MSCs - Gene Delivery Tool

Subjects: Cell Biology
Contributor: Gustavo Puras

To clearly define MSCs, and develop universal criteria for such cell population, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposed a set of standards for pre-clinical research studies. The minimal criteria of MSCs as determined by the ISCT are the following ones: The MSCs population must be plastic-adherent when maintained in tissue culture vessels under standard culture conditions.

Keywords: non-viral gene delivery ; 3D-bioprinting ; transfection ; mesenchymal stem cell ; cell therapy ; gene therapy ; niosome ; COVID-19

1.Introduction of MSCs

MSCs is a common acronym used to describe mesenchymal stemcell, Mesenchymal Stromal Cell, orMedicinalSignalingCell. However, the debate is still ongoing over which of these long names best describes MSCs [1]. They are an example of “adult” stem cells that could be derived from various tissue types.

MSCs have been isolated from almost all tissues [1] and have been reported to play critical roles in many physiological processes, such as tissue homeostasis, immunomodulation, and tissue regeneration [2].

Since the famous publications by Alexander Friedenstein et al., on MSCs, half a century ago, mounting evidence has been accumulating that bone marrow (BM)-derived MSCs are capable of differentiating into other cells of mesenchymal lineage (e.g., adipocytes, osteoblasts, chondroblasts, myocytes, and tenocytes, etc.,) [3][4]. The authors were able to isolate the plastic-adherent spindle-shaped cells that were capable of self-renewal and showed a multi-differentiation potential.

Later on, more reports unveiled potential pluripotency where these cells can transdifferentiate into cells of other lineages, endodermal (e.g., muscle, lung, and gut cells, etc.), and ectodermal (e.g., epithelial, and neural cells) Another interesting feature of MSCs is their homing ability, meaning that they can migrate into injured tissues where they can contribute to the physiological processes in ways more than one. They can differentiate into various local cell types at the injured sites, (ii) they can secrete chemokines, cytokines, and growth factors that help in tissue regeneration, (iii)

In addition to BM, MSCs can be obtained from various sources such as, adipose connective tissue, synovial fluid, hair follicles, dental pulp, salivary glands, amniotic fluid and membranes, endometrial lining, peripheral and menstrual blood, placenta and fetal membranes, umbilical cord blood, and Wharton’s jelly [5]. Therefore, due to the above-mentioned appealing features, MSCs have quickly made the transition from benchtop to bedside [6].

To clearly define MSCs, and develop universal criteria for such cell population, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposed a set of standards for pre-clinical research studies [7].

The minimal criteria of MSCs as determined by the ISCT are the following ones:

The MSCs population must be plastic-adherent when maintained in tissue culture vessels under standard culture conditions.

Nevertheless, such historical criteria have not been always correlated with the applicability of these cells in various biomedical purposes. For instance, while CD markers might stay consistent over successive passages, MSCs tend to lose their differentiation or immunomodulatory capabilities [8][9].

Despite the aforementioned criteria, ISCT now suggests considerable flexibility, particularly when it comes to MSCs the lack of expression of the HLA Class II marker is conditionally expressed once stimulated by specific cytokines.

Therefore, it is crucial to have the process of MSCs characterization well-standardized to enable accurate comparison of study outcomes and to guarantee safety and efficacy in the field. Unfortunately, to date, no single marker has been identified as being exclusively expressed by MSCs [10]. Yet, the number of MSCs markers (positive and negative) is expanding over time to help researchers verifying the MSCs features, thus increasing the confidence in the obtained/transplanted cells.

In addition, various research teams have developed and expanded innovative molecular markers (e.g., proteomic and epigenetic markers, transcriptome analysis, gene signature, etc.,). Despite all these trials to address the thorny question about MSCs identity, there is still little consensus on these characterization methods. Therefore, Arnold I. Caplan [11] has recently suggested the insignificance of characterizing every cell in every MSCs population in vitro. The author believes that most of the propagated MSCs populations have become culture-adapted and can no longer display their innate (in vivo) features, nor their therapeutic behavior, once transplanted.

2. MSCs as a Gene Delivery

The Food and Drug Administration (FDA) has defined gene therapy as “the administration of genetic material to modify or manipulate the expression of a gene product or to alter the biological properties of living cells for therapeutic use.” An essential aspect of gene therapy depends on designing a suitable gene delivery system to convey the cargo gene into the target cells. More than half a century after their introduction as a novel therapeutic approach, and despite some adverse effects seen in clinical trials, the concept of gene therapy remains to be acknowledged as a promising therapeutic alternative for various clinical disorders. However, the obstacles encountered have fueled research efforts that led to the improvement of gene carriers in terms of their efficacy and safety profiles.

Over the past decades, genetically engineered stem cells were feasibly used in cell-based gene delivery, providing long-term therapeutic effects. Furthermore, continuous research efforts have been directed toward understanding the behavior of individual stem cells in different tissue microenvironments, in vivo [12]. In parallel, the implementation of more accurate assays for MSCs and enhancement in gene vehicles have increased gene transfer efficiency. Nevertheless, quality control of the protocols applied in human gene therapy remains crucial, especially when cells are used as a gene carrier for the treatment of hereditary and acquired diseases.

For successful gene delivery to MSCs, the proper choice of the deliverable nucleic acid, as well as the delivery carrier/method, will determine the transfection outcome. Therefore, in the following section, we will review different types of exogenous nucleic acid cargo along with various non-viral nanocarriers used with MSCs.

Nucleic acids act as drugs that aim to treat and/or prevent countless intractable diseases, such as cancer, cardiovascular, neurodegenerative diseases by adding, replacing, editing, or even inhibiting specific target genes or their products [13]. Currently, therapeutic nucleic acids could be roughly classified according to their different structures into DNA and RNA drugs. Therefore, various therapeutics were developed and are now commercially available for various diseases (summarized in table **1**).

Table 1. FDA-approved RNA therapeutics for the treatment of human diseases in chronological order, adapted from [14] [15].

Drug Name	Drug Class	Brand Name	Company	Target Disease	Mechanism of Action	Year of Approval	Current Status
Fomivirsen	**ASO**	**Vitravene**	**Novartis**	**Cytomegalovirus retinitis**	**Binds to and blocks translation of IE2 mRNA.**	**1998**	**Withdrawn due to decreased need**
Pegaptanib	**Aptamer**	**Macugen**	**OSI Pharmaceuticals**	**Age-related macular degeneration (wet type)**	**Binds to and blocks the 165 isoform of VEGF.**	**2004**	**Continuous**
Mipomersen	**ASO**	**Kynamro**	**Genzyme Corporation**	**Homozygous familial hypercholesterolemia**	**Binds to ApoB mRNA and induces its degradation by RNase H.**	**2013**	**Discontinued due to side effects**

Drug Name	Drug Class	Brand Name	Company	Target Disease	Mechanism of Action	Year of Approval	Current Status
Nusinersen	**ASO**	**Spinraza**	**Cold Spring Harbor Laboratory and Ionis Pharmaceuticals**	**Spinal muscular atrophy**	**Binds to SMN2 mRNA and alters its splicing.**	**2016**	**Continuous**
Eteplirsen	**ASO**	**Exondys 51**	**Sarepta Therapeutics, Inc.**	**Duchenne muscular dystrophy**	**Binds to exon 51 and alters splicing of dystrophin pre-mRNA.**	**2016**	**Continuous**
Patisiran	**siRNA**	**Onpattro**	**Alnylam Pharmaceuticals Inc.**	**Polyneuropathy in patients with hereditary transthyretin-mediated amyloidosis.**	**Binds to transthyretin (TTR) mRNA to decrease hepatic production of TTR protein**	**2018**	**Continuous**
Inotersen	**ASO**	**Tegsedi**	**Ionis Pharmaceuticals**	**Nerve damage in adults with hereditary transthyretin-mediated amyloidosis.**	**Binds to TTR mRNA and induces its degradation by RNase H**	**2018**	**Continuous**
Givosiran	**siRNA**	**Givlaari**	**Alnylam Pharmaceuticals Inc.**	**Acute hepatic porphyria**	**Reduces the hepatic production of ALASI protein through interference with ALASI mRNA.**	**2019**	**Continuous**
Golodirsen	**ASO**	**Vyondys**	**Sarepta Therapeutics, Inc.**	**Duchenne muscular dystrophy**	**Binds to exon 53 of dystrophin pre-mRNA to alter splicing.**	**2019**	**Continuous**

Note: Antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs).

Despite such achievements, myriad challenges remain to be overcome before their impact on patient's care is fully understood. In this section, we have discussed some of the most popular nucleic acids used to transfect MSCs, highlighting their advantages and disadvantages (Summarized in**Table 2**)

Nucleic Acid	DNA/RNA	Examples	Pros	Cons	Ref
Plasmids	**DNA**	• pCMS-EGFP • pUNO1-hBMP-7	• Large DNA packaging capacity. • Easy to handle. Stable at RT for long periods of time.	• Efficient nuclear transport is required. • Plasmid backbone elements can induce intracellular inflammation and transgene silencing	[16] [17]
Mini circles	**DNA**	• McCMV-fLuc2A-EGFP • McCMV-CXCR4	• High safety profile. • Persistent transgene expression (compared to pDNA).	• Efficient nuclear transport is required. • Sustainable scale-up with clinical-grade quality is still needed.	[18] [19] [20]

Nucleic Acid	DNA/RNA	Examples	Pros	Cons	Ref
mRNA	**RNA**	• ΔLNGFR mRNA	• No need for nuclear transport. • Higher transfection efficiency (compared to pDNA). • No risk of genome integration.	• Transient expression • Repeated dosing required.	[21]
Oligonucleotides/ ASO	**DNA/RNA**	• PyNTTTTGT ONs • Smurf1 GapmeR	• Transient and specific regulation of gene expression. • No risk of genome integration	• They need delivery carriers. • Natural ONs are degraded by nucleases. • Binding to off-target RNA. • Inability to cross BBB. • Could be immunogenic.	[22] [23] [24]
Aptamers	**DNA/RNA**	• HM69 • Seq3	• High binding affinity to target molecules. • Batch-to-batch consistency. Small sizes allowing them to penetrate tissues. • Non-immunogenic.	• Irrelevant interactions with biomolecules in vivo. • Quick excretion via the kidneys.	[25] [26]
RNAi/siRNAs	**RNA**	• siRNA-Runx2 • siRNA–REST • TOP2B_5 • TOP2B_6	• Transient and specific regulation of gene expression. • No risk of genome integration.	• They need delivery carriers.	[27]
MiRNAs	**RNA**	• miR-133 agomir • miR-100–5p • miR-143–3p	• Transient and specific regulation of gene expression. • No risk of genome integration.	• They need delivery carriers.	[28] [29]
Ribozymes and Deoxy ribozymes	**DNA/RNA**	• Rzpol1a1	• Transient and specific regulation of gene expression. • No risk of genome integration.	• They need delivery carriers. • Off-target effects.	[27] [30]

Nucleic Acid	DNA/RNA	Examples	Pros	Cons	Ref
Short hairpin RNA (shRNA)	**RNA**	• TIMP-1-shRNA • shRNF2-1 • shNRF2-2	• Specific regulation of gene expression.	• Vector-dependent.	[31] [32] [33]

Table 2. A summary of nucleic acids used to transfect MSCs: The advantages and disadvantages.

Plasmid-based gene therapy was attempted to correct single-gene disorders. On a molecular level, plasmids are circular, double-stranded DNA constructs varying in size from <1000 to >200 000 bp containing transgenes. Therefore, plasmid design can dramatically influence transgene expression [34]. [16][35] genes.

The decreased backbone size was shown to be directly correlated with the levels and extent of transgene expression in mammalian cells [18]. When compared to pDNA, Maria Florian et al., demonstrated that angiopoietin 1 (ANGPT1) encoded in mcDNAs -transfected MSCs could attain notably higher and prolonged secretion levels of ANGPT1 protein, resulting in superior therapeutic effects animals with acute lung injury [20]. On the other side, Serra J and team reported insignificant differences in transfection results in BM-MSCs with mcDNAs Efficient nuclear transport is still required to achieve notable transfection efficiency [18].

Nevertheless, the protein expression takes place for a shorter duration, which demands repeated transfection. To this end, BM-MSCs were transfected with mRNAs encoding several reprogramming factors (e.g., Oct4, Klf4, Sox2, cMyc, and Lin28) resulting in the formation of iPSC colonies [36]. Moreover, mRNA transfection is being used to simultaneously express multiple proteins such as in the study of Wenbin Liao et al. Such breakthrough would not have been possible without critical recent innovations in the production of high-quality mRNA as well as the development of safe and efficient materials for in vivo delivery.

3. Applications of Engineered MSCs

As mentioned above, there are various approaches through which genetically modified MSCs can be applied to achieve therapeutic impact in different clinical conditions. MSCs were used to deliver a myriad of growth factors [37][38], cytokines [39], transcription factors [40], or even suicide gene [41][42] with various potential clinical purposes. Some of these applications are reviewed next and summarized in Table 3.

Table 3. Applications of genetically modified MSCs in vivo.

Delivery System	Carrier		Nucleic Acid		Cell Vehicles	Application	Model/Host	Ref
	Type	Composition	Vector	Delivered Gene/siRNA				
Non-viral	Liposomes	Lipofectamine Plus®	Plasmid DNA	hTERT	MSC line derived from fetal porcine pancreas	Hyperglycemia	Diabetic model/Kunbai strain mice	[43]
	Polymer	PEI	Plasmid DNA	TRAIL	BM-MSCs	Melanoma	Melanoma model/e C57BL/6 mice	[44]
	Polymer	Chitosan	Plasmid DNA	BMP-2	BM-MSCs	Bone regeneration	Calvarial defect model/Rats	[45]
	Polymer	PEI	Plasmid DNA	BMP-2	BM-MSCs deriver MVs within gene-activated scaffold (DBM/MVs-PEI/phBMP2)	Bone regeneration	Femoral condylar defect/New Zealand white rabbits	[46]
	Polymer	Alginate GAM	Plasmid DNA	BMP-2	Rat BM-MSCs	Bone regeneration	Orthotopic spinous process defect/Fischer 344 inbred rats	[47]
	Polymer	LPEI	Plasmid DNA	VEGF	BM-MSCs	Myocardial infarction	MI model/SD rats	[48]
	Polymer	Cationized pullulan	Plasmid DNA	Suicide gene (CMV-TK)	Rat BM-MSCs	Melanoma	Pulmonary melanoma metastasis model/C57BL6 mice	[41]
	Polymer	LPEI	Plasmid DNA	CDY::UPRT	AT-MSCs	GDEPT: Chemo-resistant glioblastoma	Temozolomide resistant U-251MG cells/Nude mice	[49]
	Polymers	PEI-PLGA	Plasmid DNA and siRNA	coSOX9-pDNA/Cbfa-1-siRNA	hMSCs encapsulated in fibrin hydrogels	Chondrogenic differentiation	Nude BALB/c mice	[50]
	Polymers	PLL-PEI	Plasmid DNA	HSV-TK and TRAIL	rMSCs	Glioblastoma	Glioma model/SD rats	[51]
	Polymeric NPs	BA-PEI	Plasmid DNA	VEGF	BM-MSCs	Myocardial infarction	MI model/SD rats	[52]
	Plasmid-activated scaffolds	Chitosan-gelatin andnHA	Plasmid DNA	TGF-β1 and BMP-2	BM-MSCs	Regeneration of articular cartilage and subchondral bone	Knee osteochondral defect model/Rabbits	[53]
	nHA dual gene-activated scaffold	nHA and PEI	Plasmid DNA	BMP-2 and VEGF	rMSCs	Bone regeneration	Critical-sized cranial bone defect model/Rats	[54]
	Peptide conjugated NPs	Cationic AuNPs and PEP	Plasmid DNA	VEGF	Rat BM-MSCs	Antimicrobial and wound healing properties	Infected full thickness skin defect model/Mice	[55]

Delivery System	Carrier Type	Carrier Composition	Nucleic Acid Vector	Nucleic Acid Delivered Gene/siRNA	Cell Vehicles	Application	Model/Host	Ref
		AAV		IL-10	hBM-MSCs	Cerebral ischemia	MCAO I/R model/SD rats	[39]
		Adenovirus		HSV-TK/GCV	BM-MSCs	Intracranial gliomas	Intracranial human U87 glioma model/Nude mice	[56]
		Adenovirus		HGF	hBM-MSCs	Spinal cord injury	Spinal cord injury model/ SD rats	[37]
		Adenovirus		EGFR	Murine BM-MSCs	Brain tumors	Intracranial GL261 glioma or B16 melanoma/C57BL/6 mice	[57]
		Adenovirus		IFN-β	hBM-MSCs	Pancreatic cancer	Transplant PANC-1 cancer model/SCID mice	[58]
		Fiber-modified adenovirus		kringle1-5/EGFP	hPMSCs in Matrigel plugs	Suppression of angiogenesis	SHXY/HGA-2021 loaded matrigel plugs/BALB/c nude	[59]
		Gamma -Retrovirus		IL7-IL12	hBM-MSCs	Colorectal cancer	Transplant LS174T colorectal cancer model/NSG mice	[60]
	Viral	Gamma-retrovirus		HSV-TK	hBM-MSCs	Gastrointestinal/ hepatopancreatobiliary adenocarcinoma	Phase I and II clinical trial	[42]
		HSV-1		HGF	rBM-MSCs	Cerebral ischemia	MCAO I/R model/Wistar rats	[38]
		Lentivirus		miR-126	BM-MSCs	Myocardial infarction	MI model/Mice	[61]
		Lentivirus		HGF	UCB-MSCs	Myocardial infarction	MI model/SCID mice	[62]
		Lentivirus		FGF21	Mouse BM-MSCs	Brain Injury	Lateral fluid percussion traumatic brain injury model/C57BL/6 mice	[63]
		Lentivirus		Cxcr4	rBM-MSCs	Cerebral ischemia	MCAO I/R model/SD rats	[64]
		Recombinant adenovirus		VEGF	BM-MSCs	Cerebral ischemia	MCAO I/R model/rats	[65]
		Retrovirus		AKT	Mouse BM-MSCs	Myocardial infarction	MI model/C57BL/6 mice	[66]
		Adenovirus/liposome		Ad-hEndo	hPMSCs	Ovarian cancer	Transplant A2780 ovarian cancer model/ Nude mice	[67]
	Hybrid	Adenovirus/CPP		stTRAIL	hUCB-MSCs	Glioblastoma	Intracranial xenograft human glioma model/Athymic nude mice	[68]
		Adenovirus/4HP4		IL-12M	rBM-MSCs	Melanoma and cervical cancer	B16F10 melanoma and TC-1 cervical cancer models/SCID mice	[69]

Note: 4HP4: tetrameric form of cell-permeable peptide; CPP: cell-permeable peptide; HSV: herpes simplex virus; tTATop-BMP-2: tetracycline transactivator and BMP-2 cDNAs; BA-PEI: bile acid-modified polyethyleneimine; PMAA: polymethacrylate acid; CMV: cytomegalovirus; AT-MSCs: adipose tissue-derived MSCs; HIF-1 α: hypoxia-inducible factor-

References

1. Attia, N.; Mashal, M. Mesenchymal Stem Cells: The Past Present and Future; Spinger: New York, NY, USA, 2020.
2. Mehanna, R.A.; Nabil, I.; Attia, N.; Bary, A.A.; Razek, K.A.; Ahmed, T.A.; Elsayed, A. The effect of bone marrow-derived mesenchymal stem cells and their conditioned media topically delivered in fibrin glue on chronic wound healing in rats. BioMed Res. Int. 2015, 2015.
3. Afanasyev, B.V.; Elstner, E.E.; Zander, A.R. AJ Friedenstein, founder of the mesenchymal stem cell concept. Cell Ther. Transpl. 2009, 1, 35–38.
4. Friedenstein, A.J.; Petrakova, K.V.; Kurolesova, A.I.; Frolova, G.P. Heterotopic transplants of bone marrow. Transplant 1968, 6, 230–247.
5. Attia, N.; Khalifa, Y.H.; Rostom, D.M.; Mashal, M. Mesenchymal stem cells versus their extracellular vesicles in treatment of liver fibrosis: Is it possible to compare? Med. Res. Arch. 2021, 9, 9.
6. James, R.; Namitha, H.; Kaushik, D.D. Clinical applications of mesenchymal stem cells. In Biointegration of Medical Implant Materials (Second Edition); Sharma, C.P., Ed.; Elsevier: Amsterdam, Netherlands, 2020; pp. 101–116.
7. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.C.; Krause, D.S.; Deans, R.J.; Keating, A.; Prockop, D.J.; Horwitz, E.M. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006, 8, 315–317.
8. Yang, Y.-H.K.; Ogando, C.R.; See, C.W.; Chang, T.-Y.; Barabino, G.A. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. Stem Cell Res. Ther. 2018, 9, 1–14.
9. Yu, K.-R.; Lee, J.Y.; Kim, H.-S.; Hong, I.-S.; Choi, S.W.; Seo, Y.; Kang, I.; Kim, J.-J.; Lee, B.-C.; Lee, S.; et al. A p38 MAPK-Mediated Alteration of COX-2/PGE2 Regulates Immunomodulatory Properties in Human Mesenchymal Stem Cell Aging. PLoS ONE 2014, 9, e102426.
10. Sotirov, R.; Kostadinova, M.; Pashova, S.; Kestendjieva, S.; Vlahetova, K.; Abadjieva, D.; Stoyanova, E.; Oreshkova, T.; Kistanova, E.; Mourdjeva, M. Morphology of Mesenchymal Stem Cells in 3D spheroids. Acta Morphol. Anthropol. 2018, 25, 90–96.
11. Caplan, A.I. There Is No “Stem Cell Mess”. Tissue Eng. Part. B Rev. 2019, 25, 291–293.
12. Satija, N.; Singh, V.K.; Verma, Y.K.; Gupta, P.; Sharma, S.; Afrin, F.; Sharma, M.; Sharma, P.; Tripathi, R.P.; Gurudutta, G.U. Mesenchymal stem cell-based therapy: A new paradigm in regenerative medicine. J. Cell. Mol. Med. 2009, 13, 4385–4402.
13. Sridharan, K.; Gogtay, N.J. Therapeutic nucleic acids: Current clinical status. Br. J. Clin. Pharmacol. 2016, 82, 659–672.
14. Yu, A.-M.; Choi, Y.H.; Tu, M.-J. RNA Drugs and RNA Targets for Small Molecules: Principles, Progress, and Challenges. Pharmacol. Rev. 2020, 72, 862–898.
15. Kim, Y.-K. RNA Therapy: Current Status and Future Potential. Chonnam Med. J. 2020, 56, 87–93.
16. Attia, N.; Mashal, M.; Grijalvo, S.; Eritja, R.; Zárate, J.; Puras, G.; Pedraz, J.L. Stem cell-based gene delivery mediated by cationic niosomes for bone regeneration. Nanomed. Nanotechnol. Biol. Med. 2018, 14, 521–531.
17. Hamann, A.; Nguyen, A.; Pannier, A.K. Nucleic acid delivery to mesenchymal stem cells: A review of nonviral methods and applications. J. Biol. Eng. 2019, 13, 1–16.

CD: cytosine deaminase; UPRT: uracil phosphoribosyltransferase; GAM: gene-activated matrix; GDEPT: gene-directed enzyme prodrug therapy; MVs: microvesicles; DBM: demineralized bone matrix; PLL-PEI: polylysine-modified polyethylenimine; hPMSCs: human placenta-derived MSCs; HSV: herpes simplex virus; MCAO I/R: middle cerebral artery occlusion ischemia/reperfusion; SD: Sprague-Dawley; MI: myocardial infarction; hEndo: human endostatin; UCB-MSCs: umbilical cord blood-derived MSCs.

18. Gaspar, V.P.; de Melo-Diogo, D.; Costa, E.; Moreira, A.; Queiroz, J.; Pichon, C.; Correia, I.; Sousa, F. Minicircle DNA vectors for gene therapy: Advances and applications. Expert Opin. Biol. Ther. 2014, 15, 353–379.
19. Mun, J.-Y.; Shin, K.K.; Kwon, O.; Lim, Y.T.; Oh, D.-B. Minicircle microporation-based non-viral gene delivery improved the targeting of mesenchymal stem cells to an injury site. Biomaterials 2016, 101, 310–320.
20. Florian, M.; Wang, J.-P.; Deng, Y.; Souza-Moreira, L.; Stewart, D.J.; Mei, S.H.-J. Gene Engineered Mesenchymal Stem Cells: Greater Transgene Expression and Efficacy With Minicircle Vs. Plasmid DNA Vectors in a Mouse Model of Acute Lung Injury. Stem Cell Res. Ther. 2020, 12, 1–9.
21. Wiehe, J.M.; Ponsaerts, P.; Rojewski, M.T.; Homann, J.M.; Greiner, J.; Kronawitter, D.; Schrezenmeier, H.; Hombach, V.; Wiesneth, M.; Zimmermann, O.; et al. mRNA-Mediated Gene Delivery Into Human Progenitor Cells Promotes Highly Efficient Protein Expression. J. Cell. Mol. Med. 2007, 11, 521–530.
22. Insúa, A.; Montaner, A.; Rodriguez, J.; Elías, F.; Fló, J.; López, R.; Zorzopulos, J. PyNTTTTGT oligonucleotides as tools in tissue repair procedures. Top. Tissue Eng. 2007, 3, 1–13.
23. García-García, P.; Ruiz, M.; Reyes, R.; Delgado, A.; Évora, C.; Riancho, J.A.; Rodríguez-Rey, J.C.; Pérez-Campo, F.M. Smurf1 Silencing Using a LNA-ASOs/Lipid Nanoparticle System to Promote Bone Regeneration. Stem Cells Transl. Med. 2019, 8, 1306–1317.
24. Hanagata, N.J. Structure-dependent immunostimulatory effect of CpG oligodeoxynucleotides and their delivery system. Int. J. Nanomed. 2012, 7, 2181.
25. Wang, M.; Wu, H.; Li, Q.; Yang, Y.; Che, F.; Wang, G.; Zhang, L. Novel aptamer-functionalized nanoparticles enhances bone defect repair by improving stem cell recruitment. Int. J. Nanomed. 2019, 14, 8707.
26. Zou, Y.; Wen, X.; Ling, D.; Zhang, D.; Lei, L.; Zhu, D.; Wang, H.; Wang, K.; Guo, Q.; Nie, H. Precise monitoring of mesenchymal stem cell homing to injured kidney with an activatable aptamer probe generated by cell-SELEX. Appl. Mater. Today 2021, 22, 100974.
27. Kamaci, N.; Emnacar, T.; Karakas, N.; Arikan, G.; Tsutsui, K.; Isik, S. Selective silencing of DNA topoisomerase IIβ in human mesenchymal stem cells by siRNAs (small interfering RNAs). Cell Biol. Int. Rep. 2011, 18, 15–21.
28. Chen, Y.; Zhao, Y.; Chen, W.; Xie, L.; Zhao, Z.-A.; Yang, J.; Chen, Y.; Lei, W.; Shen, Z. MicroRNA-133 overexpression promotes the therapeutic efficacy of mesenchymal stem cells on acute myocardial infarction. Stem Cell Res. Ther. 2017, 8, 1–11.
29. Carthew, J.; Donderwinkel, I.; Shrestha, S.; Truong, V.; Forsythe, J.; Frith, J. In situ miRNA delivery from a hydrogel promotes osteogenesis of encapsulated mesenchymal stromal cells. Acta Biomater. 2020, 101, 249–261.
30. Chen, Y.; Zhao, H.; Tan, Z.; Zhang, C.; Fu, X. Bottleneck limitations for microRNA-based therapeutics from bench to the bedside. Die Pharm. Int. J. Pharm. Sci. 2015, 70, 147–154.
31. Zhu, Y.; Miao, Z.; Gong, L.; Chen, W.-C. Transplantation of mesenchymal stem cells expressing TIMP-1-shRNA improves hepatic fibrosis in CCl4-treated rats. Int. J. Clin. Exp. Pathol. 2015, 8, 8912–8920.
32. Baker, N.; Zhang, G.; You, Y.; Tuan, R.S. Caveolin-1 regulates proliferation and osteogenic differentiation of human mesenchymal stem cells. J. Cell. Biochem. 2012, 113, 3773–3787.
33. Yoon, D.S.; Choi, Y.; Lee, J.W. Cellular localization of NRF2 determines the self-renewal and osteogenic differentiation potential of human MSCs via the P53–SIRT1 axis. Cell Death Dis. 2016, 7, e2093.
34. Christensen, M.D.; Nitiyanandan, R.; Meraji, S.; Daer, R.; Godeshala, S.; Goklany, S.; Haynes, K.; Rege, K. An inhibitor screen identifies histone-modifying enzymes as mediators of polymer-mediated transgene expression from plasmid DNA. J. Control. Release 2018, 286, 210–223.
35. Kim, J.Y.; Park, S.; Park, S.H.; Lee, D.; Kim, G.H.; Noh, J.E.; Lee, K.J.; Kim, G.J. Overexpression of pigment epithelium-derived factor in placenta-derived mesenchymal stem cells promotes mitochondrial biogenesis in retinal cells. Am. J. Pathol. 2021, 101, 51–69.
36. Varela, I.; Karagiannidou, A.; Oikonomakis, V.; Tzetis, M.; Tzanoudaki, M.; Siapati, E.-K.; Vassilopoulos, G.; Graphakos, S.; Kanavakis, E.; Goussetis, E. Generation of Human β-Thalassemia Induced Pluripotent Cell Lines by Reprogramming of Bone Marrow–Derived Mesenchymal Stromal Cells Using Modified mRNA. Cell. Reprogramming 2014, 16, 447–455.
37. Jeong, S.R.; Kwon, M.J.; Lee, H.G.; Joe, E.H.; Lee, J.H.; Kim, S.S.; Suh-Kim, H.; Kim, B.G. Hepatocyte growth factor reduces astrocytic scar formation and promotes axonal growth beyond glial scars after spinal cord injury. Exp. Neurol. 2012, 233, 312–322.
38. Zhao, M.-Z.; Nonoguchi, N.; Ikeda, N.; Watanabe, T.; Furutama, D.; Miyazawa, D.; Funakoshi, H.; Kajimoto, Y.; Nakamura, T.; Dezawa, M. Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF

gene transfer with HSV-1 vector. J. Cereb. Blood Flow Metab. 2006, 26, 1176–1188.

39. Nakajima, M.; Nito, C.; Sowa, K.; Suda, S.; Nishiyama, Y.; Nakamura-Takahashi, A.; Nitahara-Kasahara, Y.; Imagawa, K.; Hirato, T.; Ueda, M. Mesenchymal stem cells overexpressing interleukin-10 promote neuroprotection in experimental acute ischemic stroke. Mol. Ther. Methods Clin. Dev. 2017, 6, 102–111.
40. Raisin, S.; Morille, M.; Bony, C.; Noël, D.; Devoisselle, J.-M.; Belamie, E. Tripartite polyionic complex (PIC) micelles as non-viral vectors for mesenchymal stem cell siRNA transfection. Biomater. Sci. 2017, 5, 1910–1921.
41. Zhang, T.-Y.; Huang, B.; Yuan, Z.-Y.; Hu, Y.-L.; Tabata, Y.; Gao, J.-Q. Gene recombinant bone marrow mesenchymal stem cells as a tumor-targeted suicide gene delivery vehicle in pulmonary metastasis therapy using non-viral transfection. Nanomed. Nanotechnol. Biol. Med. 2014, 10, 257–267.
42. Niess, H.; von Einem, J.C.; Thomas, M.N.; Michl, M.; Angele, M.K.; Huss, R.; Günther, C.; Nelson, P.J.; Bruns, C.J.; Heinemann, V. Treatment of advanced gastrointestinal tumors with genetically modified autologous mesenchymal stromal cells (TREAT-ME1): Study protocol of a phase I/II clinical trial. BMC Cancer 2015, 15, 1–13.
43. Cao, H.; Chu, Y.; Zhu, H.; Sun, J.; Pu, Y.; Gao, Z.; Yang, C.; Peng, S.; Dou, Z.; Hua, J. Characterization of immortalized mesenchymal stem cells derived from foetal porcine pancreas. Cell Prolif. 2011, 44, 19–32.
44. Salmasi, Z.; Hashemi, M.; Mahdipour, E.; Nourani, H.; Abnous, K.; Ramezani, M. Mesenchymal stem cells engineered by modified polyethylenimine polymer for targeted cancer gene therapy, in vitro and in vivo. Biotechnol. Prog. 2020, 36, e3025.
45. Malek-Khatabi, A.; Javar, H.A.; Dashtimoghadam, E.; Ansari, S.; Hasani-Sadrabadi, M.M.; Moshaverinia, A. In situ bone tissue engineering using gene delivery nanocomplexes. Acta Biomater. 2020, 108, 326–336.
46. Liang, Z.; Luo, Y.; Lv, Y. Mesenchymal stem cell-derived microvesicles mediate BMP2 gene delivery and enhance bone regeneration. J. Mater. Chem. B 2020, 8, 6378–6389.
47. Loozen, L.D.; Kruyt, M.C.; Kragten, A.H.; Schoenfeldt, T.; Croes, M.; Oner, C.F.; Dhert, W.J.; Alblas, J. BMP-2 gene delivery in cell-loaded and cell-free constructs for bone regeneration. PLoS ONE 2019, 14, e0220028.
48. Kim, S.H.; Moon, H.-H.; Kim, H.A.; Hwang, K.-C.; Lee, M.; Choi, D. Hypoxia-inducible vascular endothelial growth factor-engineered mesenchymal stem cells prevent myocardial ischemic injury. Mol. Ther. 2011, 19, 741–750.
49. Ho, Y.K.; Woo, J.Y.; Tu, G.X.E.; Deng, L.-W.; Too, H.-P. A highly efficient non-viral process for programming mesenchymal stem cells for gene directed enzyme prodrug cancer therapy. Sci. Rep. 2020, 10, 1–15.
50. Jeon, S.Y.; Park, J.S.; Yang, H.N.; Woo, D.G.; Park, K.-H. Co-delivery of SOX9 genes and anti-Cbfa-1 siRNA coated onto PLGA nanoparticles for chondrogenesis of human MSCs. Biomaterials 2012, 33, 4413–4423.
51. Malik, Y.S.; Sheikh, M.A.; Xing, Z.; Guo, Z.; Zhu, X.; Tian, H.; Chen, X. Polylysine-modified polyethylenimine polymer can generate genetically engineered mesenchymal stem cells for combinational suicidal gene therapy in glioblastoma. Acta Biomater. 2018, 80, 144–153.
52. Moon, H.-H.; Joo, M.K.; Mok, H.; Lee, M.; Hwang, K.-C.; Kim, S.W.; Jeong, J.H.; Choi, D.; Kim, S.H. MSC-based VEGF gene therapy in rat myocardial infarction model using facial amphipathic bile acid-conjugated polyethyleneimine. Biomaterials 2014, 35, 1744–1754.
53. Chen, J.; Chen, H.; Li, P.; Diao, H.; Zhu, S.; Dong, L.; Wang, R.; Guo, T.; Zhao, J.; Zhang, J. Simultaneous regeneration of articular cartilage and subchondral bone in vivo using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds. Biomaterials 2011, 32, 4793–4805.
54. Curtin, C.M.; Tierney, E.G.; McSorley, K.; Cryan, S.A.; Duffy, G.P.; O'Brien, F.J. Combinatorial gene therapy accelerates bone regeneration: Non-viral dual delivery of VEGF and BMP2 in a collagen-nanohydroxyapatite scaffold. Adv. Healthc. Mater. 2015, 4, 223–227.
55. Peng, L.-H.; Huang, Y.-F.; Zhang, C.-Z.; Niu, J.; Chen, Y.; Chu, Y.; Jiang, Z.-H.; Gao, J.-Q.; Mao, Z.-W. Integration of antimicrobial peptides with gold nanoparticles as unique non-viral vectors for gene delivery to mesenchymal stem cells with antibacterial activity. Biomaterials 2016, 103, 137–149.
56. Ryu, C.H.; Park, K.Y.; Kim, S.M.; Jeong, C.H.; Woo, J.S.; Hou, Y.; Jeun, S.-S. Valproic acid enhances anti-tumor effect of mesenchymal stem cell mediated HSV-TK gene therapy in intracranial glioma. Biochem. Biophys. Res. Commun. 2012, 421, 585–590.
57. Sato, H.; Kuwashima, N.; Sakaida, T.; Hatano, M.; Dusak, J.E.; Fellows-Mayle, W.K.; Papworth, G.D.; Watkins, S.C.; Gambotto, A.; Pollack, I.F. Epidermal growth factor receptor-transfected bone marrow stromal cells exhibit enhanced migratory response and therapeutic potential against murine brain tumors. Cancer Gene Ther. 2005, 12, 757–768.
58. Kidd, S.; Caldwell, L.; Dietrich, M.; Samudio, I.; Spaeth, E.L.; Watson, K.; Shi, Y.; Abbruzzese, J.; Konopleva, M.; Andreeff, M. Mesenchymal stromal cells alone or expressing interferon-β suppress pancreatic tumors in vivo, an effect

countered by anti-inflammatory treatment. Cytotherapy 2010, 12, 615–625.

59. Chu, Y.; Liu, H.; Lou, G.; Zhang, Q.; Wu, C. Human placenta mesenchymal stem cells expressing exogenous kringle1-5 protein by fiber-modified adenovirus suppress angiogenesis. Cancer Gene Ther. 2014, 21, 200–208.
60. Hombach, A.A.; Geumann, U.; Günther, C.; Hermann, F.G.; Abken, H. IL7-IL12 engineered Mesenchymal stem cells (MSCs) improve a CAR T cell attack against colorectal cancer cells. Cells 2020, 9, 873.
61. Chen, J.-J.; Zhou, S.-H. Mesenchymal stem cells overexpressing MiR-126 enhance ischemic angiogenesis via the AKT/ERK-related pathway. Cardiol. J. 2011, 18, 675–681.
62. Zhao, L.; Liu, X.; Zhang, Y.; Liang, X.; Ding, Y.; Xu, Y.; Fang, Z.; Zhang, F. Enhanced cell survival and paracrine effects of mesenchymal stem cells overexpressing hepatocyte growth factor promote cardioprotection in myocardial infarction. Exp. Cell Res. 2016, 344, 30–39.
63. Shahror, R.A.; Linares, G.R.; Wang, Y.; Hsueh, S.-C.; Wu, C.-C.; Chuang, D.-M.; Chiang, Y.-H.; Chen, K.-Y. Transplantation of mesenchymal stem cells overexpressing fibroblast growth factor 21 facilitates cognitive recovery and enhances neurogenesis in a mouse model of traumatic brain injury. J. Neurotrauma 2020, 37, 14–26.
64. Yu, X.; Chen, D.; Zhang, Y.; Wu, X.; Huang, Z.; Zhou, H.; Zhang, Y.; Zhang, Z. Overexpression of CXCR4 in mesenchymal stem cells promotes migration, neuroprotection and angiogenesis in a rat model of stroke. J. Neurol. Sci. 2012, 316, 141–149.
65. Chen, B.; Zhang, F.; Li, Q.-Y.; Gong, A.; Lan, Q. Protective effect of Ad-VEGF-Bone mesenchymal stem cells on cerebral infarction. Turk. Neurosurg. 2016, 26, 8–15.
66. Noiseux, N.; Gnecchi, M.; Lopez-Ilasaca, M.; Zhang, L.; Solomon, S.D.; Deb, A.; Dzau, V.J.; Pratt, R.E. Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. Mol. Ther. 2006, 14, 840–850.
67. Zheng, L.; Zhang, D.; Chen, X.; Yang, L.; Wei, Y.; Zhao, X. Antitumor activities of human placenta-derived mesenchymal stem cells expressing endostatin on ovarian cancer. PLoS ONE 2012, 7, e39119.
68. Kim, S.M.; Lim, J.Y.; Park, S.I.; Jeong, C.H.; Oh, J.H.; Jeong, M.; Oh, W.; Park, S.-H.; Sung, Y.-C.; Jeun, S.-S. Gene therapy using TRAIL-secreting human umbilical cord blood–derived mesenchymal stem cells against intracranial glioma. Cancer Res. 2008, 68, 9614–9623.
69. Seo, S.; Kim, K.; Park, S.; Suh, Y.; Kim, S.; Jeun, S.; Sung, Y. The effects of mesenchymal stem cells injected via different routes on modified IL-12-mediated antitumor activity. Gene Ther. 2011, 18, 488–495.