REVIEW ARTICLE

Genetic modification of mesenchymal stem cells to enhance their anti-tumor efficacy

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Abstract

Non-hematopoietic mesenchymal stem cells (MSCs) are widely used in regenerative medicine and tissue engineering as they possess multilineage differentiation potential and self-renewal properties. MSCs can be easily isolated from several tissues and expanded following standard cell culture procedures. MSCs have the capability of mobilization to the tumor site; so, they can automatically relocate to the tumor sites through their chemokine receptors following intravenous transplantation. In this respect, they can be used for MSC-based gene therapy. In this therapeutic technique, beneficial genes are inserted by viral and non-viral methods into MSCs that lead to transgene expression in them. Genetic modifications of MSCs have been widely studied and thoroughly investigated to further enhance their therapeutic efficacy. The current strategies of MSC-based therapies emphasize the incorporation of beneficial genes, which will enhance the therapeutic ability of MSCs and have better homing efficiency. Non-viral methods produce less toxicity and immunogenicity compared to viral gene delivery methods and thus represent a promising and efficient tool for the genetic engineering of MSCs. Several non-viral gene delivery strategies have been developed in recent decades, and some of them have been used for MSCs modification. This mini review provides an overview of current gene delivery approaches used for the genetic modification of MSCs with beneficial genes including viral and non-viral vectors.

Key words Mesenchymal stem cells (MSC), cell-based therapy, genetic modification, viral and non-viral vectors, transfection, gene therapy

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Introduction

MSCs are multipotent adult stromal cells that can be isolated from different sources, including amniotic fluid, bone marrow, dental pulp, adipose tissue, and umbilical cord [1-5]. MSCs are classified as cells that can adhere to plastic, express the surface molecules CD73, CD90, and CD105 (approximately 95% positive), but lack the expression of CD45, CD19, CD14, CD34, CD11b, CD79alpha, and HLA-DR. MSCs possess specific characteristics of stem cells and differentiate into chondroblasts, adipocytes, osteoblasts, myoblasts, fibroblasts, and cardiomyoblasts under in vitro differentiation conditions [6].

Several studies have been reported on the possible therapeutic mechanisms of MSCs for the regenerative treatment of untreatable diseases. (i) The homing property of MSCs, enable them to adhere to tumor sites [7]. The homing effect of MSCs theoretically implies that, in clinical applications, MSCs can be migrated to the tumor site using only intravascular transplantation of MSCs and not surgery. (ii) Although the ratio of differentiated cells to transplanted cells has been reported to be very low, MSCs can differentiate directly into damaged cells, facilitating repair [8, 9]. (iii) MSCs can control immune responses [10-12] and, by controlling the activity of immune cells [13-15], can facilitate the regeneration of damaged tissues. (iv) It has been stated that MSCs can express different trophic factors that can inhibit immune cell function, delay cell death at damaged sites by inhibitors, and promote progenitor/stem cell proliferation and target cell differentiation [10]. (v) It is recognized that MSCs are hypoimmunogenic or immune-privileged, enabling allogeneic MSC transplantation through major barriers to histocompatibility and the development of off-the-shelf therapies consisting of MSCs grown in culture [16].

More than 1,100 clinical trials using MSCs have been reported for different diseases (https://clinicaltrials.gov) based on the therapeutic potential of MSCs. However, MSCs based therapy has not yet demonstrated adequate therapeutic effects in humans, despite several clinical trials using MSCs. Thus, MSC priming [17, 18], genetic modification [19], Three-Dimensional (3-D) culture [20], and MSC-derived exosomes [21, 22] have been studied to enhance the therapeutic potential of MSCs. Upon delivery to the damaged site, and after exposure to inflammatory cytokines, MSCs release various factors that control the function of inflammatory cells; this is followed by treatment of the damage. Consequently, it is possible to enhance the therapeutic effect of MSCs by pre-exposing them to inflammatory cytokines such as IFN-g, TNF-a and IL-1b [18]. Three-dimensional stem cell culture using different scaffolds has been reported to enhance the efficiency of stem cell proliferation and differentiation [20] and increase their therapeutic effects in liver disease, peritonitis, kidney injury, and myocardial infection [23, 24]. Owing to the therapeutic effects of various trophic factors secreted by MSCs, their regenerative therapeutic effects can be improved by using the secretome or exosome extracted from MSC. As exosomes can be processed, qualitatively regulated and repeatedly administered, they are an ideal factor, which can be used for the treatment of acute diseases. Enhancing the expression of the target gene, which plays an important role in tissue regeneration, is one way to reliably boost the therapeutic impact of MSCs. Therefore, we will address gene delivery methods in MSCs in this study, which are known to have low transformation efficiencies, and discuss the development and therapeutic efficacy of functionally enhanced MSCs.

Genetic modification of MSCs

Since the MSCs are attracted to the affected and/or tumor site makes it possible to use MSCs as a vehicle for different therapeutic agents, including genes. There are several strategies for genetically modifying MSCs, but they can be broadly divided into methods that are viral and non-viral vector systems [25]. Viral transduction of MSCs is commonly achieved using lenti-, retro-, adeno- or adeno-associated virus without affecting their stem cell properties [26, 27]. Non-viral methods of gene transfer encompass all physical and chemical methods of gene delivery. Non-viral techniques are effective because they are capable of transmitting larger transgenes than viral techniques, are more cost-effective and are ideal for production scale-up and trigger the less immune response. Single or mixed cationic lipids, peptides, surfactants, metals (gold, magnetic iron), polysaccharides, and synthetic polymers have been used in non-viral vector systems for genetic manipulation [28-30].

Lentiviral vectors

Lentiviruses have double-stranded RNA as their genetic material and can transduce both quiescent and dividing cells. After entering into the cells, they incorporate the genome of their vectors into the host genome, ensuring long-term transgenic expression [31]. These vectors are effective in converting dividing, non-dividing or slow-dividing cells, without changing their viability and differentiation potential. Non-integrating lentivirus vectors have recently been produced by viral integrase alterations or long terminal repeats and have been used for stable and safe delivery of the gene, resulting in long-term transgene expression [32]. These are one of the most commonly used vectors in mesenchymal stem cell-based gene therapy and have advantages such as large genome size, stable gene transfer, and high infection efficiency [33]. Besides, lentiviruses can be transduced into non-dividing cells and last for various generations. MSCs engineered with lentiviral vectors overexpressing HSP70 enhanced survival and resistance to apoptosis under conditions of hypoxia and ischemia without causing the morphology, viability, or differentiation capabilities of MSCs [34]. Transduction of MSCs with PGC-1α using lentiviral vectors reduced neuronal apoptosis and improved the capability of axonal regeneration in a rat model of traumatic spinal cord injury [35]. MSCs engineered with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-stimulated apoptosis in cancer cell lines, including colon, lung, pleural mesothelioma and oral squamous cancer [36]. However, the major concern with the use of lentiviral vectors is that they lack specificity, thus it can lead to the infection of cells that do not need to be transduced. Furthermore, the majority of lentiviruses that have been produced are derived from HIV, raising safety concerns for applications in in vivo gene therapy [37].

Adenoviral (AV) vectors

Adenoviruses (AVs) are non-enveloped viruses containing a double-stranded DNA genome with icosahedral nucleocapsids. Since they have a broad host range and can infect both dividing and non-dividing cells, adenoviruses are the most widely used gene delivery vector [38]. Non-pathogenicity is a significant advantage in their use as vectors for gene transfer. There is no risk of insertional mutagenesis and the payload capacity of these vectors in MSCs is high (~36Kb). The efficacy of adenovirus gene transmission is closely related to the expression of Coxsackievirus and Adenovirus Receptors (CARs) on target cells [39]. Due to a very low expression level of CARs on MSCs [40], gene delivery efficacy using these vectors is very low. To enhance the efficacy of gene delivery by these vectors into MSCs, a capsid- and a fiber-altered adenovirus has been developed [41, 42]. Moreover, in bone marrow-derived MSCs, the initial robust expression of the newly inserted gene gradually declines after 21 days; this strategy could

Table 1. Viral vectors used for the genetic modification of mesenchymal stem cells.

Viral vector	Structure	Advantages	Disadvantages
Lentiviral vectors	ds RNA	Easy manipulation and production; Easily cross nuclear membrane; infects dividing and quiescent cells; replication incompetent; no insertion into oncogene	Can generate insertional mutagenesis
Adenoviral vectors	ds DNA	High production of viral particles; lower risk of genotoxicity; large DNA inserts	Transient gene expression; immunogenic; insertional mutagenesis
Retroviral vectors	ss RNA	Easy manipulation and production; DNA incorporated into host cell genome; long-term stable gene expression	Insertional mutagenesis; can't cross nuclear membrane
Adeno-associated virus-based vectors	ss DNA	Infects dividing and quiescent cells; long-term gene expression; non-cytotoxic; non-immunogenic	Small DNA inserts

therefore only be extended to the transient expression of target genes [43]. Genetical modification of MSCs with bi-cistronic adenoviral vector encoding FGF2 and PDGF-BB has been shown to initiate collateral vessel formation and angiogenesis in a hind limb ischemia model [44]. Human placenta-derived Transduction of MSCs derived from human placenta with adenoviral vector expressing NK4, injected through the tail vein, delayed the growth of lung metastases in C-26 lung metastasis model [45]. However, adenoviruses have high immunogenicity, which restricts their use in gene therapy. CD4+, CD8+ and antigen presenting cells are triggered by direct administration of Adenoviral vectors with a transgene into the host cells. This activation of the immune response triggered by both transgenic and viral capsid protein expression can often lead to the removal of the viral particle and the silencing of the transgenes [46].

Retroviral vectors

Retroviruses are viruses of single-stranded RNA that have a lipid envelope and reverse transcriptase with receptor-binding proteins.

After binding to the receptor, the outside layer of the viral envelope integrates with the cellular membrane, internalizes the virus and releases the content into the cytoplasm. The viral RNA reverse-transcribed to DNA utilizing reverse transcriptase and integrates into the host genome [47]. There are many difficulties in using Retroviral vectors for gene therapy, despite their high tropism to host cells, such as the absence of long-term transgene expression, ineffective transduction of MSCs, induction of insertional mutagenesis, and the requirement for high vector loads to be administered in several rounds to transduce host cells [39, 42].

Adeno-associated virus-based vectors

Adeno-associated viruses (AAVs) are small, single-stranded, non-pathogenic DNA viruses that are adenovirus-dependent for replication [48]. For the following reasons, AAVs are considered to be an attractive gene therapy vector: (i) despite their ubiquity in the human population, they have no association with any disease; (ii) many human tissues can be easily infected with these vectors; (iii) AAV vectors are not capable of replication without a helper

adenovirus; (iv) these vectors can exist in an episomal form for long-term transgene expression; (v) nontoxic in clinical trials in humans [49]. Despite the significant advantages such as site-specific integration, low immunogenicity the application of AAVs is restricted due to various reasons. Although AAVs can infect a broad spectrum of cells, they show certain serotype specificity towards the cell type being used [50]. One of the major obstacles in the clinical use of AAV is the presence of antibodies to AAV in the majority of the human population, which limits vector efficiency [51]. Such immune reactions were found to be more prevalent against AAV2 in particular. Another impediment is the need for the conversion of single-stranded DNA into double-stranded DNA before integration into the genome, which is a rate-limiting step. Therefore, the molecular details of the AAV and host interaction biology need to be thoroughly investigated and develop strategies develop to resolve the limitations and make use of the benefits provided by AAVs [39].

Non-viral vectors

Different non-viral (physical and chemical) methods are widely used to introduce therapeutic genes in to MSCs. Restricted immunogenicity, improved biosafety and high loading capability are the key advantages of using non-viral vectors. Various physical methods such as electroporation [52], nucleofection [53], sonotransfection [54] and nanoparticles [55] are extensively used to transduce MSCs with target genes. However, the application of non-viral systems in gene therapy has been limited because of their low transfection efficiency and transient transgene expression [56]. In addition, transfection reagents and/or procedures can enhance the cytotoxicity of MSCs, leading to cell death or senescence. Recently, Helledie et al. shown that electroporation is a superior lipofection gene delivery method in MSCs without instigating proliferation and differentiation potential loss, whereas lipofection with Lipofectamine2000 decreased proliferation rate and increased MSC cell death [57]. Compared to most viral methods, they identified a simple and effective electroporation protocol that resulted in transfection efficiency of up to 90%, but the absolute transfection efficacy was approximately 35 percent. In another study, a novel method based on Therapeutic Ultrasound (TUS) has been developed for efficient gene delivery into MSCs [58]. MSCs were transfected using low intensity and moderate frequency TUS with plasmids encoding hemopexin-like domain fragment (PEX), an angiogenesis inhibitor. TUS-mediated PEX transfected MSCs expressed biologically active PEX without loss of stem and homing abilities and subsequently inhibited 70% of prostate tumor development in a mouse model [58]. Among conventional non-viral delivery systems, liposomes and polymers have been shown to transfect MSCs transiently [59-62]. Minicircles are tiny DNA-based vectors that are normally present in traditional plasmid vectors without prokaryotic backbone sequences. Minicircles have shown less toxicity and immunogenicity, higher transfection efficiency and longer transgene expression, in vitro and in vivo, compared to conventional plasmid vectors [63-65]. Several studies indicate that MSCs [66, 67] can be effectively and stably transfected by minicircles.

Conclusions

MSCs possess strong regenerative, pro-inflammatory, anti-inflammatory and drug delivery properties. These features introduce MSCs as attractive cell-based therapeutics for inflammatory based medical conditions. The homing property of MSCs to the affected and/or tumor site makes it possible to use MSCs to deliver various therapeutic agents, including genes. MSCs engineered with the therapeutic agents not only have

important therapeutic effects but also act predominantly in only the damaged site, reducing the expected frequency of side effects resulting from the non-selective action of the drug. Viral and non-viral vectors have been studied for gene delivery into MSCs, and MSCs equipped with genes have been reported to enhance their therapeutic effects significantly in the treatment of regenerative medicine and cancer. Viral vectors have drawbacks, such as high immunogenicity and insertional mutagenesis, but they have the benefits of the high efficiency of transfection and long-term expression of genes. In comparison, non-viral vector gene delivery has poor transfection efficiency and transient target gene expression. Therefore, to suit the therapeutic purpose, various types of vectors must be used depending on the disease being treated. In addition, to make use of the benefits of each vector and to compensate for the disadvantages, new methods must be created. In the future, if such research is carried out, it is expected that not only will the therapeutic impact of MSCs be improved, but the application of MSCs to different diseases will dramatically improve patients' quality of life.

Ethics approval and consent to participate

Not applicable.

Funding

Not applicable.

Author contributions

SW wrote the paper, GKM corrected the manuscript.

Competing interests

All authors disclose no competing interests.

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