ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 6(E), pp. 27550-27554, June, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

NT ADVANCEMENT IN DIABETES TREATMENTUSING B-CELL LINES

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2283

ARTICLE INFO

Article History:

Received 20th March, 2018
Received in revised form 27th April, 2018
Accepted 5th May, 2018
Published online 28th June, 2018

Key Words:

Beta Cell Line, *In-Vivo* Study, Sv40 T-Antigen, Transgenic Mice, Insulinoma.

ABSTRACT

Every year large number of people suffers diabetes which may also be fatal. Approaches have been made from time to time which included in vivo models but have certain limitations so it becomes the need of the hour to develop in vitro methods. In-vitro methods here comes into play with which attempts have been made to establish beta cell lines that can retain normal regulation of insulin secretion but have mostly been unsuccessful. The availability of such cell lines is limited and hence researchers have used X-rays and viruses to induce insulinomas, derivation of cells from transgenic mice or even from non-islet cells to produce immortalized cell cultures. In this article, we have briefly discuss in-vitro models over-viewing cell line techniques for producing beta cells from animal and human origin by throwing the light upon the creation of continuous cell line from beta cell of pancreas by expression of SV40 antigen which has the capability of producing transformed beta cell lines with improved response to glucose by expressing various enzymes like GLUT and glucokinase. The need is to produce a normal cell line of human origin.

INTRODUCTION

Diabetes or diabetes mellitus is the class of metabolic disorder which is caused by either inadequate production of insulin or due to desensitization of cell or both. Diabetes is a chronic condition that causes high blood sugar level. Frequent urination increase in hunger and thrust are the common symptoms various serious long term complications associated with diabetes including cardiovascular diseases, chronic kidney diseases, stoke, foot ulcer, and damage to the eyes.

Response of beta cells in different types of diabetes

Diabetes is characterized by loss of inadequate production of insulin by pancreatic beta cell which leads to insulin deficiency. It is due to destruction of beta cell by T cell mediated autoimmune attack it is partially inherited with multiple genes which include HLA genotype. In type 2 diabetes beta cell produce insulin in sufficient amount but that insulin is resist by cell. Thus, it is called non-insulin dependent or insulin resistance diabetes.Type 2 diabetesmellitus is primarily due to lifestyle factor and Genetic Riseras *et.,al* January 2008- **09** about 90% of diabetes mellitus are type 2.

Pathophysiology

Insulin is the hormone that regulates the uptake of glucose from blood into most of the cells of body Ghasemi *et.,al* Feb 2013.The body obtains glucose from three main place's viz. intestinal absorption of food, breakdown of glycogen and stored form of glucose found in lever and gluconeogenesis. Insulin is released from islet of beta cell in response to high level of blood glucose. Insulin binds to insulin receptor present on cell membrane of cells. Binding of insulin to α-subunit of receptor activates tyrosin kinase activity of β-subunit. Insulin stimulates glucose transport across cell membrane by ATP dependent translocation of glucose transporter GLUT to plasma membrane. This glucose is used as fuel by cell.

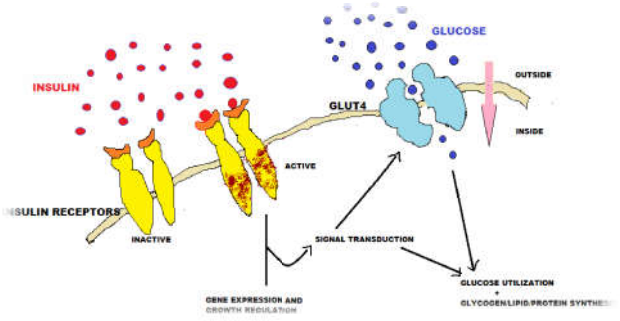

Figure 1 Diagrammatic Representation of Physiology of Bcell

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By understanding the mechanism of insulin release and its pathophysiology, development of different in-vitro and in-vivo techniques has been done in past few years for study of diabetesamong which cell line technique has been found to be the most sophisticated.

Different Experimental Models on Diabetes

In-vivo Studies of Diabetes Mellitus

Streptozotocin Induced Model of Diabetes Mellitus

Streptozotocin is an antibiotic derived from Streptomyces and structurally is glucosamine derivative of nitro urea. It has been exclusively used as a tool for induction of Diabetes Mellitus as it is cytotoxic for beta cell of pancreas.60mg/kg dose of streptozotocin to rat intravenously increase blood glucose level to 150–200 mg%. STZ enters into beta cells through glucose transporter GLUT where it causes alkylation of acid (DNA). It also causes polyadenosinediphospateribosylation and nitric oxide release, as a result beta cells are destroyed by necrosis, as it destroys beta cell and cause deficiency of insulin toinduce Type 1 diabetes mellitus Mythili et., al Apr 2004.

Alloxan induced model of Diabetes Mellitus

Alloxan is a chemical used for induction of diabetes mellitus in various animals. It is a derivative of urea which can cause selective necrosis of beta cell and produce Type 1 diabetes. Wistar or Sprague dawley rats weighing between 150-200 gmare used, subcutaneously injection 100-175 mg/kg alloxan. Pancreatic lesion has been produced by the administration of alloxan and it is reported that severity of lesion is directly proportional to the dose of the drug administered Viana *et.,al* Jun 2004.

Transgenic mice model

Schaefer et al (1994) had developed a transgenic model of chronic hyper glycemia. The extra-cellular domain of receptor of human beta cell has been expressed as stable soluble protein that is efficiently secreted and binds insulin with high affinity expression under the mouse, transferring promoter of transgene and coding a secreted derivative of human insulin receptor in transgenic mice that results in accumulation of high affinity insulin binding protein in plasma.

Obese model of type II diabetes

As type 2diabetes are closely linked with obesity mostly animal model of type 2 are obese. Monogenic model are most common. Defect in leptin induce satiety and thus lack functional leptin in animal which cause obesity. Theses model include $Lep^{ob/ob}$mouse which is deficient in leptin.

Why In-Vivo Models Fail In Diabetes

Different animal models of diabetes had been used and among which Rodent models were instrumental in discovery of insulin long ago. Thayer *et.,al* 2010While it is known that these models develop diabetes and responds to treatment but had certain limitations Atkinson *et.,al* Jun 1999. There were studies which compared different animal models with regard to change in pancreatic islet with progression of disease and they were compared with human type 1 diabetes. There were many therapies which had shown to prevent or reverse diabetes yet none of these have translated to therapies that prevent or cure Type -1 diabetes in human. This may be due to difference in autoimmunity associated with humans and rodents or is due to difference in islet morphology and function when compared to humans. Rodents are widely used for understanding the various biochemical pathways activated during diabetes. It was assumed that these animal models act like human models but they failed to express the biochemical pathways regulated in human body. According to a scientific fact. In rodents for the secretion of insulin biosynthesis of citric acid intermediates is very important and this process is called as anaplerosis, an enzyme known as pyruvate carboxylase (PC) has play very important role in this process, but in research scientist had found that human islet depends less on PC than rodents model, and the level of PC and enzyme activity was found to be very low in human isletMacDonald *et.,al* May 2011 There are various differences between the way in which animals and humans regulate their blood sugar. Due to the differences in human and animal models, advancement becomes the need for the development of new methods to test potential drug and therapy. Moreover organizations like People for Ethical Treatment of Animals (PETA) which work for ethical treatment of animals put restrictions on use of animals as models. Due to the failure in the above mentioned methods and reasons, it is required to develop certain in vitro models for testing and screening diabetes. In-vitro studies on insulin secretion include conventional antidiabetic agents which affect several pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs as well as nutrient absorption.[Poitout *et.,al* Feb 1996 ,Størling *et.,al* Jul 2005, Iwashima *et.,al* Jan 2001]. Cell line development technique have widely used now a days.

In-vitro methods of diabetes mellitus

Cell Line

Cell line is a cell culture that is derived from one cell or set of cells of the same type and which under certain conditions the cell proliferate indefinitely in the laboratory. The cell for primary cell culture is directly obtained from an animal or plant. Once cell is completely grown on to the surface of media and no longer can grow due to depletion of all the nutrients. This stage is called confluence. The cell is then sub-cultured into secondary culture. After one sub-culture whatever cell we get is called a cell line.

Cell lines used in culture

Finite cell line

The cell culture divide only a limited number of times before their growth rate declines and they eventually die. The cell line with limited number of life span known as finite cell line. They normally divide 20-100 times before extinction.

Continous cell line

They are also called immortalized cell lines which are derived from multi-cellular organisms. This type of cell line is having the characteristic of definite proliferation but evasion from normal cell senescence occurs due to mutation. The transformed cell for continuous cell line may be obtained from normal cell culture by treating them with chemical carcinogens or by infecting with oncogenic virus.

Isolation and Preparation of Transformed beta cell lines

One of the methods for the isolation of Beta cells is cervical dislocation by killing the adult mouse. Liberases are dissolved in hank's buffer solution (HBBS) and injected in pancreas of mouse. After that the pancreas is carefully removed and undergoes digestion for 10-15 min, the enzyme usually used for digestion is trypsin. For termination 10ml of HBBS is used. Centrifugation of islet is done in 3000Rpm.Generally cells have finite life span of 20-100 generation, but there may be some cell which can be continuously dividing due to having mutation and can produce a continuous cell line. Life span of the finite cells is controlled by some senescence genes by negative regulation of the sequence of cell cycle. Freshney 2000. Deletion and/or mutation of one or more oncogenes can dominate the action of senescence by resulting in the breakout of cell cycle from the negative regulation and the re-expression of telomerase.

In culture primary beta cells cannot proliferate easily. Scientists are trying to develop and establish methods to create a cell line which can retain its normal regulation of insulin secretion and have the same genotype and tissue markers that were acquired by their parental tissue. In the last few years number of approaches has been made to prevail over replicating senescence. Among all the methods expression of SV40 large Toncoprotein is the method of choice for transformation of cell.SV40 T antigen has the capability of making the primary cells grow in culture and of transforming cultured cells into tumouregenic ones Efrat *et.,al* Jan 1987 who were working on beta cell line had find out some specific amount of tumor suppressor protein 53(p53),which in distinguish to normal beta cell have expressed large Tantigen from RIP-1-TAG-transgenic mice (under the influence of rat insulin promoter SV40 large Tantigen were expressed in the islet of beta cell) where protein was undetectable.

Isolation and creation of primary beta cells using SV40 antigen

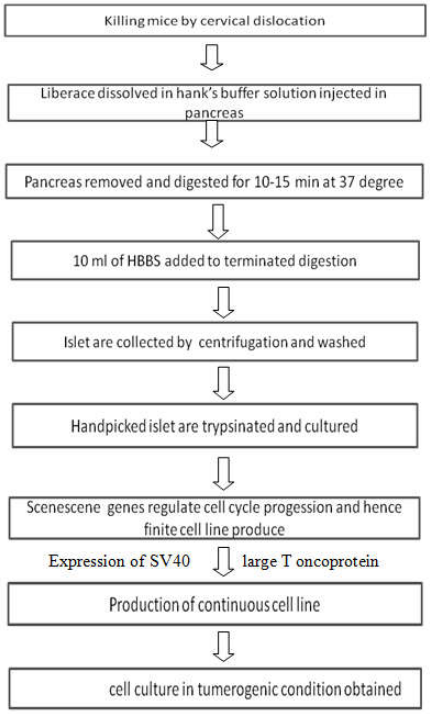

Fig 2 Flowcharts of Isolation and preparation of Beta Cell line by inducing SV40 antigen.

Physiology of Beta Cells

Though cell line technique proves to be an important and valuable tool for the study of beta cell function but they cannot perfectly mimic the way in which primary beta cell work.There is also a limited availability of primary islets and hence cell line culture acts as a potential source for this procedure. In contrary to a normal beta cell which express mainly the high-km glucokinaseisotype which trigger insulin secretion for which glucose concentration act as physiological threshold, the insulinoma derived cell lines have unlimited growth in their tissue culture exhibiting difference in their insulin secreting response to glucoseEfrat Apr2004.Significant amount of insulin up to 30% of those found in normal beta cells produced by few cell lines however show a normal response to glucose in physiological range.. Of all often used cell lines from various species MIN-6and INS-1 cell lines responds to glucose stimulation and express glucokinase and hence best reflect physiological condition.[Gilligan *et.,al* Aug 1989, Efrat *et.,al* Dec 1988, Ishihara *et.,al* Nov 1993, Radvanyi *et.,al* Jul 1993].

Insulin secreting cell lines

Only a few studies report the use of stable insulin secreting cell lines from spontaneous insulinomas of human or animal origin of which the most widely used are the radiation –or- virus induced insulinomas. Beta-hyperplastic islet-derived cells (BHC) and Hamster pancreatic beta cells(HIT) cells are the derivatives of pre-neoplastic islet cells which have been transformed with SV40, while irradiation of cell lines resulted in generation of rat insulinoma cell line (RIN) and insulinoma cell line (INS-1) . These cell lines retain some characteristics of normal beta cells and represent transformed cell lines Wollheim *et.,al* Dec .1990

RINr Cell Line

RINr and RINm were initiated from tumour that was developed in rats whose physiological characteristics differ from native beta cells. By induction of high dose of x-rays on rat insulinoma RINm5f were derived. These cell contain insulin and small amounts of glucagon and somatostatin and are one of the very popular insulin secreting beta cell line used now a days. Abnormal properties of glucose transport and or phosphorylation have also been reported for few of the derivative cell lines Halban *et.,al* May1983.

Transformed Beta Cell Lines and Insulin Secreting Beta Cell Lines Derived From Transgenic Mice

Transformed beta cell lines include invention of new cell lines from transgenic animals in which oncogenes are expressed under the control of the insulin promoter. The expression of the introduced genes may vary as a function of the insertion site of the recombinant chromosomal DNA. It should be ensured that a constant and high level of expression must result and hence constitutive promoters mostly SV40 T-antigen has been used. SV40 or Simian Vacoulating virus 40, a circular DNA of Rhesus monkey origin is a member of the polynomavirus. It is having the potential to cause tumours and infect eukaryotic cells which makes it one of the most studied viral systems. It is also having the capability of transforming a variety of cell lines including the human cells Soria *et.,al* May 2000.

Tab 1 List of Some Insulin-Secreting Cell Lines

Cell line	Cell origin	species	Method	Advantage	Disadvantage	References
MIN6	Insulinoma	mouse	SV40 T-antigen transgenic mouse	Express glucokinase. and Glut2	Treatment with nicotinamides Makes them responsive to glucose.	(Ishihara et al., 1993)
RINm	Insulinoma	Rat	Radiation induced		Insulin level decreases with Passages. Also secrete Somatostatin. Not responsive to glucose stimulation	(Gazdar et al.,1980
CM	Insulinoma	Human	from the ascetic Fluid of a patient with a liver metastasis of a malignant insulinoma.	Express GLUT2 and glucokinase concentration.	No insulin secretion in response to increasing glucose	(Baroni et al., 1999)
Blox5	Foetal Pancreas	Human,	SV40 T-antigen H-rasval12 hTERT oncogene,	Exhibit glucose responsive insulin secretion. Express glucokinase	Low insulin content	(Dufayet de la Tour . et al., 2001)
CRI-G1	Insulinomas	rat	Radiation induced		In parallel to insulin they release glucagon. Not responsive to physiological glucose conc.	(Carrington et al., 1986)
TMR-1	Foetalpancrease	human	SV40T-antigen	Express GLUT2	Express small amount of insulin and glucagone. Not responsive to glucose stimulation	(Wang et.,al 1997)
IN-111	Insulinoma	rat	BK-virus induced		Not responsive to physiological glucose conc.	(Uchida et.,al 1979)
βHC	Hyperplastic islet	mouse	SV40T antigen transgenic mouse	βHC9express GLUT and glucokinase	High hexokinase activity develops.	(Radvani et al., 1993)

Six Mouse Insulinoma Cell Line (Min-6)

MIN-6 expresses an insulin promoter/T-antigen construct and originates from a transgenic mouse. They form islet like cells. Its response to glucose within the physiological range varies. In the presence of nicotinamide it shows a good response and expresses GLUT-2 and glucokinase but sometimes due to the out growth of cells a loss in glucose induced insulin secretion had been observed Thayer *et.,al* 2010.

Beta Tumour Cells (BTC)

Beta tumour cell lines have been derived by expression of the SV40 T-antigen oncoprotein and represent the class of highly differentiated beta cell lines under the control of the insulin promoter in transgenic mice or rats. BTC produces mature insulin by processing of proinsulin I and II produced by them. BTC can be derived from primary beta cell tumours arising in the transgenic mice repeatedly.

Thus, targeted expression of an oncogene with a cell specific regulatory element can be used both to immortalize a rare cell type and to provide a selection for the maintainence of its differentiated phenotype Efrat Jun 1999.

CONCLUSION

The understanding of the mechanism that regulates insulin secretion plays a key role in the development of new therapies and in the cure and control of the disease. Use of cell line techniques open new insights to the better and improved ways of understanding the complications of the disease by the use of carcinogenic and finite cell lines in the laboratories. Expression of SV40 immortalizes cell lines and thus it provides a basis for the establishment of cell lines in vitro from which differentiated continuous cell line may be easily preserved and insulin secretion by various stimulations is possible. The treatment of diabetes with phytochemicals had not produced satisfactory response to the disease. Thus, investigators turned to the use of cell line therapy for the cure of the disease.

For the advancement of beta cell biology during last few decades, researchers have made numerous attempts to createhuman pancreatic β cell lines, which proved to be a milestone for drug discovery, and provide a pathway to β cell replacement therapy for the treatment of diabetes. Several steps of the process for the generation of rodent pancreatic β cell lines had been developed which took years of work. In one of the earlier significant studies, it was found that after inducing irradiation on islets of rats, insulin-producing pancreatic tumor appeared and could be propagated as insulinomas which were transplantable which provided a way for the passage of the insulinoma cells in vitro as RIN cells.

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How to cite this article:

Vandna Dewangan *et al*.2018, The Nt Advancement In Diabetes Treatmentusing B-Cell Lines. *Int J Recent Sci Res.* 9(6), pp. 27550-27554. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2283

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