TISSUE-SPECIFIC STEM CELLS

Introducing Transcription Factors to Multipotent Mesenchymal Stem Cells: Making Transdifferentiation Possible

RAN BARZILAY, ELDAD MELAMED, DANIEL OFFEN

Laboratory of Neurosciences, Felsenstein Medical Research Center and Department of Neurology, Rabin Medical Center, Tel Aviv University, Sackler School of Medicine, Petah-Tikva, Israel

ABSTRACT

Multipotent mesenchymal stem cells (MSCs) represent a promising autologous source for regenerative medicine. Because MSCs can be isolated from adult tissues, they represent an attractive cell source for autologous transplantation. A straightforward therapeutic strategy in the field of stem cell-based regenerative medicine is the transplantation of functional differentiated cells as cell replacement for the lost or defective cells affected by disease. However, this strategy requires the capacity to regulate stem cell differentiation toward the desired cell fate. This therapeutic approach assumes the capability to direct MSC differentiation toward diverse cell fates, including those outside the mesenchymal lineage, a process termed transdifferentiation. The capacity of MSCs to undergo functional transdifferentiation has been questioned over the years. Nonetheless, recent studies support that genetic manipulation can serve to promote transdifferentiation. Specifically, forced expression of certain transcription factors can lead to reprogramming and alter cell fate. Using such a method, fully differentiated lymphocytes have been reprogrammed to become macrophages and, remarkably, somatic cells have been reprogrammed to become embryonic stem-like cells. In this review, we discuss the past and current research aimed at transdifferentiating MSCs, a process with applications that could revolutionize regenerative medicine. STEM CELLS 2009;27:2509–2515

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Tissue-specific adult stem cells have been known for years for their capacity to differentiate along their lineage of origin. The most extensively researched adult stem cell population is found in the bone marrow and generates the hematopoetic lineage [1]. These cells are known to self-renew and differentiate along a well-defined hierarchy. Over the last few years, tissue-specific stem/progenitor cell populations have been isolated from various sources, including cord blood [2], brain [3], skin [4], fat [5], skeletal muscle [6], heart muscle [7], liver [8], and kidney [9]. Recently, it has become clear that some subpopulations of adult stem cells are capable of differentiating into mature cells not related to their original lineage, a process termed transdifferentiation. Several reports have claimed that adult stem cell populations, isolated from various compartments in the mature organism, can display this unexpected plasticity previously thought to be an exclusive feature of embryonic stem cells (ESCs). Stem cells capable of such plasticity have been isolated from brain [10, 11], bone marrow [12], skin [13], fat [14], skeletal muscle [15], umbilical cord blood [16], and other visceral organs.

To date, the best characterized adult stem cell population considered to possess transdifferentiation capacity is bone marrow mesenchymal stem cells, often referred to as multipotent stromal cells (MSCs). These cells were discovered in the 1970s when Friedenstein et al. [17] found a stem cell population in the bone marrow negative for hematopoietic markers. MSCs display their defining capacity to self-renew and differentiate along the mesenchymal lineage into bone, fat, and cartilage cells [18]. In addition, under specific conditions, MSCs are capable of differentiating into cells outside this restricted lineage, revealing plastic potential [19]. MSCs have been reported to transdifferentiate into neural cells (discussed in detail below), pancreatic cells [20], liver cells [21], and cardiomyocytes [22]. These data notwithstanding, contemporary views have raised doubts regarding the feasibility of MSC transdifferentiation, claiming that this phenomenon is, at best, marginal [23, 24].

MSCs are a focus of regenerative medicine research for two main reasons. First, because MSCs can be derived from the patient's own bone marrow, they represent a potential source for autologous cell transplantation, a replacement procedure that reduces the likelihood of immune rejection or, at least, avoids many complications associated with allogeneic transplantations. Second, using MSCs for replacement therapies circumvents the moral dilemmas of using fetal tissue as well as the ethical and political issues surrounding the use of

fertilized human eggs, which are required for generating human ESCs.

These considerations have driven scientists to keep investigating the disputed potential of MSCs to undergo transdifferentiation. Of note, transdifferentiation is sometimes referred to as lineage conversion, and henceforth these terms are used interchangeably. Here, we review past and present data relating to the plasticity of MSCs and, in light of the more recent studies, reassess the aforementioned scientific doubts concerning their plasticity. Specifically, we describe cutting-edge technologies that have enabled researchers to address the issue of plasticity, resulting in a new understanding that challenges the orthodox view of embryonic development. Indeed, genetic manipulations have revealed that cells can be reprogrammed to exhibit an alternative identity.

Key studies have already shown that a fully differentiated lymphocyte can be reprogrammed to the fate of a macrophage through the forced expression of a single transcription factor (TF) [25, 26]. Various reports have shown that forced expression of TFs in stem cells could serve as a means to induce or enhance the differentiation process. These studies, among others, have founded the basis for pioneering studies that reported the generation of ESC-like cells from somatic cells through forced expression of a number of TFs [27–29]. Considering these advances, we discuss the potential of reprogramming to promote transdifferentiation and enable MSCs to serve as a replacement source for nonmesenchymal tissues.

The Controversy of MSC Transdifferentiation

The controversial topic of MSCs neural transdifferentiation induced in vitro has been extensively reviewed elsewhere [30–34]. Such data were initially contentious because many stem cell scientists questioned more generally whether adult stem cells are capable of transdifferentiation [35–38].

Doubts were also raised regarding the interpretation of in vivo studies that asserted neural transdifferentiation of bone marrow cells [39–41]. Some researchers investigating bone marrow transplantations suggested that the supposedly transdifferentiated cells were rather a result of fusion between donor bone marrow cells and host brain cells [42]. This idea of cell fusion was endorsed by several studies that reported fusion phenomena in other organs [43–45].

Despite such initial skepticism regarding the capacity of MSCs to differentiate into neurons or glial cells, as time passed it became increasingly unreasonable to ignore the growing body of evidence supporting this phenomenon. It was discovered that MSCs express a considerable repertoire of neural genes, which likely contributes to the contested neurogenic predisposition of these cells [46–50]. Indeed, recent publications show that marrow-derived neuronal-like cells exhibit some typical neuronal electrophysiological traits [51–56]. Moreover, functional neuronal differentiation is accompanied by comprehensive upregulation of genes involved in synaptic transmission and long-term potentialization [57]. Furthermore, differentiation in vitro cannot be explained by the occurrence of fusion, and newer induction protocols have shifted from the initial use of chemical stressors to the more "physiologic" signaling molecules such as neurotrophic factors or other cytokines [58–60].

As a result of the somewhat contradictory views regarding MSC capacity to differentiate into neural lineages, it is important to try to reconcile all the inconsistencies in the early research literature that prompted so much controversy in order to gain insight into this phenomenon. To work with MSCs, they must be separated from their niche and cultured in an environment that enables propagation and expansion. This procedure generates a seemingly homogeneous cell population. One possible reason for the contradictory results is that the varying methods of MSC isolation and culture employed by different laboratories select specific populations that possess unequal transdifferentiation capacities. In support of this explanation, and despite there being clear criteria for characterizing MSC populations [61], clonal analysis of MSC cultures has revealed that, though the majority of cells share the same cell surface epitope expression profile, distinct subpopulations do exist [62, 63].

These subpopulations are functionally heterogeneous and contain undifferentiated stem/progenitors and lineage-restricted precursors with varying capacities to differentiate into assorted cell types. Recent research offers two, not incompatible, understandings of the variability present in MSC populations. First, there is evidence to suggest that some cells in MSC cultures are descendants of the neuroepithelium, hence the neural differentiation exhibited by some MSCs is not strictly transdifferentiation but rather neural differentiation along the neuroectodermal lineage [64, 65]. Second, studies from various scientific disciplines reveal that, even in clonal populations, gene expression patterns are stochastic, resulting in variability that is sometimes manifested at the cellular level and facilitates behavioral plasticity, particularly with respect to extrinsic cues. Namely, fluctuations in the transcriptional network are manifested in a cell's readiness to be directed to a certain cell fate at a certain moment. In general, the stochastic nature of gene expression is explained by oscillations in TF expression and consequent fluctuations in the transcriptional network and is considered vital for adaptability [66, 67].

Current studies are aimed at unraveling the mechanisms underlying the potential of MSCs to transdifferentiate, specifically the role of neural gene expression. As alluded to earlier, cells within MSC cultures have been observed to express genes and proteins associated with key neural behaviors, including neural development, differentiation, neurotrophic factor secretion and sensing, axon guidance, and synapse formation [46–50]. Whereas the expression of neural markers by MSCs could be interpreted as solely a reflection of a predisposition toward the neuroectodermal lineage, it is also possible that some of these genes and proteins play roles in MSC function in the bone marrow, perhaps as regulators of hematopoiesis. The bone marrow is innervated by nervous tissue organized in a circuit termed the neuroreticular complex, which allows stromal cells to receive signals from the nervous system and regulate hematopoiesis [68]. This intimate association among bone, marrow, and nervous tissue could be interpreted as a rationale for stromal cell expression of various classes of neuroregulatory proteins [23].

In conclusion, pioneering studies reporting MSC neural transdifferentiation were received enthusiastically in anticipation of finding an accessible autologous cell source suitable for replacing degenerated neurons in neurodegenerative disease. Subsequently, contradictory data led many to regard this phenomenon as having limited biological significance. However, more recently, the molecular and functional heterogeneity of MSCs, which express a broad array of regulatory proteins found in neurons, has been suggested to explain the initially diverse assessments of their neural transdifferentiation potential.

Genetic Manipulation of MSCs

Exposure to extrinsic signaling molecules can induce MSCs to transdifferentiate, as discussed above. An alternative

strategy to harness MSCs for regenerative therapy involves genetic manipulation [69]. Stem cells can be genetically manipulated in order to achieve: (a) gene delivery, whereby transplanted transgenic stem cells serve as a vehicle to deliver specific products to an affected area [70]; (b) cell reprogramming, whereby forced overexpression or knocked down expression of gene(s) serves to direct stem cell differentiation in a specified manner [71, 72]; and (c) stem cell enrichment/marking, whereby stem cells engineered to express a reporter gene enable in vitro sorting and in vivo tracing of particular stem cells [73, 74].

Genetic manipulation of stem cells is a delicate and complex task. Their remarkable proliferation capacity makes them poor candidates for nonviral episomal plasmid transfection/electroporation/nucleofection. At the same time, most stem cells are sensitive to antibiotic selection; hence, attempts to establish stably transfected stem cells are rarely successful. The method employed most commonly is viral transduction, as a result of its high infective efficiency [70]. Nonetheless, one should bear in mind the pitfalls of viral integration, because some studies have shown that tumor transformation is a prominent risk [75].

MSCs derived from bone marrow are being evaluated as potential vehicles for cell- and gene-specific therapy against disease. Their ability to home to sites of injury renders them promising candidates for the restoration or replacement of organ systems and/or the delivery of gene products [76, 77]. Comparative studies evaluating different viral transduction methods of MSCs have shown that lentiviruses are the most efficient vectors. McMahon et al. [78] have shown the superiority of lentiviral gene delivery to rat MSCs over other viral or nonviral gene delivery methods. Other studies reported the ability of lentiviral vectors to transduce human [79–83] or mouse [84] MSCs and sustain transgene expression longer than other viral and nonviral vectors. These studies have also indicated that the transduction procedure itself does not affect the growth, differentiation potential, and migration capacity of MSCs and does not induce excessive cell death.

Stem Cell Reprogramming—The End of Lineage Restriction?

It is now accepted that the requirements of a stem cell population are to be capable of self-renewal (thus maintaining the stem cell pool) and able to differentiate into and give rise to specific cell types [85]. The latest studies have shown that some cells, though not considered stem cells in the past, indeed exhibit these two traits, at least under specific conditions. Therefore, a current, still contentious, view is that there is no such thing as a stem cell, instead there is "the stem cell state" [86, 87]. This state depends on many variables: the niche, the epigenetic state, the transcriptional code, and probably other factors yet to be discovered.

There is a dramatic corollary of this revised understanding of "stemness," because it is now conceivable and feasible that stem cells can be engineered and the fate of stem cells controlled, which would revolutionize regenerative medicine. The complex processes and interactions that occur in vivo restrict, at least to date, the possibility of creating stem cells in vivo. Nonetheless, emerging in vitro molecular technologies that enable manipulation of the epigenetic state and transcriptional code have allowed realization of the once unthinkable, namely, the induction of pluripotency.

In the last 2 years, several studies have reported the generation of induced pluripotent stem (iPS) cells derived from terminally differentiated somatic cells via genetic manipulations [27–29, 88–102]. These cells were forced to express four, or in later studies, fewer than four, specific TFs and subsequently exhibited features of ESCs. This alteration in cell fate has been termed "reprogramming." These studies highlight the key role of TF expression in determining cell identity or, in other words, demonstrate that cell identity, under specific extrinsic signaling, is merely a reflection of the transcriptional profile. The exact mechanisms that underlie the pluripotent state and govern cell fate decisions remain enigmatic, but studying iPS cells has revealed that key TFs influence the epigenetic state [103].

Despite the great promise of stem cell reprogramming for regenerative medicine, possible risks and complications of genetic manipulations must be born in mind. In particular, as mentioned above, viruses are presently the most successful vehicles for genetic manipulation, especially retroviruses and lentiviruses. These viruses are used because of their high infectivity and ability to integrate into the DNA of the host cell, which ensures stable expression. However, the latter viral feature represents a double-edged sword because gene integration into some DNA regions can cause malignant transformation [75, 88]. Because integration is random and cannot, to date, be targeted to specific genomic sites, the risk of inducing malignancy cannot be overlooked. In light of these concerns, recent innovative studies have attempted to engineer TF expression without the use of retroviruses [104–106]. For example, novel technologies aim to deliver TFs in the form of proteins, which would avoid the neoplastic risks associated with genetically manipulating stem cells [107].

Delivery of TFs into MSCs Promotes Transdifferentiation

Studies in ESCs and somatic cells have highlighted the rationale of inducing the expression of TFs, via genetic manipulation, as a means to induce and maintain differentiation. Specifically, Thomas Graf's pioneer studies in blood cells have shown that forced expression of a single TF is sufficient to trigger a specialized B cell to transdifferentiate into a macrophage [25]. Notably for MSCs, delivery of TFs through genetic manipulation (alone or combined with a cocktail of extrinsic signaling molecules) may be exploited to enhance differentiation and widen the spectrum of MSC plasticity (Fig. 1).

To date, various TFs have been delivered into MSCs with diverse outcomes. The first MSC/TF studies employed TF delivery as a means to enhance MSC differentiation toward the osteogenic or chondrogenic fate [108, 109]. A later study found that forced expression of the Smad8 TF, when combined with expression of the bone morphogenic protein 2, was sufficient in vitro and in vivo to generate neotendon from MSCs [109]. Similarly, the migratory [110] and proangiogenic [111] properties of MSCs were shown to be enhanced by forced expression of specific TFs.

Subsequently, more demanding TF/MSC experiments attempted to induce MSCs to transdifferentiate into cell types outside the mesenchymal lineage. Encouraging results showing efficient generation of functional insulin-producing cells were reported following overexpression of the pancreatic β-cell-specific TF pancreatic duodenal homeobox 1 (PDX1) [71, 112]. Importantly, using animal models of diabetes, these studies also demonstrated that TF-expressing MSCs could serve as a source for β-cell replacement therapy.

Other TF/MSC studies have focused on enhancing neuronal transdifferentiation [72, 113, 114]. The proneural TF nerogenin 1 (Ngn1) has been found to promote neuronal
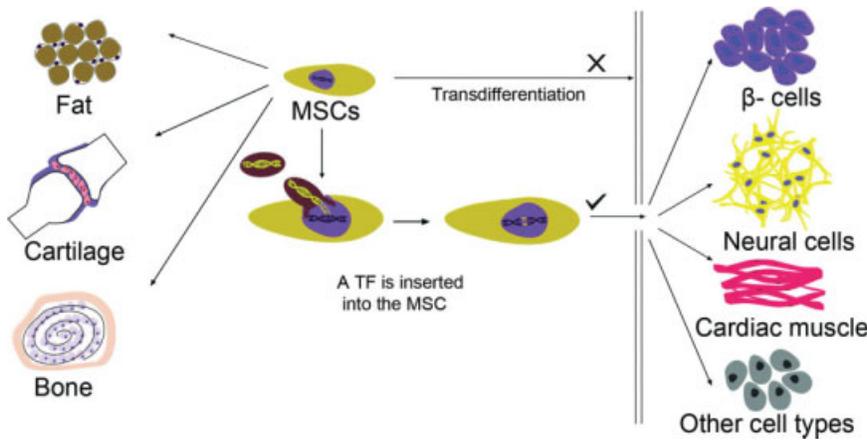
Figure 1. Introduction of transcription factors expands mesenchymal stem cell (MSC) plasticity. Abbreviation: TF, transcription factor.

differentiation of MSCs. Briefly, Ngn1 expression was sufficient to convert MSCs to the neuronal cell fate, such that the Ngn1-expressing MSCs expressed neuron-specific proteins and the voltage-gated Ca^{2+} and Na^+ channels absent in parental MSCs. Importantly, in line with the aforementioned PDX1 studies, Ngn1-expressing MSCs were demonstrated, in an animal stroke model, to serve as replacement neural cells, because their transplantation improved motor function dramatically when compared with unmanipulated MSCs [72]. Taking a slightly different approach, some studies reported that silencing a TF, namely, neuron-restrictive silencer factor, can promote the neural differentiation of MSCs [113, 114]. Specifically, the manipulated MSCs exhibited superior electrophysiological properties.

In our laboratory, we recently achieved lentiviral delivery of LMX1a into human MSCs [115]. *LMX1a* is considered to be a master gene controlling midbrain mesodiencephalic dopaminergic neuron development [116, 117]. Analysis of the transduced MSCs, which were also exposed to extrinsic signaling molecules, revealed that specific dopaminergic developmental genes were upregulated in a manner typical of developing midbrain dopaminergic neurons. Moreover, transduced cells expressed higher levels of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, and secreted significantly higher levels of dopamine relative to nontransduced cells.

In summary, forced expression of single TFs in MSCs can promote transdifferentiation into pancreatic β-cells, endothelial cells, and neuronal cells [71, 72, 111, 112, 115]. Remarkably, transdifferentiated cells exhibit their identity long term, even when removed from culture plates and transplanted into animals. Of note, in our study, as in the other MSC/TF studies described above, the intention was to avoid any promotion of the innate tendency of MSCs to differentiate into mesenchymal tissues, such as fat, bone or cartilage. In addition, and importantly, induction of pluripotency was not desirable because then the manipulated cells could divide and differentiate into unwanted fates.

Delivery of TFs Into MSCs—Direct Lineage Conversion Versus Generation of iPS Cells

The reprogramming of MSCs into iPS cells was described in previous reports [93, 101]. In line with the pluripotent capacity of iPS cells, these reprogrammed MSCs can be induced to differentiate into all three germ layer cell types. This raises the question, when using MSCs to generate a specific cell type: what is the better method—transdifferentiation/lineage conversion induced by TF delivery or reprogramming to become an iPS cell followed by differentiation? Because

these two approaches have not been compared directly yet, the answer to this question is a matter for speculation. Nevertheless, as outlined below, the available data lead us to expect that the former approach will prove more successful with regard to replacement therapy.

First, considering the relatively low yield in generating iPS cells, and considering data indicating the potential of lineage conversion when expressing TFs in cells from a source similar to the target cell type [25, 26, 118], MSCs seem to present an attractive candidate for this strategy of inducing transdifferentiation. This is results from the complexity of their transcriptome, because MSCs express regulatory genes that play a role in the specification of diverse cell fates and cell functions [23] that may account for the plasticity of MSCs. Namely, if one aims to direct an MSC to transdifferentiate into a neuron, for example, it is possible that passing through the ESC-like state is redundant. Rather, it is possible that manipulation of the balance between specific TFs in the transcriptome may direct the cell to the desired neural fate at a higher magnitude than through the iPS cell state. Second, when considering future clinical applications, there are at least two advantages to the lineage conversion approach. Technically, lineage conversion is a simpler protocol. More importantly, however, the iPS cell, though theoretically only an intermediary in the reprogramming process, resembles an ESC and is prone to form teratomas upon transformation [119]. Bearing this risk in mind with the reportedly low tendency of MSCs to form tumors, we suggest that TF delivery to promote transdifferentiation will prove to be a simpler and safer approach to sourcing replacement therapy.

CONCLUSION

Numerous reports credit MSCs with therapeutic potential because they represent a renewable source for autologous stem cell transplantation. Until recently, the beneficial effect of transplanted MSCs was considered to be supportive rather than substitutive as a result of the limited evidence of transdifferentiation or of functionally replacing diseased/lost cells. However, a growing body of research suggests that it is possible to force tissue-specific stem cells to overcome the limits of lineage restriction by inducing expression of TFs via genetic manipulation. Indeed, using this approach, MSCs have been directed to differentiate into cells resembling dopaminergic neurons, pancreatic β-cells, and other specific cell types.

Although these data are promising, future studies must establish whether such cells can serve as a safe and functional autologous source when treating diseases such as Parkinson's disease or diabetes mellitus.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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