Gene Therapy: New Therapeutic approach to Diabetes Mellitus

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Abstract

In the past decade there has been a great deal of enthusiasm and high expectations for cell transplantation and genetic engineering. Type 1 diabetes mellitus (T1DM) is an autoimmune disorder characterized by T cell-mediated self-destruction of insulin-secreting islet β cells. Management of T1DM is challenging and complicated especially with conventional medications. One of the potential therapeutic alternatives to treat T1DM is emerged with Gene therapy. This review primarily focuses on the current status and the future perspectives of gene therapy in the management of T1DM. A number of the studies which are reported on gene therapy for the management of T1DM are performed in animal models and in preclinical studies. In addition, the safety of such therapies is yet to be established in humans. Currently, there are several gene level interventions and options that are being investigated, notably, would be the overexpression of genes and proteins needed against T1DM, transplantation of cells that express the genes against T1DM, stem-cells mediated gene therapy, genetic vaccination, immunological precursor cell-mediated gene therapy and vectors.

Keywords: *Autoimmune disease, Gene therapy, Insulin, Type 1 diabetes mellitus, Proteins*

INTRODUCTION

To treat disease with cells is not a new concept. Many of the genes responsible have been identified and studies being carried out as to how they might be used as engineering tools for therapeutic purposes [1].Gene therapy is the technique of delivering or manipulating genetic material inside the cell as a therapeutic approach to treat disease [2]. It aims to correct defective genes that are responsible for disease development and effectively prevents disease onset or halts its progression. The three main intervention techniques in gene therapy include, a) introducing a new gene into the body, b) replacing faulty genes with functional genes and c) by inactivating defective genes causing the disease [3, 4]. There are two common types of gene therapy, namely somatic gene therapy, as the name implies, targets on somatic cells which in this case refers to the diseased cells, whereas, germline gene therapy

targets on reproductive cells to prevent disease development in subsequent generations [3]. Gene therapy has emerged as one of the current trends in therapeutics for its potential to treat various diseases such as autoimmune diseases, diabetes, cancers and heart diseases that cannot be cured using conventional therapies [4].

Diabetes mellitus is usually classified as type 1 or type 2 diabetes. Type 1 results from a b-cell defect, often due to an autoimmune process. Type 2 diabetes is characterized by insulin resistance which is often combined with an insulin secretory defect. The number of people suffering from diabetes is growing at an alarming rate [5]. T1DM is an autoimmune disease characterized by T cell-mediated self-destruction of insulin-secreting islet β cells in the pancreas [6]. Like any other autoimmune diseases, the etiology of T1DM is complex and can result from both environmental and genetic factors [7]. During the past few decades, researchers have successfully identified several genes that are responsible for the development of T1DM [8]. Dinesh et al. reviewed the literature in terms of over expression of genes and proteins needed against T1DM using gene therapy, transplantation of cells expressing gene against T1DM or stem-cells mediated gene therapy, genetic vaccination, immunological precursor cell mediated gene therapy and vectors used in gene therapy for T1DM [9].

Gene transfer methods

A number of various gene transfer methods have been used. These include non-viral methods such as calcium phosphate co-precipitation, lipofection, direct microinjection, electroporation and biolistics, as well as gene transfer via viral vectors.

Non-viral methods. Calcium phosphate co-precipitation is a simple and non-expensive method for genetically modifying pancreatic cells. When calcium chloride with the DNA of interest is added to buffered saline/phosphate solution, a precipitate forms. Cells can endocytose or phagocytose the DNA containing precipitate. This method has been tested in a variety of cell types and can produce either transiently transfected cells or cells that are able to stably express the transgene. Liposomes have also been used as high efficiency transfection agents of cells both in vivo and in vitro; unlike calcium phosphate co-precipitation, which is conducted in vitro. The advantage of in vivo lipofection is that the liposomes may be injected into the bloodstream and is less invasive than other treatments, such as transplantation. Liposomes containing DNA have minimal positive charges which improve their interaction with target cells and the consequent transfection efficiency [10]. Directly injecting DNA into cells is an effective method for transfecting cells. However, as each cell needs to be targeted individually, this is a labor intensive technique and is not suited for the targeting of large cell numbers. Electroporation creates permeable membranes for gene transfer by applying high voltages to cells; and in many cases, causes cell death. To allow efficient gene transfer to surviving b-cells the islets need to be dissociated from the tightly clustered sacs of cells into single cell suspensions. Without the maintenance of their morphology, the dissociated islets may be non-functional. Although it is possible for gene transfer into the cell, electroporation cannot efficiently integrate DNA into the host genome [11]. In comparison to both lipofection and calcium phosphate co-precipitation, biolistic transfection produces higher transfection efficiencies. Biolistics is the use of a “gene gun” to transfect cells with a transgene [11]. The “gene gun” rapidly discharges DNA—microprojectiles into cells.

Viral vectors

The choice of an appropriate vector requires careful consideration. In order to be successful vectors need to be simple to manufacture in large numbers, have the ability to be targeted to a specific site, be able to transduce both

dividing and non-dividing cells, result in high transduction efficiency, not elicit a strong immune response and allow for long term expression of the transgene[12]. For transgene delivery into islets, the vector is required to pass through the islet membrane and transducer the sac of cells within. Studies by Leibowtz et al. have previously shown that successful transduction of the cells within islets only occur at the periphery of the islet (approximately 10% of cells) and cells in the core of the islet are not transduced [13]. The main disadvantage of retroviral transduction is that they are only able to transduce cells that are currently dividing—non-dividing islets cannot be transduced by retroviral vectors [14]. There may also be random integration of the transgene into the host genome, resulting in insertional mutagenesis [15]. Adenoviral vectors have the advantage over retroviral vectors in that they are able to transduce both dividing and non-dividing cells [14] and can be prepared in high titres [16]. Adenoviruses can infect insulin-secreting cells [13] and have been shown to be able to transduce rodent islets. [17-19] Barbu et al. have shown that by confocal sectioning of intact islets transduced with GFP that expression on the cells was in fact only on the periphery of the islets and as such transduction efficiencies are approximately only 30% [20]. The weaknesses of this type of gene transfer are that the vector antigens elicit potent immune responses [21] and the inserted DNA is episomal, resulting in short term transgene expression [22]. Lentiviral vectors have similar characteristics to both retroviral vectors and adenoviral vectors. The retroviral characteristics are the ability to integrate the transgene into host chromosomal DNA and to alter the surface envelope proteins. Lentiviral vectors are able to transduce primary and post-mitotic cells—such as neurons, liver, muscle cells, primary endothelial cells and islets;[13],[23] and to transduce dividing and non-diving cells without the potent immune responses that adenoviral vectors elicit [16].

Conclusion and future perspectives

T1DM is a worldwide epidemic where a significant number of patients are suffering from it. The primary goal of any therapy for T1DM is to achieve near normal BG levels and gene therapy is a strategy employed to maintain a near normal BG level in an efficient, safe and specific way. In this review, the essential genes and proteins that can be overexpressed to treat T1DM via gene therapy were discussed, each one with their own advantages and limitations. Gene therapy is employed for this purpose, as the expression of genes is impossible to modulate by any surgical or instrumental approaches. The field of genetic engineering is also crucial in this regard for incorporation of genes into cells and development of other novel techniques of gene therapy. In addition, transplantation of cells expressing genes against T1DM was also reviewed. Various types of cells expressing different genes were discussed in this review with their advantages and limitations. Transplantation of stem cells expressing genes against T1DM is evolving slowly as a potential therapeutic approach for T1DM. Advantages and drawbacks of different types of stem cells are presented in this review. Besides, genetic vaccination also has a promising scope for the treatment of T1DM, as it offers a great flexibility in controlling the nature of T-cell response.

Different strategies used in DNA vaccination are pDNA and viral-vector based vaccinations. Overall, genetic vaccination offers favourable outcomes in preventing or reversing T1DM. Furthermore, immunological interventions using gene therapy is also a therapeutically potential approach for T1DM. Immunological interventions might be able to prevent beta cell from autoimmune destruction and reduce patients' dependence on insulin. Different types of immune interventions such as immunoregulatory and anti-inflammatory strategies are reviewed, each with their own outcomes and limitations. Several systems of

viral and non-viral vectors are also discussed in this review with each system having their own advantages and limitations. Vectors are used to achieve a safe and efficient delivery of gene to targeted site and thus play a crucial role in gene therapy. The choice of vectors used should be based on the therapeutic application. More studies are required to be carried out on non-viral vectors as non-viral vectors lack antigenicity D.K. Chellappan **et al**. and is safer to be used in humans. Selection of a suitable vector is crucial in any of such interventions and further optimization of viral vectors is required to diminish the common adverse effects of using viral vectors, such as insertional mutagenesis and host immune-genicity.

The construction of non-viral vectors should also be further investigated in detail to improve the transfection efficiency and utility of non-viral systems in the near future. More studies are also required to investigate the possibilities of how the sensitivity of stem cells towards glucose levels could be enhanced. For the case of gene vaccination, a more efficient combination of DNA vaccine need to be studied for as this approach is relatively new and there may be more effective combinations of DNA vaccines that have not yet been developed. In addition, more in depth studies are required to establish the effectiveness of combined immunological interventions as there is little evidence available and a better understanding of the biology of cytokines involved in T1DM is also important for development of safe and effective immunotherapy. Lastly, there is a need to explore for potential genes and proteins to minimize the potential adverse effects, thus giving a possibility to develop a safe and novel treatment for T1DM.

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