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Stem cells with potential to generate insulin-producing cells in man

Henryk Zulewski

Division of Endocrinology, Diabetes and Clinical Nutrition and Department of Research, University Hospital, Basel, Switzerland

Summary

Replacement of insulin-producing cells represents an almost ideal treatment for patients with diabetes mellitus type 1. Transplantation of pancreatic islets of Langerhans – although successful in experienced centres – is limited by the lack of donor organs. Generation of insulin-producing cells from stem cells represents an attractive alternative. Stem cells with the potential to differentiate into insulin-producing cells include embryonic stem cells (ESC) as well as adult stem cells from various tissues including the pancreas, liver, central nervous system, bone marrow and adipose tissue. The use of human ESC is hampered by ethical concerns and the inability to create patient specific ESC with therapeutic cloning. Among adult stem cells mesenchymal stem cells appear to have a particular developmental plasticity ex vivo that include their ability to adopt a pancreatic endocrine phenotype. The present review summarises the current knowledge on the development of insulin-producing cells from stem cells with special emphasis on human mesenchymal stem cells isolated from the pancreas and adipose tissue.

Key words: stem cells; diabetes mellitus; pancreas

Introduction

Diabetes mellitus is a debilitating metabolic disease caused by absent (juvenile or type 1) or insufficient (type 2) insulin production from pancreatic β cells. With an actual prevalence of 5.9% diabetes is affecting 15.7 millions people in the USA and approximately 400,000 in Switzerland (data for CH calculated). Diabetes is associated with serious long-term complications, such as cardiovascular disorders, kidney disease, and blindness. The actual treatment strategies for type 1 diabetes (5–10% of all diabetics) are based on insulin replacement by several injections daily combined with meticulous blood glucose monitoring and life style adaptations. However, even under best circumstances type 1diabetic patients are faced with a therapeutic dilemma. A good metabolic control that is prerequisite in order to avoid long-term complications is associated with a high frequency of severe hypoglycaemia (DCCT) [1]. Replacement of pancreatic β-cells would represent an almost ideal treatment that could overcome this therapeutic dilemma. Transplantation of islets of Langerhans was shown to be successful in experienced centres but, due to shortage of organs and life long immunosuppression this therapy can be offered to a very limited number of patients only [2, 3]. Insulin secreting cells generated from stem cells could represent an attractive alternative [4].

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Embryonic stem cells

Embryonic stem cells (ESC) have the potential for almost unlimited supply of β-cells [5] but there are considerable ethical concerns regarding the use of human ESC. The generation of individual, patient specific stem cell-derived β-cells for transplantation purposes requires therapeutic cloning of human ESC. Cloning of embryonic stem cells is already accomplished in mice and other mammals [6]. The only paper so far that claimed to have cloned human ESC reported fabricated data [7]. Another problem with ESC is related to their enormous proliferative capacity and the risk for development of teratocarcinoma. Therefore, many scientists continue to evaluate

the not yet exhausted potential of adult stem/progenitor cells. Nevertheless, research with human ESC may help to decipher some crucial steps in development of pancreatic endocrine cells in vitro, since almost all data available on pancreas development were obtained from animal models. Recently, one important step towards differentiation and isolation of endodermal cells, the presumed precursors for pancreatic cells, was achieved in mouse and human ESC [8, 9].

Potential sources for adult stem cells within the pancreas

Stem/progenitor cells with the potential to differentiate into insulin-producing cells in vitro and/or in vivo were described in pancreatic islets [10–12], pancreatic ducts [13], among the population of pancreatic acinar cells [14–16] and within adult or foetal pancreas without further specification [17–19]. In some instances progenitor cells were postulated in pancreatic ducts that would expand and differentiate into insulin-producing cells in response to specific stimuli [13]. Whereas in the case of acinar cells a de-differentiation appears to be the first step followed by re-differentiation into β-cells. The origin of the bona fide stem cell however, remains somewhat elusive and a recent report questioned the entire concept of β-cell stem/progenitor cells with studies using genetic lineage tracing experiments [20]. With this approach it has been shown that pre-existing β-cells rather than adult stem/progenitor cells retained a proliferative capacity and may thus represent the major source of new β-cells in adult life, at least in mice [20]. In this study the authors almost excluded the possibility of stem or progenitor cells to play a role in β-cell replacement in adult life. This extreme position however may not be justified by the data, given the fact that the study was not designed to identify precursor cells per se but rather to provide evidence for or against their participation in β-cell regeneration. And, this study is in conflict with numerous recent in vivo and in vitro studies suggesting the existence of pancreatic stem/precursor cells [14–16, 19, 21]. Today, it is not evident which of the concepts will pass the test of time.

Nestin-expressing mesenchymal stem cells from human islets of Langerhans

The development of normal pancreas is the result of close interaction between mesenchymal and epithelial cells that form the initial buds. Signals from mesenchymal cells direct pancreatic development towards endocrine or exocrine fate ([22] for review). Mesenchymal cells of the developing pancreas express the transcriptional factor islet 1 (Isl-1) [23] that is also expressed in pancreatic epithelial cells, as well as nestin [24], a neural stem cell marker [25]. Nestin-positive cells have been also described in human and rat islets of Langerhans including the hypothesis that these cells may represent a stem cell population [11]. It has been shown that cultured nestin-expressing cells from adult human islets can be differentiated into insulin-expressing cells [11, 12], and nestin-positive cells isolated from human foetal pancreas can be expanded and differentiated into insulin-expressing islet-like clusters that reversed hyperglycaemia in diabetic mice [18]. A recent study described clonal multipotential precursors from adult mouse pancreas that were able to generate neural and pancreatic lineages [19]. The respective precursor cells were generated initially from both nestin$^+$ and nestin$^-$ cells, but all of them expressed nestin during the expansion period indicating that nestin may be a marker of proliferating stem cells. Nestin is believed to play an important role in the selective, unequal partitioning of cytoplasmic components during the division of stem cells so as to maintain one daughter cell as a stem cell and the other daughter cell as a "differentiated" cell [26]. Others described that nestin-expressing cells in the pancreas may be part of vascular endothelial cells or pancreatic stellate cells [27, 28]. In addition, studies including cell lineage analyses indicate that during development nestin-expressing cells may give rise to exocrine pancreatic cells [29, 30] but not endocrine lineages [29–32]. A very recent report however, described expression of exocrine as well as endocrine markers in nestin-positive cells isolated from the developing pancreas of nestin/EGFP transgenic mice [33]. Thus, the developmental potential of nestin-positive cells may also include endocrine cells.

Even though it has been shown that nestin-expressing cells are part of mesenchymal cells of the developing pancreas [24, 34] their fate or function in postnatal life are unknown. Stem cells with a mesenchymal phenotype have been recently shown to develop in human islet cultures *in vitro* by de-differentiation of epithelial β cells induced by powerful growth factors like epidermal growth factor (EGF) or fibroblast growth factor (FGF) [35]. This phenomenon is named epithelial to mesenchymal transition (EMT) [35]. The islet-

derived mesenchymal cells express nestin and vimentin and were able to re-differentiate into insulin-producing cells given the appropriate stimuli [35].

In our current research project we isolated and immortalised on a single cell basis nestin-positive cells from cultured human islets. Interestingly, these cells were also positive for Isl-1 that is known for its critical role in the development of pancreatic endocrine cells. In addition, the isolated nestin- and Isl-1-positive cells displayed a mesenchymal phenotype as mirrored by their ability to differentiate into adipocytic and osteocytic phenotypes given the appropriate stimuli [36]. These cells were negative for insulin and the insulin promoter factor 1 (Ipf-1). Upon differentiation with serum free medium supplemented with a cocktail of differentiation stimulating factors they could be induced to form islet-like cluster and to express several pancreatic developmental genes. This includes the transcription factors Ipf-1, Isl-1, Pax-4, Pax-6, Ngn-3, Nkx-2.2 and Nkx-6.1, as well as the pancreatic endocrine genes insulin, glucagon and somatostatin [36] (figure 1). Glucagon and c-peptide-positive cells were also identified by immunocytochemistry and electron microscopy (figure 2).

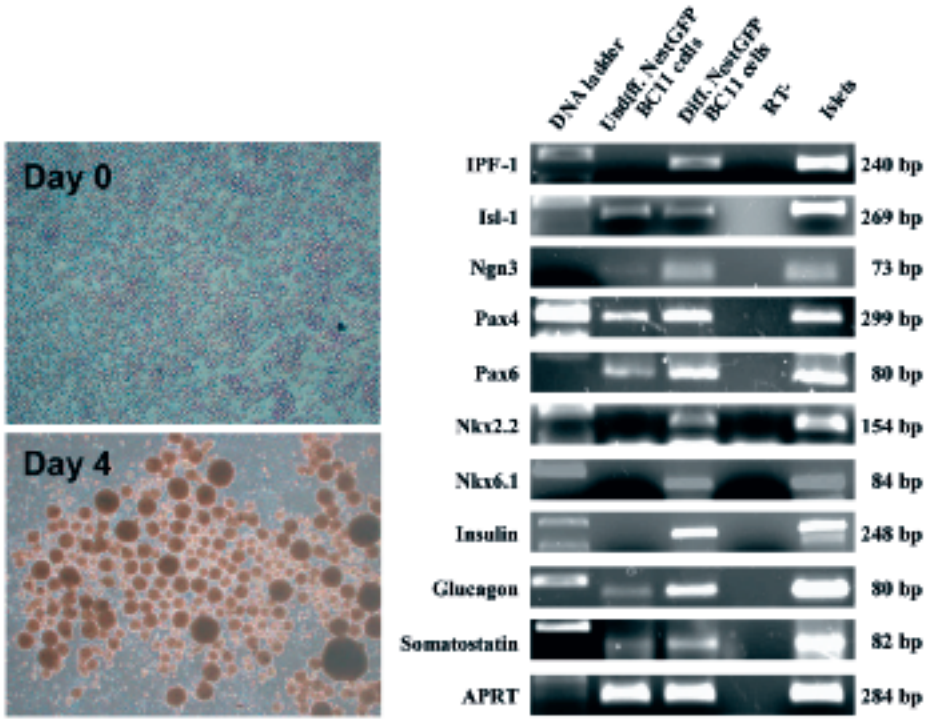

Figure 1

Differentiation of reversibly immortalised islet-derived nestin-positive stem cells (Nest GFP-BC11 cells) into an endocrine phenotype. After 4 days of culture in differentiation medium the cells formed islet-like clusters (left panels). Expression of pancreatic endocrine transcription factors including Ipf-1, Isl-1, Ngn3, Pax4, Pax6, Nkx2.2, and Nkx6.1 as well as the mRNA transcripts of the islet genes insulin, glucagon and somatostatin (right panel). After Eberhardt et al. [75]. Reproduction with permission of the publisher.

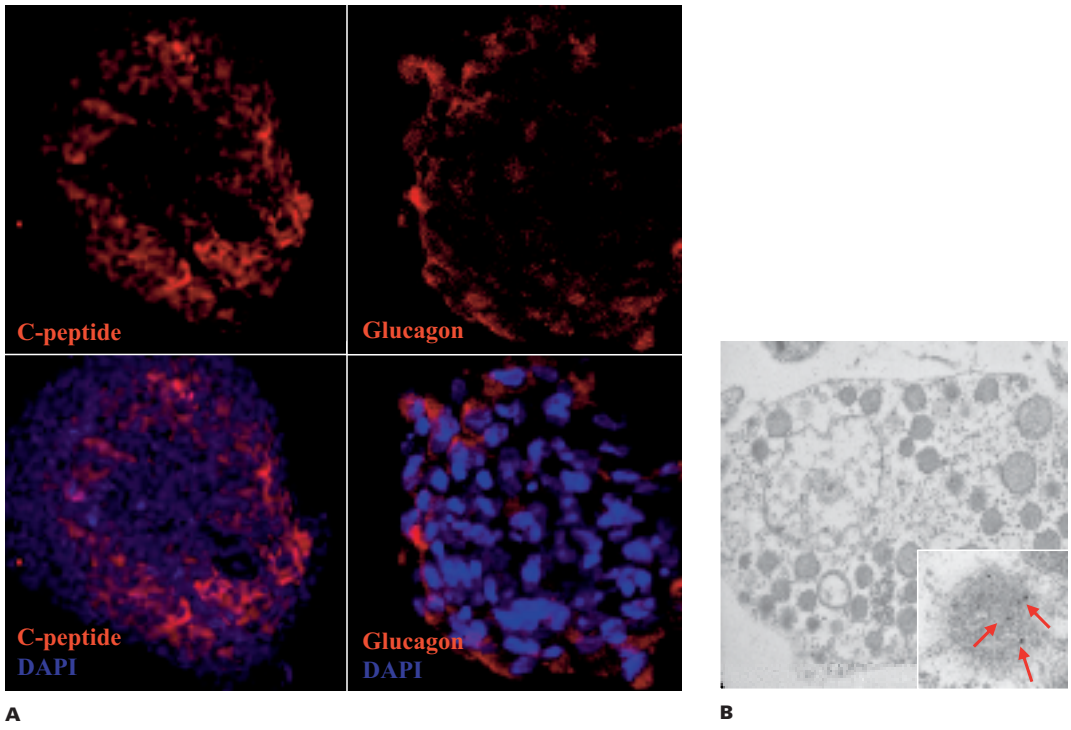

Figure 2

Immunofluoresence staining of differentiated reversibly immortalised islet-derived mesenchymal stem cells.
A. Islet-like clusters were stained with specific antibodies against C-peptide or glucagon. Nuclei staining was performed with DAPI. Image was obtained with a laser scanning confocal microscope (Zeiss LSM 510). Original magnification x 200 for C-peptide image and, x 400 for glucagon.
B. Electron microscopy studies revealed formation of granules in differentiated cells in contrast to undifferentiated cells. Red arrows indicate immunogold labelling for C-peptide (inset). After Eberhardt et al. [75]

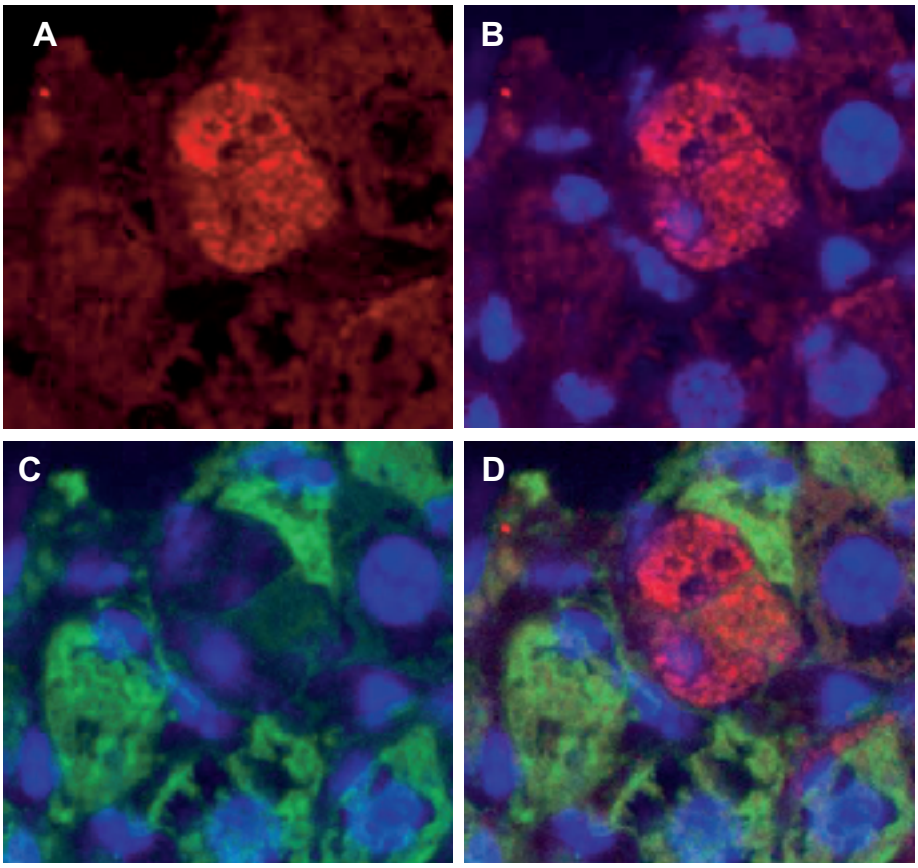

Figure 3

Engraftment of cultured human islet-derived cells into mouse liver. Fluorescence-immunohistochemistry with human and mouse specific antibodies against albumin using confocal microscopy with 630 fold magnification. Panel (A) shows one cell stained with antibodies against human albumin, (B) the same cell with additional DAPI staning for cell nuclei, (C) albumin staining with antibodies against mouse albumin, (D) digital overlay of human and mouse albumin staining showing no co-staining for mouse albumin in the human albumin-positive cell. From von Mach et al. [37], reproduction with permission of the publisher.

C-peptide is part of the proinsulin peptide and thus indicator of de-novo insulin synthesis. Although these results are encouraging and indicate that such cells may have the potential to become functioning β-cells the actual differentiation efficacy is limited and the cells are not yet able to secrete insulin in response to glucose.

There is however evidence that cultured nestin-positive cells from human islets are multipotent as they can adopt a hepatic phenotype *in vivo*. We have recently shown that nestin-positive islet-derived cells differentiate in human albumin-producing cells if grafted directly into mouse liver (figure 3) [37]. Similar results were obtained with our immortalised nestin- and Isl-1-positive cells [36]. Interestingly, human bone marrow-derived mesenchymal stem cells (MSC) were also recently described to differentiate into a hepatic phenotype *in vivo* without evidence for cell fusion [38]. These data suggest that cells with a mesenchymal phenotype may share properties of hepatic precursor/stem cells.

Stem cells outside the pancreas as potential source for β-cell replacement

Stem cells with the potential to differentiate into insulin-producing cells have been also described in the liver [39, 40], the central nervous system [41], the spleen [42] and bone marrow [43–46]. Some of the *in vivo* reports however are controversial and were not confirmed by others [47–51]. Another hypothesis generated by recent data suggests that at least *in vivo* bone marrow-derived cells could play a supportive role in pancreas regeneration rather than participate in the differentiation of endocrine cells themselves [48]. Such developmental potential of bone marrow-derived MSC however was described *in vitro*. MSC from mouse and rat bone marrow were shown to harbour the potential to differentiate into insulin-secreting cells *in vitro* and to reverse hyperglycaemia in an animal model of diabetes [44, 46]. Recently human bone marrow-derived MSC were described to express at low level the islet transcription factor Nkx-6.1 and to differentiate into insulin-expressing cells upon adenoviral transduction with vectors encoding the transcription factors Ipf-1, Hlxb-9 or Foxa-2 [52]. The mechanisms underlying this apparent developmental plasticity of MSC are unknown. Interestingly, MSC were also shown to bear the potential to adopt a neural phenotype *in vitro* and *in vivo* [53–56] in rodents and humans [55] suggesting a neuro-endocrine developmental capacity of these cells. Expanding MSC express several stem cell marker-like stem cell factors (SCF) and Thy-1 [57, 58] but also nestin [55, 56, 59]. Neural precursor cells express besides nestin the side population stem cell marker ABCG2 [60, 61]. ABCG2 expression was described in nestin-positive islet-derived precursor cells [62]. Nestin and possibly ABCG2 expression

could therefore represent a possible link between MSC and their ability to differentiate into neuroendocrine cells. In addition to bone marrow-derived MSC another cell type of haematopoietic origin was recently discovered as potential source for pluripotent stem cells. Human peripheral blood monocyte-derived subset of proliferating cells appear to have the potential to differentiate *in vitro* in different cell lineages including neural, hepatic [63] and pancreatic phenotype with induction of insulin production [64]. There is an increasing body of evidence suggesting that adult stem cells may indeed be converted into insulin-producing cells although the efficacy needs substantial improvements and the mechanisms responsible for this phenomenon are poorly understood.

Mesenchymal stem cells from human adipose tissue

MSC from human bone marrow and adipose tissue represent a very similar cell population with comparable phenotypes [65–68]. Thus, MSC with the potential to adopt a pancreatic endocrine phenotype could also exist in human adipose tissue. We isolated human adipose tissue-derived MSC (adMSC) from four donors and expanded the cells in FGF containing culture medium. Proliferating adMSC expressed the stem cell markers ABCG2, nestin, SCF and Thy-1 but also Isl-1 mRNA. Using immunocytochemistry we found Isl-1 protein in the nuclei of approximately 10% of cultured adMSC [69]. When subjected to serum-free differentiation medium an up-regulation of the transcription factors Ipf-1, Isl-1 and Ngn-3 and the islet genes insulin, glucagon and somatostatin was observed (figure 4a–c). In parallel, c-peptide-positive cells were found after differentiation (figure 4d) [69].

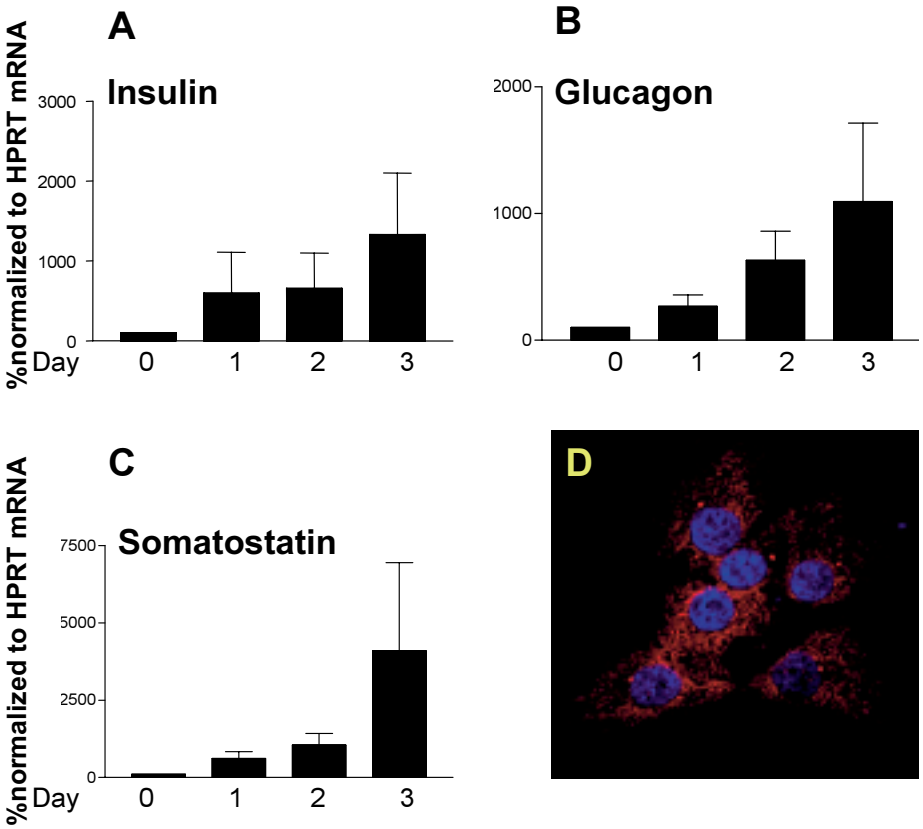

Figure 4

A–C: Induction of the islet genes insulin, glucagon and somatostatin in adipose tissue-derived MSC in response to defined culture conditions. D: Immunocytochemistry for C-peptide adipose tissue-derived MSC (magnification x 200). After Timper et al. [69], reproduction with permission of the publisher.

Expression of Isl-1 in mesenchymal stem cells

The phenotype of mesenchymal stem cells that are able to turn on Isl-1 and other early transcription factors in response to defined culture conditions is unknown and needs further studies. Expression of Isl-1 is together with Ipf-1 one of the earliest pancreatic markers detected in the pancreatic anlage at embryonic day E9 in the mouse [23]. Isl-1 is crucial for the generation of pancreatic endocrine cells and explants of the pancreatic anlage from Isl-1 knock-out mice were unable to give rise to insulin- and glucagon-positive cells as did the wild-type controls [23]. Isl-1 expression in our

MSC could thus reflect the first crucial step towards induction of other developmental transcription factors and pancreatic islet genes. But, Isl-1 is also involved in the development of the central nervous system, especially motorneurons [70, 71] and it has been recently also shown to play a role in heart development [72, 73]. Therefore, at least some of the Isl-1-positive MSC may equally represent potential precursors for these organs.

Potential and limitations of adult mesenchymal stem cells

It has been shown that human bone marrow-derived MSC can be differentiated into hepatocytes *in vivo* without evidence for cell fusion if xenografted directly into the rat liver [38]. This demonstrates an unexpected developmental potential of these cells. Liver and pancreas are believed to origin from similar endodermal precursors during development. Pancreatic stem/precursor cells can give rise to hepatocyte and vice versa [37, 39, 74, 75]. It is therefore tempting to speculate that human adipose tissue- as well as bone marrow-derived MSC harbour the potential to adopt a pancreatic endocrine phenotype and give rise to functioning insulin-secreting cells. We have shown that human MSC from pancreatic islets as well as adipose tissue can be induced to activate pancreatic developmental genes including Isl-1, Ipf-1, Ngn-3, Pax-4, Pax-6, Nkx-2.2, Nkx-6.1, as well as the islet genes insulin, glucagon and somatostatin. This differentiation was achieved without genetic modifications of the cells. However, we are still far away from production of clinically meaningful amounts of insulin.

Although, impressive knowledge accumulated in recent years regarding pancreas development and especially the role of crucial transcription factors required for proper differentiation of pancreatic β-cells [76], there is considerable lack of information on factors needed for in vitro differentiation of stem cells; ESC and adult stem cells alike. There is no unequivocally accepted protocol for differentiation of stem cells into insulin-producing cells and there is little general agreement on the cell type that should be studied. The overall potential of adult cells to change their fate in vitro and differentiate into almost every tissue was again demonstrated recently, using adult fibroblasts that were converted into cells with ESC-like phenotype by manipulating just 4 important factors [77]. There is substantial circumstantial evidence suggesting that MSC may have the potential to differentiate into various tissues including insulin-producing cells in vitro. But, the candidate cells among the MSC that harbour this potential need to be identified together with a considerable improvement of current differentiation protocols in order to achieve significant advancements in the search for stem cell-based therapies for diabetes mellitus type 1.

Correspondence:
Dr. Henryk Zulewski
Departement Forschung
Klinik für Endokrinologie,
Diabetologie und klin. Ernährung
Universitätskliniken
Hebelstrasse 20
CH-4031 Basel
Switzerland
henryk.zulewski@unibas.ch

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