

Review Article

Genetic Engineering of Mesenchymal Stem Cells to Induce Their Migration and Survival

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Mesenchymal stem cells (MSCs) are very attractive for regenerative medicine due to their relatively easy derivation and broad range of differentiation capabilities, either naturally or induced through cell engineering. However, efficient methods of delivery to diseased tissues and the long-term survival of grafted cells still need improvement. Here, we review genetic engineering approaches designed to enhance the migratory capacities of MSCs, as well as extend their survival after transplantation by the modulation of prosurvival approaches, including prevention of senescence and apoptosis. We highlight some of the latest examples that explore these pivotal points, which have great relevance in cell-based therapies.

1. Introduction

Interest in stem cell-based regenerative medicine is growing. Furthermore, implementation of genetic engineering methods is capable of further enhancing the therapeutic potential of stem cells [1]. Mesenchymal stem cells (MSCs) are very promising because they are easy to isolate and they have a broad range of differentiation capabilities, either naturally or through cell engineering [2]. However, when considering the use of MSCs in therapy, many practical problems should be resolved, among which is proper and efficient delivery and keeping the cells alive at the sites of action. Under hypoxic conditions, endogenous MSCs have an increased ability to migrate and influence the factors secreted from the damaged tissue. As a response to the reduced partial pressure of oxygen in tissues surrounding the injured area, MSCs change their membrane receptors and are capable of migration toward the site of the damage [3]. This occurs, however, exclusively within the damaged and surrounding regions; thus, the migration refers only to MSCs that are relatively close to the site(s) of injury and can be effectively activated.

Therefore, the number of recruited cells is limited. For this reason, it was proposed that the therapeutic effect could be enhanced by the administration of exogenous MSCs to the sites of injury, and this was, indeed, confirmed [4].

To date, local injection of MSCs is the most prevalent cell delivery method, but local injection has many drawbacks. In particular, the deposition of a bulk cell suspension in very delicate organs, such as the brain, tears the complex tissue structure, causes pressure on local structures, and frequently results in microbleeding, which triggers inflammatory responses and may augment the host reaction against the graft (Figure 1(a)) [5]. In addition, the needle insertion into acutely damaged brain tissue, as in the case of stroke or traumatic brain injury, is life-threatening due to the risk of hematoma formation. Thus, substantial effort has been devoted to avoiding such stressful conditions which can be detrimental to both graft and host. An attractive alternative to intraparenchymal injection is deposition of cells in fluid compartments, from which they could migrate toward injured/diseased areas without causing any strain on the intact tissue (Figure 1(b)). There are multiple studies

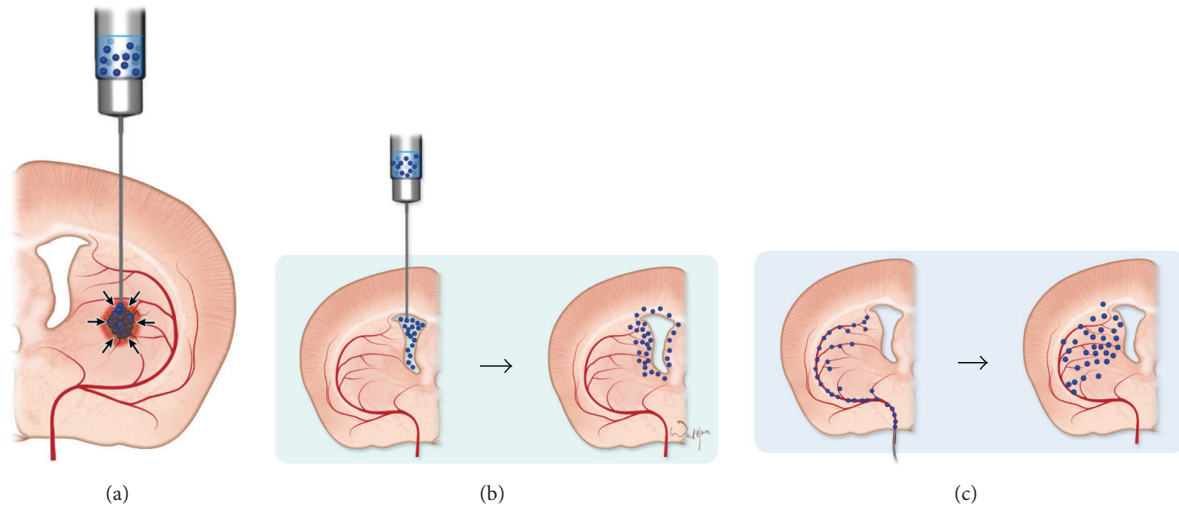


FIGURE 1: The role of migration in various routes of cell delivery. (a) Intraparenchymal injection triggers inflammatory responses and may augment the host reaction against the graft (arrows). Single stem cell infiltration of brain parenchyma after intraventricular (b) and intra-arterial (c) infusion.

with transplantation of cells into cerebrospinal fluid spaces [6, 7] but inadequate intraparenchymal migration limited the therapeutic effect [8].

Intravascular injection is another way to use body fluids for efficient delivery of cells to large body areas, including the brain. The intravenous route is noninvasive, but wide, whole-body cell distribution might limit the amount of cells that reach the diseased area. The intra-arterial route is especially interesting as it is still minimally invasive but allows targeting of specific body areas, including particular brain regions, and in that case the cells are expected to extravasate and disperse within a brain parenchyma (Figure 1(c)). Moreover, the safety issues have been recently extensively investigated and addressed [9, 10].

However, MSCs are not equipped to allow for efficient migration from cerebrospinal fluid or the blood to the brain parenchyma. While the precision of intraparenchymal injection can be very high, due to the fragility of damaged tissue, cells must be deposited at some distance from the disease site to avoid further injury. Implanted cells are then required to migrate a certain distance to reach the diseased tissue. Despite the fact that native MSCs showed some migration toward injured areas after administration [11], there is a need to further increase that migration ability after engraftment of exogenous MSCs, and that can be achieved by genetic cell engineering [12].

However, an important aspect of MSC-based therapies is the maintenance of their proliferative and differentiation capacities. Prolonged culture of MSCs results in an inevitable senescence, consequently leading to the loss of their proliferative activity [13]. To address this issue, several efforts have been made to increase the expression of stemness-related genes in such a way that the beneficial properties of MSCs are maintained and even increased by extending their *in vitro* expansion potential.

Furthermore, it is important to note that, in the majority of cases, the delivered therapeutic cells encounter adverse conditions after transplantation into injured target tissues with a hostile microenvironment. High levels of oxidative stress, local hypoxia, and proapoptotic cytokines all contribute to the elimination of the transplanted therapeutic cells, which, in turn, limits their therapeutic activity. Thus, prosurvival approaches are needed to prolong the engraftment time of exogenous cells.

2. MSCs Engineered to Increase Migratory Properties

SDF-1 is one of the most potent chemokines involved in the process of cell migration [14]. Under physiological conditions, SDF-1 is produced within the damaged tissue and is released from the injured area, exerting chemoattractive signals for the cells that express the CXCR4 receptor on their outer cell membrane [15, 16]. CXCR4 basal protein presence in outer MSC membranes differs between various MSCs. There are some data that unmodified bone marrow-derived mouse [17] and human [18] MSCs do not possess CXCR4 in their outer membranes or present low levels, that is, rat MSCs [16, 19, 20] and human adipose-derived MSCs [15]. However, other studies bring contrary observations for rat MSCs [21, 22]. Moreover, CXCR4 presence is altered during *in vitro* culture [15]. MSCs could heavily increase the presence of CXCR4 particularly when exposed to a low concentration of oxygen [20, 22] or after adequate stimulation to elicit the endogenous CXCR4 gene overexpression [15, 23]. Despite this, there have been numerous reports of engineering MSCs to increase the expression of the CXCR4 gene, which have resulted in a higher density of the CXCR4 receptor and effectively increased the migration of MSCs toward SDF-1 [24–26] (Figure 2).

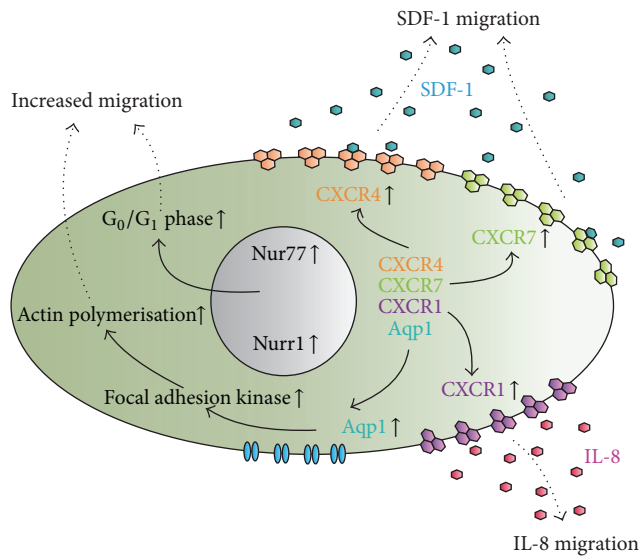


FIGURE 2: MSCs engineered to enhance migratory properties. Increased MSC migration could be accomplished by membrane-bound receptor engineering (i.e., CXCR1, CXCR4, and CXCR7), water channel receptors (Aqp1), or the upregulation of defined nuclear receptors (i.e., Nur77 and Nurrl1).

The beneficial immunoregulatory effects of CXCR4-expressing MSCs were observed in a study on kidney transplantation [27]. In a different study, CXCR4-engineered MSCs had a positive impact on early liver regeneration, which was attributed to their enhanced homing to liver grafts, with an emphasis on the contribution to the improvement of hepatocyte proliferation [28]. Another example of the beneficial effects of CXCR4-overexpressing MSCs was enhanced tissue repair in an acute kidney injury model [29]. In this case, CXCR4-MSCs homed to the lesion site with enhanced affinity, compared to the control MSCs, exhibiting beneficial paracrine actions. Another example is the data on the use of CXCR4-MSCs in the healing of skin wounds. As in the above cases, it was shown that CXCR4-engineered MSCs migrated with higher affinity to the sites of injuries, accelerating the process of wound healing [30]. In the case of a rat cerebral ischemia model, the delivered CXCR4-MSCs possessed higher mobilization and enhanced neuroprotection compared to the control cells [31]. In addition to the CXCR4 element from the SDF-1-CXCR4 signal axis, MSCs were also engineered to overexpress SDF-1. Nakamura et al. presented the results of SDF-1-overexpressing MSCs that had enhanced migration properties in *in vitro* migration assays, and SDF-1-MSCs were used in the *in vivo* experiments for wound healing. It was observed that SDF-1-MSCs contributed to a significant wound size decrease, which raised expectations that modified MSCs could be used in the treatment of skin injuries [32].

In addition to the CXCR4 binding abilities of SDF-1, CXC chemokine receptor 7 (CXCR7) was observed to bind SDF-1 as well [33], so that the SDF-1/CXCR7 signaling axis was used to engineer the MSCs. Wang et al. used CXCR7-overexpressing MSCs in a cerebral ischemia-reperfusion rat

hippocampus model. It was proven that the overexpressed CXCR7 receptor promoted the migration of MSCs toward an SDF-1 gradient, acting jointly with the SDF-1/CXCR4 signaling axis [34]. Overexpression of the CXCR7 receptor in MSCs resulted in their enhanced migration toward the secondary lymphoid organs, potentially inhibiting the immune system response in graft-versus-host disease and thus decreasing clinical symptoms [35].

Another CXC chemokine receptor selected to enhance the migratory properties of MSCs was the CXC chemokine receptor 1 (CXCR1). The CXCR1 is a receptor for IL-8, which, in turn, was shown to be expressed and released in gliomas [36]. This phenomenon was used to improve targeting of MSC toward gliomas [37]. In a different study, CXCR1-MSCs were shown to accumulate in the infarcted myocardium with high affinity, where the survival and engraftment of exogenously delivered CXCR1-MSCs were elevated, providing a putative new strategy for the injured myocardium [38].

The migratory properties of MSCs were also manipulated via modification of the aquaporin-1 (Aqp1) gene. Overexpression of Aqp1 resulted in an increase of the migration capabilities of Aqp1-MSCs toward the sites of injury [39]. Aqp1 is a water channel molecule that transports water across the cell membrane. It was shown that Aqp1 interacted with β -catenin, which was an important regulator of cell migration [40].

Two nuclear receptors, Nur77 and Nurrl1, were also brought into play to improve the migratory capabilities of MSCs [41]. The high expression of Nur77 and Nurrl1 was characteristic of the cells with enhanced cellular migration properties [42, 43]. In that case, it was proven that the overexpression of these two transcription factors promoted the migration of MSCs.

The migration of cells through a vessel wall constitutes a distinct challenge. It was shown that adhesion molecules play an important role in this process [44–46]. It has been reported that viral transduction of ITGA-4 was sufficient to increase the homing of MSCs to bone marrow [47]. However, it is not clear whether this phenomenon could be accomplished when targeting the brain parenchyma, although there is encouraging data from *in vitro* studies [48].

Finally, there are examples of MSCs dual target engineering in order to enhance vessel wall migration. In that case, MSCs simultaneously modified with two mRNAs for PSGL-1 and SLeX were compelled to produce functional ligands for P-selectins and E-selectins, which altogether resulted in improved inflamed tissue homing, like inflamed ear [49] and spinal cord [50].

3. Modifications of MSCs to Combat Senescence

Two transcription factors, Sox2 and Oct4, are involved in maintenance of the pluripotency and self-renewal abilities of embryonic stem cells [51, 52]. Previously, both factors were used to reprogram adult somatic cells into induced pluripotent stem cells [53]. In addition, there are several reports that these two transcription factors were efficiently applied to engineer MSCs. Fan et al. found that bone

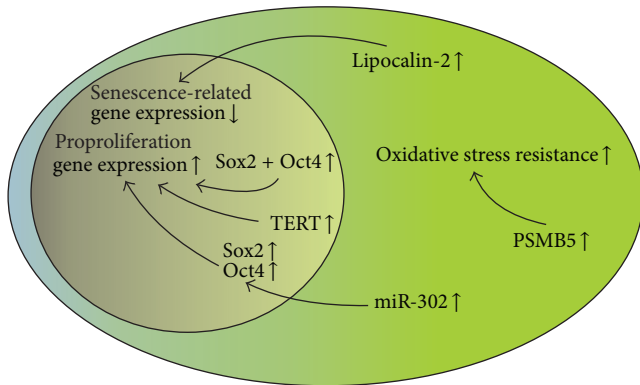


FIGURE 3: Proliferative and differentiation potential of MSC engineering. An improvement in the proliferative hallmarks of MSCs could be accomplished by the stimulation of proliferation genes regulated by Sox2 and Oct4 transcription factors. Furthermore, senescence-related genes could be silenced (i.e., lipocalin-2 production), or oxidative stress resistance might be enhanced (i.e., PSMB5).

marrow-derived MSCs simultaneously overexpressing Sox2 and Oct4 genes were characterized by improved proliferative and differentiation potential compared to control cells [54] (Figure 3). Similar beneficial effects related to Sox2 and Oct4 overexpression were reported for adipose-derived MSCs [55]. In that case, the transduced cells were more proliferative than controls, with increased differentiation abilities for adipocytes and osteoblasts. However, in a different study, bone marrow-derived MSCs were found to be efficiently engineered with the Sox2 gene, successfully retained in an undifferentiated state, but, in this case, the osteogenic and adipogenic differentiation potential of engineered cells was inhibited [56]. It has been shown that overexpression of the Oct4 gene in MSCs resulted in an increased expression of other stemness genes, such as Sox2 [57]. The overexpression of the Sox2 and Oct4 genes could also be achieved by concomitant treatment with leukemia inhibitory factor (LIF) and transfection with one of the stem cell-specific miRNAs, miR-302 [58]. What is more, miR-302 reportedly induced proliferation and inhibited oxidant-induced cell death in human adipose-derived MSCs [59].

Telomerase reverse transcriptase (TERT) gene transfection is yet another strategy to prevent senescence in cultured MSCs. TERT is an RNA-dependent DNA polymerase, which synthesizes and extends telomeric DNA, thus sustaining the immortal phenotype of stem cells [60]. It has been previously shown that MSCs lack TERT gene expression during *in vitro* expansion [61]; for this reason, the TERT gene engineering was converted into an interesting approach to reverse senescence in cultured MSCs. In addition, the differentiation potential of TERT-transfected MSCs toward osteogenic and neural lineages was improved compared to native MSCs [62].

In a different study, TERT immortalized MSCs had enhanced proliferative capabilities, and the cell-cycle-related gene expression factors were elevated, preventing the transfected MSCs from cell-cycle arrest [63]. Since the proteasomal pathway is important in the maintenance of cellular

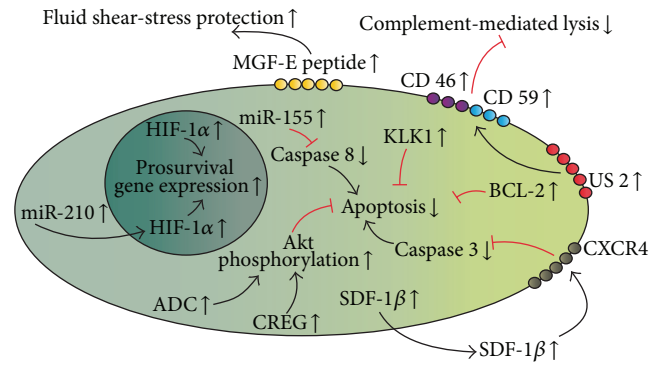


FIGURE 4: MSC pro-survival engineering. Pro-survival strategies primarily target the apoptosis process by downregulation of the elements involved in the apoptotic cascade (i.e., caspase 8 inhibition by miR-155). Other approaches involve the induction of pro-survival genes (i.e., HIF-1 α), while still others ensure protection from fluid-stress or complement-mediated lysis.

homeostasis and its dysfunction may lead to replicative senescence, transfection of MSCs with the β -subunit of the mammalian proteasome complex (PSMB5) also resulted in inhibition of cellular senescence [64, 65]. Yet, two other examples of preventing the cellular senescence of MSCs include transfection with small interfering RNAs against the glucocorticoid receptor gene [66] and lipocalin-2 gene overexpression, which protects the pluripotency of MSCs under hypoxic conditions [67].

Finally, the proliferative capabilities of MSCs could be improved by overexpressing growth factor genes. However, certain growth factors could severely impair the therapeutic properties of MSCs [68].

4. Engineering of MSCs to Improve Survival

Physiologically, MSCs migrate to the sites of injury, which are under hypoxic conditions, but, despite this physiological behavior, MSCs are also sensitive to the harsh local conditions encountered in the areas of their putative therapeutic action [69]. The survival of therapeutic cells is particularly important in injuries associated with hypoxia in the damaged tissue, such as myocardial infarction and stroke. For this reason, diverse pro-survival strategies have been developed to modify MSCs in order to prolong their survival in the target organ, giving them sufficient time to elicit beneficial effects (Figure 4).

Interestingly, SDF-1 β was found to be a pro-survival player that enhanced cellular autophagy and decreased apoptosis in the SDF-1 β -producing MSCs cultivated *in vitro* [70]. In addition, in a low oxygen concentration, a cardiac-type fatty acid binding protein was useful for the survival of the modified MSCs; however, the cell growth and proliferation of those cells were negatively affected [71].

Hypoxia-inducible factor-1 α (HIF-1 α) is a major regulator of the changes in the cellular metabolism caused by hypoxia [72]. HIF-1 α regulates the activation of a broad range of genes involved in angiogenesis, erythropoiesis, cell proliferation, differentiation, and apoptosis in order to facilitate the adaptation of the cells to hypoxic conditions [73]. Therefore, the

HIF-1 α gene could be worth consideration as a target in the pro-survival approaches for MSC therapies, since beneficial results were seen in HIF-1 α -engineered MSCs in trials with a mouse hind-limb ischemia model [74] and in a rat myocardial infarction model [75]. Furthermore, miRNA technology also has an application in this regard, since MSCs were modified to overexpress miR-210, which favored HIF-1 α protein activity in the positive feedback regulatory loop that fosters the survival of the modified MSCs under hypoxic conditions [76, 77].

Apart from HIF-1 α , other engineering solutions have been used to target selected proteins from defined signaling pathways, with a specific emphasis on apoptosis. There are several examples of MSC engineering approaches to produce proteins that could inhibit apoptotic signaling in the therapeutic cells by providing antiapoptotic signals mediated by Bcl-2 [78], a cellular repressor of E1A-stimulated genes (CREG) [79, 80], kallikrein (KLK1) [81, 82], angiotensin-converting enzyme 2 [83], arginine decarboxylase (ADC) [84], integrin-linked kinase (ILK) [85, 86], or protein kinase G α [87]. The antiapoptotic effects could be obtained because of the silencing properties of small hairpin RNAs (shRNA) as well. For instance, the expression of the well-known proapoptotic factor caspase 8 gene was abolished after pre-miRNA-155-designed caspase 8 shRNA transfection into MSCs [88].

An attractive option that could be viewed as a group of pro-survival solutions is the protection of therapeutic MSCs from the negative influence of extravasated blood [89]. For example, the use of mechanogrowth factor E (MGF-E) peptide in the membranes of the MSCs might protect the transfected cells from improper fluid shear stress [90]. Another example is the data reporting that MSCs could be protected from the complement-mediated damage by overexpression of US2 protein from the human cytomegalovirus [91].

5. Future Perspectives

MSCs emerge as a very attractive cell type for clinical applications because of their availability in comparison to other cells and there are no elevated ethical problems associated with their harvesting. Additionally, these cells may be quite easily propagated in *in vitro* environments. The use of autologous MSCs would be one of the most convenient solutions, which would obviate many of the problems associated with the immunological aspects and adequate cell donor match.

However, in the case of autologous cells, a scant starting material might pose a serious problem. This issue could be addressed by the use of genetic engineering techniques aimed at the increase of the mitotic properties. In practice, this means avoiding senescence, which inevitably occurs during prolonged *in vitro* culture. Due to these techniques, a sufficient number of cells at the moment of transplantation could be achieved that, in turn, would pave the way to the enhanced therapeutic outcome.

In practice, the problem with an insufficient number for cells for autologous transplantation is caused by the fact that the need of cell therapy occurs mainly in the elderly. In these patients, the therapeutic potential and number of MSCs that might be obtained from biopsy is reduced, compared

to young patients [92]. However, an appropriate stimulation can modulate these cells to convert them into suitable therapeutic material in spite of their old age. Once again, in the field of genetic engineering, methods could contribute to the increase in the cell number and enhancement of their therapeutic potential.

In addition, another group of problems is related to the proper targeting of therapeutic cells to ensure that the least amount of these valuable cells would be lost during the administration step and to ensure that as many of them as possible will be delivered to the site of action. It seems that one of the less traumatic ways of cell administration is an intravascular delivery route. In this case, securing MSCs from the negative influence of blood seems to be pivotal, followed by proper tissue targeting, in order to prevent additional cell loss due to lung and lymphoid organ entrapment.

Subsequently, since these cells have to be used to treat injured areas, wherein native cells are heavily damaged, and in a location that is full of destructive factors, therapeutic cell protection at this stage seems to be also very important. Therefore, it is extremely important to ensure the prolonged survival of these cells after administration, so that they could remain long enough at a target site to have more time to act.

Finally, it appears that for the effective practical cell therapy, particularly for autologous transplants, all of the above mentioned elements should be incorporated at the same time. This would imply a need to use advanced techniques [93], modifying MSCs with multiple genes, at the same time ensuring the effective expression of each of them, and perhaps in some cases providing some sort of sequential induction of introduced gene expression. This aim might be achieved by the employment of different genetic material loaded nanostructures that, depending on the nanostructure composition, would potentially release their contents into the cytoplasm with different speed [94, 95].

6. Conclusions

The aim of this review was to bring attention to the importance of promigratory and pro-survival aspects when considering MSC-based therapies. The successful therapeutic use of MSCs depends on efficient cell delivery and adequate survival of therapeutic cells, as well as the unhindered differentiation capacity of engineered MSCs. It seems that, in practice, there would be a need for the simultaneous implementation of a combination of the presented genetic engineering solutions presented here, in order to achieve truly therapeutic goals. The coupling of modified genes might be potentially tailored to the needs of specific patients and contribute to personalized medicine.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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