J. Cell. Mol. Med. Vol 12, No 4, 2008 pp. 1155-1168

Mesenchymal stem cells: biology and clinical potential in type 1 diabetes therapy

Meng Liu, Zhong Chao Han*

State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences & Peking Union Medical College; National Engineering Research Center of Cell Products, Tianjin, PR China

Received: November 1, 2007; Accepted: February 18, 2008

- **Introduction**
- **Biological characterization of mesenchymal stem cells**
  - **Isolation and culture of human MSCs**
  - **Phenotypic properties of MSCs**
  - **Multi-potent differentiation of MSCs**
  - **Immunomodulatory effects of MSCs**
- **Aetiology and current treatment of type 1 diabetes**
- **Mesenchymal stem cells in type 1 diabetes therapy**
  - **MSCs with potential to differentiate into insulin-producing cells**
  - **MSCs as cellular vehicle for insulin gene therapy**
  - **Induction of IPCs from stem cells by protein transduction technology**
  - **Minimum requirements for replacement β-cells**
  - **MSCs for therapeutic neovascularization in type 1 diabetes**
- **Concluding remarks**

Abstract

Mesenchymal stem cells (MSCs) can be derived from adult bone marrow, fat and several foetal tissues. *In vitro*, MSCs have the capacity to differentiate into multiple mesodermal and non-mesodermal cell lineages. Besides, MSCs possess immunosuppressive effects by modulating the immune function of the major cell populations involved in alloantigen recognition and elimination. The intriguing biology of MSCs makes them strong candidates for cell-based therapy against various human diseases. Type 1 diabetes is caused by a cell-mediated autoimmune destruction of pancreatic β-cells. While insulin replacement remains the cornerstone treatment for type 1 diabetes, the transplantation of pancreatic islets of Langerhans provides a cure for this disorder. And yet, islet transplantation is limited by the lack of donor pancreas. Generation of insulin-producing cells (IPCs) from MSCs represents an attractive alternative. On the one hand, MSCs from pancreas, bone marrow, adipose tissue, umbilical cord blood and cord tissue have the potential to differentiate into IPCs by genetic modification and/or defined culture conditions *in vitro*. On the other hand, MSCs are able to serve as a cellular vehicle for the expression of human insulin gene. Moreover, protein transduction technology could offer a novel approach for generating IPCs from stem cells including MSCs. In this review, we first summarize the current knowledge on the biological characterization of MSCs. Next, we consider MSCs as surrogate β-cell source for islet transplantation, and present some basic requirements for these replacement cells. Finally, MSCs-mediated therapeutic neovascularization in type 1 diabetes is discussed.

Keywords: mesenchymal stem cells • type 1 diabetes • islet transplantation • insulin-producing cells • cell-based therapy

Introduction

Mesenchymal stem cells (MSCs) were first identified by Friedenstein and his colleagues [1], who described bone-forming progenitor cells from rat bone marrow. In addition to postnatal bone marrow, MSCs can also be isolated from adipose tissues, foetal liver, blood, bone marrow, lung, cord blood, placenta and umbilical cord [2–7]. Several lines of evidence have shown that under appropriate environments, MSCs are able to differentiate into mesodermal, endodermal and even ectodermal cells. Another intriguing feature of MSCs is that they escape immune recognition and inhibit immune responses, consequently are called hypoimmunogenic cells. Therefore, MSCs appear to be a very promising tool for regenerative and immunoregulatory cell therapy.

*Correspondence to: Prof. Zhong Chao HAN,
State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences & Peking Union Medical College, 288 Nanjing Road, Tianjin 300020, P. R. China.
Tel.: +86-22-27210717
Fax: +86-22-66211430
E-mail: tihzchan@public.tpt.tj.cn

doi:10.1111/j.1582-4934.2008.00288.x

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

Diabetes mellitus is a devastating metabolic disease, which falls into two categories. Type 1 diabetes results from autoimmune-mediated destruction of β cells in the islets of Langerhans of the pancreas, while type 2 diabetes is due to systemic insulin resistance and reduced insulin secretion by islet β cells. In comparison with conventional or intensive insulin treatment, islet transplantation is the only therapy for type 1 diabetes that achieves an insulin-independent, constant normoglycemic state and avoids hypoglycemic episodes. However, the application of this treatment is restricted by the limited availability of primary human islets from heart-beating donors. Some recent studies indicate that MSCs can differentiate into insulin-producing cells by genetic and/or microenvironmental manipulation *in vitro*. Thus, MSCs provide an alternative β-cell source for islet transplantation.

In this review, we will summarize the major biological features of MSCs, and their possible applications in the treatment of type 1 diabetes.

Biological characterization of mesenchymal stem cells

Isolation and culture of human MSCs

Standard conditions for generation of bone marrow derived mesenchymal stromal cultures have been reported [8, 9]. However, the property of plastic adherence itself is not sufficient to obtain purified MSCs, some investigators have tried different methods for isolation of homogenous cell populations [10, 11]. Besides adult bone marrow, researchers in our laboratory have also successfully isolated MSCs from other origins such as foetal lung [12], pancreas, skin, muscle, bone marrow, cord blood and umbilical cord [13]. MSCs in culture have a fibroblastic morphology and adhere to the tissue culture substrate. Under current *in vitro* culture conditions MSCs obtained from young donors can grow to 24–40 population doublings and the proliferative potential of the cells obtained from older donors is more compromised [14]. Afterwards, MSCs enter growth arrest, a phenomenon termed replicative senescence [15]. Replicative senescence is a common characteristic of cultured diploid cells, it is caused by several factors including progressive telomere shortening during continuous subculture *in vitro* [14, 16] due to absence of telomerase activity [17, 18]. Some studies have demonstrated that forced ectopic expression of human telomerase reverse transcriptase (hTERT) in MSCs can dramatically extend their lifespan to $>$260 population doublings, while maintaining their osteogenic, chondrogenic, adipogenic, neurogenic and stromal differentiation potential [17, 19, 20]. Thus, telomerase activation is a potential strategy for obtaining large number of biologically competent MSCs for clinical application. Unexpectedly, the extensive cell proliferation *in vitro* led to genetic instability and resulted in MSCs transformation [21]. It seems that controllable expression of hTERT gene is very necessary.

Phenotypic properties of MSCs

Considerable progress has been made towards characterizing the cell surface antigenic profile of human bone marrow-derived MSC populations using fluorescence activated cell sorting (FACS) and magnetic bead-sorting techniques. Nevertheless, to date there is no specific marker or combination of markers that specifically identifies MSCs. Therefore, MSCs have been defined by using a combination of phenotypic markers and functional properties. It is generally agreed that adult human MSCs express Stro-1 [10, 22–23], CD105 (SH2) [24] and CD73 (SH3/4) [25] as well as some cell adhesion molecules including integrins (α1, α2, α3, α5, α6, α_V, β1, β3, β4) [26], intercellular adhesion molecule-1, -2 (ICAM-1,-2), vascular cell adhesion molecule-1 (VCAM-1), lymphocyte function-associated antigen 3 (LFA-3), CD72, and activated leucocyte-cell adhesion molecule (ALCAM) [9, 27, 28–30]. They also express human leucocyte antigen (HLA) class I but not class II molecules on cell surface [31]. Additionally, MSCs lack the expression of typical haematopoietic antigens CD45, CD34 and CD14 [27]. (See Table 1 for details).

Multi-potent differentiation of MSCs

A large number of studies demonstrate that bone marrow-derived MSCs from human, canine, rabbit, rat and mouse have the capacity to differentiate into mesenchymal tissues both *in vitro* and *in vivo*, including bone [8, 32], cartilage [33], fat [34, 35], tendon [36, 37], muscle [38, 39] and haematopoietic supporting stroma [35]. In addition, MSCs can differentiate into tissues of ectodermal (*e.g.* neurons) [40] and endodermal (*e.g.* hepatocytes) origin [41].

Individual colonies derived from single MSC precursors have been reported to be heterogeneous in terms of their multi-lineage differentiation potential [27, 42]. The heterogeneity of adult MSCs could be explained by the notion that in bone marrow, the MSC pool comprises not only putative MSCs, but also subpopulations at different stages of differentiation. Notwithstanding the multipotentiality of MSCs is a basis for using them to generate different cells and tissues for replacement therapy, the molecular mechanisms that govern MSCs differentiation are incompletely understood. Based on the genetic and genomic information provided by various studies, Baksh *et al.* [43] propose a model for the regulation of adult stem cell differentiation, which incorporates two continuous yet distinct compartments ('stem cell compartment' and 'commitment compartment'). The commitment and differentiation of MSCs to specific mature cell types is a tightly and temporally controlled process, involving the activities of various transcription factors, cytokines, growth factors, and extracellular matrix molecules. Global gene expression profiling using DNA microarray technology has already been used successfully to identify genes that regulate osteogenic, adipogenic and chondrogenic differentiation of MSCs [44, 45], which has greatly facilitated our effort to elucidate the mechanism controlling adult stem cell differentiation. The traditional view of linear hierarchical progression of stem cells

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

from one differentiation stage to the next during their phenotypic determination has been challenged by the recent findings [46–48]. Using an *in vitro* differentiation strategy, Song *et al.* [49] showed that MSC-derived, fully differentiated osteoblasts, adipocytes and chondrocytes can switch their phenotypes to other mesenchymal lineages in response to specific extracellular stimuli. Taken together, it could be concluded that both pre-committed progenitor cells and terminally differentiated cells retain the multi-potency, and that their plasticity can be preserved during differentiation and be required under defined, appropriate microenvironmental circumstances.

Immunomodulatory effects of MSCs

MSCs have been shown to suppress immune reactions both *in vitro* and *in vivo* in a non-MHC restricted manner [50]. These stem cells are considered to be hypoimmunogenic, displaying low expression levels of HLA class I and no expression of costimulatory molecules, such as B7-1 (CD80), B7-2 (CD86) and CD40 [26, 51, 52]. *In vitro*, MSCs are able to suppress T lymphocyte proliferation induced by alloantigens [50, 51, 53, 54], mitogens [50, 55–58], as well as activation of T cells by CD3 and CD28 antibodies [51, 59, 60]. Suppression of T cell proliferation by MSCs has no immunological restriction, similar suppressive effects being observed with cells that were autologous or allogeneic to the responder cells [50, 52, 53, 61]. Another level at which MSCs modulate immune responses is through the induction of regulatory T cells. MSCs have been reported to induce formation of $CD8^+$ regulatory T cells that were responsible for inhibition of allogeneic lymphocyte proliferation [58]. Furthermore, an increase in the population of $CD4^+CD25^+$ regulatory T cells has been demonstrated in mitogen-stimulated peripheral blood mononuclear cell (PBMCs) cultures in the presence of MSCs [60–61]. However, depletion of $CD4^+CD25^+$ regulatory T cells had no effect on the suppression of T cell proliferation by MSCs [59]. Apart from naive and memory T cells [59], MSCs can also inhibit several functions of B cells [62], natural killer cells [63, 64] and monocyte-derived dentritic cells [65, 66]. Although the exact mechanism underlying the immunosuppressive effects of MSCs has not been fully clarified, most studies supported that soluble factors are involved. These factors include transforming growth factor (TGF)-β1 [56, 67], hepatocyte growth factor (HGF) [56, 63], prostaglandin E2 (PGE2) [60, 63] and indoleamine 2,3-dioxygenase (IDO) [68–70]. Additionally, it is well-established that IFN-γ plays an important role in the enhancement of MSCs' suppressive activity [31, 60, 68, 69].

The immunomodulatory capacity of MSCs has also been evaluated *in vivo*. First, intravenous administration of MSCs derived from BM of baboons prolonged the survival of allogeneic skin grafts [53]. Subsequently, murine MSCs have been demonstrated to prevent experimental autoimmune encephalomyelitis (EAE) in mice [71]. In phase I studies, Lazarus *et al.* [72, 73] estimated the feasibility of transplanting autologous or allogeneic MSCs to improve engraftment of HSCs, as well as to reduce graft-versus-host disease (GVHD). Another clinical trial also displayed that third party haplo-identical (mother-derived) MSCs can be safely infused to treat severe acute GVHD that is refractory to conventional immunosuppressive therapy [74]. In contrast, infusion of MSCs had no beneficial effects on collagen-induced arthritis (CIA) as tested in a murine model of rheumatoid arthritis (RA) [75]. Grinnemo *et al.* [76] observed that after transplantation of human MSCs into experimentally induced ischaemic rat myocardium, MSCs induced significant lymphocyte proliferation in PBMC cultures of immunized rats. Moreover, there was prominent infiltration of macrophages in the area of injection in immunocompetent rats. Therefore, though MSCs have been shown to be transplantable across allogeneic barriers, xenogeneic transplant rejection may occur.

Table 1 Phenotypic properties of mesenchymal stem cells

CD locus	Other names	Detection	References
	Stro-1	Positive	[10, 22, 23]
CD105	SH2	Positive	[24]
CD73	SH3/4	Positive	[25]
CD49a	α1 integrin	Positive	[26]
CD49b	α2 integrin	Positive	[26]
CD49c	α3 integrin	Positive	[26]
CD49e	α5 integrin	Positive	[26]
CD49f	α6 integrin	Positive	[26]
CD51	αV integrin	Positive	[26]
CD29	β1 integrin	Positive	[26]
CD61	β3 integrin	Positive	[26]
CD104	β4 integrin	Positive	[26]
CD54	ICAM-1	Positive	[9, 27, 28–30]
CD102	ICAM-2	Positive	[9, 27, 28–30]
CD106	VCAM-1	Positive	[9, 27, 28–30]
CD58	LFA-3	Positive	[9, 27, 28–30]
CD72		Positive	[9, 27, 28–30]
CD166	ALCAM	Positive	[9, 27, 28–30]
	HLA-I	Positive	[31]
	HLA-II	Negative	[31]
CD45		Negative	[27]
CD34		Negative	[27]
CD14		Negative	[27]

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

Aetiology and current treatment of type 1 diabetes

In the year 2000, 150 million people worldwide were found to be affected by diabetes mellitus, and this number is considered to double in 2025 [77]. Type 1 diabetes is characterized by the selective destruction of pancreatic β-cells caused by an autoimmune attack, and it accounts for 5–10% of all causes of diabetes mellitus. Autoimmune destruction of β-cells is due to multiple genetic predispositions and is also related to environmental factors that are still poorly defined [78]. When clinical symptoms are observed the autoimmune process is markedly advanced. It is reported that 60–80% of the β-cell mass have been destroyed at the time of diagnosis [79].

Since 1920s, insulin therapy has changed diabetes from a rapidly fatal disease to a chronic disease associated with significant secondary complications, such as renal failure, cardiovascular disease, retinopathy and neuropathy. It is now well-established that the risk of diabetic complications is dependent on the degree of glycaemic control in diabetic patients. Long-term studies strongly suggest that tight control of blood glucose achieved by conventional or intensive insulin treatment, self blood glucose monitoring, and patient education can significantly prevent the development and retard the progression of chronic complications of this disease [80–82]. While aggressive insulin therapy that maintains glucose levels near the normal range reduces the risk of secondary complications, patients often find such control difficult to achieve and suffer an increased risk of hypoglycaemia [83]. This is caused by the fact that external insulin injection can not mimic the physiological control that pancreatic β cell-derived insulin secretion exerts on the body's glycaemia. By contrast, replacement of a patient's islets of Langerhans either by whole pancreas transplantation or by isolated islet transplantation is the only treatment of type 1 diabetes that achieves an insulin-independent, constant normoglycaemic state and avoids hypoglycaemic episodes [84, 85]. Nonetheless, due to shortage of organs and lifelong immunosuppression this therapy can be offered to a very limited number of patients. What is now required is an essentially infinite supply of a physiologically competent substitute for primary human pancreatic islets, and generation of insulin-producing cells from stem cells represents an attractive alternative [86].

Mesenchymal stem cells in type 1 diabetes therapy

MSCs with potential to differentiate into insulin-producing cells

Among adult stem cells, MSCs appear to have a particular developmental plasticity *ex vivo* that include their ability to adopt a pancreatic endocrine phenotype. It has been demonstrated that MSCs residing in various tissues and organs are able to differentiate into functional insulin-producing cells, such as MSCs from pancreas, bone marrow, adipose tissue, cord blood and cord tissue. This will help to meet the demand of β cells for islet transplantation, and the goal of a permanent cure for type 1 diabetes will be realized.

The mature pancreas has two functional compartments: the exocrine portion (99%), including acinar and duct cells, and the endocrine portion (1%), including the islets of Langerhans. Islets are composed of four cell types that synthesize and secrete distinct peptidic hormones: β-cells (insulin), α-cells (glucagon), δ-cells (somatostatin) and PP-cells (pancreatic polypeptide). It has been described that adult rat and human islets of Langerhans contain nestin-positive progenitor cells, which can be differentiated into insulin-expressing cells *ex vivo* [87]. In another study, Ramiya *et al.* [88] displayed how pluripotent stem cells isolated from the pancreatic ducts of adult pre-diabetic non-obese diabetic (NOD) mice differentiate to form glucose-responsive islets that can reverse insulin-dependent type 1 diabetes after being implanted into diabetic NOD mice. Simultaneously, duct tissue from human pancreas was expanded and directed to differentiate into functional islet tissue *in vitro* [89]. Then, Bonner-Weir *et al.* [90] considered that ductal epithelial cells are likely to be the pancreatic progenitors which can add new β cells by the process of neogenesis. The clonal identification of multi-potent precursor cells from adult mouse pancreas that generate endocrine β-like cells were also performed [91]. Recently, several studies have indicated that MSCs are likely to exist within pancreatic duct and islet. Zhang *et al.* [92] showed that nestin-positive cells isolated from human foetal pancreas possess the characteristics of pancreatic progenitor cells since they have highly proliferative potential and the capability of differentiation into insulin-producing cells *in vitro*. Huang *et al.* [93] further proved that after differentiation the islet-like cell clusters (ICCs) displayed the ability to reverse hyperglycaemia in diabetic mice. Additionally, these nestin-positive pancreatic progenitor cells share many phenotypic markers with MSCs derived from bone marrow [92]. In agreement with these findings, another group [94] successfully isolated pancreatic stem cells from adult human pancreatic duct, these cells not only express nestin and pdx-1 but also exhibit the identical markers of MSCs. Moreover, Seeberger and his colleagues [95] reported the expansion of MSCs from adult human pancreatic ductal epithelium. In addition to expression of the same surface antigens as MSCs from human bone marrow, adipose and umbilical cord blood [11, 96, 97], they demonstrated that pancreatic MSCs could be differentiated into mesodermal cells including osteocytes, adipocytes and chondrocytes. Their preliminary data also suggest that these cells have the potential to derive β-cells. An earlier study has established that fibroblast-like precursor cells derived from adult human islets are generated by epithelial-to-mesenchymal transition (EMT) [98]. However, in a recent paper, researchers verified that EMT does not underlie the appearance of fibroblast-like cells in mouse islet cultures, but that fibroblast-like cells appear to represent MSC-like cells akin to MSCs isolated from bone marrow [99]. More recently, it has been revealed that human islet-derived precursor cells (hIPCs), which do not express the insulin gene, nonetheless exhibit transcriptionally active epigenetic marks. These findings in hIPCs may be an indication of the 'committed state' of hIPCs as endocrine pancreas precursor cells [100].

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

In conclusion, MSCs in human pancreas could serve as a competent candidate for generating insulin-producing cells.

Bone marrow is an important source of easily accessible adult stem cells, and bone marrow transplantation (BMT) is considered to be effective for the treatment of autoimmune type 1 diabetes. However, there is a great debate on the issue of the fate of transplanted bone marrow stem cells. Ianus *et al.* [101] showed that mouse bone marrow-derived cells can differentiate into pancreatic endocrine β cells with glucose-dependent and incretin-enhanced insulin secretion when transplanted into lethally irradiated mice. By using a CRE-LoxP system, the authors also ruled out cell fusion events. Many controversial observations still exist. Hess *et al.* [102] reported that transplantation of c-kit positive mouse bone marrow-derived stem cells initiated endogenous pancreatic regeneration and improved blood glucose level in streptozocin (STZ)-induced diabetic mice *via* enhanced endothelial proliferation by donor cells. In a similar study, Lee *et al.* [103] demonstrated that transplanted MSCs from human bone marrow lowered blood glucose levels in diabetic immunodeficient mice by promoting repair of mouse pancreatic islets. Furthermore, independent studies by Choi *et al.* [104], Lechner *et al.* [105] and Taneera *et al.* [106] showed little evidence for significant transdifferentiation of bone marrow cells (BMCs) into pancreatic β cells, even in pancreatic injury models of mice. Lately, cotransplantation of syngeneic BMCs and syngeneic or allogeneic MSCs into diabetic mice resulted in rapid recovery of blood glucose and serum insulin levels accompanied with efficient tissue regeneration. Researchers suggested that two aspects operate parallelly and synergistically in this model. First, BMCs and MSCs induce the regeneration of recipient derived pancreatic insulin-secreting cells. Second, MSCs inhibit T cell-mediated immune responses against newly formed β-cells. Their work offers a novel potential therapeutic protocol for type 1 diabetes [107]. On the other hand, recent studies illustrated that when cultured *in vitro*, bone marrow derived-cells obtained from mice [108] and rats [109] could be differentiated into insulin-producing cells. Multi-potent adult progenitor cells (MAPCs) or MSCs within bone marrow are intriguing candidates that can give rise to insulin-positive cells. In 2002, Jiang *et al.* [110] proposed the existence of pluripotent MSCs derived from adult marrow. Chen *et al.* [111] and Wu *et al.* [112] isolated MSCs from rat bone marrow, and successfully induced their differentiation into islet-like cells. Moreover, transplantation of these islet-like cells could alleviate the hyperglycaemia in diabetic rats. Subsequently, a group of researchers [113] proved that treatment of rat pancreatic extract can differentiate rat marrow mesenchymal cells into insulin-producing cells *in vitro*. In another study, Moriscot *et al.* [114] indicated that human bone marrow MSCs are able to differentiate into insulin-expressing cells by infection with adenoviruses coding for several transcription factors of the β-cell developmental pathway and coculture with islet tissue or islet-conditioned medium. Recently, two studies [115, 116] have presented evidence that pancreatic duodenal homeobox-1 (PDX-1) gene-modified human bone marrow-derived MSCs can be induced to differentiate into functional insulin-producing cells. In addition, Sun *et al.* [117] demonstrated that bone marrow-derived MSCs from diabetic patients can differentiate into IPCs under appropriate conditions *in vitro*. Their results provide the direct evidence for the feasibility of using patient's own BM-MSCs as a source of IPCs for beta-cell replacement therapy.

MSCs from human bone marrow and adipose tissue represent very similar cell populations with comparable phenotypes [2, 96, 118–119]. Thus, MSCs with the potential to adopt a pancreatic endocrine phenotype could also exist in human adipose tissue. Timper *et al.* [120] isolated human adipose tissue-derived MSCs and expanded them in basic fibroblast growth factor (bFGF) containing culture medium. Proliferating MSCs expressed the stem cell markers nestin, ABCG2, SCF, Thy-1 as well as the pancreatic endocrine transcription factor Isl-1 mRNA. When subjected to defined differentiation medium, a down-regulation of ABCG2 and an up-regulation of transcription factors Isl-1, Ipf-1 and Ngn3 were observed together with induction of the islet genes insulin, glucagon and somatostatin. Consequently, adipose tissue-derived MSCs could be an alternative source of pancreatic β-cells.

Human umbilical cord blood (HUCB) is another source of stem cells with the potential to develop into insulin-producing cells. A few *in vivo* studies give support to this point. In one study [121], transplantation of HUCB cells resulted in the improvement of blood glucose levels and survival rate in type 2 diabetic mice. Furthermore, a regression of glomerular hypertrophy and tubular dilatation, common complications attributed to diabetes, was observed in HUCB-treated mice. In another study [122], transplantation of HUCB cells into type 1 diabetic mice led to a dose-dependent reduction in blood glucose levels and the degree of autoimmune insulitis. A recent report [123] has focused on the *in vivo* capacity of HUCB-derived cells to generate insulin-producing cells. Following transplantation of HUCB cells into NOD/SCID/β_2m^{null} mice, IPCs of human origin were found in recipient pancreatic islets. Double FISH analysis using species-specific probes further indicated that HUCB cells can give rise to insulin-producing cells by fusion-dependent and -independent mechanisms. The number of HUCB cells that transdifferentiated and the rate of such an event are critical aspects. The proportion of HUCB-derived insulin-producing cells per total number of islet cells [123] was less than in the case of BM-derived insulin-producing cells [101]. However, under diabetic conditions, the demand for the neogenesis of insulin-producing cells might increase and the rate of HUCB cell differentiation could become higher in order to compensate for the regeneration of β-cell mass. On the other hand, the stem cell type in HUCB responsible for generation of insulin-producing cells remains unclear. Since MSCs have been identified in the cord blood [124] and HUCB-derived USSC (unrestricted somatic stem cell) share most of the cell markers and properties with MAPCs [125], it should be considered that MSCs may take part in the differentiation of HUCB cells towards a β-cell phenotype. In addition to HUCB, the Wharton's jelly of the human umbilical cord is rich in mesenchymal stem cells (UC-MSCs) that fulfil the criteria for MSCs. Recently, Chao *et al.* [126] successfully differentiated UC-MSCs into mature ICCs, and these ICCs possess insulin-producing ability *in vitro* and *in vivo*. Moreover, they indicated that UC-MSCs seem to be the preferential source of stem cells to convert into IPCs, because of the large potential donor pool, its rapid availability, no risk of discomfort for the donor, and low risk of rejection.

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

MSCs as cellular vehicle for insulin gene therapy

MSCs are a promising target population for cell-based gene therapy against a variety of different diseases [127]. The apparently high self-renewal potential makes them strong candidates for delivering genes and restoring function of organs and tissues. The ability to genetically modify MSCs provides a means for durable expression of therapeutic genes. Following the development of better assays for stem cells and improvements in vector biology, gene transfer efficiencies into MSCs have increased prominently. To assess the capacity of MSCs to produce heterologous proteins, many transgenes were expressed in MSCs *in vitro*. The proteins included coagulation factors VIII [128], IX [129], IL-3 [130], human growth hormone [131], human erythropoietin (hEPO) [132] and so on. As a result, MSCs could act as platforms for recombinant protein production *in vivo* to treat acquired and inherited disorders. As far as type 1 diabetes is concerned, insulin gene therapy using MSCs is an alternative treatment.

Human insulin gene is located on chromosome 11p15.5 [133]. Insulin synthesis and release from islet β-cells is complex and tightly regulated. Glucose affects insulin at all levels, including transcription, translation and release. Mature insulin results from a processing pathway which starts at the rough endoplasmic reticulum and ends at the Golgi apparatus. Translation of insulin mRNA yields preproinsulin, which is sequentially cleaved by endoproteinases PC1 and PC2/PC3 to give pro-insulin first and mature insulin plus C-peptide second. In the secretory granule, six insulin molecules are coordinated by a Zn atom, which is demonstrated under microscopy by dithizone staining. Some researchers have begun to set foot in the field of MSCs-based insulin gene therapy for type 1 diabetes. In one study [134], human bone marrow MSCs transduced with adeno-associated virus (AAV) containing furin-cleavable human preproinsulin gene produce increased amount of insulin and C-peptide compared to the control group. In another study [135], retrovirus vector pLNCX was used to transfer the human insulin gene into human BM-MSCs. The transfected MSCs expressed the insulin gene and stably secreted insulin into culture media. More recently, Xu *et al.* [136] showed that experimental diabetes in mice could be relieved effectively for up to 6 weeks by intrahepatic transplantation of bone marrow-derived murine MSCs infected with the recombinant retrovirus-carrying human insulin gene. However, implantation of engineered cells using diabetic animal models and evaluation of therapeutic effect should be performed with more tests of efficacy and safety of engineered human MSCs as surrogate β-cells in further study. In addition, other researchers [137] are working with a modified herpes I virus as a vector for the human insulin gene. The theoretical advantages of the herpes I virus are: (*i*) the large capacity to accommodate a construct; (*ii*) the ability of the virus to infect primary and second cell lines *in vitro*; (*iii*) although the virus enters the nucleus it does not integrate with the host DNA and is therefore not likely to unmask oncogenes, it functions separate to the host DNA as an episome; (*iv*) most patients have already had contacts with the herpes I virus, which normally resides in a quiescent state in neurological tissue; (*v*). immune reaction against the virus is relatively mild; (*vi*) established antiviral treatment against the herpes virus is available. In consequence, the modified herpes I virus could serve as a new vector for human insulin gene delivery into MSCs. (Table 2)

Induction of IPCs from stem cells by protein transduction technology

New technology, known as protein transduction technology, has been recently developed. A variety of peptides, known as protein transduction domains (PTDs) or cell-penetrating peptides (CPPs), have been characterized for their ability to translocate into live cells. Proteins and peptides can be directly internalized into cells when synthesized as recombinant fusion proteins or covalently cross-linked to PTDs. There are numerous examples of biologically active full-length proteins and peptides that have been delivered to cells both *in vitro* and *in vivo*. The most commonly studied PTDs are homeodomain transcription factors such as Antennapedia (Antp), HSV type 1 protein VP22 and HIV-1 transactivator TAT protein. The mechanism of PTD-mediated protein transduction is mainly *via* endocytosis followed by passage from the vesicle into the cytoplasm [138].

It has been suggested that protein transduction technology is useful for the treatment of diabetes, because this technology facilitates the differentiation of stem cells into insulin-producing cells. First, PDX-1 protein and BETA2/NeuroD protein, two pancreatic endocrine transcription factors, both have a PTD sequence in their structure. Noguchi *et al.* demonstrated that PDX-1 [139] or BETA2/NeuroD [140] protein induced insulin expression in pancreatic ductal progenitor cells. Similarly, Domínguez-Bendala *et al.* [141] showed that TAT-mediated neurogenin 3 (ngn3) protein transduction stimulated pancreatic endocrine differentiation *in vitro*. In another research, Gräslund's group [142] reported that the third helix of the homeodomain of transcription factor Isl-1 internalized into cells. Thus, delivery of exogenous transcription factors (PDX-1, BETA2/NeuroD, ngn3, Isl-1, etc.) by protein transduction technology could be a novel strategy for generating IPCs from stem/progenitor cells without requiring gene transfer technology. We propose MSCs as strong candidate stem cells for this new approach.

Minimum requirements for replacement β-cells

As mentioned above, insulin-producing cells generated either by transdifferentiation of MSCs or by delivery of insulin gene into MSCs are able to act as replacement β-cells for the transplantation therapy of type 1 diabetes. These MSCs-derived IPCs may solve the donor shortage issue for islet cell transplantation and provide a cure for this disease. Nevertheless, any substitute for primary islets of Langerhans will require some minimum essential properties. The basic requirements for surrogate β-cells are described as follows [143].

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

Table 2 Cell-based treatment protocols in experimental diabetes models

Study	Cell source for transplantation	Therapeutic effects in diabetic animal models
Ramiya *et al.* [88]	Islets generated from mouse pancreatic stem cells	Insulin-independent, blood glucose levels return to near-normal levels
Huang *et al.* [93]	ICCs derived from NIPs residing in human foetal pancreas	Reverse hyperglycaemia
Hess *et al.* [102]	mouse c-kit$^+$ BM-derived cells	Reduce hyperglycaemia, accompanied by a proliferation of recipient pancreatic cells
Lee *et al.* [103]	Human BM-MSCs	Lower blood glucose levels, promote repair of pancreatic islets and renal glomeruli
Urbán *et al.* [107]	Mouse syngeneic BMCs and syngeneic or allogeneic MSCs	Rapid recovery of blood glucose and serum insulin levels accompanied with efficient pancreatic tissue regeneration
Tang *et al.* [108]	IPCs obtained from mouse bone marrow	Reverse hyperglycaemia, improve metabolic profiles
Oh *et al.* [109]	IPCs transdifferentiated from rat BMCs	Lower blood glucose levels, maintain comparatively normal glucose levels
Chen *et al.* [111] and Wu *et al.* [112]	Islet-like cells differentiated from rat marrow MSCs	Lower glucose levels
Li *et al.* [115] and Karnieli *et al.* [116]	IPCs generated from PDX-1 gene-modified human BM-MSCs	Reduction of hyperglycaemia
Ende *et al.* [121, 122]	HUCB mononuclear cells	Improve blood glucose levels, survival rate, glomerular hypertrophy, tubular dilatation and insulitis
Chao *et al.* [126]	ICCs derived from human UC-MSCs	Alleviate hyperglycaemia and glucose intolerance significantly
Xu *et al.* [136]	Mouse BM-MSCs infected with recombinant retrovirus-carrying human insulin gene	Improvement of body weight, blood glucose and serum insulin levels

Abbreviations: ICCs, islet-like cell clusters; NIPs, nestin-positive islet-derived progenitor cells; MSCs, mesenchymal stem cells; BMCs, bone marrow cells; IPCs, insulin-producing cells; PDX-1, pancreatic duodenal homeobox-1; HUCB, human umbilical cord blood; UC, umbilical cord.

First, to make any significant therapeutic impact vast numbers of replacement β-cells will be required. Current transplantation protocols use up to 1×10^6 primary human islets per recipient, equivalent to approximately $2–4 \times 10^9$ β-cells. As a result, the ability of MSCs to replicate and to differentiate toward pancreatic endocrine phenotype makes them attractive candidates for producing replacement β-cells. Secondly, the replacement cells must have the ability to synthesize, store and release insulin in response to changes in the ambient glycaemia. Understanding β-cell function at the molecular level will likely facilitate to manufacture physiologically competent insulin-producing cells from MSCs. Thirdly, the proliferative capacity of the replacement cells must be tightly controlled to avoid the development of hyperinsulinemic hypoglycaemia as the β-cell mass expands *in vivo*. Excluding proliferative cells from the transplant material will help to overcome this problem. In the case of insulin gene transferred MSCs, the possibility of tumour formation has to be considered. Finally, the transplanted cells must avoid destruction by the recipient's immune system. Two major mechanisms are involved in the immune attack against replacement β-cells, one is transplant rejection and the other is recurrence of autoimmunity. In addition to appropriate immunosuppressive treatment, autologous transplantation of MSCs-derived IPCs will circumvent the immune rejection dilemma. On the other hand, Burt *et al.* [144] indicated that HSC transplantation may re-introduce tolerance to islet cells in type 1 diabetics. Thus, cotransplantation of MSCs-derived IPCs and HSC from the same donor (autologous or allogeneic) could evade the risks of recurring autoimmunity. Furthermore, the pathways of β-cell differentiation *in vitro* may differ significantly from those *in vivo* [145], and it is also possible that current *in vitro* differentiation protocols do not generate β-cells, but cells that have some phenotypic and functional similarity to authentic β-cells. Since IPCs generated from MSCs are developmentally and immunologically distinct from primary β-cells, they may escape the recipient's autoimmune assault.

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

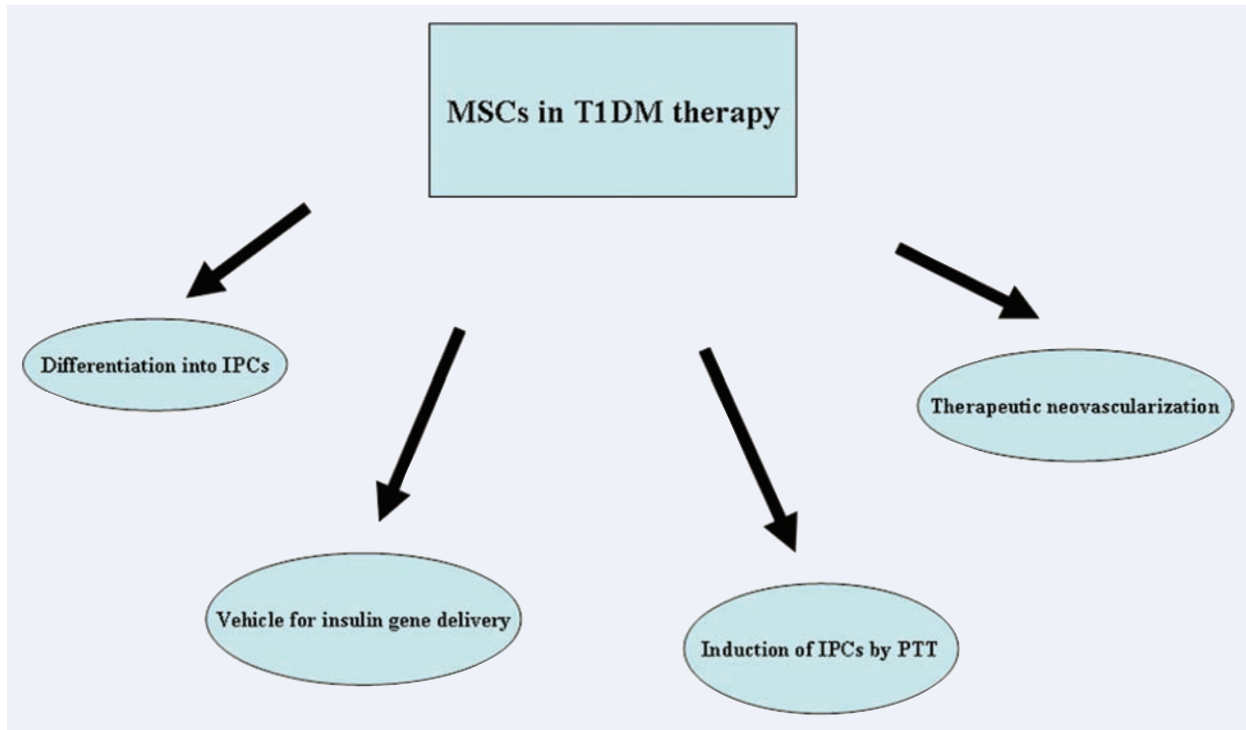

Fig. 1 Mesenchymal stem cells in the treatment of type 1 diabetes. The clinical potentials of mesenchymal stem cells (MSCs) in type 1 diabetes therapy are illustrated. Abbreviations: T1DM, type 1 diabetes mellitus; IPCs, insulin-producing cells; PTT, protein transduction technology.

MSCs for therapeutic neovascularization in type 1 diabetes

It has been demonstrated that endothelial progenitor cells (EPCs) are responsible for postnatal vasculogenesis in physiological and pathological neovascularization [146]. Ischaemia and tissue injury are potent stimuli for neovascularization. We have reported that autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells (M-PBMSCs) improves critical limb ischaemia (CLI) in diabetes [147]. Further investigation indicated that local transplantation of M-PBMNCs achieved therapeutic neovascularization *via* supply of abundant angioblasts (EPCs) and angiogenic factors [148]. However, EPCs in type 1 diabetic patients are dysfunctional, and their dysfunction may contribute to the pathogenesis of vascular complications in type 1 diabetes [149]. Our group also proved that M-PBMNCs from diabetic patients augment neovascularization in ischaemic limbs but with impaired capability [150]. Clinically, allogenic transplantation of normal M-PBMNCs may be more effective, but such transplanted cells are likely to encounter immune rejection. Therefore, autologous transplantation of diabetic M-PBMNCs is still a good-albeit compromised and not perfect-approach for CLI in diabetes. On the other hand, the pancreatic islets of Langerhans are well vascularized throughout life. Signals from the endothelium may play a role in postnatal islet cell proliferation and neogenesis. Mathews *et al.* [151] provided evidence that transplanted bone marrow-derived EPCs are recruited to the pancreas in response to STZ-induced islet injury and that EPC-mediated neovascularization of the pancreas could in principle facilitate the recovery of non-terminally injured β-cells. Neovascularization of the pancreas is likely to be an adaptive response to β-cell injury in type 1 diabetes.

MSCs have been shown to promote angiogenesis both *in vivo* [152] and *in vitro* [153]. Yet the underlying mechanism of this action remains elusive. Oswald *et al.* [154] showed the differentiation of expanded adult human BM-MSCs into cells with phenotypic and functional features of endothelial cells. However, Kinnaird *et al.* [155] demonstrated that MSCs secrete a wide array of arteriogenic cytokines and they contribute to collateral re-modelling in ischaemic limb *via* paracrine mechanisms. Recently, another two studies suggest that BM-MSCs enhance angiogenesis in wounds of diabetic mice through paracrine effects [156, 157]. An increasing bulk of evidence supports that release of angiogenic factors rather than endothelial transdifferentiation is accountable for MSCs-mediated strengthened angiogenesis. MSCs express genes encoding a broad spectrum of arteriogenic/angiogenic cytokines including vascular endothelial growth factor (VEGF),

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

fibroblast growth factor (FGF), Angiopoetin-1 (Ang-1), matrix metalloproteinase (MMPs), transforming growth factor-β (TGF-β) and so on [158]. For example, MSCs have been reported to generate sufficient quantities of VEGF to enhance survival and differentiation of endothelial cells [159]. In addition to stimulating the *in situ* proliferation of endothelial cells, VEGF has also been shown to promote neovascularization by mobilizing bone marrow-derived EPCs [160]. Thus, transplanted MSCs may initiate angiogenesis in diabetic ischaemic limbs or injured pancreas by producing angiogenic factors. Neovascularization will become a new direction for the application of MCSs in type 1 diabetes therapy. (Fig. 1)

Concluding remarks

In the past few years, there has been dramatic progress in our understanding of the biology of MSCs. Data in the literature concerning cell expansion, phenotypic characterization of MSCs as well as their multi-potency and immunomodulatory properties, are vast and sometimes contradictory. Although the precise identity of MSCs remains a challenge, this has not hampered the beginning of considerable investigation aiming at their potential clinical applications. It is generally accepted that type 1 diabetes is now curable by islet transplantation therapy, and MSCs offer a starting material for generating the large numbers of surrogate β-cells required. The most difficult and yet unsolved issue are how to manufacture physiologically functional insulin-producing cells from MSCs. Moreover, the angiogenic effect of MSCs could also be utilized for diabetes treatment. In conclusion, the prospect of MSCs in treating type 1 diabetes seems to be very promising. However, we should realize that much work needs to be done before pushing the MSC-based therapy from bench to bedside.

Acknowledgement

This study was supported by National Natural Science Foundation of China (30570357 and 30600238), 863 project of the Ministry of Science and Technology of China (2006AA02A110), Tianjin Municipal Science and Technology Commission, China (06YFSZSF01300 and 05YFJZJC01500) and Jerome Lejuene Foundation, France.

References

1. **Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV.** Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol.* 1966; 16: 381–90.
2. **De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhaim P, Chen I, Fraser J, Hedrick MH.** Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs.* 2003; 174: 101–9.
3. **Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM.** Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood.* 2001; 98: 2396–402.
4. **Noort WA, Kruysselbrink AB, in't Anker PS, Kruger M, van Bezooijen RL, de Paus RA, Heemskerk MH, Löwik CW, Falkenburg JH, Willemze R, Fibbe WE.** Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34+ cells in NOD/SCID mice. *Exp Hematol.* 2002; 30: 870–8.
5. **Erices A, Conget P, Minguell JJ.** Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol.* 2000; 109: 235–42.
6. **In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH.** Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells.* 2004; 22: 1338–45.
7. **Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC, Chen CC.** Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells.* 2004; 22: 1330–7.
8. **Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N, Kadiyala S.** Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. *J Orthop Res.* 1998; 16: 155–62.
9. **Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL.** Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (mesenchymal stem cells) and stromal cells. *J Cell Physiol.* 1998; 176: 57–66.
10. **Simmons PJ, Torok-Storb B.** Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood.* 1991; 78: 55–62.
11. **Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM.** Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. *Blood.* 2001; 98: 2615–25.
12. **Fan CG, Tang FW, Zhang QJ, Lu SH, Liu HY, Zhao ZM, Liu B, Han ZB, Han ZC.** Characterization and neural differentiation of fetal lung mesenchymal stem cells. *Cell Transplant.* 2005; 14: 311–21.
13. **Lu LL, Liu YJ, Yang SG, Zhao QJ, Wang X, Gong W, Han ZB, Xu ZS, Lu YX, Liu D, Chen ZZ, Han ZC.** Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. *Haematologica.* 2006; 91: 1017–26.
14. **Stenderup K, Justesen J, Clausen C, Kassem M.** Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone.* 2003; 33: 919–26.
15. **Kassem M, Ankersen L, Eriksen EF, Clark BF, Rattan SI.** Demonstration of cellular aging and senescence in serially passaged long-term cultures of human trabecular osteoblasts. *Osteoporos Int.* 1997; 7: 514–24.
16. **Rattan SIS.** Aging outside the body: usefulness of the Hayflick system. In: Kaul SC, Wadwha R, editors. Aging of cells in and outside the body. London: Kluwer Academic Publishers; 2003. pp. 1–8.
17. **Simonsen JL, Rosada C, Serakinci N, Justesen J, Stenderup K, Rattan SI, Jensen TG, Kassem M.** Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. *Nat Biotechnol.* 2002; 20: 592–6.

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

18. **Zimmermann S, Voss M, Kaiser S, Kapp U, Waller CF, Martens UM.** Lack of telomerase activity in human mesenchymal stem cells. *Leukemia.* 2003; 17: 1146–9.
19. **Shi S, Gronthos S, Chen S, Reddi A, Counter CM, Robey PG, Wang CY.** Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. *Nat Biotechnol.* 2002; 20: 587–91.
20. **Kobune M, Kawano Y, Ito Y, Chiba H, Nakamura K, Tsuda H, Sasaki K, Dehari H, Uchida H, Honmou O, Takahashi S, Bizen A, Takimoto R, Matsunaga T, Kato J, Kato K, Houkin K, Niitsu Y, Hamada H.** Telomerized human multipotent mesenchymal cells can differentiate into haematopoietic and cobblestone area-supporting cells. *Exp Hematol.* 2003; 31: 715–22.
21. **Serakinci N, Guldberg P, Burns JS, Abdallah B, Schr¯dder H, Jensen T, Kassem M.** Adult human mesenchymal stem cell as a target for neoplastic transformation. *Oncogene.* 2004; 23: 5095-98.
22. **Simmons PJ, Gronthos S, Zannettino A, Ohta S, Graves SE.** Isolation, characterization and functional activity of human marrow stromal progenitors in hemopoiesis. *Prog Clin Biol Res.* 1994; 389: 271–80.
23. **Simmons PJ, Torok-Storb B.** CD34 expression by stromal precursors in normal human adult bone marrow. *Blood.* 1991; 78: 2848–53.
24. **Barry FP, Boynton RE, Haynesworth S, Murphy JM, Zaia J.** The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). *Biochem Biophys Res Commun.* 1999; 265: 134–9.
25. **Barry F, Boynton R, Murphy M, Haynesworth S, Zaia J.** The SH-3 and SH-4 antibodies recognize distinct epitopes on CD73 from human mesenchymal stem cells. *Biochem Biophys Res Commun.* 2001; 289: 519–24.
26. **Majumdar MK, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR, Mosca JD.** Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci.* 2003; 10: 228–41.
27. **Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR.** Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999; 284: 143–7.
28. **Conget PA, Minguell JJ.** Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol.* 1999; 181: 67–73.
29. **Deans RJ, Moseley AB.** Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol.* 2000; 28: 875–84.
30. **De Ugarte DA, Alfonso Z, Zuk PA, Elbarbary A, Zhu M, Ashjian P, Benhaim P, Hedrick MH, Fraser JK.** Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. *Immunol Lett.* 2003; 89: 267–70.
31. **Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O.** HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol.* 2003; 31: 890–6.
32. **Bruder SP, Jaiswal N, Haynesworth SE.** Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem.* 1997; 64: 278–94.
33. **Kadiyala S, Young RG, Thiede MA, Bruder SP.** Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential *in vivo* and *in vitro*. *Cell Transplant.* 1997; 6: 125–34.
34. **Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI.** A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res.* 1999; 14: 700–9.
35. **Prockop DJ.** Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science.* 1997; 276: 71–4.
36. **Young RG, Butler DL, Weber W, Caplan AI, Gordon SL, Fink DJ.** Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. *J Orthop Res.* 1998; 16: 406–13.
37. **Awad HA, Butler DL, Boivin GP, Smith FN, Malaviya P, Huibregtse B, Caplan AI.** Autologous mesenchymal stem cell-mediated repair of tendon. *Tissue Eng.* 1999; 5: 267–77.
38. **Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F.** Muscle regeneration by bone marrow-derived myogenic progenitors. *Science.* 1998; 279: 1528–30.
39. **Galmiche MC, Koteliansky VE, Brière J, Hervé P, Charbord P.** Stromal cells from human long-term marrow cultures are mesenchymal cells that differentiate following a vascular smooth muscle differentiation pathway. *Blood.* 1993; 82: 66–76.
40. **Woodbury D, Schwarz EJ, Prockop DJ, Black IB.** Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res.* 2000; 61: 364–70.
41. **Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP.** Bone marrow as a potential source of hepatic oval cells. *Science.* 1999; 284: 1168–70.
42. **Muraglia A, Cancedda R, Quarto R.** Clonal mesenchymal progenitors from human bone marrow differentiate *in vitro* according to a hierarchical model. *J Cell Sci.* 2000; 113: 1161–6.
43. **Baksh D, Song L, Tuan RS.** Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *J Cell Mol Med.* 2004; 8: 301–16.
44. **Doi M, Nagano A, Nakamura Y.** Molecular cloning and characterization of a novel gene, EMILIN-5, and its possible involvement in skeletal development. *Biochem Biophys Res Commun.* 2004; 313: 888–93.
45. **Qi H, Aguiar DJ, Williams SM, La Pean A, Pan W, Verfaillie CM.** Identification of genes responsible for osteoblast differentiation from human mesodermal progenitor cells. *Proc Natl Acad Sci USA.* 2003; 100: 3305–10.
46. **LaBarge MA, Blau HM.** Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell.* 2002; 111: 589–601.
47. **Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC.** Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp Neurol.* 2002; 174: 11–20.
48. **Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM.** Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol.* 2002; 30: 896–904.
49. **Song L, Tuan RS.** Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. *FASEB J.* 2004; 18: 980–2.
50. **Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringdén O.** Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol.* 2003; 57: 11–20.
51. **Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC.** Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation.* 2003; 75: 389–97.

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

52. **Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, Deans RJ, McIntosh KR.** T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J Biomed Sci.* 2005; 12: 47–57.
53. **Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R.** Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. *Exp Hematol.* 2002; 30: 42–8.
54. **Potian JA, Aviv H, Ponzio NM, Harrison JS, Rameshwar P.** Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. *J Immunol.* 2003; 171: 3426–34.
55. **Maitra B, Szekely E, Gjini K, Laughlin MJ, Dennis J, Haynesworth SE, Koc ON.** Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. *Bone Marrow Transplant.* 2004; 33: 597–604.
56. **Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM.** Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood.* 2002; 99: 3838–43.
57. **Le Blanc K, Rasmusson I, Götherström C, Seidel C, Sundberg B, Sundin M, Rosendahl K, Tammik C, Ringdén O.** Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes. *Scand J Immunol.* 2004; 60: 307–15.
58. **Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, NoÎl D, Jorgensen C.** Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood.* 2003; 102: 3837–44.
59. **Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F.** Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood.* 2003; 101: 3722–9.
60. **Aggarwal S, Pittenger MF.** Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood.* 2005; 105: 1815–22.
61. **Maccario R, Podestà M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, Ibatici A, Piaggio G, Pozzi S, Frassoni F, Locatelli F.** Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. *Haematologica.* 2005; 90: 516–25.
62. **Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A.** Human mesenchymal stem cells modulate B-cell functions. *Blood.* 2006; 107: 367–72.
63. **Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M.** Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells.* 2006; 24: 74–85.
64. **Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L.** Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood.* 2006; 107: 1484–90.
65. **Zhang W, Ge W, Li C, You S, Liao L, Han Q, Deng W, Zhao RC.** Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. *Stem Cells Dev.* 2004; 13: 263–71.
66. **Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N.** Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood.* 2005; 105: 4120–6.
67. **Angoulvant D, Clerc A, Benchalal S, Galambrun C, Farre A, Bertrand Y, Eljaafari A.** Human mesenchymal stem cells suppress induction of cytotoxic response to alloantigens. *Biorheology.* 2004; 41: 469–76.
68. **Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, Romagnani P, Maggi E, Romagnani S, Annunziato F.** Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells.* 2006; 24: 386–98.
69. **Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D.** Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase mediated tryptophan degradation. *Blood.* 2004; 103: 4619–21.
70. **Plumas J, Chaperot L, Richard MJ, Molens JP, Bensa JC, Favrot MC.** Mesenchymal stem cells induce apoptosis of activated T cells. *Leukemia.* 2005; 19: 1597–604.
71. **Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A.** Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T cell anergy. *Blood.* 2005; 106: 1755–61.
72. **Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI.** *Ex vivo* expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. *Bone Marrow Transplant.* 1995; 16: 557–64.
73. **Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR, Moseley AB, Bacigalupo A.** Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant.* 2005; 11: 389–98.
74. **Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O.** Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet.* 2004; 363: 1439–41.
75. **Djouad F, Fritz V, Apparailly F, Louis-Plence P, Bony C, Sany J, Jorgensen C, Noël D.** Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis. *Arthritis Rheum.* 2005; 52: 1595–603.
76. **Grinnemo KH, Månsson A, Dellgren G, Klingberg D, Wardell E, Drvota V, Tammik C, Holgersson J, Ringdén O, Sylvén C, Le Blanc K.** Xenoreactivity and engraftment of human mesenchymal stem cells transplanted into infracted rat myocardium. *J Thorac Cardiovasc Surg.* 2004; 127: 1293–300.
77. **Zimmet P, Alberti KG, Shaw J.** Global and societal implications of the diabetes epidemic. *Nature.* 2001; 414: 782–7.
78. **American Diabetes Association.** Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2004; 27: S5–10.
79. **Notkins AL, Lernmark A.** Autoimmune type 1 diabetes: resolved and unresolved issues. *J Clin Invest.* 2001; 108: 1247–52.
80. **The Diabetes Control and Complications Trial Research Group.** The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med.* 1993; 329: 977–86.

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

81. **Wang PH, Lau J, Chalmers TC.** Meta-analysis of effects of intensive blood-glucose control on late complications of type-1 diabetes. *Lancet.* 1993; 341: 1306–9.
82. **Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group.** Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA.* 2003; 290: 2159–67.
83. **Steffes MW, Sibley S, Jackson M, Thomas W.** Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care.* 2003; 26: 832–6.
84. **Sutherland DE, Gores PF, Farney AC, Wahoff DC, Matas AJ, Dunn DL, Gruessner RW, Najarian JS.** Evolution of kidney, pancreas, and islet transplantation for patients with diabetes at the University of Minnesota. *Am J Surg.* 1993; 166: 456–91.
85. **Bretzel RG, Browatzki CC, Schultz A, Brandhorst H, Klitscher D, Bollen CC, Raptis G, Friemann S, Ernst W, Rau WS, Hering BJ.** Clinical islet transplantation in diabetes mellitus-report of the Islet Transplant Registry and the Giessen Center experience. *Diab Stoffw.* 1993; 2: 378-390.
86. **Scharfmann R.** Alternative sources of beta cells for cell therapy of diabetes. *Eur J Clin Invest.* 2003; 33: 595–600.
87. **Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, M¸ller B, Vallejo M, Thomas MK, Habener JF.** Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate *ex vivo* into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes.* 2001; 50: 521–33.
88. **Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG.** Reversal of insulin-dependent diabetes using islets generated *in vitro* from pancreatic stem cells. *Nat Med.* 2000; 6: 278–82.
89. **Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, O'Neil JJ.** *In vitro* cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci USA.* 2000; 97: 7999–8004.
90. **Bonner-Weir S, Sharma A.** Are there pancreatic progenitor cells from which new islets form after birth. *Nat Clin Pract Endocrinol Metab.* 2006; 2: 240–1.
91. **Seaberg RM, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, Wheeler MB, Korbutt G, van der Kooy D.** Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol.* 2004; 22: 1115–24.
92. **Zhang L, Hong TP, Hu J, Liu YN, Wu YH, Li LS.** Nestin-positive progenitor cells isolated from human fetal pancreas have phenotypic markers identical to mesenchymal stem cells. *World J Gastroenterol.* 2005; 11: 2906–11.
93. **Huang H, Tang X.** Phenotypic determination and characterization of nestin-positive precursors derived from human fetal pancreas. *Lab Invest.* 2003; 83: 539–47.
94. **Lin HT, Chiou SH, Kao CL, Shyr YM, Hsu CJ, Tarng YW, Ho LL, Kwok CF, Ku HH.** Characterization of pancreatic stem cells derived from adult human pancreas ducts by fluorescence activated cell sorting. *World J Gastroenterol.* 2006; 12: 4529–35.
95. **Seeberger KL, Dufour JM, Shapiro AM, Lakey JR, Rajotte RV, Korbutt GS.** Expansion of mesenchymal stem cells from human pancreatic ductal epithelium. *Lab Invest.* 2006; 86: 141–53.
96. **Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH.** Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002; 13: 4279–95.
97. **Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH.** Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood.* 2004; 103: 1669–75.
98. **Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM.** Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science.* 2004; 306: 2261–4.
99. **Chase LG, Ulloa-Montoya F, Kidder BL, Verfaillie CM.** Islet-derived fibroblast-like cells are not derived via epithelial-mesenchymal transition from Pdx-1 or insulin-positive cells. *Diabetes.* 2007; 56: 3–7.
100. **Mutskov V, Raaka BM, Felsenfeld G, Gershengorn MC.** The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. *Stem Cells.* 2007; 25: 3223–33.
101. **Ianus A, Holz GG, Theise ND, Hussain MA.** *In vivo* derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest.* 2003; 111: 843–50.
102. **Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen S, Gray DA, Bhatia M.** Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol.* 2003; 21: 763–70.
103. **Lee RH, Seo MJ, Reger RL, Spees JL, Pulin AA, Olson SD, Prockop DJ.** Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. *Proc Natl Acad Sci USA.* 2006; 103: 17438–43.
104. **Choi JB, Uchino H, Azuma K, Iwashita N, Tanaka Y, Mochizuki H, Migita M, Shimada T, Kawamori R, Watada H.** Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. *Diabetologia.* 2003; 46: 1366–74.
105. **Lechner A, Yang YG, Blacken RA, Wang L, Nolan AL, Habener JF.** No evidence for significant transdifferentiation of bone marrow into pancreatic beta-cells *in vivo*. *Diabetes.* 2004; 53: 616–23.
106. **Taneera J, Rosengren A, Renstrom E, Nygren JM, Serup P, Rorsman P, Jacobsen SE.** Failure of transplanted bone marrow cells to adopt a pancreatic beta-cell fate. *Diabetes.* 2006; 55: 290–6.
107. **Urbán VS, Kiss J, Kovács J, Gócza E, Vas V, Monostori E, Uher F.** Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. *Stem Cells.* 2008; 26: 244–53.
108. **Tang DQ, Cao LZ, Burkhardt BR, Xia CQ, Litherland SA, Atkinson MA, Yang LJ.** *In vivo* and *in vitro* characterization of insulin-producing cells obtained from murine bone marrow. *Diabetes.* 2004; 53: 1721–32.
109. **Oh SH, Muzzonigro TM, Bae SH, LaPlante JM, Hatch HM, Petersen BE.** Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. *Lab Invest.* 2004; 84: 607–17.
110. **Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM.** Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature.* 2002; 418: 41–9.
111. **Chen LB, Jiang XB, Yang L.** Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells. *World J Gastroenterol.* 2004; 10: 3016–20.
112. **Wu XH, Liu CP, Xu KF, Mao XD, Zhu J, Jiang JJ, Cui D, Zhang M, Xu Y, Liu C.** Reversal of hyperglycemia in diabetic rats by portal vein transplantation of islet-like cells generated from bone marrow

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

mesenchymal stem cells. *World J Gastroenterol.* 2007; 13: 3342–9.
113. **Choi KS, Shin JS, Lee JJ, Kim YS, Kim SB, Kim CW.** *In vitro* trans-differentiation of rat mesenchymal cells into insulin-producing cells by rat pancreatic extract. *Biochem Biophys Res Commun.* 2005; 330: 1299–305.
114. **Moriscot C, De Fraipont F, Richard MJ, Marchand M, Savatier P, Bosco D, Favrot M, Benhamou PY.** Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation *in vitro*. *Stem Cells.* 2005; 23: 594–603.
115. **Li Y, Zhang R, Qiao H, Zhang H, Wang Y, Yuan H, Liu Q, Liu D, Chen L, Pei X.** Generation of insulin-producing cells from PDX-1 gene-modified human mesenchymal stem cells. *J Cell Physiol.* 2007; 211: 36–44.
116. **Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S.** Generation of Insulin-producing Cells From Human Bone Marrow Mesenchymal Stem Cells By Genetic Manipulation. *Stem Cells.* 2007; 25: 2837–44.
117. **Sun Y, Chen L, Hou XG, Hou WK, Dong JJ, Sun L, Tang KX, Wang B, Song J, Li H, Wang KX.** Differentiation of bone marrow-derived mesenchymal stem cells from diabetic patients into insulin-producing cells *in vitro*. *Chin Med J.* 2007; 120: 771–6.
118. **Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, Bae YC, Jung JS.** Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. *Cell Physiol Biochem.* 2004; 14: 311–24.
119. **Dicker A, Le Blanc K, Aström G, van Harmelen V, Götherström C, Blomqvist L, Arner P, Rydén M.** Functional studies of mesenchymal stem cells derived from adult human adipose tissue. *Exp Cell Res.* 2005; 308: 283–90.
120. **Timper K, Seboek D, Eberhardt M, Linscheid P, Christ-Crain M, Keller U, M¸ller B, Zulewski H.** Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun.* 2006; 341: 1135–40.
121. **Ende N, Chen R, Reddi AS.** Transplantation of human umbilical cord blood cells improves glycemia and glomerular hypertrophy in type 2 diabetic mice. *Biochem Biophys Res Commun.* 2004; 321: 168–71.
122. **Ende N, Chen R, Reddi AS.** Effect of human umbilical cord blood cells on glycemia and insulitis in type 1 diabetic mice. *Biochem Biophys Res Commun.* 2004; 325: 665–9.
123. **Yoshida S, Ishikawa F, Kawano N, Shimoda K, Nagafuchi S, Shimoda S, Yasukawa M, Kanemaru T, Ishibashi H, Shultz LD, Harada M.** Human cord blood-derived cells generate insulin-producing cells *in vivo*. *Stem Cells.* 2005; 23: 1409–16.
124. **Bieback K, Kern S, Klüter H, Eichler H.** Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. *Stem Cells.* 2004; 22: 625–34.
125. **Koblas T, Harman SM, Saudek F.** The application of umbilical cord blood cells in the treatment of diabetes mellitus. *Rev Diabet Stud.* 2005; 2: 228–34.
126. **Chao KC, Chao KF, Fu YS, Liu SH.** Islet-Like Clusters Derived from Mesenchymal Stem Cells in Wharton's Jelly of the Human Umbilical Cord for Transplantation to Control Type 1 Diabetes. *PLoS ONE.* 2008; 3: e1451.
127. **Hamada H, Kobune M, Nakamura K, Kawano Y, Kato K, Honmou O, Houkin K, Matsunaga T, Niitsu Y.** Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy. *Cancer Sci.* 2005; 96: 149–56.
128. **Chuah MK, Van Damme A, Zwinnen H, Goovaerts I, Vanslembrouck V, Collen D, Vandendriessche T.** Long-term persistence of human bone marrow stromal cells transduced with factor VIII-retroviral vectors and transient production of therapeutic levels of human factor VIII in nonmyeloablated immunodeficient mice. *Hum Gene Ther.* 2000; 11: 729–38.
129. **Cherington V, Chiang GG, McGrath CA, Gaffney A, Galanopoulos T, Merrill W, Bizinkauskas CB, Hansen M, Sobolewski J, Levine PH, Greenberger JS, Hurwitz DR.** Retroviral vector-modified bone marrow stromal cells secrete biologically active factor IX *in vitro* and transiently deliver therapeutic levels of human factor IX to the plasma of dogs after reinfusion. *Hum Gene Ther.* 1998; 9: 1397–407.
130. **Lee K, Majumdar MK, Buyaner D, Hendricks JK, Pittenger MF, Mosca JD.** Human mesenchymal stem cells maintain transgene expression during expansion and differentiation. *Mol Ther.* 2001; 3: 857–66.
131. **Hurwitz DR, Kirchgesser M, Merrill W, Galanopoulos T, McGrath CA, Emami S, Hansen M, Cherington V, Appel JM, Bizinkauskas CB, Brackmann HH, Levine PH, Greenberger JS.** Systemic delivery of human growth hormone or human factor IX in dogs by reintroduced genetically modified autologous bone marrow stromal cells. *Hum Gene Ther.* 1997; 8: 137–56.
132. **Bartholomew A, Patil S, Mackay A, Nelson M, Buyaner D, Hardy W, Mosca J, Sturgeon C, Siatskas M, Mahmud N, Ferrer K, Deans R, Moseley A, Hoffman R, Devine SM.** Baboon mesenchymal stem cells can be genetically modified to secrete human erythropoietin *in vivo*. *Hum Gene Ther.* 2001; 12: 1527–41.
133. **Ohneda K, Ee H, German M.** Regulation of insulin gene transcription. *Semin Cell Dev Biol.* 2000; 11: 227–33.
134. **Kim JH, Park SN, Suh H.** Generation of insulin-producing human mesenchymal stem cells using recombinant adeno-associated virus. *Yonsei Med J.* 2007; 48: 109–19.
135. **Lu Y, Wang Z, Zhu M.** Human bone marrow mesenchymal stem cells transfected with human insulin genes can secrete insulin stably. *Ann Clin Lab Sci.* 2006; 36: 127–36.
136. **Xu J, Lu Y, Ding F, Zhan X, Zhu M, Wang Z.** Reversal of diabetes in mice by intrahepatic injection of bone-derived GFP-murine mesenchymal stem cells infected with the recombinant retrovirus-carrying human insulin gene. *World J Surg.* 2007; 31: 1872–82.
137. **Calne R.** Cell transplantation for diabetes. *Philos Trans R Soc Lond B Biol Sci.* 2005; 360: 1769–74.
138. **Noguchi H, Matsumoto S.** Protein transduction technology offers a novel therapeutic approach for diabetes. *J Hepatobiliary Pancreat Surg.* 2006; 13: 306–13.
139. **Noguchi H, Kaneto H, Weir GC, Bonner-Weir S.** PDX-1 protein containing its own antennapedia-like protein transduction domain can transduce pancreatic duct and islet cells. *Diabetes.* 2003; 52: 1732–7.
140. **Noguchi H, Bonner-Weir S, Wei FY, Matsushita M, Matsumoto S.** BETA2/NeuroD protein can be transduced into cells due to an arginine- and lysine-rich sequence. *Diabetes.* 2005; 54: 2859–66.
141. **Domínguez-Bendala J, Klein D, Ribeiro M, Ricordi C, Inverardi L, Pastori R, Edlund H.** TAT-mediated neurogenin 3 protein transduction stimulates pancreatic endocrine differentiation *in vitro*. *Diabetes.* 2005; 54: 720–6.
142. **Kilk K, Magzoub M, Pooga M, Eriksson LE, Langel U, Gräslund A.** Cellular internalization of a cargo complex with a novel

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

peptide derived from the third helix of the islet-1 homeodomain. Comparison with the penetratin peptide. *Bioconjug Chem.* 2001; 12: 911–6.
143. **Burns CJ, Persaud SJ, Jones PM.** Stem cell therapy for diabetes: do we need to make beta cells? *J Endocrinol.* 2004; 183: 437–43.
144. **Burt RK, Oyama Y, Traynor A, Kenyon NS.** Hematopoietic stem cell therapy for type 1 diabetes: induction of tolerance and islet cell neogenesis. *Autoimmun Rev.* 2002; 1: 133–8.
145. **Houard N, Rousseau GG, Lemaigre FP.** HNF-6-independent differentiation of mouse embryonic stem cells into insulin-producing cells. *Diabetologia.* 2003; 46: 378–85.
146. **Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM.** Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res.* 1999; 85: 221–8.
147. **Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC.** Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. *Diabetes Care.* 2005; 28: 2155–60.
148. **Zhou B, Liu PX, Lan HF, Fang ZH, Han ZB, Ren H, Poon MC, Han ZC.** Enhancement of neovascularization with mobilized blood cells transplantaion: Supply of angioblasts and angiogenic cytokines. *J Cell Biochem.* 2007; 102: 183–95.
149. **Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ.** Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes.* 2004; 53: 195–9.
150. **Zhou B, Bi YY, Han ZB, Ren H, Fang ZH, Yu XF, Poon MC, Han ZC.** G-CSF-mobilized peripheral blood mononuclear cells from diabetic patients augment neovascularization in ischemic limbs but with impaired capability. *J Thromb Haemost.* 2006; 4: 993–1002.
151. **Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS, Graubert TA.** Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. *Diabetes.* 2004; 53: 91–8.
152. **Al-Khaldi A, Eliopoulos N, Martineau D, Lejeune L, Lachapelle K, Galipeau J.** Postnatal bone marrow stromal cells elicit a potent VEGF-dependent neoangiogenic response *in vivo*. *Gene Ther.* 2003; 10: 621–9.
153. **Gruber R, Kandler B, Holzmann P, Vögele-Kadletz M, Losert U, Fischer MB, Watzek G.** Bone marrow stromal cells can provide a local environment that favors migration and formation of tubular structures of endothelial cells. *Tissue Eng.* 2005; 11: 896–903.
154. **Oswald J, Boxberger S, Jørgensen B, Feldmann S, Ehninger G, Bornhäuser M, Werner C.** Mesenchymal stem cells can be differentiated into endothelial cells *in vitro*. *Stem Cells.* 2004; 22: 377–84.
155. **Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S, Fuchs S, Epstein SE.** Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation.* 2004; 109: 1543–9.
156. **Javazon EH, Keswani SG, Badillo AT, Crombleholme TM, Zoltick PW, Radu AP, Kozin ED, Beggs K, Malik AA, Flake AW.** Enhanced epithelial gap closure and increased angiogenesis in wounds of diabetic mice treated with adult murine bone marrow stromal progenitor cells. *Wound Repair Regen.* 2007; 15: 350–9.
157. **Wu Y, Chen L, Scott PG, Tredget EE.** Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells.* 2007; 25: 2648–59.
158. **Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE.** Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms. *Circ Res.* 2004; 94: 678–85.
159. **Kaigler D, Krebsbach PH, Polverini PJ, Mooney DJ.** Role of vascular endothelial growth factor in bone marrow stromal cell modulation of endothelial cells. *Tissue Eng.* 2003; 9: 95–103.
160. **Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM.** VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.* 1999; 18: 3964–72.

© 2008 The Authors
Journal compilation © 2008 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd