

## Regenerative medicine of the pancreatic $\beta$ cells

SATOKO YAMADA and ITARU KOJIMA

Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-8512, Japan

### Abstract

Diabetes mellitus is a metabolic disorder that affects millions of people. The number of patients suffering from diabetes continues to increase all over the world. Both type 1 and type 2 diabetes result from an inadequate mass of functioning  $\beta$  cells. To achieve the ultimate goal of curing diabetes in the future, the mechanism of the regenerative process of the adult human pancreas must be elucidated. In this review, we first summarize the regenerative processes of the pancreas observed in animal models *in vivo*, and approaches to promote the regeneration of the pancreas *in vivo*. Next we consider other new approaches, such as stem cell research and cell-based therapy, for the cure of diabetes in the future. Based on the innovative success of the Edmonton protocol, islet transplantation has been considered to be a new therapeutic option for the treatment of diabetes. However, a serious shortage of donor pancreata is a critical problem. We suggest that the following issues should be solved in order to realize cell-based therapy. The first is to establish a source of stem/progenitor cells that will multiply easily *in vitro* and maintain their property as progenitor cells. The probable use of adult stem cells will circumvent potential ethical problems, and autotransplantation will become possible. The most difficult and as yet unsolved issue is how to differentiate these cells and acquire fully functional islets. Further investigations to understand the regenerative process of the adult pancreas and the appropriate induction of stem cell differentiation will help to establish cell-based therapy in diabetes.

**Key words** Diabetes · Regenerative process of pancreas · Cell-based therapy

### Introduction

The number of patients suffering from diabetes is now increasing rapidly all over the world. Blood glucose

levels can be controlled to some extent by multiple injections of insulin or by oral hypoglycemic agents, but the ideal glycemic control has not yet been perfectly achieved by these conventional treatments. Most of all, type 1 diabetes is a chronic metabolic disorder in which pancreatic islet  $\beta$ -cells are irreversibly destroyed by autoimmunity. In these patients, an almost complete loss of functional islet  $\beta$  cells leads to a long-lasting, absolute deficiency of insulin secretion. They are suffering from unstable glycemic control, and incomplete compensation for glucose homeostasis leads to irreversible diabetic complications. Frequent, recurrent hypoglycemia unawareness is extremely dangerous and can be fatal. The real cure for type 1 diabetes is the replacement of pancreatic  $\beta$  cells. In this regard, the surgical treatment of diabetes, i.e., successful pancreas transplantation, has the possibility to cure diabetes. In a recent report, the worldwide, 3-year organ survival rate for simultaneous kidney and pancreas transplantation had improved to approximately 70%–80%.<sup>1</sup> These results were obtained in a highly selected group of type 1 diabetic patients who had severe difficulties in achieving glycemic control. Nowadays, the American Diabetes Association recommends pancreas transplantation for patients with unacceptably poor metabolic control and quality of life despite optimum medical treatment. An innovative success for pancreatic islet transplantation (the Edmonton protocol) was reported in 2000,<sup>2</sup> and islet transplantation has also been considered to be a new therapeutic option for the treatment of diabetes. Ryan et al.<sup>3</sup> summarized the best outcomes of reproductive Edmonton treatment in 2002. In 17 consecutive Edmonton protocol-treated subjects, all became insulin-independent, and 14 subjects have maintained demonstrable C-peptide secretion and kept stable glycemic control. No death or life-threatening infection occurred. This new protocol is relatively safe and efficient for the reduction of exogenous insulin and the prevention of recurrent hypoglycemia, and as a result an improvement

Offprint requests to: I. Kojima

Received: February 25, 2005 / Accepted: March 2, 2005

in the quality of life. However, the major remaining problem is that the minimum number of islets required for insulin independence is large: about 9000 islets/kg. The demand for pancreas and islet transplantation far exceeds the number available, even if the indication is limited to adult patients with type 1 diabetes who have recurrent hypoglycemia with poor symptom recognition. This limited supply restricts the availability of this promising treatment. Physiologically competent substitutes for human pancreatic islets are required.

In recent promising studies, substantial evidence has been accumulated demonstrating that stem cells must also exist in adult organs, and which are capable of differentiating into various cell types beyond tissue lineage boundaries.<sup>4</sup> The isolation and expansion of stem/progenitor cells and their subsequent differentiation into pancreatic islet cells will help to meet the demand for cell-based therapy, and the goal of a permanent cure for diabetes will be realized.

In this review, we describe the mechanism of regeneration of pancreatic  $\beta$  cells, and then focus on the possible candidates for adult stem/progenitor cells found to date and discuss cell-based therapy for diabetes in the near future.

### **The regeneration process of pancreatic $\beta$ cells**

Pancreatic  $\beta$  cells always turn over even in normal conditions.<sup>5</sup> The pancreas senses the functional mass of  $\beta$  cells by some means, and maintains the total  $\beta$  cell number by balancing the loss and proliferation of these cells. When the demand for insulin increases (as in the case of pregnancy, obesity, and various pancreatic injuries), a compensatory mechanism operates by promoting the proliferation or hypertrophy of the  $\beta$  cells. In persons with a risk of diabetes, these compensatory mechanisms are impaired, and the functioning  $\beta$  cell mass is a critical factor for the onset of diabetes.<sup>6</sup> Understanding the precise mechanism for the regulation of regeneration and maintenance of the pancreatic  $\beta$  cell mass is one of the most important issues for the establishment of new therapeutic approaches for diabetes.

Experimental models for pancreatic regeneration have been examined in the rodent pancreas, which has a strong  $\beta$  cell regenerative potential. Several models have been studied as models of diabetes: ex. 90% pancreatectomy,<sup>7–13</sup> treatment with streptozotocin (STZ, a toxin that induces hyperglycemia due to rapid and massive  $\beta$  cell death),<sup>14–16</sup> duct ligation,<sup>17</sup> cellophane wrapping,<sup>18,19</sup> and overexpression of interferon- $\gamma$ .<sup>20</sup> Several differences have been observed between these models, but  $\beta$  cell neogenesis does occur in numerous sites even in the adult pancreas.

In summary, regenerative processes of the pancreas observed in animal models *in vivo* are composed of four complex mechanisms. These are: (1)  $\beta$  cell neogenesis in residual islets (by intraislet progenitor cells or by dedifferentiation of somatostatin-expressing  $\delta$  cells), (2) the proliferation of duct cells and subsequent differentiation into new  $\beta$  cells, which may include progenitor cells in and/or around the expanding duct, (3) dedifferentiation of acinar cells and differentiation into  $\beta$  cells or transdifferentiation of acinar cells to islet cells, and (4) replication and proliferation of preexisting  $\beta$  cells in the residual islets. Based on the observations in these *in vivo* regeneration models, it is clear that pancreatic stem/progenitor cells exist within the adult pancreas (ducts, acini, and islets). In this regard, using a genetic lineage tracing method, Dor et al.<sup>21</sup> recently showed that adult pancreatic  $\beta$  cells are formed by self-duplication rather than by stem cell differentiation. Their experiment showed that the normal turnover of  $\beta$  cells occurs through replication of preexisting  $\beta$  cells. More intriguingly, newly formed beta cells are also derived from preexisting  $\beta$  cells after 70% pancreatectomy. They postulate that mature  $\beta$  cells have important roles in  $\beta$  cell regeneration. It should be noted, however, that 70% pancreatectomy does not cause a severe reduction of the  $\beta$  cell mass. Therefore, their results do not rule out the existence of the pancreatic stem cells and the involvement of the stem cells in more severe pancreatic injury and diabetes.

### **Approaches to promote regeneration of the pancreas *in vivo***

To search for growth and/or differentiation factors that can expand pancreatic stem/progenitor cells and differentiate them into mature pancreatic islet cells is the most imperative task for the establishment of a curative treatment for diabetes. To date, a large number of investigations have used these *in vivo* pancreatic regeneration models.

Glucagon-like peptide-1 (GLP-1) is an intestine-derived insulinotropic hormone that stimulates glucose-dependent insulin secretion from pancreatic  $\beta$  cells.<sup>22</sup> GLP-1 has more diverse pleiotropic actions that are favorable for diabetic states. List and Habener<sup>22</sup> summarized the action of GLP-1 in the development and growth of pancreatic  $\beta$  cells. They confirmed that GLP-1 possesses properties that can stimulate  $\beta$  cell proliferation and increase the  $\beta$  cell mass via the inhibition of  $\beta$  cell apoptosis, stimulation of  $\beta$  cell proliferation, and stimulation of stem/progenitor cell differentiation into endocrine cells. The biological half-life of GLP-1 is short, and a long-acting analog of GLP-1 exendin-4,<sup>23</sup> or inhibitors of dipeptidyl peptidase

IV (which rapidly cleaves and inactivates GLP-1)<sup>24</sup> are the most promising factors for the treatment of diabetes, although their mechanisms of actions remain to be fully elucidated.

Activin, a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily, is well known to regulate the growth and differentiation of cells in multiple organs.<sup>25</sup> In the development of the pancreas, activin- $\beta_B$  secreted from the notochord, together with fibroblast growth factor-2 (FGF2), suppresses the expression of sonic hedgehog (Shh), permits the expression of PDX-1 in the dorsal endoderm, and regulates the prepancreatic endoderm to form pancreatic buds at E8.5.<sup>26</sup> Activin and its receptors ActRIIA and ActRIIB are broadly expressed in the pancreatic epithelium at E12.5.<sup>27</sup> Activin promotes the development and differentiation of endocrine cells via both autocrine and paracrine mechanisms.<sup>28</sup> Follistatin from mesenchymal tissue suppresses endocrine differentiation and promotes exocrine differentiation via blockage of the activin signal.<sup>29</sup> Moreover, in pancreatic AR42J cells, which possess both endocrine and neuroendocrine properties, activin A induces morphological changes and converts them into pancreatic polypeptide (PP)-expressing neuron-like cells.<sup>30</sup> We also examined the expression of activin in the adult pancreas.<sup>31</sup> Both  $\beta_A$  and  $\beta_B$  subunits of activins and ActRIIB receptor are expressed in the pancreatic duct epithelium in adult mice. The expression of both activin subunits is markedly up-regulated after pancreatic injury by streptozotocin injection or partial pancreatectomy. These observations strongly suggest that activins are involved in the process of differentiation and regeneration of the pancreatic endocrine cells.

Betacellulin (BTC) is a polypeptide growth factor originally isolated from a conditioned medium of insulinoma cells.<sup>32</sup> BTC has been shown to be a mitogen for retinal epithelial cells and vascular smooth muscle cells. Immunoreactive BTC is expressed in endocrine cells in the fetal pancreas<sup>33</sup> as well as in the regenerating pancreas.<sup>34</sup> We have reported that BTC, acting in coordination with activin A, converted pancreatic AR42J cells into insulin-producing cells.<sup>30</sup> Because AR42J cells resemble amphicrine transitional cells in the regenerating pancreas, we strongly suggest that BTC can also promote the growth and differentiation of pancreatic  $\beta$  cells in the regenerative pancreas. For these reasons, we have systematically investigated the effect of BTC on pancreatic regeneration models.<sup>16,35,36</sup> In the 90%-pancreatectomized rat model, the numbers of islet cell-like clusters (ICCs) and BrdU/insulin double-positive cells in ICCs and islets were significantly higher in BTC-treated rats.<sup>35</sup> The  $\beta$  cell mass and the insulin content of the pancreas were also greater. However, the number of the PDX-1-positive cells in the ducts soon after pancreatectomy was not significantly different. We suggest

that BTC increased the replication of preexisting  $\beta$  cells in the islet and the promotion of the early steps of  $\beta$  cell differentiation from PDX-1-positive duct/progenitor cells. In the STZ-treated mice model, BTC mainly acts on intraislet precursor cells.<sup>16</sup> BrdU/insulin double-positive cells, PDX-1/somatostatin double-positive cells, and the number of  $\beta$  cells in the islet were significantly increased in BTC-treated mice. In neonatal STZ-treated rats, we investigated the effects of activin A and/or BTC.<sup>36</sup> Combined treatment with activin A and BTC significantly increased the DNA synthesis in preexisting  $\beta$  cells, ductal cells, and  $\delta$  cells. The numbers of ICCs, islets, and insulin/somatostatin-positive cells were significantly increased. The combination of activin A and BTC showed a greater increase. The presumptive sites of action of activin A and BTC are as follows: promotion of  $\beta$  cell replication, ductal cell proliferation,  $\beta$  cell neogenesis from duct cells, and transdifferentiation of  $\delta$  cells to  $\beta$  cells. These *in vivo* studies indicate that BTC can promote the regeneration of  $\beta$  cells by acting in multiple steps, and the combination of activin A and BTC may be a more effective treatment.

However, because activins and BTC have pleiotropic actions in various organs,<sup>25</sup> the clinical application of these differentiation-promoting factors seems difficult. The induction of apoptosis in various cells is an unfavorable action of activin. Recently, Umezawa et al.<sup>37</sup> screened the compounds that can induce insulin expression in AR42J cells and identified a low-molecular weight (794) compound conophylline. Conophylline is a vinca alkaloid extracted from the tropical plant *Ervatamia microphylla*. Like activin A, conophylline can differentiate AR42J cells into cells with a neuron-like morphology, and eventually into insulin-positive cells in the presence of HGF. In particular, the viability of differentiated cells treated by conophylline is better than of those treated by activin A. We investigated the effect of conophylline on the differentiation of pancreatic progenitor cells in *in vitro* organ culture of the fetal pancreas and in *in vivo* neonatal STZ-induced diabetes rats.<sup>38</sup> In organ culture, pancreatic epithelium expansion was scarce, but the expression of the insulin gene and the numbers of insulin- and PDX-1-positive cells were markedly increased in conophylline-treated tissues. This differentiation-promoting effect of conophylline was stronger than that of activin A. In the neonatal STZ-induced diabetes rat model, the insulin content and the  $\beta$ -cell mass, and the presence of PDX-1-positive ductal cells were significantly increased by the conophylline treatment. However, the numbers of BrdU-positive duct cells and  $\beta$  cells were not affected. We suggest that conophylline mimics the effect of activin A and promotes the differentiation of pancreatic progenitor cells into  $\beta$  cells.

With regards to BTC, Dunbar and Goddard<sup>39</sup> recently cloned and characterized a novel BTC splicing variant. In this variant, a 147bp deletion preceding the fifth cysteine of the EGF motif led to the generation of an unusual BTC precursor in which the C-loop of the EGF domain and the transmembrane domain are deleted. In a preliminary study, we investigated the effect of BTC- $\delta 4$  in AR42J cells (Ogata et al., submitted for publication). Treatment with activin A and BTC- $\delta 4$  induced insulin-positive cells, but a large number of the differentiated cells became apoptotic. It is suggested that BTC- $\delta 4$  lacks antiapoptotic action, presumably owing to the lack of the EGF motif. The differentiation-promoting action of BTC- $\delta 4$  may not be exerted via the EGF or a related receptor.

For the establishment of a permanent cure for diabetes in the future, one hopeful approach is to promote an *in vivo* regenerative process in the pancreas. One way to do this is by using favorable growth and differentiation factors in combination. The plausible site that could enhance the regenerative process of the pancreas may be divided into several steps: (1) a triggering step to initiate the replication of pancreatic stem progenitor cells, (2) the proliferation of progenitor cells for physiological demand, and (3) differentiation of the progenitors into fully functional  $\beta$  cells. The precise mechanisms of step (1) have not yet been elucidated, and the factors which act on this step have not been identified. Several factors enhancing steps (2) and (3) have been elucidated, but further investigations of effective factors are needed in future studies.

Another approach involves a gene delivery method which has attempted to promote *in vivo* regeneration. Taniguchi et al.<sup>40</sup> performed adenoviral vector-mediated gene delivery of transcription factors PDX-1 and *ngn3* in the mouse pancreas by retrograde intracommun bile ductal injection. The gene delivery of PDX-1 in the duct induced the proliferation of pancreatic ductal cells and neogenesis of insulin-producing cells. This new approach may provide a potential new method for the treatment of diabetes.

### Approaches to cell-based therapy in diabetes

Recent progress in islet transplantation has brought about the possibility of a cure for diabetes. However, the scarcity of donor islet  $\beta$ -cells still remains a major obstacle. Vast numbers of functional  $\beta$  cells will be required to produce any significant therapeutic effect. Although preexisting  $\beta$  cells may be the major source of new  $\beta$  cells *in vivo* (Dor et al.<sup>21</sup>), mature  $\beta$  cells have a very low proliferative capacity and it is difficult to make them maintain their mature function during *in vitro* expansion. As a result, to establish methods to isolate

and expand stem/progenitor cells and subsequently differentiate these cells into fully functional pancreatic endocrine cells should be the major strategy for the supply of cell-based therapy in the future.

Stem cells are defined by their dual capacity for self-replication and differentiation into multiple specialized cell lineages. They can be classified into two main categories, embryonic stem (ES) cells and adult stem cells. Understanding the process of stem cell differentiation and identifying the markers of adult stem cells will lead to the isolation and establishment of pancreatic stem cells.

### Embryonic stem cells

Embryonic stem cells (ES cells) are derived from the inner mass of the mammalian blastocyst.<sup>41</sup> They can proliferate in unlimited numbers in an undifferentiated state, and differentiate into all lineages of cells under appropriate conditions and thus are pluripotent. It has been reported by many groups that ES cells can differentiate into insulin-producing cells *in vitro*.<sup>42–44</sup> After selection for nestin-expressing ES cells, the treatment of growth inhibitors<sup>45</sup> or the induction of PDX-1,<sup>46</sup> for example, promoted them to differentiate into insulin-producing cells. Moreover, a feeder-free ES cell line-harboring system has recently been developed which can avoid possible contamination by murine feeder cells.<sup>47</sup> Recent reports have stated that the method based on the selection of nestin-positive cells irreproducible (because the cells are already committed to a neural fate) and therefore is not suitable for the generation of pancreatic  $\beta$ -cells from ES cells.<sup>48</sup> A new strategy to generate insulin-producing cells without nestin-expressing cells has been shown. In either case, ES cells may be able to serve as a potential source of insulin-producing cells. However, a significant number of problems still remain unsolved in terms of their clinical applications: for example, how to optimize the conditions to generate more insulin-positive islet cells, at the same time how to stop the cells differentiating into another lineage, and how to avoid the risk of teratoma formation if they are implanted *in vivo*. The ethical issue is another major obstacle to the clinical use of ES cells.

### Adult pancreatic stem cells

As mentioned above, there exist many lines of substantial evidence demonstrating that adult pancreatic stem/progenitor cells are retained in or around the pancreatic duct and in the islets. In practice, several *in vitro* studies have shown that insulin-producing cells can be generated

from adult pancreatic ductal tissues. Ramiya et al.<sup>49</sup> first reported that functioning islets could be produced from the long-term culture of isolated mouse adult duct epithelial cells. The transplantation of these islets into diabetic mice could reverse insulin-dependent diabetes for about 2 months. Bonner-Weir et al.<sup>50</sup> also cultured human adult ductal tissues as a monolayer with a thin layer of Matrigel and observed the formation of islet-like buds consisting of CK19-positive duct cells and insulin-positive cells. The islet buds showed glucose-dependent insulin secretion to some extent. In another study reported by Heremans et al.,<sup>51</sup> adult human pancreatic duct cells were converted into insulin-expressing cells after the ectopic adenovirus-mediated expression of neurogenin 3, which is a critical transcription factor in the determination of differentiation into the endocrine lineage in pancreas development. These studies show that adult pancreatic duct cells might be a potential source for cell-based therapy. However, several problems still remain unsolved. Are there true pancreatic stem cells in the duct tissue which differentiate into endocrine cells? Or do adult duct cells have the potential to lose their ductal phenotype and then convert into insulin-positive cells? The specific markers that can identify pancreatic stem cells have not yet been established. Gao et al.<sup>52</sup> tried to characterize the endocrine progenitor cells as follows: some of the CK19-positive duct epithelial cells differentiated into endocrine cells, and a serum-free culture was an absolute requirement for differentiation. By combining flow cytometry and clonal analysis, Suzuki et al.<sup>53</sup> showed that a possible candidate for the pancreatic progenitor cells might express HGF receptor c-met, but will not express CD45, TER119, c-kit, or Flk-1. The identification and isolation of pancreatic stem cells have remained elusive.

Substantial evidence suggests that other potential stem cells might be in the islets. To date, nestin-positive cells<sup>54</sup> and small cells<sup>55</sup> have been reported as possible candidates. Zulewski et al.<sup>54</sup> isolated nestin-positive cells within rat and human islets that could proliferate in vitro and differentiate into multiple cell lineages (liver, exocrine pancreas, ductal/endocrine phenotype). Whether or not nestin is a possible marker for pancreatic stem cells is still under debate. Nestin is an intermediate filament protein involved in cytoskeletal formation, cell migration, and mitosis. It has been reported that nestin can be used as a marker for neuronal stem cells. In the pancreas, nestin is expressed heterologously in the pancreatic duct, acini, islets, mesenchyme, and vessels. For these reasons, nestin is not a suitable marker for pancreatic stem cells, although it may have some functional roles in islet differentiation. In human and canine pancreatic islets, novel cells called "small cells" were identified by Petropavlovskaja and Rosenberg.<sup>55</sup> These cells formed small clusters and were

positive for all endocrine hormones and PDX-1, but negative for CK19 or nestin. Intra-islet precursor cells are also of interest, but their capacity for growth and proliferation might be insufficient for cell expansion.

Seaberg et al.<sup>56</sup> clonally identified pancreas-derived multipotent precursors (PMPs) from adult mouse pancreatic islet and duct cells which could generate neural and pancreatic lineages. PMPs derived from nestin<sup>+</sup> and nestin<sup>-</sup> cells ultimately coexpressed markers of both neural (nestin) and pancreatic precursors (PDX-1). They can differentiate into all types of neural progeny and both pancreatic endocrine and exocrine cells. The problems which still remain to be solved are that the self-renewal capacity of PMPs is limited, and how the differentiation of PMPs toward  $\beta$ -cells can be properly regulated. Recently, Gershengorn et al.<sup>57</sup> reported that human islet precursor cells (hIPCs) derived from adult human postmortems that exhibit a mesenchymal phenotype donated islets. Rather surprisingly, hIPCs are derived directly from the transition of insulin or glucagon-expressing epithelial islet cells into mesenchymal phenotype. Removal of serum from the culture medium leads to a re-differentiation of hIPCs into proinsulin-expressing endocrine cells. This study shows that even differentiated adult endocrine cells have remarkable plasticity and may be a putative source for generating more  $\beta$ -cells in vitro.

Pancreatic acinar cells are not classified as stem cells. Nevertheless, as shown in a rat duct ligation model, acinar cells dedifferentiate into cells forming duct-like structures and new  $\beta$  cells are produced from them.<sup>17</sup> This suggests that pancreatic acinar cells are also a probable source of  $\beta$  cells. Rooman et al.<sup>58</sup> also observed direct acinoductal transdifferentiation in vitro. Cultured rat exocrine cells lost their acinar phenotype and began to express ductal cell markers CK7, CK20, and Flk-1, and also PDX-1 and PGP9.5. This suggests that transdifferentiated cells showed similar characteristics to pancreatic endocrine precursor cells. Recent reports by Song et al.<sup>59</sup> showed a few insulin-positive cells coexpressing cytokeratins in cultured adult pancreatic acinar cells. This represents the first evidence that adult pancreatic acinar cells differentiate into insulin-expressing cells in vitro. Adult exocrine tissues are useful sources of  $\beta$  cells.

### Adult hepatic and intestinal stem cells

During embryogenesis, the pancreas, liver, and gastrointestinal tract are all derived from the anterior endoderm. In particular, the ventral foregut endoderm differentiates into the ventral pancreas as a default pathway, except that it differentiates into liver when FGF signaling from cardiac mesoderm acts on it.<sup>60</sup>

Transdifferentiation of the pancreas to liver (and vice versa) has been observed in several pathological conditions in adult humans and animals.<sup>61,62</sup> In addition, PDX-1-expressing endodermal cells differentiate into intestinal cells in the absence of Ptf1a.<sup>63</sup> Given these data, common stem/progenitor cell populations may exist in the adult and differentiate into each tissue.

Hepatic oval cells appear in severe liver injury when mature hepatocytes cannot proliferate to repair the liver damage.<sup>64</sup> Oval cells express immature liver cell marker  $\alpha$ -fetoprotein (AFP), and also express hepatocyte lineage marker albumin and biliary epithelium marker CK19. Given that these cells can differentiate into both types of mature liver cells, they are considered to be hepatic stem cells. Yang et al.<sup>65</sup> reported that oval cells can transdifferentiate into pancreatic endocrine cells when cultured in high glucose. They can synthesize and secrete insulin when stimulated with glucose. However, since oval cells can only be induced by severe liver injury, they may not be suitable candidates for a  $\beta$  cell source. We used another type of adult hepatic stem-like cell, namely HSL cells, and converted them to insulin-producing cells.<sup>66</sup> HSL is an epithelial cell line obtained from normal adult rat liver which can be converted to both hepatocytes<sup>67</sup> and biliary epithelial cells. We consider HSL cells to be more immature progenitor cells. This is because HSL cells do not express albumin and CK19. The rapid growth and easy handling of HSL cells are their major merit for future clinical use. More committed hepatocyte progenitor cells, called small hepatocytes, can also be induced to generate insulin-producing cells.<sup>68</sup> Rat hepatic cell line named WB-1 which stably expressed the active form of PDX-1 can differentiate into functional insulin-producing cells.<sup>69</sup> Adult liver stem cells obtained from normal liver will be probable sources for autologous cell transplantation therapy for diabetes.

Suzuki et al.<sup>70</sup> induced insulin production in fetal intestinal epithelial progenitors by using GLP-1 (1-37). PDX-1 and/or Isl-1 induction of immature rat intestinal stem cells, IEC-6, into insulin-producing cells has also been reported.<sup>71,72</sup> In the adult gastrointestinal tract, there is substantial evidence that multipotential stem cells reside within gastric glands and the intestinal crypt.<sup>73</sup> These adult intestinal stem cells are also probable candidates for generating  $\beta$  cells based on the advantages of access to these cells.

### **Multipotent adult progenitor cells**

Bone marrow is an important source of easily accessible adult stem cells. Bone marrow-derived stem cells can reconstitute the hematopoietic system, and a significant number of bone marrow transplantations have been

performed over many years. Several recent studies suggest that transplanted bone marrow-derived stem cells can generate into multiple lineage cells, including liver, brain, lung, gastrointestinal tract, and skin.<sup>74,75</sup> Ianus et al.<sup>76</sup> observed that bone marrow-derived cells populate pancreatic islets 4–6 weeks after transplantation. They identified male donor-derived insulin-positive cells by EGFP expression by using a CRE-LoxP system when the insulin gene is actively transcribed in the recipient female mice. Isolating these EGFP<sup>+</sup>/Y chromosome<sup>+</sup> cells showed glucose-dependent insulin secretion and incretin-enhanced insulin secretion. This suggests that bone marrow-derived stem cells may contribute to islet neogenesis under physiological conditions. They also showed that these  $\beta$  cells were generated from transdifferentiation of bone marrow-derived donor cells, not by cell fusion. Many controversial observations still exist. Hess et al.<sup>77</sup> showed that the transplantation of c-kit (stem cell marker)<sup>+</sup>-bone marrow-derived stem cells initiated endogenous pancreatic regeneration and improved blood glucose level in STZ-induced diabetic mice via enhanced endothelial proliferation by donor cells. On the other hand, independent studies by Choi et al.<sup>78</sup> and Lechner et al.<sup>79</sup> showed little evidence for a significant transdifferentiation of bone marrow cells into pancreatic  $\beta$  cells, even in pancreatic injury models of mice. They established stable bone-marrow chimerism first, and then induced pancreatic injury. Given all these data,  $\beta$  cell neogenesis from only bone marrow-derived cells might be rare, but the existence of multipotent adult stem cells that can differentiate into  $\beta$  cells has never been denied. Jiang et al.<sup>80</sup> proposed the existence of pluripotent mesenchymal stem cells derived from adult marrow. Recent studies showed that adult bone marrow-derived cells obtained from mice<sup>81</sup> and rats<sup>82</sup> could differentiate into pancreatic  $\beta$  cells both in vitro and in vivo. Bone marrow cells may also provide a potential source for the future treatment of diabetes.

In addition to the above-mentioned cells, other types of tissue-derived stem cells, including salivary gland progenitor cells<sup>83,84</sup> and amnion cells,<sup>85</sup> can also be used for the generation of insulin-producing cells. Potent adult stem cells reported to be able to differentiate insulin-producing cells are summarized in Table 1.

### **Cell-based therapy in diabetes for the future**

To establish cell-based therapy for diabetes in the future, we propose that the following issues be solved and some goals be clarified. The first is to establish the source of stem/progenitor cells that can easily be expanded in vitro while maintaining their properties as progenitor cells. The probable use of adult tissue stem

**Table 1.** Potential adult stem cells that differentiate to insulin-producing cells

Cell source	Animals	Reference
Pancreatic stem cells		
Ductal stem cells	m, h, p	49, 50, 51, 52, 56
Intraislet stem cells	h, r, canine	54, 55, 56
Acinar cells	r	59
Liver stem cells		
Oval cells	r	65
Liver-epithelial cells	r	66
Small hepatocytes	r	68
Intestinal epithelial cells	m	70
Bone marrow-derived cells	m, h, r	76, 77, 81, 82
Duct cells of the salivary gland	r, m	83, 84
Amniotic epithelial cells	h	85

m, mouse; h, human; r, rat

cells will become possible for autotransplantation. The most difficult and unsolved issue is how to differentiate them and acquire fully functional islets. For clinical applications, many more problems, including, for example, autoimmunity and tumorigenesis, remain to be carefully investigated.

Recently, it has been reported that islet regeneration can lead to the permanent reversal of diabetes in NOD mice. An injection of live male donor splenocytes and complete Freund's adjuvant eliminates autoimmunity and permanently restores normoglycemia in diabetic NOD female mice.<sup>86</sup> Donor splenocytes contain cells that can rapidly differentiate into islet and ductal cells within the pancreas. This report shows another intriguing possible cure for autoimmune diabetes without the need to generate exogenous functional islets.

In either case, a new age is now dawning which will bring about a cure for diabetes by new approaches. Further studies to understand the mechanisms of adult human pancreatic regeneration and the appropriate induction of stem cell differentiation will help to establish more effective cell-based therapy.

## References

1. Rovertson RP (2004) Islet transplantation as a treatment for diabetes — a work in progress. *N Engl J Med* 350:694–705
2. Shapiro AMJ, Lakey JRT, Ryan EA, Korbitt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238
3. Ryan EA, Lakey JRT, Paty BW, Imes S, Korbitt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AMJ (2002) Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–2157
4. Poulson R, Alison MR, Forbes SJ, Wright NA (2002) Adult stem cell plasticity. *J Pathol* 197:441–456
5. Bonner-Weir S (2001)  $\beta$ -cell turnover. *Diabetes* 50:S20–S24
6. Weir GC, Laybutt DR, Kaneto H, Bonner-Weir S, Sharma A (2001)  $\beta$ -cell adaptation and decompensation during the progression of diabetes. *Diabetes* 50:S154–S159
7. Bonner-Weir S, Trent DF, Weir GC (1983) Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 71:1544–1553
8. Brockenbrough JS, Weir GC, Bonner-Weir S (1988) Discordance of exocrine and endocrine growth after 90% pancreatectomy in rats. *Diabetes* 37:232–236
9. Sharma A, Zangen DH, Reitz P, Taneja M, Lissauer ME, Miller CP, Weir GC, Habener JF (1999) The homeodomain protein IDX-1 increases after an early burst of proliferation during pancreatic regeneration. *Diabetes* 48:507–513
10. Terazono K, Uchiyama Y, Ide M, Watanabe T, Yonekura H, Yamamoto H, Okamoto H (1990) Expression of reg protein in rat regenerating islets and its co-localization with insulin in the beta cell secretory granules. *Diabetologia* 33:250–252
11. Smith FE, Rosen KM, Villa-Komaroff L, Weir GC, Bonner-Weir S (1991) Enhanced insulin-like growth factor I gene expression in regenerating rat pancreas. *Proc Natl Acad Sci USA* 88:6152–6156
12. Kim SW, Kim KH, Park SJ, Her HH, Jang JY, Park YH (2001) Endogenous gastrin stimulates regeneration of remnant pancreas after partial pancreatectomy. *Dig Dis Sci* 46:2134–2139
13. Miyasaka K, Ohta M, Masuda M, Funakoshi A (1997) Retardation of pancreatic regeneration after partial pancreatectomy in a strain of rats without CCK-A receptor gene expression. *Pancreas* 14:3991–3999
14. Like AA, Rossini AA (1976) Streptozotocin-induced pancreatic insulinitis: new model of diabetes mellitus. *Science* 193:415–418
15. Fernandes A, King LC, Guz Y, Stein R, Wright CVE, Teitelman G (1997) Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* 138:1750–1762
16. Li L, Seno M, Yamada H, Kojima I (2003) Betacellulin improves glucose metabolism by promoting conversion of intraislet precursor cells to  $\beta$  cells in streptozotocin-treated mice. *Am J Physiol Endocrinol Metab* 285:E577–E583
17. Wang RN, Kloppel G, Bouwens L (1995) Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. *Diabetologia* 38:1405–1411
18. Rosenberg L, Brown RA, Duguid WP (1983) A new model for the development of duct epithelial hyperplasia and the initiation of nesidioblastosis. *J Surg Res* 35:63–72
19. Rafaeloff R, Pittenger GL, Barlow SW, Qin XF, Yan B, Rosenberg L, Duguid WP, Vinik AI (1997) Cloning and sequencing of the pancreatic islet neogenesis-associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. *J Clin Invest* 99:2100–2109

20. Gu D, Sarvetnick N (1993) Epithelial cell proliferation and islet neogenesis in IFN- $\gamma$  transgenic mice. *Development* 118:33–46
21. Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic  $\beta$  cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429:41–46
22. List JF, Habener JF (2004) Glucagon-like peptide 1 agonists and the development and growth of pancreatic  $\beta$  cells. *Am J Physiol Endocrinol Metab* 286:E875–E881
23. Xu G, Stoffers DA, Habener JF, Bonner-Weir S (1999) Exendin-4 stimulates both  $\beta$ -cell replication and neogenesis, resulting in increased  $\beta$ -cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48:2270–2276
24. Pospisilik JA, Martin J, Doty T, Ehses JA, Pamir N, Lynn FC, Piteau S, Demuth HU, McIntosh CH, Pederson RA (2003) Dipeptidyl peptidase IV inhibitor treatment stimulates  $\beta$ -cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* 52:741–750
25. Massague J, Chen YG (2000) Controlling TGF- $\beta$  signaling. *Genes Dev* 14:627–644
26. Hebrok M, Kim SK, Melton DA (1998) Notochord repression of endodermal sonic hedgehog permits pancreatic development. *Genes Dev* 12:1705–1713
27. Furukawa M, Eto Y, Kojima I (1995) Expression of immunoreactive activin A in fetal rat pancreas. *Endocr J* 42:63–68
28. Sanvito F, Herrera PL, Huarte J, Nichols A, Montesano R, Orci L, Vassalli JD (1994) TGF- $\beta$ 1 influences the relative development of the exocrine and endocrine pancreas in vitro. *Development* 120:3451–3462
29. Miralles F, Czernichow P, Scharfmann R (1998) Follistatin regulates the relative proportions of endocrine versus exocrine tissue during pancreatic development. *Development* 125:1017–1024
30. Mashima H, Ohnishi H, Wakabayashi K, Mine T, Miyagawa J, Hanafusa T, Seno M, Yamada H, Kojima I (1996) Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. *J Clin Invest* 97:1647–1654
31. Zhang YQ, Zhang H, Maeshima A, Kurihara H, Miyagawa J, Takeuchi T, Kojima I (2002) Up-regulation of the expression of activins in the pancreatic duct by reduction of the  $\beta$ -cell mass. *Endocrinology* 143:3540–3547
32. Shing Y, Christofori G, Hanahan D, Ono Y, Sasada R, Igarashi K, Folkman J (1993) Betacellulin: a mitogen from pancreatic  $\beta$ -cell tumors. *Science* 259:1604–1607
33. Miyagawa J, Hanafusa T, Sasada R, Yamamoto K, Igarashi K, Yamamori K, Seno M, Tada H, Nammo T, Li M, Yamagata K, Nakajima H, Namba M, Kuwajima M, Matsuzawa Y (1999) Immunohistochemical localization of betacellulin, a new member of the EGF family, in normal human pancreas and islet tumor cells. *Endocr J* 46:755–764
34. Li M, Miyagawa J, Moriwaki M, Yuan M, Yang Q, Kozawa J, Yamamoto K, Imagawa A, Iwahashi H, Tochino Y, Yamagata K, Matsuzawa Y (2003) Analysis of expression profiles of islet-associated transcription and growth factors during  $\beta$ -cell neogenesis from duct cells in partially duct-ligated mice. *Pancreas* 27:345–355
35. Li L, Seno M, Yamada H, Kojima I (2001) Promotion of  $\beta$ -cell regeneration by betacellulin in ninety percent-pancreatectomized rats. *Endocrinology* 142:5379–5385
36. Li L, Yi Z, Seno M, Kojima I (2004) Activin A and betacellulin. Effect of regeneration of pancreatic  $\beta$  cells in neonatal streptozotocin-treated rats. *Diabetes* 53:608–615
37. Umezawa K, Hiroki A, Kawakami M, Naka H, Takei I, Ogata T, Kojima I, Koyano T, Kowithayakom T, Pang HS, Kam TS (2003) Induction of insulin production in rat pancreatic acinar carcinoma cells by conophylline. *Biomed Pharmacother* 57:341–350
38. Ogata T, Li L, Yamada S, Yamamoto Y, Tanaka Y, Takei I, Umezawa K, Kojima I (2004) Promotion of  $\beta$ -cell differentiation by conophylline in fetal and neonatal rat pancreas. *Diabetes* 53:2596–2602
39. Dunbar AJ, Goddard C (2000) Identification of an alternatively spliced mRNA transcript of human betacellulin lacking the C-loop of the EGF motif and the transmembrane domain. *Growth Factors* 18:169–175
40. Taniguchi H, Yamato E, Tashiro F, Ikegami H, Ogihara T, Miyazaki J (2003)  $\beta$ -cell neogenesis induced by adenovirus-mediated gene delivery of transcription factor pdx-1 into mouse pancreas. *Gene Ther* 10:15–23
41. Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292:154–156
42. Lumelsky N, Blondel O, Laeng P, Velasco I, Raven R, McKay R (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to islets. *Science* 292:1389–1394
43. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F (2000) Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 49:157–162
44. Assady S, Maor G, Amit M, Itskovitz-Elder J, Skorecki KL, Tzukerman M (2001) Insulin production by human embryonic stem cells. *Diabetes* 50:1–7
45. Hori Y, Rulifson IC, Tsai BC, Heit JJ, Cahoy JD, Kim SD (2002) Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. *Proc Natl Acad Sci USA* 99:16105–16110
46. Miyazaki S, Yamato E, Miyazaki J (2004) Regulated expression of pdx-1 promotes in vitro differentiation of insulin-producing cells from embryonic stem cells. *Diabetes* 53:1030–1037
47. Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, Carpenter MK (2001) Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol* 19:971–974
48. Blyszczuk P, Asbrand C, Rozzo A, Kania G, St-Onge L, Rupnic M, Wobus AM (2004) Embryonic stem cells differentiate into insulin-producing cells without selection of nestin-expressing cells. *Int J Dev Biol* 48:1095–1104
49. Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Gornelius JG (2000) Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Nat Med* 6:278–282
50. Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, O'Neil JJ (2000) In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci USA* 97:7999–8004
51. Heremans Y, Van De Casteele M, in't Veld P, Gradwohl G, Serup P, Madsen O, Pipeleers D (2002) Recapitulation of embryonic neuroendocrine differentiation in adult human pancreatic duct cells expressing neurogenin 3. *J Cell Biol* 159:303–312
52. Gao R, Ustinov J, Pulkkinen MA, Lundin K, Korsgren O, Otonkoshi T (2003) Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. *Diabetes* 52:2007–2015
53. Suzuki A, Nakauchi H, Taniguchi H (2004) Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting. *Diabetes* 53:2143–2152
54. Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Muller B, Vallejo M, Thomas MK, Habener JF (2001) Multipotent nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 50:521–533
55. Petropavlovskaja M, Rosenberg L (2002) Identification and characterization of small cells in the adult pancreas: potential progenitor cells? *Cell Tissue Res* 310:51–58
56. Seaberg RM, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, Wheeler MB, Korbitt G, van der Kooy D (2004) Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol* 22:1115–1124
57. Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels BM, Raaka BM (2004) Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science* 306:2261–2264

58. Rooman I, Heremans Y, Heimberg H, Bouwens L (2000) Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. *Diabetologia* 43:907–914
59. Song KH, Ko SH, Ahn YB, Yoo SJ, Chin HM, Kaneto H, Yoon KH, Cha BY, Lee KW, Son HY (2004) In vitro transdifferentiation of adult pancreatic acinar cells into insulin-expressing cells. *Biochem Biophys Res Commun* 316:1094–1100
60. Kim SK, MacDonald RJ (2002) Signaling and transcriptional control of pancreatic organogenesis. *Curr Opin Genet Dev* 12:540–547
61. Rao MS, Dwivedi RS, Yeldandi AV, Subbarao V, Tan XD, Usman MI, Scarpelli DG, Nemali MR, Yeldandi A, Thangada S, Kumar S, Reddy JK (1989) Role of periductal and ductular epithelial cells of the adult rat pancreas in pancreatic hepatocyte lineage. A change in the differentiation commitment. *Am J Pathol* 134:1069–1086
62. Terada T, Nakanuma Y (1993) An immunohistochemical survey of amylase isoenzymes in cholangiocarcinoma and hepatocellular carcinoma. *Arch Pathol Lab Med* 117:160–162
63. Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, Wright CV (2002) The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* 32:128–134
64. Fausto N, Campbell JS (2003) The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev* 120:117–130
65. Yang L, Li S, Hatch H, Ahrens K, Cornelius JG, Petersen BE (2002) In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. *Proc Natl Acad Sci USA* 99:8078–8083
66. Yamada S, Terada K, Ueno Y, Sugiyama T, Seno M, Kojima I (2005) Differentiation of adult hepatic stem-like cells into pancreatic endocrine cells. *Cell Transplantation* (in press)
67. Nagai H, Terada K, Watanabe G, Ueno Y, Aiba N, Shibuya T, Kawagoe M, Kameda T, Sato M, Senoo H, Sugiyama T (2002) Differentiation of liver epithelial (stem-like) cells into hepatocytes induced by coculture with hepatic stellate cells. *Biochem Biophys Res Commun* 293:1420–1425
68. Nakajima-Nagata N, Sakurai T, Mitaka T, Katakai T, Yamato E, Miyazaki J, Tabata Y, Sugai M, Shimizu A (2004) In vitro induction of adult hepatic progenitor cells into insulin-producing cells. *Biochem Biophys Res Commun* 318:625–630
69. Cao LZ, Tang DQ, Horb ME, Li SW, Yang LJ (2004) High glucose is necessary for complete maturation of PDX1-VP16-expressing hepatic cells into functional insulin-producing cells. *Diabetes* 53:3168–3178
70. Suzuki A, Nakauchi H, Taniguchi H (2003) Glucagon-like peptide 1 (1-37) converts intestinal epithelial cells into insulin-producing cells. *Proc Natl Acad Sci USA* 100:5034–5039
71. Kojima H, Nakamura T, Fujita Y, Kishi A, Fujimiya M, Yamada S, Kudo M, Nishio Y, Maegawa H, Haneda M, Yasuda H, Kojima I, Seno M, Wong NC, Kikkawa R, Kashiwagi A (2002) Combined expression of pancreatic duodenal homeobox 1 and islet factor 1 induces immature enterocytes to produce insulin. *Diabetes* 51:1398–1408
72. Yoshida S, Kajimoto Y, Yasuda T, Watada H, Fujitani Y, Kosaka H, Gotow T, Miyatsuka T, Umayahara Y, Yamasaki Y, Hori M (2002) PDX-1 induces differentiation of intestinal epithelioid IEC-6 into insulin-producing cells. *Diabetes* 51:2505–2513
73. Brittan M, Wright NA (2002) Gastrointestinal stem cells. *J Pathol* 197:492–509
74. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105:369–377
75. Brazelton TR, Rossi FM, Keshet GI, Blau HM (2002) From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 290:1775–1779
76. Ianus A, Holz GG, Theise ND, Hussain MA (2003) In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 111:843–850
77. Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen S, Gray DA, Bhatia M (2003) Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol* 21:763–770
78. Choi JB, Uchino H, Azuma K, Iwashita N, Tanaka Y, Mochizuki H, Migita M, Shimada T, Kawamori R, Watada H (2003) Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. *Diabetologia* 46:1366–1374
79. Lechner A, Yang YG, Blacken RA, Wang L, Nolan AL, Habener JF (2004) No evidence for significant transdifferentiation of bone marrow into pancreatic  $\beta$  cells in vivo. *Diabetes* 53:616–623
80. 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 (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418:41–49
81. Tang DQ, Cao LZ, Burkhardt BR, Xia CQ, Litherland SA, Arkinson MA, Yang LJ (2004) In vivo and in vitro characterization of insulin-producing cells obtained from urine bone marrow. *Diabetes* 53:1721–1732
82. Oh SH, Muzzonigro TM, Bae SH, LaPlante JM, Hatch HM, Petersen BE (2004) Adult bone marrow-derived cells transdifferentiating into insulin-producing cells for the treatment of type I diabetes. *Lab Invest* 84:607–617
83. Okumura K, Nakamura K, Hisatomi Y, Nagano K, Tanaka Y, Terada K, Sugiyama T, Umeyama K, Matsumoto K, Yamamoto T, Endo F (2003) Salivary gland progenitor cells induced by duct ligation differentiate into hepatic and pancreatic lineages. *Hepatology* 38:104–113
84. Hisatomi Y, Okumura K, Nakamura K, Matsumoto S, Satoh A, Nagano K, Yamamoto T, Endo F (2004) Flow cytometric isolation of endodermal progenitors from mouse salivary gland differentiate into hepatic and pancreatic lineages. *Hepatology* 39:667–675
85. Wei JP, Zhang TS, Kawa S, Aizawa T, Ota M, Akaike T, Kato K, Konishi I, Nikaido T (2003) Human amnion-isolated cells normalize blood glucose in streptozotocin-induced diabetic mice. *Cell Transplant* 12:545–552
86. Kodama S, Kuhlreiber W, Fujimura S, Dale EA, Faustman DL (2003) Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* 302:1223–1227