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Engineering mesenchymal stem cells for regenerative medicine and drug delivery

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ABSTRACT

Researchers have applied mesenchymal stem cells (MSC) to a variety of therapeutic scenarios by harnessing their multipotent, regenerative, and immunosuppressive properties with tropisms toward inflamed, hypoxic, and cancerous sites. Although MSC-based therapies have been shown to be safe and effective to a certain degree, the efficacy remains low in most cases when MSC are applied alone. To enhance their therapeutic efficacy, researchers have equipped MSC with targeted delivery functions using genetic engineering, therapeutic agent incorporation, and cell surface modification. MSC can be genetically modified virally or non-virally to overexpress therapeutic proteins that complement their innate properties. MSC can also be primed with non-peptidic drugs or magnetic nanoparticles for enhanced efficacy and externally regulated targeting, respectively. Furthermore, MSC can be functionalized with targeting moieties to augment their homing toward therapeutic sites using enzymatic modification, chemical conjugation, or non-covalent interactions. These engineering techniques are still works in progress, requiring optimization to improve the therapeutic efficacy and targeting effectiveness while minimizing any loss of MSC function. In this review, we will highlight the advanced techniques of engineering MSC, describe their promise and the challenges of translation into clinical settings, and suggest future perspectives on realizing their full potential for MSC-based therapy.

1. Introduction

Mesenchymal stem cells (MSC) are adult stem cells capable of self-renewal and differentiation into multiple lineages including cartilage, adipose, and bone. MSC are characterized by their ability to adhere to plastics under standard cell culture conditions, expressing CD44, CD73, CD90, CD105, but not CD45, CD34, and CD14 [1].

Friedenstein first reported MSC merely as proliferating fibroblastic cells from bone marrow capable of differentiating into osteoblasts, chondrocytes and adipocytes. Along with their self-renewal property, MSC secrete factors, such as growth factors, both in an autocrine and paracrine fashion, which affect the surrounding microenvironment to promote angiogenesis, decrease inflammation, and enhance tissue repair. Moreover, MSC exert strong immunosuppressive properties, allowing them to be transplanted without any pre- or post-treatment. Additionally, they are easy to expand in culture and have multi-lineage differentiation potential and tropism toward neo-angiogenic, tumor, and inflammatory sites. MSC also pose no risk of teratoma formation, nor are there

Abbreviations: MSC, mesenchymal stem cells; BM, bone marrow; UC, umbilical cord; iPSC, induced pluripotent stem cells; UC-MSC, UC-derived MSC; BM-MSC, BM-derived MSC; NP, nanoparticle; AAV, adeno-associated virus; ASC, adipose-derived stem cells; ZFN, zinc finger nuclease; Epo, erythropoietin gene; TALENS, transcription activator-like effector nucleases; CRISPR, clustered regularly interspaced short palindromic repeats; MBs, microbubbles; PEI, poly(ethylenimine); Akt1, protein kinase B; Bcl-2, B-cell lymphoma-2; HO-1, heme oxygenase-1; CXCR4, chemokine (C-X-C motif) receptor 4; CCR-1, C-C chemokine receptor type-1; BMP2, bone morphogenetic protein-2; TGF-β, transforming growth factor-β; IGF-1, insulin like growth factor-1; VEGF, vascular endothelial growth factor; Runx-2, runt-related transcription factor-2; PLL, poly-L-lysine; PCL, poly-ε-caprolactone; ILs, interleukins; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; scFvCD20-TRAIL, CD20-specific single chain Fv antibody TRAIL fusion; FIX, Factor IX; PLA, poly(D,L-lactide); PLMA, poly(D,L-lactic acid-co-α,β-malic acid); SPIO, superparamagnetic iron oxide; PTX, paclitaxel; HSC, hematopoietic stem cells; HCELL, hematopoietic cell E-/L-selectin ligand; PSGL-1, P-selectin glycoprotein ligand-1; CB, cord blood; FTVI, fucosyltransferase VI; SLe^X, Sialyl-Lewis X; HSPC, hematopoietic stem/progenitor cells; NHS, N-hydroxy-succinimide; SDF-1, stromal cell-derived factor-1; VCAM-1, vascular cell adhesion molecule-1; Ale, alendronate; ICAM-1, intercellular adhesion molecule-1; MAdCAM, mucosal vascular addressin cell adhesion molecule.

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any ethical issues associated with the cell source. All of these properties collectively make MSC an attractive candidate for cell-based therapies.

MSC have been isolated from a wide range of sources including bone marrow (BM) [2], umbilical cord (UC), adipose tissue [3], liver [4], multiple dental tissues [5], and induced pluripotent stem cells (iPSC) [6]. Each of these sources has its own advantages and disadvantages. BM is the most characterized and documented source of MSC. However, the collection of MSC from BM is painful, invasive, and characterized by a low yield [2]. MSC in the UC can be obtained from Wharton jelly, veins, arteries, the UC lining, and the subamnion and perivascular regions. UC-derived MSC (UC-MSC) can be obtained through a painless collection method and have fewer associated ethical issues. They also renew faster than BM-derived MSC (BM-MSC) [7]. Adipose tissue is another popular source, mainly because a large number of MSC can be obtained through minimally invasive methods [3]. In all cases, these cells need to be monitored regularly to ascertain their quality. While there are many sources for MSC, the quality of the MSC is highly variable from donor to donor and is significantly affected by age and aging disorders. Furthermore, extended handling of MSC *in vitro* reduces their differentiation potential. To circumvent these issues, MSC were recently derived from iPSC [6]. These cells have the same *in vitro* and *in vivo* characteristics of BM-MSC, such as the potential for adipogenesis, osteogenesis, and chondrogenesis. MSC derived from iPSC also display higher capacity for proliferation and stronger telomerase activity, leading to better engraftment and survival after transplantation. Additionally, they display superior capabilities in repairing tissue ischemia compared to BM-MSC [6]. In addition to tissue regeneration, MSC have been used to treat type-1 diabetes [8], myocardial infarction [9], graft-versus-host disease [10], inflammatory bowel disease [11], and cancer [12]. Currently, there are 395 ongoing or completed clinical trials worldwide using MSC or mesenchymal stromal cells [13], indicating the popularity of MSC for cell-based therapies.

In this review, we will highlight the advanced techniques used to engineer MSC for tissue engineering and drug delivery applications. The challenges and advantages of each technique will also be analyzed and discussed. Numerous clinical trials have established the safety of using MSC for cell-based therapies. However, the efficacy of MSC *in vivo* is still low due to poor survival, retention, and engraftment of the cells. The first section of this paper focuses on genetic modification to enhance the survival, migration, and secretion of growth factors for their application in the field of regenerative medicine. This is followed by a discussion of MSC applications in cancer therapy and gene therapy. Although genetic modification is a powerful tool, only protein-based drugs can be delivered using this approach. Additionally, the genetic modification could potentially affect the innate properties of MSC. Hence, over the last few years, nanoparticle (NP)-based MSC delivery systems have gained increasing attention. While numerous synthetic NP platforms have been designed and some have even shown promising clinical outcomes, obstacles (including toxicity, specificity, and delivery efficiency) remain to be overcome before translation. In contrast, MSC offer intrinsic homing properties, low toxicity, and low immunogenicity, which could lead to higher delivery efficiency compared to conventional nanomedicine platforms. The second section of the paper focuses on combining conventional NP platforms with MSC-based therapies. The various methods used to load the therapeutic agents onto MSC, release the therapeutic agents from MSC, and the applications of such MSC-NP combination are analyzed in detail. However, NP-based MSC therapy must ensure that the NP does not compromise the cell's native properties and it can deliver a suitable release profile. To deal with these issues, researchers have used surface modification of MSC as an alternative. Using various engineering approaches (enzymatic modification, chemical modification, and non-covalent interactions), researchers immobilize targeting moieties onto the cell surface to direct MSC to the therapeutic site. As surface modification confers only transient expression of targeting molecules on MSC, it does not significantly affect the cells' phenotype. The last section will suggest future perspectives for translating MSC-based therapies.

2. Techniques for engineering MSC

2.1. Genetic modifications

The clinical application of MSC is often hampered by inadequate *in vivo* performance with respect to survival, retention, and engraftment. Genetic engineering is one approach to improve the *in vivo* performance of MSC. MSC are genetically engineered to secrete factors that can protect MSC from apoptosis, increase their survivability in hypoxic conditions, and enhance other innate properties, such as migration, cardiac protection, and differentiation to a particular lineage. Moreover, genetic modifications have also been used to engineer MSC to produce therapeutic proteins for treating diseases like hemophilia and diabetes, and for repairing musculoskeletal disorders. Genetic modification of MSC is usually achieved *via* viral vectors although the use of non-viral vectors is on the rise.

2.1.1. Viral transduction

MSC are readily amenable to viral modification. Standard protocols can lead to 90% transduced cells with no effect on lineage differentiation or the quality of the progeny [14,15]. Viral transduction can also offer a long-term and stable production of the protein of interest. The most common vectors include retrovirus, lentivirus, baculovirus, and adeno-associated virus (AAV) [16]. Retrovirus leads to integration of the transgene into the host genome. While this results in a stable expression, it could also lead to insertional mutagenesis and activation of oncogenes [17]. Retrovirus is used when long-term protein production is desirable, such as treatment of genetic diseases. Lentivirus also enables stable transgene expression through integration into the genome. Non-integrating lentiviral vectors have also been designed that can circumvent the problems associated with integration [18]. Baculovirus, on the other hand, is non-toxic; it neither replicates nor integrates into the host genome and is capable of transducing with high efficiency. Baculovirus can transduce adipose-derived stem cells (ASC) with 95% efficiency and minimal toxicity [19]. Finally, AAV is one of the most promising vectors as it is non-pathogenic to humans and results in long-term gene expression. However, a large fraction of the human population have neutralizing antibodies against AAV, which drastically reduces their *in vivo* efficacy [20]. To circumvent the issue of activating oncogenes and to achieve targeted integration, Benabdallah et al. used zinc finger nuclease (ZFN) to add erythropoietin gene (Epo) into the chemokine (C-C motif) receptor-5 gene locus of MSC. ZFN was delivered to MSC using adenovirus while Epo was delivered using integrase-defective lentiviral vectors. The MSC derived from human BM, adipose tissue, and UCB was transduced with Epo by the ZFN-driven targeted gene addition. When these cells were injected into the peritoneum of non-obese diabetic severe combined immunodeficient interleukin-2Rγ null mice, the hematocrit levels rose from an average of 49% to more than 60% at day 10 [21]. This study clearly demonstrates the potential of site directed insertion (Fig. 1A) compared to the conventional random integration using viral vectors. Site-directed integration can also be achieved using transcription activator-like effector nucleases (TALENS), and clustered regularly interspaced short palindromic repeats (CRISPR).