



## Concise Review: Mesenchymal Stem Cells for Diabetes

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### ABSTRACT

Mesenchymal stem cells (MSCs) have already made their mark in the young field of regenerative medicine. Easily derived from many adult tissues, their therapeutic worth has already been validated for a number of conditions. Unlike embryonic stem cells, neither their procurement nor their use is deemed controversial. Here we review the potential use of MSCs for the treatment of type 1 diabetes mellitus, a devastating chronic disease in which the insulin-producing cells of the pancreas (the  $\beta$ -cells) are the target of an autoimmune process. It has been hypothesized that stem cell-derived  $\beta$ -cells may be used to replenish the islet mass in diabetic patients, making islet transplantation (a form of cell therapy that has already proven effective at clinically restoring normoglycemia) available to millions of prospective patients. Here we review the most current advances in the design and application of protocols for the differentiation of transplantable  $\beta$ -cells, with a special emphasis in analyzing MSC potency according to their tissue of origin. Although no single method appears to be ripe enough for clinical trials yet, recent progress in reprogramming (a biotechnological breakthrough that relativizes the thus far insurmountable barriers between embryonal germ layers) bodes well for the rise of MSCs as a potential weapon of choice to develop personalized therapies for type 1 diabetes. *STEM CELLS TRANSLATIONAL MEDICINE* 2012;1:59–63

### INTRODUCTION

Type 1 diabetes results from the autoimmune destruction of the insulin-producing  $\beta$ -cells of the pancreas, termed islets of Langerhans. Predisposition to developing the disease has a strong genetic background, but environmental factors not yet fully understood are thought to trigger its onset. As a consequence of the ablation of  $\beta$ -cells, hormonal substitutive therapy (insulin administration) is required to control blood sugar levels. Albeit life-saving, this intervention is insufficient to prevent long-term complications that often include vascular degeneration, blindness, and kidney failure. The search for alternative approaches is therefore of paramount clinical interest. Islet transplantation is one such approach that has proven successful at establishing that cell therapies can revert, sometimes for years, the symptoms of the disease [1]. However, both the need for immunosuppression and the scarcity of organs available for processing and transplantation has hindered the widespread use of this therapy. Mesenchymal stem cells (MSCs) from different sources may indeed help us tackle the issue from both fronts: not only do they possess immunomodulatory properties that could aid in the development of interventions designed to minimize immune rejection but there is evidence that they may also be coaxed to

differentiate into glucose-responsive, insulin-producing cells. By describing the state of the art in the definition of protocols for their efficient specification along the  $\beta$ -cell lineage, this short review will focus on the latter aspect of MSC-related research.

Although first described as the chief component of the stroma within the bone marrow hematopoietic niche, MSCs have been found in a multitude of tissues, including fat, umbilical cord Wharton's jelly and blood, periodontal ligament, skeletal muscle, and others [2]. Despite their heterogeneity (which has been observed even among different clones from the same donor [3]), MSCs must satisfy a series of criteria to be considered as such, including adherence to plastic in culture, proven ability to differentiate along common connective lineages, and the simultaneous presence and absence of specific markers [4]. Although the choice of these mesodermal cells as a substrate for differentiation protocols whose endpoint is an endodermal derivative (such as the insulin-producing  $\beta$ -cell) would appear counterintuitive, their ease of procurement and their low tumorigenicity when compared with pluripotent stem cells are factors that have made MSCs the workhorse of adult stem cell-based research for diabetes [5]. Whether or not it is true that  $\beta$ -cells can be derived from MSCs

may end up being no more than an academic discussion, as long as their derivatives are proven to secrete insulin in a safe and glucose-responsive manner.

Most strategies applied thus far to induce MSCs along the  $\beta$ -cell lineage make use of chemical methods, that is, approaches in which soluble factors known to influence pancreatic development are added sequentially and/or in combination to the culture medium. Unlike for human embryonic stem (hES) cells [6, 7], there is no “standard” protocol for MSCs, and much of what has been described lacks the in-depth rationale that has been the guiding force behind methods based on the differentiation of the former toward definitive endoderm [8]. Only recently have steps been taken to this end, by means of the adaptation of hES strategies to primitive populations of MSCs [9]. Indeed, the fact that some MSC populations are responsive to certain protocols whereas others are not may be a reflection of their intrinsic potential, which in turn may depend on a plethora of factors. Because the most obvious one is their provenance, we will organize the rest of this review based on the biological source from which MSCs are obtained.

### Pancreatic Islets

It would hardly be a stretch to start looking at the pancreas for MSCs with potential to give rise to insulin-producing  $\beta$ -cells. Although the putative islet progenitors that might be at least partially responsible for  $\beta$ -cell regeneration are not expected to be mesenchymal in nature [10], the hypothesis that resident pancreatic MSCs may be more amenable than others to adopting a pancreatic fate *in vitro* is one certainly worth exploring. Thus, Zulewski and collaborators reported the isolation of nestin-positive islet-derived progenitor cells from rat pancreatic islets. These fibroblast-looking cells could be partially induced *in vitro* towards pancreatic endocrine, exocrine, and hepatic phenotypes [11]. This study was limited in depth and scope but nevertheless opened a new path that is now the subject of active research. Thus, Huang and Tang reported the ability of similarly derived cells at reversing hyperglycemia in diabetic NOD-SCID mice [12]. These findings prompted a search for the human counterparts of these cells, whose results were first reported by Zhang et al. [13]. Once again, however, the analysis of the ability of these cells to commit along the pancreatic endocrine lineage was not substantial enough, and the results were inconclusive. Looking for a biologically plausible explanation for the putative role of mesenchymal-like cells in  $\beta$ -cell regeneration, Gershengorn and colleagues contended that  $\beta$ -cells could dedifferentiate into MSCs and then redifferentiate under certain conditions through a process termed epithelial-to-mesenchymal transition [14]. Not only was such hypothesis disproven by subsequent studies [15, 16] but the claim that the “transitioned” cells were true  $\beta$ -cells had little support in the observation that the insulin content of the former was 2 orders of magnitude lower than that typically observed in the latter. Whether or not a physiological phenomenon was behind the reported behavior of these cells *in vitro*, refinements in the original method did result in more robust levels of insulin expression [17, 18].

A different subset of islet-derived MSCs, possibly related to intraislet CD90+/CD105+ progenitors and to cells of pericytic nature, was recently characterized [19]. This is consistent with the findings of Crisan et al., who reported the prospective isolation of MSCs from multiple organs by means of pericytic markers [20]. In summary, although these findings are suggestive that MSCs of pancreatic origin may indeed have the potential to differentiate along

the  $\beta$ -cell lineage, to date there is little evidence to support their purported superiority to MSCs derived from other tissues.

### Pancreatic Ductal and Acinar Tissue

The hypothesis that islet progenitor cells may reside in the pancreatic ducts is rooted in the consistent and well documented observation that islets frequently appear to coalesce following waves of migration and differentiation described as “ductal budding” [21]. When placed in culture, ductal cells often exhibit a combination of mesenchymal- and pancreatic progenitor-like phenotypes [22, 23]. Others have reported that, although MSC-like ductal cells differentiate along their canonical (adipogenic, chondrogenic) routes, they express endocrine lineage markers such as Isl1, Nkx2.2, Nkx6.1, Ngn3, Pdx1, and NeuroD throughout their expansion. However, attempts at differentiating them into  $\beta$ -cells yielded the often reported mix of promise and disappointment, perhaps because of the simplicity of the induction methods [24]. Indeed, Sordi and colleagues went as far as suggesting that contaminating cells of endodermal origin could be responsible for those observations [25].

### Adipose Tissue-Derived MSCs

Because of its ease of procurement and our ability to bank cells from the prospective patient, the adipose tissue has garnered significant attention in the field over the past few years [2, 26]. As seen with MSCs obtained from other tissues, adipose-derived MSCs respond to the usual generic cues (nicotinamide, exendin-4, betacellulin, etc.) by differentiating into cells that express some key pancreatic markers but remain unable to regulate insulin secretion [27]. Adipose-derived stromal cells (ADSCs), a heterogeneous population somewhat resembling MSCs, responded to a stagewise differentiation protocol *in vitro* by yielding endocrine-like cells with some degree of functionality [28]. Although ectopic Pdx1 expression did not dramatically improve the differentiation of ADSCs in a transgenic setting *in vitro*, an *in vivo* diabetic microenvironment seemed to result in better maturation, and long-term reversal of hyperglycemia was reported upon transplantation [29]. From a therapeutic perspective, ongoing clinical trials have already shown the safety of fat-derived MSC-like cells, although the results are difficult to interpret because of the lack of characterization of the cell populations used [30].

### Bone Marrow

The bone marrow has been historically the prime source of MSCs. Early work by Ianus and colleagues [31] showed that labeled cells from the bone marrow were able to contribute, even if just partially, to the pancreatic endocrine lineage. However, as similar experiments have yielded contradicting results [32, 33], the jury is still out as to whether nondifferentiated MSCs can be specified towards  $\beta$ -cells upon infusion *in vivo*. Differences in the way bone marrow is processed (and therefore in the composition of the infused preparation) may account for some of these differences. As for *in vitro* approaches, numerous chemical-based methods involving the usual differentiation cocktails (nicotinamide, betacellulin, exendin-4, etc.) have failed to show that bone marrow-derived MSCs are inherently superior to those extracted from other sources in terms of differentiation yield or functionality [34–36]. However, another study on human bone marrow-derived MSCs showed that *in vivo* maturation was able to compensate for the poor efficiency of *in vitro* differentiation,

leading to normoglycemia in diabetic mice [37]. Transgenic strategies, chiefly those that make use of Pdx1, have also given somewhat positive results, especially when combined with transplantation in a diabetic microenvironment [38, 39]. Interestingly, although bone marrow-derived MSCs from diabetic patients respond to differentiation cues in a manner that is comparable to that observed in cells obtained from healthy subjects [40], there are some differences that reflect the uniqueness of the diabetic microenvironment [41]. Whether such differences could end up being significant in the context of the development of potential autologous therapies remains to be ascertained.

### Umbilical Cord Blood and Placenta

Other MSC-rich, easily bankable tissues that could potentially be used for the treatment of diabetes are the umbilical cord (blood and Wharton's jelly) and the placenta [42, 43]. Fetally derived cells from these sources are thought to be more malleable and potent than most of their adult counterparts [9]. MSC-like cells adhere to plastic and form colonies not only from the stroma of the cord proper, the chorion, and the amniotic membrane but also from the blood itself [44–46]. The first report that umbilical cord blood-derived cells expressed basal levels of genes involved in the progression of pancreatic endocrine development [47] has been later confirmed by others [9], lending support to the intriguing notion that these tissues may contain cells that are somehow primed to give rise to  $\beta$ -cells. Several groups have already shown that umbilical cord blood-derived cells can be coaxed into producing insulin, albeit not with the same efficiency as true  $\beta$ -cells [44, 48–50]. More recently, Prabakar et al. [9] established that a population of umbilical cord blood-derived cells that basally expressed high levels of both pluripotent stem cell (chiefly Oct3/4) and pancreatic endodermal (Pdx1, Ngn3, Nkx6.1) markers could effectively recapitulate all the stages of the standard protocol used to differentiate  $\beta$ -cells from hES cells, including the first critical step of definitive endoderm specification. Human C-peptide could be detected systemically months after the transplantation of the differentiated cells into immunodeficient mice. Despite the unequivocal mesenchymal nature of these cells, these findings demonstrate that MSCs from the umbilical cord blood may behave like hES cells under the appropriate circumstances.

### Amniotic Fluid

One of the latest sources to join the ever-growing armamentarium of tissue-specific MSCs is the amniotic fluid. These fibroblastic-like cells, shed by the fetus to the surrounding liquid as it develops, share many properties with placental, chorionic, and cord blood/tissue MSCs [51, 52]. They can be procured with relative ease, although not routinely, through standard prenatal diagnostic procedures. De Coppi and colleagues recently described a subpopulation of amniotic fluid cells with multi-germ layer (mesoderm, ectoderm, and endoderm) specification potential [53]. Like umbilical cord blood cells, they could be expanded very significantly while expressing hES cell markers (telomerase, Oct3/4, SSEA-4, etc.). Trovato and colleagues [54] have already reported preliminary attempts at differentiating amniotic fluid-derived cells into insulin-producing cells, although more work is required to validate their potential in this direction.

### CONCLUSION

Because of their ease of procurement and differentiation into a variety of connective tissues, MSCs have taken an early lead over

other stem cell types and as such are highly represented in ongoing clinical trials for a variety of conditions. Whether diabetes could be one of these in the near future (at least from the  $\beta$ -cell replenishment perspective) remains to be established. The evidence herein reviewed indicates that we are not there yet. Few would dispute that, being mesodermal in origin, MSCs have a steeper hill to climb than known pluripotent cell types such as hES and induced pluripotent stem cells to become  $\beta$ -cells. However, a lesson that we have learned over the past decade is that cells do not always respond in “canonical” fashion when given *in vitro* stimuli to which they would have never been exposed in their physiological niches. A high degree of epigenetic plasticity has been demonstrated time and again since the advent and subsequent refinement of reprogramming interventions [55–57], and therefore the old dogma that cell commitment is cast in stone no longer holds true. Although the available data thus far would seem to indicate that more “undifferentiated” MSCs such as those found in connection to embryonic tissues (umbilical cord blood, placenta, chorion, and amnions) are more powerful than those obtained from adult tissues (fat and bone marrow), the last few years have given us the novel perspective that potency is not an intrinsic, immutable trait but rather a circumstantial—and therefore subject to manipulation—state. If terminally differentiated cells can travel back in time to reacquire full pluripotency, we can surely envision MSCs of different origins adopting pancreatic fates. In view of this new paradigm, factors such as the ease of collection and expansion, perhaps from the very same patient we intend to treat, may end up weighing more than the relative potency at the time of isolation.

The definition of a  $\beta$ -cell specification standard, similar to what the ViaCyte protocol represents for hES cells [7, 8], is perhaps the top priority that we identify in the field. Generic differentiation agents typically used in this context (nicotinamide, exendin-4, etc.) have already taken us as far as they could, which is nowhere near the phenotype of a true  $\beta$ -cell. Recent efforts at driving MSCs through the same developmental stages that hES cells (or embryonic pancreatic progenitors, for that matter) go through in their differentiation [9] are a significant step forward that may pave the way to sounder, more robust methods potentially applicable to MSCs of different origins.

There is ongoing discussion on whether to use allogeneic or human leukocyte antigen (HLA)-matched MSCs for a number of therapies. Although some claim a purported immunoprivilege of MSCs primarily through the inhibition of effector functions [58] (which would prevent their rejection in an allotransplantation setting [59]), others report that MSCs lose their immune privilege upon differentiation [60] or are rejected right away [61]. Thus, it has been suggested that MSC effects on the recipient's immune system may be affected not only by cell-to-cell interactions but also by environmental factors not fully understood yet [58]. Regardless of the potential immune privilege of these cells in an HLA-mismatched context, their use for  $\beta$ -cell replenishment in type 1 diabetes is likely to require immunological interventions or physical immunoisolation to avoid recurrence of autoimmunity.

Be it as it may, by now the clinical prospects of MSCs seem to be solidly established. Beyond their role as building blocks for new  $\beta$ -cells, their immunomodulatory [62] and proangiogenic [63] properties make them ideal candidates for combination

therapies. MSCs are known to produce and secrete in their vicinity, among others, vascular endothelial growth factor, brain-derived neurotrophic factor, nerve growth factor, basic fibroblast growth factor, insulin-like growth factor-1, and hepatocyte growth factor [64]. Their concerted action may prove of great value to create a favorable niche for engraftment by interacting with local microenvironments and inducing morphogenetic, anti-apoptotic, mitogenic, and angiogenic effects [65, 66].

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#### AUTHOR CONTRIBUTIONS

J.D.-B.: conception and design, data analysis and interpretation, manuscript writing; G.L.: provision of study material, assembly of data, data analysis and interpretation, manuscript writing; L.I.: conception and design, manuscript writing; C.R.: conception and design, final approval of manuscript.

#### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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