R E V I E W

Genome Editing and Human Pluripotent Stem Cell Technologies for in vitro Monogenic Diabetes Modeling

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Abstract: Diabetes is a metabolic disease characterized by chronic hyperglycemia. Polygenic diabetes, which encompasses type-1 and type-2 diabetes, is the most prevalent kind of diabetes and is caused by a combination of different genetic and environmental factors, whereas rare phenotype monogenic diabetes is caused by a single gene mutation. Monogenic diabetes includes Neonatal diabetes mellitus and Maturity-onset diabetes of the young. The majority of our current knowledge about the pathogenesis of diabetes stems from studies done on animal models. However, the genetic difference between these creatures and humans makes it difficult to mimic human clinical pathophysiology, limiting their value in modeling key aspects of human disease. Human pluripotent stem cell technologies combined with genome editing techniques have been shown to be better alternatives for creating in vitro models that can provide crucial knowledge about disease etiology. This review paper addresses genome editing and human pluripotent stem cell technologies for in vitro monogenic diabetes modeling.

Keywords: monogenic diabetes, MODY, NDM, genome editing, pluripotent stem cell

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by pancreatic β cell loss and hyperglycemia due to altered insulin production and/or action. Type 1 diabetes mellitus (T1DM) is an autoimmune disorder characterized by destruction of pancreatic β cells,[1] whereas the pathogenesis that defines Type 2 diabetes mellitus (T2DM) is insulin resistance in the insulin-target tissues and β cell dysfunction.[2] Polygenic diabetes, which encompasses T1DM and T2DM, is the most prevalent kind of diabetes and involves alteration in different genes, whereas rare phenotype monogenic diabetes (MD) is caused by a single gene mutation linked to pancreatic development and β cell activity.[3]

Increased glucose across the GLUT 2 transporter is processed by the enzyme glucokinase in the normal pancreatic beta-cell, resulting in increased ATP generation. This results in the closing of the K_{ATP} channel, which depolarizes the cell membrane and activates calcium influx through voltage-gated calcium channels, allowing insulin granule exocytosis.[4] The inner subunit (Kir6.2) of the K_{ATP} channel is encoded by KCNJ11, while the outer subunit (ABCC8) is encoded by ABCC8 (SUR1).[5] Even in the presence of hyperglycemia, mutations in either gene cause the K_{ATP} channels to remain abnormally "stuck open". The cell membrane cannot properly depolarize without channel closure, and hence insulin cannot be released from the beta-cell.[5,6]

For many years, animal studies have been employed extensively in biomedical research to acquire insights into the normal and aberrant biological processes that occur in humans. However, the variation in disease conditions between animal models and humans limits their utility in mimicking crucial aspects of human disease.[7,8] Human pluripotent stem cells (hPSCs), which include human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs), can be used as an alternative for disease modeling because of their ability to differentiate into all cell types.[9,10]

ESCs and iPSCs have provided a critical opportunity for biological research, allowing us to address problems that were previously unanswerable due to a lack of a human model and replicating the development process with differentiation techniques to generate cell populations of therapeutic significance.[11,12] Furthermore, the recent discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene manipulation enables the correction or introduction of specific pathogenic mutations, the generation of isogenic cell lines, and the investigation of the same genetic variant in multiple genetic backgrounds.[13,14] The purpose of this review paper is to describe recent achievements in modeling monogenic diabetes with genome editing and pluripotent stem cell technology.

Monogenic Diabetes

MD is linked to mutations in a variety of genes involved in pancreatic development and ß cell activity.[15] MD can be divided into two categories: Neonatal diabetes mellitus (NDM) and Maturity-onset diabetes of the young (MODY).[16] Depending on the time of diagnosis NDM is diagnosed within the first six months of life, whereas MODY is diagnosed before the age of 25.[17]

MODY

MODY is the most frequent kind of monogenic diabetes, accounting for 1–5% of all diabetes mellitus cases.[18] It is a clinically and genetically heterogeneous collection of endocrine diseases caused by mutations in a single gene implicated in pancreatic beta cell function.[19] These abnormalities are mostly caused by reduced glucose phosphorylation, decreased activity of pancreatic beta cell expressed transcription factors or altered insulin biosynthesis.[20] MODY is non-insulin-dependent diabetes that is often diagnosed before the age of 25 and is autosomal dominant. Multiple kinds of monogenic diabetes, each with a different clinical presentation, have been linked to mutations in more than ten different genes.[20,21]

MODY is thought to be caused by a variety of genetic mutations, including missense, frameshift, splice site, nonsense, indel, deletion, and insertion.[22–24] According to their underlying molecular etiology, MODY subtypes may be divided into five groups: transcriptional regulatory disorders, enzyme disorders, protein misfolding disorders, ion channel disorders, and signal transduction disorders.[25] Table 1 shows the several types of mutations linked to MODY, as well as the affected chromosomes, subtypes, and molecular etiology.

NDM

NDM is a rare type of monogenic diabetes compared to MODY and it is diagnosed within the first six months of life, includes a variety of clinically and genetically diverse disorders.[26,27] NDM can be permanent (PNDM) and require lifetime therapy, or transient (TNDM), with insulin reliance for the first few months and spontaneous remission of diabetes by the age of 18 months.[26,28–30]

NDM is genetically diverse, with over 25 identified genetic causes that are linked to a variety of pancreatic and non-pancreatic characteristics.[31,32] Mutations in KCNJ11/Kir6.2 and ABCC8/SUR1, which encode the two subunits that make up the hetero-octameric ATP-dependent K+ (K_{ATP}) channel, in INS and ZFP57 genes, or chromosomal 6q24 abnormalities are the most common NDM genetic subtypes.[31,33]

One of the main causes of TNDM is the overexpression of genes on chromosome 6q24. On chromosome 6q24, TNDM is associated with the overexpression of at least two imprinted genes, Pleomorphic adenoma gene-like 1 (PLAGL1) and Hydatidiform Mole Associated and Imprinted (HYMAI).[34,35] Paternal uniparental chromosome 6 (UPD6), paternal allele 6q24 duplication, or a loss of methylation mutation (LOM) within the differentially methylated region (DMR) could all be genetic mechanisms causing 6q24-related neonatal diabetes.[35]

Another key gene linked to NDM is the INS gene. It is the second most prevalent cause of newborn diabetes that is permanent.[36] In most cases, mutations in the insulin gene appear to result in insulin protein misfolding.[36] These proteins appear to aggregate in numerous subcellular compartments, causing endoplasmic reticulum (ER) stress and beta-cell death.[37] Table 2 shows monogenic causes of neonatal diabetes with associated clinical features.

Table 1 Molecular Pathogenesis of Different Types of MODY

MODY Subtype	Gene	Chromosomal Locus	Types of Mutations	Category of Disorder	Pathophysiology	Treatment	Reference
MODY1	HNF4A	20q13.12	Missense, frameshift, splice site, nonsense, indel, deletion, insertion	Transcriptional regulation disorder	Beta cell dysfunction	SU	[99–102]
MODY 2	GCK	7p13	Missense, frameshift, splice site, nonsense, indel, deletion, insertion	Enzyme disorder	Glucose sensing defect	No medication required	[103,104]
MODY 3	HNF1A	12q24.31	Missense, frameshift, splice site, nonsense, indel, deletion, insertion	Transcriptional regulation disorder	Beta cell dysfunction	SU	[57,97,105]
MODY 4	PDX1/IPF1	13q12.2	Missense, nonsense, deletion, insertion	Transcriptional regulation disorder	Beta cell dysfunction	Diet or OAD or Insulin	[94]
MODY 5	HNF1B	17q12	Missense, frameshift, splice site, nonsense, indel, deletion, insertion	Transcriptional regulation disorder	Beta cell dysfunction	Insulin	[106,107]
MODY 6	NEUROD1	2q32	Missense, frameshift, nonsense, indel, deletion, insertion	Transcriptional regulation disorder	Beta cell dysfunction	OAD or Insulin	[108]
MODY 7	KLF11	2p25	Missense	Transcriptional regulation disorder	Beta cell dysfunction	OAD or Insulin	[18,109]
MODY 8	CEL	9q34	Missense, frameshift, indel, deletion, insertion	Protein misfolding disorder	Pancreatic exocrine and endocrine dysfunction	OAD or Insulin	[110]
MODY 9	PAX4	7q32	Missense, splice site, deletion	Transcriptional regulation disorder	Beta cell dysfunction	Diet or OAD or Insulin	[111,112]
MODY10	INS	11p15.5	Missense, splice site, nonsense, indel, insertion	Protein misfolding disorder	Insulin biosynthesis defect	OAD or Insulin	[59]
MODY11	BLK	8p23	Missense	Transcriptional regulation disorder	Insulin secretion defect	Diet or OAD or Insulin	[113]
MODY12	ABCC8	11p15	Missense	Ion channel disorder	Insulin secretion defect	Insulin SU	[114,115]
MODY13	KCNJ11	11p15	Missense	Ion channel disorder	Insulin secretion defect	Insulin SU	[87,94,116]
MODY14	APPL1	3p14.3	Missense, nonsense	Signal transduction disorder	Insulin secretion defect	Diet or OAD or Insulin	[117]

Abbreviations: ABCC8, ATP-binding cassette transporter subfamily C member 8; Akt2, Akt serine/threonine kinase 2; APPL1, Adaptor protein, phosphotyrosine interaction, PH domain, and leucine zipper-containing protein 1; BLK, B-lymphocyte specific tyrosine kinase; CEL, Carboxyl-ester lipase; GCK, Glucokinase; HNFs, Hepatocyte nuclear factors; HNF1A, Hepatocyte nuclear factor 1 alpha; HNF4A, Hepatocyte Nuclear Factor 4 alpha; HNF1B, Hepatocyte Nuclear Factor 1 beta; INS, Insulin; IPF1, Insulin promoter factor 1; IRS, Insulin receptor substrate; KCNJ11, Potassium channel inwardly rectifying subfamily J member 11; KLF11, Krueppel-like factor 11; NEUROD1, Neurogenic differentiation 1; MODY, Maturity-onset diabetes of the young; PAX4, Paired box gene 4; PDX1, Pancreas/duodenum homebox1.

Table 2 Monogenic Causes of Neonatal Diabetes Mellitus

Gene	Chromosomal Locus	Transient Vs Permanent	Clinical Features	Inheritance Pattern	Treatment	References
KCNJ11	11p15.1	Either	Low birth weight, developmental delay, seizures (DEND syndrome), may have other neurologic Features	Spontaneous (80%), AD (20%)	Insulin SU	[116]
ABCC8	11p15.1	Either	Low birth weight	Spontaneous, AD	Insulin SU	[6]
PLAGL1	6q24.2	Transient	Low birth weight, possible IUGR, diagnosed earlier than channel mutations (closer to birth), relapsed cases may respond to SU	Spontaneous, AD for paternal duplications	Insulin	[34]
INS	11p15.5	Either	Low birth weight	Spontaneous (80%), AD (20%) AR (rare: T or P)	Insulin	[118]
GATA6	18q11	Permanent	Pancreatic hypoplasia or agenesis, exocrine insufficiency, cardiac defect	Spontaneous, AD	Insulin	[119,120]
EIF2AK3	2p12	Permanent	Wolcott-Rallison syndrome, skeletal dysplasia (1–2 y old) Episodic acute liver failure, exocrine Pancreatic insufficiency	Spontaneous, AR	Insulin	[121,122]
GCK	7p13	Permanent	Low birth weight	Spontaneous, AR (neonatal diabetes), AD (GCK-MODY)	Insulin	[123]
PTF1A	10p12	Permanent	Neurologic abnormalities, exocrine insufficiency, kidney involvement		Insulin	[124,125]
FOXP3	Xp11.23	Permanent	Autoimmune thyroid disease, exfoliative dermatitis, enteropathy (IPEX syndrome)	X-linked	Insulin	[126]
ZFP57	6p22.1	Transient	Variable phenotype Low birth weight, macroglossia, developmental delay	Spontaneous, maternal Hypomethylation Imprinting	Insulin	[30]
GLIS3	9p24.2	Permanent	Hypothyroidism, kidney cysts, glaucoma, hepatic fibrosis	Spontaneous, AR	Insulin	[127]
PDX1	13q12.1	Permanent	Pancreatic hypoplasia or agenesis, exocrine Insufficiency	Spontaneous, AR (neonatal diabetes), AD (PDX1-MODY)	Insulin	[128]
SLC2A2	3q26.2	Either	Fanconi-Bickel syndrome (hepatomegaly, RTA)	Spontaneous, AR	Insulin	[129]
SLC19A2	1q24.2	Permanent	Neurologic deficit (stroke, seizure) Visual disturbance; cardiac abnormality	Spontaneous, AR	Insulin Thiamine (rarely)	[130]

(*Continued*)

Table 2 (Continued).

Gene	Chromosomal Locus	Transient Vs Permanent	Clinical Features	Inheritance Pattern	Treatment	References
GATA4	8p23	Permanent	Pancreatic hypoplasia or agenesis, exocrine insufficiency, cardiac defect	Spontaneous, AR	Insulin	[131,132]
NEUROD1	2q31.3	Permanent	Neurologic abnormalities (later), learning difficulties, sensor neural deafness	Spontaneous, AR	Insulin	[133,134]
NEUROG3	10q22.1	Permanent	Diarrhea (due to lack of entero-endocrine cells)	Spontaneous, AR	Insulin	[131,135]
NKX2-2	20p11.22	Permanent	Neurologic abnormalities (later), very low birth weight	Spontaneous, AR	Insulin	[136]
RFX6	6q22.2	Permanent	Low birth weight, intestinal atresia, gall bladder hypoplasia, diarrhea	Spontaneous, AR	Insulin	[137]
IER3IP1	18q21.1	Permanent	Microcephaly, infantile epileptic encephalopathy	Spontaneous, AR	Insulin	[138]
MNX1	7q36	Permanent	Neurologic abnormalities (later)	Spontaneous, AR	Insulin	[139]
HNF1B	17q21	Transient	Pancreatic atrophy, abnormal kidney, and Genitalia development	Spontaneous, AD	Insulin	[140]

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; DEND, developmental delay, epilepsy, and neonatal diabetes; DM, diabetes mellitus; IUGR, intrauterine growth restriction; MODY, maturity onset diabetes of the young; RTA, renal tubular acidosis; SGA, small for gestational age.

In-Vitro Modeling of MD Using hPSCs
Overview of hPSCs
Stem cells are distinguished by their key property to self-renew and differentiate into various cell types based on their potency. Based on their ability to differentiate into a wide range of cell lineages they are classified as pluripotent, multipotent, or unipotent.[38] Human ESCs and iPSCs are examples of hPSCs. They can, in principle, grow into any somatic cell present in the human body and might be used as a disease model to examine various molecular mechanisms involved in disease causation.[39,40]

Despite the fact that ESCs have a high potential for use in biological research to better understand fundamental biological processes, they have certain limitations in future therapeutic settings because of the same rejection issues that current organ transplants encounter. Individual members of society are also concerned about using human embryos for research objectives.[41,42] On the other hand iPSC technology enables the generation of pluripotent cells from nearly any individual, and protocols are being designed to differentiate those cell lines into a wide range of cell types present in the body. IPSCs are being utilized to investigate the impact of genetic variations in disease pathogenesis in a variety of derived cell types, such as pancreatic beta like cells and various kinds of neurons.[43,44]

hPSCs Models of MD
Because pancreatic beta cells are the primary cells affected in all kinds of diabetes, a robust strategy for generating functional cells in vitro is required for a better understanding of the pathophysiology associated with different types of diabetes. Small molecules and growth factors are used in in-vitro differentiation techniques to enhance or inhibit certain pathways at each stage of the differentiation process.[10,45] Pancreatic beta cells are known to be derived from pancreatic progenitors that express PDX1 and NKX6.1.[46,47] In-vitro, hPSCs are differentiated into several stages of pancreatic β cells. The expression of essential transcription factors (TFs) that regulate β cell development and functionality distinguishes each stage.[15]

While ESCs are derived from cells isolated from the blastocyst's inner cell mass,[48] iPSCs allow for the production of pluripotent cells by transcriptional reprogramming of terminal cells from adult patients.[49] The capacity to produce patient-specific human tissue in vitro has enabled genetic disease models which can mimic the disease phenotype in humans.

Various methodologies might be used to generate relevant cell types from hPSCs for modeling MD. The most popular strategy is to develop iPSCs from affected patients' somatic cells containing a specific mutation/variant (patient-derived iPSCs) and compare them to iPSCs generated from healthy controls (Ctr-iPSCs). Another option is to employ genome-editing methods to induce MD-relevant mutations into existing hPSC lines (ESCs and iPSCs) in order to explore the function of the genes linked with MD.[50]

Patient-Derived iPSCs for Modeling MD

Takahashi K. and Yamanaka S. reported the generation of the first induced pluripotent stem cells (iPSCs) in mouse fetal fibroblasts by transduction of four transcription factors (Oct3/4, Sox2, c-Myc, and Klf4), revealing that somatic cells can be converted to a pluripotent stage iPSCs. The iPSCs formed have the characteristics of ESCs and may be utilized to develop into different cell types.[49] The generation of iPSCs from patients and their differentiation into disease-relevant cell types has enormous potential for in vitro disease modeling.[51] Keratinocytes, dermal fibroblasts, adipocytes, and peripheral blood cells are among the human somatic cells that may be reprogrammed. Retroviruses, lentiviruses, plasmids, adenoviruses, Sendai viruses, transposons, protein, modified RNA, and miRNA are being used in reprogramming.[52]

Rezania et al developed a seven-stage protocol for generating reprogrammed hiPSC lines into insulin-producing cells. During static in vitro incubations, stage (S) 7 cells exhibited critical markers of mature pancreatic beta cells and secreted glucose-stimulated insulin identical to human islets. This can be generalized to in-vitro models of monogenic diabetes.[53]

Diabetes stem cell models should allow for the investigation of specific processes leading to human β-cell failure as well as the testing of techniques to retain or restore β-cell function. Teo et al were the first to report the successful generation of hiPSCs from patients with five different MODY conditions (MODY1, MODY2, MODY3, MODY5, and MODY8) as a valuable resource for studying the role of these MODY genes/transcription factors in the development of human pancreas and beta cells, as well as the regulation of beta-cell secretory function.[54]

Shang et al used induced pluripotent stem cells derived from skin fibroblasts of Wolfram syndrome patients and discovered that these WFS1 mutant cells have insulin processing and secretion in response to various secretagogues comparable to healthy controls, but with a lower insulin content and increased activity of UPR pathways.[55] Mutations in the WFS1 gene (wolframin), which is abundantly expressed in human islets as well as the heart, brain, placenta, and lung, can cause childhood-onset insulin-dependent diabetes.[56]

Other researchers investigated the influence of MODY1/HNF4A mutation on the development of the foregut lineage in humans using a hiPSCs-based disease modeling method. Their findings indicate that in MODY1, liver and pancreas development is disrupted early on, contributing to patients' altered hepatic proteins and β-cell abnormalities.[57] Conditional ablation of Hnf4a in mouse pancreatic β-cells, on the other hand, did not result in a diabetic phenotype, despite reduced glucose-stimulated insulin secretion (GSIS),[58] showing that rodent models do not adequately reproduce the MODY1 phenotype in humans.

Balboa et al developed an in-vitro model using hiPSCs from patients with INS mutations and showed that the INS mutations cause proinsulin misfolding, higher symptoms of ER stress, and decreased proliferation in INS mutant beta-like cells compared to corrected controls.[59] Other researchers generated hiPSCs from fibroblasts of a patient with PNDM and undetectable insulin at birth owing to a homozygous mutation in the insulin gene's translation start site in another recent study. Their findings reveal that INS mutant cells differentiated into hormone-negative hiPSCs.[60]

Application of Genome Editing Technologies for Monogenic Diabetes Modeling Using hPSCs

Methods in Genome Editing

Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly inter-spaced short palindromic repeats-associated Cas protein system (CRISPR/Cas) are three cutting-edge gene technologies that use enzyme "scissors" to cut DNA at specified gene loci.[61] The mode of action is common for the three methods, in which

these enzymes bind to a sequence of interest in the genomic DNA and induce targeted double-strand break (DSB) followed by DNA repair mechanisms, either homologous recombination (HR) or non-homologous end-joining (NHEJ).[62]

ZFNs

ZFNs are artificial structures created by combining restriction endonucleases with zinc-finger-binding domain proteins designed to target user-specified sequences within the genome.[62] ZFNs are made up of N-terminal DNA binding domain that has been custom-engineered linked together via a linker peptide to a C-terminal endonuclease.[63] The endonuclease is a non-specific cleavage domain from the bacterial FokI endonuclease that dimerizes to cleave DNA, whereas the DNA binding domain is an array of several zinc-finger domains, each of which recognizes a distinct three base pair sequence of DNA.[64]

When there are DSBs in the genome, an intracellular signaling cascade known as the DNA damage response is activated to repair the double-strand breaks caused by ZFNs.[65] The mechanisms include the following:[1] ZFNs containing FokI endonucleases and protein-binding domains are introduced into the cell,[2] FokI and protein-binding domains are released to enter the nucleus,[3] protein-binding domains attach with DNA fragment to be removed,[4] FokI cuts out the identified DNA segment by creating double-stranded DNA break, and[5] the desirable DNA segment is inserted and integrated into the DNA sequence.[61]

TALENs

TALENs, like ZFNs, have a modular structure with N-terminal transcription activator-like effector (TALE) DNA-binding domain fused to a C-terminal FokI endonuclease domain.[66,67] TALENs are somewhat more site-specific than ZFNs, with fewer off-target effects because TALE repeats can target single base pair (bp), but Zinc fingers recognize DNA triplets.[68]

TALE DNA binding domain is composed of 34-residue repeat domains.[69] At positions 12 and 13, the repeat variable residues (RVDs) make contact with DNA.[69] For constructing synthetic TALE arrays, the most often employed RVDs are NI for adenine, HD for cytosine, NG for thymine, and NN or HN for guanine or adenine.[70,71] TALEs are commonly built to recognize 12- to 20-bps of DNA, with more bases resulting in greater genome-editing specificity.[72] TALE DNA-binding domains may be built in a variety of ways, the easiest being Golden Gate assembly.[66]

Recent TALEN assembly improvements have focused on the development of strategies to improve their performance, such as specificity profiling to uncover nonconventional RVDs that increase TALEN activity.[73,74] The FokI cleavage domain, which cuts on the inside of a 12- to 19-bp spacer region that separates each TALE binding site, mediates dimerization of TALEN proteins.[67] Targeted nucleases cause DSBs, which are repaired by NHEJ or, in the presence of a donor template, HDR.[75]

CRISPR/Cas9

Bacteria have a distinct adaptive immune system that destroys foreign DNA using an RNA-guided DNA endonuclease.[76] The three processes of CRISPR immunity are spacer acquisition, CRISPR RNA (crRNA) synthesis, and interference.[77] CRISPR/Cas evolved from prokaryotes' adaptive resistance to bacteriophages, invading plasmids, and viruses. A–T-rich leader sequences are found right adjacent to 27–42 bp palindromic repeats in the bacterial genome. The palindromes are divided by "interspaced" DNA known as protospacers, which is a template of previously identified bacteriophage DNA and acts as a marker of earlier infection.[78,79]

Past foreign DNA fragments are used as spacers in the CRISPR genomic locus. CRISPR RNAs (crRNAs) are transcribed from CRISPR loci and contain a unique sequence complementary to target DNA (called protospacer) as well as a repeat region that hybridizes to a short RNA termed transactivated crRNA (tracrRNA). The crRNA then assembles with Cas proteins to form the effector complex; Cas proteins are guided to break target DNA by the crRNA and tracrRNA combination.[79] Furthermore, crRNA and tracrRNA may be combined into a single-guide RNA (sgRNA) that can target and activate Cas9 endonuclease activity.[80]

The CRISPR/Cas9 genome editing system may be broken down into three steps: recognition, cleavage, and repair.[81] Through its 5' crRNA complementary base pair component, the designed sgRNA drives Cas9 and detects the target sequence in the gene of interest. In the absence of sgRNA, the Cas9 protein stays inactive. Cas9 nuclease causes DSBs three base pairs upstream of Protospacer Adjacent Motif (PAM).[82] The PAM sequence is a short (2–5 bp) conserved DNA sequence downstream of the cut site that changes in length depending on the bacterial species. Cas9 protein, the

most extensively used nuclease in genome editing tools, detects the PAM sequence at 5-NGG-3 (N can be any nucleotide base). The Cas9 protein is then activated for DNA cleavage.[83,84] The HNH domain cleaves the complementary strand of target DNA, whereas the RuvC domain cleaves the non-complementary strand, resulting in primarily blunt-ended DSBs. Finally, the host cellular machinery repairs the DSB.[85]

CRISPR/Cas-9 Based Genetic Manipulation of hPSCs for Modeling MD

The use of hPSCs and in vitro differentiation protocols that mimic in vivo pancreatic development is well suited to investigate pancreatic monogenic diseases,[86,87] as are genome editing tools such as CRISPR/Cas9 to modify hPSCs for human in vitro disease modeling to understand the role of MD relevant mutations in pancreatic development and beta-cell function.[88,89]

Several pancreatic differentiation protocols have been employed for modeling MD using hPSCs.[47,90,91] hPSCs can be successfully differentiated into definitive endoderm, which can then be shaped into a posterior-foregut-like population capable of upregulating PDX1 expression in response to retinoic acid and fibroblast growth factor 10 (FGF10), as well as inhibitors of the bone morphogenetic protein (BMP) and hedgehog signaling pathways.[47]

The genome editing of a particular cell line that differentiates rapidly to beta-cells may be utilized to develop knock-out models of pancreatic development genes and used to understand their function.[92] The CRISPR-Cas9 nuclease system may be used to introduce various genomic changes. The following steps are taken to alter hPSCs for modeling MD using the CRISPR-Cas9 nuclease system: (a) sgRNA design, (b) sgRNA synthesis, (c) single or multiplex sgRNA transfection in hPSCs, (d) assessment of Indel frequency, and (e) clonal propagation of knockout lines.[93]

Huangfu et al use TALEN and CRISPR-Cas-mediated gene editing in combination with hPSC-directed differentiation to model MD in vitro. This study examines the involvement of PDX1 and seven other pancreatic transcription factors (RFX6, PTF1A, GLIS3, MNX1, NGN3, HES1, and ARX) in pancreatic cell commitment.[94] This phenotype correlates with the observation that patients with heterozygous PDX1 mutations develop diabetes at a young age, and it also validates that low levels of PDX1 can cause beta-cell dysfunction, a decrease in beta-cell mass during fetal development, and/or the maintenance of beta-cell mass in adults.[95,96]

Cardenas-Diaz et al employed the CRISPR-Cas9 technology to genetically alter ESCs to ablate one or two alleles of HNF1A and differentiate these stem cell lines into pancreatic beta-like cells in MODY3, one of the most frequent forms of MDs caused by mutations in the transcription factor HNF1A. Their findings imply that HNF1A plays an important role in endocrine cell development since deletion of HNF1A results in increased production of alpha cell markers such as glucagon and reduced expression of PAX4, a transcription factor that regulates beta cell development.[97]

Shi et al employed CRISPR/Cas9 to generate hPSCs with frameshift mutations in GATA6, either alone or in conjunction with mutations in GATA4. GATA6± haploinsufficiency changes pancreatic progenitor cell development, resulting in a lower proportion of glucose-responsive beta-like cells, according to their findings.[88] Chia et al studied the role of GATA6 using both gene-edited and patient-derived hPSCs and discovered that GATA6 heterozygous hPSCs had a slight decrease in endoderm development, but GATA6-null hPSCs can only form mesoderm-like cells. According to their findings, GATA6 appears to be upstream of the endoderm program in humans.[98]

Conclusion

Monogenic diabetes is an interesting disease category to model in vitro using hPSCs since it is caused by a single mutation. Over the last decade, great progress in the field of hPSCs has been made in terms of developing hPSC-based models and differentiation processes for studying diabetes pathogenesis. By modifying the genotype of the initial hPSCs or producing hiPSCs from patients with monogenic diabetes, researchers are seeking to understand the mechanisms behind the development of various types of monogenic diabetes. Detailed understanding of the molecular underpinnings behind pancreatic beta-cell development and function, as well as using reliable models that can precisely mimic the effects in humans, can considerably improve the chances of developing effective diabetes treatments.

Disclosure

The authors report no conflicts of interest in relation to this work.

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