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Genetically Engineered Mesenchymal Stem Cells using Viral Vectors: A New Frontier in Anti-Angiogenic Therapy

(Sel Stem Mesenkima Kejuruteraan Genetik menggunakan Vektor Virus: Suatu Sempadan Baharu dalam Terapi Anti-Angiogenik)

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ABSTRACT

Mesenchymal stem cells (MSCs) are adult stem cells that possess the remarkable ability to self-renew and differentiate into various cell lineages. Due to their regenerative potential, MSCs have emerged as the most commonly used stem cell type in clinical applications. Angiogenesis, the formation of new blood vessels, plays a critical role in several pathological conditions, including ocular neovascular diseases, cancer, and inflammatory disorders. Conventional anti-angiogenic therapies face limitations such as frequent visits for repeated doses, off-target effects and resistance development. Recent advances in genetic engineering techniques have opened up novel avenues in regenerative medicine. Genetically engineering MSCs using viral vectors presents a promising strategy to specifically target angiogenesis and enhance anti-angiogenic therapies' efficacy. Viral vectors, including lentiviruses, adeno-associated viruses and adenoviruses, provide an effective means of delivering therapeutic genes into MSCs, allowing the expression of a wide range of therapeutic agents, including anti-angiogenic proteins. This review explores the frontier of using genetically engineered MSCs delivered through viral vectors as a potent anti-angiogenic therapeutic approach. By leveraging the unique properties of MSCs and the targeted delivery capabilities of viral vectors, this approach initiates the potential to revolutionize anti-angiogenic therapy, offering new possibilities for the treatment of angiogenesis-related diseases.

Keywords: Angiogenesis; anti-angiogenic therapy; genetic engineering; mesenchymal stem cells; viral vectors

ABSTRAK

Sel stem mesenkima (MSCs) adalah sel stem dewasa yang memiliki keupayaan luar biasa untuk memperbaharui diri dan berubah menjadi pelbagai barisan sel. Disebabkan potensi regeneratif mereka, MSCs telah menjadi jenis sel stem yang paling biasa digunakan dalam aplikasi klinikal. Angiogenesis, pembentukan saluran darah baru, memainkan peranan penting dalam beberapa keadaan patologi, termasuk penyakit neovaskular okular, kanser dan penyakit keradangan. Terapi anti-angiogenesis konvensional mempunyai kekurangan seperti lawatan kerap untuk dos berulang, kesan di luar sampingan dan pembangunan rintangan. Kemajuan terkini dalam teknik kejuruteraan genetik telah membuka peluang baharu dalam perubatan regeneratif. Kejuruteraan genetik MSCs menggunakan vektor virus merupakan strategi yang berpotensi untuk menyerang angiogenesis secara khusus dan meningkatkan keberkesanan terapi anti-angiogenesis. Vektor virus termasuk lentivirus, virus adeno-terkait dan adenovirus menyediakan cara yang berkesan untuk menghantar gen terapi ke dalam MSCs, membolehkan ekspresi pelbagai agen terapeutik, termasuk protein anti-angiogenesis. Kajian ini meneroka hala tuju penggunaan MSCs yang direka bentuk secara genetik yang dihantar melalui vektor virus sebagai pendekatan terapeutik anti-angiogenesis yang berkuasa. Dengan memanfaatkan sifat unik MSCs dan keupayaan penghantaran yang dituju oleh vektor virus, pendekatan ini berpotensi untuk mengubah terapi anti-angiogenesis, menawarkan kemungkinan baru untuk rawatan penyakit berkaitan angiogenesis.

Kata kunci: Angiogenesis; kejuruteraan genetik; sel stem mesenkima; terapi anti-angiogenesis; vektor virus

Introduction

Mesenchymal stem cells (MSCs), as mesenchymal stromal cells, are adult stem cells that can self-renew and differentiate into multiple lineages. They were first discovered in bone marrow but later in other tissues such as adipose tissue, muscle, peripheral blood, hair follicles, teeth, placenta, and umbilical cord (Ding, Shyu & Lin 2011). Mesenchymal stem cells have emerged as the predominantly utilized stem cell type in clinical settings. MSCs can migrate to injury sites in response to environmental signals and promote tissue regeneration by releasing paracrine factors with pleiotropic effects and different source and multilineage differentiation potential (Hmadcha et al. 2020).

Angiogenesis, the formation of new blood vessels, plays a crucial role in various pathological conditions, including ocular neovascular diseases, cancer, and rheumatoid arthritis. Uncontrolled angiogenesis is a hallmark of numerous diseases, often leading to the progression and spread of tumors or contributing to the pathogenesis of inflammatory disorders. Conventional anti-angiogenic therapies have limitations, such as frequent visits for repeated doses, off-target effects and the development of resistance. Genetically engineering MSCs using viral vectors offers a novel strategy to target angiogenesis and improve the efficacy of anti-angiogenic therapies specifically (Hu et al. 2008).

Recent advances in genetic engineering techniques have paved the way for novel approaches in regenerative medicine. One such strategy involves using MSCs, a population of multipotent cells with immunomodulatory and tissue repair properties (Damasceno et al. 2020). MSCs have a unique ability to home to sites of inflammation and injury, making them appealing candidates for targeted therapeutic delivery. MSCs can be genetically modified to improve their therapeutic properties and explicitly tailored for anti-angiogenic therapy (Pawitan et al. 2020). Viral vectors, which are derived from naturally occurring viruses, are an effective way to deliver exogenous genes into target cells such as MSCs (Hodgkinson et al. 2010). These vectors can be programmed to express a wide range of therapeutic agents, including anti-angiogenic proteins and peptides (Javan, Khosrojerdi & Moazzeni 2019). Viral vectors, including lentiviruses, adeno-associated viruses and adenoviruses, have been extensively employed to introduce therapeutic genes into MSCs (Varkouhi et al. 2020).

This review explores the novel frontier of using genetically engineered MSCs delivered through viral vectors as a potent anti-angiogenic therapeutic approach. We discuss the advantages and challenges associated with this strategy, highlight the recent progress made in preclinical and clinical studies, and shed light on the prospects of this emerging field.

By harnessing the unique properties of MSCs and the targeted delivery capabilities of viral vectors, this approach holds the potential to revolutionize anti-angiogenic therapy, opening up new avenues for the treatment of angiogenesis-related diseases.

MSCs as a Gene Delivery Vehicle

Mesenchymal stem cells (MSCs) are stromal cells that can self-renew and differentiate into many lineages (Via, Frizziero & Oliva 2012). The International Society for Cellular Therapy defines MSCs as cells with a specific immunophenotype, *ex vivo* plastic-adherent growth, and multilineage differentiation (Dominici et al. 2006). Although MSCs have a wide range of anti-inflammatory and immune-modulatory properties, as shown in the clinical trials using MSCs, the properties of cultured MSCs *in vitro* suggest they can have broader applications (Pittenger et al. 2019). MSCs' multipotent features make them an appealing candidate for developing pre-clinical and clinical trials (Ding, Shyu & Lin 2011).

In gene therapy, the delivery of foreign genetic material into host cells is crucial for the success of the treatment. There are three main categories of gene delivery methods: Mechanical methods such as microinjection or electroporation, chemical methods involving lipid or nanoparticle carriers, and biological methods using viral, bacterial, or cell-based vectors (Ramamoorth & Narvekar 2015). The success of gene therapy hinges upon the efficacy of the gene delivery vehicle to the MSCs. It must be able to carry a sufficient amount of genetic material to the targeted cells and facilitate efficient gene expression. The ideal gene delivery vehicle to the MSCs should possess several characteristics, such as the ability to sustain gene expression for the desired period, low or non-immunogenicity, and safety for human use (Mali 2013).

Benefits of Using MSCs as a Gene Delivery Vehicle

In current research on gene therapy, viral vectors and synthetic liposomes have become the preferred gene delivery vehicle options for clinical applications. However, the major drawback of using viral vectors is that they have been shown to trigger immunogenicity (Seow & Wood 2009). Hence, introducing genes into MSCs to serve as a gene delivery vehicle might overcome the limitations that arise from viral vectors.

MSCs can be an excellent choice of delivery vehicle due to their relative ease of isolation from various human tissues, such as bone marrow, Wharton's jelly from the umbilical cord, adipose tissue, and dental tooth pulp (Mansoor et al. 2019). MSCs can be propagated extensively through *in vitro* expansion without losing differentiative capacity (Porada & Almeida-Porada 2010). This accessibility facilitates their use in various therapeutic applications. Furthermore, MSCs can migrate and home to damaged tissues and tumors, known as homing (Gao et al. 2013; Lan et al. 2012). This homing ability is crucial for therapeutic applications, as it suggests that MSCs can be directed or recruited to sites of injury, allowing them to participate in tissue repair and regeneration, as demonstrated in studies involving corneal injury and tumor microenvironments. Lan et al. (2012) demonstrated this homing effect by showing a 2-fold increase of MSC circulation towards corneal injury sites in mice within 48 hours, but not in normal cornea. Marofi et al. (2017) reviewed that MSCs migration to the tumor site is strongly associated with generating inflammatory chemokines and growth factors within the tumor microenvironment. A wide range of adhesion molecules and toll-like receptors on the surface of MSCs strongly suggest their responsibility for tumor tropism.

Another feature of MSCs worth highlighting is their high capability to be genetically manipulated through *in vitro* applications (D'souza et al. 2015). Genetic manipulation can be performed using various vectors to express therapeutic proteins and then secrete these proteins into the damaged tissues or tumor sites. This opens up avenues for targeted and localized delivery of therapeutic agents. Wen et al. (2012) explored the use of allogeneic MSCs with adenoviral vector genetic modification that overexpressed the hepatocyte growth factor (HGF) gene. Transplantation of HGF-transgenic MSCs was performed one week after traumatic osteonecrosis of the femoral head (ONFH) in a rabbit model. The results showed recovery with decreased empty lacunae and increased vascular endothelial growth factor (VEGF) expression. The ability of genetically modified MSCs to promote recovery, as seen in studies involving traumatic osteonecrosis and hepatocyte growth factor (HGF) overexpression, highlights their therapeutic potential in diverse diseases. With engineered MSCs as a promising new treatment method for various diseases, continuous research and clinical trials can further explore their application in various medical conditions.

Moreover, MSCs have a low immunogenicity property. The low immunogenicity of MSCs is closely associated with the low expression levels of MHC class I and class II molecules, along with co-stimulatory molecules (García-Bernal et al. 2021; Hu et al. 2010). This unique property allows them to be used as an allogeneic transplant without HLA matching. In short, the unique immunologic tolerance of MSCs allows them to engraft into xenogeneic environments while preserving their ability to perform therapeutic effects toward targeted tissues or tumor sites (Esmaeilzadeh & Farshbaf, 2015). Expanding research in this area could lead to breakthroughs in developing effective treatments without the need for strict matching criteria.

Table 1 summarizes the *in vitro*, *in vivo* and up-to-date clinical trials using MSCs as gene delivery vehicles. The summarized clinical trials using MSCs as gene delivery vehicles demonstrate the translation of these findings into clinical investigations. Both clinical trials used autologous bone marrow derived MSCs. Continued efforts in conducting robust clinical trials, considering different sources of MSCs and targeting various critical diseases, will be essential to validate the safety and efficacy of MSCs-based gene therapies.

Despite the promising attributes of MSCs as gene delivery vehicles, several research gaps warrant further exploration. While the homing ability of MSCs to damaged tissues and tumors is acknowledged, a more comprehensive understanding of the underlying mechanisms is needed. Unraveling the intricate signaling pathways and factors influencing MSCs homing will contribute to enhancing their therapeutic efficacy.

MSCs have previously proven to be safe (Jung, Bauer & Nolta 2012; Sun et al. 2018); however, the long-term safety assessments of MSCs-based gene therapies are lacking. Comprehensive studies are needed to evaluate the durability of transgene expression, potential risks of insertional mutagenesis, and any off-target effects associated with prolonged exposure to genetically modified MSCs. Further advancements in enhancing the targeted delivery of therapeutic proteins are essential. This involves exploring innovative strategies to improve the specificity and efficiency of MSCs homing to specific tissues or tumor microenvironments. Also, gene editing technologies should be pursued to optimize genetic manipulation methods and ensure controlled and regulated expression of therapeutic genes.

The future direction of research in MSC-based gene delivery should focus on refining techniques, deepening mechanistic insights, ensuring long-term safety, and exploring innovative strategies for personalized and combination therapies. Collaborative efforts across disciplines, rigorous clinical trials, and advancements in translational research will be vital to unlocking the full therapeutic potential of MSCs in gene therapy.

TABLE 1. Summary of *in vitro*, *in vivo* and clinical studies that involved MSCs as gene delivery vehicles

Source of MSCs	Gene of interest & vector	Route of administration & period	Dose concentration	Multiplicity of Infection (MOI)	Disease Model	Result	References
			in vitro studies				
Human Wharton's Jelly	IL-35 in Lentiviral vector	Induction through 96- well plate for 4 days	1×10^6	50	Mouse splenocytes to isolate CD4+ T cells	IL-35 managed to induce the proliferation of Treg cell, reduces the activity of Th17 and Th1 in CD4+ T cells. This brings function to reduce inflammation and autoimmune diseases	(Amari et al. 2015)
Human umbilical cord	IL-18 in Lentiviral vector	Invasion assay through 24-well Transwell chamber for 5 days	5×10^4	70	MCF-7 and HCC1937 cells (Breast cancer cells)	IL-18 significantly suppressed the proliferation, migration and invasion of the MCF-7 and HCC1937 cells. This involved the induction of G1-phase to S-phase arrest of the breast cancer cells	(Liu et al. 2015)
Adipose	IL-27 in Lentiviral vector	Co-culture & 1 day assessment	$1.5 - 2.0 \times 10^6$ cells	-	NaiveT cells from mouse	IL-27 demonstrated overexpression of IL-10 in T cells that can reduce inflammation and autoimmune diseases	(Hajizadeh- Sikaroodi et al. 2014)
			in vivo studies				
Allogeneic Bone Marrow	Hepatocyte Growth Factor (HGF) in Adenoviral vector	Transplantation & 4 weeks assessment	1×10^6 cells/ 100 uL	300	Osteonecrosis of the femoral head (ONFH) in rabbit	Recovery with decreased empty lacunae and hematopoietic tissue	(Wen et al. 2012)
Mouse Bone Marrow	IL-25 by lipofection	Syngeneic system transplantation	-	-	pancreatic tumor in mice	IL-25 able to induce apoptosis in pancreatic tumoural cells	(Piri, Esmaeilzadeh& Hajikhanmirzaei 2012)

			Clinical trials				
Autologous Bone Marrow MSCs	Hepatocyte Growth Factor (HGF) in Plasmid	Intravenously administered weekly for 3 consecutive weeks	2×10^6 cells/kg	Not applicable	4 patients with pulmonary silicosis	• cough and chest distress symptoms improved post-therapy of 6 months • significant improvement of pulmonary function • The ratios of the peripheral CD4- and CD8- positive cell concentrations were increased • serum IgG levels in these patients were normalized • CT scans showed partial absorption of the nodular and reticulonodular lung lesions during follow-up of at least 12 months	(Liu et al. 2015)
Autologous Bone Marrow MSCs	Gamma-retroviral vector	Intravenously administered weekly for 3 consecutive weeks	1.5×10^6 cells/kg	1-4	6 patients in Phase I & 16 patients in Phase II with Adenocarcinoma	This published paper is a summary of study protocol. In phase I of the study, the safety of the investigational medicinal product (IMP) is tested in six patients by three treatment cycles consisting of re-transfusion of MSCs at different concentrations followed by administration of the prodrug Ganciclovir. In phase II of the study, sixteen patients will be enrolled in receiving IMP treatment. A subgroup of patients that qualifies for surgery will be treated preoperatively with the IMP to verify homing of the MSCs to tumors as to be confirmed in the surgical specimen	(Niess et al. 2015)

GENE THERAPY PROCESS USING GENETICALLY MODIFIED MSCS AS A DELIVERY VEHICLE FOR TARGETED TREATMENT

Gene therapy utilizing genetically modified MSCs represents an innovative approach for the targeted treatment of various diseases. MSCs, classified as the second generation, offer distinct advantages as delivery vehicles, combining the regenerative properties of stem cells with the precision of genetic modifications. Second-generation MSCs refer to those engineered or genetically modified to enhance specific characteristics. In the context of gene therapy, these modifications often involve introducing therapeutic genes, allowing MSCs to express and deliver targeted therapeutic proteins. This classification distinguishes them from unmodified or first-generation MSCs.

Figure 1 represents the gene therapy process using second-generation MSCs as the gene delivery vehicle. The first step in this process involves identifying the mutated or malfunctioned gene responsible for the targeted disease, followed by the production of therapeutic genes for treatment. There are four main types of therapeutic genes, including functional genes, silencing genes, suicide genes, and marker genes, depending on the specific method of treating the disease (Marofi et al. 2017). The loading of the therapeutic gene into MSCs can be achieved through several methods, including viral vectors such as adeno- associated virus, lentivirus or retrovirus, non-viral vectors such as plasmids, or physical methods such as RNAi, liposomes, or electroporation. Therapeutic genes are introduced into MSCs, enabling them to express specific proteins with therapeutic effects. The expression of the introduced genes is verified through molecular assays and imaging techniques. This step ensures the successful incorporation of the therapeutic genes into the MSCs and confirms their ability to produce the desired therapeutic proteins. The genetically modified MSCs undergo further expansion to achieve the required cell number for effective therapeutic delivery. This step is crucial for generating a clinically relevant cell population while maintaining the characteristics of the modified MSCs.

These genetically engineered MSCs are administered to the patient, either locally or systemically, depending on the therapeutic target. In some cases, a personalized approach may be adopted, tailoring the gene therapy to the individual patient's specific genetic profile or disease characteristics. This may involve using patient-derived MSCs for genetic modification. The homing ability of MSCs directs them to the specific tissues or sites of injury, facilitating targeted delivery of therapeutic proteins. Once the therapeutic gene has arrived at the nucleus of the targeted cell, it integrates with the DNA and corrects the mutated or malfunctioning gene (Ramamoorth & Narvekar 2015). Genetically modified MSCs exert their therapeutic effects by expressing and secreting therapeutic proteins. This may involve promoting tissue repair, modulating the immune response, or inhibiting the growth of tumors, depending on the specific therapeutic genes introduced.

The gene therapy process utilizing genetically modified MSCs as the second generation of delivery vehicles holds great promise for targeted and personalized treatments. Advances in genetic engineering and stem cell biology continue to propel this field forward, offering new avenues for addressing complex diseases with high precision and efficacy.

BIODISTRIBUTION OF MSCs AS A GENE DELIVERY VEHICLE

Safety is a crucial consideration in developing cell-based gene therapy using MSCs. The choice of administration route can impact the biodistribution of MSCs in various organs, which may have different effects. Therefore, this section will thoroughly discuss the potential impact of MSC deposition in different organs. Furthermore, an in-depth review of the toxicity study for MSCs will also be presented to ensure the safety and efficacy of the therapy.

The administration route significantly influences the distribution of MSCs within the body, with implications for both safety and clinical outcomes. The commonly used routes of administration for MSCs are intravenous, intraarterial, and intralesional (Sanchez-Diaz et al. 2021). Table 2 summarises the *in vivo* and clinical studies on MSCs biodistribution that are relevant to intravenous, intraarterial, intralesional and subconjunctival using MSCs as an administration pathway. Intravenous administration of MSCs results in initial accumulation in the lungs, a common observation reported by Kim et al. (2016) and Schubert et al. (2018).

They were subsequently redistribution to the liver, spleen, and kidneys, indicating a systemic distribution pattern. Understanding this trajectory is crucial for predicting potential effects on organs involved in filtration and clearance. Intraarterial administration, on the other hand, bypasses the pulmonary filter, allowing MSCs to distribute more widely into other organs.

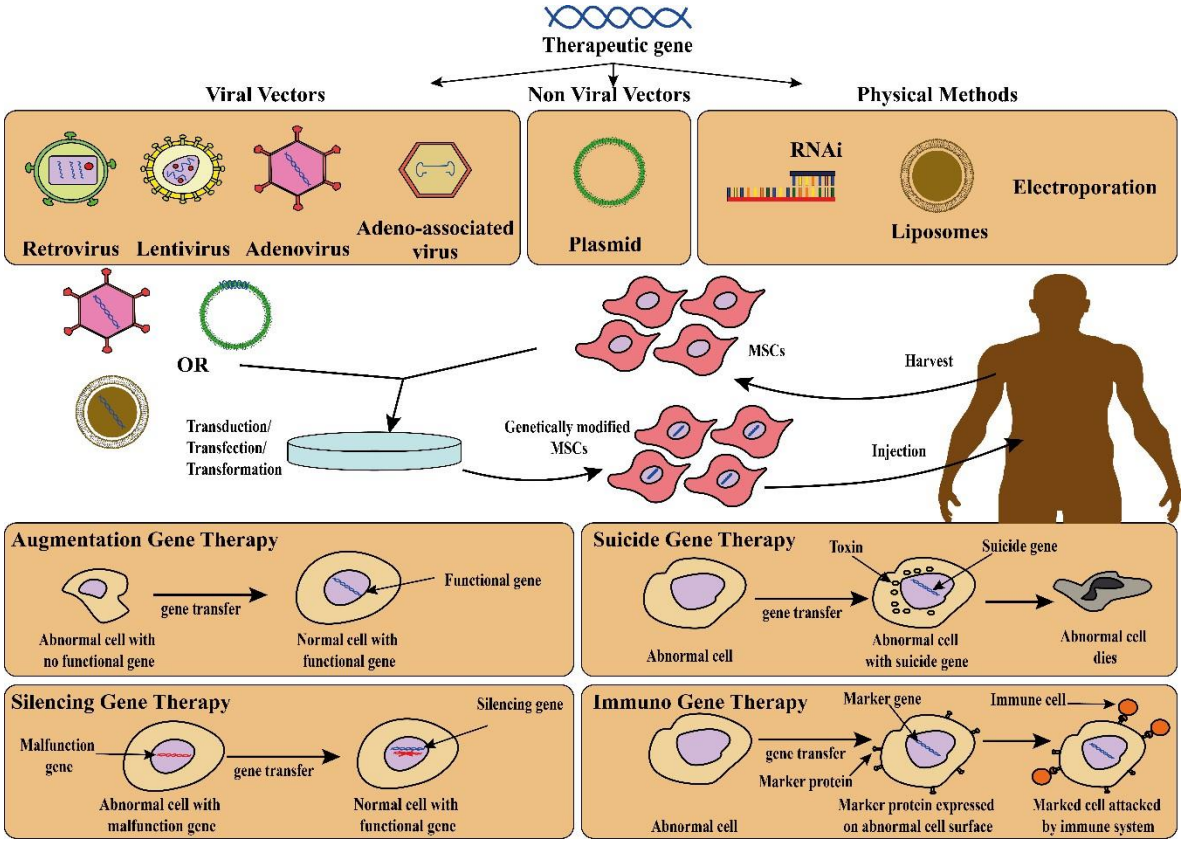

FIGURE 1. Gene therapy process using genetically engineered MSCs as a delivery vehicle for targeted treatment

Espinosa et al. (2016) observed homogeneous distribution through the entire distal limb, including within the hoof, after intraarterial selective infusion via the median artery. This route provides an alternative to intravenous delivery, potentially influencing the therapeutic impact by targeting specific organs or tissues more efficiently. In contrast, intralesional injection leads to localized distribution, where MSCs remain at the injection site without systemic migration. This route offers a targeted approach and may be advantageous when localized therapeutic effects are desired, minimizing systemic exposure. Khan et al. (2018) demonstrated MSCs distributed throughout the tendon synovial sheath but restricted to the synovial tissues, with no systemic biodistribution observed. Another study (Zhang et al. 2021) reported no labeled cells infiltrating the cornea when injected into the subconjunctival on Day 28.

Thorough biodistribution and toxicity studies are crucial to ensure the safety of MSC-based therapy in clinical applications. These studies aim to investigate the potential adverse effects of MSCs on the host organism. Table 2 describes the crucial findings of *in vivo* biodistribution studies on vast host organisms ranging from small animals (mice, rats, and rabbits) to large animals (horses, dogs, and sheep). Understanding the interaction between MSCs and host organs are crucial for predicting and mitigating potential toxicities. Factors such as host immune response, inflammatory reactions, and any off-target effects need to be thoroughly investigated to ensure the overall safety of the therapy. Long-term effects of MSC administration, including any potential accumulation or persistence in specific organs, should be a focus of toxicity studies. A clinical trial has been found to assess the biodistribution of MSCs at 8 months and 28 months post-injection (Henriksson et al. 2019). MSCs were found to be persistent enough to be detected at 8 months post-injection but not detected at 28 months. However, such longer term evaluation studies are limited.

Assessing the durability of the therapeutic effects and the resolution of any adverse events over time is essential for the clinical translation of MSC-based gene therapies. Thus, the inclusion of longitudinal monitoring in clinical trials is recommended to track the biodistribution of MSCs over time. This will provide valuable insights into the persistence of therapeutic effects and any potential late-onset adverse events.

The insights gained from studying the biodistribution of MSCs and conducting toxicity assessments have direct implications for the clinical use of MSC-based gene therapies. Understanding where MSCs accumulate and assessing potential risks informs the selection of administration routes and dosage regimens for optimal therapeutic outcomes. Optimization efforts should focus on selecting the most effective route that balances targeted delivery with minimized systemic exposure, aligning with the desired clinical outcomes. Additionally, the integration of advanced imaging techniques, such as positron emission tomography (PET) or magnetic resonance imaging (MRI), can enhance the accuracy of biodistribution studies. Integrating these techniques into preclinical and clinical research allows accurate, real-time visualization and quantification of MSCs distribution. The critical evaluation of MSCs biodistribution and toxicity is foundational for ensuring the safety and efficacy of gene therapies. Recommendations for optimization, longitudinal monitoring, and advanced imaging contribute to advancing the field toward safe and effective clinical applications.

VIRAL GENE DELIVERY INTO MSCs

The field of viral gene transfer has advanced significantly through a deep comprehension of the life cycle of viruses, which involves two critical stages: infection and replication. Gene transfer has focused on manipulating the viral genome to abrogate its replication ability and, instead, introducing a heterologous gene of interest through transduction (Vannucci et al. 2013). This allows for the targeted delivery of genetic information to a specific cell. To achieve this, modified viral vectors are introduced to mesenchymal stem cells, which then act as effective gene delivery agents when administered to the patient. By modifying MSCs with various beneficial genes, the therapeutic potential of these cells can be significantly enhanced, leading to an increase in survival rates. Lentiviruses, adenoviruses, adeno-associated viruses, and retroviruses are among the viral vectors that are currently employed for viral gene transfer into MSCs.

LENTIVIRUSES

Lentiviruses have garnered significant attention as gene delivery agents, owing to their unique ability to infect non-dividing or slow-proliferating cells, such as MSCs, without cell division (Zahler et al. 2000). This efficiency is attributed to the pre-integration complex, which allows the lentiviral vectors to infect target cells efficiently. While lentiviral vectors are derived from HIV-1, their modifications have been developed based on the HIV-1 vector system, as opposed to the HIV-2 vector system, due to their enhanced efficacy (Dissen et al. 2012). Lentiviral vectors enter the target cell via endocytosis and undergo endosomal escape, allowing their genome to be reversed transcribed to double-stranded DNA (dsDNA) and subsequently integrated into the host cell chromatin. Lentiviral vectors can integrate up to a maximum size of 9kb and are widely used in gene therapy research (McGinley et al. 2011). Figure 2(a) demonstrates the mechanism of infection from entering the cell and integrating the targeted gene into the nucleus.

ADENOVIRUSES

Adenovirus is a non-enveloped virus with double-stranded DNA genomes that encode genes ranging from 26 to 45kb. It consists of icosahedral capsids with 12 vertices and 7 surface proteins, and its DNA genome encodes 30 proteins (San Martín 2012). Adenovirus enters host cells through various receptors, including the commonly known integrin receptor, inducing endocytosis for internalization of the virus, as shown in Figure 2(b). The virus then proceeds through the endosomal rupture process, known as cytoplasmic transport, to the nuclear envelope for nuclear pore complex attachment (Greber & Flatt 2019). Adenovirus can transfer the gene of interest to the nucleus of the host cell without integrating with the host chromatin (Nowakowski et al. 2013). It can transfect both dividing and non-dividing cells, with a maximum insert size of up to 36kb. However, adenovirus has several disadvantages, including high immunogenicity, potential insertional mutagenesis, and a short expression duration.

TABLE 2. Summary of *in vivo and clinical* studies that involved MSCs in biodistribution study

Source of MSCs	MSCs marking techniques	Route of administration	Dose concentration	*In vivo* Model	Result	References
			in vivo			
Human bone marrow derived MSCs	Near-infrared fluorescent dye labeled MSCs, evaluated with bioluminescence and fluorescence imaging, qPCR & histologic examinations	Intravenous (Tail vein)	1×10^6 cells	1 normal mice & 2 mice with brain tumor (n=3/group)	Imaging techniques were performed at 1 h and 4 h, Day 1, Day 4, Day 7 and Day 14. MSCs resided predominantly in the lung until Day 1 and the signal intensity decreased over time. Many cells moved from the lung toward other organs (liver and spleen) after Day 1, and the signal remained stable in these regions for 14 days. From Day 1 to Day 14, MSCs were clearly detectable in the tumor area	(Kim et al. 2016)
Murine autogenic adipose-derived MSCs	Luciferase transgenic mice's MSCs, evaluated with bioluminescence imaging and qRT-PCR	Intravenous (Tail vein)	0.5×10^6 cells/100 μL	8 normal mice & 16 mice with cisplatin- induced acute kidney injury	Imaging was performed on Day 1, Day 3 and Day 6. qRT-PCR was performed in kidney, lung, liver tissue and blood on Day 6. Bioluminescence showed a high distribution of MSCs to lungs on Day 1, which disappeared on Day 3 and Day 6. RT-PCR on Day 6 showed variable amounts of MSCs-mRNA in blood, liver and kidneys	(Schubert et al. 2018)
Allogenic MSCs, human decidual stromal cells (DSCs)	^{111}In-oxinate labeled MSCs, evaluated with SPECT-TC imaging	Selective Intraarterial (superior mesenteric artery) & Intravenous	5×10^6 cells	6 adult male New Zealand White rabbits	SPECT-TC was performed at 6 h, 24 h, 48 h, and 120 h post infusion. Intravenous administration resulted in early and long distribution of MSCs to the lungs. In contrast, selective intraarterial injections resulted in the distribution of MSCs in the intestine and the liver	(Arnberg et al. 2016)

Allogenic MSCs	^{99m}Tc-HMPAO labelled MSCs, evaluated with Scintigraphic images.	Intraarterial selective infusion (median artery)	35×10^6 cells suspended in 2 mL normal saline	6 Horses	Images were taken at the time of injection and at 1 h, 6 h and 24 h post injection. Homogeneous distribution of ^{99m}Tc-HMPAO labeled MSC was observed through the entire distal limb, including within the hoof	(Espinosa et al. 2016)
Autogenic MSCs, bone marrow MSCs	5-bromo-2-deoxyuridin labeled MSCs, evaluated with histologic examinations	Intraarterial	$5 - 10 \times 10^6$ cells / mL	12 Beagle dogs induced Osteonecrosis of the femoral head	Histologic examinations were performed 8 weeks after cell infusion. Organs had an uneven distribution of MSCs: Heart, liver, gallbladder, kidney and stomach had the majority of MSCs	(Jin et al. 2016)
Autogenic MSCs, bone marrow MSCs	Fluorescent-conjugated magnetic iron- oxide nanoparticles (MIONs) labeled MSCs, evaluated with MRI, histology and flow cytometry	Intralesional	5×10^6 cells / 1 mL PBS	50 adult female sheep induced with tendon injury	MSCs are distributed throughout the tendon synovial sheath but restricted to the synovial tissues, with no MSCs detected in the tendon or surgical lesion. No systemic biodistribution was observed	(Khan et al. 2018)
Allogenic MSCs, adipose-derived MSCs	Luciferase labeled MSCs, evaluated with bioluminescence imaging	Intralesional	4×10^6 cells	32 Lewis male rats induced fistula (Crohn's disease)	Imaging was performed at Day 0, 2, 7, 14 and 30. MSCs distributed only in the injection site, with a high reduction of luminescence by Day 2. MSCs were detectable up to Day 30	(Ryska et al. 2017)
Bone marrow-derived MSCs	DiI labeled MSCs, evaluated with fluorescence microcope camera	Subconjunctival injection	2×10^6 cells / 100 µL PBS	60 rats induced corneal chemical burn	Imaging was performed at Day 7, 14 and 28. Cells located in the injection site (conjunctival sac) at Day 28 and no labeled cells infiltrated the cornea	(Zhang et al. 2021)
Human limbal MSCs & Bone marrow- derived MSCs	CellTracker™ CM-DiI MSCs, evaluated with fluorescence microscopy	Subconjunctival injection	5×10^3 cells / 200 µL	12 Rabbits induced partial corneal/ limbal chemical burn	Four weeks after transplantation, the movement of cells was clearly visualized towards the cornea under fluorescence microscopy	(Li et al. 2018)

Clinical trials

Autogenic MSCs	^{111}In-oxine labeled MSCs, evaluated with Dual head gamma camera and SPECT imaging	Intravenous	$250-400 \times 10^6$ cells	4 patients with liver cirrhosis	MSCs were detected at 2 h, 4 h, 6 h, 24 h, 48 h, 7th and 10th days after administration. Pre-48 h images showed a large majority of cells distributed in the lungs. Later images showed a drastic decrease in lung area, with a higher amount of MSCs distributed in the spleen and liver	(Gholamrezanezhad et al. 2011)
Allogenic MSCs, Adult- derived human liver stem cells	^{111}In-oxine labeled MSCs, evaluted with SPECT imaging	Intravenous	1 initial infusion of 35×10^6 cells labeled with ^{111}In-oxine, followed by 1 infusion of 125×10^6 cells the next day and 3 infusions of 250×10^6 cells every 2 weeks thereafter (total infusion period, 50 days)	1 patient with Hemophilia A	Total body imaging was performed at 1 h, 4 h, 24 h, 48 h, 72 h, and 144 h post administration. MSCs were initially (1 h) trapped in the lungs and liver. At 144 h, lungs signal decreased and liver signal increased	(Sokal et al. 2017)
Autogenic bone marrow MSCs	18-FDG labeled MSCs, evaluated with PET-TC imaging	Selective Intraarterial (pancreaticoduodenal artery and splenic artery) & Intravenous	$490 \pm 310 \times 10^6$ cells for pancreaticoduodenal artery. $1204 \pm 484 \times 10^6$ cells for splenic artery. $688 \pm 230 \times 10^6$ cells for intravenous	21 patients with Type 2 diabetes mellitus	Images were taken at 30 min and 90 min post administration. Selective intraarterial administration led to MSCs homing in pancreas head (pancreaticoduodenal artery) or body (splenic artery). For intravenous group, MSCs distributed to lungs at 30 min with significant clearance at 90 min image, with no distribution to pancreas	(Sood et al. 2017)
Autogenic MSCs	Iron sucrose labeled MSCs, evaluated with histologic examination	Intralesional	1×10^6 cells	4 patients with intervertebral disc degeneration	Intravertebral discs were explanted at 8 months (3 patients) and 28 months (1 patient) post injection. MSCs were detected at 8 months, but not at 28 months. Detected MSCs had differentiated into chondrocyte-like cells	(Henriksson et al. 2019)

ADENO-ASSOCIATED VIRUS (AAV)

Adeno-associated virus (AAV) is a single-stranded DNA virus capable of integrating its genome into human chromosome 19. Its viral genome comprises two genes, each producing multiple polypeptides: rep for viral genome replication and cap for encoding proteins (Dissen et al. 2012). AAV can serve as a viral vector by introducing separate plasmids flanking therapeutic genes and adding a helper such as adenoviruses and herpes simplex viruses.

The transduction pathway of AAV initiates by binding to a specific receptor-mediated endocytosis on the cell surface to commence infection (Desfarges & Ciuffi 2012). Heparin sulfate proteoglycan receptor (HSPG) promotes clathrin-mediated endocytosis and forms the endosome, involving αvβ5 integrin (Dissen et al. 2012). The virus subsequently undergoes endosomal escape and gradually traverses the nuclear pore complex into the nucleus. Inside the nucleus, the virus's capsid protein degrades, and its genome undergoes replication by relying on the host cell polymerase, forming an episome for the expression of the desired protein. Figure 2(c) illustrates the brief mechanism of the transduction pathway of AAV to the host cell for transferring therapeutic genes.

AAV is a virus that can transfer its genome to the host cell's nucleus and integrate with host chromatin or act as an extrachromosomal DNA. It elicits a low immune response in host cells and has efficient transfection, providing a good length of expression *in vivo* (Nowakowski et al. 2013). However, AAV has a few significant disadvantages, such as its small size, which can only accommodate a maximum insert size of 4.5kb, and safety concerns due to potential insertional mutagenesis (Johnson 2010).

RETROVIRUSES

Retroviruses have a unique transcription mechanism, allowing them to integrate with the host genome and transfer therapeutic genes to host cells, making them an effective vector for gene therapy. Retrovirus infection involves endocytosis of the virus into the host cell, followed by endosomal escape and fusion with the transmembrane protein at the virus membrane (Sandrin, Russell & Cosset 2003). The resulting fused membrane flips inside out, allowing the viral gene to enter the host cell cytoplasm. Eventually, reverse transcription occurs, and the resulting double-stranded DNA enters the nucleus for expression to express the desired protein. (Figure 2(d)).

Despite their high transfection efficiency and low immune response in host cells, retroviruses have limitations, such as a payload size limit of 8kb and low transfection efficiency *in vivo* studies, as well as safety concerns related to insertional mutagenesis (Johnson 2010). To address these limitations, retroviruses have been modified into various vectors, including retroviral bicistronic vectors and murine stem cell retroviral vectors. These modified vectors have shown promising results in treating myocardial infarction by limiting the infarct area's size or promoting angiogenesis and cell survival.

Retroviral bicistronic vectors, based on Internal Ribosome Entry Site (IRES), have been utilized to transfer genes to MSCs for modifying them into gene delivery agents (Martin et al. 2006). Meanwhile, murine stem cell retroviral vectors, based on the retroviral bicistronic vector, have presented high efficiency transduction and long-term gene expression in MSCs (Sandrin, Russell & Cosset 2003). Both vectors have shown promising results in treating myocardial infarction by limiting the infarct area's size or promoting angiogenesis and cell survival. Overview of the key characteristics of each viral vector is summarized in Table 3.

ANTI-ANGIOGENESIS

Angiogenesis is a physiological process that involves the formation of new blood vessels from pre-existing ones, often in response to tissue hypoxia or insufficient tissue oxygenation. This results in the accumulation of hypoxia-inducible factor (HIF-1α) and overexpression of vascular endothelial growth factor (VEGF) (Adams & Alitalo 2007; Hirota & Semenza 2006). The angiogenesis process involved: (a) signalling, (b) detachment and sprouting, (c) migration and proliferation, (d) tube/lumen formation, (e) pericyte recruitment, and (f) vessel maturation and remodeling (Van Hove & Benoit 2015).

Angiogenesis is a complex biological process that begins with releasing pro- angiogenic signals from ischemic tissues, creating a growth factor gradient primarily involving HIF-1α and VEGF (Hirota & Semenza 2006). The subsequent interaction between endothelial cells (EC) and pericytes results in pericyte destabilization and detachment, causing further degradation of the extracellular matrix (ECM) and the formation of sprouts towards ischemic tissues. ECs then

migrate towards ischemic tissues while proliferating in response to factors such as VEGF, FGF, and SDF-1 (Kuhlmann et al. 2005; Lieu et al. 2011). The resulting immature vessels are composed of ECs assembled to enable cell-cell contact to form tube/lumen-like structures. Finally, recruited pericytes interact with ECs and are stabilized by factors such as Ang1 and PDGF.

TABLE 3. Overview of the key characteristics of each viral vector

Virus Type	Similarity	Differences	Advantages	Disadvantages
Lentiviruses	Gene delivery vectors; RNA viruses	Stable integration; infects both dividing an non-dividing cells	Stable integration; infects both dividing and non-dividing cells	Risk of insertional mutagenesis
Adenoviruses	Gene delivery vectors; double- stranded DNA viruses	Transient expression; does not integrate into host genome	Large transgene capacity; does not integrate into host genome	High immunogenicity
AAV	Gene delivery vectors; double- stranded DNA viruses	Versatile tropism, potential for stable integration	Stable transgene expression, reduced immunogenicity	Limited packaging capacity
Retrovirus	Gene delivery vectors; RNA viruses	Stable integration; infects both dividing and non-dividing cells	Stable transgene expression, only infect dividing cells	Risk of insertional mutagenesis

However, an overexpression of pro-angiogenic factors can lead to an excess of new blood vessel formation, contributing to various diseases such as ocular disorders, cancer, psoriasis, and arthritis (Dreyfuss, Giordano & Regatieri 2015; Van Hove & Benoit 2015). Therefore, anti- angiogenesis factors, which are angiogenesis inhibitors that block the formation of new blood vessels, are critical for preventing or treating such diseases. Angiogenesis inhibitors can function by inhibiting angiogenic signaling pathways, such as VEGF and its receptors, tyrosine kinase, or other growth factors involved, by inhibiting the interaction between ECs and ECM through integrin inhibition, or by inhibiting pericytes. The process through which MSCs modulate angiogenesis is elucidated in Figure 3.

Therefore, genetically MSCs have emerged as a promising therapeutic option for diseases involving angiogenesis. These MSCs are engineered to overexpress anti-angiogenic markers, enabling targeted delivery to the site of interest. Such advancements pave the way for novel and effective treatments for angiogenesis-related diseases.

ANTI-ANGIOGENESIS IN ENGINEERED MSCs USING VIRAL VECTORS

Genetically engineered MSCs utilizing viral vectors have emerged as a promising approach for anti-angiogenesis therapy. Genetically modified MSCs have presents compelling anti- angiogenic effects in various preclinical models. Table 4 summarizes findings on the potential of engineered MSCs using various viral vectors to inhibit angiogenesis both directly and indirectly.

The application of engineered MSCs using lentivirus have been found in various disease models. For instance, in acute lung injury, Chen et al. (2013) showed improvement in pulmonary microvascular permeability and total severity scores significantly reduced in lipopolysaccharide (LPS)-induced lung injury using

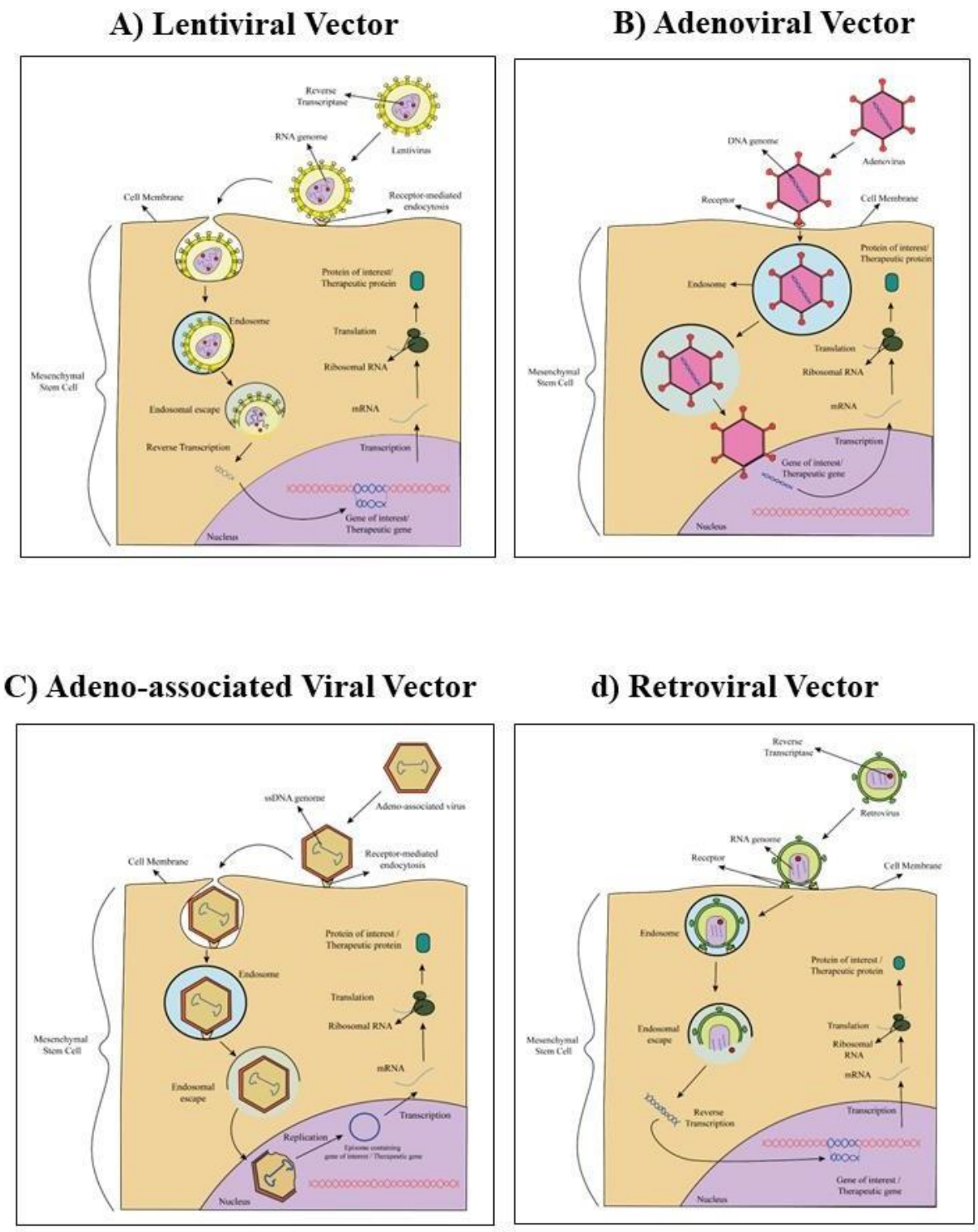

FIGURE 2. Mechanism of infection of a) lentiviral, b) adenoviral, c) adeno-associated viral and d) retroviral vector into MSC

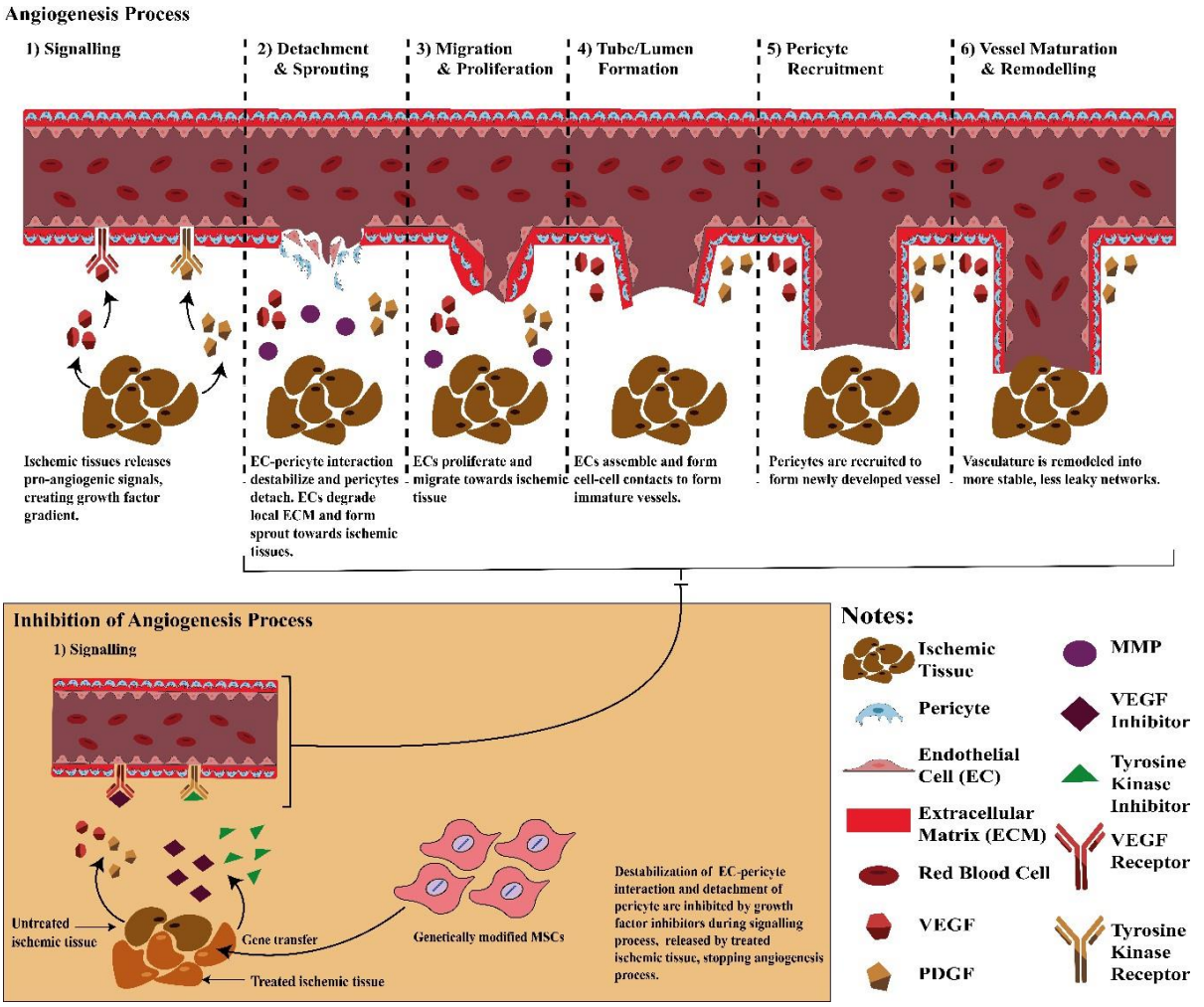

FIGURE 3. The modulation of angiogenesis by mesenchymal stem cells. Genetically modified MSCs released growth factor inhibitors that inhibited the destabilization EC- pericyte interaction and detachment of pericyte at the treated ischemic tissue, and subsequently hinder the angiogenesis process

BALB/C mouse bone marrow derived- MSC. Another study demonstrated similar anti-angiogenic effects on LPS- induced lung injury using Angiopoietin-1 (Ang1) using C57BL/6 mice bone marrow-derived MSCs (Xu et al. 2008). Li et al. (2017) demonstrated that the overexpression of anti- angiogenic factors by BALB/C mouse bone marrow derived-MSC can inhibit endothelial cell proliferation in tube formation assay in hepatocellular carcinoma. The authors further confirmed the inhibition of microvessel density and hepatocellular carcinoma (HCC) tumour formation *in vivo*. In a different study, Bone marrow derived MSCs were engineered to express thrombospondin-1 (TSP-1) via lentivirus transduction (LV-TSP-1-BM- MSCs) to treat Glioblastoma multiforme (GBM) (Choi et al. 2015). The study inhibited angiogenesis by suppressing brain endothelial cells during angiogenesis. This diversity highlights the versatility of MSC-based anti-angiogenic therapies across different pathological contexts.

Furthermore, the use of engineered MSCs has contributed to therapeutic impact in tumorigenesis. Another study treated the same HCC disease model with

MSCs engineered specific anti-angiogenic factors, sFlt1, using adenoviruses (Niu et al. 2016). The engineered MSCs in combination with low-dose doxorubicin and overexpressing sFlt1 demonstrate promising results in inhibiting tumor growth. This combination therapy approach enhances the therapeutic impact, potentially mitigating the need for high doses of cytotoxic agents. Chu et al. (2014) reported using adenoviruses to engineer human placenta derived MSCs by overexpressing kringle1-5 gene to suppress angiogenesis effects *in vitro* and *in vivo*. The authors showed promising findings on inhibiting microvessel growth in aortic rings *in vitro*. Human placenta derived MSCs was engineered by Zhang et al. (2014) to express endostatin by using adenoviral vector. The findings indicated a significant reduction in blood vessel and tumour cell proliferation. MSCs can be home to angiogenic sites and act as cellular carriers for the targeted delivery of anti-angiogenic agents. Engineered MSCs exhibit the ability to home to angiogenic sites, enabling targeted delivery of anti-angiogenic agents. This homing effect is showcased in studies where MSCs engineered with sFlt1 resulted in decreased lung metastases and inhibited angiogenesis, underscoring the clinical significance of targeted therapies (Hu et al. 2008). Another anti- angiogenesis study on the tumour model performed by Wang et al. (2013) showed MSCs engineered with pigment epithelium-derived factor (PEDF) using adeno-associated virus (AAV). These engineered MSCs improved tumour migration *in vitro* by infiltrating the vessels surrounding the tumour site and inhibited glioma cells significantly in a xenograft model.

Other studies focus on paracrine effects via extracellular vesicles. MSCs demonstrate the capacity to release extracellular vesicles containing anti-angiogenic miRNAs or proteins, exerting paracrine effects on nearby endothelial cells (Hmadcha et al. 2020). This paracrine modulation further contributes to the suppression of angiogenesis, showcasing the multifaceted mechanisms of MSC-mediated anti-angiogenic effects.

Studies using various viral vectors have consistently demonstrated the *in vivo* efficacy of engineered MSCs in inhibiting angiogenesis. These findings hold clinical significance as they provide a basis for exploring MSCs-based therapies in human trials, particularly in cancer and other angiogenesis-related disorders. In a clinical setting, interferon-β (IFN-β) has been used for inhibiting tumor growth due to its potency in anti-angiogenesis through the suppression of endothelial growth factors (Takano et al. 2014). Ren et al. (2008b) transduced MSCs with recombinant AAV encoding mouse IFN- β to investigate the therapeutic effect on prostate cancer lung metastasis. Results indicated a suppression of blood vessel counts and tumour cell proliferation. The authors also evaluated interferon-α (IFN-α) using recombinant AAV (rAAV) on the lung metastasis model of melanoma (Ren et al. 2008a). The transduced MSCs with rAAV- IFN-α were intravenously injected and immunohistochemistry demonstrated a decrease in blood vasculature and proliferation.

The promising outcomes in preclinical models warrant translating engineered MSCs-based anti-angiogenic therapies into clinical trials. However, a research gap exists in understanding the long-term safety and durability of MSCs-based anti-angiogenic therapies. Longitudinal studies assessing potential off-target effects, the persistence of therapeutic effects, and the emergence of late-onset adverse events are crucial for a comprehensive safety profile. Also, rigorous clinical investigations are essential to validate the safety, efficacy, and feasibility of these approaches in human subjects.

Further research can explore comparative preclinical studies and combination therapies in clinical settings. Comparative studies comparing the efficacy of different viral vectors and their impacts on MSC function could provide valuable insights for optimizing vector selection. Innovations in viral vector design, including the development of next- generation vectors, should be pursued. Advancements in vector design and delivery methods may further refine the precision of engineered MSCs for anti-angiogenic therapies. Additionally, exploring combination therapies with conventional treatments may enhance the overall therapeutic potential.

The use of viral vector-engineered MSCs for anti-angiogenesis therapy is a promising avenue with significant clinical potential. Continued research, translation to clinical trials, and addressing existing research gaps will be crucial for realizing the full therapeutic impact of this innovative approach in various disease contexts.

TABLE 4. Summary of MSCs as delivery vehicles using viral vectors with anti-angiogenesis property in various disease models

Viral vector	Disease	Transfected product	MSC Source	Multiplicities of infection (MOI)	Subject	Administration route	Number of cells injected/dosage	Result	References
Lentivirus	Hepatocellular carcinoma (HCC)	sFlt-1	BALB/C mouse bone marrow derived-MSC	50	BALB/C mice	Intravenous	6×10^5 cells/0.1 mL PBS	Tumor weight decrease 0.322±0.0008 g compared to PBS treatment which is 0.286±0.012 g	(Li et al. 2017)
	Acute lung injury (ALI)	KGF	C57BL/6 mouse MSC	20	C57BL/6 mice	Intravenous	5×10^5 cells, 200 mL total volume	MSC-kgf provides higher survival rate (i.e., 70% - 100%) within 168 hours compared to NS, MSCs and MSCs-vec and ALI improved by MSCs-kgf is more apparent than others	(Chen et al. 2013)
	ALI	Ang-1	C57BL/6 mice bone marrow-derived MSCs	20	C57BL/6 mice	Intravenous	NA	Ang-1 protein expression in MSC-Ang1 showed a decrement temporally and gradually recover and increase significantly at day 14 and show a major improvement for lung histopathology and total severity score of lung injury reduce	(Xu et al. 2008)

	human glioblastoma (GBM) cell lines	mice	Human bone marrow derived MSCs (BM- MSC)	NA	thrombospondin-1 (TSP-1)	lentivirus	NA	*in vitro* – inhibited formation of branch points in human brain microvascular endothelial cells (HBMVEC) *in vivo* – inhibits angiogenesis and sensitizes brain endothelial cells	(Choi et al. 2015)
	Glioblastoma (tumourbearing mice)	MSC	Bone-marrow mesenchymal stem cells	NA	Viral – pLVX-CMV-Puro (puromycin) expression vector	Intravenous	NA	*in vivo* – reduced capillaries	(Shi et al. 2019)
Adenovirus	HCC	sFlt-1(with continuous low-dose doxorubicin treatment)	BALB/c nu/nu mice MSC	100	BALB/c nu/nu mice	Intravenous (intraperitoneally injected with doxorubicin)	1×10^5 cells (1 mg/kg for doxorubicin)	MSC.sFlt1 + continuous low-dose doxorubicin shown a major decrease in tumor volume (mm^3) for 5 weeks compared to NS, continuous low-dose doxorubicin, MSC. sFlt1	(Niu et al. 2016)
	Tumor neovascularization	Human Kringle1-5	Healthy donor mothers	50	BALB/c nude mice	Subcutaneous	1×10^6 cells mixed with 500 µl Matrigel solution	K1-5-HPMSCs and Mock- HPMCs have no significant difference in EGFP expression but K1-5 protein expression is higher in K1-5-	(Chu et al. 2014)

								HPMSCs compared to others and result results to a decrease of tube length of neovessels and able to arrest neovascularization *in vivo*	
	mouse tumor model	Mouse bone-marrow derived MSCs (BM-MSCs)	Tumour metastases	3000	soluble vascular endothelial growth factor receptor-1 (sFlt- 1)	adenovirus	NA	induce the inhibition of angiogenesis. Very few newborn microvessels were observed	(Hu et al. 2008)
	Colorectal cancer	mouse	Human placenta derived MSCs (PM-SCs)	3000	endostatin human umbilical vein endothelial cells (HUVECs)	adenovirus	NA	*in vitro* – inhibition of endothelial cell tube formation *in vivo* – decrease in microvessels densities, inhibition of angiogenesis	(Zhang et al. 2014).
Adeno- associated virus	Glioma	PEDF	Human MSCs (hM-SCs)	500	BALB/c-nu/nu mice	Intravenous	1×10^6 cells in 200µl of DMEM.	Survival of mice with MSC-PEDF is 10.52 days which longer compared to PBS and unmodified MSCs and MSC-PEDF also leads to reduction of tumour tissue size, 30.5±7.1 compared to unmodified MSCs	(Wang et al. 2013)

							or PBS, 65.3±4.9 and 51.8±4.8, respectively	
Melanoma lung metastasis	IFN-α	C57BL/6 mice bone marrow- derived MSC	1000	C57BL/6 mice	Intravenous	5×10^5 cells in 200μL	MSC producing IFNα that were transduced with rAAV-IFN-α reduce the growth of melanoma cell significantly and prolonged the survival of mice	(Ren et al. 2008a)
Prostate cancer lung metastasis	IFN-β	C57BL/6 mice bone marrow- derived MSC	1000	C57BL/6 mice	Intravenous	5×10^5 cells in 200μL	MSC-IFNβ that transduced by rAAV-IFN-β provide a superior antitumor effect compared to IFN-β plasmid- transfected MSCs with more blood vessel growth in IFN-β plasmid- transfected MSCs	(Ren et al. 2008b)

Conclusion

The utilization of MSCs for anti-angiogenesis therapy holds immense promise, yet the field faces formidable challenges. First and foremost, the optimal selection of anti-angiogenic genes demands careful consideration, as different diseases may necessitate specific gene sets for effective therapeutic outcomes. The intricate task of identifying genes that strike a balance between efficacy in inhibiting angiogenesis and long-term safety requires an in-depth understanding of disease-specific pathways. Furthermore, the design of efficient vectors for delivering these genes into MSCs poses challenges related to stability, payload capacity, and targeted delivery, with a critical need to address safety concerns such as the risk of insertional mutagenesis. Equally crucial is the safety of genetically modified MSCs, encompassing issues like potential immunogenicity and unintended off-target effects. The immune response triggered by genetically modified MSCs could lead to rejection or inflammatory reactions, necessitating thorough evaluation. Additionally, ensuring that genetic modifications do not result in unintended consequences requires rigorous testing for specificity and safety. Long- term effects and the potential development of resistance to anti-angiogenic therapies using genetically engineered MSCs also warrant extensive investigation. Continuous monitoring, multidisciplinary collaboration, and comprehensive preclinical studies are essential to overcome these challenges and pave the way for the safe and effective application of genetically engineered MSCs in anti-angiogenesis therapy.

In conclusion, the application of genetically engineered mesenchymal stem cells (MSCs) using viral vectors for anti-angiogenesis has shown significant potential in inhibiting the formation of blood vessels and suppressing tumour growth in various types of cancer. Studies on mice and *in vitro* tests have demonstrated successful inhibition of angiogenesis through the expression of angiogenic inhibitors, such as endostatin, tumstatin, and sFlt-1, as well as kringle1-5 protein and thrombospondin-1. These genetically altered MSCs have shown promising results in inhibiting angiogenesis in various types of cancers such as prostate cancer, colorectal cancer, and glioblastoma multiforme. These findings suggest that genetically engineered MSCs could potentially serve as a promising therapeutic option for anti-angiogenesis treatment in cancer. However, more extensive research, including preclinical and clinical studies, is required to validate the safety, efficacy, and translation of genetically engineered MSCs for anti-angiogenesis therapies. With continued advancements in gene therapy and MSC research, genetically modified MSCs hold significant promise for the future development of targeted anti-angiogenic treatments.

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