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Tools for Diabetes Research and Their Use in Drug and Gene Therapy

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Abstract

Diabetes mellitus is now a world-wide health problem and much attention has paid on the issue by medical scientists to find out novel treatment protocols to deal with the problem. As we know, one new therapy method in clinic depended on the basic research and the tools for research work were the core issue that the scientists cared much. In this review, we first describe the tools including cellular and animal tools for diabetes research, in which their history, development and use in drug treatment as well as in gene therapy were emphasized. Much attention is paid on the latest developments that by using these models creates the novel possible protocols to treat diabetes of both type 1 and type 2 diseases.

Keywords: Diabetes mellitus;Drug; Gene Therapy

Introduction

Nowadays, the morbidity of diabetes mellitus as a metabolic disease is rising rapidly worldwide and this increases the burden of people in economy and health in the society[1-2]. The number of people with diabetes is estimated to further increase to about 642 million by 2040 [3]. Diabetes mellitus can be divided into two types according to the pancreas function in delivering insulin. Type 2 diabetes mellitus (T2DM) characterized by excess blood sugar levels accounts for around 90% of the cases and type 1 diabetes mellitus (T1DM) characterized by insulin deficiency in pancreas accounts for only 10% of the cases [3]. Both of the T1DM and T2DM can cause severe damage to body systems such as kidneys, eyes, and the heart, as well as the vascular system more generally. Thereafter, people have pay much attention to researches dealing with diabetes in many aspects such as environment, diet, lifestyle, pathophysiology, treatment and even gene therapy. However, the escalating burdens of T1DM and T2DM indicated that past prevention efforts via interventions to lifestyle such as increasing physical activity and promoting healthy diet have not led to most population improvement. Still nowadays, the scientists use various cellular and animal models to study diabetes and hope to find novel methods to conquer this disease. In this review, we describe the general models, both cellular and animal, used in diabetes research and indicate how these models were used in drug treatment, drug development and gene therapy in diabetes.

Cellular models and their application

Mouse models

Mouse cellular models were usually from mouse insulinoma that was induced by SV40 T-antigen gene transformation. These models include MIN, Beta-TC, IgSV195, Beta-HC and NIT. Among the models, some of them can be further classified into sub-lines such as HIT-T15, MIN6, Beta-TC1, Beta-TC6, NIT-1 and so on. Most of the models can produce proinsulin or insulin under the glucose stimulation and can be used as ideal models in diabetes research concerning not only physiology but also pathology.

Beta-TC6

This cell line is insulin-secreting and often used in the research of insulin secretion mechanism. One of the novel anti-diabetes drugs now used in clinic, GLP-1 (glucagon-like peptide-1) receptor agonist, was developed on the basis of this mechanism [4]. GLP-1 could stimulate the beta-TC6 cells in which calcium ions concentration increased rapidly and followed by insulin secretion [5]. It was verified that many other drugs or chemicals also had the effects on stimulating intracellularly the calcium increase in the beta-TC6 cell line. This is the common mechanism underlying the effects of them to secrete insulin [6-7]. In pancreatic cancer patient, altered intracellular calcium fluxes in pancreatic cancer cells could induce diabetes mellitus [8]. Also, this cellular model was used to screen some phytochemicals to observe if they could stimulate insulin secretion to lower glucose levels [9-10]. This is the basic way to discover new compounds which maybe in the following development to become novel anti-diabetes drugs.

Beta-HC9

The beta-HC9 cell line which was derived from pancreatic islets with beta-cell hyperplasia is characterized by a normal concentration-dependency curve for glucose-stimulated insulin release, whereas the beta-TC6 cell line, derived from pancreatic beta-cell tumors, shows a marked leftward shift of this curve [11]. Maximum velocity and the Michaelis-Menten constant of glucose uptake in beta-HC9 and beta-TC6 cells were similar, especially GLUT-2 (Glucose Transporter Protein-2) transporters were expressed in these two cell lines. In this cell lines, the kinetic characteristics of glucose usage, glucose oxidation, and glucose-induced oxygen consumption was similar to those of glucose phosphorylation, indicating that the kinetics of glucose metabolism from the glucose phosphorylation step in the cytosol to the mitochondrial process of oxidative phosphorylation are determined by the glucose-phosphorylating enzyme, that is, by glucokinase in beta-HC9 cells. All of these mean that the cell line express both GLUT-2 and glucokinase and thus it provides an opportunity for the quantitative analysis of glucose metabolism, the associated generation of coupling factors, and other essential beta-cell functions involved in glucose sensing and insulin secretion [12]. In the following research, the abnormality of glucokinase was studied [13] and some other factors beside glucose such as lipid or amino acid were studied on the influence of insulin secretion in this cell line [14-15]. However, it was actually seldom to use this cell line model to study the function of GLUT-2 and usually primary islet cells and rat cell models were used in GLUT-2 function studies.

*NIT-*1

NIT-1 is a pancreatic beta-cell line established from one of the transgenic mice harboring a hybrid rat insulin-promoter/SV40 large T-antigen gene spontaneously following by developed beta-cell adenomas [16]. Immunocytochemical staining of the cells showed most contained insulin, with less of them containing glucagon, and none containing pancreatic polypeptide or somatostatin. Glucagon content assayed by radioimmuno-method in cell extracts was only 0.27% of the insulin content. As responding to glucose stimulation, insulin secretion in two-hour period at 16.5 mM glucose stimulation was 638 ng/106 cells (41% of intracellular content) compared to only 1.3 ng glucagon (32% of intracellular content) in the same cell numbers. Stimulated insulin secretion was consistently observed in response to 11 and 16.5 mM glucose in many passages of the cells. Specifically at passage 19, both theophylline and tolbutamide could stimulate insulin secretion at 5.5 mM glucose. Northern-blot analysis confirmed that high levels of insulin mRNA but only few trace glucagon mRNA and undetectable somatostatin mRNA were expressed. Using this model, it was discovered that immune-system could regulate the insulin secretion [17-19]. And now, people have discovered that some chemical agents through the unique genes could regulate insulin secretion in this cell line model [20-22]. As described above, it is clear that immune-system in our body has take part in insulin regulation as is verified as inflammation in the pancreas. Some genes through miRNA also participate the regulation of insulin secretion. This opened the way on which people could follow the clue to find out the real causes leading to the diabetes and develop new drugs to treat diabetes.

*MIN*6

The cell line MIN6 has been established from insulinomas obtained by targeted expression of the simian virus 40 T antigen gene in transgenic mice [23]. This cell line produces insulin and T antigen and has morphological characteristics of pancreatic beta cells. MIN6 cells exhibit glucose-inducible insulin secretion comparable with cultured normal mouse islet cells and produce liver-type glucose transporter (GLUT-2) mRNA at high level suggesting that exclusive expression of the liver-type GLUT-2 is related to glucose-inducible insulin secretion. MIN6 cells do not express either major histocompatibility (MHC) class I or class II antigens on the cell surface. However, treatment with interferon-gamma induces high levels of MHC class I antigens, and a combination of interferon-gamma and tumor necrosis factor-alpha induces a MHC class II antigen on the cell surface. These results emphasize that the MIN6 cell line retains physiological characteristics of normal beta cells. The MIN6 cell line will be especially useful to analyze the molecular mechanisms by which beta cells regulate insulin secretion in response to extracellular glucose concentrations. This superfusion system of a pancreatic beta-cell line, MIN6, when loaded with fura-2, also allowed simultaneous measurement of cytosolic free calcium concentration and insulin secretion. MIN6 cells released insulin in response to high glucose, thus resembling events in normal islet cells. Especially, cytosolic free calcium concentration and insulin secretion rapidly increased and the increase was suppressed by mannoheptulose or by sodium azide. This increase was also suppressed by lowering the temperature of the medium. Cytosolic free calcium concentration and the insulin secretion induced by leucine were not influenced by mannoheptulose but were inhibited by sodium azide. There was a close relation between the rise in cytosolic free calcium concentration and insulin secretion in all cases. This findings provided a direct evidence that a rise in cytosolic free calcium concentration depended on glucose metabolism and was a primary signal for insulin secretion [24]. Nowadays, the studies indicated that the insulin secretion was closely related with inflammation and some factors could influence the inflammation process [25-29]. All of these researches mean that diabetes is actually an inflammatory disease which at first stage is caused by inflammation in the pancreatic islets. Therefore, the MIN6 model can be used to observe some inflammatory factors by which to influence the insulin secretion in the cells and figure out which one could lead to diabetes as the result occurred in the pancreas. In recent years, miRNA regulation to the function of insulin secretion appeared more and more important. It was documented that not only miRNA but also lncRNA could regulate insulin secretion in MIN6 cells [30-32]. Insulin secretion is coded by insulin gene and its gene expressing is regulated by miRNA or lncRNA. This kind of gene regulation opened the way by which epigenetic gene therapy could find its target in diabetes treatment.

Rat models

RIN

The origin of the RIN cell cultures were initiated from a transplantable islet cell tumor induced by high-dose x-irradiation in an inbred NEDH (New England Deaconess Hospital) rat. The tumor was maintained by serial transplantation in NEDH rats. After nine transplants, it was successfully heterotransplanted into athymic nude mice with BALB/c background. Continuous cell lines were then derived either from rat transplants or from nude mouse heterotransplants. These cell lines were named RIN-r and RIN-m respectively [33]. These cell lines revealed a wide range of insulin secretion, from undetectable to relatively high concentrations. Preliminary data indicate that at least some clones secrete somatostatin. The presence of glucagon in the supernatant fluids of the parent lines and clones could not be unequivocally demonstrated, because the values obtained were near the lower limit of detectability of the assay (15 pg/ml of supernatant fluid). So, the cell lines are mainly insulin-secreting cells and are used as pancreatic insulin research. Later, Cells grown in culture from rat islet cell tumor (parent cells) and clones obtained from them were used to establish the RINm5F cell line [34]. In the establishment process, parent cells secreted primarily insulin and somatostatin with very small quantities of glucagon. The clones, based on hormone content and secretion, were divided into three phenotypic groups: insulin secreting, somatostatin secreting, and nonsecreting clones. Specific receptors for insulin, glucagon, and somatostatin were demonstrated on parent cells and clones. Actually, the RINm5F cell line is a subclone of original RINm and RINr cell lines and used as a cell but no glucose stimulation effect [35-36]. This no response to glucose to stimulate insulin secretion was related with intracellular calcium oscillation [37-39]. This characteristics made the cell line become the best model to study glucose tolerance and insulin resistance [40-41]. Nowadays, it is shown that the RIN cell models are used to study how to protect pancreatic cells from oxidative damage and the mechanism underlying the damage [42-46]. It is well-known that inflammation occurred in pancreas could lead to diabetes and such inflammation is usually caused by oxidative damage to pancreatic cells. Thereafter, the cell line RINm5F is a good model in this research.

*BRIN-BD*11

This insulin-secreting cell line (BRIN-BD11) was established after electrofusion of RINm5F cells with New England Deaconess Hospital rat pancreatic islet cells [47]. The fusion mixture was with insulin output 5-10 times greater than parent RINm5F cells and then was subcultured until with eventual establishment of the clones named BRIN-BD11. Morphological studies indicated that these cells growed as monolayers with epithelioid characteristics, maintaining stability in tissue culture for many passages. Culture of these cells for 24 h at higher glucose revealed a 1.8- to 2.0-fold increase of insulin secretion compared with lower glucose simulation. Dynamic insulin release was recorded in response to 16.7 mmol/l glucose, resulting in a rapid threefold insulin secretory peak followed by a sustained output slightly above basal. Stimulation of insulin secretion with 16.7 mmol/l glucose was abolished by mannoheptulose or diazoxide. In contrast, glyceraldehydes and K^+ evoked 1.7- to 9.0-fold insulin responses. Besides, L-Alanine also evoked a twofold secretory response, which was potentiated 1.4-fold by increasing the Ca^{2+} concentration in the media. Forskolin and phorbol 12-myristate 13-acetate both increased insulin secretion in the presence of L-alanine. Western blotting confirmed that BRIN-BD11 cells expressed the GLUT2 glucose transporter. This, coupled with a high glucokinase/hexokinase ratio in the cells, confirms an intact glucose sensing mechanism. High-performance liquid chromatography analysis demonstrated that insulin was the major product secreted under stimulatory conditions. All of the data indicate that the BRIN-BD11 cell line represents an important stable glucose-responsive insulin-secreting beta-cell line for diabetes studies. Later, the characteristics of this cell line was in detail studied [48-49]. At present, the cell line was used to study the GLP (Glucagon-like Peptide) receptors on the cells and the effect of glucagon receptor antagonist on the cells [50-52]. It is discovered that some peptides or proteins such as neuropeptide-Y and gastrin family peptides can influence or regulate the function of pancreatic beta-cells [53-56]. And other factors which could influence the pancreas function were studied [57]. These results indicate that in our body the neuro-endocrine system has taken part in the regulation of pancreas function and insulin secretion can be influenced by the neuro-endocrine system. This has broadened our mind to think about the novel protocol to treat diabetes of both type 1 and type 2 diseases.

*INS-*1 *and INS-*1*E*

The insulin-secreting cell line (INS-1) was established from cells isolated from an x-ray-induced rat transplantable insulinoma [58]. The continuous growth of these cells was dependent on the reducing agent 2-mercaptoethanol and removal of this thiol compound caused a 15-fold drop in total cellular glutathione levels. These cells proliferated slowly at the population doubling time about 100 hrs and showed morphological characteristics typical of native beta-cells. Most cells stained positive for insulin and did not react with antibodies against the other islet hormones. The content of immunoreactive insulin was about 8 mg/106 cells which corresponding to 20% of the native beta-cell content. These cells synthesized both proinsulin I and II and made conversion of the two precursor hormones similar to those observed in rat islets. However, glucose failed to stimulate the proinsulin biosynthesis. In static incubations, glucose stimulated insulin secretion from floating cell clusters or from attached cells in the media and under perifusion conditions, higher concentrations of glucose enhanced secretion 2.2-fold insulin. In the presence of forskolin and 3-isobutyl-1-methylxanthine, increase of glucose concentration caused a 4-fold enhancement of the rate of secretion. Glucose also depolarized INS-1 cells and raised the concentration of cytosolic Ca2+. This suggests that glucose is still capable of eliciting part of the ionic potential change

at the plasma membrane, then leads to insulin secretion. The structural and functional characteristics of INS-1 cells remained unchanged over a period of 2-year culture passage. INS-1 cells retain beta-cell surface antigens, as revealed by reactivity with the anti-ganglioside monoclonal antibodies R2D6 and A2B5. These indicate that INS-1 cells have remained stable status and retained a high degree of differentiation which make this cell line a suitable model for studying various aspects of beta-cell function.

Rat insulinoma-derived INS-1 cells become a widely used beta-cell surrogate. However, due to their nonclonal nature, INS-1 cells are heterogeneous and are not homogeneous over extended culture periods. Then clonal INS-1E cells from parental INS-1 were isolated based on both their insulin content and their secretory responses to glucose [59]. The stable differentiated INS-1E beta-cell phenotype over hundred passages can be kept and safely cultured in which insulin contents were about 2.30 mg/million cells. Glucose-induced insulin secretion was dose-related and similar to rat islet responses. Secretory responses to amino acids and sulfonylurea were similar to those of islets. Moreover, INS-1E cells retained the amplifying signaling pathway as judged by glucose-evoked augmentation of insulin release in a high-depolarized state in the cells. INS-1E cells exhibited glucose dose-dependent elevations of cytosolic Ca^{2+} and mitochondrial Ca^{2+} levels by which mitochondrial membrane potential and cell membrane potential were all fully activated by glucose. Using the perforated patch clamp technique, a potasium current was identified in whole cell voltage clamp by diazoxide or tolbutamide stimulation. As in native beta-cells, tolbutamide usually induced electrical activity of potassium conductance that is important in setting the resting potential of the cells. And some concentration of glucose also elicited electrical activity to some degree that is the action potential of the cells to secrete insulin. Therefore, INS-1E cells represent a stable and valuable beta-cell model for insulin secretion research. Interestingly, it was discovered that glutamate, a central neuronal transmitter, had the effect to regulate the cell line to secrete insulin [60-62]. This indicates a kind of axes regulation of neuro-endocrine system to pancreatic insulin secretion. It was verified that some factors related with neuronal protein or hormone could regulate insulin secretion in this cell model and have the future to treat diabetes [63-64]. Nowadays, more and more data have shown that some genes can regulate insulin secretion by using this model [65-66]. Besides, people have studied many chemical agents in which some have stimulating, but some have inhibiting or toxic effects on pancreatic beta-cells in this model [67-70]. From the results, it is clear that chemical agents could exert their positive or negative regulation to pancreatic function and this explains the occurrence of side effects during diabetes drug treatment.

Human

CM

In the human pancreatic cell models, although TRM-1 and Blox5 were established from fetal pancreas [71-72], CM was the most used cell model in the category. In the cell line, it have been evaluated the expression of different beta-cell markers, islet molecules and auto-antigens relevant in diabetes autoimmunity in order to define its similarities with native beta cells and to discover whether it could be considered as a model for studies on immunological aspects of Type 1 diabetes [73]. The positivity of the CM cell line for known markers of neuro-endocrine derivation was determined by means of immunocytochemical analysis using different anti-islet monoclonal antibodies reacting with islet gangliosides and antibody binding to an islet glycoprotein. Then, the expression and characteristics of glutamic acid decarboxylase (GAD) and of GM2-1 ganglioside, both known to be islet autoantigens in diabetes autoimmunity and expressed by human native beta cells, were investigated in the CM cell line. The results showed that beta cell markers identified by anti-islet monoclonal antibodies were expressed by CM cells and islet molecules such as GAD and GM2-1 ganglioside were present and possessed similar characteristics to those found in native beta cells. The pattern of expression of other gangliosides by CM cells was also identical to human pancreatic islets. The beta cell autoantigens reacting with antibodies present in diabetic islet cell were also detectable in this insulinoma cell line. Therefore, CM cells show close similarities to native beta cells with respect to the expression of neuro-endocrine markers, relevant beta cell autoantigens in Type 1 diabetes (GAD, GM2-1, ICA antigen), and other gangliosides. This insulinoma cell line may be an ideal model for studies of autoimmune phenomena occurring in Type 1 diabetes. Later, this human insulinoma cell line CM was shown to retain beta-cell function, particularly the expression of constitutive beta-cell genes (insulin, the glucose transporters GLUT1 and GLUT2, glucokinase), intracellular and secreted insulin, beta-cell granules, and cAMP content [74]. It was showed that CM cells from an early-passage expressed specific beta-cell genes in response to glucose stimulation, in particular the insulin and GLUT genes. However, such capacity was lost at later passages when cells were cultured at standard glucose concentrations. If cultured at lower glucose concentration for a longer time, CM cells re-acquired the capacity to respond to glucose stimulation as shown by the increased expression of beta-cell genes (insulin, GLUT2, glucokinase). these data show that the human insulinoma cell line CM, at both early-passage and late-passage, posseses a functional glucose-signalling pathway and insulin mRNA expression similar to normal beta-cells, representing, therefore, a good model for studies concerning the signalling and expression of beta-cells. The physical method such as ultrasound could simulate insulin secretion in this cell model and this arouses the possibility to use the ultrasound to treat diabetes [75-76]. Besides, people have used this model to test the stem cell therapy in diabetes and the results are promising [77-78]. This human pancreatic cell line was from the ascetic fluid of a patient with liver metastasis of a malignant insulinoma and was real human origin. However, because of its no insulin secretion in response to increasing glucose, it is hindered to its use in the insulin secretion research.

Animal models and their application

As to the diabetes animal models, there are many different kinds of animal whole body models used in the research, such as mouse models and rat models in which some are induced by chemicals and some are made by genetic methods. All of these animal models represent human type 1 and type 2 diabetes in their phathophysiology and response to drug treatment. Therefore, these animal models cover almost the whole conditions expressed in the diabetes process and complications in different organs of the disease [79-81]. Our focus of this review is put on how these animal models are used to study the pathophysiology of diabetes and the mechanism of novel drugs against diabetes, especially some advances using these models in the field of diabetes research.

Type 1 Diabetes Animal Models

BB Rats

In 1974, spontaneous autoimmune diabetes was identifed in rat animal and Biobreeding (BB) rat was derived from outbred Wistar rats. [82]. Then, immunologically and genetically distinct BB rat substrains were derived from several tertiary Biobreeding (BB) rat colonies. Additionally, BB rats resistant to diabetes have also been bred to act as controls. In the model, Biobreeding (BB) rats develop diabetes with a similar incidence between males and females after puberty, in which with about 90% of rats developing diabetes between eight and sixteen weeks of age. The rat diabetic phenotype is quite severe and characterized by hyperglycemia, hypoinsulinemia, weight loss and ketonuria. The rat is requiring insulin therapy for survival. The early histological abnormalities in the islets of rat pancreas indicate the latter clinical and metabolic symptoms of the rat as the model. Therefore, it is a good model to study diabetes pathophysiology, especially the immunopathology leading to the disease [83]. At present, this rat model was used to reveal the pathogenesis of diabetes [84]. More and more attentions have been paid on the autoimmunity mechanisms causing diabetes by using this model in research [85-87] and it has been thrown light on this issue of diabetes [88]. As the result, it is gradually clear that because of some genetic factors in the body, some autoantigens are produced in the body and immune cells are activated to produce some cytokines which in turn lead to inflammation occurred in islets of pancreas. The damage of beta-cells in pancreas by such inflammation in the end makes the islets losing the ability to secrete insulin and hyperglycemia occurs. The protocols to treat autoimmuno-diabetes fall into three main methods: autoantigen treatment, inflammation treatment and immunomodulatory treatment.

Nonobese Diabetic Mouse(NOD)

When this type of mouse model was developed at Shionogi Research. Laboratories in Osaka of Japan in 1974, it was found that insulitis in the mice appeared at around 3rd or 4th week of their age [89]. During this pre-diabetic phase, the islets of the pancreas in mice were infiltrated with CD4+ , CD8+ lymphocytes, also with NK and B cells. This infiltration of innate immune cells into the islets, then which become adaptive CD4+ and CD8+ T cell subsets into the islets leading to at last diabetes development about from approximately 4-6 weeks of age. Because of insulitis leads to the destruction of beta cells, the onset of overt diabetes usually appears when approximately 90% of the pancreatic insulin secretion is lost at around 10-14 weeks and the mice can lose weight rapidly requiring insulin treatment. Unlike other models used in autoimmunity studies, Nonobese Diabetic (NOD) mouse is one of the most commonly used models to study type 1 diabetes (T1D), which developing spontaneous diabetes very similar to humans in pathophysiology. People have made the identifcation of several autoantigens and biomarkers in the model that are similar in humans and enabled the model to be found the therapeutic targets for development of novel drugs treating diabetes. Now, as we know, both in NOD mice and in humans, the most important genetic factor that contributes to T1D susceptibility is the major histocompatibility complex (MHC) known as insulin dependent susceptibility 1 (idd1) in mice and insulin dependent diabetes mellitus1 (IDDM1) in humans [90-91]. In later studies, more than 40 genetic loci discovered in both NOD mice and humans have been shown to play an important role in mediating T1D susceptibility, including genes related to immune system function and regulation as well as pancreatic beta cell function. MHC class 2 proteins in NOD mice share structural similarities to those in humans, which may confer resistance or susceptibility to the diabetes in both NOD mice and humans. The similarity in the genes of type 1 diabetes (T1D) between NOD mice and humans has provided a potentially suitable model for finding therapies in which modulation of the autoimmune response is being targeted. Nowadays, this model is still active in use to study the pathophysiology of diabetes and some new advances have been made in the autoimmunity field [92-93].

As to the new development, more and more researches concerning vaccines and antibodies in treating T1D with this model [94-95]. That indicates as an autoimmune disease, T1D can be treated with immunological protocols. Interestingly, it is discovered that the homeostasis of gut microbiota could regulate the autoimmunity in T1D in the model [96-98]. Through regulating the gut microbiota people could control the immune response occurred in gut or even in blood stream, then control the autoimmunity that may lead to diabetes. Autoimmunity can have both tumor-promoting and tumor-suppressing impacts on an organ such as pancreas. However, the net effects of autoimmunity are likely to vary based on the tumor type, the susceptibility to T cell activating and the environmental stimuli to the tumor cells. By using the NOD mice model, it is clear that after inoculating the Ela1-TAg transgenic vector, NOD mice display delayed formation of pancreatic cancer and substantially slower tumor growth rates, suggesting that elevated autoimmunity and autoreactivity limit local tumor development and growth [99].

Akita Mouse

The Akita mouse diabetes model was initially established in Akita of Japan from a C57BL/6NSlc mouse strain due to a spontaneous mutation in insulin-2 gene. This mutated mice caused incorrect proinsulin processing and the aggregation of this misfolded proteins leading to subsequent endoplasmic reticulum (ER) stress and beta-cell death in pancreas [100-101]. Usually, these alterations resulted in pronounced insulin dependent diabetes at an onset of three to four weeks of age. The resulting rodent model exhibits characteristics of hyperglycemia, hypoinsulinemia, polyuria and polydipsia. It has been well utilized as a model of type 1 diabetes (T1D) and as a tool to investigate potential alleviators of ER stress in the islets. At present, this model is still used in revealing the pathophysiology and complications of diabetes such as neuronal damage, retinal damage, renal damage as well as cardiac damage [102-105]. Especially, in recent years, the emphases is put on the researches concerning nephropathy and retinopathy caused by diabetes [106-109]. All of these data reflect the mouse model to be an original diabetes model suitable for the disease study compared with the conditions similar with human suffering the diabetes.

Type 2 Diabetes Animal Models

Zucker Diabetic Fatty (ZDF) Rats

In 1961, after a cross-match between rats of Merck (M-strain) and Sherman rats, it was discovered of this type of rats in which they are characterized by a mutated leptin receptor that induces hyperphagia and the rats become obese at the four weeks of age [110]. Because of homozygous mutation (fa/fa) of the leptin hormone receptor, the rats developed type 2 diabetes in male animals when they were fed a high-energy rodent diet and these rats were expressed of hyperinsulinemic, hyperlipidemic and hypertensive as well as impaired glucose tolerance phenotypes. Later, these rats developed advanced insulin resistance and glucose intolerance between three and eight weeks of age and turn overtly diabetic rats between eight and ten weeks of age with high glucose levels in the feeding state. There is evidence that suggests a good consistency between the increase DNA content in islet and serum insulin levels indicating that islet hyperplasia plays a vital role in the development of hyperinsulinemia in the rat model known as Zucker Diabetic Fatty (ZDF) rats. Meanwhile, triglycerides and cholesterol levels in obese rats are higher than those observed in lean rats. This is because in skeletal muscle and pancreatic islets, excessive non-beta-oxidative metabolism of fatty acid is attributed to lipo-toxicity to these tissues. The common complications in the model such as obesity, insulin resistance, cardiovascular disease and diabetes are believed to be caused by high levels of these metabolic products by disrupting islet cell function and ultimately by promoting programmed cell death in pancreas. In addition, very high lipid levels can also be induced in obese Zucker Diabetic Fatty (ZDF) rats feeding high saturated fat and sucrose-containing diets. Therefore, this is a good model for studying the obese-type diabetes. In recent years, this model is often used to test some drugs effects in treating diabetes [111-114] and the model will contribute to the novel drug development [115-118]. In contrast to the type 1 diabetic models, type 2 diabetic models, even also used in studies of pathophysiology of the disease, are mainly used in the researches concerning pharmaceutical and pharmacological effects of drugs dealing with diabetes and often correlate the studies with the clinical situations.

Goto-Kakizaki Rats

This type 2 diabetes (T2D) rat model originates from Wistar (W) rat established by repeated inbreeding with the rats at the upper limit concentration of normal glucose tolerance [119-120]. This kind of the most likely glucose intolerance maybe due to impaired islet cell mass and the background function of a polygenic inheritance in the rats. Following chronic exposure to hyperglycemia (called gluco-toxicity) may further impair cell function and insulin action, and at last contribute to the development of diabetes with persistent hyperglycemia. However, insulin resistance produced may likely make contribution to the secondary cause in the development of hyperglycemia in this model. In Goto-Kakizaki (GK) rat model, the pancreatic islets structure may be disrupted with clear fibrosis making the islets resemble the appearance of a starfish. This structure change makes the islet cells mass decreased and beta-cells proliferation inhibited resulting the insulin secretion decreased by almost 60%. Therefore, this is a glucose-induced non-obese diabetes rat model and is good and suitable for T2D research [121]. At present, This model is often used to study signaling pathway in diabetes cells or tissues [122-124]. And this model is also used to study the possible muscle damage such as cardiac muscle or skeletal muscle caused by diabetes [125-127]. Now, some questions have been put forward as to the genetic differences in this model as compared with high-fat diet feeding induced diabetes model and this will makes clear how much part of the genetics has taken part in the development of the disease [128-129]. In contrast to the ZDF model which is a spontaneous genetic type 2 diabetes model and the high-fat diet feeding induced type 2 diabetes model, this Goto-Kakizaki (GK) model belongs to the middle of the former two models in which genetic, in part, takes the role in diabetes development and provides a wide model selection when doing research work.

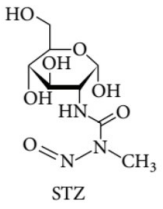
Chemically Induced Diabetes Models

Streptozotocin (STZ) Induced Model

In chemical structure, STZ belongs to a nitrosourea analogue (Fig.1), in which there is linkage between the N-methyl-N-nitrosourea (MNU) moiety and carbon-2 of hexose [130]. Generally, the mode of action of toxicity of STZ to islet cells depends on the DNA alkylating activity of its methyl-nitrosourea moiety. In the pancreatic cells, the transfer of the methyl group from STZ to the DNA molecules in cells causes the damage along a chain of cellular events and at last leads to cellular DNA fragmentation (apoptosis).

STZ-induced diabetes is classified into types: the adult type and neonatal type. In the adult type, rats weighing 140 to 300 g af-

ter an overnight fast were given a single streptozotocin (STZ) injection (45-70 mg/kg) intraperitoneally, with the drug solution dissolved in 0.1 mole/L citrate buff er (pH 4.5). In the neonatal type of STZ-induced diabetes, neonatal Wistar (W) rats of 2-4 days of age are intraperitoneally injected with 65-100 mg/kg of STZ, and the neonatal rats are kept with their mother during the lactation period until week four. Usually, in these two types of rats, type 2 diabetes (T2D) can be induced by STZ and can be used as good models for diabetes research. In this STZ-induced model, whether STZ is good or bad to the diabetes heart in the rats has been studied through the ischemia-reperfusion method by preconditioning or by postconditioning protocols [131]. Nowadays, STZ-induced diabetes model is still used in the studies concerning the relation between gut microbiota and diabetes [132-134]. Some studies show that in this diabetes model, some factors or agents could protect liver function damaged by the disease and emphasize the relationship between diabetes and liver function [135-137]. Some anti-oxidation agents such as resveratrol, curcumin and gallic acid show effective in ameliorating diabetes using this rat model [138-140]. STZ-induced diabetes model is simple-manipulated and easy-controlled, thereafter it is widely used in experimental and pre-clinical researches of diabetes. Alloxan Induced Diabetes Model. In chemistry, al-



STZ

Figure 1. The chemical structure of streptozotocin (STZ)

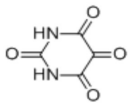


Figure 2. The chemical structure of alloxan

loxan is 2,4,5,6-Tetraoxohexahydropyrimidine and a very old molecule (Fig.2).

In making the model, alloxan was used to induce diabetes in fasting male. Wistar (W) rats weighing 200-250 g and the drug was given subcutaneously at the dosage of 125 mg/kg of alloxan. It is well known that alloxan possesses two pathological eff ects on pancreatic cells. In one aspect, it can selectively inhibit glucokinase in islets, which is in charge of secretion of insulin induced by glucose. In another aspect, it induces reactive oxygen species (ROS) formation in the islets causing a selective necrosis of beta cells, then creates a state of insulin dependent diabetes by the model [141]. Usually, this chemical can induce animal diabetes in mouse, rat, rabbit and cat. In recent years, this alloxan-induced diabetes model is used in the studies using plant components or extracts to treat the disease [142-146]. Steroid compounds such as sex hormones and vitamin D or E are found to be effective in ameliorating diabetes in this model [147-150]. The model is especially easy to be induced in mice and induced diabetes falling into type 1 or type 2 is attributed to the alloxan dosage to be used on the mice. Therefore, alloxan-induced diabetes is more suitable for small animal models.

Perspectives

Stem Cell Application in the Models

As we know, diabetes mellitus (DM) is a complicated metabolic disease in which, nowadays the treatment maybe diet control, insulin injections, or islet and pancreas transplantation. However, all of which are limited because exogenous insulin injections fail to successfully simulate normal insulin secretion in islet beta-cells and islet transplantation lacks suitable organ donors. So far, stem cells with highly self-renewal and multi-directional differentiation potential have become a new protocol for the treatment of diabetes. Various stem cell types are being explored to serve as an alternative source of islets in treating diabetes. This study was conducted to evaluate the ability of in-house developed human embryonic stem (hES) cells-derived pancreatic progenitors to ameliorate diabetic symptoms in mice [151]. Pancreatic progenitors were packed in macro-capsules and transplanted into Swiss mice and some mice were taken as controls. Thirty days post-transplantation, diabetes was induced by streptozotocin treatment and mice were then followed up for handred days and body weight and blood glucose levels were regularly monitored. It was observed that control mice lost weight, maintained high glucose levels and did not survive beyond 40 days, whereas transplanted group maintained body weight and lowered blood glucose levels. In some research [152], rat Muscle-derived satellite cells (MDSCs) were separated, cultivated in vitro and inducted into insulin-producing cells verified by using dithizone staining and so on. Then, T1D rat model induced with Streptozocin (STZ) was built, and MDSCs induced insulin-producing cells labeled by Dil and control cells were transplanted respectively. Transplantation of these cells into streptozotocin (STZ)-induced diabetic rats resulted in lower blood glucose, lower urine glucose, higher body weight, higher glucose tolerance and less water intake and urine output than control rats. Histological examination revealed that the transplanted cells reached the pancreas and repaired damaged tissues. The above research conclusions provide good prospect for the treatment of diabetes. In T1D, successful treatment need to restore the function of pancreatic beta-cells that are destroyed by the self immune system and overcome the further destruction of insulin-producing cells.

Gene Therapy Application in the Models

Over the past years, genetic and pigenetic therapies (gene therapy) have been developed to treat many human diseases, including diabetes, cancer, autoimmunity, and genetic disorders. Most of these approaches have relied on drugs that ubiquitously alter the genetic or epigenetic marks (e.g., DNA binding, DNA interference, DNA methylation or histone modifications). However, these protocols nowadays translate gradually the drug gene therapy into biolog-

ical gene therapy with gene vectors such as retro-virus (RV) or adeno-associated virus (AAV) [153]. In one of these studies [154], it was report that a robust system for in vivo activation of endogenous target genes through trans-epigenetic remodeling was established. The system relies on recruitment of Cas9 and transcriptional activation complexes to target loci by modified single guide RNAs. In this study, they used this technology to treat mouse models of diabetes and other diseases and demonstrated that CRISPR/Cas9-mediated target gene activation could be achieved in vivo leading to measurable lower glucose level phenotypes and amelioration of diabetes symptoms. This has established new avenues for developing targeted epigenetic therapies against human diabetes. In another research [155-156], adeno-associated virus (AAV) carrying Pdx1 and MafA expression cassettes was transduced into the diabetes model and it was through the pancreatic duct to reprogram alpha-cells into functional beta- cells, then normalized blood glucose in both beta-cell toxin-induced diabetic mice and in autoimmune non-obese diabetic (NOD) mice. This gene therapy strategy also induced alpha to beta-cell conversion in toxin-treated human islets as well as restoring blood glucose levels in NOD/SCID mice upon transplantation. This strategy represented a new therapeutic approach to bolster endogenous insulin production and provides a potential basis for treating human type 1 diabetes.

Artificial Intelligence Application in the Models

Artificial Intelligence (AI) in medicine is a general term that implies the use of a computer to model intelligent behavior in cells or in body with minimal human intervention. AI is generally accepted as having started with the invention of robots such as the term derives from the Czech word robota, the biosynthetic machines used as forced labor and the Leonardo Da Vinci's lasting heritage, named after him, the burgeoning use of robotic-assisted surgery for complex urologic and gynecologic operation procedures. AI, described as the science and engineering of making intelligent machines was officially born in 1956 and the term is applicable to a broad range of items in medicine such as robotics, medical diagnosis, medical statistics, and human biology including today's "omics" in bioinformatics. AI in medicine usually has two main branches that are virtual and physical. In the virtual branch, it includes informatics approaches from machine deep learning system to health management systems control, such as electronic health records and physicians' treatment decisions. In the physical branch, it is represented by robots used to assist the elderly patient nursing or the attending surgeon operation. Also in this branch are targeting nano-robots and checking micro-robots, a unique new drug delivery system or a new dynamic diagnostic system [157]. Nowadays, these applications have also found their ways in diabetes research and treatment. In one of the studies [158], a deep learning system (DLS) was built that was a machine learning technology with potential for screening diabetic retinopathy and related eye diseases such as vision-threatening diabetic retinopathy, possible glaucoma, and age-related macular degeneration (AMD) in diabetes patients. Diagnostic performance of a DLS for diabetic retinopathy and related eye diseases were evaluated using 494-661 retinal images. Then, DLS was trained for detecting diabetic retinopathy, possible glaucoma, and AMD. Area under the receiver operating characteristic curve (AUC) and sensitivity and specificity of the DLS with professional graders were as the reference standard. In the primary validation dataset for this evaluation of retinal images from multiethnic cohorts of patients with diabetes, the DLS had high sensitivity and specificity for identifying diabetic retinopathy and related eye diseases. This research provides necessary applicability of the DLS in diabetes compared with health care settings and the utility of the DLS could be helpful to improve vision outcomes. In another research [159], the predictive models for detecting undiagnosed diabetes using data from the Longitudinal Study of Adult Health database were built and used to compare the performance of different machine-learning algorithms in this task. After selecting a subset of 27 candidate variables from the literature, models were built and validated in four sequential steps: (1) parameter tuning with tenfold cross-validation, repeated three times; (2) automatic variable selection using forward selection, a wrapper strategy with four different machine-learning algorithms and tenfold cross-validation, to evaluate each subset of variables; (3) error estimation of model parameters with tenfold cross-validation, repeated ten times; and (4) generalization testing on an independent dataset. The models were created with the following machine-learning algorithms: logistic regression, artificial neural network, naïve Bayes, K-nearest neighbor and random forest. The best models were created using artificial neural networks and logistic regression. These achieved mean areas under the curve of, respectively, 75.24% and 74.98% in the error estimation step and 74.17% and 74.41% in the generalization testing step. Most of the predictive models produced similar results and demonstrated the feasibility of identifying individuals with highest probability of having undiagnosed diabetes through easily-obtained clinical data.

The Close Concerns (http://www.closeconcerns.com), is a healthcare information company focused exclusively on diabetes and obesity care. Close Concerns was established by Ann M. Carracher, Payal H. Marathe, and Kelly L. Close and publishes Closer Look, a periodical that brings together news and insights in diabetes areas [160]. From the latest developments relevant to researchers and treatments, it is shown that some light has been thrown on the pave that leading to curing the troublesome disease-diabetes of both type 1 and type 2 classifications.

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