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Genetic Modification of Stem Cells in Diabetes and Obesity

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Abstract

Genetic modification, or gene transfer, represents a method of treatment for several diseases. It has been used extensively in the context of cardiovascular diseases; however, its role in the context of metabolic diseases, such as diabetes and obesity, has remained largely unexplored. In this chapter, we will review the use of adult stem cells, focusing on endothelial progenitor cells (EPCs) and mesenchymal stromal cells (MSCs), in the context of diabetes. We have highlighted the use of viral vectors, particularly DNA viruses, as a tool for genetic modification to help stem cells survive and resist apoptosis in a hyperglycemic environment. We then discuss genetic modification of EPCs and MSCs to treat complications of diabetes and obesity. Although there are several unanswered questions in the field of metabolic diseases, the future application of gene transfer technology along with genetic modification of stem cells prior to the therapy holds significant therapeutic promise.

Keywords: gene transfer, endothelial dysfunction, apoptosis, vascular complications, superoxide dismutase, stem cells, diabetes, viral vectors, mesenchymal stromal cells, endothelial progenitor cells

1. Introduction

In the past few decades, there have been many important advances in the treatment of diabetes and its associated complications that have dramatically improved the lives of patients. Despite this progress, there are still many unresolved issues with pharmaceutical therapies and, therefore, a cure is still elusive. The estimated number of adults worldwide with diabetes is

over 400 million and it has been predicted that between 2010 and 2030, there will be an increase in the number of people with diabetes in both developing and developed countries (69 and 20%, respectively) [1, 2]. With this alarming rise, there is also a dramatic increase in patients who have cardiovascular comorbidities [3].

Currently, injections of exogenous insulin are still the mainstay therapy for type 1 diabetes. Although whole pancreas or islet transplantations have become clinically available, this approach is limited by the short number of donors, the adverse effects of immunosuppressive therapy and possibility of acute and chronic transplant rejection is high. For type 2 diabetes, there are a multitude of medications on the market, yet a tight glycemic control remains a challenge for patients.

Here, we will discuss the use of two different adult human stem cells: endothelial progenitor cells (EPCs) and mesenchymal stromal cells (MSCs) (**Table 1**) and the role they can play to treat diabetes and its complications.

Adult stem cells:	EPCs	MSCs
Origin	Bone marrow and hematopoietic cells	Any mesenchymal tissue
Harvested from	Peripheral blood or bone marrow	Fat, bone, cartilage, muscle, liver, bone marrow
Surface markers*	CD34/133/ KDR (+) or combination, CD45(-)	CD73/90/ 105 (+) and negative for CD34/45/ 11b/14/19 and HLA class II
Immunogenicity	High incidence of rejection	Low, cells can be transplanted across species
Cell shape/size	Size of a white blood cell, circular	Similar in size to a muscle cell, spindle shaped
Responds to	Acute injury, necessary for vasculogenesis	Chronic need of the body to maintain mesenchymal tissues
Helps to form	Endothelium <i>Progenitor cell</i>	Any adult mesenchymal tissue: fat, bone, cartilage, muscle. <i>Multipotent</i>
Gluko-toxicity	High incidence of apoptosis	Chronic injury, leads to intra-cellular ROS accumulation

*As per, International Society for Cellular Therapy (ISCT).

Table 1. Characteristics of EPCs and MSCs.

EPCs, which can be harvested from peripheral blood and from bone marrow, are broadly classified as hematopoietic stem cells. These cells come in the circulation in increased numbers from bone marrow (BM), in response to an acute ischemia or injury. MSCs, on the other hand, are resident in all mesenchymal tissue and bone marrow and unlike EPCs, these cells are multipotent [4]. In adults, EPCs are acute response stem cells which increase in number in order to increase tissue perfusion, whereas MSCs are cells that are necessary for chronic regeneration in a mesenchymal tissue. Both EPCs and MSCs have been used for the therapy of multiple disease states. For the purpose of this review, we will discuss therapies involving MSCs and EPCs in the context of diabetes, obesity and myocardial ischemia including the use of genetically modified stem cells where genetic modification has been carried out using viral vectors.

2. Endothelial progenitor cells

As previously mentioned, EPCs are precursors of the endothelium. The specific markers that have been used to define EPCs are CD34+, CD34/KDR+, and CD133+ [5–7].

The endothelium plays an important role in the regulation of vascular tone and homeostasis through its paracrine properties [8, 9]. The roles of the endothelium include the following: forming a blood vessel wall monolayer, maintain vasodilation and promote angiogenesis. Endothelial dysfunction leads to vasoconstriction and inflammation [10]. When the endothelial function is impaired, there is a reduced production of nitric oxide (NO) and an increased production of reactive oxygen species (ROS) [11]. This leads to cellular apoptosis and aggravated inflammation which promotes atherosclerosis through platelet aggregation, adhesion and plaque formation [10, 12]. Therefore, endothelial dysfunction is a major cause of cardiovascular diseases (CVD) such as stroke, myocardial infarction (MI) and peripheral vascular disease (PVD) [13, 14].

A prime cause of endothelial dysfunction is diabetes mellitus, which is caused by hyperglycemia and glucose intolerance due to insulin deficiency/resistance [15]. Chronic hyperglycemia, associated with both type I and type II diabetes mellitus, leads to endothelial dysfunction and cardiovascular disease (CVD) [16]. A hyperglycemic state damages the endothelium and impairs EPC number and function. This interferes with vasculogenesis, poor healing and impaired overall endothelial function [17]. Therefore, preventing hyperglycemic environment induced damage to endothelium and EPCs is very important in order to prevent endothelial dysfunction and associated cardiovascular disease.

One approach to treat endothelial dysfunction in diabetes is non-pharmacological therapy. Lifestyle changes, such as diet and exercise can help to reduce body weight and improve endothelial function. We have previously reported a significant improvement in endothelial function, measured by flow mediated dilatation and endothelial progenitor cells (EPCs), specifically CD34+ cell number, function and gene expression after 6 weeks of aerobic exercise [18].

Pharmacological therapies that improve endothelial function include calcium antagonist, beta blocker, angiotensin-converting-enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), statins, insulin resistance reducing drugs and even erythropoietin [19].

Another manner in which endothelium dysfunction can be treated is with EPCs themselves. Several studies were performed to treat endothelial dysfunction by transplantation of EPCs and it was determined that these cells could be a powerful tool for cell-based therapy primarily through their paracrine properties. EPCs from bone marrow (BM) can circulate in peripheral blood and repair damaged endothelium either by transforming into mature endothelium or through their paracrine properties [20, 21]. These cells can also be used as a biomarker for endothelial dysfunction [18].

Unfortunately, the number of EPCs found in diabetic patients is reportedly lower than their healthy patient counterpart [22]. In addition, the EPCs also lose their ability to migrate to

damaged areas [16]. In order to prevent that scenario, researchers have looked into modifying the EPC genes themselves. Di Stefano and Cols. reported that a gene deletion can reverse the high-glucose caused defects of BM-derived EPCs and increase angiogenesis by reducing oxidative stress-related apoptosis [22]. Manipulation of the gene expression of EPCs may facilitate an increased efficacy of diabetic patient's endothelial progenitor cells [22]. These cells could then be used to treat many of the side effects associated with diabetes.

One of the major side effects of high glucose exposure, or diabetes, is ischemia. Ischemia could be responsible for poor cardiac function as well as peripheral vascular disease (PVD). We have previously demonstrated the use of genetically modified rat EPCs to treat myocardial infarction (MI)-related complications in Sprague-Dawley rats. Adeno-associated virus (AAV) mediated IGF-1 expression reportedly increased cardiac function by increasing cardiomyocyte proliferation and capillary density in the myocardium and by decreasing cardiomyocyte apoptosis in a myocardial infarction rat model [7]. Both adenovirus and recombinant AAV are DNA virus which, remains as an episomal character without integrating with host genome. This reduces chances of mutagenesis and avoids unnecessary prolonged over-expression [4, 23]. Another study showed that genetically modified human EPCs (hEPCs) with a tissue kallikrein (TK) gene upregulation helps hEPCs to minimize oxidative stress-related apoptosis, as well as, enhances vascularization and offers protection against ischemia-induced MI [24]. Similarly, VEGF-165 gene upregulated EPCs also significantly improved cardiac function by promoting angiogenesis in rats with an ischemic myocardium [25]. In order to address peripheral vascular disease, Goto and cols. examined the overexpression of beta integrin in EPCs and found that it helped to promote angiogenesis and improve blood flow in mouse hind limbs [26]. In order to combat the increase in apoptosis caused by high glucose environments, the silencing of apoptotic genes p53 has been explored. Silencing p53 showed better survivability of the EPCs in high glucose. When p53 silenced EPCs were transplanted into a diabetic mouse model, an increased blood flow and vascularization was found [27, 28]. Another study also demonstrated increased blood flow in ischemic mouse hind limb post-genetic modification of EPCs. This study showed that the inhibition of glycogen synthase kinase-3beta (GSK3beta) boosts survival of EPCs and increases migration of vascular endothelial cells to the ischemic hind limb—thereby improving angiogenesis [29].

Systemic inflammation is directly related to diabetes. It has therefore been hypothesized that a reduction in inflammation could positively affect endothelial dysfunction. An interesting study showed that the genetic modification of EPCs, to overexpress A20 (an anti-inflammatory protein), decreases endothelial inflammation. This could be a cell-based therapy to reduce inflammation caused by diabetes [30].

Erectile dysfunction is another complication of diabetes that can manifest due to vasculopathy and neuropathy that could also be treated with genetic modification. Cell-based treatment for diabetes mellitus-induced erectile dysfunction (DMED) is possible by over expressing human telomerase reverse transcriptase (hTERT) EPCs. Endothelial nitric oxide synthase (eNOS) expression increased significantly after EPCs-hTERT treatment as well as the reduction in apoptosis and the resistance to oxidative stress. Therefore, EPC-hTERT treatments helped to improve erectile function in DMED rats [31].

3. Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) have attracted scientific and clinical interest for the role they could play to establish an effective therapy in regenerative medicine. Considering the regenerative and immunomodulatory properties of MSCs, these cells have been considered as a promising cell-based therapy to treat diabetes [32]. MSCs can be obtained from different sources such as umbilical cord blood, bone marrow, adipose tissue, pancreatic islet, fetal liver, lung and other tissues [33–35]. These cells are easily expanded in culture. The specific markers that are used to define the MSCs are CD44, CD73, CD90, CD105 but not CD31, CD34, CD45 [36]. MSCs can be differentiated into osteoblasts, adipocytes, myocytes and chondrocytes [37].

When transplanted into a streptozotocin (STZ)-induced diabetic mouse model, bone marrow-derived MSCs were able to improve β -cell mass and increased insulin production, which resulted in a reversal of hyperglycemia [38–41]. Additionally, similar results were found when bone marrow-derived MSCs were obtained from donors with newly (6 weeks) diagnosed type I diabetes [41].

Regarding the use of MSCs to treat type 2 diabetes and obesity the most promising results come from experiments performed in obese diabetic mouse models. Diet-induced obese (DIO) mice fed with high fat diet (60% of calories from fat) for several weeks do become obese with a decreased glucose tolerance and insulin sensitivity. However, after mouse adipose tissue-derived MSC transplantation, DIO mice demonstrated a reduction in blood glucose levels and improved glucose disposal [42]. Interestingly, the DIO mice that had received MSCs showed reduced body weights, a decrease in serum triacylglycerol coupled concomitantly with an increase in HDL levels [42]. The mechanisms by which MSCs can reverse the complications caused by diabetes and obesity are still unclear. The literature indicates that MSCs can migrate to an injured pancreas, suppress pro-inflammatory cytokines and prevent β -cell apoptosis. However, one cannot exclude the fact that MSCs could also differentiate into pancreatic β -cells [42, 43].

Recently, our group has demonstrated that high glucose level, as typically observed in diabetic patients, promotes adipogenesis, increases accumulation of intracellular reactive oxygen species (ROS), upregulates inflammatory genes and decreases cellular oxygen consumption rate (OCR) in human adipose-derived MSCs [44]. Nevertheless, these effects promoted by hyperglycemia on MSCs can be minimized or reversed by upregulation of mitochondrial (rather than cytosolic, or extracellular) antioxidants such as superoxide dismutase-2 (SOD-2) also known as manganese-dependent superoxide dismutase (Mn-SOD).

In our study, we induced SOD-2 overexpression in human MSCs with the use of an adenoviral vector serotype 5 (AdSOD-2), a DNA virus, by gene transduction. As mentioned before the adenovirus remains as an episomal character, not integrating with the host genome and is therefore considered a safe approach to induce the over-expression of antioxidants in human cells for translational research [4].

Remarkable results were observed when SOD-2 upregulated MSCs were transplanted in db/db leptin receptors deficient obese diabetic mice. First, we noted that when human MSCs

are delivered intra-peritoneally (IP), they reach distal intra-peritoneal fat pockets. Therefore, when injecting SOD2 upregulated MSCs intraperitoneally (one single infusion), we expected the cells to reach these distal fat pockets and cause a reduction in ROS and thus help to reduce inflammation. Following a reduction in ROS in the local adipocyte pockets, the next step was to verify whether this approach could increase insulin sensitivity and reduce fat mass as well as blood glucose levels. In fact, at four weeks post-delivery of SOD-2 upregulated MSCs, a significant improvement in glucose tolerance and total body weight was found in db/db mice. The control for this experiment was db/db mice that received green fluorescent protein (GFP) transduced MSCs (AdGFP upregulated human adipo-derived MSCs) [44]. It was not elucidated whether the benefits promoted by genetically modified MSCs are restricted to local adipocyte pockets or if there is systemic improvement. However, the overall effect of improvement in glucose tolerance is clear. Different studies suggest that the “homing-in” and benefits of MSCs (genetically modified or not) depends on the route of cell delivery. Thus, the target of this cell-based therapy might change considering intra-peritoneal versus cells delivered into the tail vein [42, 44].

Injection of MSCs into the tail vein may help cells to reach the hepato-biliary system rather than fat in the peritoneal space. Additionally, injecting via intrasplenic route was found to be more effective at reversing hyperglycemia than the intrapancreatic route in a STZ-induced diabetic mouse model [45]. The effects of upregulation of other antioxidants on MSCs to treat diabetes in different high fat diet induced obese diabetic mouse models are under investigation in our laboratory [46].

4. Conclusion

In this review, we have briefly described promising studies that have used genetically modified EPCs and MSCs to reduce endothelial dysfunction and rejuvenate mesenchymal tissue (i.e. adipose tissue), respectively, in disease states such as diabetes and obesity. We have described the use of these stem cells post-genetic modification that can lead to a novel, yet safe therapy to improve the lives of the diabetic and obese population. Finally, we have outlined the use of these cells as a disease and therapy bio-marker in order to predict the disease progression of both prediabetes and diabetes.

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