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REVIEW ARTICLE

REGENERATION THERAPY FOR DIABETES MELLITUS: A REVIEW

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ABSTRACT

D*ecrease in beta cell mass and its function are common observations in both type 1 and type 2 diabetes mellitus. Therefore recovery of these cells may be an option for treating diabetes mellitus permanently. Islet transplantation has shown some advantages such as insulin independency and restoration of normal metabolic functions. The major hurdle in this technique is insufficient number of islet donors. This strategy involves the differentiations of cell by either in vivo or in vitro regeneration of insulin producing cells. Adult or embryonic stem cells, acinar cells, progenitor cells and other endocrine cells have been used in this technique. Development of functionally mature beta cell and safety of cells producing insulin are major hurdles for clinical application of this strategy. This article reviews the various recent strategies for major hurdles in management of diabetes.*

Keywords: *Diabetes mellitus, beta cell, transplantation, islets of langerhans, insulin, regeneration.*

INTRODUCTION

Diabetes mellitus(DM) is a metabolic disorder in which blood glucose exceeds the normal limit because of either inadequate supply of insulin which many occur due to degradation of cells producing insulin or resistance to insulin.[1-3] To control this exceed blood glucose level, various preparations of insulin and oral hypoglycemic agents(OHA) have been used. But these treatments are not permanently effective for diabetes mellitus and there is also lack of maintenance of DM, overdose may cause hypoglycemia. There is need to develop better management for treatment of DM. Beta cell mass is one of exciting approach for the treatment of DM, which is linked with Stem Cell Therapy.

Some of the successful methods for regaining functions of beta cell mass are Islet transplantation and whole pancreas transplantation. However limited availability of islets or whole pancreas is one of the drawback for this therapy. Another drawback is it requires immunosuppressant therapy, which produces some serious side effects for example transplant rejection. Various techniques which provides solutions for these drawbacks, such as investigation of alternative for beta cells i.e. some non-beta cells have been developed to produce insulin. In addition to this, in-vivo regeneration of beta cells is also investigated.

In this review we will describe recent development on islet cell replacement and various strategies for regeneration therapy for diabetes mellitus.

DEVELOPMENT OF PANCREAS

Before discussing about regeneration therapy for diabetes we need to understand normal development of pancreas. In humans Pancreas is an outgrowth of duodenum which is a part of small intestine. Ductal cells plays main role in the development of both exocrine and endocrine system. Endocrine part consist of groups of cells called islets of langerhans, which are cellular masses consisting several to several hundred cells lying in the interstitial tissues of pancreas. There function is to maintain appropriate level of different nutrients in the cellular depots and in blood. It consists of alpha cells (15-20%) releasing glucagon, beta cells (60-90%) releasing insulin, delta cells (3-9%) releasing somatostatin, pancreatic polypeptides (1%) and very small amount of special cells which releases gastrin. In humans insulin secretion and differentiation of pancreatic beta cells takes place by activation of insulin promoter factor (IPF-1). After the fourth week of embryonic development isolated clumps of endodermal cells bud forms the tubules and accumulated within the mesoderm to form the islet cells. After 10^{th} week they secrete insulin. New beta cells formation takes place precursors secrete IPF-1 in pancreatic duct. Pancreatic cells are developed from ductal cells. Ductal cells

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can be differentiated from endocrine cells by genes as they express. Ductal cells express gene *cytokeratin-9* (CK-9) which is necessary for encoding structural proteins. On the other hand beta cells of pancreas express gene PDX-1 which encodes transcription from insulin gene. IPF-1 is most important factor for beta cell renewal and growth. Other factors such as epidermal growth factor, transforming growth factor and growth hormone are also responsible for beta cell renewal and development. In addition to these factors glucose is also responsible for beta cell proliferation and destruction.

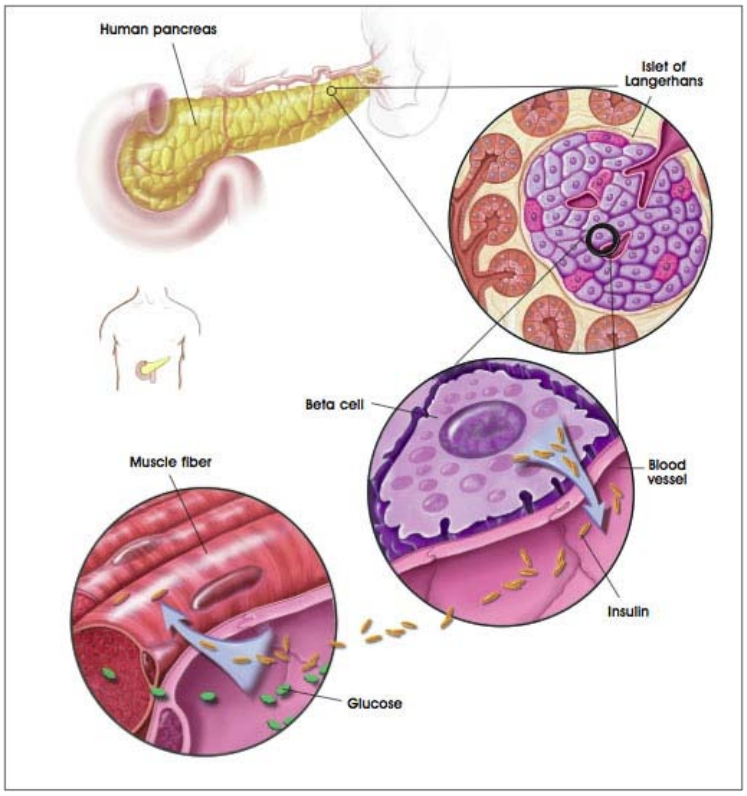

Fig. 1. Insulin Production in the Human Pancreas.

The pancreas is, adjacent to the duodenum (the first portion of the small intestine), located in the abdomen. A cross-section of the pancreas shows the functional unit of the endocrine pancreas i.e. the islet of Langerhans which is. Encircled is the beta cell that located adjacent to blood vessels, synthesizes and secretes insulin and can easily control the glucose concentration in the body. Insulin utilizes glucose by absorbing in the tissue.
(© 2001 Terese Winslow, Lydia Kibiuk)

Techniques for Regeneration Therapy of DM

1) Islet transplantation
2) Insulin producing non-beta cells development
3) Stem cell derived beta cells
4) In-vivo regeneration

Islet Transplantation:

Pancreas or Islet transplantation is a technique to control metabolic function of pancreas which cannot be achieved by exogenous insulin or oral hypoglycemic agents. This technique has advantages that, complications associated with diabetes are permanently removed and there is no need to take daily insulin or OHA and brings life to normal.
Islet transplantation was first performed on rodent model. Later it was performed on type-1 diabetic patients. Transplantation needs much attention to prevent transplantation rejection and recurrence of autoimmune responses to beta cells which is more expensive and reduces quality of life. To overcome these drawbacks needs to do further study on development in method of the isolation of islets and to develop effective immunosuppressant therapy.[6] Immunosuppressant therapy without steroids called “Edmonton Protocol” has been developed to avoid side effects.

The Edmonton Protocol is a method used to implant pancreatic islets for the treatment of type 1 diabetes mellitus. Edmonton is a name of city of Canada where the method was developed in the late 1990s. Difference between this procedure and other procedure is that it doesn’t utilize glucocorticoids for immunosuppression. It involves isolating islets donor pancreas by using mixture of enzymes Liberase (Roche). Then it is requires to infuse isolated islets cells into the portal vein of recipient then two immunosuppressants (sirolimus and tacolimus) as well as a monoclonal antibody drug is given to the patient.

Recently it is observed that 70% of islet transplants are successful.[7]Various strategies are developed, to prevent transplant rejection are as follows.

i. Prevention of antigen presenting cell activation.
ii. Shifting of pathogenic effector cells to protective regulatory cells and removing of co-stimulatory pathway which is replacement for immune attack inside the body.
iii. Develop beta cells which are immune attack resistant.
iv. By changing genes.
v. Microencapsulation of beta cells by synthetic polymers. These polymers allow small molecules to pass through passages such as insulin glucose and amino acids. Larger molecules like immune cells and antibodies can't be entering inside the encapsulated area. But this strategy has two drawbacks its lack of biocompatibility and oxygen deprivation.[9]

Lack of donor for islet cells or whole pancreas is a major drawback for this technique, due to which there is no fulfillment of demand of patients. Fetal, embryonic, neonatal or adult porcine islets are other possible sources for cell transplantation. Pigs have many similarity with humans.[10, 11] one major drawback of pig as a source for islet is that it has Gal-α-Gal antigen. Primary beta source is another possible source for islet transplantation which is obtained by transduction with oncogenes for expansion. But this strategy has drawbacks like tumorogenecity and impaired insulin secretion.

In future, there is need to improve immunosuppressive regimes, to modify the microencapsulation strategy, to tolerate the islet transplant, prevent the transplant rejection. One area which is very much important for this technique is availability of suitable source for islet, which should fulfill the demand of patients.

INSULIN PRODUCING NON-BETA CELLS

It is very interesting strategy in which some non-beta cells are engineered as they produce insulin. This strategy removes the problem of autoimmune system activation which causes transplant rejection. Various cells such as hypatocytes, fibroblast, muscle cells neuroendocrine cells have been engineered which can produce insulin.[8] In case of diabetes not only pancreas is affected but other organs such as liver and kidney also gets damaged. Liver has glucose sensing activity similar to pancreas therefore researchers created much attention on hepatocyte as a source of insulin. In a preclinical model it was tried that transplantation of primary hepatocytes by some modification to generate glucose sensing system like pancreatic beta cells. It was transduced with glucose sensing promoter gene.[14] In this study hepatocytes were isolated surgically then ex-vivo electropolated by an insulin expression plasmid. Intestinal K-cells are another source for insulin secretion because they have the necessary enzyme which can convert proinsulin to insulin as well as they have exocytotic mechanism. A murine enteroendocrine cell line is another successful non-beta cell source for insulin secretion. When it is transplanted, it not only reverses complications of diabetes but also has a glucose sensing mechanism like beta cells of langerhans. One more non-beta cell source for insulin secretion is bone marrow mesenchymal stromal cells which are successful in treating diabetes in mice.

All these above described sources are good for insulin secretion but they can't be totally mimicking the functions of islet cells. Islet cells mainly have glucose sensing system, peptidase and secretory granules that can produce insulin by exocytosis. Therefore it is very difficult to engineer non-beta cells that totally mimicking beta cells functions. There is need to develop a non-beta cell source by combining features of two or more source to avoid drawbacks of single source.

STEM CELL DERIVED BETA CELLS

Various studies are performed on stem cells and it is proved that stem cells and progenitor cells have the ability to differentiate in-vitro as well as produce insulin. Adult stem cells are completely mature cells and are most important source for various types of models. Another advantage by using stem cell is that these cells are not considered for ethical issues. Many studies shown that stem cells or progenitor cells, which produce insulin, are present in bone marrow, liver, spleen, neuronal tissue, umbilical cord blood, adipose tissue, intestine, salivary glands and messengial cells.[19]

Some researchers have been found fetal tissues as a source for islet progenitor cells, for example some has uses mice and compared implanted insulin content with several sources of stem cells such as purified human islets, fresh human fetal pancreatic tissue and cultured islet tissues. They found that insulin content was initially higher but with its concentration was decreased. When cultured islets implanted in patients however the content of insulin increased for three months. From this point it was concluded that precursor cells in cultured islets able to proliferate and differentiate into functioning islet tissue but further after certain period of time they could not proliferate. So researchers found that it is difficult to expand culture of fetal islet progenitor cells in culture.

Some researchers have given attention on culturing islet cells from human adult cadavers for the development of transplantable material. However differentiated beta cells are difficult to proliferate and culture. Some have got success in this technique, for example Fred Levine and colleagues at University of California had developed islet cells from

human cadavers, they added special gene which stimulates cell proliferation. After that they further engineered beta cells which express PDX-1 gene for stimulation of insulin producing gene. When they transplanted it into deficient mice insulin secretion takes place due to presence of glucose. But they reported that these cells didn't stimulate and secrete insulin as normal beta cells. Major difficulty in this technique was maintaining balance between differentiation and growth. Those cells producing insulin efficiently did not proliferate well and those proliferate well couldn't produce insulin efficiently.

Method for isolation and culturing of embryonic stem cells is another valuable discovery that renewed hopes of doctors, researchers and diabetic patients. This technique may be helpful in finding treatment for type1 diabetes and for type2 as well. In this technique the cells are produced in a manner that they should avoid immune rejection. Before transplantation cells were kept in non-immunogenic material.

Bernat Soria and colleagues added DNA part of insulin gene to embryonic stem cell from mice. This insulin gene was linked to another gene so that it rendered the mice resistant to antibiotic drugs. In these conditions the cells which only activating insulin gene were able to survive, then these cells were cultured in low glucose concentration for differentiation. Then they implanted it into the spleen of diabetic mice and found successful reversal of diabetes.

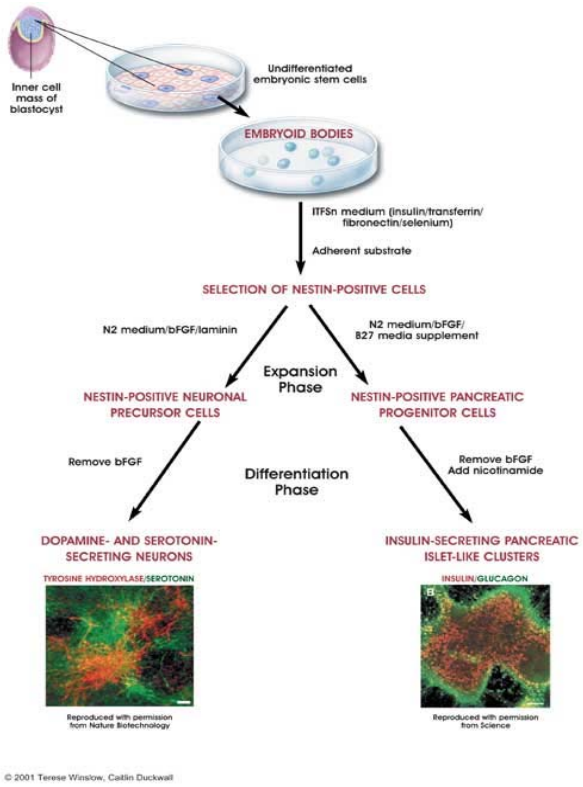

Fig.2. Series of experiment by Ran McKay and colleagues

Ron McKay and colleagues has done series of experiments in which they developed insulin secreting from embryonic stem cells which was similar to pancreatic islets. First they had taken embryoid bodies. They then selected the cells which expresses the neural maker nestin. By using 5 step culturing technique they convert it to islet like cultures that can give response to glucose for secretion of insulin.

But these cells were less active than normal islet cells and when these cells injected into diabetic mice they were not able to reverses diabetic symptoms. Then they further expanded and differentiated and characterized when was grown in culture, they observed 3 dimensional structure which was similar to pancreatic islets, then they implanted it into shoulder of mice and they observed that techniques was successfully reverse symptoms of diabetes.

Further study is continued to induce PDX-1 gene and to develop human embryonic stem cell system that can trap into differentiating active insulin producing islets that may possible soon.

IN-VIVO REGENERATION

To provoke replication of beta cells along with induction of neogenesis are the key approaches for study of in vivo regeneration of the same. Beta cell mass gets augmented in case of pregnancy and other diabetologic stimuli like glucose, free fatty acids (FFA). This implies that the beta cells can be grown artificially. Use of growth factor like vit A, hepatocyte growth factor have been used along with epidermal growth factor, gastin, BTC etc.

In addition, the regeneration process is orchestrated by Reg protein, one of the member of regenerating protein family, and islet neogenesis gene associated protein (INGAP). These two are investigated and made a potential therapy for diabetes. Restored cell mass can be attained along with normoglycemia in autoimmune non-obese diabetic by the combination of EGF and GLP-1.

Clinical studies are going on using Exendin-4, immune suppressors, EGF, gastrin analogs. In phase 2, INGAP did not show any treatment effects on glucose level. Transcription factors are expressed with or without signaling molecule, being a successful method for beta cell regeneration. streptozotocin-induced diabetic rats undergone delivery of the Pdx-1 gene along with the BTC gene into the pancreas. This showed normalized blood insulin and C-peptide levels. Along with that, blood glucose levels were also maintained below 200 mg/dl.

Pdx-1, Ngn3, and musculoaponeurotic fibrosarcoma oncogene homolog A, together expressed a reduced hyperglycemia. They also conferred beta cell-like characteristics to nonislet tissue, like liver and the intestine. Liver made to receive Pdx-1 gene or NeuroD and BTC genes, which showed insulin being expressed from the liver cells. Same genes showed downregulated hyperglycemia in mice. Likewise, intestine also studied for the expression of insulin. In intestinal epithelia forced expression of Pdx-1 [38], MafA [39], or GLP-1 was done with the help of adenovirus-mediated gene transfer.

CONCLUSION

Cell based therapy normally has some more risks than other therapy because it provides external cells to the body which may defend by autoimmune system of the body. Out of all above explained methods, islet transplantation is an effective technique for regaining of power of islet cells. This method not only gives stable blood glucose level but also relieves from repeated exogenous insulin administration. But shortage of islet donor is major hurdle for this strategy. To overcome from this, the differentiation of progenitor or pluripotent cells into insulin generating cells has been developed. But still it is not clinically applied. Most of the data are obtained from preclinical studies. Clinical applicability is negligible in this area. There is need to understand mechanism for normal regeneration process in adults. This may increase its clinical application.

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