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Research status and prospect of stem cells in the treatment of diabetes mellitus

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Beta cell mass and function are decreased to varying degrees in diabetes. Islet cell replacement or regenerative therapy may offer great therapeutic promise to people with diabetes. In addition to primary pancreatic β cells, recent studies on regeneration of functional insulin producing cells (IPCs) revealed that several alternative cell sources, including embryonic stem cells, induced pluripotent stem cells and adult stem cells, can generate IPCs by differentiation, reprogramming, and trans-differentiation. In this review, we discuss stem cells as a potential alternative cell source for the treatment of diabetes.

cell therapy, diabetes, stem cells, insulin producing cells

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Diabetes mellitus is a glucose metabolism disorder that is caused by insulin secretion deficiency or dysfunction, and is characterized by the elevation of blood glucose. At present, there are approximately 346 million adult diabetic patients worldwide. By 2030, the number of diabetic patients is expected to reach 4 billion [1]. Based on etiology, diabetes is divided into three categories. (i) Type I diabetes (T1DM) is an autoimmune endocrine disease mediated by T cells. It is generally believed that immune dysfunction is the main pathogenesis of T1DM, and that T1DM has a strong genetic predisposition. When the susceptible population is exposed to environmental factors, T cells alter their function and secrete large amounts of interleukin-2. Cytokines such as γ-interferon trigger an inflammatory response in the pancreatic islets, which damages β cells, resulting in dysfunction and insulin secretion deficiency [2], thus triggering T1DM [2]. (ii) Type II diabetes mellitus (T2DM) consists of hyperinsulinemia and insulin resistance. Under normal conditions, insulin reduces blood glucose levels via activation of insulin signaling pathways in the muscle, liver and adipose tissues. However, in patients with T2DM, the insulin sensitivity is decreased in such peripheral tissues. Persistent high blood glucose levels and stimulation of β cells will damage β cells, leading to serious defects of β cell function and impairment of insulin secretion. Insulin resistance and the incidence of T2DM may result from heredity, obesity, poor lifestyle and other factors. However, its molecular mechanism remains unknown [3]. Although the pathogenesis of T1DM differs from that of T2DM, the two exhibit similar pathological changes including glucose intolerance, hyperglycemia, hyperlipidemia, and related organ complications. (iii) Gestational diabetes mellitus is a special type of diabetes mellitus. Pregnancy leads to physiological insulin resistance, increased hormone secretion under various anti-insulin effects, and corresponding increased insulin requirements with peaks in the second trimester of pregnancy. Because of the limitation of insulin secretion, if compensa-

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tory physiological changes are not maintained, pregnant women may develop hyperinsulinemia or hyperglycemia. Following childbirth, blood glucose levels return to normal. Gestational diabetes mellitus significantly increases neonatal and intrauterine fetal mortality, and affects the health of both the mother and baby.

Although modern medicine has made considerable progress in the treatment of diabetes, lifelong exogenous insulin therapy is still required for management of T1DM and T2DM, thereby increasing the occurrence of infection, ketoacidosis, hypoglycemia, chronic complications, as well as retinal, nervous, renal, cardiovascular and cerebrovascular illnesses [4]. Although pancreas and pancreatic islet transplantation have made remarkable progress in establishment of an endogenous insulin secretion system [5,6], the current significant shortage of donor organs has hindered its extensive development. Therefore, the development of stem cell transplantation may be the ideal choice for the treatment of T1DM.

Stem cells are a type of cell with strong self-renewal ability and the potential for differentiation. In theory, pluripotent stem cells have the ability to differentiate into all cell types of the body. Therefore, they are an ideal cell source for regenerative medicine and tissue engineering. It has been found that stem cells are capable of replacing aging, damaged or dead cells in mature organs. Therefore, stem cells have been increasingly used in the treatment of various end-stage refractory diseases (e.g., myocardial infarction, severe burns, and degenerative diseases of the nervous system). This article reviews the research progress made in recent years regarding stem cell-derived insulin-producing cells (IPCs) for the treatment of diabetes mellitus.

1 Research status of stem cell replacement therapy for diabetes mellitus

Since insulin was first discovered and isolated in 1920, exogenous insulin replacement has become the main treatment for the control of plasma glucose levels. Diabetes is caused by the loss of a specific cell function. In theory, pancreas transplantation may be used as a cell replacement therapy to compensate for the loss of β cell function. From 1966 to 2008, a total of more than 30000 pancreas transplantations were performed [7]. Because pancreas transplantation is complex and accompanied by many postoperative complications, researchers have studied islet transplantation, and confirmed increased levels of serum C-peptide after transplantation. Islet transplantation is a simple and minimally invasive process. In the Edmonton protocol of 2000, seven patients refrained from insulin therapy for an average of 11.9 months [8]. Although pancreatic and pancreatic islet transplantation has achieved positive therapeutic effects, the significant shortage of donor organs remains an issue.

Even though pancreas transplantation and islet transplantation are not the conventional treatment for diabetes mellitus, successful transplantation of β cells has resulted in a new breakthrough in stem cell therapy. With the potential of multiple differentiating and self-renewal, stem cells have demonstrated superiority in cell replacement therapy, as well as unique roles in developmental biology and other fields of study. Stem cell therapy has the following advantages for the treatment of diabetes mellitus. It is not limited by the donor source, and can provide a long-term source for functional β cells. Stem cell-derived islet cells can avoid allograft rejection, thereby reducing the necessity for immunosuppressive therapy. They are also capable of secreting a variety of cytokines and improving the local microenvironment of pancreatic lesions, thereby improving the prognosis. The stem cells used for β cell replacement include pancreatic stem cells, hepatic stem cells, mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs).

1.1 Pancreatic stem cells

The islet contains four types of functional cells: glucagon-secreting α cells, insulin-secreting β cells, somatostatin-secreting δ cells, and pp cells. Promoting the proliferation of existing β cells is the most direct and convenient source of new β cells. During postnatal development, β cells proliferate in great numbers with physiological changes, such as pregnancy and obesity, suggesting that these cells are capable of self-renewal. Studies have shown that, after adult rats undergo resection of 90% of the pancreas, pancreatic tissues and β cells can regenerate efficiently [9]. However, it remains controversial whether new β cells are derived from the proliferation of existing β cells or the differentiation of pancreatic stem cells.

Wang [10] found that ligation of the pancreatic duct of rats results in the formation of a large number of new islets, which mainly consist of β-like cells, and that the formation of new β cells cannot be completely attributed to the proliferation of existing β cells. The researchers therefore assumed that new β cells are derived from stem cells, which may originate from the differentiation and proliferation of pancreatic duct cells. Xu [11] found that neurogenin 3 (NGN3)-positive cells are present after pancreatic duct ligation, and under suitable conditions, these cells can differentiate into IPCs in vitro. Inada [12] also supported the theory that the pancreatic duct, acinus and islet are postnatally derived from pancreatic duct epithelial cells. Nevertheless, whether the pancreatic duct epithelium is the source of β cells remains controversial. Furayama [13] performed several experiments *in vivo* to stimulate pancreatic regeneration, and found no pancreatic progenitor cell-derived β cells using a lineage tracing method. During embryonic development, it is assumed that endocrine cells are derived from the pancreatic duct epithelial cells and separate from SRY-related high mobility group-box gene 9-positive progenitor cells, and

that β cells are sustained by self-renewal of existing β cells. Of course, the lineage tracing methods currently used have limitations. Because the labeling efficiency may be very low, most of the ductal cells are not labeled. Therefore, it is uncertain whether β cell progenitors are present among the ductal cells.

Scientists have also discovered pluripotent cells in the pancreatic islets of humans and rats, which may be categorized into various types of pancreatic endocrine cells. However, Bonner [14] believed that, to a certain degree, the processes of β cell growth and regeneration do not involve specific progenitor cells, but rather slow replication of mature β cells. Brennand [15] confirmed that β cells are homogenous cells that can sustain themselves through slow replication. So directly promoting the proliferation rate of β cell maybe the best way to obtain enough β cell for cell therapy. However, although it has been found that a large number of factors promote β cell proliferation through different mechanisms, such as glucose transporter protein 1 and corticotropin-releasing factor receptor type 1 [16], the detailed mechanisms remain unclear. Elaborating the mechanism of β cell self-renewal is important to obtain new β cells from existing β cells. However, at present, many difficulties still remain for obtaining adequate numbers of new β cells from the existing β cells. Although the sources of pancreatic stem cells are controversial. Simon et al. [17] isolated pancreatic-duodenal homeobox 1 $(PDX1)^+$/$insulin^+$/glucose transporter isoform 2^- cells from the pancreatic duct and pancreatic islet tissues of transgenic mice by lineage tracing and cell sorting. These cells formed spheres and expanded clonally *in vitro*, and could differentiate into various types of pancreatic cells. Transplantation of this cell population ameliorated diabetes in streptozocin (STZ)-treated mice. Thus, the authors defined this group of cells as pancreatic progenitor cells.

Exocrine gland cells, the major cell type in the pancreas, are one of the alternative sources of β cells. PDX1 is a specific transcription factor of pancreatic progenitor cells and a gene with spontaneous induction and self-enhancing expression. Similar to endocrine cells, exocrine cells also originate from PDX1-positive pancreatic progenitor cells, and under suitable conditions, can be induced to possess the phenotypic characteristics of endocrine cells [18]. In 2008, Zhou [19] used three transcription factors, PDX1, NGN3 and macrophage activating factor A, to convert pancreatic exocrine cells into β-like cells. Although these cells are very different from intrinsic β cells in terms of size, shape and ultrastructure, they are capable of secreting insulin and alleviate the symptoms of hyperglycemia.

1.2 Embryonic stem cells and induced pluripotent stem cells

Early studies of ESCs mainly focused on mouse ESCs to avoid ethical controversies. Through in vitro differentiation, a small amount of pancreatic cells (1%–3%) can be obtained [20]. However, although in vitro differentiation can generate pancreatic endocrine cells, it is not possible to precisely control the differentiation or determine whether the insulin in the cells is derived from synthesis or uptake from the induction medium [21]. It is generally accepted that the maintenance of β cell function depends on the microenvironment of the pancreatic islet, and especially on the interaction among β, α and δ cells. Kahan [22] found that mouse ESCs can only maintain an optimal state of differentiation in the presence of islet cells, and particularly emphasized the importance of the microenvironment in the differentiation of ESCs [23]. Based on this perspective, researchers have transplanted ESC-derived pancreatic endocrine progenitor cells into mice to observe their maturation and therapeutic effects.

Brolen [21] used human ESCs to obtain pancreatic progenitor cells. Although the cells expressing PDX1 were confirmed to be pancreatic progenitor cells, they were unable to secrete insulin and showed no glucose reactivity. However, these cells continued to differentiate and mature after transplantation into a mouse, thereby possessing the abilities of insulin synthesis and processing. Together with similar results obtained by Kahan, this study showed that human ESCs are capable of responding to environmental signals in the body and differentiating into endocrine islet cells. These results also provide evidence for the relationship between the mouse in vivo environment and human cells, suggesting that a conserved mechanism of islet development exists in humans and mice.

The findings of D'Amour [23] are an important milestone for the investigation of human ESC-derived IPCs. They first differentiated ESCs to IPCs in five stages: definitive endoderm, forgut, hindgut, pancreatic endoderm, and then endocrine cells. The steps of the differentiation protocol are based on the developmental biology of the pancreas. The insulin content of these cells is similar to that in human fetal islet cells. The cells are capable of secreting insulin under stimulation by potassium chloride and various other secretagogue agents, but unfortunately do not respond to glucose stimulation. Glucose-stimulated insulin secretion is an important indicator to evaluate the functional maturity of β cells. The neonatal islet has normal functions at about 4 weeks after birth, indicating that the endocrine cells obtained by D'Amour were still at the immature islet stage. Because of the current lack of understanding of the mechanism of functional islet maturation *in vivo*, an effective method to promote islet maturity in vitro has yet to be found. To verify the maturity and therapeutic effects of these cells [24], ESCs have been induced to pancreatic progenitor cells, which were equivalent to fetal 6- to 9-week pancreatic tissue, and then transplanted into mice for further maturation. At 40 days after transplantation, the cells were stimulated

by glucose, and C-peptide release was detected in recipient mice, suggesting de novo synthesis of human insulin in mice. More importantly, these cells were able to correct STZ-induced hyperglycemia in mice. Using an appropriate induction protocol, these studies have shown that insulin-secreting islet-like cells obtained in vitro and then transplanted in vivo may continue their maturation, and achieve the normal functions of β cells. Following the study of D'Amour, researchers began focusing more on improving the efficiency of ESC differentiation into pancreatic progenitor cells. Chen [25] found that the small molecule indolactam V is capable of promoting the differentiation of human ESCs into PDX1-positive cells both in vitro and *in vivo*, which has provided a new method to obtain a large number of ESC-derived β cells.

In addition to ESCs, iPSCs also provide hope for the treatment of diabetes. Bar-Nur [26] differentiated β cell-derived iPSCs into IPCs. These cells can secrete insulin upon stimulation by glucose and after transplantation into T2DM mice to normalize glycated hemoglobin levels and correct high blood glucose, thus providing evidence for the clinical application of iPSCs in T1DM/T2DM treatments. Currently, the most common strategy for iPSC induction involves genome-integrating viruses that carry an intrinsic risk of tumor formation because of the spontaneous reactivation of viral transgenes. With the improvement of research methods, the safety of iPSCs and their possible clinical applications have been greatly enhanced. Stadtfeld [27] established a method to produce iPSCs using an adenovirus. In this method, exogenous genes are briefly and highly expressed within the cells, but not integrated into the genome, thus ensuring the safety of iPSCs in clinical application. Another method to produce iPSCs is the addition of appropriate growth factors to the culture medium of spermatogonial stem cells for reprogramming to iPSCs that can differentiate into cells of all three germ layers [28]. Because exogenous genes are not used in the conversion process, there is no exogenous gene integration or its related risks. In 2010, at the annual meeting of the American Society for Cell Biology, it was shown that spermatogonial stem cell-derived iPSCs can be induced to β-like cells that react to physiological levels of glucose *in vitro* and reduce the glucose levels of diabetic mice *in vivo*.

Despite the encouraging results, these studies are still in the early stages of using ESCs and iPSCs in treatments for diabetes mellitus. The direct differentiation of pancreatic endocrine cells and the mechanism of functional islet maturation remain unclear. Furthermore, another issue of concern among the current studies is the lack of observation of the long-term efficacy and safety analyses of ESCs and iPSCs in treatments for diabetes, which signifies that a large amount of research must be performed before human clinical trials can be conducted.

1.3 Mesenchymal stem cells

Bone marrow contains two different types of stem cells, namely hematopoietic stem cells (HSCs) and MSCs. Investigators successfully differentiated these two stem cell types into various types of cells, suggesting that they possess multipotency. Because bone marrow stem cells are easily obtained by clinical procedures, bone marrow cells have become one of the research focuses of cell therapy for diabetes [29].

At present, research focusing on the treatment of diabetes with bone marrow stem cells includes the following findings [30]. Peripheral intravenous injection of MSCs can inhibit autoreactive T cells and alleviate the TIDM autoimmune reaction. Urban [31] showed that the immunomodulatory effect of MSCs is one of the mechanisms that assist β cell regeneration. Investigators injected a mixture of HSCs and MSCs into the bone marrow of mice with STZ/radiation-induced injury, which successfully enabled the high blood glucose levels of the mice to return to normal. It is worth noting that the investigators found that MSCs were able to inhibit the proliferation of pancreatic β cell-specific T cells, thereby reducing the damage caused by T cells on new β cells and increasing β cell regeneration to a certain degree. Combined with islet transplantation, this method enables the implant to evade immune surveillance, and improves the survival rate of the implant. Bone marrow MSCs have been shown to inhibit autoimmunization and alloimmunization. Ding [32] took advantage of this feature to transplant bone marrow MSCs and allogeneic islets, a method that may prolong the survival time of the transplanted islets and maintain normal blood glucose for a significant period of time. It was assumed that MSCs play a role in immune evasion and promote the survival of the transplanted islet by synthesis and secretion of matrix metalloproteinase 2 and 9 into the extracellular matrix. Bone marrow MSCs have also been differentiated into IPCs for cell replacement therapy. In early studies, researchers focused on the differentiation potential of bone marrow stem cells for their therapeutic effects. It was confirmed that bone marrow MSCs can differentiate into IPCs *in vitro* and *in vivo*, and correct high blood glucose levels in diabetic mice [33,34]. However, scientists questioned the ability of bone marrow MSCs to differentiate into IPCs because differentiated bone marrow cells only account for a small portion of the transplanted cells [35]. It is also important to observe decreased levels of plasma glucose and increased levels of insulin before the presence of MSC derived IPCs. Therefore, the success of bone marrow MSCs in the treatment of diabetes is likely to be achieved by endocrine or paracrine mechanisms that assist the existing β cells, stimulate proliferation and insulin secretion, and effectively control blood glucose levels, rather than by direct differentiation into β cells.

These *in vivo* studies elaborate on the potential of bone marrow stem cells to stimulate β cell regeneration in the injured pancreatic tissue, and various research perspectives have revealed different mechanisms of the effects of bone marrow stem cells. Although not all mechanisms can be elaborated in detail, bone marrow stem cells are able to stimulate the regeneration of damaged pancreatic β-cells, or become an alternative β cell source after induction. There is no doubt that bone marrow stem cells have therapeutic effects on diabetes. Bone marrow stem cells are an ideal choice for future cell therapy and the treatment of diabetes.

1.4 Hepatic stem cells

In developmental biology, because the liver and pancreas originate from the endoderm and possess common progenitor cells, it has been speculated that liver cells can be used as an alternative source of β cells [36]. Exogenous PDX1 and NGN3 have been expressed in the mouse liver by adenoviral transduction, which in turn induced pancreatic endocrine and exocrine gene expression in liver cells. The transdifferentiated cells can survive in the liver for a long period of time. New pancreatic tissue clusters formed around the central veins of the liver, where they released insulin, but did not affect the normal liver function. More importantly, these insulin-secreting liver tissues corrected STZ-induced hyperglycemia to maintain normal blood glucose levels for a period of 8 months [37,38]. However, Yang [39] found that, in some cases, PDX1-reprogrammed liver tissue is only capable of forming pancreatic β progenitor cells that only further differentiate into functional IPCs under a state of high glucose *in vitro* or *in vivo*. To date, no evidence has been found which shows that the modified liver cells proliferate *in vitro* and can reach a sufficient number of functional cells for transplantation therapy.

Nevertheless, these liver tissues still have good prospects, and are not controversial like pancreatic stem cells. Because of its easy collection from biopsy and strong regenerative capacity, liver tissue has become an ideal source of seed cells for transdifferentiation. In the future, an effective method for *in vitro* expansion of cells transdifferentiated from liver will be required. The safety of transdifferentiated liver tissue induced in the human body also requires further exploration.

2 Problems and prospects of cell therapy for diabetes mellitus

Although the results of many studies have confirmed that stem cells can enable diabetic patients to maintain normal blood sugar levels independently from insulin administration for a period of several years, many problems exist for the application of stem cells in the clinical treatment of diabetes. The first of these challenges is the cell source. Each recipient requires approximately 100000 pancreatic islets for transplantation, and a large number of alternative cells must also be prepared *in vitro*. Despite the fact that several research teams have used various methods to differentiate stem cells into β-like cells, when used in clinical application, these cells are far from meeting the functional requirements because of relatively low differentiation efficiency to the target cells. Therefore, based on further characterization of pancreatic islet development and differentiation, a relatively effective method for promoting the differentiation of stem cells into functional β cells should be developed, including large-scale production of transplantable cells. Second, there are numerous side effects associated with stem cell therapy, which cannot be ignored. Kroon [24] has shown that the probability of generating teratomas or other tissue components exceeds 15% after transplantation of human ESC-derived pancreatic cells in mice. Other studies showed that in vitro proliferation of MSCs increases the risk of tumor formation and metastasis [40,41]. These safety issues must be carefully considered and evaluated before cell therapy can be used in clinical trials, and appropriate countermeasures must be performed. In addition, there is the problem of immune rejection that is associated with stem cell therapy [42]. After receiving allogeneic cell therapy, patients must undergo immunosuppressive therapy. However, immunosuppressive drugs have a role in insulin resistance and inhibition of insulin secretion, thus increasing the difficulty of implanting cells to maintain proper blood sugar levels. In addition, immunosuppressive drugs increase the risk of malignancy, although this effect is only found in kidney transplantation. For the treatment of T1DM, after transplantation of alternative autologous cells, the cells will be quickly damaged by autoantibodies unless a method to protect such cells is found. For the treatment of T2DM, the cells will be scrutinized because the dysfunction of β cells is typically closely related to genetic abnormalities or changes. Transplantation of β-like cells from T2DM patients may not be able to control blood glucose levels for a significant amount of time.

Any new treatment method for clinical applications will face many problems. Although these problems still remain, we have every reason to believe that islet cell replacement therapy and regenerative medicine will eventually be widely used in clinical practice, and that these methods will result in new clinical efficacy or possibly even a cure for diabetes, thus mitigating the suffering and reducing the social burden of diabetic patients.

Table 1 Summary of cell therapies for diabetes mellitus[a)]

Type		Source	Induce strategies	Transplantation site & cell number	*In vivo* GRIR	Advantages	Limitations
ES cells, e.g. CyT49,H8,H9 [24,25]		Inner cell mass	Stepwise differentiation based on the developmental biology	(2–7)×10^6 renal capsular space; 1.5×10^6–1×10^7 epididymal fat pads	Yes	Unlimited supply	Risk of formation teratoma; ethical controversy
iPS cells [26,43]		Skin cells; pancreatic islet beta cells; spermatogonial stem cells	Stepwise differentiation based on the developmental biology	(2–7)×10^6 renal capsular space	Yes	Autologous source; unlimited supply; avoiding ethical controversy	Epigenetic memory
Adult stem cell	Hepatic cells [38,44,45]	Hepatic oval cell; biliary tree stem cell; WB cell line	Adenovirus transfection; stepwise differentiation	(2–7)×10^6 renal capsular space	Yes	Autologous source; easy harvest; endodermal origin	Difficulty achieving sufficient *in vitro* cell mass for use inbtransplant therapy; epigenetic memory
	Pancreatic stem cells [10,17,19]	Pancreatic exocrine cells, ductal cells and islet cells	PDX1+cells by FACS; adenovirus transfection	350–500 PMP spheres, renal capsular space	Yes	Autologous source	Difficult proliferation
	Bone MSCs [33–35]	Bone marrow, adipose tissue, umbilical cord, periosteum, etc.	Stepwise differentiation; differentiation *in vivo*	4.2×10^7 tail vein; 2×10^6 renal capsular space and splenic vein	Yes	Easy harvest; promoting vascularization; induce regeneration of pancreatic IPC in pancreas; immunoprotective	Risk of malignant transformation *in vitro*; risk of tumor development after transplantation; difficulty differentiation directly

a) GRIR, glucose responsive insulin release; ES cells, embryonic stem cells; MSCs, mesenchymal stem cells; iPS cells, induced pluripotent stem cells.

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