in Tissue Regeneration

Many previous studies have explored the ability of GFs to regulate MSC growth in vitro via adding these GFs to cell culture media and/or by inhibiting their cognate receptors, allowing for the study of concentration-dependent effects. When MSCs are treated with GFs including FGF-2, PDGF-B, TGF-β1, and VEGF-A, this has been shown to result in enhanced production and secretion of GFs by MSCs.[49] As such, overexpressing target GFs in MSCs may be able to yield similar therapeutic effects to those observed upon adding recombinant GFs to MSC cultures, although they can also affect the biology of cells in a therapeutically uncertain manner. Indeed, the secretion of exosomes and GFs such as HGF, FGF-B, and VEGF is potentially key to the regenerative abilities of MSCs.[23] When these GFs are overexpressed, this is associated with significant enhancement of MSC-mediated regeneration of tissues, making

Table 2 Summary of Common Vectors Used for GF Expression in MSCs

Types of Vectors	Commonly Used Examples	Transduction Efficiency in MSCs	Advantages	Disadvantages	Preclinical or Clinical Application	Ref
Viral vector	Retrovirus	74.8–85.6%	Long-term stable gene expression	Insertional mutagenesis and activation of oncogenes	Preclinical	[32]
	Adenovirus	76.2–80%	Lower risk of genotoxicity	Transient gene expression	Preclinical	[35]
	Lentivirus	96.3–99.1%	Long-term stable gene expression	Insertional mutagenesis	Clinical	[32]
	Adeno-associated virus	≥ 65%	Long-term gene expression; non-immunogenic	Limited transport capacity	Preclinical	[36]
Nonviral vector	Physical methods					
	Electroporation	68.0–80.0%	Moderate transfection efficiency	Low cell viability	Preclinical	[34,37]
	Nucleofection	51.0–88.0%	Moderate/High transfection efficiency	Low cell viability	Preclinical	[34,38]
	Chemical methods					
	Lipid and polymeric agents	2.0–35.0%	Low immunogenicity	Low transfection levels, cytotoxic	Preclinical	[33,38]]
	Dendrimers	10.0–17.0%	Low cytotoxicity and immunogenicity	Low transfection levels	Clinical	[33,39]
	Inorganic nanoparticles	25.0–75.0%	Wider availability, controlled delivery, low toxicity	Only moderate transfection efficiencies	Preclinical	[31,33]

Abbreviations: GF, growth factor; MSC, Mesenchymal stem cell; Ref, references.

such GF overexpression strategies a focus of key therapeutic interest. A general overview of the therapeutic utilization of GF gene-modified MSCs is shown in Figure 1. First, MSCs are extracted from humans or animals, identified, and amplified. Second, the GF gene of interest is integrated into the vector and jointly introduced into the MSCs. Third, GF modified MSCs are delivered to the target tissues of the recipient organism wherein they can play a therapeutic role via secreting GFs, promoting angiogenesis, and enhancing homing functions.

The Selection of GFs in MSC Modification

Initially selection of GFs used to treat MSCs was based on prior understanding of the role of these GFs in cellular differentiation and morphogenesis, with experiments being aimed at exploring the ability of these GFs to drive MSC differentiation towards particular lineages. For example, HGF is a multifunctional factor produced by MSCs which can bind to its cognate receptor c-Met on cells of the vascular endothelium.[50] Studies using mice lacking expression of HGF in specific tissues highlighted the ability of this GF to support tissue repair and regeneration,[51] and the implantation of MSCs overexpressing HGF led to enhanced left ventricular remodeling,[52] reductions in neurological deficits,[53] and enhanced liver function.[43] Similarly, MSCs engineered to overexpress VEGF have been shown to enhance the viability of cells in the context of in vitro hypoxia and can also improve capillary formation in animal models of myocardial infarction,[54] hind limb ischemia,[49] and skin defects.[55] Certain GFs exhibit similar repair effects in MSCs for many tissue types. For instance, angiopoietin-1 (Ang-1) is a growth factor that specifically acts on endothelial cells and can drive angiogenesis.[56] MSCs overexpressing Ang-1 have been shown to inhibit cardiac remodeling and to drive improved myocardial angiogenesis and arteriogenesis relative to

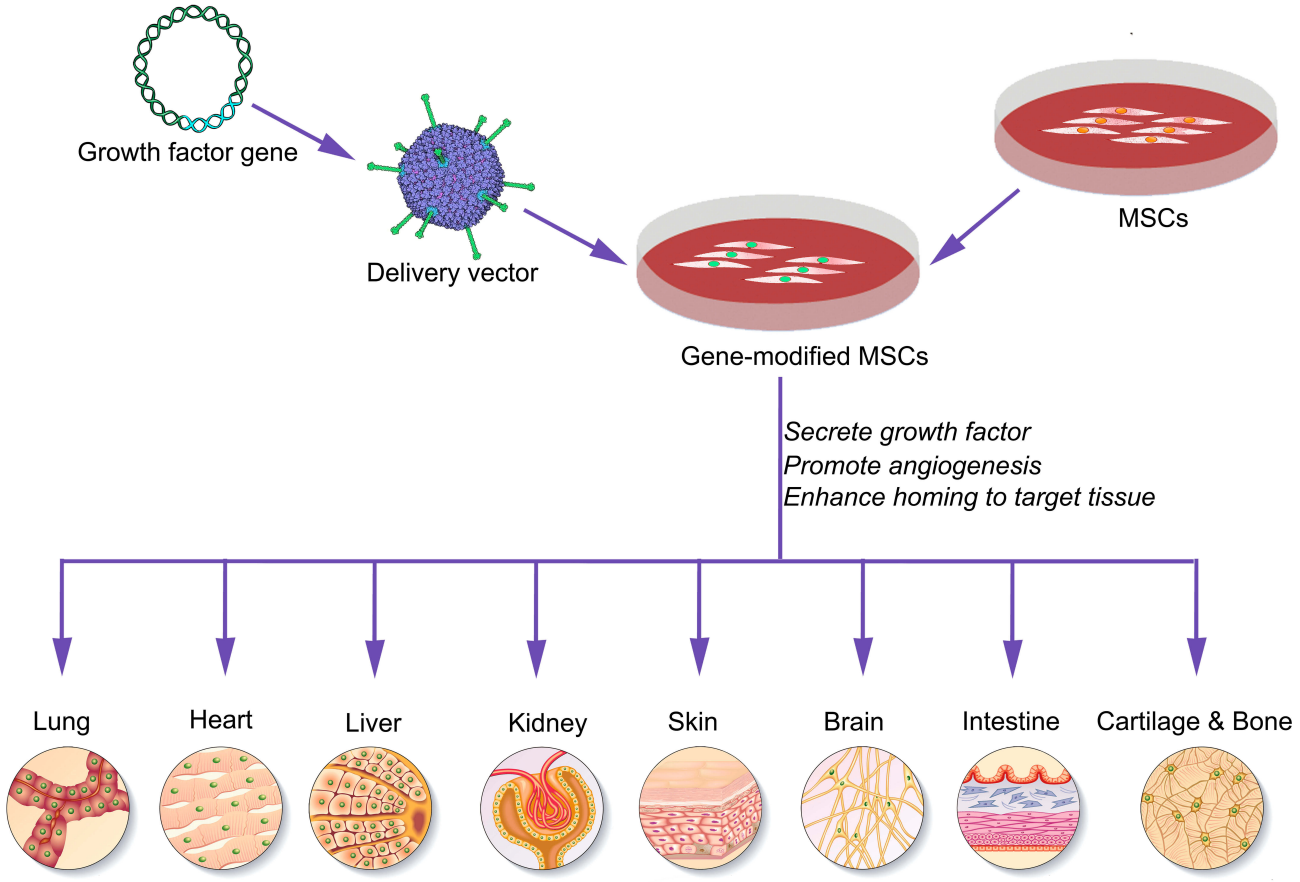

Figure 1 An overview of the therapeutic utilization of GF gene-modified MSCs.
Abbreviation: GF, growth factor; MSC, Mesenchymal stem cell.

control MSCs.[57] Such cells were also able to markedly reduce pulmonary inflammation[58] and to facilitate tissue repair.[59] MSCs overexpressing Ang-1 have also been shown to improve wound healing in a rat model system, enhancing angiogenesis in addition to dermal and epidermal tissue regeneration.[60] Tissue-specific repair factor modifications enhance the repair capabilities of MSCs in specific tissues. For example, BDNF promotes the survival and differentiation of neuronal tissue by acting on receptor kinases,[61] and BDNF-MSCs have primarily been used to promote the survival of neurons in the context of brain injury.[62,63] Similarly, TGF family proteins are closely linked to MSC survival and differentiation.[64,65] In particular, TGF-β superfamily genes are often used to drive MSC chondrogenic differentiation.[66] Therefore, TGF-β1 has been chosen to engineer rat MSCs to support enhanced regeneration of cartilage.[42] Hence, the selection of a particular GF for use in the modification of MSCs depends upon the effect of growth factors on MSCs and also on the response of the damaged tissue itself.

MSCs Overexpressing Multiple GF Genes Exhibit Therapeutic Utility

The primary mechanism whereby such gene-modified MSCs contribute to tissue repair is via the secretion of these multifactorial GFs rather than via their ability to differentiate into particular cell types, with these cells serving key roles in inhibiting fibrosis and inflammation while promoting angiogenesis.[49] Some studies have modified MSCs to express multiple synergistic genes in an effort to enhance their therapeutic utility. For example, IGF-1 is a GF that promotes cell survival,[67] whereas HGF promotes angiogenesis while suppressing fibrosis and inflammation.-[18] In a rat model of myocardial infarction, human adipose-derived stem cells that continuously produced IGF-1 and HGF were able to achieve a 1.3-fold increase in medium-sized blood vessel density at the infarct border zone relative to control cells.[68] In another study using a porcine model of myocardial infarction, such MSCs overexpressing HGF and IGF-1 were able to drive angiogenesis and suppress

inflammation more effectively than other cells, although these cells also exhibited enhanced fibrosis suggesting that combined IGF-1 and HGF exposure over extended periods of time can induce both beneficial and counterproductive effects.[69] This suggests that the preparation of MSCs secreting both IGF-1 and HGF may not be an effective means of synergistically effective cardiac repair, with the elevated levels of either factor in the local environment potentially contributing to this effect.

The Role of Exosomes Derived from GF-Modified MSCs in Tissue Regeneration

Multiple studies have indicated a role for MSCs in regenerative medicine through their paracrine effects and ability to produce exosomes.[17,70] Encapsulated with a lipid bilayer, exosomes can protect their contents from degradation and can transport a variety of small biomolecules including mRNAs, miRNAs, and proteins to surrounding cells. Moreover, MSC sources and culture conditions have been shown to influence the regenerative responses induced by exosomes, as a number of GFs can be detected in MSC-derived exosomes, including HGF, IGF family members, FGF2, and platelet-derived growth factor-AA (PDGF-AA).[69] As natural vesicles suitable for gene delivery, MSC-derived exosomes exhibit a broad range of therapeutic effects, and can mediate tissue repair, immunological regulation, and inflammatory control.[70,72] Moreover, recent studies have revealed that MSC-derived exosomes can mediate therapeutic benefits in animal disease models, with previous studies of bone fracture, cutaneous wound, myocardial infarction, and acute hepatic injury all having demonstrated the clinical utility of such exosomes.[16,17,70,73] Exosomes can modulate the differentiation and migration of MSCs in a targeted manner, offering an opportunity to promote tissue regeneration in a cell-free manner. Genetic manipulation can also be used to control the levels of such GFs in these exosomes, as in studies in which huc-MSCs were engineered to secrete GFs in a controlled fashion over an extended period.[71] Such genetically modified MSC-derived exosomes may thereby be able to mediate tissue regenerative benefits, making them ideal for future therapeutic regenerative regimens.

Preclinical Use of GF-Modified MSCs in Tissue Regeneration

The therapeutic value of MSCs stems largely from their ability to mediate angiogenesis and tissue regeneration,[75] secreting GFs and exosomes to achieve therapeutic efficacy and homing to target tissue sites.[9,16,17] A number of preclinical studies to date have sought to use genetically-modified MSCs that secrete GFs in order to treat a wide range of conditions associated with tissue injuries. A detailed overview of the uses of GF-modified MSCs in preclinical tissue repair studies is given in Table 3.

Central Nervous System (CNS) Lesions

Occlusive cerebrovascular diseases can result in cerebral ischemia and significant neuropathology, leading to the exploration of many modes of treating such diseases including the application of MSC-based therapies. One of the keys to treating CNS lesions is to maintain the integrity of the blood-brain barrier and to reduce edema in the context of ischemia, thus reducing the severity of injury. Importantly, MSCs can home to the CNS in vivo, allowing them to improve functional recovery following stroke owing to their ability to drive angiogenesis and neurogenesis while suppressing local inflammation via GF and chemokine secretion.[88] MSCs overexpressing specific GFs can also help to facilitate efficient CNS tissue regeneration. For example, MSCs overexpressing HGF have shown superior efficacy in reducing neurological deficits in the rat middle cerebral artery occlusion (MCAO) model relative to unmodified MSCs.[53] Su et al[89] found that BMSCs engineered to overexpress GDNF using a lentivirus were able to protect against injury in PC12 cells, highlighting their potential therapeutic value in the context of Parkinson's disease. However, there are still many obstacles to the widespread use of this technique in CNS lesions. Intracerebral injection remains particularly difficult if the lesions are widespread and numerous. In addition, intra-arterial injection will increase risk of embolic events,[90] and intravenous injections typically result in few cells reaching the target sites.[91,92]

Ischemic Heart Disease (IHD)

In many nations, the primary cause of morbidity and mortality is myocardial infarction (MI),[93] and as such it is one of the most common targets of therapeutic efforts to engineer MSCs to facilitate tissue repair. Indeed, a number of genes have been proposed as targets for MSC-mediated delivery in the context of MI including HO-1,[94] IGF-1,[69] Ang-1,[57,81] SVV,[95] Bcl-2[96] and Akt1.[97,98] Over 30 clinical studies to date have been registered using MSCs for the treatment of MI, but these studies have suggested the need for improved therapeutic efficacy of these MSCs. Angiogenesis mediates clinical benefits via the formation, remodeling, and maturation of blood vessels in injured tissues, making GF engineering an ideal

Table 3 Preclinical Studies of the Use of Genetically Engineered MSCs in Tissue Repair

Disease	Therapeutic Modification	Vector	Cell Type	Cell Counts/ per Animal	Method of Administration	Effects	Ref
Transient MCAO	FGF-2	Replication-incompetent HSV-1 vector	Rat BMMSC	1×10^6/Rats	Administered intracerebrally	Enhanced survival, reduced infarction volume, improve functional recovery	[76]
Transient MCAO	BDNF	Adenoviral vector	Human MSC	5×10^5/Rat	Intracerebrally injection	Promotes the survival and differentiation, reduced infarct size	[63]
Transient MCAO	HGF	Multimutated herpes simplex virus type-1 vector	Rat BMMSC	1×10^6/Rat	Injected into the right striatum	Improved neurological deficits, reduced infarction volume	[53]
Huntington's disease	BDNF	Lentiviral vector	Human BMMSC	5×10^5 cells per hemisphere/ Mouse	Injected bilaterally into the striata with vehicle	Decreased striatal atrophy, reduced anxiety, induced increase in neurogenesis-like activity.	[77]
Parkinson's disease	BDNF	Electroporation	Rat BMMSC	5×10^6/Rat	Lateral ventricular injection	Reduce the DA metabolic rate, improve the level of DA, and improve the behavior of PD rats	[78]
Traumatic Brain Injury	BDNF	Adenoviral vector	Rat BMMSC	1×10^6/Rat	Intraventricular injection	Increased BDNF levels, attenuated neuronal injury	[62]
Myocardial infarction	VEGF	Bile acid-modified polyethyleneimine	Human BMSC	1×10^6/Rat	Injected intramyocardially into the contracting wall bordering the infarct	Improved cell viability, enhanced capillary formation in the infarcted region, attenuated left ventricular remodeling	[54]
Myocardial infarction	HGF	Retroviral vector	Rat BMMSC	2×10^6/Rat	Injected into three points around the infarct area	Improved left ventricular function, decreased infarcted scar area, and increased angiogenesis.	[52]
Acute myocardial infarction	HGF and IGF-1	Lentiviral vectors	Pig MSC from adipose tissue (paMSC)	50×10^6/Pig	7–8 injections surrounding the infarcted area	Reduced inflammation, promoted angiogenic processes	[69]
Myocardial fibrosis	HGF	Lentiviral vector	Rat BMMSC	2×10^7/Rat	Injected into the border zone of infarcted heart tissue	Enhance cell survival, improve cardiac function, stimulate angiogenesis, and reduce myocardial fibrosis	[79]
Myocardial infarction	VEGF	Adenovirus vector	Rat BMMSC	5×10^6/Rat	Injected into the border zone surrounding the infarcted area	Induced myocardial angiogenesis and cardiomyocyte regeneration, prevented progressive scar formation and heart dysfunction	[80]

(*Continued*)

Table 3 (Continued).

Disease	Therapeutic Modification	Vector	Cell Type	Cell Counts/ per Animal	Method of Administration	Effects	Ref
Acute myocardial infarction	Ang-1	Recombinant adenoviruses	Rat BMMSC	5×10^6/Rat	Injected into the border zone surrounding the infarct anteriorly and laterally	Increased capillary density and reduced infarct size	[57]
Ischemic myocardium	Angiopoietin	Adenovirus vector	Rat BMMSC	3×10^6/Rat	Injected into the ischemic myocardium	Induced differentiation, promoted angiogenesis, improved cardiac function	[81]
Myocardial infarction	Survivin	Lentiviral vector	Rat BMMSC	2×10^6/Rat	Intra-myocardial injections	Increased capillary density reduced the infarct size, inhibited collagen deposition, and further improved cardiac function	[82]
Radiation-induced intestinal injury	HGF	Adenoviral vectors	Human UC-MSCs	2×10^6/Mouse	Injected intravenously via the tail vein	Improved intestinal histopathology, reduced inflammation, and increased the proliferation and decreased the apoptosis of intestinal epithelial cells.	[83]
Radiation-wound injury	PDGF-A and BD-2	Adenoviral vector	Rat BMMSC	1×10^7/Rat	Injected into the wound bed and margin of the excisional wound.	Resulted in better granulation formation/maturation and skin appendage, promoted wound healing regeneration	[84]
Radiation-induced lung injury	TGF-β type II receptor	Adenoviral vector	Mouse BMMSC	5×10^5/Mouse	Intravenously injected	Attenuated early lung injury and improved survival and lung fibrosis	[85]
Radiation-induced liver injury	NGF	Plasmid vector	Mouse BMMSC	5×10^{10}/Mouse	Intravenously injected	Inhibited the apoptosis of mouse hepatic cells induced by radiation, improved the survival rate of mice	[86]
Cartilage defects	TGF-β1	Pullulan–spermine (nonviral gene vector)	Rat BMMSC three-dimensional (3D) reverse transfection system	1×10^6 cells per scaffold/Rat	TGF-β1 gene-transfected MSC seeded gelatin sponge was implanted to the full-thickness cartilage defect	Promoted chondrogenesis of MSCs, and improved cartilage regeneration.	[42]
Osteopenia	BMP-2	Adeno-associated virus	Mouse BMMSC	1×10^6/Mouse	Intravenously injected	Increased bone mineral density and bone mineral content, promoted proliferative capabilities of cells	[87]
Limb ischemia	VEGF	Lentiviral vectors	Human BMSC	1×10^6/Mouse	Injected into the tail vein	Induced the migration of endothelial cells and enhanced blood flow restoration	[49]

(*Continued*)

Table 3 (Continued).

Disease	Therapeutic Modification	Vector	Cell Type	Cell Counts/ per Animal	Method of Administration	Effects	Ref
Skin defect wound	VEGF	Recombinant adenovirus	Human UC-MSC	_	Injection into the wound	Promotes vascular endothelial cell division, proliferation and induction of vascularization	[55]
Chronic skin wound	Ang-1	Recombinant adenovirus vector	Rat BMMSC	1×10^6/Rat	Injected intradermally at the margin of the excisional wound at four injection sites	Promoted wound healing with increased epidermal and dermal regeneration, and enhanced angiogenesis	[60]
Periodontal regeneration	FGF-2	Plasmid - liposome complexes	Dog BMMSC	1×10^7/Dog	Transplanted into root furcation defects	Accelerated the proliferation, induced morphogenesis	[44]
Liver fibrosis	HGF	Plasmid vector	Human UCB-MSCs	1×10^6/Rat	Injected via the caudal vein	Migrated into the injured liver and expressed hHGF, improved the liver morphology and reversed liver damage	[43]
Lung injury.	Ang-1	Lentiviral vector	Mouse BMMSC	-	Intravenously administered via the jugular vein	Attenuated the inflammatory reaction and vascular leakage, improved lung histopathological changes	[59]
Severe acute lung injury	Ang-1	Nuclear-targeting electroporation	Murine BMMSC	2.5×10^5/Mouse	Infused via a jugular venous canula	Reduced pulmonary inflammation, improved both alveolar inflammation and permeability	[58]
Acute kidney injury	HGF	Adenovirus vector	Human UC-MSC	$1x10^6$/Rat	Injected via the left carotid artery	Promoted proliferation, decreased apoptosis and inflammation, and promoted the amelioration of renal function	[50]

Abbreviation: MSC, Mesenchymal stem cell; MCAO, middle cerebral artery occlusion; FGF, fibroblast growth factor; HSV, herpes simplex virus; BMMSC, bone marrow-derived mesenchymal stem cell; BDNF, brain-derived neurotrophic factor; HGF, hepatocyte growth factor; DA, dopamine; PD, Parkinson's disease; VEGF, vascular endothelial growth factors; IGF, insulin-like growth factor; Ang, angiopoietin; PDGF, platelet-derived growth factor; BD, beta-defensin; TGF-β, transforming growth factor-beta; NGF, nerve growth factor; BMP, bone morphogenetic protein; UC-MSC, umbilical cord-derived mesenchymal stromal cell; UCB-MSC, umbilical cord blood-derived mesenchymal stem cell.

means of achieved such angiogenic efficacy in a therapeutic setting. Moreover, among angiogenic growth factors, the HGF/Met pathway is a key mediator of cardiovascular remodeling following tissue injury,[99] with HGF mediating the migration and expression of cardiac-specific markers in MSCs.[100] Many studies have utilized murine, rat, and porcine models of MI to confirm the ability of such HGF-expressing MSCs to enhance cardiac function, drive angiogenesis, and decrease myocardial fibrosis.[79,101-103] In addition, human BMSCs expressing HGF have been shown to have enhanced anti-arrhythmic properties.[104] Following the delivery of these modified cells to the infarcted region, low local nutrient and oxygen levels can result in poor survival and engraftment efficiency. VEGF is known to enhance the survival of these and other cell types upon transplantation in damaged tissues.[105] Normally, angiogenesis in the infarcted tissue is not sufficient to meet the needs of the remaining viable myocardial tissue, thereby compromising contractile compensation.[80] Moon et al[54] found that MSCs overexpressing VEGF were able to induce a 1.4-fold increase in VEGF expression upon hypoxic exposure relative to cells grown under normoxic conditions, and these modified MSCs were able to facilitate the enhanced microvascularization of infarcted myocardial tissues.

Musculoskeletal Defects and Skin Injuries

Bone, muscle, and skin are all highly metabolized tissues with a relatively high vascular supply, based on the homeostasis of biomaterial structures that need to be studied for

growth and remodeling.[106] Kumar et al[87] found that mice transplanted with MSCs engineered to overexpress bone morphogenetic protein 2 (BMP2) exhibited increased bone mineral density and content and improved BMSC proliferation relative to control animals, with a corresponding improvement in bone formation. Dental pulp stem cells overexpressing HGF have also been shown to prevent bone loss in the early phase of ovariectomy-induced osteoporosis.[107] MSCs engineered to overexpress Ang-1 are also able to facilitate wound healing as well as dermal and epidermal regeneration and angiogenesis.[60] In addition, tissue engineering is usually achieved via inserting stem cells into three-dimensional scaffolds that are induced to generate new cells.[6,108] GF-modified MSCs have been widely used in this innovative treatment for musculoskeletal defects and skin wounds, with many studies having explored optimal tissue engineering approaches to improving the efficiency of cells, scaffolds, and bioactive factors.[33] The most commonly studied technique is to add supplemental growth factors that locally provide signals that mimic the process of bone regeneration.[109] It is therefore important to design systems that provide this biological cue in a time-controlled manner so as to mimic the normal bone healing process. Brunger et al attempted to develop a system using poly-L-lysine to immobilize a lentivirus encoding TGF-β3 in a 3D woven poly scaffold to induce robust and sustained cartilaginous extracellular matrix formation by hMSCs.[110]

Radiation Injury

Certain tissues including the lungs, intestines, and bone marrow are highly radiation sensitive. While hematopoietic stem cells can regenerate the bone marrow, strategies to mediate similar regeneration of lung and intestinal tissue are limited. GF-overexpressing MSCs may therefore represent an ideal approach to regenerating tissues following radiation injury and associated damage. For example, in a model of radiation-induced lung fibrosis, MSCs overexpressing HGF were shown to home to damaged lung tissue wherein they could promote epithelial cell proliferation and survival, thereby decreasing local inflammation and fibrosis.[104] Similarly, MSCs engineered to overexpress TGF-β2 using an adenoviral vector were able to reduce lung injury and protect alveolar type II cells from radiation-induced apoptosis and DNA damage while reducing local inflammation, highlighting the benefits of GF production by MSCs in a paracrine manner.[85] BMSCs engineered to express VEGF were similarly able to improve radiation-induced tissue injury repair owing to their ability to drive angiogenesis and regeneration of muscle fibers.[111] BMSCs modified to express both BD2 and PDGF-A using an adenoviral vector were also able to improve wound healing in a model of radiation-induced wounding.[84] MSCs overexpressing HGF suppress local inflammation and enhance small intestinal recovery in a murine model of radiation induced intestinal injury.[83] Irradiation of cardiac tissue can result in late cardiovascular complications, and HGF can reduce such radiation-induced cardiac injury in a model of irradiation-induced heart disease.[112] Adenoviral-mediated overexpression of HGF can also prevent radiation-induced hematopoietic damage[113] and can reduce radiation induced hepatic damage in a rat model system.[114]

Other Tissue Injuries and Diseases

In addition to the diseases mentioned above, MSCs modified to overexpress GFs have been employed to treat a wide range of tissue injuries and diseases in preclinical studies. Studies have shown that MSCs overexpressing HGF and Ang-1, respectively, can improve therapeutic outcomes in ischemia/reperfusion injury in the lung[115] and in a Phosgene-induced model of lung injury owing to their ability to decrease pulmonary inflammation and endothelial permeability.[116] Furthermore, MSCs modified to overexpress HGF have been shown to improve such AKI in a rat model of ischemia/reperfusion injury via reducing kidney inflammation and apoptotic cell death, thus making these cells of value to human therapeutic implementation.[50] Moreover, MSCs expressing HGF can also enhance liver regeneration, making them viable for the treatment of those patients suffering from liver fibrosis or cirrhosis.[43]

Clinical Trials Utilizing Genetically Modified MSCs

Given the number of preclinical studies demonstrating the potential utility of genetically modified MSCs, it is perhaps unsurprising that a number of clinical trials have been or are currently being conducted exploring the clinical value of such therapeutic approaches. To date over one thousand MSC-based trials have been conducted globally as reported in the US National Institute of Health database (ClinicalTrial.gov) in order to evaluate the safety and efficacy of either autologous or allogeneic MSCs. These trials are primarily focused on treating human diseases such as cancer,[117] metabolic and inflammatory diseases such as chronic obstructive pulmonary disease,[118] or adult respiratory distress syndrome.[119] These studies are primarily reliant upon the use of unmodified MSCs for

these clinical efforts, with very few studies to date utilizing genetically modified MSCs. In 2006, Ripa et al published the results of a trial initiated in 2003 piloting the combination of VEGF gene therapy and stem cell mobilization in patients with severe chronic ischemic heart disease, finding this approach to be safe in humans[120] (NCT00135850). Another relevant study aims to explore the use of MSCs overexpressing BDNF for the treatment of Huntington's disease (HD) patients in a pre-cellular therapy observational study[121] (NCT01937923). At present, however, this study has only enrolled a cohort of individuals early-stage HD in order to characterize their clinical and biomarker findings at baseline for comparisons to a planned future Phase 1 trial safety and tolerability trial applying these BDNF-modified MSCs. This trial has been submitted as an Investigational New Drug application to the Food and Drug Administration.[122]

A number of challenges still face the clinical implementation of GF gene-modified MSC-based therapies. Of particular difficulty is the production of clinical grade therapeutic products, as such cellular and gene therapies differ from traditional pharmaceutical compounds, instead representing a form of heterogenous biological product that can vary in response to a wide range of culture conditions. Modified MSCs also have the potential to become malignant upon transplant, and the use of recombinant viral vectors to manipulate these cells poses a significant safety concern.[109] Minimizing variability in sample preparation while still remaining cost-effective thus represents a significant challenge. Therefore, the production of modified MSCs for clinical applications must comply with the Good Manufacturing Practice (GMP) standards for medicinal products. These recommended approaches include product safety, cell characterization, and manufacturing process control.[123] Cell donors must be screened carefully and the stem cells expanded in the GMP production facilities should be tested using standardized procedures to assess their viability, sterility, genetic stability, tumorigenicity, adventitious agents, pyrogenicity, and mycoplasma infection status. Modified MSCs also have to be verified with respect to their identities, purity, stability and potency. In addition, the biological activity and toxicity of stem cell products must be tested in an applicable animal model under Good Laboratory Practice (GLP) conditions prior to administration into humans.[124] To ensure product efficacy, however, it is essential that these standardized production procedures do not compromise therapeutic efficacy. The fate of modified MSCs upon intravenous injection is also uncertain, as previous trials of unmodified MSCs have achieved limited efficacy owing to their quick elimination from circulation.[125] Therefore, to achieve significant functional benefits, this strategy requires a defined selection of the number and type of stem cells to be delivered, an explicit vector application method, and fixed transduction efficiency and time of administration. In addition, the design of novel bioactive materials such as three-dimensional spheroids[126] and nano-active scaffolds[109] to bolster stem cell survival, signaling, and function at the target site can also help to increase the cost-effectiveness of the applications of modified MSCs for tissue repair.

At present it remains unclear as to whether it will be legally permissible to utilize genetically modified MSCs for clinical treatment. The potential consequences of utilizing such cells in humans are not well understood, and as such the safety of these approaches needs to be more thoroughly examined in animal model systems in order to identify means overcoming any potential safety issues. In addition, many of the ethical issues associated with genetically-modified MSC research are similar to those arising in other MSC-based interventions. Efforts to address these issues typically focus upon minimizing the risk of harm, emphasizing the importance of informed consent and information disclosure, reducing the potential for overpromising, limiting excessive expectations and therapeutic misconceptions, and avoiding pressure from commercial entities and disease constituencies to move quickly into the clinic.[125,127] In addition, justice is a necessary consideration given that stem cell interventions can be extraordinary costly and labor-intensive,[123] as can many other novel biotechnologies. Justice necessitates that additional attention be paid to the cost of genetically modified MSC interventions in an effort to make them available, effective, and safe, with the goal of reducing unfair disparities in treatment accessibility. These ethical considerations continue to provide crucial guidance for the clinical application of these approaches not only for the trials specifically considered, but also for investigators exploring new translational medicine pathways.

Current Challenges and Future Prospects

The therapeutic utility of GF gene-modified MSCs has been a focus of increasing research interest in recent years owing to their enhanced ability to suppress inflammation, home to target tissues, regulate immune responses, and facilitate tissue repair. Several preclinical and clinical

studies have utilized MSC-based therapeutic strategies for treating a range of disorders and injuries. While efforts to modify MSCs to overexpress defined GFs are still in their early stages and are far from clinical application, although they offer a potentially ideal means of directed tissue regeneration. MSCs alone are limited in their ability to home to and survive in injured tissues, making the modification of MSCs to express such GF genes vital in order to facilitate more robust regenerative medicine approaches. While the outcomes of many of the studies reported in this review are promising, there remain many challenges which must be overcome. These include the need to optimize delivery strategies in human patients while simultaneously preventing immunogenicity or tumor formation. Preclinical findings highlight the safety and therapeutic efficacy of these GF-modified MSCs for the treatment of tissue damage.

In addition, large-scale, multi-center clinical trials are needed to conclusively demonstrate the long-term beneficial effects of such therapies.

Further ongoing clinical studies and efforts to demonstrate the long-term beneficial effects will help to ensure that these promising therapeutic tools soon become available to patients as a novel and efficacious form of regenerative medicine.

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Disclosure

The authors report no conflicts of interest in this work.

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