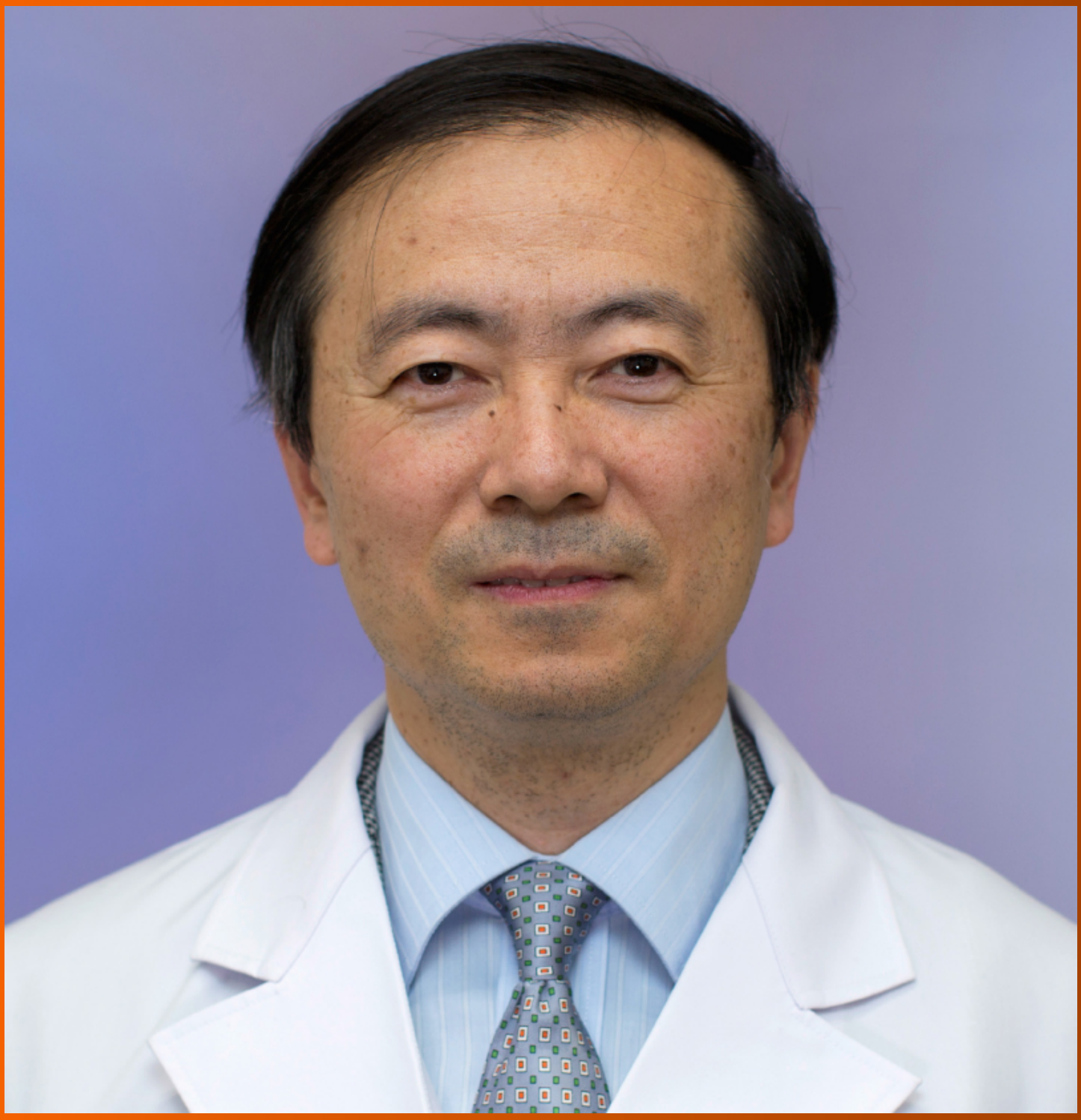
ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2021 March 26; 13(3): 193-235



Published by Baishideng Publishing Group Inc

WJSC

World Journal of Stem Cells

Contents

Monthly Volume 13 Number 3 March 26, 2021

REVIEW

MINIREVIEWS

World Journal of Stem Cells

Monthly Volume 13 Number 3 March 26, 2021

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Stem Cells*, Yun-Wen Zheng, PhD, Associate Professor, Department of Gastrointestinal and Hepato-Biliary-Pancreatic Surgery, University of Tsukuba Faculty of Medicine, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8575, Japan. ywzheng@md.tsukuba.ac.jp

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells* (*WJSC*, *World J Stem Cells*) is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc*.

INDEXING/ABSTRACTING

The *WJSC* is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, BIOSIS Previews, Scopus, PubMed, and PubMed Central. The 2020 Edition of Journal Citation Reports® cites the 2019 impact factor (IF) for *WJSC* as 3.231; IF without journal self cites: 3.128; Ranking: 18 among 29 journals in cell and tissue engineering; Quartile category: Q3; Ranking: 113 among 195 journals in cell biology; and Quartile category: Q3. The *WJSC*'s CiteScore for 2019 is 4.9 and Scopus CiteScore rank 2019: Histology is 15/60; Genetics is 124/324; Genetics (clinical) is 35/90; Molecular Biology is 177/381; Cell Biology is 143/274.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Shengwen Calvin Li, Tong Cao, Carlo Ventura

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-0210/editorialboard.htm

PUBLICATION DATE
March 26, 2021

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2021 March 26; 13(3): 193-207

DOI: 10.4252/wjsc.v13.i3.193

ISSN 1948-0210 (online)

REVIEW

Functional maturation of immature β cells: A roadblock for stem cell therapy for type 1 diabetes

Zi-Yi Sun, Ting-Yan Yu, Fang-Xu Jiang, Wei Wang

ORCID number: Zi-Yi Sun 0000-0003-0236-4396; Ting-Yan Yu 0000-0003-4538-3335; Fang-Xu Jiang 0000-0003-3172-0476; Wei Wang 0000-0002-7453-7391.

Author contributions: Sun ZY wrote the paper; Yu TY drew the figures; Jiang FX and Wang W proposed the ideas, revised the manuscript, and finally approved the submission.

Supported by the National Natural Science Foundation of China, No. 81471081; the Natural Science Foundation of Fujian Province, China, No. 2019J01010; Xiamen Research Foundation for Science and Technology Project No. 3502Z20194037; and Scientific Research Foundation for Advanced Talents, Xiang'an Hospital of Xiamen University, No. PM201809170005.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Zi-Yi Sun, Ting-Yan Yu, Fang-Xu Jiang, Wei Wang, Department of Endocrinology, Xiang'an Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen 361100, Fujian Province, China

Fang-Xu Jiang, School of Biomedical Science, University of Western Australia, Nedlands 6009, Australia

Fang-Xu Jiang, School of Health and Medical Sciences, Edith Cowan University, Perth 6000, Australia

Corresponding author: Wei Wang, MD, Doctor, Department of Endocrinology, Xiang'an Hospital of Xiamen University, School of Medicine, Xiamen University, No. 2000 Xiang'an East Road, Xiang'an District, Xiamen 361100, Fujian Province, China.
wwei19742007@hotmail.com

Abstract

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease caused by the specific destruction of pancreatic islet β cells and is characterized as the absolute insufficiency of insulin secretion. Current insulin replacement therapy supplies insulin in a non-physiological way and is associated with devastating complications. Experimental islet transplantation therapy has been proven to restore glucose homeostasis in people with severe T1DM. However, it is restricted by many factors such as severe shortage of donor sources, progressive loss of donor cells, high cost, *etc.* As pluripotent stem cells have the potential to give rise to all cells including islet β cells in the body, stem cell therapy for diabetes has attracted great attention in the academic community and the general public. Transplantation of islet β-like cells differentiated from human pluripotent stem cells (hPSCs) has the potential to be an excellent alternative to islet transplantation. In stem cell therapy, obtaining β cells with complete insulin secretion *in vitro* is crucial. However, after much research, it has been found that the β-like cells obtained by *in vitro* differentiation still have many defects, including lack of adult-type glucose stimulated insulin secretion, and multi-hormonal secretion, suggesting that *in vitro* culture does not allows for obtaining fully mature β-like cells for transplantation. A large number of studies have found that many transcription factors play important roles in the process of transforming immature to mature human islet β cells. Furthermore, PDX1, NKX6.1, SOX9, NGN3, PAX4, *etc.*, are important in inducing hPSC differentiation *in vitro*. The absent or deficient expression of any of these key factors may lead to

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: August 29, 2020
Peer-review started: August 29, 2020
First decision: January 7, 2021
Revised: January 19, 2021
Accepted: February 25, 2021
Article in press: February 25, 2021
Published online: March 26, 2021

P-Reviewer: Saad K
S-Editor: Zhang H
L-Editor: Wang TQ
P-Editor: Xing YX

the islet development defect *in vivo* and the failure of stem cells to differentiate into genuine functional β-like cells *in vitro*. This article reviews β cell maturation *in vivo* and *in vitro* and the vital roles of key molecules in this process, in order to explore the current problems in stem cell therapy for diabetes.

Key Words: Stem cell therapy; Type 1 diabetes mellitus; β cell; Maturation

Core Tip: Type 1 diabetes is a metabolic disease characterized by absolute lack of insulin. Current insulin replacement therapy supplies insulin in a non-physiological way and is associated with devastating complications. Diabetes stem cell therapy with insulin-producing β-like cells differentiated *in vitro* from human pluripotent stem cells has recently attracted great interest in the academic community and the general public. Although great progress has been made, the β-like cells differentiated *in vitro* still have many defects. Here we summarize the latest knowledge on β-cell maturation *in vivo* and *in vitro* and the vital roles of key molecules in this process, in order to explore the current problems in diabetes stem cell therapy.

Citation: Sun ZY, Yu TY, Jiang FX, Wang W. Functional maturation of immature β cells: A roadblock for stem cell therapy for type 1 diabetes. *World J Stem Cells* 2021; 13(3): 193-207
URL: https://www.wjgnet.com/1948-0210/full/v13/i3/193.htm
DOI: https://dx.doi.org/10.4252/wjsc.v13.i3.193

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the absolute deficiency of β-cell function. Disorders of the immune system cause destruction of β-cells, resulting in the absolute lack of insulin secretion and the inability to properly regulate blood glucose homeostasis[1,2]. This process is mediated by autoimmunity, with the participation of both innate and adaptive immunity[3,4]. Due to insufficient insulin secretion, blood glucose rises rapidly in the short term. This can cause life-threatening conditions such as hypoglycemia unawareness, diabetic ketoacidosis, or diabetic hyperosmolar coma. Long-term hyperglycemia will damage the cardiovascular and cerebrovascular systems and microcirculation in varying degrees[5], resulting in complications including eye disease, nephropathy, peripheral neuropathy, and coronary atherosclerotic heart disease. Significantly, T1DM is also associated with some other chronic autoimmune diseases, such as celiac disease[6,7]. Finally, as most patients with T1DM have had the condition since childhood, long-term insulin use is not only an inconvenience to daily life, but also an economic burden on society. Therefore, research on new treatment methods for T1DM is crucial.

Since several decades ago, T1DM has been experimentally treated by whole pancreas and then islet transplantation[8,9]. However, due to its unfeasibly high costs and insufficient donor sources for the increasing number of T1DM patients, this treatment cannot be widely implemented in practice. To resolve this problem, the ultimate goal is to develop a stem cell therapy for diabetes, namely, differentiate islet β-like cells from human pluripotent stem cells (hPSCs) capable of glucose stimulated insulin secretion (GSIS) similar to mature β cells, and effectively regulating blood glucose homeostasis in the body after transplantation. Great efforts have thus been concentrated on discovering technologies in how to effectively differentiate hPSCs into genuine β-like cells that could maintain the long-term survival and functional stability if transplanted. This review article summarizes the latest progresses on the β-cell development and functional maturity *in vivo* and on the differentiation of insulin-secreting β-like cells *in vitro* from human pluripotent or multiple stem cells.

BRIEF SUMMARY OF AUTOIMMUNITY IN T1DM

The etiology and pathogenesis of T1DM are not fully understood, but they are generally believed to be related to genetic and environmental factors. Although most patients do not have a family history of T1DM, genetic susceptibility is an important factor. A combination of epigenetics such as DNA methylation and histone modification, altered microRNA profiles, and other pathological mechanisms may also be related to the development of T1DM and affects the immune reaction on islet β cells. Studies have shown that the main genetic risk factors for T1DM are located in the major histocompatibility complex class II human leukocyte antigen (HLA) region, and the genetic polymorphism in this region largely determines the genetic risk of T1DM[10-12].

The autoimmunity in T1DM patients is manifested by the presence of circulating islet autoantibodies and autoreactive T cells. The human body temporally establishes an immune balance after birth and the pancreatic islet self-reactive T cells are regulated and suppressed from becoming active[13,14]. CD4+ helper cells and CD8+ cytotoxic T lymphocytes play an important role in the pathogenesis of T1DM by producing autoantibodies and recognizing β-cell proteins as autoantigens[15]. HLA molecules induce the proliferation of pathogenic T cells by presenting self-antigens to naive T cells, and producing self-reactive CD4+ T cells. These activated CD4+ T cells then produce cytokines, which in turn activate β cell-specific cytotoxic CD8+ T cells. Subsequently, these activated T cells are recruited to pancreatic islets and stimulate macrophages and other T cells. This leads to the destruction of pancreatic β cells[16,17].

DEFECTS OF CURRENT T1DM TREATMENT

Patients with T1DM need lifelong insulin replacement therapy. Exogenous insulin supplementation is not only a cumbersome process but is also associated with acute hypoglycemia unawareness episodes. It can lead to chronic devastating complications such as heart and kidney failures, blindness, foot necrosis, and cancers. Although islet transplantation can replace destroyed β cells and exert insulin secretion function in the human body, this method has many limitations, such as a shortage of donors, high costs, strong immune system rejection after transplantation, and long-term use of anti-rejection drugs[18,19]. Immunotherapy includes non-self-antigen-specific and self-antigen specific therapies. The former involves regulatory T cell replacement therapy that aims to self-reactivate T cells, B cells, and inflammatory cytokines, while the latter mainly targets the regulation and inactivation of self-antigen. Unfortunately, a T1DM immunotherapy that can totally replace the standard insulin replacement therapy has not yet been developed[20]. Researchers have also tested the possibility of mysenchymal stem cells (MSCs) as an innovative treatment for autoimmune diseases. MSCs are a class of multipotent stem cells with the ability to self-replicate. Their inherent self-renewal potential and immune regulation ability are considered to be an exciting starting point for the treatment of autoimmune diseases[21]. For example, MSCs may have the ability to prevent the autoimmune destruction of β cells in T1DM animal models and generate functional β cells to maintain blood glucose homeostasis[22,23].

At present, there are several issues in the clinical application of stem cell therapy, including selection of appropriate encapsulation materials and transplantation site, and the need for further research on improving immune regulation and new blood vessel formation methods. However, the most pressing issue is how to obtain fully functional and mature β cells through *in vitro* culture. In order to solve these problems, it is critical that the maturation process of islet β cells *in vivo* and *in vitro*, and major functioning transcription factors and other critical molecules are better understood.

MATURATION PROCESS OF β CELLS *IN VIVO*

The pancreas consists largely of exocrine glands and in a smaller proportion, endocrine glands[24]. The exocrine glands are composed of pancreatic acinar tissues and pancreatic ducts, and the endocrine glands are composed of cell clusters of different sizes, known as the islets of Langerhans[25]. The islet is an endocrine micro-organ, consisting of at least five types of endocrine cells: α cells (15%-20%), β cells (60%-80%), δ cells (5%-10%), ε cells (< 1%), and pancreatic polypeptide-secreting (PP) cells (2%)[26]. Observed by optical projection tomography, there are about 1000 islets in the pancreas of 8-wk-old mice, with each islet containing an average of 800 β cells. There are about

1000000 islets in the human pancreas, each containing about 400-600 β cells. The β cells as polygonal cells have a diameter of 13-18 μm, and each contains about 100000 insulin secretory vesicles[27,28]. Insulin is stored in a crystallized form in these vesicles that are ultimately released through exocytosis[29]. The exocytosis of insulin granules is controlled by the ATP-sensitive K (K_{ATP}) channel and requires calcium ions to flow into the cells through the cell membrane calcium channel. When the blood glucose concentration rises, the glucose uptake and metabolism in β cells also increase, which leads to an increase in ATP production. These changes in adenine nucleotide concentrations cause the K_{ATP} channel to close, triggering calcium influx and insulin secretion[30].

Embryonic pancreatic development begins with ventral and dorsal pancreatic buds. In mice, the dorsal bud appears on day 9.0 of the embryo development (E9) and the ventral bud appears on E9.5 along the dorsal and ventral surfaces of the posterior foregut endoderm (Figure 1). The first transformation of mouse pancreatic morphology begins from E9.5 to E12.5, during which time pancreatic progenitor cells rapidly proliferate to form the pancreatic endoderm. At E12 to E13, the ventral and dorsal buds contact and fuse together. At E13, pancreatic endodermal cells proliferate, and pancreatic progenitor cells give rise to neurogenin 3 (NGN3) positive progenitor cells, which then form mature endocrine cells[31-33]. By E14.5, the developing islets consist of many insulin-producing β and glucagon-producing α cells, and δ cells that secrete somatostatin appear for the first time. PP cells begin to appear before birth. At birth, β cells in mice do not have adult-type insulin secretion function, but gradually mature within 2-3 wk after birth[34].

During human embryonic development, dorsal pancreatic buds appear around the fourth week of gestation, followed by abdominal buds. In contrast to the early presence of glucagon-expressing cells in mouse pancreatic buds, human endocrine-expressing cells are not detected until G7.5–8w after the dorsal buds grow for 3 wk in early embryonic pancreas. These endocrine cells are derived from NGN3+ endocrine progenitor cells, and among them, the first to appear are insulin-producing β cells. The transcription factors PAX6, PAX4, NKX2.2, NKX6.1, HLXB9, *etc.* are involved in the process of differentiation from endocrine progenitor to insulin-producing β cells[35]. β-cell replication is easily detectable at G9w and peaks around G14-16w[36-38]. At this time, embryonic β cells are multi-hormonal cells that produce insulin, glucagon, and growth hormone, as they are still in an immature state. Immature β cells have strong proliferative ability, but they do not have the functions of mature β cells.

The hallmark feature of functional β cells is mature GSIS, which means when postprandial blood glucose increases, pancreatic β cells secrete a sufficient amount of insulin to prevent hyperglycemia, and inhibit insulin secretion under fasting conditions to prevent hypoglycemia. This is also known as the biphasic model that is established after β-cell maturation. Human studies have shown that neonatal β cells do not have this biphasic secretion function, because these β cells are not fully mature at this stage. After birth, pancreatic islet cells gradually lose their proliferative capacity and develop highly sensitive and powerful GSIS capacity under the control of transcription factors such as MafA[39]. There is no definite conclusion about the time point at which β cells fully mature in humans, but it is closely aligned to the time when a newborn begins to take food supplements. According to the experiment of Otonkoski *et al*[40], human islet β cells obtain mature insulin secretion function at about 26-44 wk of age. However, further studies are required to confirm the stage of β-cell maturation.

KEY MARKERS IN THE PROCESS OF β CELL MATURATION

At present, due to the limitations on human studies, most of the understanding of β-cell maturation comes from rodent studies. In mice, the β cells in the fetal stage are immature and highly proliferative. At this time, the β cells can already generate insulin granules and show high basal insulin levels, but the regulated mechanism of insulin secretion remains to be established. After birth, the β cells have not yet obtained a mature phenotype to respond to the stimulation of changing glucose concentration to properly secrete insulin[41,42]. The first mature wave in mice appears 2 wk after birth. At this time, β cells are still proliferative, but this characteristic is gradually lost since β cells follow the biphasic maturation model and need to adapt to the dietary changes of the newborn. The second maturation wave occurs in the third week after birth, which coincides with the weaning period. This is also true for the human newborn[43-45]. During this period, the proliferative property of β cells gradually

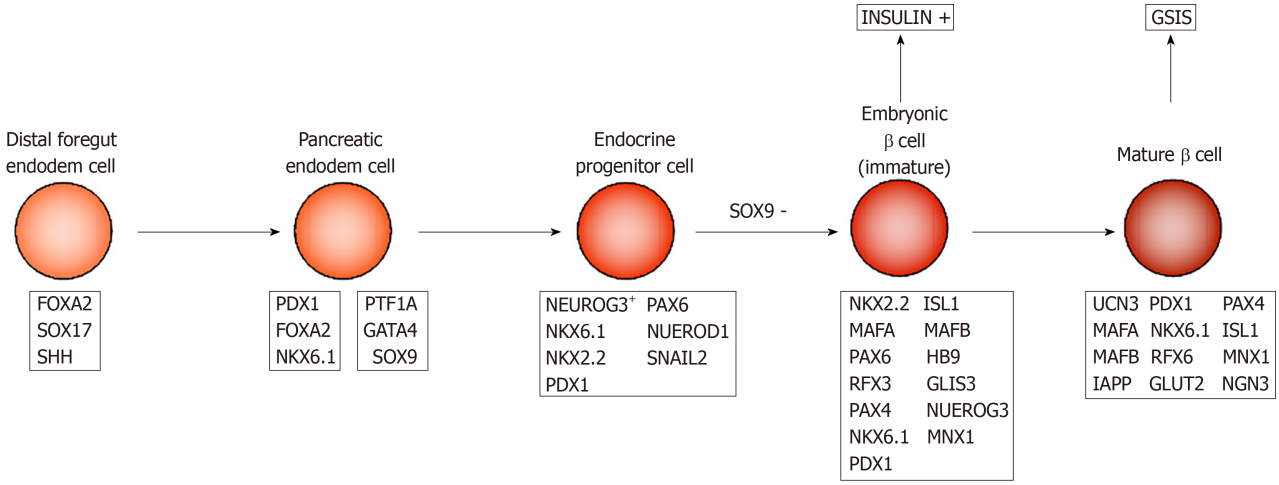


Figure 1 β-cell development and maturation *in vivo*. Important stages of β cell development and maturation *in vivo*, which mainly include distal foregut endoderm, pancreatic endoderm, endocrine progenitor, immature β cells, and mature β cells, and key transcription factors involved are described. GSIS: Glucose stimulated insulin secretion.

disappears, and is replaced by an adult GSIS feature. The GSIS contains a variety of cellular processes, in which β cells sense changes in glucose concentration through specific glucose transporters (GLUT1 & GLUT2)[46,47]. Subsequently, glucose stimulation causes the mitochondria to actively participate in the control and enhancement of insulin secretion in the mature GSIS process. Finally, the insulin granules fuse with the cytoplasmic membrane and secrete insulin *via* the exocytosis[48].

There are many key transcription factors involved in the process of β cell maturation. For example, the transcription factors MAFA and MAFB play important roles in the development and maturation of β cells, respectively. MAFB is expressed earlier than MAFA, and appears in mouse pancreatic epithelial cells at E10.5[49], while MAFA is originally expressed in insulin+ cells at E13.5[50]. At E15.5, 50% and 90% of cells with insulin secretion ability express MAFA and MAFB, respectively. However, in adult mice, MAFA is only expressed in β cells while MAFB is in α cells[51], suggesting the transformation from MAFB to MAFA signals β cell maturation. Critical for the development of immature insulin+ cells, MAFB is expressed in most insulin+ cells at E15.5 and E18.5. However, it is only expressed in a small amount of P14 mouse β cells, and by P28, MAFB expression is completely absent[52]. In contrast, the level of MAFA in islet β cells in immature P2 mice is only 7% of that in adult mice, in which the GSIS properties of these β cells are also poor. MAFA overexpression in P2 β cells can substantially enhance the GSIS ability[53]. Other experiments indicate that the expression level of MAFA in mature β cells is significantly higher than that in immature cells and is positively related to insulin secretion capacity[54]. Taken together, the increased expression of MAFA and the disappearance of MAFB signal the maturation of β cell function and are important links for β cells to obtain adult GSIS.

Many other transcription factors are not direct markers of β-cell transformation from immaturity to maturity, but play important roles in the process of β-cells achieving functional maturity. During endocrine cell formation, NGN3 regulate the early differentiation of islet cells and formation of endocrine cells during development[55]. All pancreatic endocrine cells are derived from NGN3 expressing endocrine progenitor cells. Individuals lacking NGN3 will not be able to produce any functional pancreatic endocrine cells and will subsequently die from diabetes[56,57]. Individuals lacking NGN3 expression can still produce embryonic β cells and produce insulin, however, they cannot respond to glucose stimulation and eventually obtain functionally mature β cells[58], suggesting that NGN3 expression is essential for β-cell maturation. The transcription factor PDX1 is activated in the mouse foregut endoderm at E8.5 and expressed in multipotent pancreatic progenitor cells of early pancreatic buds[59]. In 5-wk-old male mice, the lack of PDX1 expression results in changes in the expression of genes that control β-cell function and proliferation status (such as *MAFA* and *GLUT2*), leading to decreased insulin secretion levels[60]. The β cell glucose tolerance of PDX1-deficient mice is impaired, plasma insulin levels are reduced, and the adult-type GSIS is impaired[61].

In addition to the above, the transcription factors NEUROD, MNX1, PAX4, NKX6.1, *etc*. also play important roles in the process of β-cells becoming functionally

mature[62-65].

UCN3 can be used as a marker of β cell maturity[66]. UCN3 is a member of the CRF (corticoltropin release-factor) family[67,68]. As an endogenous ligand of the CRF receptor 2, it is closely related to the regulation of energy balance and/or glucose metabolism in the body[69]. It is also expressed as a secreted protein in local areas of the brain and the pancreas. In mouse islets, UCN3 first appears in β cells at E17.5 and is expressed as a characteristic marker of β cells from P7 to the entirety of the adult period. Given that in P14, UCN3 and insulin expression completely overlap, Blum *et al*[70] used Western blot and immunohistochemistry to analyze the protein expression levels and found that UCN3 expression in mature β cells is 7 times higher than in immature cells. Immunofluorescence staining indicates that UCN3 expression is high in all adult β cells; however, this was not detected in embryonic islets at E18.5[70].

The functional maturation of β cells involves the switching of cell signals from mTORC1 to AMPK (5' adenosine monophosphate activated protein kinase)[71-73] (Figure 2). mTOR is a nutrition-sensitive kinase and essential for regulating the proliferation and growth of postnatal pancreatic β cells[74,75]. Studies have shown that mTORC1 promotes β-cell proliferation in embryonic and neonatal stages by regulating cyclins D2 and D3 and CDK4. The specific loss of mTORC1 in mouse β-cells can lead to severe glucose intolerance, which is related to an insufficient number of β-cells[76,77]. AMPK is an effective inhibitor of mTORC1, and its kinase activity is regulated by the intracellular ratio of ATP to AMP/ADP[78]. Loss of LKB1 (AMPK upstream activator) can increase β-cell proliferation and mass by inducing mTORC1, resulting in increased insulin output[79,80]. Helman *et al*[81] found that the function of β-cells after birth is closely related to changes in the nutritional environment, which is mainly due to amino acid-stimulated insulin secretion and GSIS and through the mTORC1 signaling pathway. These researchers found that under two nutritional conditions, there was no difference in the expression of PDX1, NKX6.1, UCN3, MAFA, and other transcription factors in β cells, indicating that the switch to adult-type GSIS is not affected by the expression of these markers. Instead, changes in glucose reactivity are related to the activation of mTORC1 after changes in nutritional conditions, and there is a positive correlation between insulin secretion and mTORC1 activation. Disrupting the nutritional sensitivity of mTORC1 in mature β cells will cause their insulin secretion to return to a functional immature state[82].

Synaptotagmin 4 (Syt4) may play an important role in the maturation of β cells[83]. As a non-Ca^{2+} binding paralog of the β cell Ca^{2+} sensor Syt7, it increases approximately 8-fold during β-cell maturation, and the absence of Syt4 will increase the secretion of basal insulin in newborn mice. The role of this protein is to reduce the sensitivity of immature β cells to calcium ions that directly regulate the exocytosis of insulin granules and influence the normal secretory process of insulin[84].

Recently, a Wnt/Plane cell polarity effector protein Flattop (Fltp) was found to distinguish immature (Fltp-) and terminally mature (Fltp+) β cells[85]. Fltp+ cells have higher expression levels of β-cell functional genes (*i.e.*, *SLC2A2*, *NKX6.1*, *UCN3*, *MAFA*, *etc.*), and it can be observed that the number of mature secreted granules is significantly increased, the mitochondrial physiological function is enhanced, and the static GSIS is higher[86].

Other studies showed that the microenvironment is also important for obtaining mature β cells[87,88]. Freshly isolated β cells in suspension culture release much less insulin than scattered β cells that re-aggregate into islets, suggesting that the composition of pancreatic islets, cell polarity, contact between homotype cells, contact between heterotype cells, and interaction with the surrounding tissues and environment can all lead to differences in glucose reactivity and insulin secretion.

Among them, paracrine regulation plays an important role in β cell function (Figure 3). Even if the islets are dispersed to the cellular level, most β cells still retain the link with α cells[89,90], which suggests the co-evolution of the two types of endocrine cells is necessary for the pancreatic islet development, and may be of great significance to the pancreatic islet maturation. Islet paracrine signals from one cell type can regulate others in the same pancreatic islet by spreading through the gap or circulating through intra-islet blood vessels[91]. For example, glucagon secreted from α cells inhibits insulin secretion from β cells (Figure 3B). Insulin receptors are found on both α and β cell membranes, which further confirms the existence of the paracrine effect of islet cells[92]. Insulin secreted from β cells activates the GABAA receptor on the α cell membrane, leading to a large influx of Cl- and inhibiting the secretion of glucagon[93]. β cells can electrically couple to surrounding α and δ cells through the gap, to secrete synchronously, thereby generating insulin secretion pulse[94]. Moreover, secretory molecules of pancreatic islet pericytes and local macrophages have nutritional effects on β cells[95,96].

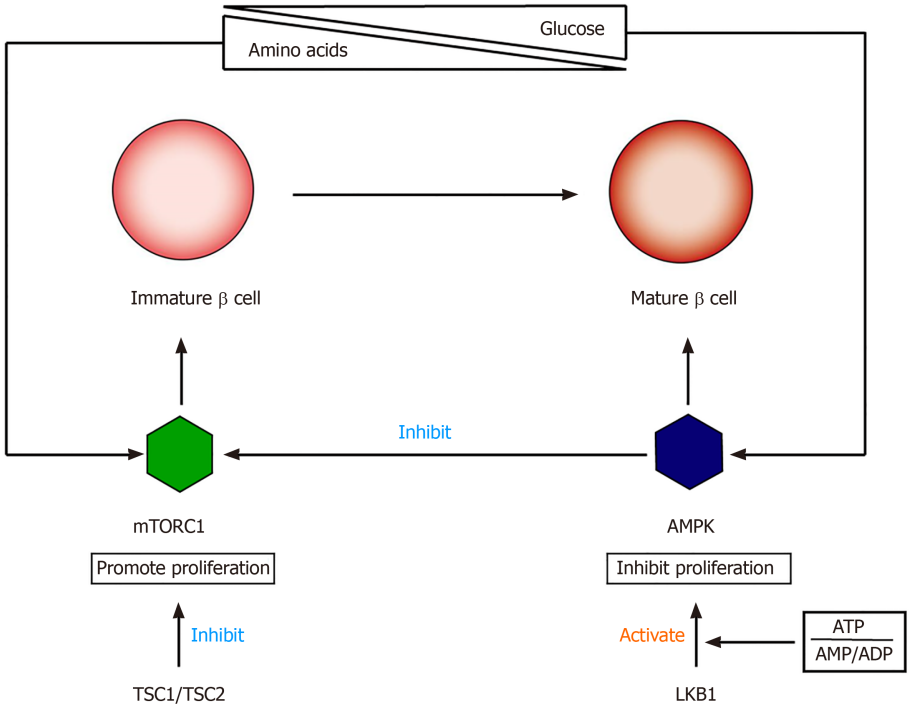


Figure 2 Relationship between mTORC1 and AMPK in the process of β cell transformation from immature to mature. Immediately after birth, the nutritional substrates are mainly amino acids, which activate the nutritionally sensitive mTORC1 and promote cell proliferation. mTORC1 is inhibited by AMPK and upstream regulatory factors TSC1/TSC2. When the nutritional substrate changes from amino acids to glucose, AMPK activity is stimulated. AMPK is activated by the upstream factor LKB1 under the regulation of intracellular ratio of ATP *vs* AMP/ADP, inhibits β cell proliferation, and promotes maturation, resulting in the establishment of adult-type glucose stimulated insulin secretion.

At low glucose concentrations, the islet α cells secrete glucagon that activates the glucagon receptor on the β cell membrane in a paracrine manner and inhibits insulin secretion[97]. Additionally, ghrelin locally released by ε cells in pancreatic islets can inhibit GSIS. This process may involve the activation of GHSR-coupled Gi, the opening of voltage-dependent K^+ channels, and the inhibition of Ca^{2+} influx[98]. Finally, enteroendocrine hormones released after food ingesting participate in the GSIS. The most important hormones are glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)[99]. Studies have confirmed the expression of GLP-1 and GIP receptors in pancreatic β cells. After activation, these two hormones bind to the receptor coupled to the heterotrimeric Gs protein, thereby activating adenylyl cyclase, increasing intracellular cAMP, and enhancing GSIS[97].

INDUCTION OF β CELLS *IN VITRO* AND CURRENT PROBLEMS

Due to the increasing demand for *in vitro* differentiation of hPSCs into β cells to treat T1DM, researchers have actively explored ways to obtain functionally β cells *in vitro*. hPSCs include human embryonic stem cells (ESCs) and induced pluripotent stem cells (hiPSCs). These cells are considered a reliable source for β-cell replacement due to their ability to self-renew and differentiate into all major somatic lineages[100,101]. After experimenting many methods, researchers found that the induction of genuine β cells *in vitro* must follow the same differentiation process *in vivo*. Using Matrigel or low-density mouse embryonic fibroblasts as the culture platform, hPSCs can finally differentiate to β-like cells following the sequence of definitive endoderm, primitive intestinal canal pancreatic progenitor cells, endocrine progenitor cells, and hormone-expressing endocrine cells, when regulated by specific doses and sequences of growth factors and signaling molecules (such as retinoic acid, BMP pathway inhibitors, FGF10, and FGF7)[102]. However, β-like cells produced *in vitro* by this method are mainly insulin positive multi-hormonal cells. They will only exhibit limited GSIS *in vitro* due to their lack of expression of key transcription factors of β-cells. Once transplanted into mice, they lose the ability to respond to glucose concentration stimuli[103].

Pagliuca *et al*[104] used a scalable suspension-based culture system to generate and cultivate hPSCs, and cell clusters (about 100-200 mm in diameter, each cluster contains

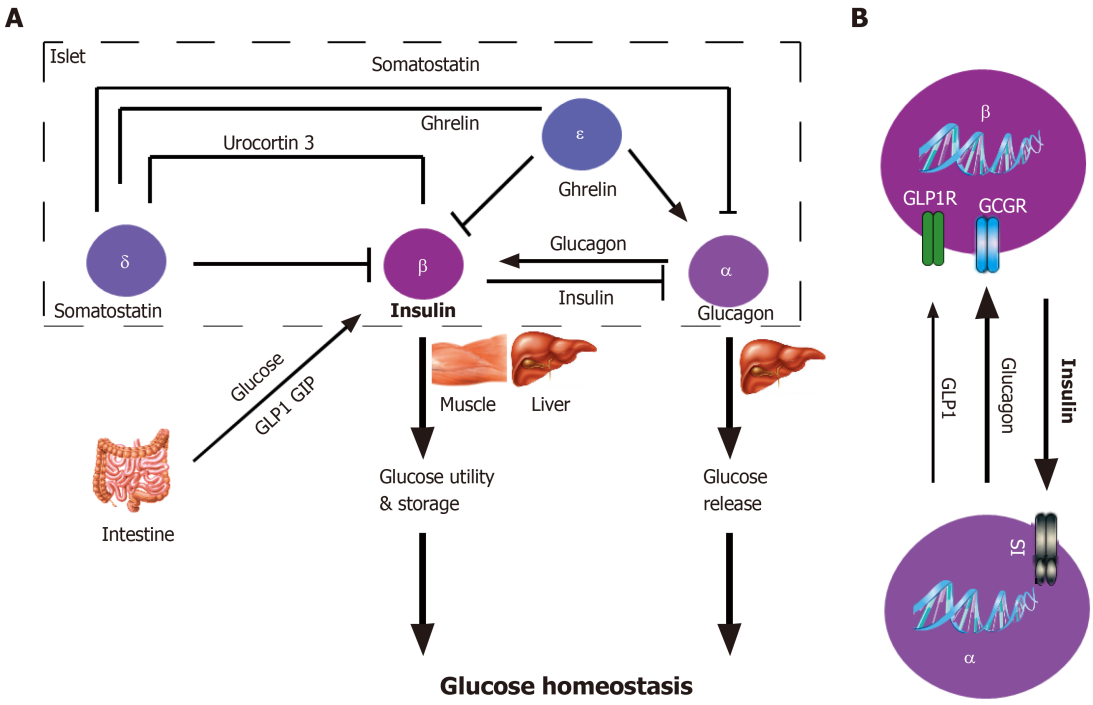


Figure 3 Paracrine regulations within the pancreatic islets. A: There are at least 5 paracrine regulatory circuits within the islets, among which α and β cells are two important cells that fine-tune the secretion of islet hormones that regulate blood glucose homeostasis. At the same time, δ and ε cells can also regulate the secretion of insulin and glucagon through the paracrine interactions. Meanwhile, the enteroendocrine hormones GLP1 and GIP secreted from the intestinal endocrine cells can also regulate β-cell insulin secretion by binding to receptors on the cell membrane; B: More detailed interactions between α and β cells.

hundreds of cells) from hESC line (HUES8) or hiPSC line (hiPSC-1 and hiPSC-2) were induced to transform into definitive endoderm (> 95% SOX17+ cells, DE), and subsequently differentiated into early pancreatic progenitor cells (> 85% PDX1+ cells, PP1). Culturing PP1 for 5 d under KGF or FGF7, retinoic acid, and SANT1 (sonic hedgehog signaling pathway antagonists) conditions can lead to forming pancreatic progenitor cells (PP2) expressing PDX1+/NKX6.1+, and producing functional β cells 3-4 mo after transplantation into mice. After testing the insulin secretion of these β-like cells, researchers found that their function is similar to that of adult β cells.

These stem cell (SC)-derived β-like cells are known as SC-β cells[104] that are arguably the most advanced β-like cells induced *in vitro* from hPSCs. The increased level of UCN3 expression was found to coincide with the functional maturation of the SC-β cells[105,106]. In SC-β cells, the nutritional regulation of mTORC1 activity is mainly determined by the amount of amino acids, not glucose, which is very similar to fetal β cells in the body[107]. This is consistent with the fact that SC-β cells are not mature at this time. After reducing the amount of amino acids in the culture medium, the SC-β cells can be induced to be more mature[108]. In adults, the supply of nutrients is periodic, so the activity of mTORC is also dynamic, which requires the participation of AMPK, TSC1, TSC2 (the upstream inhibitors of mTOR), *etc.*

Davis *et al*[109] found that SC-β cells have lower GSIS levels than cadaver islet β cells. They found that glucose metabolism is a restricting factor that inhibits the formation of mature GSIS in SC-β cells. Abnormal mitochondrial metabolism is also associated with the immature GSIS in SC-β cells that can be used to explore the metabolic processes and quantify their ability to transport glucose. Furthermore SC-β cells can sense and respond to changes in metabolic flux, but the metabolism of glyceraldehyde 3-phosphate is the key "defect" or "bottleneck". The activities of the enzymes GAPDH and PGK1 are significantly lower in SC-β cells than in cadaver islets, and these two enzymes can catalyze the enzymatic conversion of 3-phosphoglyceraldehyde to 3-phosphoglycerate. When the enzyme concentrations are reduced, the slow glycolysis flux in SC-β cells inhibits the production of phosphoenolpyruvate in mitochondria, resulting in restrictions on GSIS promoted by downstream mitochondrial phosphoenolpyruvate carboxykinase. Therefore, bypassing the above-mentioned defect in the glycolysis process that is unique to the *in vitro* culture process can drastically increase the intracellular PEP and make the cells have mature islet-like insulin secretion characteristics[110,111]. The researchers proposed that treating differentiated SC-β cells with metabolized, cell-permeable intermediates that skip

these enzymatic steps in glycolysis can result in islet-like insulin secretion and acts through the same mechanisms that underlie glucose sensing in functional islets. Taken together, these data suggest that the *in vitro* functional maturation of SC-β cells can be achieved by improving the nutritional conditions in the culture medium. However, further exploration is required as to how this bottleneck is formed in the differentiation process *in vitro*.

In the process of differentiation of β cells *in vitro*, it is necessary to ensure the generation of other islet cells. Indeed, other cells may also be differentiated in addition to SC-β cells. Veres *et al*[112] showed that in SC-islets derived from hPSCs, there are also α-like cells expressing GCG, ARX, IRX2, and INS and enterochromaffin cells that express CHGA, TPH1, LMX1A, and SLC18A1. These cells are multi-hormonal cells and when transplanted, they can improve the function of β cells through local interactions or autocrine signaling in SC islets. Furthermore, CD49a was found as a surface marker of SC-β cells, and it was showed that pure SC-β cell clusters can be obtained by magnetic separation.

Protein transduction technology for delivering targeted transcription factors is also used to obtain insulin-producing cells from stem cells *in vitro*[113]. Protein transduction domains (PTD) or cell penetrating peptides can be directly internalized into cells when the protein is synthesized as a recombinant fusion molecule or covalently crosslinked to the PTDs, the mechanism of PTD-mediated protein transduction through endocytosis as a vesicle into the cytoplasm[114]. Thus, PTD may provide a new strategy of generating insulin-secreting cells from stem/progenitor cells without transferring foreign transcription factor genes such as *PDX-1*, *B2/NEUROD*, *NGN3*, and *ISL-1*[115,116].

In addition to hPSCs, multipotent stem cells including hematopoietic stem cells, mesenchymal stromal cells/MSCs, and adipose-derived stem cells are also possible sources for generation of insulin-producing cells[117]. MSCs from various tissues and organs and the umbilical cord blood can be differentiated into islet-like cells or insulin-producing cells (IPC) that express key transcription factors such as PAX6 and ISL1[118]. Besides IPC differentiation, MSCs may also secrete various cytokines and growth factors to help regenerate endogenous islet β cells[119]. Si *et al*[120] found that injection of MSCs into diabetic rats can lead to significant endogenous β-cell regeneration. Meanwhile, Ianus *et al*[121] found that approximately 1.7% to 3% of regenerated islet β cells originated from transplanted MSCs.

CONCLUSION

T1DM is an autoimmune disease, which is generally early onset and is characterized by an absolute lack of insulin secretion. Therefore, the current treatment is to supplement the required insulin from an external source. However, there are many problems with this treatment method, including cumbersome procedures and associated devastating complications. If patients forget to take their medicine, acute complications are immediately developed, such as diabetic ketoacidosis. Therefore, in recent years, researchers have proposed other treatments for T1DM, such as islet transplantation, immunotherapy, and stem cell therapy. Among them, stem cell therapy is the most promising treatment method, but it still faces many obstacles such as generation of matured β-like cells in achieving the clinical application. First, the changes in the nutritional conditions, the surrounding microenvironment, and the related molecular mechanisms may all play a part in the maturation of β-like cells. Second, abnormal mitochondrial function in glucose metabolism is closely related to immature GSIS of β-like cells. Developing normal mitochondrial function in SC-β cells must be achieved in the next few years to generate mature β-like cells. Third, the microenvironment for *in vitro* differentiation also appears to be crucial for the functional maturation of β cells as isolated β cells cannot have adult-type GSIS, which is related to the paracrine effects among pancreatic islet cells as described above. This suggests that the reconstruction of islet-like structures, for example 3D bioprinting with *in vitro* differentiated SC-β and other endocrine cells, is necessary for generating mature SC-β cells. Finally, critical molecules/compounds to mature the reconstructed islet-like structures need to be discovered. It is hoped that in the near future, stem cell therapy can ultimately become a viable curative treatment for most T1DM patients.

REFERENCES

1 **Tyka K**, Jörns A, Turatsinze JV, Eizirik DL, Lenzen S, Gurgul-Convey E. MCPIP1 regulates the sensitivity of pancreatic beta-cells to cytokine toxicity. *Cell Death Dis* 2019; **10**: 29 [PMID: 30631045 DOI: 10.1038/s41419-018-1268-4]

2 **Shao L**, Zhou HJ, Zhang H, Qin L, Hwa J, Yun Z, Ji W, Min W. SENP1-mediated NEMO deSUMOylation in adipocytes limits inflammatory responses and type-1 diabetes progression. *Nat Commun* 2015; **6**: 8917 [PMID: 26596471 DOI: 10.1038/ncomms9917]

3 **Cobo-Vuilleumier N**, Lorenzo PI, Rodríguez NG, Herrera Gómez IG, Fuente-Martin E, López-Noriega L, Mellado-Gil JM, Romero-Zerbo SY, Baquié M, Lachaud CC, Stifter K, Perdomo G, Bugliani M, De Tata V, Bosco D, Parnaud G, Pozo D, Hmadcha A, Florido JP, Toscano MG, de Haan P, Schoonjans K, Sánchez Palazón L, Marchetti P, Schirmbeck R, Martín-Montalvo A, Meda P, Soria B, Bermúdez-Silva FJ, St-Onge L, Gauthier BR. LRH-1 agonism favours an immune-islet dialogue which protects against diabetes mellitus. *Nat Commun* 2018; **9**: 1488 [PMID: 29662071 DOI: 10.1038/s41467-018-03943-0]

4 **Silva C**, Sampaio-Pinto V, Andrade S, Rodrigues I, Costa R, Guerreiro S, Carvalho E, Pinto-do-Ó P, Nascimento DS, Soares R. Establishing a Link between Endothelial Cell Metabolism and Vascular Behaviour in a Type 1 Diabetes Mouse Model. *Cell Physiol Biochem* 2019; **52**: 503-516 [PMID: 30897318 DOI: 10.33594/000000036]

5 **Yin L**, Zeng C, Yao J, Shen J. Emerging Roles for Noncoding RNAs in Autoimmune Thyroid Disease. *Endocrinology* 2020; **161** [PMID: 32270194 DOI: 10.1210/endocr/bqaa053]

6 **Needell JC**, Zipris D. The Role of the Intestinal Microbiome in Type 1 Diabetes Pathogenesis. *Curr Diab Rep* 2016; **16**: 89 [PMID: 27523648 DOI: 10.1007/s11892-016-0781-z]

7 **Rezaei-Tavirani S**, Rostami-Nejad M, Vafaee R, Khalkhal E, Keramatinia A, Ehsani-Ardakani MJ, Razzaghi M. Introducing tumor necrosis factor as a prominent player in celiac disease and type 1 diabetes mellitus. *Gastroenterol Hepatol Bed Bench* 2019; **12**: S123-S129 [PMID: 32099612]

8 **Espona-Noguera A**, Ciriza J, Cañibano-Hernández A, Orive G, Hernández RMM, Saenz Del Burgo L, Pedraz JL. Review of Advanced Hydrogel-Based Cell Encapsulation Systems for Insulin Delivery in Type 1 Diabetes Mellitus. *Pharmaceutics* 2019; **11** [PMID: 31726670 DOI: 10.3390/pharmaceutics11110597]

9 **Cahill D**, Zamboni F, Collins MN. Radiological Advances in Pancreatic Islet Transplantation. *Acad Radiol* 2019; **26**: 1536-1543 [PMID: 30709732 DOI: 10.1016/j.acra.2019.01.006]

10 **Ding H**, Wang F, Shi X, Ma H, Du Y, Hou L, Xing N. LncRNA MALAT1 induces the dysfunction of β cells *via* reducing the histone acetylation of the PDX-1 promoter in type 1 diabetes. *Exp Mol Pathol* 2020; **114**: 104432 [PMID: 32243891 DOI: 10.1016/j.yexmp.2020.104432]

11 **Dvornikova KA**, Bystrova EY, Platonova ON, Churilov LP. Polymorphism of toll-like receptor genes and autoimmune endocrine diseases. *Autoimmun Rev* 2020; **19**: 102496 [PMID: 32062033 DOI: 10.1016/j.autrev.2020.102496]

12 **Noble JA**. Immunogenetics of type 1 diabetes: A comprehensive review. *J Autoimmun* 2015; **64**: 101-112 [PMID: 26272854 DOI: 10.1016/j.jaut.2015.07.014]

13 **Scherm MG**, Serr I, Zahm AM, Schug J, Bellusci S, Manfredini R, Salb VK, Gerlach K, Weigmann B, Ziegler AG, Kaestner KH, Daniel C. miRNA142-3p targets Tet2 and impairs Treg differentiation and stability in models of type 1 diabetes. *Nat Commun* 2019; **10**: 5697 [PMID: 31836704 DOI: 10.1038/s41467-019-13587-3]

14 **Song J**. Development of Auto Antigen-specific Regulatory T Cells for Diabetes Immunotherapy. *Immune Netw* 2016; **16**: 281-285 [PMID: 27799873 DOI: 10.4110/in.2016.16.5.281]

15 **Xie Z**, Chang C, Zhou Z. Molecular mechanisms in autoimmune type 1 diabetes: a critical review. *Clin Rev Allergy Immunol* 2014; **47**: 174-192 [PMID: 24752371 DOI: 10.1007/s12016-014-8422-2]

16 **Ilonen J**, Lempainen J, Veijola R. The heterogeneous pathogenesis of type 1 diabetes mellitus. *Nat Rev Endocrinol* 2019; **15**: 635-650 [PMID: 31534209 DOI: 10.1038/s41574-019-0254-y]

17 **Morran MP**, Vonberg A, Khadra A, Pietropaolo M. Immunogenetics of type 1 diabetes mellitus. *Mol Aspects Med* 2015; **42**: 42-60 [PMID: 25579746 DOI: 10.1016/j.mam.2014.12.004]

18 **Pathak V**, Pathak NM, O'Neill CL, Guduric-Fuchs J, Medina RJ. Therapies for Type 1 Diabetes: Current Scenario and Future Perspectives. *Clin Med Insights Endocrinol Diabetes* 2019; **12**: 1179551419844521 [PMID: 31105434 DOI: 10.1177/1179551419844521]

19 **Wu Q**, Zheng S, Qin Y, Zheng X, Chen H, Yang T, Zhang M. Efficacy and safety of stem cells transplantation in patients with type 1 diabetes mellitus-a systematic review and meta-analysis. *Endocr J* 2020; **67**: 827-840 [PMID: 32321876 DOI: 10.1507/endocrj.EJ20-0050]

20 **Ni Q**, Pham NB, Meng WS, Zhu G, Chen X. Advances in immunotherapy of type I diabetes. *Adv Drug Deliv Rev* 2019; **139**: 83-91 [PMID: 30528629 DOI: 10.1016/j.addr.2018.12.003]

21 **Viswanathan C**, Sarang S. Status of stem cell based clinical trials in the treatment for diabetes. *Curr Diabetes Rev* 2013; **9**: 429-436 [PMID: 24160356 DOI: 10.2174/15733998113096660082]

22 **Kakkar A**, Sorout A, Tiwari M, Shrivastava P, Meena P, Saraswat SK, Srivastava S, Datt R, Pandey S. Current Status of Stem Cell Treatment for Type I Diabetes Mellitus. *Tissue Eng Regen Med* 2018; **15**: 699-709 [PMID: 30603589 DOI: 10.1007/s13770-018-0143-9]

23 **Sordi V**, Pellegrini S, Krampera M, Marchetti P, Pessina A, Ciardelli G, Fadini G, Pintus C, Pantè G, Piemonti L. Stem cells to restore insulin production and cure diabetes. *Nutr Metab Cardiovasc Dis* 2017; **27**: 583-600 [PMID: 28545927 DOI: 10.1016/j.numecd.2017.02.004]

24 **Rorsman P**, Ashcroft FM. Pancreatic β-Cell Electrical Activity and Insulin Secretion: Of Mice and Men. *Physiol Rev* 2018; **98**: 117-214 [PMID: 29212789 DOI: 10.1152/physrev.00008.2017]

25 **Baron M**, Veres A, Wolock SL, Faust AL, Gaujoux R, Vetere A, Ryu JH, Wagner BK, Shen-Orr SS, Klein AM, Melton DA, Yanai I. A Single-Cell Transcriptomic Map of the Human and Mouse Pancreas Reveals Inter- and Intra-cell Population Structure. *Cell Syst* 2016; **3**: 346-360. e4 [PMID: 27667365 DOI: 10.1016/j.cels.2016.08.011]

26 **Benitez CM**, Qu K, Sugiyama T, Pauerstein PT, Liu Y, Tsai J, Gu X, Ghodasara A, Arda HE, Zhang J, Dekker JD, Tucker HO, Chang HY, Kim SK. An integrated cell purification and genomics strategy reveals multiple regulators of pancreas development. *PLoS Genet* 2014; **10**: e1004645 [PMID: 25330008 DOI: 10.1371/journal.pgen.1004645]

27 **Olofsson CS**, Göpel SO, Barg S, Galvanovskis J, Ma X, Salehi A, Rorsman P, Eliasson L. Fast insulin secretion reflects exocytosis of docked granules in mouse pancreatic B-cells. *Pflugers Arch* 2002; **444**: 43-51 [PMID: 11976915 DOI: 10.1007/s00424-002-0781-5]

28 **Jiang FX**, Morahan G. Insulin-secreting β cells require a post-genomic concept. *World J Diabetes* 2016; **7**: 198-208 [PMID: 27226815 DOI: 10.4239/wjd.v7.i10.198]

29 **Lemaire K**, Ravier MA, Schraenen A, Creemers JW, Van de Plas R, Granvik M, Van Lommel L, Waelkens E, Chimienti F, Rutter GA, Gilon P, in't Veld PA, Schuit FC. Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. *Proc Natl Acad Sci USA* 2009; **106**: 14872-14877 [PMID: 19706465 DOI: 10.1073/pnas.0906587106]

30 **Ashcroft FM**, Rorsman P. Diabetes mellitus and the β cell: the last ten years. *Cell* 2012; **148**: 1160-1171 [PMID: 22424227 DOI: 10.1016/j.cell.2012.02.010]

31 **Murtaugh LC**. Pancreas and beta-cell development: from the actual to the possible. *Development* 2007; **134**: 427-438 [PMID: 17185316 DOI: 10.1242/dev.02770]

32 **Jennings RE**, Berry AA, Strutt JP, Gerrard DT, Hanley NA. Human pancreas development. *Development* 2015; **142**: 3126-3137 [PMID: 26395141 DOI: 10.1242/dev.120063]

33 **Al-Khawaga S**, Memon B, Butler AE, Taheri S, Abou-Samra AB, Abdelalim EM. Pathways governing development of stem cell-derived pancreatic β cells: lessons from embryogenesis. *Biol Rev Camb Philos Soc* 2018; **93**: 364-389 [PMID: 28643455 DOI: 10.1111/brv.12349]

34 **Collombat P**, Hecksher-Sørensen J, Krull J, Berger J, Riedel D, Herrera PL, Serup P, Mansouri A. Embryonic endocrine pancreas and mature beta cells acquire alpha and PP cell phenotypes upon Arx misexpression. *J Clin Invest* 2007; **117**: 961-970 [PMID: 17404619 DOI: 10.1172/JCI29115]

35 **Evans-Molina C**, Vestermark GL, Mirmira RG. Development of insulin-producing cells from primitive biologic precursors. *Curr Opin Organ Transplant* 2009; **14**: 56-63 [PMID: 19337148 DOI: 10.1097/MOT.0b013e3283186fc1]

36 **Pan FC**, Brissova M. Pancreas development in humans. *Curr Opin Endocrinol Diabetes Obes* 2014; **21**: 77-82 [PMID: 24569548 DOI: 10.1097/MED.0000000000000047]

37 **Jennings RE**, Berry AA, Kirkwood-Wilson R, Roberts NA, Hearn T, Salisbury RJ, Blaylock J, Piper Hanley K, Hanley NA. Development of the human pancreas from foregut to endocrine commitment. *Diabetes* 2013; **62**: 3514-3522 [PMID: 23630303 DOI: 10.2337/db12-1479]

38 **Lyttle BM**, Li J, Krishnamurthy M, Fellows F, Wheeler MB, Goodyer CG, Wang R. Transcription factor expression in the developing human fetal endocrine pancreas. *Diabetologia* 2008; **51**: 1169-1180 [PMID: 18491072 DOI: 10.1007/s00125-008-1006-z]

39 **Hang Y**, Yamamoto T, Benninger RK, Brissova M, Guo M, Bush W, Piston DW, Powers AC, Magnuson M, Thurmond DC, Stein R. The MafA transcription factor becomes essential to islet β-cells soon after birth. *Diabetes* 2014; **63**: 1994-2005 [PMID: 24520122 DOI: 10.2337/db13-1001]

40 **Otonkoski T**, Andersson S, Knip M, Simell O. Maturation of insulin response to glucose during human fetal and neonatal development. Studies with perifusion of pancreatic isletlike cell clusters. *Diabetes* 1988; **37**: 286-291 [PMID: 3286329 DOI: 10.2337/diab.37.3.286]

41 **Bastidas-Ponce A**, Scheibner K, Lickert H, Bakhti M. Cellular and molecular mechanisms coordinating pancreas development. *Development* 2017; **144**: 2873-2888 [PMID: 28811309 DOI: 10.1242/dev.140756]

42 **Sharon N**, Chawla R, Mueller J, Vanderhooft J, Whitehorn LJ, Rosenthal B, Gürtler M, Estanboulieh RR, Shvartsman D, Gifford DK, Trapnell C, Melton D. A Peninsular Structure Coordinates Asynchronous Differentiation with Morphogenesis to Generate Pancreatic Islets. *Cell* 2019; **176**: 790-804. e13 [PMID: 30661759 DOI: 10.1016/j.cell.2018.12.003]

43 **Stolovich-Rain M**, Enk J, Vikesa J, Nielsen FC, Saada A, Glaser B, Dor Y. Weaning triggers a maturation step of pancreatic β cells. *Dev Cell* 2015; **32**: 535-545 [PMID: 25662175 DOI: 10.1016/j.devcel.2015.01.002]

44 **Chen TY**, Lines D, Dickson C, Go C, Kirkwood RN, Langendijk P. Elevating glucose and insulin secretion by carbohydrate formulation diets in late lactation to improve post-weaning fertility in primiparous sows. *Reprod Domest Anim* 2016; **51**: 813-818 [PMID: 27548995 DOI: 10.1111/rda.12760]

45 **Cappelli APG**, Zoppi CC, Silveira LR, Batista TM, Paula FM, da Silva PMR, Rafacho A, Barbosa-Sampaio HC, Boschero AC, Carneiro EM. Reduced glucose-induced insulin secretion in low-protein-fed rats is associated with altered pancreatic islets redox status. *J Cell Physiol* 2018; **233**: 486-496 [PMID: 28370189 DOI: 10.1002/jcp.25908]

46 **Kang T**, Jensen P, Huang H, Lund Christensen G, Billestrup N, Larsen MR. Characterization of the Molecular Mechanisms Underlying Glucose Stimulated Insulin Secretion from Isolated Pancreatic β-

cells Using Post-translational Modification Specific Proteomics (PTMomics). *Mol Cell Proteomics* 2018; **17**: 95-110 [PMID: 29113996 DOI: 10.1074/mcp.RA117.000217]

47 **Meda P**, Schuit F. Glucose-stimulated insulin secretion: the hierarchy of its multiple cellular and subcellular mechanisms. *Diabetologia* 2013; **56**: 2552-2555 [PMID: 24114115 DOI: 10.1007/s00125-013-3073-z]

48 **Salinno C**, Cota P, Bastidas-Ponce A, Tarquis-Medina M, Lickert H, Bakhti M. β-Cell Maturation and Identity in Health and Disease. *Int J Mol Sci* 2019; **20** [PMID: 31671683 DOI: 10.3390/ijms20215417]

49 **Artner I**, Le Lay J, Hang Y, Elghazi L, Schisler JC, Henderson E, Sosa-Pineda B, Stein R. MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. *Diabetes* 2006; **55**: 297-304 [PMID: 16443760 DOI: 10.2337/diabetes.55.02.06.db05-0946]

50 **Matsuoka TA**, Artner I, Henderson E, Means A, Sander M, Stein R. The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. *Proc Natl Acad Sci U S A* 2004; **101**: 2930-2933 [PMID: 14973194 DOI: 10.1073/pnas.0306233101]

51 **Nishimura W**, Kondo T, Salameh T, El Khattabi I, Dodge R, Bonner-Weir S, Sharma A. A switch from MafB to MafA expression accompanies differentiation to pancreatic beta-cells. *Dev Biol* 2006; **293**: 526-539 [PMID: 16580660 DOI: 10.1016/j.ydbio.2006.02.028]

52 **Artner I**, Hang Y, Mazur M, Yamamoto T, Guo M, Lindner J, Magnuson MA, Stein R. MafA and MafB regulate genes critical to beta-cells in a unique temporal manner. *Diabetes* 2010; **59**: 2530-2539 [PMID: 20627934 DOI: 10.2337/db10-0190]

53 **Hang Y**, Stein R. MafA and MafB activity in pancreatic β cells. *Trends Endocrinol Metab* 2011; **22**: 364-373 [PMID: 21719305 DOI: 10.1016/j.tem.2011.05.003]

54 **Aguayo-Mazzucato C**, Koh A, El Khattabi I, Li WC, Toschi E, Jermendy A, Juhl K, Mao K, Weir GC, Sharma A, Bonner-Weir S. Mafa expression enhances glucose-responsive insulin secretion in neonatal rat beta cells. *Diabetologia* 2011; **54**: 583-593 [PMID: 21190012 DOI: 10.1007/s00125-010-2026-z]

55 **Ediger BN**, Lim HW, Juliana C, Groff DN, Williams LT, Dominguez G, Liu JH, Taylor BL, Walp ER, Kameswaran V, Yang J, Liu C, Hunter CS, Kaestner KH, Naji A, Li C, Sander M, Stein R, Sussel L, Won KJ, May CL, Stoffers DA. LIM domain-binding 1 maintains the terminally differentiated state of pancreatic β cells. *J Clin Invest* 2017; **127**: 215-229 [PMID: 27941246 DOI: 10.1172/JCI88016]

56 **Scavuzzo MA**, Hill MC, Chmielowiec J, Yang D, Teaw J, Sheng K, Kong Y, Bettini M, Zong C, Martin JF, Borowiak M. Endocrine lineage biases arise in temporally distinct endocrine progenitors during pancreatic morphogenesis. *Nat Commun* 2018; **9**: 3356 [PMID: 30135482 DOI: 10.1038/s41467-018-05740-1]

57 **Churchill AJ**, Gutiérrez GD, Singer RA, Lorberbaum DS, Fischer KA, Sussel L. Genetic evidence that Nkx2.2 acts primarily downstream of Neurog3 in pancreatic endocrine lineage development. *Elife* 2017; **6** [PMID: 28071588 DOI: 10.7554/eLife.20010]

58 **Zhu Z**, Li QV, Lee K, Rosen BP, González F, Soh CL, Huangfu D. Genome Editing of Lineage Determinants in Human Pluripotent Stem Cells Reveals Mechanisms of Pancreatic Development and Diabetes. *Cell Stem Cell* 2016; **18**: 755-768 [PMID: 27133796 DOI: 10.1016/j.stem.2016.03.015]

59 **Kropp PA**, Dunn JC, Carboneau BA, Stoffers DA, Gannon M. Cooperative function of Pdx1 and Oc1 in multipotent pancreatic progenitors impacts postnatal islet maturation and adaptability. *Am J Physiol Endocrinol Metab* 2018; **314**: E308-E321 [PMID: 29351489 DOI: 10.1152/ajpendo.00260.2017]

60 **Spaeth JM**, Gupte M, Perelis M, Yang YP, Cyphert H, Guo S, Liu JH, Guo M, Bass J, Magnuson MA, Wright C, Stein R. Defining a Novel Role for the Pdx1 Transcription Factor in Islet β-Cell Maturation and Proliferation During Weaning. *Diabetes* 2017; **66**: 2830-2839 [PMID: 28705881 DOI: 10.2337/db16-1516]

61 **Guo L**, Inada A, Aguayo-Mazzucato C, Hollister-Lock J, Fujitani Y, Weir GC, Wright CV, Sharma A, Bonner-Weir S. PDX1 in ducts is not required for postnatal formation of β-cells but is necessary for their subsequent maturation. *Diabetes* 2013; **62**: 3459-3468 [PMID: 23775765 DOI: 10.2337/db12-1833]

62 **Romer AI**, Singer RA, Sui L, Egli D, Sussel L. Murine Perinatal β-Cell Proliferation and the Differentiation of Human Stem Cell-Derived Insulin-Expressing Cells Require NEUROD1. *Diabetes* 2019; **68**: 2259-2271 [PMID: 31519700 DOI: 10.2337/db19-0117]

63 **Mu C**, Wang T, Wang X, Tian H, Liu Y. Identification of microRNAs regulating Hlxb9 gene expression during the induction of insulin-producing cells. *Cell Biol Int* 2016; **40**: 515-523 [PMID: 26801823 DOI: 10.1002/cbin.10586]

64 **Sun J**, Xu M, Ortsäter H, Lundeberg E, Juntti-Berggren L, Chen YQ, Haeggström JZ, Gudmundsson GH, Diana J, Agerberth B. Cathelicidins positively regulate pancreatic β-cell functions. *FASEB J* 2016; **30**: 884-894 [PMID: 26527065 DOI: 10.1096/fj.15-275826]

65 **Rezania A**, Bruin JE, Xu J, Narayan K, Fox JK, O'Neil JJ, Kieffer TJ. Enrichment of human embryonic stem cell-derived NKX6.1-expressing pancreatic progenitor cells accelerates the maturation of insulin-secreting cells in vivo. *Stem Cells* 2013; **31**: 2432-2442 [PMID: 23897760 DOI: 10.1002/stem.1489]

66 **van der Meulen T**, Xie R, Kelly OG, Vale WW, Sander M, Huising MO. Urocortin 3 marks mature human primary and embryonic stem cell-derived pancreatic alpha and beta cells. *PLoS One* 2012; **7**: e52181 [PMID: 23251699 DOI: 10.1371/journal.pone.0052181]

67 **Bhargava A**. CRF and urocortins: a challenging interaction between family members. *Gastroenterology* 2011; **140**: 1391-1394 [PMID: 21447312 DOI: 10.1053/j.gastro.2011.03.024]

68 **Novembri R**, Carrarelli P, Toti P, Rocha AL, Borges LE, Reis FM, Piomboni P, Florio P, Petraglia F. Urocortin 2 and urocortin 3 in endometriosis: evidence for a possible role in inflammatory response. *Mol Hum Reprod* 2011; **17**: 587-593 [PMID: 21454316 DOI: 10.1093/molehr/gar020]

69 **Jamieson PM**, Cleasby ME, Kuperman Y, Morton NM, Kelly PA, Brownstein DG, Mustard KJ, Vaughan JM, Carter RN, Hahn CN, Hardie DG, Seckl JR, Chen A, Vale WW. Urocortin 3 transgenic mice exhibit a metabolically favourable phenotype resisting obesity and hyperglycaemia on a high-fat diet. *Diabetologia* 2011; **54**: 2392-2403 [PMID: 21667214 DOI: 10.1007/s00125-011-2205-6]

70 **Blum B**, Hrvatin S, Schuetz C, Bonal C, Rezania A, Melton DA. Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. *Nat Biotechnol* 2012; **30**: 261-264 [PMID: 22371083 DOI: 10.1038/nbt.2141]

71 **Balcazar N**, Sathyamurthy A, Elghazi L, Gould A, Weiss A, Shiojima I, Walsh K, Bernal-Mizrachi E. mTORC1 activation regulates beta-cell mass and proliferation by modulation of cyclin D2 synthesis and stability. *J Biol Chem* 2009; **284**: 7832-7842 [PMID: 19144649 DOI: 10.1074/jbc.M807458200]

72 **Blandino-Rosano M**, Chen AY, Scheys JO, Alejandro EU, Gould AP, Taranukha T, Elghazi L, Cras-Méneur C, Bernal-Mizrachi E. mTORC1 signaling and regulation of pancreatic β-cell mass. *Cell Cycle* 2012; **11**: 1892-1902 [PMID: 22544327 DOI: 10.4161/cc.20036]

73 **Jaafar R**, Tran S, Shah AN, Sun G, Valdearcos M, Marchetti P, Masini M, Swisa A, Giacometti S, Bernal-Mizrachi E, Matveyenko A, Hebrok M, Dor Y, Rutter GA, Koliwad SK, Bhushan A. mTORC1 to AMPK switching underlies β-cell metabolic plasticity during maturation and diabetes. *J Clin Invest* 2019; **129**: 4124-4137 [PMID: 31265435 DOI: 10.1172/JCI127021]

74 **Maiese K**. New Insights for nicotinamide: Metabolic disease, autophagy, and mTOR. *Front Biosci (Landmark Ed)* 2020; **25**: 1925-1973 [PMID: 32472766]

75 **Riahi Y**, Israeli T, Yeroslaviz R, Chimenez S, Avrahami D, Stolovich-Rain M, Alter I, Sebag M, Polin N, Bernal-Mizrachi E, Dor Y, Cerasi E, Leibowitz G. Inhibition of mTORC1 by ER stress impairs neonatal β-cell expansion and predisposes to diabetes in the *Akita* mouse. *Elife* 2018; **7** [PMID: 30412050 DOI: 10.7554/eLife.38472]

76 **Blandino-Rosano M**, Barbaresso R, Jimenez-Palomares M, Bozadjieva N, Werneck-de-Castro JP, Hatanaka M, Mirmira RG, Sonenberg N, Liu M, Rüegg MA, Hall MN, Bernal-Mizrachi E. Loss of mTORC1 signalling impairs β-cell homeostasis and insulin processing. *Nat Commun* 2017; **8**: 16014 [PMID: 28699639 DOI: 10.1038/ncomms16014]

77 **Ni Q**, Gu Y, Xie Y, Yin Q, Zhang H, Nie A, Li W, Wang Y, Ning G, Wang W, Wang Q. Raptor regulates functional maturation of murine beta cells. *Nat Commun* 2017; **8**: 15755 [PMID: 28598424 DOI: 10.1038/ncomms15755]

78 **Lin SC**, Hardie DG. AMPK: Sensing Glucose as well as Cellular Energy Status. *Cell Metab* 2018; **27**: 299-313 [PMID: 29153408 DOI: 10.1016/j.cmet.2017.10.009]

79 **Fu A**, Ng AC, Depatie C, Wijesekara N, He Y, Wang GS, Bardeesy N, Scott FW, Touyz RM, Wheeler MB, Screaton RA. Loss of Lkb1 in adult beta cells increases beta cell mass and enhances glucose tolerance in mice. *Cell Metab* 2009; **10**: 285-295 [PMID: 19808021 DOI: 10.1016/j.cmet.2009.08.008]

80 **Granot Z**, Swisa A, Magenheim J, Stolovich-Rain M, Fujimoto W, Manduchi E, Miki T, Lennerz JK, Stoeckert CJ Jr, Meyuhas O, Seino S, Permutt MA, Piwnica-Worms H, Bardeesy N, Dor Y. LKB1 regulates pancreatic beta cell size, polarity, and function. *Cell Metab* 2009; **10**: 296-308 [PMID: 19808022 DOI: 10.1016/j.cmet.2009.08.010]

81 **Helman A**, Cangelosi AL, Davis JC, Pham Q, Rothman A, Faust AL, Straubhaar JR, Sabatini DM, Melton DA. A Nutrient-Sensing Transition at Birth Triggers Glucose-Responsive Insulin Secretion. *Cell Metab* 2020; **31**: 1004-1016. e5 [PMID: 32375022 DOI: 10.1016/j.cmet.2020.04.004]

82 **Saxton RA**, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 2017; **169**: 361-371 [PMID: 28388417 DOI: 10.1016/j.cell.2017.03.035]

83 **Huang C**, Walker EM, Dadi PK, Hu R, Xu Y, Zhang W, Sanavia T, Mun J, Liu J, Nair GG, Tan HYA, Wang S, Magnuson MA, Stoeckert CJ Jr, Hebrok M, Gannon M, Han W, Stein R, Jacobson DA, Gu G. Synaptotagmin 4 Regulates Pancreatic β Cell Maturation by Modulating the Ca^{2+} Sensitivity of Insulin Secretion Vesicles. *Dev Cell* 2018; **45**: 347-361. e5 [PMID: 29656931 DOI: 10.1016/j.devcel.2018.03.013]

84 **Andersson SA**, Olsson AH, Esguerra JL, Heimann E, Ladenvall C, Edlund A, Salehi A, Taneera J, Degerman E, Groop L, Ling C, Eliasson L. Reduced insulin secretion correlates with decreased expression of exocytotic genes in pancreatic islets from patients with type 2 diabetes. *Mol Cell Endocrinol* 2012; **364**: 36-45 [PMID: 22939844 DOI: 10.1016/j.mce.2012.08.009]

85 **Gegg M**, Böttcher A, Burtscher I, Hasenoeder S, Van Campenhout C, Aichler M, Walch A, Grant SG, Lickert H. Flattop regulates basal body docking and positioning in mono- and multiciliated cells. *Elife* 2014; **3** [PMID: 25296022 DOI: 10.7554/eLife.03842]

86 **Bader E**, Migliorini A, Gegg M, Moruzzi N, Gerdes J, Roscioni SS, Bakhti M, Brandl E, Irmler M, Beckers J, Aichler M, Feuchtinger A, Leitzinger C, Zischka H, Wang-Sattler R, Jastroch M, Tschöp M, Machicao F, Staiger H, Häring HU, Chmelova H, Chouinard JA, Oskolkov N, Korsgren O, Speier S, Lickert H. Identification of proliferative and mature β-cells in the islets of Langerhans. *Nature* 2016; **535**: 430-434 [PMID: 27398620 DOI: 10.1038/nature18624]

87 **Roscioni SS**, Migliorini A, Gegg M, Lickert H. Impact of islet architecture on β-cell heterogeneity, plasticity and function. *Nat Rev Endocrinol* 2016; **12**: 695-709 [PMID: 27585958 DOI: 10.1038/nrendo.2016.147]

88 **Sakhneny L**, Rachi E, Epshtein A, Guez HC, Wald-Altman S, Lisnyansky M, Khalifa-Malka L, Hazan A, Baer D, Priel A, Weil M, Landsman L. Pancreatic Pericytes Support β-Cell Function in a Tcf7 L2-Dependent Manner. *Diabetes* 2018; **67**: 437-447 [PMID: 29246974 DOI: 10.2337/db17-0697]

89 **Dolenšek J**, Rupnik MS, Stožer A. Structural similarities and differences between the human and the mouse pancreas. *Islets* 2015; **7**: e1024405 [PMID: 26030186 DOI: 10.1080/19382014.2015.1024405]

90 **Briant LJB**, Reinbothe TM, Spiliotis I, Miranda C, Rodriguez B, Rorsman P. δ-cells and β-cells are electrically coupled and regulate α-cell activity *via* somatostatin. *J Physiol* 2018; **596**: 197-215 [PMID: 28975620 DOI: 10.1113/JP274581]

91 **Rodriguez-Diaz R**, Tamayo A, Hara M, Caicedo A. The Local Paracrine Actions of the Pancreatic α-Cell. *Diabetes* 2020; **69**: 550-558 [PMID: 31882565 DOI: 10.2337/dbi19-0002]

92 **Muller D**, Huang GC, Amiel S, Jones PM, Persaud SJ. Identification of insulin signaling elements in human beta-cells: autocrine regulation of insulin gene expression. *Diabetes* 2006; **55**: 2835-2842 [PMID: 17003350 DOI: 10.2337/db06-0532]

93 **Xu E**, Kumar M, Zhang Y, Ju W, Obata T, Zhang N, Liu S, Wendt A, Deng S, Ebina Y, Wheeler MB, Braun M, Wang Q. Intra-islet insulin suppresses glucagon release *via* GABA-GABAA receptor system. *Cell Metab* 2006; **3**: 47-58 [PMID: 16399504 DOI: 10.1016/j.cmet.2005.11.015]

94 **Head WS**, Orseth ML, Nunemaker CS, Satin LS, Piston DW, Benninger RK. Connexin-36 gap junctions regulate *in vivo* first- and second-phase insulin secretion dynamics and glucose tolerance in the conscious mouse. *Diabetes* 2012; **61**: 1700-1707 [PMID: 22511206 DOI: 10.2337/db11-1312]

95 **Sasson A**, Rachi E, Sakhneny L, Baer D, Lisnyansky M, Epshtein A, Landsman L. Islet Pericytes Are Required for β-Cell Maturity. *Diabetes* 2016; **65**: 3008-3014 [PMID: 27388217 DOI: 10.2337/db16-0365]

96 **Banaei-Bouchareb L**, Gouon-Evans V, Samara-Boustani D, Castellotti MC, Czernichow P, Pollard JW, Polak M. Insulin cell mass is altered in Csf1op/Csf1op macrophage-deficient mice. *J Leukoc Biol* 2004; **76**: 359-367 [PMID: 15178709 DOI: 10.1189/jlb.1103591]

97 **Ahrén B**. Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. *Nat Rev Drug Discov* 2009; **8**: 369-385 [PMID: 19365392 DOI: 10.1038/nrd2782]

98 **Koh DS**, Cho JH, Chen L. Paracrine interactions within islets of Langerhans. *J Mol Neurosci* 2012; **48**: 429-440 [PMID: 22528452 DOI: 10.1007/s12031-012-9752-2]

99 **Meier JJ**, Nauck MA. Incretins and the development of type 2 diabetes. *Curr Diab Rep* 2006; **6**: 194-201 [PMID: 16898571 DOI: 10.1007/s11892-006-0034-7]

100 **Zhang Y**, Zhang Z, Chen P, Ma CY, Li C, Au TYK, Tam V, Peng Y, Wu R, Cheung KMC, Sham PC, Tse HF, Chan D, Leung VY, Cheah KSE, Lian Q. Directed Differentiation of Notochord-like and Nucleus Pulposus-like Cells Using Human Pluripotent Stem Cells. *Cell Rep* 2020; **30**: 2791-2806. e5 [PMID: 32101752 DOI: 10.1016/j.celrep.2020.01.100]

101 **Gaertner B**, Carrano AC, Sander M. Human stem cell models: lessons for pancreatic development and disease. *Genes Dev* 2019; **33**: 1475-1490 [PMID: 31676735 DOI: 10.1101/gad.331397.119]

102 **Kunisada Y**, Tsubooka-Yamazoe N, Shoji M, Hosoya M. Small molecules induce efficient differentiation into insulin-producing cells from human induced pluripotent stem cells. *Stem Cell Res* 2012; **8**: 274-284 [PMID: 22056147 DOI: 10.1016/j.scr.2011.10.002]

103 **Bruin JE**, Erener S, Vela J, Hu X, Johnson JD, Kurata HT, Lynn FC, Piret JM, Asadi A, Rezania A, Kieffer TJ. Characterization of polyhormonal insulin-producing cells derived *in vitro* from human embryonic stem cells. *Stem Cell Res* 2014; **12**: 194-208 [PMID: 24257076 DOI: 10.1016/j.scr.2013.10.003]

104 **Pagliuca FW**, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, Peterson QP, Greiner D, Melton DA. Generation of functional human pancreatic β cells in vitro. *Cell* 2014; **159**: 428-439 [PMID: 25303535 DOI: 10.1016/j.cell.2014.09.040]

105 **van der Meulen T**, Huising MO. Maturation of stem cell-derived beta-cells guided by the expression of urocortin 3. *Rev Diabet Stud* 2014; **11**: 115-132 [PMID: 25148370 DOI: 10.1900/RDS.2014.11.115]

106 **van der Meulen T**, Donaldson CJ, Cáceres E, Hunter AE, Cowing-Zitron C, Pound LD, Adams MW, Zembrzycki A, Grove KL, Huising MO. Urocortin3 mediates somatostatin-dependent negative feedback control of insulin secretion. *Nat Med* 2015; **21**: 769-776 [PMID: 26076035 DOI: 10.1038/nm.3872]

107 **Melnik BC**. The pathogenic role of persistent milk signaling in mTORC1- and milk-microRNA-driven type 2 diabetes mellitus. *Curr Diabetes Rev* 2015; **11**: 46-62 [PMID: 25587719 DOI: 10.2174/1573399811666150114100653]

108 **Russ HA**, Parent AV, Ringler JJ, Hennings TG, Nair GG, Shveygert M, Guo T, Puri S, Haataja L, Cirulli V, Blelloch R, Szot GL, Arvan P, Hebrok M. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *EMBO J* 2015; **34**: 1759-1772 [PMID: 25908839 DOI: 10.15252/embj.201591058]

109 **Davis JC**, Alves TC, Helman A, Chen JC, Kenty JH, Cardone RL, Liu DR, Kibbey RG, Melton DA. Glucose Response by Stem Cell-Derived β Cells In Vitro Is Inhibited by a Bottleneck in Glycolysis. *Cell Rep* 2020; **31**: 107623 [PMID: 32402282 DOI: 10.1016/j.celrep.2020.107623]

110 **Motté E**, Szepessy E, Suenens K, Stangé G, Bomans M, Jacobs-Tulleneers-Thevissen D, Ling Z, Kroon E, Pipeleers D; Beta Cell Therapy Consortium EU-FP7. Composition and function of macroencapsulated human embryonic stem cell-derived implants: comparison with clinical human islet cell grafts. *Am J Physiol Endocrinol Metab* 2014; **307**: E838-E846 [PMID: 25205822 DOI: 10.1152/ajpendo.00219.2014]

111 **Robert T**, De Mesmaeker I, Stangé GM, Suenens KG, Ling Z, Kroon EJ, Pipeleers DG. Functional Beta Cell Mass from Device-Encapsulated hESC-Derived Pancreatic Endoderm Achieving Metabolic Control. *Stem Cell Reports* 2018; **10**: 739-750 [PMID: 29503087 DOI: 10.1016/j.stemcr.2018.01.040]

112 **Veres A**, Faust AL, Bushnell HL, Engquist EN, Kenty JH, Harb G, Poh YC, Sintov E, Gürtler M, Pagliuca FW, Peterson QP, Melton DA. Charting cellular identity during human *in vitro* β-cell differentiation. *Nature* 2019; **569**: 368-373 [PMID: 31068696 DOI: 10.1038/s41586-019-1168-5]

113 **Vanikar AV**, Trivedi HL, Thakkar UG. Stem cell therapy emerging as the key player in treating type 1 diabetes mellitus. *Cytotherapy* 2016; **18**: 1077-1086 [PMID: 27424148 DOI: 10.1016/j.jcyt.2016.06.006]

114 **Noguchi H**, Bonner-Weir S, Wei FY, Matsushita M, Matsumoto S. BETA2/NeuroD protein can be transduced into cells due to an arginine- and lysine-rich sequence. *Diabetes* 2005; **54**: 2859-2866 [PMID: 16186386 DOI: 10.2337/diabetes.54.10.2859]

115 **Domínguez-Bendala J**, Klein D, Ribeiro M, Ricordi C, Inverardi L, Pastori R, Edlund H. TAT-mediated neurogenin 3 protein transduction stimulates pancreatic endocrine differentiation in vitro. *Diabetes* 2005; **54**: 720-726 [PMID: 15734848 DOI: 10.2337/diabetes.54.3.720]

116 **Kilk K**, Magzoub M, Pooga M, Eriksson LE, Langel U, Gräslund A. Cellular internalization of a cargo complex with a novel peptide derived from the third helix of the islet-1 homeodomain. Comparison with the penetratin peptide. *Bioconjug Chem* 2001; **12**: 911-916 [PMID: 11716681 DOI: 10.1021/bc0100298]

117 **Bosco D**, Armanet M, Morel P, Niclauss N, Sgroi A, Muller YD, Giovannoni L, Parnaud G, Berney T. Unique arrangement of alpha- and beta-cells in human islets of Langerhans. *Diabetes* 2010; **59**: 1202-1210 [PMID: 20185817 DOI: 10.2337/db09-1177]

118 **Cho J**, D'Antuono M, Glicksman M, Wang J, Jonklaas J. A review of clinical trials: mesenchymal stem cell transplant therapy in type 1 and type 2 diabetes mellitus. *Am J Stem Cells* 2018; **7**: 82-93 [PMID: 30510843]

119 **Pokrywczynska M**, Krzyzanowska S, Jundzill A, Adamowicz J, Drewa T. Differentiation of stem cells into insulin-producing cells: current status and challenges. *Arch Immunol Ther Exp (Warsz)* 2013; **61**: 149-158 [PMID: 23283518 DOI: 10.1007/s00005-012-0213-y]

120 **Si Y**, Zhao Y, Hao H, Liu J, Guo Y, Mu Y, Shen J, Cheng Y, Fu X, Han W. Infusion of mesenchymal stem cells ameliorates hyperglycemia in type 2 diabetic rats: identification of a novel role in improving insulin sensitivity. *Diabetes* 2012; **61**: 1616-1625 [PMID: 22618776 DOI: 10.2337/db11-1141]

121 **Ianus A**, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 2003; **111**: 843-850 [PMID: 12639990 DOI: 10.1172/JCI16502]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2021 March 26; 13(3): 208-220

DOI: 10.4252/wjsc.v13.i3.208

ISSN 1948-0210 (online)

MINIREVIEWS

Mesenchymal stromal cell-dependent immunoregulation in chemically-induced acute liver failure

Jia-Hang Zhou, Xuan Lu, Cui-Lin Yan, Xin-Yu Sheng, Hong-Cui Cao

ORCID number: Jia-Hang Zhou 0000-0003-0623-517X; Xuan Lu 0000-0003-2893-6268; Cui-Lin Yan 0000-0001-8415-3490; Xin-Yu Sheng 0000-0002-0984-2592; Hong-Cui Cao 0000-0002-6604-6867.

Author contributions: Zhou JH contributed to the study design, data collection, and manuscript writing; Lu X, Yan CL and Sheng XY contributed to the data collection; Cao HC conducted the conception, design, and manuscript writing; All authors have read and approved the final manuscript.

Supported by National Natural Science Foundation of China, No. 81971756; and Stem Cell and Translational Research from National Key Research and Development Program of China, No. 2016YFA0101001.

Conflict-of-interest statement: Th authors declare having no conflict of interests for this article.

Jia-Hang Zhou, Xuan Lu, Cui-Lin Yan, Xin-Yu Sheng, Hong-Cui Cao, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China

Hong-Cui Cao, Zhejiang Provincial Key Laboratory for Diagnosis and Treatment of Aging and Physic-chemical Injury Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China

Corresponding author: Hong-Cui Cao, MD, PhD, Professor, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, No. 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China. hccao@zju.edu.cn

Abstract

Drug-induced liver injury (DILI), which refers to liver damage caused by a drug or its metabolites, has emerged as an important cause of acute liver failure (ALF) in recent years. Chemically-induced ALF in animal models mimics the pathology of DILI in humans; thus, these models are used to study the mechanism of potentially effective treatment strategies. Mesenchymal stromal cells (MSCs) possess immunomodulatory properties, and they alleviate acute liver injury and decrease the mortality of animals with chemically-induced ALF. Here, we summarize some of the existing research on the interaction between MSCs and immune cells, and discuss the possible mechanisms underlying the immunomodulatory activity of MSCs in chemically-induced ALF. We conclude that MSCs can impact the phenotype and function of macrophages, as well as the differentiation and maturation of dendritic cells, and inhibit the proliferation and activation of T lymphocytes or B lymphocytes. MSCs also have immunomodulatory effects on the production of cytokines, such as prostaglandin E2 and tumor necrosis factor-alpha-stimulated gene 6, in animal models. Thus, MSCs have significant benefits in the treatment of chemically-induced ALF by interacting with immune cells and they may be applied to DILI in humans in the near future.

Key Words: Mesenchymal stromal cell; Immune response; Drug-induced liver injury; Acute liver failure; Dendritic cell

Manuscript source: Unsolicited manuscript

Specialty type: Cell and tissue engineering

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: October 6, 2020
Peer-review started: October 6, 2020
First decision: December 24, 2020
Revised: January 8, 2021
Accepted: February 15, 2021
Article in press: February 15, 2021
Published online: March 26, 2021

P-Reviewer: Cao T, Philips CA
S-Editor: Gao CC
L-Editor: Filipodia
P-Editor: Xing YX

Core Tip: Drug-induced liver injury (DILI) is a crucial cause of acute liver failure (ALF). Although mesenchymal stromal cells (MSCs) have not been applied to DILI in clinical trials, their efficacy has been proven in various animal models of chemically-induced ALF. Immune system disorders play key roles in chemically-induced ALF, and MSCs are able to regulate the immune system through soluble factors and cell-to-cell contact, and eventually improve liver damage. We, herein, discuss the immunomodulatory properties of MSCs in different animal models that mimic the pathology of DILI in humans.

Citation: Zhou JH, Lu X, Yan CL, Sheng XY, Cao HC. Mesenchymal stromal cell-dependent immunoregulation in chemically-induced acute liver failure. *World J Stem Cells* 2021; 13(3): 208-220
URL: https://www.wjgnet.com/1948-0210/full/v13/i3/208.htm
DOI: https://dx.doi.org/10.4252/wjsc.v13.i3.208

INTRODUCTION

Drug-induced liver injury (DILI), the most common cause of acute liver failure (ALF) in developed countries, accounts for approximately 50% of ALF cases[1]. In patients with hypersensitivity or reduced tolerance due to special constitutions, the immune-privileged state of the liver can be disrupted by drugs and chemicals or their metabolites, such as reactive intermediate species[2], resulting in unbalanced immune cell infiltration and liver injury[3].

Mesenchymal stromal cells (MSCs) are widely studied adult pluripotent stem cells. They possess not only all of the common characteristics of stem cells but also immunomodulatory properties. They have been extensively researched due to their wide range of sources and easy availability. Since the first MSC transplantation in a pediatric patient experiencing grade IV treatment-refractory acute graft *vs* host disease (GVHD) in 2004[4], there have been an increasing number of studies demonstrating that MSC transplantation can effectively modulate the immune system in several immune-related disorders. In addition to the ability of MSCs to migrate to damaged liver sites and undergo proliferation and differentiation into hepatocytes, the therapeutic mechanism of MSCs in ALF mainly depends on their potential immunomodulatory nature[5].

The main immune cells consist of neutrophils, T cells, B cells, natural killer (NK) cells, monocytes/macrophages, and dendritic cells (DCs). MSCs alter macrophages from a regularly activated (M1) phenotype to an either/or activated (M2) phenotype, resulting in reduced secretion of the proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interferon-γ (IFN-γ) and interleukin (IL)-1, and increased secretion of the anti-inflammatory cytokine IL-10, which to a great extent is dependent on cell-to-cell contact or soluble factors, such as prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase (IDO), and TNF-α-stimulated gene 6 (TSG-6)[6]. MSCs impact two stages of DCs: differentiation and maturation. When co-cultured with MSCs, DC precursors and immature DCs express lower levels of major histocompatibility complex class II (MHCII) and costimulatory molecules cluster of differentiation (CD) 86, CD80, and CD40, which result in a weakened ability to stimulate T cell proliferation. However, the immunosuppressive capacity of MSCs in mature DCs remains controversial[7].

Several studies have shown the inhibitory effects of MSCs on T cell activation, proliferation, and differentiation to T helper 17 (Th17) cells through PGE2, programmed cell death protein 1 (referred to as PD-1), and IL-10[8]. Additionally, MSCs can stimulate the generation and proliferation of immunosuppressive regulatory T cells (Tregs)[9]. Similarly, MSCs suppress the proliferation, activation, and antibody production ability of B cells and induce the B regulatory cells (Bregs)[10].

MSCs have been studied as a prospective therapy for the treatment of DILI and ALF due to their immunomodulatory ability. Several animal models of chemically-induced ALF have been used to study the mechanisms of DILI and the mechanisms of potentially novel therapies[3]. MSCs can alleviate ALF by interacting with different

immune cells because the main pathogenic immune cells differ in these animal models, and these discoveries in animal models will contribute to the clinical application of MSC-based strategies for the treatment of human DILI.

In this review, we summarize a number of existing studies on the interplay of MSCs and the immune system, and discuss some possible mechanisms underlying the immunomodulatory ability of MSCs in chemically-induced ALF. MSC-based therapy may be applied to DILI in humans in the near future.

IMMUNE SYSTEM RESPONSE TO CHEMICALLY-INDUCED ALF

The liver is an organ that is dominated by metabolic functions. It is inevitably exposed to the metabolites of various foods or drugs in the blood from the portal vein, which requires this organ to have high immune tolerance and self-repair abilities[2]. Chemically-induced liver injuries refer to liver damage caused by chemical hepatotoxic substances, including alcohol, drugs, traditional Chinese medicines, chemical poisons from food, and organic and inorganic poisons in industrial production. On the one hand, the immune system of the liver has to tolerate the heavy antigenic load of daily food residues from the portal vein in a healthy state; on the other hand, it must respond efficiently to numerous viruses, bacteria, parasites, and chemical hepatotoxic substances[11]. Excessive inflammation often contributes to morbidity and mortality in chemically-induced ALF (Figure 1).

Innate immune cells in response to chemically-induced ALF

In DILI, necrotic hepatocytes show many damage-associated molecular patterns (referred to as DAMPs), such as high-mobility group box-1 protein, DNA fragments, and heat shock proteins[12], and these factors can be identified by Toll-like receptors (commonly known as TLRs) on innate immune cells. Then, proinflammatory factors recruit inflammatory immune cells into the liver, activating them to remove necrotic cell debris[13].

Liver macrophages mainly include two cell types: resident Kupffer cells and infiltrating monocyte-derived macrophages. Although of different cellular origins, both types of macrophages can phagocytose microorganisms and metabolic waste in liver sinusoids. The numbers of liver macrophages become greatly increased in any type of liver injury, due to the self-renewal ability of Kupffer cells and the infiltration of monocyte-derived macrophages[14]. In the early stages of liver injury, Kupffer cells recognize DAMPs derived from damaged hepatocytes and then secrete several proinflammatory cytokines and chemokines to attract neutrophils, NK cells, and bone-marrow-derived monocytes to the regions of inflammation[15]. Ly6C$^+$ monocytes and Ly6C$^-$ monocytes exist in the blood of mice, and can differentiate into hepatic macrophages. Infiltrating Ly6C$^+$ monocytes promote inflammation and induce organ impairment, but eventually maturate with the downregulation of Ly6C expression, at which point they acquire the ability to restore liver integrity[16].

Neutrophils are the first-line immune cells that have the fastest response when inflammation occurs. However, uncontrolled neutrophil infiltration and activation lead to excessive inflammation in chemically-induced liver injuries. The expression levels of C-X-C motif chemokine ligand (referred to as CXCL) 1, IL-6, TNF-α, and monocyte chemoattractant protein-1 in the injured liver are significantly increased to regulate the infiltration and activation of neutrophils[17]. Tissue-resident phagocytes, including macrophages and DCs, release a variety of proinflammatory mediators and establish a chemoattractant gradient, triggering neutrophil recruitment into tissues. Neutrophils express receptors (G protein-coupled receptor, Fc-receptors, adhesion molecules, TLRs, C-type lectins) that can recognize these signals and then release granules (myeloperoxidase), generate reactive oxygen species, and form neutrophil extracellular traps[18,19].

DCs in ALF engage in the innate immune response involving macrophages and neutrophils, with antigen recognition by pattern-recognition receptors. However, the most important effect of DCs is initiation of the adaptive immune response. DCs reside in organs such as the liver as immature cells, which are very effective at antigen recognition, capture and processing, and then circulate in the blood or lymph fluid to peripheral immune organs where they can achieve terminal maturation with the ability of efficient antigen presentation as well as activation of T cells[20].

In the healthy state, hepatocytes express MHC-I, which binds to inhibitory receptors on NK cells, preventing NK cell activation[21]. In contrast, infected hepatocytes lacking MHC-I can be recognized and eliminated by NK cells[22].

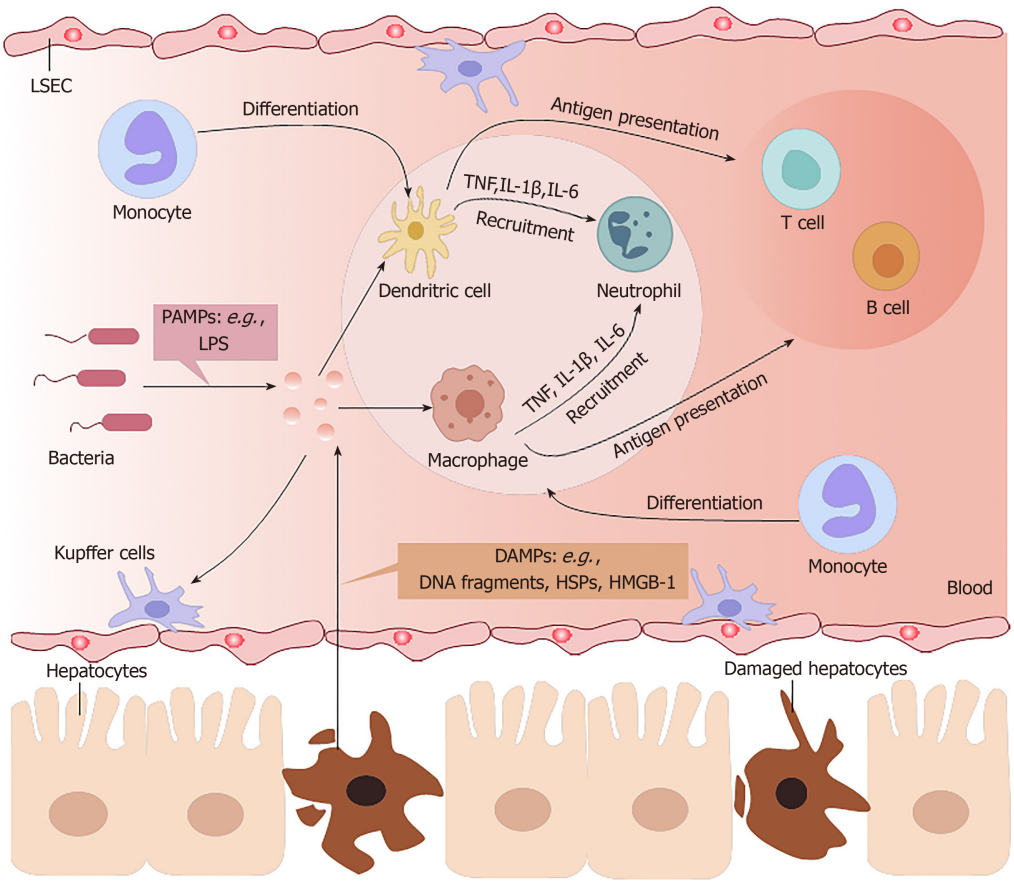


Figure 1 Regulation of immune cells in response to chemically-induced acute liver failure. Necrotic hepatocytes release many damage-associated molecular patterns (DAMPs), such as high-motility group box-1 (HMGB-1), DNA fragments, and heat shock proteins (HSPs), and pathogen-associated molecular patterns (PAMPs) from the blood can be identified by Toll-like receptors on innate immune cells. Proinflammatory factors recruit inflammatory immune cells, such as monocytes, into the liver. On the one hand, macrophages and dendritic cells are activated and produce tumor necrosis factor (TNF), interleukin (IL)-1β, and IL-6 to recruit more neutrophils to remove necrotic cell debris. On the other hand, the two types of antigen presenting cells can present antigens to T cells and B cells to activate acquired immunity. LPS: Lipopolysaccharide; LSECs: Liver sinusoidal endothelial cells.

Adaptive immune cells in response to chemically-induced ALF

Contrary to innate immune responses, which induce acute liver injury (ALI) in experimental animal models[13], adaptive immune responses play an undefined secondary role in DILI[23]. In homeostasis, liver sinusoidal endothelial cells and Kupffer cells constitutively express IL-10, prostaglandins, TNF-α, and transforming growth factor-beta (TGF-β) to expand Tregs, attenuate T cell activation, and induce liver immune tolerance[12]. In some ALI models induced by some special types of drugs or chemicals, T cells are important. Yu *et al*[24] found that IL-1β is upregulated in the AAGL (Agrocybe aegerita galectin) model and is crucial to recruit T cells from peripheral blood into the injured liver; treatment with IL-1β antibody can significantly alleviate hepatocyte damage. The possible mechanism may be inhibition of p38 or nuclear factor-kappa B (NF-κB) signaling pathways and subsequently reduced infiltration of T cells into the liver. Heymann *et al*[25] applied a concanavalin A (Con A)-induced liver injury model to mimic immune reactions observed in humans, trigger an inflammatory cascade by activating resident Kupffer cells, initiate neutrophil infiltration, and increase $CD4^+$ T cell infiltration and activation[26]. Although Tiegs *et al*[27] showed that $CD4^+$ T cells play a more critical role than $CD8^+$ T cells in Con A-induced liver injury in wild-type mice, $CD8^+$ T cells played an important role in T cell-transferred Rag2-knockout mouse (in which T cells cannot mature) challenged with Con A. Both IL-33 released by the injured liver and perforin secreted by $CD8^+$ T cells were crucial components in their study.

INTERACTION BETWEEN MSCS AND IMMUNE CELLS

Immune characteristics of MSCs

Quiescent MSCs display immune homeostatic features biased towards suppression. When MSCs are induced by various proinflammatory cytokines, these immunosuppressive properties can be considerably enhanced, resulting in polarization to immunosuppressive phenotypes of MSCs. IDO and inducible nitric oxide synthase (referred to as iNOS) are the key to the immune regulatory functions of MSCs, with a series of potential complementary suppressor pathways, including heme oxygenase-1, soluble human leukocyte antigen-G5, TGF-β, PGE2, galectin, and TSG-6[28]. MSCs possess a pro-inflammatory or anti-inflammatory phenotype by contacting immune cell responses in different situations, and regulate the immune response by secreting soluble factors or direct cell contact[6].

Potential mechanisms underlying the immunoregulatory properties of MSCs through innate immune cells

MSCs can regulate the proliferation and activation of Kupffer cells, macrophages, DCs, neutrophils, and NK cells (Figure 2). MSCs reportedly transfer to injury sites in response to large amounts of inflammatory factors, such as IL-6 and TNF-α secreted by activated Kupffer cells[29]. MSCs, in turn, inhibit the phenotype transition of activated Kupffer cells to M1 and stimulation of the NF-κB pathway in lipopolysaccharide (commonly known as LPS)-treated Kupffer cells[30]. Several studies have revealed the mechanism by which MSCs cause immunosuppression *via* the interaction of macrophages. MSCs can interact directly and physically with innate immune cells. Upregulated CD54 on human MSCs (referred to here as hMSCs) co-cultured with M1 macrophages in an *in vitro* co-culture system increased IDO activity and inhibited the proliferation of T cells[31]. Similarly, upregulated CD200 on mouse bone marrow stromal cells (BMSCs) in contact with M1 macrophages can also enhance the immunotherapeutic effects of MSCs to reprogram proinflammatory macrophages[32]. On the other hand, soluble factors secreted by MSCs can contribute to the immunoregulatory properties of MSCs. Corneal-derived MSCs can secrete pigment epithelium-derived factor and then modulate the immunophenotype and angiogenic function of macrophages[33]. TSG-6 and PGE2 secreted by MSCs have also been widely studied, due to their immunoregulatory effects on MSCs and macrophages[32,34].

In addition, MSC-derived exosomes, which are rich in proteins, mRNAs, and microRNAs (designated as miRs), have been used as a therapy for liver diseases in recent years. In a study of experimental autoimmune hepatitis, BMSC-derived exosomes, which are rich in miR-223, effectively alleviated liver injury by downregulating formation of the NLR family pyrin domain containing 3 (referred to as NLRP3) inflammasome[35]. In another study, miR-17 derived from adipose tissue-derived MSC (AMSC)-derived exosomes was shown to ameliorate LPS/D-galactosamine-induced ALI by inhibiting activation of the TXNIP/NLRP3 inflammasome of macrophages[36]. MSCs can limit neutrophil recruitment or infiltration and inhibit neutrophil activation to prevent an excessive inflammatory response. MSCs ameliorate the hepatic inflammatory response by reducing the release of neutrophil chemoattractant CXCL2 and attenuating neutrophil chemotaxis *via* downregulation of C-X-C motif chemokine receptor 2 expression in neutrophils[37]. In a septic mouse model, MSCs optimally balanced the distribution of circulating and tissue-infiltrated neutrophils, maximizing bacterial killing and minimizing liver injury[34].

Many studies have focused on the effects of MSCs on DCs *in vitro*, especially through soluble factors. One showed that TSG-6 secreted by mouse BMSCs suppressed the maturation and activation of DCs by inactivating mitogen-activated protein kinase and NF-κB signaling pathways[38]. In addition, IL-6 reportedly participates in the immune regulation mechanism mediated by the murine MSC line *via* inhibition of DCs[39]. Regarding hMSCs, Spaggiari *et al*[40] demonstrated that human BMSCs can secrete PGE2 to inhibit differentiation of monocyte-derived DCs. Selleri *et al*[41] showed that human umbilical-cord-derived MSCs can secrete lactate to induce granulocyte-macrophage colony-stimulating factor/IL-4-treated monocytes to differentiate into M2 macrophages rather than DCs by metabolic reprogramming. A study by Liu *et al*[42] showed that MSCs derived from mouse embryonic fibroblasts can induce a type of novel regulatory DCs that express low levels of CD11c and Ia and are phenotypically different from immature and mature DCs *via* IL-10.

In addition, direct cell contacts are as important as soluble molecules in MSC/DC interactions. hMSCs can inhibit the proliferation ability and differentiation capability

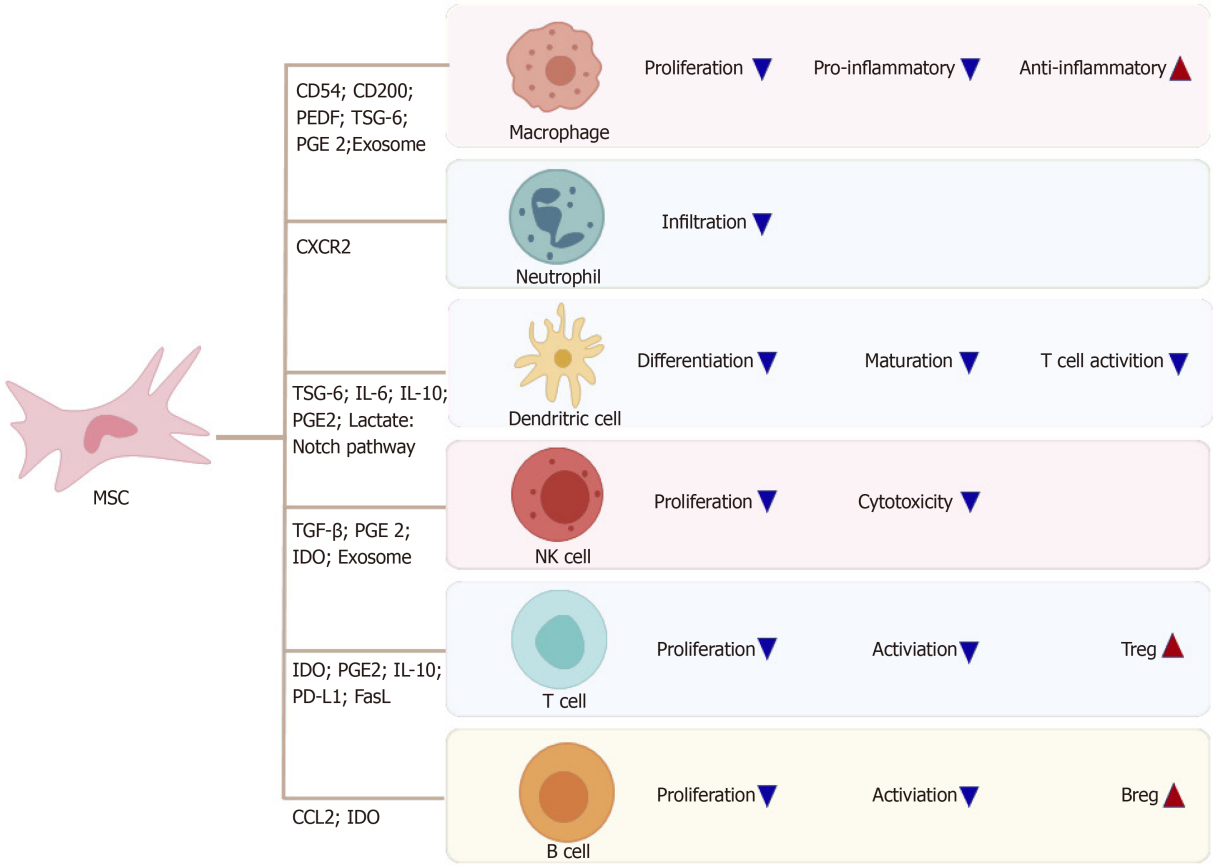


Figure 2 Mesenchymal stromal cells regulate innate and adaptive immune cells. Mesenchymal stromal cells (MSCs) regulate innate and adaptive immune cells through soluble factors and direct cell-to-cell contact. Breg: Regulatory B cell; CXCL2: C-X-C motif chemokine ligand 2; CXCR2: C-X-C motif chemokine receptor 2; IDO: Indoleamine 2,3-dioxygenase; IL: Interleukin; PEDF: Pigment epithelium-derived factor; PGE2: Prostaglandin E2; Treg: Regulatory T cell; TSG-6: Tumor necrosis factor-alpha-stimulated gene-6.

of $CD34^+$ hemopoietic progenitor cells into interstitial DCs but cannot inhibit maturation of $CD34^+$ hemopoietic progenitor cell-derived DCs, and the inhibitory effect is associated with the Notch pathway[43]. Cahill *et al*[44] demonstrated that mouse MSC induction of functional tolerogenic DCs that can induce Tregs *in vitro* requires Notch signaling. This hypothesis was confirmed in an animal model treated with Jagged-1 knockdown MSCs. In another study, mature DCs cocultured with MSCs expressing Jagged-2 acquired tolerogenic properties[45]. MSCs can influence the proliferation capacity, cytokine release, phenotypic conversion, and cytotoxicity of IL-2-induced NK cells[46]. The mechanism may include TGF-β[47], PGE2[48], IDO[49] and exosomes[50]. Moreover, NK cells can stimulate MSC recruitment by secreting neutrophil-activating peptide 2[51], and activated NK cells can efficiently lyse MSCs[46].

Potential mechanisms of the immunoregulatory properties of MSCs through adaptive immune cells

MSCs can inhibit proliferation, activation, and differentiation of T cells, induce apoptosis of T cells and induce recruitment of Tregs. MSCs can also induce cell cycle arrest by downregulating cyclin D2 and upregulating p27kip1 in T cells, resulting in division anergy of activated T cells[52]. Of note, MSCs can inhibit T cell function by inducing apoptosis of activated T cells. Plumas *et al*[53] showed that this apoptosis can be associated with transformation of tryptophan to kynurenine by IDO expressed by MSCs in the presence of IFN-γ. Akiyama *et al*[54] revealed that BMSCs may trigger apoptosis of transient T cells through the Fas ligand (FasL)-dependent pathway, and that apoptotic T cells can induce production of TGF-β in macrophages, thereby upregulating Tregs. In some experiments *in vitro*, MSCs were shown to inhibit allogeneic T cell responses in mixed lymphocyte reactions by IDO after MSCs were activated by IFN-γ[55]. Recently, MSCs were shown to inhibit activation of $CD4^+$ T cells and reduce secretion of IL-2 *via* PD-1 ligands[56].

Several investigators have highlighted that MSCs can effectively inhibit Th17 differentiation. Duffy *et al*[57] demonstrated that this inhibition requires cyclooxygenase-2 induction, which is dependent on cell contact, leading to direct Th17 inhibition by PGE2. Qu *et al*[58] also showed that MSCs inhibit Th17 cell differentiation, and suggested that increased secretion of IL-10 may play a role. One important aspect of the immunomodulatory effect of MSCs is the recruitment and influence of Tregs[59]. MSCs can reinforce the regulatory function of $CD8^+CD28^-$ Treg cells by increasing expression of IL-10 and FasL[60]. They can also induce cell cycle arrest in the G0/G1 phase instead of induction of B-cell apoptosis in a soluble factor-dependent manner[61]. Furthermore, MSCs inhibit proliferation and activation of B cells by modifying the phosphorylation levels of the extracellular signal-related kinase 1/2 or p38 pathways[62]. Rafei *et al*[63] clarified that MSC-derived chemokine (C-C motif) ligand 2 (referred to as CCL2) can suppress secretion of immunoglobulin (referred to as Ig) in plasma cells and induce proliferation of plasmablasts, leading to IL-10-mediated blockade *via* inactivation of signal transducer and activator of transcription 3 (referred to as STAT3) and induction of paired box 5 *in vitro*. MSCs can enhance the survival and proliferation rates of $CD5^+$ Bregs in an IDO-dependent manner, increasing IL-10 expression and ameliorating refractory, chronic GVHD[10]. Similarly, AMSCs reduce plasmablast formation or induce IL-10-producing $CD19^+CD24^{high}CD38^{high}$ B cells[64]. Park *et al*[65] elucidated the effect of human AMSCs on the proliferation of Bregs in an animal model of systemic lupus erythematosus.

MSC TRANSPLANTATION TO TREAT CHEMICALLY-INDUCED ALI

Viral infection remains the main cause of ALF in developing countries, whereas DILI is more common in developed countries[1]. DILI accounts for 50% of ALF cases in the United States[66] and Europe, and the main drug responsible is acetaminophen[67]. Several animal models induced by chemical substances have been used to study the mechanisms of ALF[3]. Chemical substances, such as Con A, α-galactosylceramide (commonly known as α-GalCer) and carbon tetrachloride (CCl_4), have been used in ALF animal models in which MSCs have been demonstrated to alleviate the symptoms of liver injury effectively; although, the mechanisms are complex and not fully understood (Table 1).

Volarevic *et al*[68-70] conducted several studies on the immunoregulation of MSCs in ALF induced by Con A. Their previous studies showed that Con A-induced ALF is an excellent murine model of immune-mediated liver injury. $CD8^+$ T cells, $CD4^+$ T cells, NK T (NKT) cells, NK cells, and macrophages are reportedly related to this model and can be transferred to injured liver sites and secrete many cytokines. Meanwhile, the authors confirmed the efficacy of AMSCs for ALF induced by Con A[71]. Gazdic *et al*[72] researched the MSC-NKT cell interaction in Con A- and α-GalCer-induced murine models of ALF, and elucidated that MSCs protect hepatocytes from the cytotoxicity of liver NKT cells by attenuating their ability to produce inflammatory factors, such as TNF-α, IFN-γ and IL-4, in an iNOS- and IDO-dependent manner. In a recent study of α-GalCer-induced ALF, Ito *et al*[73] demonstrated that MSCs can increase IL-10 in Tregs, which in turn, attenuates the hepatotoxicity of liver NKT cells[9].

Milosavljevic *et al*[74] revealed another mechanism of MSC-NKT cell interaction, specifically that MSCs can attenuate CCl_4-induced ALF by downregulating IL-17 in liver NKT cells. Their findings highlighted the reduction of liver NKT cell cytotoxicity and the critical importance of increased regulatory cells (Tregs and NK Tregs) in MSC-mediated attenuation of ALF, indicating the importance of MSC-induced regulatory cells as a prospective cell-based ALF therapy. Liu *et al*[75] demonstrated through a high-dimensional analysis that MSCs significantly ameliorated CCl_4-induced ALF and regulated the immune system of the liver. In this model, MSCs regulated different immune cells in two phases. During the injury stage, MSCs reduced the numbers of $Ly6C^{low}CD8^+$ resident memory T cells (referred to as TRM) cells, conventional NK cells, and IgM^+IgD^+ B cells but increased the quantity of immunosuppressive monocyte-derived macrophages. During the recovery stage, MSCs enhanced the retention of $Ly6C^{low}CD8^+$ TRM cells and maintained the immunosuppressive ability of monocyte-derived macrophages. To reveal alterations in immune cell subsets of CCl_4-induced ALF after MSC transplantation, the authors detected the metabolomic profile of the immune system. Using high-performance chemical isotope labeling liquid chromatography-mass spectrometry, they confirmed 256 metabolites as candidate biomarkers of the immune response in CCl_4-induced ALF animal models, and 114 metabolites as candidate biomarkers of the immune response after MSC

Table 1 Immunoregulatory mechanisms of mesenchymal stromal cells in alleviating chemically-induced acute liver failure

Species of MSCs	Source	Dose	Model	Reagents for model	Immune cell	Mechanism	Effect	Ref.
Mouse	Bone marrow	5×10^5	Mouse	α-GalCer	Treg	Increase the population of Tregs and their capacity to produce IL-10; attenuate hepatotoxicity of NKT cells in an IDO-dependent manner	Attenuate ALF	Gazdic *et al*[9]
Mouse	Bone marrow	1×10^6	Mouse	TAA	Macrophage/T cell	Inhibit macrophage infiltration; reduce Th1 and Th17 cells and increase Tregs	Ameliorate FHF and reduces mortality	Huang *et al*[78]
Rat	Bone marrow	1×10^7	Rat	D-GalN/LPS	Neutrophil	Reduce the number and activity of neutrophils in both peripheral blood and liver	Improve the liver function	Zhao *et al*[77]
Mouse	Bone marrow	5×10^5	Mouse	Con A/α-GalCer	NKT cell	Attenuate the cytotoxicity and capacity of liver NKT in an iNOS- and IDO-dependent manner	Attenuate ALF	Gazdic *et al*[72]
Mouse	Bone marrow	5×10^5	Mouse	CCl_4/a-GalCer	NKT cell	Reduce IL-17-producing NKT cells and increase the presence of IL-10-producing NKT regulatory cells in an IDO-dependent manner	Attenuate ALF	Milosavljevic *et al*[74]
Mouse	Adipose tissue	AMSC-Exo, 400 μg	Mouse	LPS/D-GalN	Macrophage	Reduce NLRP3 inflammasome activation in macrophages by targeting TXNIP	Attenuate ALF	Liu *et al*[36]

α-GalCer: α-Galactosylceramide; ALF: Acute liver failure; AMSC-Exo: Adipose-derived mesenchymal stem cell-derived exosomes; CCl_4: Carbon tetrachloride; Con A: Concanavalin A; D-GalN: D-galactosamine; FHF: Fulminant hepatic failure; IDO: Indoleamine 2,3-dioxygenase; IL: Interleukin; iNOS: Inducible nitric oxide synthase; LPS: Lipopolysaccharide; NKT: Natural killer T; NLRP3: NOD2 activates (NOD)-like receptor pyrin domain-containing 3; TAA: Thioacetamide; Th1: T helper 1; Th17: T helper 17; Treg: Regulatory T cell; TXNIP: Thioredoxin-interacting protein.

transplantation. However, the potential immunomodulatory role of metabolites needs further investigation[76]. MSCs have exhibited positive effects in a rat model of D-GalN/LPS-induced ALF by inhibiting the recruitment and activity of neutrophils. Compared with monotherapy, combination of MSCs and anti-neutrophil serum can inhibit cell apoptosis more efficiently, ameliorate liver function, and reduce the mortality rate[77]. In a mouse model of thioacetamide-induced ALF, both MSCs and MSC-conditioned medium treatment reduced the incidence of death. MSC-treated livers showed less hepatocellular apoptosis and more liver regeneration, as well as downregulation of macrophage infiltration and alteration of $CD4^+$ T cells to an anti-inflammatory phenotype[78].

CONCLUSION

Increasing evidence has shown that MSCs have immunosuppressive capacities to regulate the function of immune cells in ALI as well as promote internal environmental homeostasis in chemically-induced ALF. MSCs can interact with both innate and adaptive immune systems *via* cell-to-cell interactions and the paracrine pathway, coordinating an integrated response to liver injury and preventing hepatocyte necrosis. However, there are still some deficiencies in the research of MSC-dependent immunoregulation in chemically-induced ALF. For example, the pathogenesis of liver injury models and the role of the immune system are still unclear. There has not been enough extensive and in-depth research on MSC-dependent immunoregulation in chemically-induced ALF.

Different sources and different pretreated MSCs have varying therapeutic effects on liver injury. To date, there is no uniform standard for MSC applications in animal models[79]. Thus, the results from different studies cannot be compared or repeated in different laboratories under different conditions[80].

MSCs have been widely studied for their differentiation and immunomodulation abilities. However, in one study, researchers focused on a single capability of MSCs, ignoring comparisons of their various capabilities. Future studies are needed to determine which MSC capability dominates.

There have been no clinical trials on DILI treated by MSCs. Clinical trials on MSC treatment are often applied to chronic diseases such as GVHD, diabetes, and malignant blood disease[81]. MSCs are rarely used in DILI, which has a rapid onset and high mortality rate, and more conventional and conservative treatments tend to be used. Clinical trials can be conducted only if the efficacy and safety of MSCs are supported by sufficient research. The two main obstacles to translating the results from animal experiments into clinical practice are that the pathogenicity of ALI caused by clinical drugs differs from that of animal models[6], and that the immune system of animals, such as mice, is different from that of humans, so the results demonstrated in mice are not necessarily applicable to humans. Possible solutions to these issues are to verify the results obtained in animal experiments in organoids derived from human liver, and to identify animal models with similar pathogenicity to DILI in humans. Further studies are needed to reveal the therapeutic mechanisms of MSCs.

In conclusion, MSC transplantation can efficiently reduce the high mortality rate of chemically-induced ALF and may become a prospective therapy in clinical practice. More prospective randomized studies are needed to ensure the therapeutic effects of MSCs.

REFERENCES

1 **Bernal W**, Wendon J. Acute liver failure. *N Engl J Med* 2013; **369**: 2525-2534 [PMID: 24369077 DOI: 10.1056/NEJMra1208937]

2 **Gu X**, Manautou JE. Molecular mechanisms underlying chemical liver injury. *Expert Rev Mol Med* 2012; **14**: e4 [PMID: 22306029 DOI: 10.1017/S1462399411002110]

3 **Gazdic M**, Arsenijevic A, Markovic BS, Volarevic A, Dimova I, Djonov V, Arsenijevic N, Stojkovic M, Volarevic V. Mesenchymal Stem Cell-Dependent Modulation of Liver Diseases. *Int J Biol Sci* 2017; **13**: 1109-1117 [PMID: 29104502 DOI: 10.7150/ijbs.20240]

4 **Le Blanc K**, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004; **363**: 1439-1441 [PMID: 15121408 DOI: 10.1016/S0140-6736(04)16104-7]

5 **Hu C**, Li L. Improvement of mesenchymal stromal cells and their derivatives for treating acute liver failure. *J Mol Med (Berl)* 2019; **97**: 1065-1084 [PMID: 31197403 DOI: 10.1007/s00109-019-01804-x]

6 **Bernardo ME**, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* 2013; **13**: 392-402 [PMID: 24094322 DOI: 10.1016/j.stem.2013.09.006]

7 **Đokić JM**, Tomić SZ, Čolić MJ. Cross-Talk Between Mesenchymal Stem/Stromal Cells and Dendritic Cells. *Curr Stem Cell Res Ther* 2016; **11**: 51-65 [PMID: 26337378 DOI: 10.2174/1574888x10666150904114035]

8 **Haddad R**, Saldanha-Araujo F. Mechanisms of T-cell immunosuppression by mesenchymal stromal cells: what do we know so far? *Biomed Res Int* 2014; **2014**: 216806 [PMID: 25025040 DOI: 10.1155/2014/216806]

9 **Gazdic M**, Markovic BS, Arsenijevic A, Jovicic N, Acovic A, Harrell CR, Fellabaum C, Djonov V, Arsenijevic N, Lukic ML, Volarevic V. Crosstalk between mesenchymal stem cells and T regulatory cells is crucially important for the attenuation of acute liver injury. *Liver Transpl* 2018; **24**: 687-702 [PMID: 29500914 DOI: 10.1002/lt.25049]

10 **Peng Y**, Chen X, Liu Q, Zhang X, Huang K, Liu L, Li H, Zhou M, Huang F, Fan Z, Sun J, Ke M, Li X, Zhang Q, Xiang AP. Mesenchymal stromal cells infusions improve refractory chronic graft *vs* host disease through an increase of CD5+ regulatory B cells producing interleukin 10. *Leukemia* 2015; **29**: 636-646 [PMID: 25034146 DOI: 10.1038/leu.2014.225]

11 **Doherty DG**. Immunity, tolerance and autoimmunity in the liver: A comprehensive review. *J Autoimmun* 2016; **66**: 60-75 [PMID: 26358406 DOI: 10.1016/j.jaut.2015.08.020]

12 **Iorga A**, Dara L. Cell death in drug-induced liver injury. *Adv Pharmacol* 2019; **85**: 31-74 [PMID: 31307591 DOI: 10.1016/bs.apha.2019.01.006]

13 **Jaeschke H**, Williams CD, Ramachandran A, Bajt ML. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. *Liver Int* 2012; **32**: 8-20 [PMID: 21745276 DOI: 10.1111/j.1478-3231.2011.02501.x]

14 **Krenkel O**, Tacke F. Liver macrophages in tissue homeostasis and disease. *Nat Rev Immunol* 2017; **17**: 306-321 [PMID: 28317925 DOI: 10.1038/nri.2017.11]

15 **Luedde T**, Schwabe RF. NF-κB in the liver--linking injury, fibrosis and hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 108-118 [PMID: 21293511 DOI: 10.1038/nrgastro.2010.213]

16 **Tacke F**, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. *J Hepatol* 2014; **60**: 1090-1096 [PMID: 24412603 DOI: 10.1016/j.jhep.2013.12.025]

17 **Wang XZ**, Zhang SY, Xu Y, Zhang LY, Jiang ZZ. The role of neutrophils in triptolide-induced liver injury. *Chin J Nat Med* 2018; **16**: 653-664 [PMID: 30269842 DOI: 10.1016/S1875-5364(18)30105-5]

18 **Futosi K**, Fodor S, Mócsai A. Neutrophil cell surface receptors and their intracellular signal transduction pathways. *Int Immunopharmacol* 2013; **17**: 638-650 [PMID: 23994464 DOI: 10.1016/j.intimp.2013.06.034]

19 **Alvarenga DM**, Mattos MS, Araújo AM, Antunes MM, Menezes GB. Neutrophil biology within hepatic environment. *Cell Tissue Res* 2018; **371**: 589-598 [PMID: 29127519 DOI: 10.1007/s00441-017-2722-9]

20 **Szabo G**, Mandrekar P, Dolganiuc A. Innate immune response and hepatic inflammation. *Semin Liver Dis* 2007; **27**: 339-350 [PMID: 17979071 DOI: 10.1055/s-2007-991511]

21 **Morvan MG**, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. *Nat Rev Cancer* 2016; **16**: 7-19 [PMID: 26694935 DOI: 10.1038/nrc.2015.5]

22 **Liu P**, Chen L, Zhang H. Natural Killer Cells in Liver Disease and Hepatocellular Carcinoma and the NK Cell-Based Immunotherapy. *J Immunol Res* 2018; **2018**: 1206737 [PMID: 30255103 DOI: 10.1155/2018/1206737]

23 **Kim SH**, Naisbitt DJ. Update on Advances in Research on Idiosyncratic Drug-Induced Liver Injury. *Allergy Asthma Immunol Res* 2016; **8**: 3-11 [PMID: 26540496 DOI: 10.4168/aair.2016.8.1.3]

24 **Yu W**, Lan X, Cai J, Wang X, Liu X, Ye X, Yang Q, Su Y, Xu B, Chen T, Li L, Sun H. Critical role of IL-1β in the pathogenesis of Agrocybe aegerita galectin-induced liver injury through recruiting T cell to liver. *Biochem Biophys Res Commun* 2020; **521**: 449-456 [PMID: 31676068 DOI: 10.1016/j.bbrc.2019.10.087]

25 **Heymann F**, Hamesch K, Weiskirchen R, Tacke F. The concanavalin A model of acute hepatitis in mice. *Lab Anim* 2015; **49**: 12-20 [PMID: 25835734 DOI: 10.1177/0023677215572841]

26 **Elshal M**, Abu-Elsaad N, El-Karef A, Ibrahim T. Retinoic acid modulates IL-4, IL-10 and MCP-1 pathways in immune mediated hepatitis and interrupts CD4+ T cells infiltration. *Int Immunopharmacol* 2019; **75**: 105808 [PMID: 31419710 DOI: 10.1016/j.intimp.2019.105808]

27 **Tiegs G**, Hentschel J, Wendel A. A T cell-dependent experimental liver injury in mice inducible by concanavalin A. *J Clin Invest* 1992; **90**: 196-203 [PMID: 1634608 DOI: 10.1172/JCI115836]

28 **Krampera M**, Galipeau J, Shi Y, Tarte K, Sensebe L; MSC Committee of the International Society for Cellular Therapy (ISCT). Immunological characterization of multipotent mesenchymal stromal cells--The International Society for Cellular Therapy (ISCT) working proposal. *Cytotherapy* 2013; **15**: 1054-1061 [PMID: 23602578 DOI: 10.1016/j.jcyt.2013.02.010]

29 **Yang X**, Liang L, Zong C, Lai F, Zhu P, Liu Y, Jiang J, Yang Y, Gao L, Ye F, Zhao Q, Li R, Han Z, Wei L. Kupffer cells-dependent inflammation in the injured liver increases recruitment of mesenchymal stem cells in aging mice. *Oncotarget* 2016; **7**: 1084-1095 [PMID: 26716516 DOI: 10.18632/oncotarget.6744]

30 **Liang X**, Li T, Zhou Q, Pi S, Li Y, Chen X, Weng Z, Li H, Zhao Y, Wang H, Chen Y. Mesenchymal stem cells attenuate sepsis-induced liver injury *via* inhibiting M1 polarization of Kupffer cells. *Mol Cell Biochem* 2019; **452**: 187-197 [PMID: 30178273 DOI: 10.1007/s11010-018-3424-7]

31 **Espagnolle N**, Balguerie A, Arnaud E, Sensebé L, Varin A. CD54-Mediated Interaction with Pro-inflammatory Macrophages Increases the Immunosuppressive Function of Human Mesenchymal Stromal Cells. *Stem Cell Reports* 2017; **8**: 961-976 [PMID: 28330617 DOI: 10.1016/j.stemcr.2017.02.008]

32 **Li Y**, Zhang D, Xu L, Dong L, Zheng J, Lin Y, Huang J, Zhang Y, Tao Y, Zang X, Li D, Du M. Cell-cell contact with proinflammatory macrophages enhances the immunotherapeutic effect of mesenchymal stem cells in two abortion models. *Cell Mol Immunol* 2019; **16**: 908-920 [PMID: 30778166 DOI: 10.1038/s41423-019-0204-6]

33 **Eslani M**, Putra I, Shen X, Hamouie J, Tadepalli A, Anwar KN, Kink JA, Ghassemi S, Agnihotri G, Reshetylo S, Mashaghi A, Dana R, Hematti P, Djalilian AR. Cornea-Derived Mesenchymal Stromal Cells Therapeutically Modulate Macrophage Immunophenotype and Angiogenic Function. *Stem Cells* 2018; **36**: 775-784 [PMID: 29341332 DOI: 10.1002/stem.2781]

34 **Németh K**, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. Bone marrow stromal cells attenuate sepsis *via* prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 2009; **15**: 42-49 [PMID: 19098906 DOI: 10.1038/nm.1905]

35 **Chen L**, Lu FB, Chen DZ, Wu JL, Hu ED, Xu LM, Zheng MH, Li H, Huang Y, Jin XY, Gong YW, Lin Z, Wang XD, Chen YP. BMSCs-derived miR-223-containing exosomes contribute to liver protection in experimental autoimmune hepatitis. *Mol Immunol* 2018; **93**: 38-46 [PMID: 29145157 DOI: 10.1016/j.molimm.2017.11.008]

36 **Liu Y**, Lou G, Li A, Zhang T, Qi J, Ye D, Zheng M, Chen Z. AMSC-derived exosomes alleviate lipopolysaccharide/d-galactosamine-induced acute liver failure by miR-17-mediated reduction of TXNIP/NLRP3 inflammasome activation in macrophages. *EBioMedicine* 2018; **36**: 140-150 [PMID: 30197023 DOI: 10.1016/j.ebiom.2018.08.054]

37 **Li S**, Zheng X, Li H, Zheng J, Chen X, Liu W, Tai Y, Zhang Y, Wang G, Yang Y. Mesenchymal Stem Cells Ameliorate Hepatic Ischemia/Reperfusion Injury *via* Inhibition of Neutrophil Recruitment. *J Immunol Res* 2018; **2018**: 7283703 [PMID: 30622980 DOI: 10.1155/2018/7283703]

38 **Liu Y**, Yin Z, Zhang R, Yan K, Chen L, Chen F, Huang W, Lv B, Sun C, Jiang X. MSCs inhibit bone marrow-derived DC maturation and function through the release of TSG-6. *Biochem Biophys Res*

Commun 2014; **450**: 1409-1415 [PMID: 25014173 DOI: 10.1016/j.bbrc.2014.07.001]
39 **Djouad F**, Charbonnier LM, Bouffi C, Louis-Plence P, Bony C, Apparailly F, Cantos C, Jorgensen C, Noël D. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. *Stem Cells* 2007; **25**: 2025-2032 [PMID: 17510220 DOI: 10.1634/stemcells.2006-0548]
40 **Spaggiari GM**, Abdelrazik H, Becchetti F, Moretta L. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. *Blood* 2009; **113**: 6576-6583 [PMID: 19398717 DOI: 10.1182/blood-2009-02-203943]
41 **Selleri S**, Bifsha P, Civini S, Pacelli C, Dieng MM, Lemieux W, Jin P, Bazin R, Patey N, Marincola FM, Moldovan F, Zaouter C, Trudeau LE, Benabdhalla B, Louis I, Beauséjour C, Stroncek D, Le Deist F, Haddad E. Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. *Oncotarget* 2016; **7**: 30193-30210 [PMID: 27070086 DOI: 10.18632/oncotarget.8623]
42 **Liu X**, Qu X, Chen Y, Liao L, Cheng K, Shao C, Zenke M, Keating A, Zhao RC. Mesenchymal stem/stromal cells induce the generation of novel IL-10-dependent regulatory dendritic cells by SOCS3 activation. *J Immunol* 2012; **189**: 1182-1192 [PMID: 22753940 DOI: 10.4049/jimmunol.1102996]
43 **Li YP**, Paczesny S, Lauret E, Poirault S, Bordigoni P, Mekhloufi F, Hequet O, Bertrand Y, Ou-Yang JP, Stoltz JF, Miossec P, Eljaafari A. Human mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway. *J Immunol* 2008; **180**: 1598-1608 [PMID: 18209056 DOI: 10.4049/jimmunol.180.3.1598]
44 **Cahill EF**, Tobin LM, Carty F, Mahon BP, English K. Jagged-1 is required for the expansion of CD4+ CD25+ FoxP3+ regulatory T cells and tolerogenic dendritic cells by murine mesenchymal stromal cells. *Stem Cell Res Ther* 2015; **6**: 19 [PMID: 25890330 DOI: 10.1186/s13287-015-0021-5]
45 **Zhang B**, Liu R, Shi D, Liu X, Chen Y, Dou X, Zhu X, Lu C, Liang W, Liao L, Zenke M, Zhao RC. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. *Blood* 2009; **113**: 46-57 [PMID: 18832657 DOI: 10.1182/blood-2008-04-154138]
46 **Spaggiari GM**, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood* 2006; **107**: 1484-1490 [PMID: 16239427 DOI: 10.1182/blood-2005-07-2775]
47 **Sotiropoulou PA**, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells* 2006; **24**: 74-85 [PMID: 16099998 DOI: 10.1634/stemcells.2004-0359]
48 **Chatterjee D**, Marquardt N, Tufa DM, Beauclair G, Low HZ, Hatlapatka T, Hass R, Kasper C, von Kaisenberg C, Schmidt RE, Jacobs R. Role of gamma-secretase in human umbilical-cord derived mesenchymal stem cell mediated suppression of NK cell cytotoxicity. *Cell Commun Signal* 2014; **12**: 63 [PMID: 25266361 DOI: 10.1186/s12964-014-0063-9]
49 **Spaggiari GM**, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood* 2008; **111**: 1327-1333 [PMID: 17951526 DOI: 10.1182/blood-2007-02-074997]
50 **Fan Y**, Herr F, Vernochet A, Mennesson B, Oberlin E, Durrbach A. Human Fetal Liver Mesenchymal Stem Cell-Derived Exosomes Impair Natural Killer Cell Function. *Stem Cells Dev* 2019; **28**: 44-55 [PMID: 30328799 DOI: 10.1089/scd.2018.0015]
51 **Almeida CR**, Caires HR, Vasconcelos DP, Barbosa MA. NAP-2 Secreted by Human NK Cells Can Stimulate Mesenchymal Stem/Stromal Cell Recruitment. *Stem Cell Reports* 2016; **6**: 466-473 [PMID: 27052313 DOI: 10.1016/j.stemcr.2016.02.012]
52 **Glennie S**, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 2005; **105**: 2821-2827 [PMID: 15591115 DOI: 10.1182/blood-2004-09-3696]
53 **Plumas J**, Chaperot L, Richard MJ, Molens JP, Bensa JC, Favrot MC. Mesenchymal stem cells induce apoptosis of activated T cells. *Leukemia* 2005; **19**: 1597-1604 [PMID: 16049516 DOI: 10.1038/sj.leu.2403871]
54 **Akiyama K**, Chen C, Wang D, Xu X, Qu C, Yamaza T, Cai T, Chen W, Sun L, Shi S. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. *Cell Stem Cell* 2012; **10**: 544-555 [PMID: 22542159 DOI: 10.1016/j.stem.2012.03.007]
55 **Meisel R**, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; **103**: 4619-4621 [PMID: 15001472 DOI: 10.1182/blood-2003-11-3909]
56 **Davies LC**, Heldring N, Kadri N, Le Blanc K. Mesenchymal Stromal Cell Secretion of Programmed Death-1 Ligands Regulates T Cell Mediated Immunosuppression. *Stem Cells* 2017; **35**: 766-776 [PMID: 27671847 DOI: 10.1002/stem.2509]
57 **Duffy MM**, Pindjakova J, Hanley SA, McCarthy C, Weidhofer GA, Sweeney EM, English K, Shaw G, Murphy JM, Barry FP, Mahon BP, Belton O, Ceredig R, Griffin MD. Mesenchymal stem cell inhibition of T-helper 17 cell- differentiation is triggered by cell-cell contact and mediated by

prostaglandin E2 *via* the EP4 receptor. *Eur J Immunol* 2011; **41**: 2840-2851 [PMID: 21710489 DOI: 10.1002/eji.201141499]

58 **Qu X**, Liu X, Cheng K, Yang R, Zhao RC. Mesenchymal stem cells inhibit Th17 cell differentiation by IL-10 secretion. *Exp Hematol* 2012; **40**: 761-770 [PMID: 22634392 DOI: 10.1016/j.exphem.2012.05.006]

59 **Prevosto C**, Zancolli M, Canevali P, Zocchi MR, Poggi A. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. *Haematologica* 2007; **92**: 881-888 [PMID: 17606437 DOI: 10.3324/haematol.11240]

60 **Liu Q**, Zheng H, Chen X, Peng Y, Huang W, Li X, Li G, Xia W, Sun Q, Xiang AP. Human mesenchymal stromal cells enhance the immunomodulatory function of CD8(+)CD28(-) regulatory T cells. *Cell Mol Immunol* 2015; **12**: 708-718 [PMID: 25482073 DOI: 10.1038/cmi.2014.118]

61 **Corcione A**, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006; **107**: 367-372 [PMID: 16141348 DOI: 10.1182/blood-2005-07-2657]

62 **Tabera S**, Pérez-Simón JA, Díez-Campelo M, Sánchez-Abarca LI, Blanco B, López A, Benito A, Ocio E, Sánchez-Guijo FM, Cañizo C, San Miguel JF. The effect of mesenchymal stem cells on the viability, proliferation and differentiation of B-lymphocytes. *Haematologica* 2008; **93**: 1301-1309 [PMID: 18641017 DOI: 10.3324/haematol.12857]

63 **Rafei M**, Hsieh J, Fortier S, Li M, Yuan S, Birman E, Forner K, Boivin MN, Doody K, Tremblay M, Annabi B, Galipeau J. Mesenchymal stromal cell-derived CCL2 suppresses plasma cell immunoglobulin production *via* STAT3 inactivation and PAX5 induction. *Blood* 2008; **112**: 4991-4998 [PMID: 18812467 DOI: 10.1182/blood-2008-07-166892]

64 **Franquesa M**, Mensah FK, Huizinga R, Strini T, Boon L, Lombardo E, DelaRosa O, Laman JD, Grinyó JM, Weimar W, Betjes MG, Baan CC, Hoogduijn MJ. Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. *Stem Cells* 2015; **33**: 880-891 [PMID: 25376628 DOI: 10.1002/stem.1881]

65 **Park MJ**, Kwok SK, Lee SH, Kim EK, Park SH, Cho ML. Adipose tissue-derived mesenchymal stem cells induce expansion of interleukin-10-producing regulatory B cells and ameliorate autoimmunity in a murine model of systemic lupus erythematosus. *Cell Transplant* 2015; **24**: 2367-2377 [PMID: 25506685 DOI: 10.3727/096368914X685645]

66 **Reuben A**, Koch DG, Lee WM; Acute Liver Failure Study Group. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology* 2010; **52**: 2065-2076 [PMID: 20949552 DOI: 10.1002/hep.23937]

67 **Bernal W**, Auzinger G, Dhawan A, Wendon J. Acute liver failure. *Lancet* 2010; **376**: 190-201 [PMID: 20638564 DOI: 10.1016/S0140-6736(10)60274-7]

68 **Volarevic V**, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, Mitrovic S, Pejnovic N, Arsenijevic N, Lukic ML. Protective role of IL-33/ST2 axis in Con A-induced hepatitis. *J Hepatol* 2012; **56**: 26-33 [PMID: 21703183 DOI: 10.1016/j.jhep.2011.03.022]

69 **Volarevic V**, Paunovic V, Markovic Z, Simovic Markovic B, Misirkic-Marjanovic M, Todorovic-Markovic B, Bojic S, Vucicevic L, Jovanovic S, Arsenijevic N, Holclajtner-Antunovic I, Milosavljevic M, Dramicanin M, Kravic-Stevovic T, Ciric D, Lukic ML, Trajkovic V. Large graphene quantum dots alleviate immune-mediated liver damage. *ACS Nano* 2014; **8**: 12098-12109 [PMID: 25415137 DOI: 10.1021/nn502466z]

70 **Volarevic V**, Misirkic M, Vucicevic L, Paunovic V, Simovic Markovic B, Stojanovic M, Milovanovic M, Jakovljevic V, Micic D, Arsenijevic N, Trajkovic V, Lukic ML. Metformin aggravates immune-mediated liver injury in mice. *Arch Toxicol* 2015; **89**: 437-450 [PMID: 24770553 DOI: 10.1007/s00204-014-1263-1]

71 **Kubo N**, Narumi S, Kijima H, Mizukami H, Yagihashi S, Hakamada K, Nakane A. Efficacy of adipose tissue-derived mesenchymal stem cells for fulminant hepatitis in mice induced by concanavalin A. *J Gastroenterol Hepatol* 2012; **27**: 165-172 [PMID: 21649723 DOI: 10.1111/j.1440-1746.2011.06798.x]

72 **Gazdic M**, Simovic Markovic B, Vucicevic L, Nikolic T, Djonov V, Arsenijevic N, Trajkovic V, Lukic ML, Volarevic V. Mesenchymal stem cells protect from acute liver injury by attenuating hepatotoxicity of liver natural killer T cells in an inducible nitric oxide synthase- and indoleamine 2,3-dioxygenase-dependent manner. *J Tissue Eng Regen Med* 2018; **12**: e1173-e1185 [PMID: 28488390 DOI: 10.1002/term.2452]

73 **Ito H**, Hoshi M, Ohtaki H, Taguchi A, Ando K, Ishikawa T, Osawa Y, Hara A, Moriwaki H, Saito K, Seishima M. Ability of IDO to attenuate liver injury in alpha-galactosylceramide-induced hepatitis model. *J Immunol* 2010; **185**: 4554-4560 [PMID: 20844202 DOI: 10.4049/jimmunol.0904173]

74 **Milosavljevic N**, Gazdic M, Simovic Markovic B, Arsenijevic A, Nurkovic J, Dolicanin Z, Djonov V, Lukic ML, Volarevic V. Mesenchymal stem cells attenuate acute liver injury by altering ratio between interleukin 17 producing and regulatory natural killer T cells. *Liver Transpl* 2017; **23**: 1040-1050 [PMID: 28481005 DOI: 10.1002/lt.24784]

75 **Liu J**, Feng B, Xu Y, Zhu J, Feng X, Chen W, Sheng X, Shi X, Pan Q, Yu J, Zeng X, Cao H, Li L. Immunomodulatory effect of mesenchymal stem cells in chemical-induced liver injury: a high-dimensional analysis. *Stem Cell Res Ther* 2019; **10**: 262 [PMID: 31443686 DOI: 10.1186/s13287-019-1379-6]

76 **Shi X**, Liu J, Chen D, Zhu M, Yu J, Xie H, Zhou L, Li L, Zheng S. MSC-triggered metabolomic alterations in liver-resident immune cells isolated from CCl_4-induced mouse ALI model. *Exp Cell Res*

2019; **383**: 111511 [PMID: 31362001 DOI: 10.1016/j.yexcr.2019.111511]

77 **Zhao X**, Shi X, Zhang Z, Ma H, Yuan X, Ding Y. Combined treatment with MSC transplantation and neutrophil depletion ameliorates D-GalN/LPS-induced acute liver failure in rats. *Clin Res Hepatol Gastroenterol* 2016; **40**: 730-738 [PMID: 27637473 DOI: 10.1016/j.clinre.2016.04.003]

78 **Huang B**, Cheng X, Wang H, Huang W, la Ga Hu Z, Wang D, Zhang K, Zhang H, Xue Z, Da Y, Zhang N, Hu Y, Yao Z, Qiao L, Gao F, Zhang R. Mesenchymal stem cells and their secreted molecules predominantly ameliorate fulminant hepatic failure and chronic liver fibrosis in mice respectively. *J Transl Med* 2016; **14**: 45 [PMID: 26861623 DOI: 10.1186/s12967-016-0792-1]

79 **Nauta AJ**, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 2007; **110**: 3499-3506 [PMID: 17664353 DOI: 10.1182/blood-2007-02-069716]

80 **Gazdic M**, Volarevic V, Arsenijevic N, Stojkovic M. Mesenchymal stem cells: a friend or foe in immune-mediated diseases. *Stem Cell Rev Rep* 2015; **11**: 280-287 [PMID: 25592610 DOI: 10.1007/s12015-014-9583-3]

81 **Poltavtseva RA**, Poltavtsev AV, Lutsenko GV, Svirshchevskaya EV. Myths, reality and future of mesenchymal stem cell therapy. *Cell Tissue Res* 2019; **375**: 563-574 [PMID: 30456646 DOI: 10.1007/s00441-018-2961-4]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2021 March 26; 13(3): 221-235

DOI: 10.4252/wjsc.v13.i3.221

ISSN 1948-0210 (online)

MINIREVIEWS

Insulin resistance in diabetes: The promise of using induced pluripotent stem cell technology

Ahmed K Elsayed, Selvaraj Vimalraj, Manjula Nandakumar, Essam M Abdelalim

ORCID number: Ahmed K Elsayed 0000-0001-5887-9255; Selvaraj Vimalraj 0000-0001-6951-4569; Manjula Nandakumar 0000-0002-9732-9798; Essam M Abdelalim 0000-0001-7764-6772.

Author contributions: Elsayed AK, Vimalraj S and Nandakumar M wrote and reviewed the manuscript; Abdelalim EM conceptualized the idea, wrote and revised the manuscript; all authors approved the final version of the manuscript.

Supported by the Qatar National Research Fund, No. NPRP10-1221-160041.

Conflict-of-interest statement: The authors declare that they have no conflict of interest

Ahmed K Elsayed, Manjula Nandakumar, Essam M Abdelalim, Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha 34110, Qatar

Selvaraj Vimalraj, Centre for Biotechnology, Anna University, Chennai 600025, India

Essam M Abdelalim, College of Health and Life Sciences, Hamad Bin Khalifa University, Qatar Foundation, Doha 34110, Qatar

Corresponding author: Essam M Abdelalim, DVM, MSc, PhD, Professor, Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Al Rayyan, Doha 34110, Qatar. emohamed@hbku.edu.qa

Abstract

Insulin resistance (IR) is associated with several metabolic disorders, including type 2 diabetes (T2D). The development of IR in insulin target tissues involves genetic and acquired factors. Persons at genetic risk for T2D tend to develop IR several years before glucose intolerance. Several rodent models for both IR and T2D are being used to study the disease pathogenesis; however, these models cannot recapitulate all the aspects of this complex disorder as seen in each individual. Human pluripotent stem cells (hPSCs) can overcome the hurdles faced with the classical mouse models for studying IR. Human induced pluripotent stem cells (hiPSCs) can be generated from the somatic cells of the patients without the need to destroy a human embryo. Therefore, patient-specific hiPSCs can generate cells genetically identical to IR individuals, which can help in distinguishing between genetic and acquired defects in insulin sensitivity. Combining the technologies of genome editing and hiPSCs may provide important information about the genetic factors underlying the development of different forms of IR. Further studies are required to fill the gaps in understanding the pathogenesis of IR and diabetes. In this review, we summarize the factors involved in the development of IR in the insulin-target tissues leading to diabetes. Also, we highlight the use of hPSCs to understand the mechanisms underlying the development of IR.

Key Words: Type 2 diabetes; Insulin target tissues; Human pluripotent stem cells; Induced pluripotent stem cells; Genetic factors; Disease modeling

Manuscript source: Invited manuscript

Specialty type: Developmental biology

Country/Territory of origin: Qatar

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: November 18, 2020
Peer-review started: November 18, 2020
First decision: January 25, 2021
Revised: February 7, 2021
Accepted: March 11, 2021
Article in press: March 11, 2021
Published online: March 26, 2021

P-Reviewer: Ballini A, Grawish ME, Shan L
S-Editor: Zhang H
L-Editor: A
P-Editor: Xing YX

Core Tip: The genetic factors involved in the development of insulin resistance (IR), associated with type 2 diabetes remains largely unknown due to the polygenic nature of IR and lack of the appropriate human model. In this review, we summarize and discuss the use of human pluripotent stem cell technology in studying the genetic defects underlying IR development as well as highlight the potential use of patient-derived pluripotent stem cell for *in vitro* IR modeling.

Citation: Elsayed AK, Vimalraj S, Nandakumar M, Abdelalim EM. Insulin resistance in diabetes: The promise of using induced pluripotent stem cell technology. *World J Stem Cells* 2021; 13(3): 221-235
URL: https://www.wjgnet.com/1948-0210/full/v13/i3/221.htm
DOI: https://dx.doi.org/10.4252/wjsc.v13.i3.221

INTRODUCTION

Insulin resistance (IR) is a hallmark of type 2 diabetes (T2D) and other related metabolic disorders. Several hereditary and environmental factors are known to be involved in the development of IR in individuals at risk for T2D. Persons at genetic risk for T2D tend to develop IR several years before glucose intolerance[1]. Diabetes is associated with several complications, such as diabetic ketoacidosis, nonketotic hyperosmolar coma or death, heart disease, stroke, kidney failure, foot ulcers, *etc*. Glucose metabolism is regulated by a feedback loop between islet β-cell and insulin-target tissues. Under IR condition, the β-cells control normal glucose tolerance by increasing the level of insulin secretion[2]. IR in different tissues (adipose tissue, skeletal muscle, liver, brain, gut, pancreas, vasculature, and kidney) leads to several metabolic disorders, including T2D, cardiovascular diseases, hypertension, polycystic ovary syndrome (PCOS), fatty infiltration of the liver, non-alcoholic fatty liver disease (NAFLD), apnea, a sleep disorder, arthritis, skin diseases, and cancers. Previous studies showed alterations in the gene expression profile between individuals with a family history of T2D and those without a family history of the disease. Those defects were mainly observed in the genes related to mitochondrial function and fat metabolism[3]. However, it is difficult to distinguish whether the alterations in the gene profiles are due to genetic or environmental factors. Although several genetic and environmental factors are known to be involved in the development of IR, the molecular and cellular mechanisms underlying IR development and its progression to T2D remain not completely understood. This is due to the lack of appropriate human models to study the pathophysiology of different forms of IR.

The establishment of induced pluripotent stem cell (iPSC) technology has allowed the generation of pluripotent stem cells (PSCs) from somatic cells and has led to the establishment of *in vitro* models to study the genetic factors involved in the development of human diseases[4]. The fact that iPSCs can be produced without the need of a human embryo enables us to avoid ethical concerns that restricted researchers, for decades, to use human embryonic stem cells (hESCs) in stem cell research studies. iPSCs have unlimited proliferative ability and a great potential to differentiate into all cell types of the body[4]. Therefore, iPSCs provide a source of a human model to study the IR in insulin target tissues and pancreatic β-cell dysfunction. iPSCs can generate cells genetically identical to insulin-resistant individuals, which can help in distinguishing between genetic and acquired defects in insulin sensitivity. In the current review, we mainly focus on the IR associated with diabetes and the mechanism involved. Also, we discuss the use of iPSC technology to understand and treat these disorders and explain the challenges and limitations of using the human iPSC-based models.

INSULIN RESISTANCE IN THE INSULIN-TARGET TISSUES

Insulin, secreted from pancreatic β-cells, plays a critical role in a wide range of cells and tissues in the body through its main action in regulating the cellular energy and metabolic processes of the macronutrients (carbohydrate, protein, and lipid) besides its

growth-promoting action through its mitogenic effect[5]. Impairment in insulin secretion and/or action can influence the functionality of most organs and affects the normal physiology of the whole body. The insulin performs its functions in insulin-target tissues through its binding to the insulin receptor (INSR), which activates its downstream signaling pathways (Figure 1). Insulin has an anabolic effect as it promotes glycogen, lipid, and protein synthesis as well as inhibits gluconeogenesis and lipolysis processes[6]. It also facilitates the intracellular glucose uptake through translocation of the insulin-dependent glucose transporter (GLUT4) as in skeletal muscle and adipose tissue[7]. This metabolic action of insulin is achieved through the phosphatidylinositol 3 (PI3K) kinase pathway[8]. The growth-promoting action of insulin is achieved through INSR and insulin growth factor receptor, leading to activation of Ras/MAP kinase pathways and subsequently activates the transcription factors of cell division and proliferation[9].

IR is the condition in which the cells respond inappropriately to the circulating insulin. In other terms, it is the impaired sensitivity to insulin-mediated actions[10]. It is known that insulin controls energy production mainly through glucose oxidation and inhibiting other sources such as lipolysis, protein catabolism, glycogenolysis, and gluconeogenesis. However, under IR conditions, these processes are activated as an alternative source of glucose. These catabolic processes are accompanied by the accumulation of toxic metabolic byproducts and inflammatory factors, which have harmful effects on the insulin-target tissues. In skeletal muscles, the muscular glycogen and protein synthesis are impaired with a decrease in the glucose uptake leading to sarcopenia[9]. Under IR conditions, the lipolysis is enhanced leading to the release of triglyceride, free fatty acids (FFAs), and inflammatory cytokines (*e.g.*, IL-6, TNFα, Leptin) into the circulation[11]. The metabolic toxic derivatives of FFAs and the inflammatory cytokines influence the functionality of most of the tissues either directly through its lipo-toxic and lipo-apoptotic effects or indirectly through an impairment in the insulin signaling pathway[12,13]. The liver responds to IR and the demand of other cells to glucose by stimulation of the glycogenolysis process to produce more glucose[14]. Under IR conditions, the released FFAs from fat are transported to the liver and cause NAFLD (steatohepatitis), which is subsequently followed by liver cirrhosis[15]. This impairment in liver function leads to a decrease in insulin clearance with hyperinsulinemia. Furthermore, IR induces impaired mitochondrial oxidative metabolism and endocrine disorders like PCOS, adrenal disorders, and thyroid function abnormalities[16]. It has been reported that the insulin-sensitive brain regions include the hypothalamus, prefrontal cortex, hippocampus, and fusiform gyrus. Therefore, the IR in the brain leads to mild cognitive impairment and dementia, and Alzheimer's disease[17]. IR in the gut leads to alteration in microbiota resulting in dysregulation in the short-chain fatty acid production and gut hormone production[12,18]. The clinical disorders associated with IR include T2DM, cardiovascular diseases, hypertension, PCOS, fatty infiltration of the liver NAFLD, apnea, a sleep disorder, arthritis, skin diseases, and cancers.

β-CELL DYSFUNCTION ASSOCIATED WITH INSULIN RESISTANCE

Impairment in glucose homeostasis regulated by the feedback loop between the insulin target tissues and pancreatic β-cells leads to T2D, which is associated with an abnormal increase in blood glucose levels (Figure 2). IR and pancreatic β-cell dysfunction are the major characteristic features of T2D pathological conditions. In T2D, β-cell dysfunction occurs as a result of IR in the insulin-target tissues[19]. However, the interplay between IR and pancreatic β-cell dysfunction is still complex (Figure 2). Many metabolic insults, such as obesity, saturated FFA overconsumption, inflammatory cytokines, and oxidative stress and endoplasmic reticulum (ER) stress reduce the functionality of β-cells and dysregulate the normal physiological state of β-cells, leading to their demise[20]. Loss of insulin sensitivity or IR results in hyperglycemia, hyperinsulinemia as well as activation of the catabolic processes, which increase the level of FFAs and lipotoxic cytokines. All of these elevated elements caused by IR are responsible factors for β-cell stress and dysfunction[21]. In the early stages of IR, β-cells try to compensate and control glucose homeostasis through the production of more insulin[22]. However, with chronic prolonged exposure to hyperglycemia, β-cells secrete large quantities of insulin, leading to ER stress and exhaustion of β-cells with depletion of insulin store[23]. Moreover, hyperglycemia for a long time leads to a decrease in the activity of insulin promoter through the reduction in the binding of PDX1 and MAFA with a subsequent decrease in insulin gene

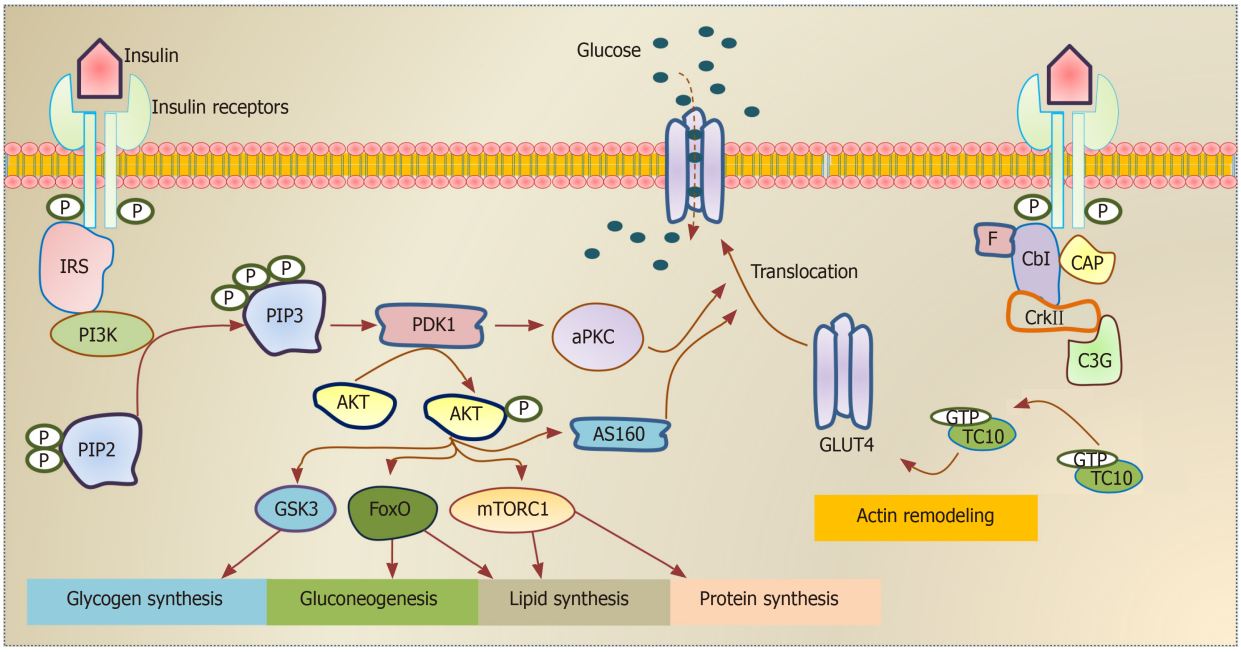


Figure 1 Schematic illustration of insulin signaling pathways. Insulin binding activates the insulin receptor (INSR), which enables the recruitment of insulin receptor substrate isoforms and subsequent activation of the phosphatidylinositol 3-kinase (PI3K). The downstream event of PI3K enhances glucose uptake by translocation of glucose transporter proteins over cell membrane, enhances glycogen, lipid and protein synthesis and regulates lipolysis and gluconeogenesis. Alternative pathway for glucose transporter type 4 (GLUT-4) translocation by insulin stimulation. Insulin binding activates the INSR, which enables F binding to CbI associated protein phosphorylates CbI and recruit CrkII/C3G complex. This complex converts guanosine diphosphate into guanosine triphosphate (GTP) on TC10. The stimulated GTP containing TC10 involved in GLUT-4 translocation by actin remodeling on GLUT-4. INSR: Insulin receptor; IRS: Insulin receptor substrate; PI3K: Phosphatidylinositol 3-kinase; PIP2: Phosphatidylinositol 4,5 bisphosphate; PIP3: Phosphatidylinositol 3,4,5 trisphosphate; PDK1: Phosphatidylinositide dependent protein kinase 1; aPKC: Atypical protein kinase C; GSK3: Glycogen synthase kinase 3; FoxO: Forkhead box O; mTORC: mTOR complex; AS160: Akt substrate 160kDa; F: Flotillin; CAP: CbI associated protein; C3G: Guanine nucleotide exchange factor; GTP: Guanosine triphosphate; GDP: Guanosine diphosphate; GLUT-4: Glucose transporter type 4.

expression and its secretion[24]. Hyperglycemia also can induce oxidative stress, inflammation, pro-apoptotic, and apoptotic genes' expression in β-cells[25]. Elevated plasma level of FFAs and glucose as a result of IR lead to glucolipotoxicity leading to β-cell failure[26]. These saturated FFAs induce both ER and oxidative stress in human β-cells and islets through the overproduction of NOS2 and NO in β-cell mitochondria[23,27] or through compromising the ER morphology and integrity[27]. In the presence of hyperglycemia, FFAs influence the biosynthesis and expression of the insulin gene, leading to suppression of adequate insulin secretion in response to glucose[28]. Increased FFAs lead to intrapancreatic and intra β-cell accumulation of triglyceride and fat droplets, triggering β-cell dysfunction and death due to an increase in the inflammation process[24,29]. Inflammation and the proinflammatory cytokines are recognized as an important contributor to β-cell dysfunction[30]. IR-associated inflammatory cytokines, such as IL6, TNFα, IFNγ, NF-κB, and others cause the dysfunctionality and death of β-cells *via* damage in the mitochondria, cellular proteins, lipids, nucleic acids, and ER stress[31]. The inflammatory cytokines and the recruited immune cells in the inflamed dysregulated pancreatic islet trigger β-cell dysfunction[32]. Proinflammatory cytokines mediate reactive oxygen species and reactive nitrogen species production and reduce ATP production and eventually lead to β-cell dysfunction[33]. The β-cell dysfunction and inadequate β-cell mass expansion can be due to the defect in the insulin signaling pathway in the pancreatic β-cells. Improper glucose sensing has been noticed in mouse β-cells, which lack the INSR or IGF1R. Additionally, loss of INSR leads to the β-cell mass reduction and early onset diabetes[34,35]. Another study showed similar diabetic phenotypes in mice with PDK1 deficiency in β-cells[36]. The impairment of cell cycle progression is hypothesized to be engaged in the β-cell mass reduction and dysfunction. It has been found that the cell cycle inhibitor, p27Kip1, is accumulated in the nucleus of the β-cells of hyperglycemic IRS2-deficient mice and the deletion of the p27Kip1 gene ameliorates β-cell proliferation and the hyperglycemia, reflecting the role of cell cycle inhibition in the β-cell function[22].

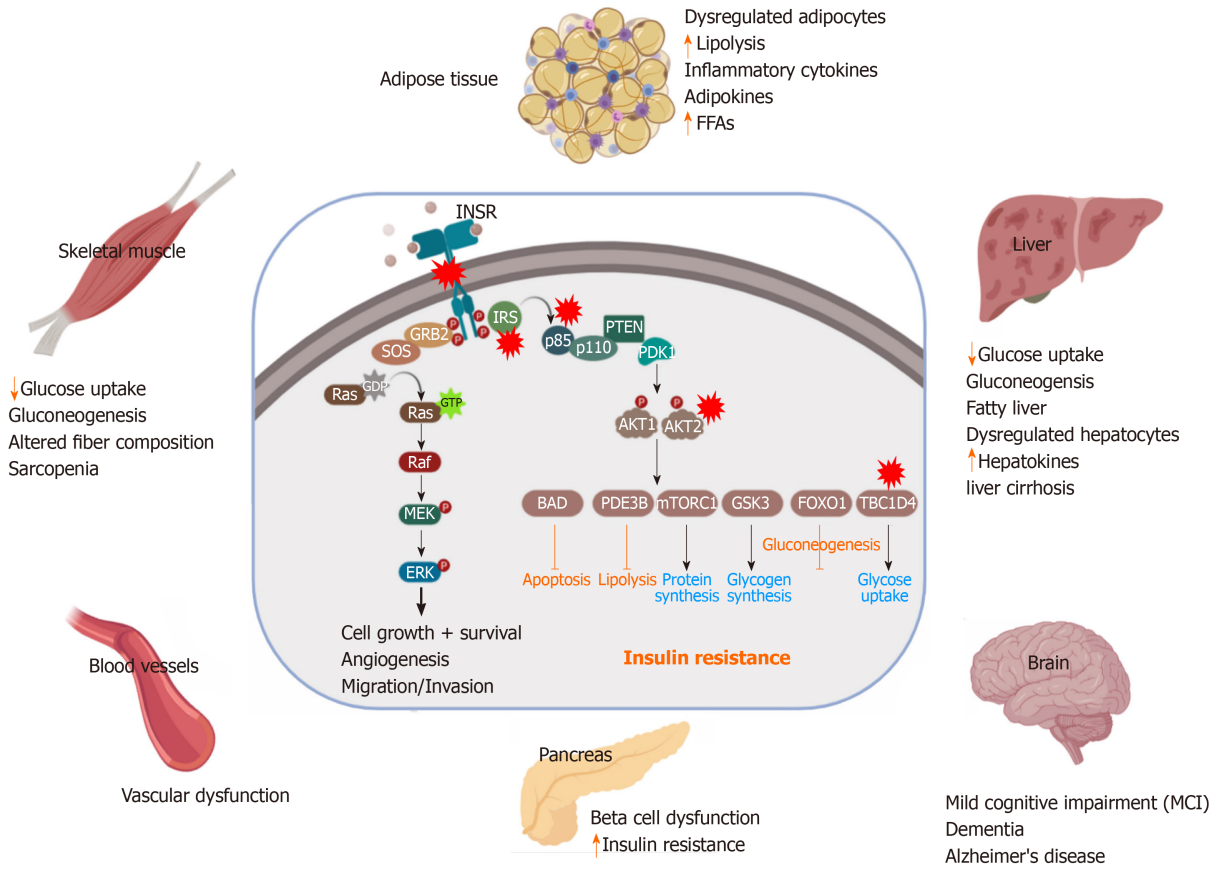


Figure 2 Pathological effect and consequence of insulin resistance. Genetic alterations and mutations in the insulin signaling pathway can lead to insulin resistance (IR) in the insulin-target tissues. The red stars indicate the reported genetically defective molecules, such as insulin receptor, insulin receptor substrates, p85, AKT and TBC1D4. In response to IR, the insulin target tissues (adipose tissue, skeletal muscle, liver, pancreas, brain and blood vessels) show activation of the catabolic processes and accumulation of toxic metabolic byproducts and the inflammatory cytokines, leading to pancreatic β-cell dysfunction and other metabolic disorders. INSR: Insulin receptor; IRS: Insulin receptor substrate; PDK1: Phosphatidylinositide dependent protein kinase 1; GSK3: Glycogen synthase kinase 3; FoxO: Forkhead box O; mTORC: mTOR complex; GTP: Guanosine triphosphate; FFAs: Free fatty acids.

STUDYING THE MECHANISMS UNDERLYING THE DEVELOPMENT OF INSULIN RESISTANCE

The IR arises as a result of the inability of the insulin-target tissues to use insulin appropriately, which is caused by many factors. The causes of IR involve genetic and environmental factors. The environmental factors can influence the insulin signaling pathway such as unhealthy diets, obesity, lack of physical activity, pharmacological agents, stress, cytokines, and hormones. These environmental factors combined with genetic factors can lead to the development of IR and diabetes[37]. Genetic predisposition causing IR may have a direct effect on the insulin signaling pathway or indirectly affecting other targets with a subsequent secondary effect on the insulin pathway[38]. The disruption and/or mutations in the genes encoding proteins involved in insulin signal transduction impair insulin action and reduce the rate of glucose uptake leading to IR[38]. Previous studies used multiple approaches to investigate the molecular mechanisms underlying the development of different forms of IR. These approaches include animal models, human insulin target tissues' explants, the human population genetic studies, and stem cells.

Animal models of insulin resistance and their limitations

Most of the animal models used to study IR are derived by inducing stress, diet, injuries, chemicals, and by different combinations mimicking IR conditions[39]. Meanwhile, there are few genetically modified IR animal models such as *IRS* knockout mouse (*IRS*$^{-/-}$) model Akt2 knockout (*Akt2*$^{-/-}$) mouse model, *Glut4*$^{+/-}$ null mutant mouse, and *Glut2*$^{-/-}$ mice[39,40]. Lipodystrophy has shown a strong association with severe IR; therefore, transgenic animals with defects in the genes regulating fatty acid metabolism are used to study IR mechanisms, such as sterol regulatory-element binding protein 1, A-ZIP/F-1, and toll-like receptor 4[39]. There are several animal

models exhibiting the features of the IR and diabetes due to mutations in specific genes either spontaneously or through selective breeding such as Leptin ob/ob and leptin db/db mouse strains, Zucker fatty (fa/fa) rat, New Zealand mice strain, OLETF rat, KK/Ay mouse and other models of the spontaneously developed IR strains[39]. Although the above-mentioned animal models provided a lot of information about the mechanisms of IR, they could not resolve all the questions related to the development of IR in human tissues and the relationship between IR and β-cell dysfunction.

Studying insulin resistance in human insulin-target tissues

The molecular and genetic basis for the development of IR in the insulin-target tissues such as skeletal muscle, adipose tissue, and liver are not fully understood. Previous studies used muscle biopsies showed that the mitochondrial DNA, mitochondrial genes, and respiratory chain subunit proteins are downregulated in IR subjects compared to healthy controls[41]. Another study showed that cultured myoblasts isolated from skeletal muscle of T2D patients have defects in glucose transport and insulin signaling[42]. In the skeletal muscle of the IR offspring of T2D parents, it has been found that the mitochondrial ATP production, mitochondrial density, and AKT activation are significantly reduced, while the IRS-1 phosphorylation (serine) is significantly increased[41,43]. The activation of PI3K pathway is required for the transport of glucose and synthesis of glycogen, which is impaired after insulin stimulation in insulin-resistant skeletal muscle[44]. The upregulation of IRS-1 phosphorylation on serine residues has been found to suppress insulin signaling in T2D[43]. Taken together, these findings indicate that the defects in insulin signaling, glucose transport, glycogen synthesis, and mitochondrial activity are the main dysfunctional features associated with the IR in the skeletal muscle.

Adipose tissue is considered the main regulator of insulin action in the body; therefore, defects in insulin signaling in adipocytes cause systemic IR, indicating that impairments in adipose insulin signaling are a common hallmark of IR[45]. Glucose transporter, GLUT4, is significantly downregulated in subcutaneous adipose cells from T2D patients and in healthy individuals with a genetic susceptibility for T2D[46]. This reduction in GLUT4 expression is associated with changes in the secretion of adipokines. Also, activation of peroxisome proliferator activated receptors (PPARs), which are responsible for adipogenesis regulation as well as lipid metabolism in adipocytes, leads to an improvement in insulin sensitivity through the induction of the expression of several genes that are related to the insulin signaling pathway. In addition to its role in adipogenesis, PPAR is also involved in regulating lipid metabolism in mature adipocytes by increasing fatty acid trapping[47]. The importance of insulin signaling in the development of adipocyte IR has been reported in T2D, where previous studies reported that the substrate of IRS-1 is involved in IR in adipocytes by inhibiting insulin-signaling[48]. Also, in T2D patients, several IRS-1 mutations have been found[49] and a reduced IRS-1 protein level has been observed in adipocytes from other T2D patients[50], relatives of T2D patients, and obese individuals[51]. In addition to insulin signaling defects, dysregulation of adipokines and the lipolysis process are linked to IR and T2D. The adiponectin gene is considered a candidate susceptibility gene for T2D as it has been detected on chromosome 3q27, which is linked to T2D and metabolic disorders[52,53].

The insulin signaling in the hepatic cells is crucial for maintaining normal liver function and regulating glucose homeostasis[54]. The liver activates glucose uptake and glycogen storage, but it inhibits glycogenolysis and gluconeogenesis. Hepatic IR progression plays a critical role in the pathogenesis of T2D. Loss of INSR signaling in hepatic tissue leads to an increase in gluconeogenesis and a decrease in lipogenesis[54,55]. The IRS-1/2 is involved in the suppression of gluconeogenesis and stimulation of lipogenesis. In the liver, insulin inhibits IRS-2 expression at the transcriptional level and doesn't influence IRS-1 expression[55,56]. Hepatic IR is characterized by the inability of insulin to inhibit the production of glucose in the liver[57]. This impairment in liver function leads to a decrease in insulin clearance with hyperinsulinemia. The released FFAs from fat tissues due to IR transports to the liver cause NAFLD (steatohepatitis), which is followed by liver cirrhosis[15]. Liver-derived proteins termed hepatokines are released into the circulation, which cause defective insulin signaling[58].

USING INDUCED PLURIPOTENT STEM CELLS (iPSCs) TO STUDY INSULIN RESISTANCE

Although animal models provided knowledge on the pathogenesis of certain forms of IR and diabetes, they cannot reflect all pathophysiological features of human diseases due to the physiological differences between humans and animals. It has been reported that the genetic makeup of the mouse model is involved in the alterations of the phenotypical outcome, even when the same mutation is created in mice with different genetic makeup. Several attempts have been made to overcome these challenges by the generation of humanized mouse models[59] or by using non-human primates[60]. Several population-based studies have been reported, in which the biopsies are isolated from both insulin-resistant and insulin-sensitive individuals to determine the mechanisms underlying IR[61]. The primary endothelial cell-based methodologies have been used to study the vascular inflammation in diabetes and atherosclerosis[30]. However, it also has certain limitations such as lack of donor availability and limited lifespan of primary cells. Furthermore, it is difficult to access human tissues, such as hepatocytes and skeletal muscles, particularly at the preclinical stages of the disease. In addition, it is difficult to distinguish between genetic and environmental factors.

In order to overcome the above-mentioned limitations, establishing a human cell model offers a great opportunity to understand the pathophysiology of human diseases. The hiPSC technology can provide human cell models to study the pathophysiology of IR. Generating patient-specific iPSCs from IR individuals with specific mutations or a family history of IR and diabetes can be used to study the genetic factors involved in the development of the disease, because the iPSCs maintain all the genetic information of the patients. Those patient-specific-iPSCs can generate all insulin target cells, such as skeletal myotubes, adipocytes, and hepatocytes as well as other cell types. The generated cell types carry the same genetic signature of the patients allowing further studies to identify the genetic defects involved in the disease progression (Figure 3).

iPSCs have been successfully generated from patients with different forms of diabetes including monogenic forms of diabetes, mitochondrial diabetes[62,63] T2D patients[64] as well as T1D patients[65]. The monogenic forms of IR can be modeled using the iPSC technology (Table 1). The IR is associated with several monogenic disorders such as Donohue and Rabson Mendenhall syndromes (INSR mutation), SHORT syndrome (PI3K mutation), Alstrom syndrome (ALMS1 mutation), Werner syndrome and Bloom syndrome (defects in DNA helicase), congenital generalized lipodystrophy (CGL) (AGPAT2 or BSCl2 mutations), and familial partial lipodystrophies (FPLD) (LMNA and PPARG mutations)[38]. In addition, several mutations associated with severe IR have been reported in AKT2, AS160 (TBC1D4), PPARG, PPP1R3A, and POLD1 encoding DNA polymerase delta[38]. Recent studies showed the generation of iPSCs from patients with IR; however, all of those studies focused on a very specific form of IR, which is due to specific mutations in INSR[66-69] (Table 1). Two of those studies generated iPSCs from patients with INSR mutations (INSR-Mut) (Donohue syndrome) focused on the effect of INSR-Mut on pluripotency and mitochondrial function in undifferentiated INSR-Mut hiPSCs[68,69]. It has been shown that INSR-Mut iPSCs are defective in their self-renewal ability because insulin and its downstream signaling are involved in regulating the unique properties of self-renewal and pluripotency in the undifferentiated iPSCs[70]. The PI3K has been shown to be crucial for the self-renewal of pluripotent stem cells[70]. Also, the INSR-Mut-iPSCs have mitochondrial dysfunctions, including alterations in the number and the size of mitochondria, and were associated with an upregulation in the expression of mitochondrial fission factor and inverted formin 2[68]. These two genes are known to enhance the mitochondrial fission as indicated in the INSR-Mut-hiPSCs[68]. Interestingly, increased mitochondrial fission has previously been detected in adult tissues, such as pancreatic β-cells and skeletal muscles of T2D patients[71]. Furthermore, it has been reported that mitochondrial DNA variation could be associated with genetic alteration, a known risk factor for T2D[72]. Also, the expression of glycolytic enzymes is downregulated, while lactate production is increased. These events lead to enhancement of ADP/ATP ratio and 5' AMP-activated protein kinase activity as well as leading to inefficient ATP and decrease in energy production with an increase in the oxidative stress[67]. All the derived INSR-Mut hiPSCs showed reduced proliferation and defective INSR phosphorylation and defects in its downstream signaling pathway such as AKT, GSK3, ERK1, and ERK2[69]. Differentiation of INSR-Mut hiPSCs towards skeletal myotubes exhibits defects in insulin signaling, glucose uptake, glycogen

Table 1 Human induced pluripotent stem cells models used to study insulin resistance

Ref.	Disease/ syndrome	iPSC models	Main findings
Iovino *et al*[69]	DS with severe insulin resistance	Three different INSR mutations (patient-derived iPSCs): (1) Compound heterozygous A897X, exon 14; (2) Homozygous A2G, exon 1; (3) Homozygous L233P, exon 3	iPSCs generated from patients with severe IR showed defects in the self-renewal of the patient-derived iPSCs
Balhara *et al*[67]		Patient-derived iPSCs with a compound heterozygous mutation in exon 14 of *INSR* (A897X)	Mesenchymal progenitor cells (MPCs) generated from patient-derived iPSCs showed defects in the insulin signaling pathway and the cellular oxidative metabolism
Burkart *et al*[68]		Four different INSR mutations (patient-derived iPSCs): (1) Compound heterozygous A897X, exon 14; (2) Homozygous (A2G), exon 1; (3) Homozygous (L233P), exon 3; (4) Homozygous (E124X), exon2	INSR-Mut hiPSCs have an impairment in the energy homeostasis as well as dysregulation of oxidative metabolism
Iovino *et al*[66]			Skeletal myotubes derived from INSR-Mut hiPSCs exhibit defects in insulin signaling, glucose uptake, and glycogen accumulation and altered insulin signaling gene expression
Mori *et al*[74]	Congenital generalized lipodystrophy(CGL)	Two different *BSCL2* mutations (patient-derived iPSCs): (1) Homozygous (E189X), exon 5; (2) Homozygous (R275X), exon 8	The adipose tissue derived from BSCL2-Mut hiPSCs exhibit notably a decrease in the lipid droplet formation as well as diffuse cytoplasmic distribution of ADRP
Friesen *et al*[75]	Familial partial lipodystrophy type 2 (FPLD2)	Heterozygous mutation in exon 1 of *LMNA* gene (R28W) (patient-derived iPSCs)	FPLD2-iPSCs recapitulate insulin resistance phenotypes and have lower capability for adipogenic differentiation with functional deficiency
Jozefczuk *et al*[78]	Insulin resistant patients with liver steatosis	Patient-derived iPSCs	iPSCs derived from IR patients recapitulate insulin resistance and showed a decrease in AKT/mTOR signaling pathway and perturbed various cellular networks
Ali *et al*[79]	Patients with psoriasis and insulin resistance	Patient-derived iPSCs	iPSCs-derived keratinocytes showed genetic alterations in the transcripts associated with IR, including IRS2 and GDF15 and glucose transporters, GLUT10 and GLUT14
Carcamo-Orive *et al*[82]	Insulin resistant patients	Patient-derived iPSCs	iPSCs derived from IR and IS individuals uncovered several IR relevant networks and identified a set of IR related driver genes

iPSCs: Induced pluripotent stem cells; DS: Donohue syndrome; INSR: Insulin receptor; IR: Insulin resistance; MPCs: Mesenchymal progenitor cells; CGL: Congenital generalized lipodystrophy; ADRP: Adipose differentiated related protein.

accumulation, and altered insulin signaling gene expression[66], indicating the genetic defects in the skeletal myotubes. Another iPSC model for IR, in which iPSCs have been generated from the fibroblasts of an insulin-resistant patient with CGL, an autosomal recessive disease due to *BSCL2* mutation[73]. These patient-specific iPSCs have been used as an *in vitro* model to study the physiopathology of lipid accumulation and lipodystrophy and its relation to IR. Adipocytes derived from these BSCL2-Mut iPSCs showed reduced lipid droplet formation and dispersed cytoplasmic distribution of adipose differentiation-related protein[74]. Another study generated iPSCs from a patient with familial FPLD2 due to mutation in the *LMNA* gene[75]. These FPLD2-iPSCs recapitulated the insulin resistance phenotype of the patient with low efficiency of *in vitro* adipogenic differentiation and less functionality[75]. Recent studies showed the ability to generate adipocytes from hESCs and hiPSCs[76,77]. It has been reported that hiPSC-derived adipocytes, transplanted into mice, are able to sustain their functional characteristics for several weeks[77], suggesting that these cells can also be used therapeutically to improve metabolic disorders in patients. Therefore, differentiation of patient-specific hiPSCs into white adipocytes can offer a large number of functional adipocytes for transplantation as a possible way to treat adipocytes-associated disorders as well as studying IR. Since the liver is an important insulin target tissue, the iPSCs have been used to understand the etiology of steatosis due to the NAFLD, which is accompanied by IR and hyperlipidemia[78]. The iPSCs generated from patients with liver steatosis showed a decrease in the AKT/mTOR signaling pathway and the IR phenotypes are observed in both liver and skin fibroblasts of the patients. Additionally, it has been shown that the transcription factor, sterol regulatory element-binding transcription factor 1, and its downstream targets, LIPIN1 (LPIN) and low-

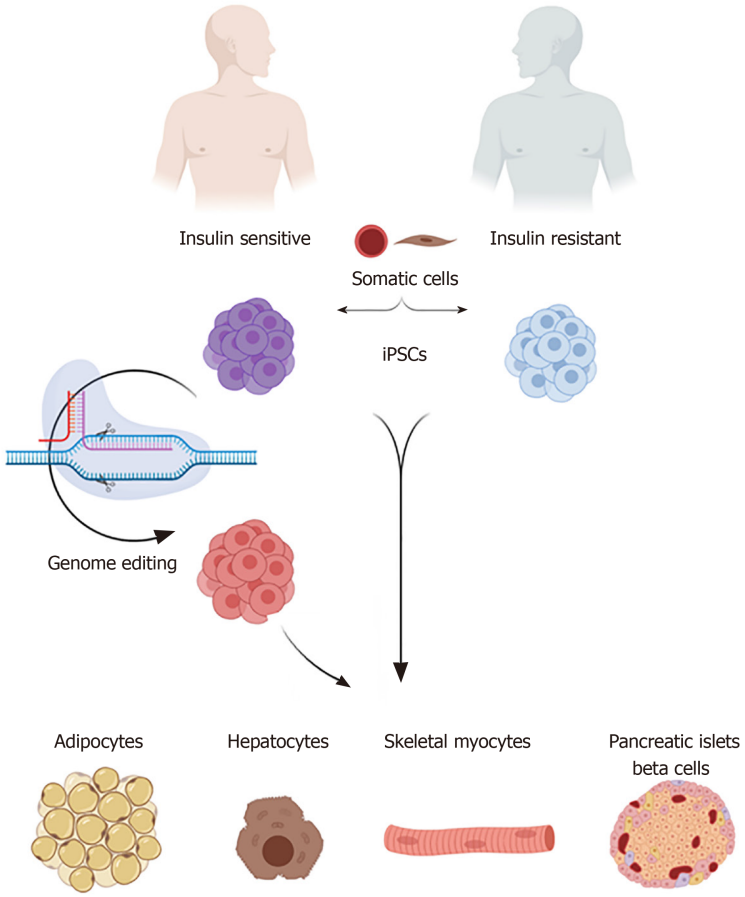


Figure 3 Using human induced pluripotent stem cells to study insulin resistance. A schematic diagram showed the possibility of using human induced pluripotent stem cells (hiPSCs) to study insulin resistance (IR) with special focus on the genetic factors. Somatic cells are reprogrammed into pluripotency to generate induced pluripotent stem cells (iPSCs) carrying the genetic signatures of insulin sensitive and IR individuals. The generated iPSCs can be differentiated *in vitro* into the main insulin-target cells, including hepatocyte, adipocyte, and skeletal myotubes as well as insulin producing β-cells. Genome editing tools can be used to correct specific mutations in the generated iPSCs to establish the isogenic iPSC control. Studying those cells can help in understanding the signaling pathways involved in the development of IR and type 2 diabetes. Also, these hiPSC-based models can be used for drug screening. iPSCs: Induced pluripotent stem cells.

density lipoprotein receptor, are involved in glycerolipid and fatty acid biosynthesis[78].

iPSCs have been also utilized to understand the polygenic form of IR. In our recent study, we used iPSC technology to understand the pathogenesis of psoriasis, skin disease, and its link to IR[79]. The results showed that keratinocytes derived from patient-specific iPSCs with psoriasis have genetic alterations in the transcripts associated with IR, including *IRS2* and *GDF15* and glucose transporters, *GLUT10* and *GLUT14*[79]. These findings indicate that patient-specific iPSCs can provide important information on the genetic predisposition of IR.

Further studies are required to fill the gaps in understanding the pathogenesis of IR and diabetes. There are several genetic factors involved in the development of IR; therefore, patient-specific iPSCs can be used to study the most common form of IR leading to T2D. Also, hiPSCs/hESCs can be genetically edited using genome editing tools, such as CRISPR/Cas9[80]. The recent genome-wide association studies (GWAS) studies have discovered several genetic defects associated with IR and diabetes development. Those defective genes can be introduced into hiPSCs/hESCs using a genome-editing tool followed by their differentiation into insulin-target cells, such as skeletal muscles, adipocytes, and hepatocytes. The combination of these technologies may provide more details about the inherited factors underlying the development of different forms of IR with a particular focus on the common form of IR. The generation of isogenic hiPSCs represents a proper human cell model to study the molecular mechanism of the newly identified candidate genes and variants through GWAS[81]. A recent study showed the generation of a large number of iPSC lines from individuals with and without IR[82]. Comparing the transcriptome profiles between iPSCs derived from 52 IR and 48 insulin sensitive individuals showed 1388 differentially expressed genes between both groups[82]. Nine of those genes (*BNIP3, CARS, IDH1, NDUFB1, HMGCR, HPN, FDPS, SLC27A1,* and *TMEM54*) have been shown to be associated with

IR and T2D[82]. These hiPSC lines are very useful to investigate the underlying mechanisms of IR and its associated metabolic disorders.

LIMITATIONS OF IPSCS AS AN *IN VITRO* MODEL TO STUDY INSULIN RESISTANCE

There are some challenges of using iPSC-based models. For example, robust protocols are needed for the differentiation of iPSCs into the desired cell types for studying metabolic diseases. Currently, the protocols are available for differentiation of hPSCs into the main insulin-target cells (adipocytes, skeletal muscles, and hepatocytes)[76,83-85] as well as pancreatic beta cells. However, most of those protocols lead to a combination of diverse cell types. The variability in the biological properties of iPSCs derived from different individuals shows the difference in the differentiation nature towards a given lineage. This reasonably affects the consistency of interpretation of the phenotypes[86]. The results obtained from populations of different genetic backgrounds may show differences. For instance, a report emphasized that population-based gene sequencing showed significant variations in the human genome[87]. Hence, generating iPSCs from patients with genetic defects, differentiating them to specific cell types, may hold a noteworthy risk[88]. Generation of an iPSC model for IR is a comparatively difficult process, because the development of metabolic diseases typically develops overtime and may show weak phenotypic changes under *in vitro* conditions[88]. Genome editing in iPSCs can be used to study candidate genes involved in IR and diabetes[87]. Also, the generation of isogenic cell lines from iPSCs could be an important strategy to overcome the problem of differences in the genetic background between different iPSC lines. The isogenic cell lines possess similar genetic milieu, epigenetic nature, and differentiation properties. This strategy may provide more consistent output and interpretation for complex diseases. The genome editing tools can make this strategy possible by engineering the genome of iPSCs. There are several reports highlight the use of genome editing tools for the generation of iPSC-based disease models[88,89].

CONCLUSION

Metabolic diseases like diabetes lead to several life-threatening complications. To develop treatments for those metabolic disorders, it is important to understand the factors involved in the development of the disease. IR is associated with several metabolic disorders; however, the genetic factors contributing to IR development are largely unknown. The human iPSC technology can provide cells genetically identical to patients with IR and diabetes. Those patient-specific iPSCs can be differentiated into all cell types involved in the IR, such as skeletal muscle, adipocytes, and hepatocytes as well as other cell types. The IR developed due to a mutation or a defect in one gene (monogenic) can be studied using iPSCs. However, the common form of IR, which is associated with genetic defects in several genes is more difficult to be studied using the iPSC technology. The recent advances in genome editing tools allow us to introduce or correct mutations in patient-specific iPSCs and hESCs as well as to generate knockout hiPSC models. Moreover, recent GWAS studies have identified several new genes involved in the development of IR and diabetes. Therefore, combining hiPSC technology, genome editing tools, and GWAS studies can help in understanding the genetic defects associated with IR. However, the common form of IR is not caused by a single gene defect, but a group of genes is involved in IR progression. Furthermore, establishing efficient differentiation protocols for the insulin-target cells is important to study the genetic defects. Currently, most of the differentiation protocols for those cells are well-established; however, the heterogeneity of the generated cells still need to be resolved. One of the solutions is to purify the target cells using surface markers or other methods. In conclusion, although there is progress in the use of hiPSC technology to study IR, a lot of work still needs to be done.

REFERENCES

1 **Shulman RG**. Nuclear magnetic resonance studies of glucose metabolism in non-insulin-dependent diabetes mellitus subjects. *Mol Med* 1996; **2**: 533-540 [PMID: 8898370 DOI: 10.1007/BF03401638]
2 **Ling C**, Rönn T. Epigenetics in Human Obesity and Type 2 Diabetes. *Cell Metab* 2019; **29**: 1028-1044 [PMID: 30982733 DOI: 10.1016/j.cmet.2019.03.009]
3 **Elgzyri T**, Parikh H, Zhou Y, Dekker Nitert M, Rönn T, Segerström ÅB, Ling C, Franks PW, Wollmer P, Eriksson KF, Groop L, Hansson O. First-degree relatives of type 2 diabetic patients have reduced expression of genes involved in fatty acid metabolism in skeletal muscle. *J Clin Endocrinol Metab* 2012; **97**: E1332-E1337 [PMID: 22547424 DOI: 10.1210/jc.2011-3037]
4 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]
5 **Bratanova-Tochkova TK**, Cheng H, Daniel S, Gunawardana S, Liu YJ, Mulvaney-Musa J, Schermerhorn T, Straub SG, Yajima H, Sharp GW. Triggering and augmentation mechanisms, granule pools, and biphasic insulin secretion. *Diabetes* 2002; **51** Suppl 1: S83-S90 [PMID: 11815463 DOI: 10.2337/diabetes.51.2007.s83]
6 **Burks DJ**, White MF. IRS proteins and beta-cell function. *Diabetes* 2001; **50** Suppl 1: S140-S145 [PMID: 11272176 DOI: 10.2337/diabetes.50.2007.s140]
7 **Saltiel AR**, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001; **414**: 799-806 [PMID: 11742412 DOI: 10.1038/414799a]
8 **Khan AH**, Pessin JE. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. *Diabetologia* 2002; **45**: 1475-1483 [PMID: 12436329 DOI: 10.1007/s00125-002-0974-7]
9 **Hunter SJ**, Garvey WT. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. *Am J Med* 1998; **105**: 331-345 [PMID: 9809695 DOI: 10.1016/s0002-9343(98)00300-3]
10 **Wilcox G**. Insulin and insulin resistance. *Clin Biochem Rev* 2005; **26**: 19-39 [PMID: 16278749]
11 **Hotamisligil GS**. Inflammatory pathways and insulin action. *Int J Obes Relat Metab Disord* 2003; **27** Suppl 3: S53-S55 [PMID: 14704746 DOI: 10.1038/sj.ijo.0802502]
12 **Artunc F**, Schleicher E, Weigert C, Fritsche A, Stefan N, Häring HU. The impact of insulin resistance on the kidney and vasculature. *Nat Rev Nephrol* 2016; **12**: 721-737 [PMID: 27748389 DOI: 10.1038/nrneph.2016.145]
13 **Gauthier MS**, Ruderman NB. Adipose tissue inflammation and insulin resistance: all obese humans are not created equal. *Biochem J* 2010; **430**: e1-e4 [PMID: 20704568 DOI: 10.1042/BJ20101062]
14 **Krauss RM**, Siri PW. Metabolic abnormalities: triglyceride and low-density lipoprotein. *Endocrinol Metab Clin North Am* 2004; **33**: 405-415 [PMID: 15158526 DOI: 10.1016/j.ecl.2004.03.016]
15 **Reaven G**. The metabolic syndrome or the insulin resistance syndrome? *Endocrinol Metab Clin North Am* 2004; **33**: 283-303 [PMID: 15158520 DOI: 10.1016/j.ecl.2004.03.002]
16 **Morrison SA**, Goss AM, Azziz R, Raju DA, Gower BA. Peri-muscular adipose tissue may play a unique role in determining insulin sensitivity/resistance in women with polycystic ovary syndrome. *Hum Reprod* 2017; **32**: 185-192 [PMID: 27827322 DOI: 10.1093/humrep/dew279]
17 **Heni M**, Kullmann S, Preissl H, Fritsche A, Häring HU. Impaired insulin action in the human brain: causes and metabolic consequences. *Nat Rev Endocrinol* 2015; **11**: 701-711 [PMID: 26460339 DOI: 10.1038/nrendo.2015.173]
18 **Burcelin R**. Gut microbiota and immune crosstalk in metabolic disease. *Mol Metab* 2016; **5**: 771-781 [PMID: 27617200 DOI: 10.1016/j.molmet.2016.05.016]
19 **Ashcroft FM**, Rorsman P. Diabetes mellitus and the β cell: the last ten years. *Cell* 2012; **148**: 1160-1171 [PMID: 22424227 DOI: 10.1016/j.cell.2012.02.010]
20 **Ferrannini E**. The stunned beta cell: a brief history. *Cell Metab* 2010; **11**: 349-352 [PMID: 20444416 DOI: 10.1016/j.cmet.2010.04.009]
21 **Prentki M**, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest* 2006; **116**: 1802-1812 [PMID: 16823478 DOI: 10.1172/JCI29103]
22 **Kasuga M**. Insulin resistance and pancreatic beta cell failure. *J Clin Invest* 2006; **116**: 1756-1760 [PMID: 16823472 DOI: 10.1172/JCI29189]
23 **Hasnain SZ**, Borg DJ, Harcourt BE, Tong H, Sheng YH, Ng CP, Das I, Wang R, Chen AC, Loudovaris T, Kay TW, Thomas HE, Whitehead JP, Forbes JM, Prins JB, McGuckin MA. Glycemic control in diabetes is restored by therapeutic manipulation of cytokines that regulate beta cell stress. *Nat Med* 2014; **20**: 1417-1426 [PMID: 25362253 DOI: 10.1038/nm.3705]
24 **Poitout V**, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr Rev* 2008; **29**: 351-366 [PMID: 18048763 DOI: 10.1210/er.2007-0023]
25 **Federici M**, Hribal M, Perego L, Ranalli M, Caradonna Z, Perego C, Usellini L, Nano R, Bonini P, Bertuzzi F, Marlier LN, Davalli AM, Carandente O, Pontiroli AE, Melino G, Marchetti P, Lauro R, Sesti G, Folli F. High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. *Diabetes* 2001; **50**: 1290-1301 [PMID: 11375329 DOI: 10.2337/diabetes.50.6.1290]
26 **van Raalte DH**, Diamant M. Glucolipotoxicity and beta cells in type 2 diabetes mellitus: target for durable therapy? *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S37-S46 [PMID: 21864750 DOI:

10.1016/S0168-8227(11)70012-2]
27 **Cnop M**, Ladrière L, Igoillo-Esteve M, Moura RF, Cunha DA. Causes and cures for endoplasmic reticulum stress in lipotoxic β-cell dysfunction. *Diabetes Obes Metab* 2010; **12** Suppl 2: 76-82 [PMID: 21029303 DOI: 10.1111/j.1463-1326.2010.01279.x]
28 **Solinas G**, Naugler W, Galimi F, Lee MS, Karin M. Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. *Proc Natl Acad Sci USA* 2006; **103**: 16454-16459 [PMID: 17050683 DOI: 10.1073/pnas.0607626103]
29 **Gastaldelli A**. Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S60-S65 [PMID: 21864753 DOI: 10.1016/S0168-8227(11)70015-8]
30 **Donath MY**, Dalmas É, Sauter NS, Böni-Schnetzler M. Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity. *Cell Metab* 2013; **17**: 860-872 [PMID: 23747245 DOI: 10.1016/j.cmet.2013.05.001]
31 **Gurgul-Convey E**, Mehmeti I, Lortz S, Lenzen S. Cytokine toxicity in insulin-producing cells is mediated by nitro-oxidative stress-induced hydroxyl radical formation in mitochondria. *J Mol Med (Berl)* 2011; **89**: 785-798 [PMID: 21487676 DOI: 10.1007/s00109-011-0747-1]
32 **Lin X**, Qin Y, Jia J, Lin T, Lin X, Chen L, Zeng H, Han Y, Wu L, Huang S, Wang M, Xie R, Liang L, Liu Y, Liu R, Zhang T, Li J, Wang S, Sun P, Huang W, Yao K, Xu K, Du T, Xiao D. MiR-155 Enhances Insulin Sensitivity by Coordinated Regulation of Multiple Genes in Mice. *PLoS Genet* 2016; **12**: e1006308 [PMID: 27711113 DOI: 10.1371/journal.pgen.1006308]
33 **Robertson R**, Zhou H, Zhang T, Harmon JS. Chronic oxidative stress as a mechanism for glucose toxicity of the beta cell in type 2 diabetes. *Cell Biochem Biophys* 2007; **48**: 139-146 [PMID: 17709883 DOI: 10.1007/s12013-007-0026-5]
34 **Kulkarni RN**, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 1999; **96**: 329-339 [PMID: 10025399 DOI: 10.1016/s0092-8674(00)80546-2]
35 **Kulkarni RN**, Holzenberger M, Shih DQ, Ozcan U, Stoffel M, Magnuson MA, Kahn CR. beta-cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter beta-cell mass. *Nat Genet* 2002; **31**: 111-115 [PMID: 11923875 DOI: 10.1038/ng872]
36 **Hashimoto N**, Kido Y, Uchida T, Asahara S, Shigeyama Y, Matsuda T, Takeda A, Tsuchihashi D, Nishizawa A, Ogawa W, Fujimoto Y, Okamura H, Arden KC, Herrera PL, Noda T, Kasuga M. Ablation of PDK1 in pancreatic beta cells induces diabetes as a result of loss of beta cell mass. *Nat Genet* 2006; **38**: 589-593 [PMID: 16642023 DOI: 10.1038/ng1774]
37 **Yaribeygi H**, Farrokhi FR, Butler AE, Sahebkar A. Insulin resistance: Review of the underlying molecular mechanisms. *J Cell Physiol* 2019; **234**: 8152-8161 [PMID: 30317615 DOI: 10.1002/jcp.27603]
38 **Semple RK**. EJE PRIZE 2015: How does insulin resistance arise, and how does it cause disease? *Eur J Endocrinol* 2016; **174**: R209-R223 [PMID: 26865583 DOI: 10.1530/EJE-15-1131]
39 **Sah SP**, Singh B, Choudhary S, Kumar A. Animal models of insulin resistance: A review. *Pharmacol Rep* 2016; **68**: 1165-1177 [PMID: 27639595 DOI: 10.1016/j.pharep.2016.07.010]
40 **Islam MS**, Loots du T. Experimental rodent models of type 2 diabetes: a review. *Methods Find Exp Clin Pharmacol* 2009; **31**: 249-261 [PMID: 19557203 DOI: 10.1358/mf.2009.31.4.1362513]
41 **Petersen KF**, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 2004; **350**: 664-671 [PMID: 14960743 DOI: 10.1056/NEJMoa031314]
42 **Krook A**, Björnholm M, Galuska D, Jiang XJ, Fahlman R, Myers MG Jr, Wallberg-Henriksson H, Zierath JR. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes* 2000; **49**: 284-292 [PMID: 10868945 DOI: 10.2337/diabetes.49.2.284]
43 **Morino K**, Petersen KF, Dufour S, Befroy D, Frattini J, Shatzkes N, Neschen S, White MF, Bilz S, Sono S, Pypaert M, Shulman GI. Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *J Clin Invest* 2005; **115**: 3587-3593 [PMID: 16284649 DOI: 10.1172/JCI25151]
44 **Beck-Nielsen H**. Mechanisms of insulin resistance in non-oxidative glucose metabolism: the role of glycogen synthase. *J Basic Clin Physiol Pharmacol* 1998; **9**: 255-279 [PMID: 10212838 DOI: 10.1515/jbcpp.1998.9.2-4.255]
45 **Adams-Huet B**, Devaraj S, Siegel D, Jialal I. Increased adipose tissue insulin resistance in metabolic syndrome: relationship to circulating adipokines. *Metab Syndr Relat Disord* 2014; **12**: 503-507 [PMID: 25162912 DOI: 10.1089/met.2014.0092]
46 **Gustafson B**, Hedjazifar S, Gogg S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. *Trends Endocrinol Metab* 2015; **26**: 193-200 [PMID: 25703677 DOI: 10.1016/j.tem.2015.01.006]
47 **Tamori Y**, Masugi J, Nishino N, Kasuga M. Role of peroxisome proliferator-activated receptor-gamma in maintenance of the characteristics of mature 3T3-L1 adipocytes. *Diabetes* 2002; **51**: 2045-2055 [PMID: 12086932 DOI: 10.2337/diabetes.51.7.2045]
48 **White MF**. IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* 2002; **283**: E413-E422 [PMID: 12169433 DOI: 10.1152/ajpendo.00514.2001]
49 **Esposito DL**, Li Y, Vanni C, Mammarella S, Veschi S, Della Loggia F, Mariani-Costantini R, Battista P, Quon MJ, Cama A. A novel T608R missense mutation in insulin receptor substrate-1 identified in a subject with type 2 diabetes impairs metabolic insulin signaling. *J Clin Endocrinol*

Metab 2003; **88**: 1468-1475 [PMID: 12679424 DOI: 10.1210/jc.2002-020933]

50 **Rondinone CM**, Wang LM, Lonnroth P, Wesslau C, Pierce JH, Smith U. Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci U S A* 1997; **94**: 4171-4175 [PMID: 9108124 DOI: 10.1073/pnas.94.8.4171]

51 **Carvalho E**, Jansson PA, Axelsen M, Eriksson JW, Huang X, Groop L, Rondinone C, Sjöström L, Smith U. Low cellular IRS 1 gene and protein expression predict insulin resistance and NIDDM. *FASEB J* 1999; **13**: 2173-2178 [PMID: 10593864 DOI: 10.1096/fasebj.13.15.2173]

52 **Kissebah AH**, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L, Blangero J, Comuzzie AG. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci U S A* 2000; **97**: 14478-14483 [PMID: 11121050 DOI: 10.1073/pnas.97.26.14478]

53 **Vasseur F**, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, Boutin P, Vaxillaire M, Leprêtre F, Dupont S, Hara K, Clément K, Bihain B, Kadowaki T, Froguel P. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet* 2002; **11**: 2607-2614 [PMID: 12354786 DOI: 10.1093/hmg/11.21.2607]

54 **Michael MD**, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 2000; **6**: 87-97 [PMID: 10949030 DOI: 10.1016/S1097-2765(05)00015-8]

55 **Thirone AC**, Huang C, Klip A. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. *Trends Endocrinol Metab* 2006; **17**: 72-78 [PMID: 16458527 DOI: 10.1016/j.tem.2006.01.005]

56 **Hirashima Y**, Tsuruzoe K, Kodama S, Igata M, Toyonaga T, Ueki K, Kahn CR, Araki E. Insulin down-regulates insulin receptor substrate-2 expression through the phosphatidylinositol 3-kinase/Akt pathway. *J Endocrinol* 2003; **179**: 253-266 [PMID: 14596677 DOI: 10.1677/joe.0.1790253]

57 **Farese RV**. Jr., Zechner R, Newgard CB, Walther TC. The problem of establishing relationships between hepatic steatosis and hepatic insulin resistance. Cell Metab 2012; 15: 570-573 [PMID: 22560209 DOI: 10.1016/j.cmet.2012.03.004]

58 **Stefan N**, Häring HU. The role of hepatokines in metabolism. *Nat Rev Endocrinol* 2013; **9**: 144-152 [PMID: 23337953 DOI: 10.1038/nrendo.2012.258]

59 **King M**, Pearson T, Rossini AA, Shultz LD, Greiner DL. Humanized mice for the study of type 1 diabetes and beta cell function. *Ann N Y Acad Sci* 2008; **1150**: 46-53 [PMID: 19120266 DOI: 10.1196/annals.1447.009]

60 **Harwood HJ Jr**, Listrani P, Wagner JD. Nonhuman primates and other animal models in diabetes research. *J Diabetes Sci Technol* 2012; **6**: 503-514 [PMID: 22768880 DOI: 10.1177/193229681200600304]

61 **Wu C**, Xu G, Tsai SA, Freed WJ, Lee CT. Transcriptional profiles of type 2 diabetes in human skeletal muscle reveal insulin resistance, metabolic defects, apoptosis, and molecular signatures of immune activation in response to infections. *Biochem Biophys Res Commun* 2017; **482**: 282-288 [PMID: 27847319 DOI: 10.1016/j.bbrc.2016.11.055]

62 **Teo AK**, Windmueller R, Johansson BB, Dirice E, Njolstad PR, Tjora E, Raeder H, Kulkarni RN. Derivation of human induced pluripotent stem cells from patients with maturity onset diabetes of the young. *J Biol Chem* 2013; **288**: 5353-5356 [PMID: 23306198 DOI: 10.1074/jbc.C112.428979]

63 **Elsayed AK**, Aghadi M, Al-Khawaga S, Hussain K, Abdelalim EM. Derivation of a human induced pluripotent stem cell line (QBRIi007-A) from a patient carrying a homozygous intronic mutation (c.613-7T>G) in the SLC2A2 gene. *Stem Cell Res* 2020; **44**: 101736 [PMID: 32146263 DOI: 10.1016/j.scr.2020.101736]

64 **Kudva YC**, Ohmine S, Greder LV, Dutton JR, Armstrong A, De Lamo JG, Khan YK, Thatava T, Hasegawa M, Fusaki N, Slack JM, Ikeda Y. Transgene-free disease-specific induced pluripotent stem cells from patients with type 1 and type 2 diabetes. *Stem Cells Transl Med* 2012; **1**: 451-461 [PMID: 23197849 DOI: 10.5966/sctm.2011-0044]

65 **Millman JR**, Xie C, Van Dervort A, Gürtler M, Pagliuca FW, Melton DA. Generation of stem cell-derived β-cells from patients with type 1 diabetes. *Nat Commun* 2016; **7**: 11463 [PMID: 27163171 DOI: 10.1038/ncomms11463]

66 **Iovino S**, Burkart AM, Warren L, Patti ME, Kahn CR. Myotubes derived from human-induced pluripotent stem cells mirror *in vivo* insulin resistance. *Proc Natl Acad Sci U S A* 2016; **113**: 1889-1894 [PMID: 26831110 DOI: 10.1073/pnas.1525665113]

67 **Balhara B**, Burkart A, Topcu V, Lee YK, Cowan C, Kahn CR, Patti ME. Severe insulin resistance alters metabolism in mesenchymal progenitor cells. *Endocrinology* 2015; **156**: 2039-2048 [PMID: 25811318 DOI: 10.1210/en.2014-1403]

68 **Burkart AM**, Tan K, Warren L, Iovino S, Hughes KJ, Kahn CR, Patti ME. Insulin Resistance in Human iPS Cells Reduces Mitochondrial Size and Function. *Sci Rep* 2016; **6**: 22788 [PMID: 26948272 DOI: 10.1038/srep22788]

69 **Iovino S**, Burkart AM, Kriauciunas K, Warren L, Hughes KJ, Molla M, Lee YK, Patti ME, Kahn CR. Genetic insulin resistance is a potent regulator of gene expression and proliferation in human iPS cells. *Diabetes* 2014; **63**: 4130-4142 [PMID: 25059784 DOI: 10.2337/db14-0109]

70 **Armstrong L**, Hughes O, Yung S, Hyslop L, Stewart R, Wappler I, Peters H, Walter T, Stojkovic P, Evans J, Stojkovic M, Lako M. The role of PI3K/AKT, MAPK/ERK and NFkappabeta signalling in

the maintenance of human embryonic stem cell pluripotency and viability highlighted by transcriptional profiling and functional analysis. *Hum Mol Genet* 2006; **15**: 1894-1913 [PMID: 16644866 DOI: 10.1093/hmg/ddl112]

71 **Anello M**, Lupi R, Spampinato D, Piro S, Masini M, Boggi U, Del Prato S, Rabuazzo AM, Purrello F, Marchetti P. Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. *Diabetologia* 2005; **48**: 282-289 [PMID: 15654602 DOI: 10.1007/s00125-004-1627-9]

72 **Pravenec M**, Hyakukoku M, Houstek J, Zidek V, Landa V, Mlejnek P, Miksik I, Dudová-Mothejzikova K, Pecina P, Vrbacky M, Drahota Z, Vojtiskova A, Mracek T, Kazdova L, Oliyarnyk O, Wang J, Ho C, Qi N, Sugimoto K, Kurtz T. Direct linkage of mitochondrial genome variation to risk factors for type 2 diabetes in conplastic strains. *Genome Res* 2007; **17**: 1319-1326 [PMID: 17693571 DOI: 10.1101/gr.6548207]

73 **Garg A**. Acquired and inherited lipodystrophies. *N Engl J Med* 2004; **350**: 1220-1234 [PMID: 15028826 DOI: 10.1056/NEJMra025261]

74 **Mori E**, Fujikura J, Noguchi M, Nakao K, Matsubara M, Sone M, Taura D, Kusakabe T, Ebihara K, Tanaka T, Hosoda K, Takahashi K, Asaka I, Inagaki N. Impaired adipogenic capacity in induced pluripotent stem cells from lipodystrophic patients with BSCL2 mutations. *Metabolism* 2016; **65**: 543-556 [PMID: 26975546 DOI: 10.1016/j.metabol.2015.12.015]

75 **Friesen M**, Cowan CA. FPLD2 LMNA mutation R482W dysregulates iPSC-derived adipocyte function and lipid metabolism. *Biochem Biophys Res Commun* 2018; **495**: 254-260 [PMID: 29108996 DOI: 10.1016/j.bbrc.2017.11.008]

76 **Karam M**, Younis I, Elareer NR, Nasser S, Abdelalim EM. Scalable Generation of Mesenchymal Stem Cells and Adipocytes from Human Pluripotent Stem Cells. *Cells* 2020; **9** [PMID: 32183164 DOI: 10.3390/cells9030710]

77 **Noguchi M**, Hosoda K, Nakane M, Mori E, Nakao K, Taura D, Yamamoto Y, Kusakabe T, Sone M, Sakurai H, Fujikura J, Ebihara K. In vitro characterization and engraftment of adipocytes derived from human induced pluripotent stem cells and embryonic stem cells. *Stem Cells Dev* 2013; **22**: 2895-2905 [PMID: 23750558 DOI: 10.1089/scd.2013.0113]

78 **Jozefczuk J**, Kashofer K, Ummanni R, Henjes F, Rehman S, Geenen S, Wruck W, Regenbrecht C, Daskalaki A, Wierling C, Turano P, Bertini I, Korf U, Zatloukal K, Westerhoff HV, Lehrach H, Adjaye J. A Systems Biology Approach to Deciphering the Etiology of Steatosis Employing Patient-Derived Dermal Fibroblasts and iPS Cells. *Front Physiol* 2012; **3**: 339 [PMID: 22969728 DOI: 10.3389/fphys.2012.00339]

79 **Ali G**, Elsayed AK, Nandakumar M, Bashir M, Younis I, Abu Aqel Y, Memon B, Temanni R, Abubaker F, Taheri S, Abdelalim EM. Keratinocytes Derived from Patient-Specific Induced Pluripotent Stem Cells Recapitulate the Genetic Signature of Psoriasis Disease. *Stem Cells Dev* 2020; **29**: 383-400 [PMID: 31996098 DOI: 10.1089/scd.2019.0150]

80 **Ding Q**, Regan SN, Xia Y, Oostrom LA, Cowan CA, Musunuru K. Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. *Cell Stem Cell* 2013; **12**: 393-394 [PMID: 23561441 DOI: 10.1016/j.stem.2013.03.006]

81 **Scott RA**, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, Mägi R, Strawbridge RJ, Rehnberg E, Gustafsson S, Kanoni S, Rasmussen-Torvik LJ, Yengo L, Lecoeur C, Shungin D, Sanna S, Sidore C, Johnson PC, Jukema JW, Johnson T, Mahajan A, Verweij N, Thorleifsson G, Hottenga JJ, Shah S, Smith AV, Sennblad B, Gieger C, Salo P, Perola M, Timpson NJ, Evans DM, Pourcain BS, Wu Y, Andrews JS, Hui J, Bielak LF, Zhao W, Horikoshi M, Navarro P, Isaacs A, O'Connell JR, Stirrups K, Vitart V, Hayward C, Esko T, Mihailov E, Fraser RM, Fall T, Voight BF, Raychaudhuri S, Chen H, Lindgren CM, Morris AP, Rayner NW, Robertson N, Rybin D, Liu CT, Beckmann JS, Willems SM, Chines PS, Jackson AU, Kang HM, Stringham HM, Song K, Tanaka T, Peden JF, Goel A, Hicks AA, An P, Müller-Nurasyid M, Franco-Cereceda A, Folkersen L, Marullo L, Jansen H, Oldehinkel AJ, Bruinenberg M, Pankow JS, North KE, Forouhi NG, Loos RJ, Edkins S, Varga TV, Hallmans G, Oksa H, Antonella M, Nagaraja R, Trompet S, Ford I, Bakker SJ, Kong A, Kumari M, Gigante B, Herder C, Munroe PB, Caulfield M, Antti J, Mangino M, Small K, Miljkovic I, Liu Y, Atalay M, Kiess W, James AL, Rivadeneira F, Uitterlinden AG, Palmer CN, Doney AS, Willemsen G, Smit JH, Campbell S, Polasek O, Bonnycastle LL, Hercberg S, Dimitriou M, Bolton JL, Fowkes GR, Kovacs P, Lindström J, Zemunik T, Bandinelli S, Wild SH, Basart HV, Rathmann W, Grallert H; DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium, Maerz W, Kleber ME, Boehm BO, Peters A, Pramstaller PP, Province MA, Borecki IB, Hastie ND, Rudan I, Campbell H, Watkins H, Farrall M, Stumvoll M, Ferrucci L, Waterworth DM, Bergman RN, Collins FS, Tuomilehto J, Watanabe RM, de Geus EJ, Penninx BW, Hofman A, Oostra BA, Psaty BM, Vollenweider P, Wilson JF, Wright AF, Hovingh GK, Metspalu A, Uusitupa M, Magnusson PK, Kyvik KO, Kaprio J, Price JF, Dedoussis GV, Deloukas P, Meneton P, Lind L, Boehnke M, Shuldiner AR, van Duijn CM, Morris AD, Toenjes A, Peyser PA, Beilby JP, Körner A, Kuusisto J, Laakso M, Bornstein SR, Schwarz PE, Lakka TA, Rauramaa R, Adair LS, Smith GD, Spector TD, Illig T, de Faire U, Hamsten A, Gudnason V, Kivimaki M, Hingorani A, Keinanen-Kiukaanniemi SM, Saaristo TE, Boomsma DI, Stefansson K, van der Harst P, Dupuis J, Pedersen NL, Sattar N, Harris TB, Cucca F, Ripatti S, Salomaa V, Mohlke KL, Balkau B, Froguel P, Pouta A, Jarvelin MR, Wareham NJ, Bouatia-Naji N, McCarthy MI, Franks PW, Meigs JB, Teslovich TM, Florez JC, Langenberg C, Ingelsson E, Prokopenko I, Barroso I. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet* 2012; **44**: 991-

1005 [PMID: 22885924 DOI: 10.1038/ng.2385]

82 **Carcamo-Orive I**, Henrion MYR, Zhu K, Beckmann ND, Cundiff P, Moein S, Zhang Z, Alamprese M, D'Souza SL, Wabitsch M, Schadt EE, Quertermous T, Knowles JW, Chang R. Predictive network modeling in human induced pluripotent stem cells identifies key driver genes for insulin responsiveness. *PLoS Comput Biol* 2020; **16**: e1008491 [PMID: 33362275 DOI: 10.1371/journal.pcbi.1008491]

83 **Su S**, Guntur AR, Nguyen DC, Fakory SS, Doucette CC, Leech C, Lotana H, Kelley M, Kohli J, Martino J, Sims-Lucas S, Liaw L, Vary C, Rosen CJ, Brown AC. A Renewable Source of Human Beige Adipocytes for Development of Therapies to Treat Metabolic Syndrome. *Cell Rep* 2018; **25**: 3215-3228. e9 [PMID: 30540952 DOI: 10.1016/j.celrep.2018.11.037]

84 **Del Carmen Ortuño-Costela M**, García-López M, Cerrada V, Gallardo ME. iPSCs: A powerful tool for skeletal muscle tissue engineering. *J Cell Mol Med* 2019; **23**: 3784-3794 [PMID: 30933431 DOI: 10.1111/jcmm.14292]

85 **Yamaguchi T**, Matsuzaki J, Katsuda T, Saito Y, Saito H, Ochiya T. Generation of functional human hepatocytes in vitro: current status and future prospects. *Inflamm Regen* 2019; **39**: 13 [PMID: 31308858 DOI: 10.1186/s41232-019-0102-4]

86 **Hockemeyer D**, Jaenisch R. Induced Pluripotent Stem Cells Meet Genome Editing. *Cell Stem Cell* 2016; **18**: 573-586 [PMID: 27152442 DOI: 10.1016/j.stem.2016.04.013]

87 **McKenna A**, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010; **20**: 1297-1303 [PMID: 20644199 DOI: 10.1101/gr.107524.110]

88 **Yu H**, Cowan CA. Minireview: Genome Editing of Human Pluripotent Stem Cells for Modeling Metabolic Disease. *Mol Endocrinol* 2016; **30**: 575-586 [PMID: 27075706 DOI: 10.1210/me.2015-1290]

89 **Park CY**, Sung JJ, Choi SH, Lee DR, Park IH, Kim DW. Modeling and correction of structural variations in patient-derived iPSCs using CRISPR/Cas9. *Nat Protoc* 2016; **11**: 2154-2169 [PMID: 27711053 DOI: 10.1038/nprot.2016.129]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com