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Engineered MSCs from Patient-Specific iPS Cells

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Abstract Mesenchymal stroma/stem cells (MSCs) represent a heterogenic cell population that can be isolated from various tissues of the body or can be generated from pluripotent stem cells by in vitro differentiation. Various promising pre-clinical and clinical studies suggest that MSCs might stimulate endogenous regeneration and/or act as anti-inflammatory agents, which could be of high therapeutic relevance for a number of diseases, including graft-versus-host disease after allogeneic hematopoietic stem cell transplantation, inflammatory bowel diseases, or some forms of liver failure. Notably, conflicting results of various studies illustrated that the source of MSCs, the cultivation condition, and the way of administration have important effects on the desired clinical effect. Some of the involved molecular pathways have recently been elucidated and an artificial modulation of these pathways by engineered MSCs might result in superfunctional MSCs for enhanced endogenous regeneration or anti-inflammatory response. In this review, we summarize important findings of conventional MSCs for applications in gastroenterology and we describe the state-of-the-art for the generation of patient-derived iPS cells that eventually might provide genetically engineered superfunctional iPS cells for advanced cell therapies.

Keywords Cell transplantation · Induced pluripotent stem cells (iPSC) · Inflammatory bowel diseases · Liver diseases · Mesenchymal stromal (stem) cells (MSC)

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Contents

1 Introduction

Mesenchymal stromal cells (MSCs) are a heterogeneous cell population that can be obtained from various tissues, such as the bone marrow or adipose tissue. Recently several studies have shown other sources of mesenchymal cells, obtained from amniotic membrane [15], dental pulp [37], and also nonmesodermal tissue origins such as spleen, liver, kidney, and lung (Anker et al. [39] with similar characteristics to bone marrow-derived MSCs, which show a characteristic surface marker profile consisting of CD-45$^-$, CD-31$^-$, and CD-90$^+$ cells. These findings might suggest a possible common niche for all of these cells, in which extracellular matrix compositions, signaling molecules, cell–cell and cell–matrix interactions, and O_2 tension would be comparable [15]. By far, the most extensively studied source of MSCs is the bone marrow, because earlier studies addressing hematopoietic stem cells within the bone marrow niche resulted in a profound insight into the biology of these mesenchymal cells. Due to the fact that MSCs harbor clonally expandable cells, which could be differentiated towards adipogenic, chondrogenic, and osteogenic tissues, these cells also could be considered as mesenchymal stem cells. It is noteworthy that the abbreviation "MSC" is not uniformly used for either the term "mesenchymal stromal cells" or the term "mesenchymal stem cells" and often both aspects are not fully distinguished in the respective publication. Considering differentiation processes and further cellular fate changes upon extended in vitro culture, a pure population of mesenchymal stem cells might be hard to obtain or even to propagate and, probably, most cultures of MSCs contain stem cells as well as more differentiated stromal cells.

MSCs are meant to be beneficial in the repair of connective tissue injuries such as wound healing, osteogenic deficiencies, and also cartilage repair [4]. However, some reports suggest that MSCs could be an alternative source to repair a variety of other degenerative tissue lesions and might allow new therapeutic strategies for

the treatment of neurological disorders [70], myocardial infarction [64], liver injuries [24, 58], and urological diseases [84]. This list of studies is by far not complete and, more important, the reproducibility as well as clinical impact of these studies is controversially discussed [55] as further described below in the context of gastrointestinal disorders.

Encouraging results from animal models and some clinical trials clearly support the research on MSCs, but also raise concerns about the lack of high cell numbers and the lack of a homogeneous cell population. Interestingly, embryonic stem cells or other pluripotent stem cells harbor the capabilities of unlimited self-renewing and differentiation potential into all somatic cell types [86], including mesenchymal stromal/stem cells. Among the various strategies to obtain MSCs from pluripotent stem cells, most protocols were first evaluated with established human embryonic stem cell lines. However, the generation of patient-derived pluripotent stem cells became feasible after pioneering studies of Shinya Yamanaka, who demonstrated that a set of four transcription factors can convert somatic cells into pluripotent stem cells [83]. Such patient-derived induced pluripotent stem cells [11] offer unique opportunities for applications in personalized medicine and allow the generation of high numbers of pluripotent starting cells, when large-scale cultivation systems were applied [62]. Such pluripotent stem cell lines can be differentiated towards functional MSC-like cells and it remains to be analyzed to which extent those MSC preparations harbor therapeutic effects and consist of a more defined homogeneous cell population.

Obviously, such engineered MSCs need to be investigated in further pre-clinical studies but may possess the potential to overcome some of the limitations that raise profound concerns of the clinical MSC applications at present.

2 Sources and Diversity of MSCs

In spite of data from over 100 trials employing MSCs in different clinical settings, correlation of MSC properties to clinical efficacy is limited due to considerable diversity of the applied MSC populations [88]. Also the broad spectrum of functional activity is not adequately reflected by the internationally agreed minimal set of consensus released criteria [22] and a defined subset of surface molecules that exactly characterize the MSCs' phenotype does not exist. All spindle-shaped cells attaching to plastic surfaces and expressing surface markers like CD-29, CD-105, CD-73, CD-44, but not hematopoietic markers (CD-34, CD-45, and CD-14) are considered as MSCs, if their ability to differentiate into mesodermal lineages (adipogenic, chondrogenic, and osteogenic differentiation) is provided. It is speculated that slightly different subtypes exist, which vary in their phenotypes due to extra-cellular or intra-cellular signaling. Nevertheless, they still possess multipotentiality as demonstrated by in vitro differentiation and in xenografts [52].

There are several cultivation protocols available that allow a proper in vitro expansion of MSCs, which makes them a readily accessible cell source for stem

cell research. Independent of the harvesting sites, namely bone marrow, fat tissue, and umbilical cord blood, MSCs show some in vitro expansion ability even to clinical scale with minimal shortfalls in stemness [15]. But there are contradicting reports concerning karyotype aberrations of in vitro expanded cells, as early as five to nine passages after MSC harvesting [59, 94].

Transcriptome analysis revealed that genes typically expressed in MSCs are cytoskeletal (vimentin and myosin) and cytolytic or extracellular proteins (Collagene I, III, VI, and different matrix metalloproteinases), cell adhesion molecules (fibronectin and integrins), cytokines (IL-11, HGF, TGF-β), and also receptors (IL-1R and IL-10R). However, among the various abilities of MSCs, their homing properties in different tissues and the sectretion of bioactive compounds such as angiogenic (VEGF), antiapoptotic (HGF), and mitogenic (IGF-I) factors [15] are important variables influencing their potential therapeutic applications. The high homing properties of MSCs are dependent on the chemokine receptor CXCR4 expression on the cell surface and its interaction with SDF-1α stimuli from injured tissue in a gradient-dependent manner that attracts MSCs and promotes further cell interactions [4]. Despite all beneficial effects of MSCs in degenerative diseases there are several issues that could interfere with the MSCs' potential to ameliorate the respective disorder. For instance, aging adversely affects MSCs self-renewal, proliferation, telomerase length, and differentiation capacity [95]. Furthermore, impaired antioxidant activity and the lack of appropriate cytoskeleton properties could lead to malfunction of MSCs in therapeutic settings [43, 45].

However, the effects of MSC therapies are transient and require repeated transplantations and therefore a high number of cells. Due to the fact that MSCs show an impaired growth and increased senescence during in vitro propagation, the proper cell amount for clinical treatment might be a major obstacle. To overcome these obstacles, several groups have reported the derivation of MSC populations from self-renewing human embryonic stem cells by numerous methods [38, 61, 89], which are discussed below.

3 Therapeutic Applications of MSCs in Gastroenterology

3.1 MSCs in Graft-Versus-Host Disease

One of the most critical side effects of allogeneic hematopoietic stem cell transplantation for the treatment of leukemia or other life-threatening hematopoietic diseases is the development of an acute graft-versus-host disease (GvHD) resulting in a high morbidity and mortality [80]. Hereby, graft-derived T cells trigger the induction of GvHD after activation by host-related major histocompatibility class I or II antigens as well as minor antigenic peptides [27]. GvHD mainly targets the skin, intestine, liver, and the hematopoietic system and is routinely treated with immunosuppressive drugs such as cyclosporine or methotrexate [79]. However, various

advanced treatment regimes were available using therapeutic antibodies against interleukin-2 [3], tumor necrosis factor α (TNFα; [46], or against CD-147 [20].

Inspired by various animal studies including baboons [8], third-party MSCs have found ready entry into a series of clinical trials for prevention of severe acute GvHD [69] and striking response rates as high as 50–90 % have been reported with noteworthy resolution of refractory intestinal GvHD [49]. It is also noteworthy that in a randomized trial patients suffering from grade II–IV acute GvHD received two transfusions of a commercially produced MSC preparation (Prochymal™, Osiris Therapeutics, Columbia, MD, USA). Of the 32 treated patients 94 % showed an initial response and as many as 77 % remained in a complete response state [44]. Despite these promising results some follow-up studies questioned the dramatic effect of MSCs on prevention and treatment of GvHD, as larger clinical trials failed to show a beneficial effect on the most common skin GvHD. However, results from GvHD phenotypes, which are more difficult to treat and which affect mainly the intestine and the liver, showed an improved response rate over placebo [2].

3.2 MSCs in Inflammatory Bowel Disease

Idiopathic inflammatory bowel disorders (IBD) such as Crohn's disease and ulcerative colitis are highly debilitating diseases of the gut that have remained largely resistant to definitive medical therapy [26, 99]. The pathophysiology of Crohn's disease includes an exaggerated infiltration of macrophages and neutrophilic granulocytes, which is triggered by activated T-helper cells. These cells produce uncontrolled amounts of inflammatory cytokines and chemokines resulting in tissue destruction of the large intestine. For instance, excessive production of IFN-γ and IL-17 by T cells and IL-12 or IL-23 by monocytes is responsible for an acute inflammation and the production of other cytokines such as TNF-α [81]. Based on the finding that an imbalance of effector T cells and suppressive regulatory T cells causes an expansion of self-reactive T cells [10], there is conclusive evidence that Crohn's disease is related to a failure of the mucosal immune system. Consequently, the therapeutic challenge applying MSCs for the treatment of IBD is twofold: curbing the inflammatory attack may be considered as the main action, but, secondly, the regeneration of a large organ such as the intestinal mucosa requires additional tissue-trophic measures to re-establish the protective mucosal barrier.

So far, the published literature on clinical evaluation of MSC-based therapy is comparatively sparse with the main evidence stemming from local application to perianal fistulas and i.v. applications pilotized in small numbers of patients [31, 71]. In a phase I clinical trial it was demonstrated that MSCs derived from the bone marrow of refractory Crohn's disease patients have identical characteristics compared to MSCs from healthy donors and have intact immunomodulatory capacities in vitro. Furthermore, administration of autologous bone marrow-derived MSCs was

safe and feasible in the treatment of refractory Crohn's disease [23]. In addition, a more recent study demonstrated the feasibility of ex vivo expanding autologous bone marrow-derived MSCs and the safety of their intra-fistular injections in patients with Crohn's disease. Moreover, the authors described a promoting effect of MSCs on in vivo differentiation of regulatory T cells [17]. Osiris Therapeutics (Columbia, MD, USA) also initiated clinical trials for Crohn's disease using their MSC preparation Proychymal^{TM}. However, the placebo group also showed improvements and the treated arm of the study failed to meet the primary endpoint [2]. In conclusion, the lack of knowledge about the direct and indirect effects of different MSC preparations hampers the evaluation of these early clinical trials and more basic research on paracrine effectors and cellular mechanisms contributing to the MSCs' immunomodulatory effect are necessary.

3.3 MSCs in Liver Regeneration

Liver, as the second largest organ in the body serves crucial roles in the human homeostasis and its malfunction could be life-threatening. The high mortality rate because of liver deformities that led to 1.4 million deaths annually has not been avoided by liver transplantation which is the most efficient therapy so far [68]. In addition to stem cell mobilizing strategies [56] and bioartificial liver devices (BAL) [78], several alternative cell-based therapies have been investigated to recover unstable conditions in chronic liver disorders as well as during metabolic or acute liver failure. In general the disorders are treated by transplantation of bone marrow hematopoietic, mesenchymal, and mononuclear cell populations (for review see [93] and [74]). The first evidence indicating MSC infusion in mice models could recover liver failure was suggested by Petersen et al. showing the presence of bone marrow-derived hepatic cells from sex-mismatched donors in the recipient mice livers [65]. These data were substantiated by findings of other groups [47, 85], but later analyses questioned the initial hypothesis of a direct transdifferentiation and rather demonstrated that the transplanted cells fuse with host hepatocytes [12, 75, 96]. Nevertheless, some studies described functional integration of MSCs into injured liver after their in vitro specification towards hepatic cells [5, 6]. On the other hand the inhibitory signals of MSCs over hepatic stellate cells (mostly responsible for extracellular matrix accumulation [29]) inhibited the proliferation and triggered their apoptosis [90]. Also secretion of anti-inflammatory cytokines such as TNF-α, IL-1, IL-10, HGF [90], and matrix metalloproteinases regulation [53] could react as an anti-fibrogenic treatment in chronic liver injuries.

Clearly, the clinical relevance of these findings is still very controversially discussed and most pre-clinical and clinical studies indicate that the MSC therapy is a transient treatment, which may have to be applied repeatedly in order to treat the respective disorders. For instance, in the case of chronic fibrogenesis the cell infusion may be crucial for preventing the turnover of new fibers [73]. Therefore,

an application of cells with similar characteristics to those of MSCs with a higher self-renewal capacity and a reduced senescence behavior would be an appropriate approach to support more long-term effects and the possibility of providing transplants from the same batch of initially transplanted cells.

4 MSCs from Patient-Derived Pluripotent Stem Cells

4.1 Generation of Patient-Specific Pluripotent Stem Cells

Pluripotent stem cells such as human embryonic stem cells (ESCs) harbor an unlimited self-renewing capability and have the potential to differentiate into all cells of the three germ layers (ectoderm, mesoderm, and endoderm) as well as into germ cells [86]. Various attempts were undertaken to derive pluripotent cells from adult individuals. The early strategies were strongly influenced by the technique of somatic cell nuclear transfer (SCNT) resulting in a cloned embryo, as first demonstrated for mammals by the birth of the sheep "Dolly" [98]. This technique, however, lacks feasibility with human cells and is ethically heavily disputed because the derivation of SCNT-derived cells implicates the destruction of a human embryo. Nevertheless, other strategies were exploited to "re-program" somatic cells towards pluripotent stem cells, either by cell fusion, by application of ESC extracts, or by using a defined set of transcription factors. In the groundbreaking study of Takahashi and Yamanaka in [83], they successfully reprogrammed mouse fibroblast by introducing ectopic defined transcription factors (Oct-4, Sox-2, Klf-4, and c-Myc known as OSKM) into the cells via retroviral transduction [83]. The oncogenic nature of c-Myc and Klf-4 urged other scientists to reprogram cells with other transcription factors such as Oct-4, Sox-2, Nanog, and Lin-28 (OSNL; [104]).

By this direct reprogramming method cell colonies with similar morphology and genetically similar information to ESCs were generated and termed induced pluripotent stem cells. Later on, numerous reprogramming studies used different cell types of ectodermal (keratinocytes [1] and neural progenitor cells [25]), mesodermal (B cells [36], or cord blood [35, 105]), and endodermal (hepatocytes [77]) origin. In addition to the cell type the composition and stoichiometry of the reprogramming factor cocktail affects successful reprogramming. Considering the stoichiometric variability caused by either high or low transgenic expression of each factor in the reprogramming cocktail, several studies ruled out the importance of a dominating Oct4 expression level [63, 87]. Therefore, a polycistronic reprogramming construct that ensures the expression of all four factors in a defined and preferential stoichiometric ratio is of high relevance for the generation of fully reprogrammed iPSC as described by various reports [13, 97]. So far several combinations of these factors along with the other factors such as Esrrb have been used for reprogramming [33]. Furthermore, small molecules, microRNAs [67], and

epigenetic modifiers are used to increase reprogramming efficacy. For instance, PD0325901 and CHIR99021 as inhibitors of MEK and GSK3 pathways increased the ratio of pluripotent cells. It has been demonstrated that members of the microRNA-290 cluster are cell cycle regulators of ESCs and could also increase iPSCs colony numbers [41]. In addition other microRNAs, such as microRNA-130b, -301b, and -721 strongly supported the generation of iPSCs [66]. Moreover, DNA methyltransferase inhibitors such as AZA and RG-180 and also histone deacetylase inhibitors such as VPA could increase reprogramming efficacy when used along with OSKM factors [33].

Direct reprogramming has provided a working methodology to induce somatic cells to go back to their embryonic state by viral integration. However, integrative and nonintegrative methods used for reprogramming is a challenging criterion for safe iPSC production. For viral integration methods classic γ-retroviral and newer lentiviral vectors are used. Because of their well-understood biology and high transduction efficacy γ-retroviral vectors are commonly used for gene transfer systems. Despite high transduction efficacy the smallest result is that γ-retroviral vectors just transduce dividing cells. However, lentiviruses, a subclass of retroviruses, can infect both dividing and nondividing cells with high transduction efficacy. Although by using these integrating vectors iPSCs could be generated very efficiently, the viral vectors' integration in the host cell genome may cause genetic mutagenesis and genomic instability [76, 103]. Nevertheless, several mouse iPSC lines were generated using integrating vectors and further applied to tetraploid embryo aggregation experiments, which resulted in fully iPSC-derived viable mice [9, 100]. To overcome potential issues with integrated reprogramming transgenes several research groups developed nonintegrating reprogramming approaches that could overcome these limitations ([60], [40], [102] and [77]) via transient viral, episomal, modified mRNA, and protein delivery.

However, reprogramming efficiency is extremely low when compared to viral transduction [102]. The same problem exists with the adenoviral delivery system, the efficiency of which is comparable to episomal vector transfection [107]. Most recently viral vectors with floxed transgenes, which could be efficiently removed [92] or piggyback transposone/transposase-based systems [42] were studied to provide clinically applicable iPSC preparations. In this line, delivery of the reprogramming factors with nonintegrating Sendai viruses seems to be a promising alternative, as high reprogramming efficiencies were obtained with this reprogramming setting [30].

4.2 Differentiation of Human iPSCs into MSCs

As outlined above, the therapeutic effect of MSC preparations may depend on the source of MSCs, the in vitro expansion of MSCs, and from batch to batch on preparation variations of MSCs. Therefore, MSCs derived from a self-renewing stem cell source may be a more suitable option. Currently the best investigated

source of nontransformed self-renewing stem cells are embryonic stem cells. A number of reports described the in vitro differentiation of human ESCs into mesenchymal cells, which were very similar to primary MSCs. Some reports just applied spontaneous differentiation approaches and basically scraped out differentiating mesenchymal cells from human ESC colonies [61]. Other groups co-cultivated human ESCs with mouse bone marrow stroma cells, namely OP9 cells [7, 89] or isolated migrating cells from embryoid bodies [38]. A more defined MSC-like cell population was obtained after sorting of CD-105^{+} and CD-24^{-} cells [50]. Also a directed differentiation using the TGFβ inhibitor SB-431542 was successfully described recently [72]. By the inhibition of the SMAD-2/3 pathway the study could show an efficient differentiation of hESCs into MSCs. Mostly, MSCs derived from hESC exhibited a normal karyotype and were very similar if not functionally identical to human bone marrow-derived MSCs concerning their immunophenotype and the thus-far investigated functions [19]. Some groups reported a favorable higher proliferation capability of ESC-derived MSCs compared to human bone marrow-derived MSCs [72, 101]. Moreover, the differentiated cells lacked the expression of remaining pluripotency markers and lost the potential of teratoma formation, when those cells were transplanted into immunodeficient mice. However, the transplanted cells produced homogeneous tissues of mesenchymal appearance [34, 48]. In contrast to bone marrow- or adipose tissue-derived MSCs the hESC-derived MSCs did not show any signs of senescence and grew for multiple passages in vitro [38]. This observation might be the determining aspect for using the cells in future cell- and gene-therapy approaches. Another advantage of ESC-derived over the adipose tissue-derived MSCs might be their increased immunosuppressive properties against T lymphocytes [72]. This observation might be important for studying allograft rejection or applying ESC-derived MSCs in inflammatory bowel diseases.

Another source of pluripotent stem cells are the ethically less concerned induced pluripotent stem cells. As discussed above, iPSCs can be derived from a variety of somatic cell types that are easily obtainable from patients. Recent studies investigated human iPSC-derived MSCs (hiPSC-MSCs) in different degenerative diseases. The first study was done by Lian et al. in [51] in which they generated MSCs from hiPSCs with similar characteristics of human bone marrow-derived MSCs in terms of surface marker expression and differentiation potential. The cells could also substitute the therapeutic ability of classical MSCs in the hind limb ischemia model in mice, where significantly attenuated injury was promoted by increased vascular and muscle regeneration [51]. Additionally, hiPSC-MSCs displayed a remarkable immunosuppressive nature by inhibiting NK-cell proliferation and allograft rejection [32].

Some other studies have used additional supplements in order to differentiate iPSCs in functional MSCs. Villa-Diaz et al. introduced a biocompatible synthetic polymer (PMEDSAH) and xenogene-free culture media for differentiation of human iPSCs towards MSCs. Those cells were then applied in a mouse model with osteogenic calvaria defects, where the integrated cells could significantly recover the defect by regenerating new bone tissue compared to the control group [91]. In

another study human ESCs and human iPSCs were differentiated into MSCs by using collagen type I coated plates [54]. In an electrophysiological study, patch clamp analysis demonstrated that hiPSCs-MSCs and human bone marrow-derived MSCs exhibited highly similar ion channel properties [106]. Recently the TGFβ inhibitor SB-431542 was successfully used in order to induce the differentiation of iPSCs into MSCs directly. Cells generated after 10 days of treatment have shown MSC characteristics in terms of immunophenotype and differentiation potential [16].

4.3 Large-Scale Cultivation

Controlled scalable expansion culture and a well-ordered differentiation process are challenges for translational clinical therapies, whenever high amounts of cells need to be transplanted. Although human iPSC-derived MSCs (hiPSC-MSCs) have generated great interest in possible clinical applications using iPSCs in regenerative medicine, the actual number of cells that could be cultivated after differentiation with traditional culture methods would be very low (Cormier et al. [18]. Currently cells aimed to be transplanted to patients are produced and cultured in static flasks. But this cultivation system results in a low amount, heterogeneity of cells, increased risk of contamination, and low cell yield due to the lack of real-time controlled parameters within culture media (including O_2 and nutrient concentration, pH, osmolarity, metabolic waste concentration, shear stress, and cell density). One probable resolution to get a sufficient amount of cells is to change the culture conditions towards suspension culture in bioreactors, which might allow the scaling up of the number of these cells in vitro. In this regard all culture parameters must be controlled in a bioreactor in order to get a tangible number of cells [28, 82]. Stirred suspension bioreactors (SSBs) have provided a dynamic condition to produce cell-based products in a safe, robust, and cost-effective manner. SSBs have been developed for many experiments, in which a large amount of cells is required and they were also successfully used for the expansion of undifferentiated pluripotent human stem cells [62, 108]. However, it is unclear if pluripotent cells that were expanded in such a bioreactor system can also be differentiated towards a MSC-like phenotype in a SSB or in another suspension culture system. To investigate these issues, further studies on robust differentiation protocols providing ESC- or iPSC-derived MSCs in suspension cultures or on the amplification of initially differentiated mesenchymal precursor cells in a bioreactor system capable of promoting MSC expansion [21], might be of high impact.

5 Conclusion and Outlook

In spite of data from over 100 trials employing MSCs in different clinical settings correlation of MSC properties to clinical efficacy is limited due to the considerable diversity of the applied MSC-populations [88]. Also the internationally agreed

minimal set of consensus criteria for the definition and characterization of MSCs is not able to reflect adequately the broad spectrum of functional activity in the diverse contexts of tissue regeneration and anti-inflammatory therapies. Thus, engineered MSCs from well-characterized iPSC lines may not only solve the problem posed by the principally limited expansion capacity of MSCs, but may also serve as a homogeneous source of MSCs with more defined therapeutic characteristics. In this regard, one could even think of artificial iPSC-derived MSCs that overexpress a distinct therapeutically relevant transgene. One example for such a therapeutic transgene could be indoleamine-2,3-dioxygenase (IDO), which is induced by interferon-gamma (IFN-gamma) and which catalyzes the conversion from tryptophan to kynurenine and has been identified as a T cell inhibitory effector pathway in professional antigen-presenting cells [57]. Engineered MSCs, which were derived from iPSC lines harboring such a constitutively expressed IDO transgene, could serve as artificial MSCs for the treatment of inflammatory disease, where IDO-mediated T cell inhibition could further support the therapeutic effect of MSCs.

As the systemic application of MSCs might be hampered by an impaired pulmonary passage, MSCs could also be genetically modified to overcome such a limitation. For example, Rap1 [14], a member of the GTPase family of proteins with regulatory effects on multiple adhesion molecules, could be knocked-down in engineered MSCs, which then should gain an enhanced bioavailability after intravenous administration due to an improved pulmonary passage.

In conclusion, engineering MSCs from pluripotent stem cells such as iPSCs could generate advanced cellular therapies for the treatment of a variety of diseases, including intestinal GvHD and inflammatory bowel diseases, as well as some forms of liver failure.

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