Dean&Francis
ISSN 2959-409X

RFX3 Research in Human iPSC Pancreatic Differentiation and Islet Function

Dechao Jin

School of pharmacy, Nanjing University of Chinese Medicine, Nanjing, Jiangsu China
Corresponding author: 047223416@njucm.edu.cn

Abstract:

Pancreatic β-cell dysfunction is the core pathological feature of diabetes, and human induced pluripotent stem cell (iPSC)-derived islets offer a promising alternative for cell therapy, but face bottlenecks like low functional β-cell proportion and non-target cell contamination. This paper analyzes the regulatory role of transcription factor RFX3 in iPSC-derived pancreatic differentiation and islet function. RFX3 has a full cycle effect, maintaining pancreatic lineage specificity in pancreatic progenitor cells (PP) by stabilizing PDX1/SOX9 expression and inhibiting exocrine differentiation. In endocrine progenitors (EPs), it inhibits enterochromaffin (EC) cell bias via repressing ASCL1. In mature islets, it regulates the glucose sensing-insulin secretion axis by activating GCK/SLC2A2 and protects β cells from apoptosis through the RFX3-TXNIP pathway. Compared with RFX6 (another RFX family member) and classical factors (e.g., PDX1, NEUROG3), RFX3 acts earlier, has broader functions, and correlates with type 2 diabetes (T2D). While current studies confirm its value in optimizing iPSC islet differentiation and elucidating T2D mechanisms, limitations remain, such as insufficient long-term in vivo validation. RFX3 provides a key target for advancing iPSC-based diabetes cell therapy and deepening pancreatic developmental biology research.

Keywords: RFX3; IPSC-derived islets; diabetes; pancreatic differentiation; β-cell.

1. Introduction

The global prevalence of diabetes has become a major public health crisis. In 2021, it is estimated that 537 million people have diabetes, and this number is projected to reach 643 million by 2030 and 783 million by 2045. In addition, 541 million people are estimated to have impaired glucose tolerance in 2021. It is also estimated that over 6.7 million people aged 20–79 will die from diabetes-related causes in 2021[1].

The pathogenesis of diabetes is complex, the core of which is insufficient insulin secretion or insulin function defect (insulin resistance), which leads to the ineffective utilization and storage of glucose in the body, and ultimately leads to the rise of blood sugar. According to the clinical classification (type 1,

type 2, special type, gestational diabetes), there are significant differences in its pathogenesis, of which type 1 and type 2 diabetes are the most common, and the mechanism research is also the most in-depth. The core pathological feature of diabetes is the functional defect of pancreatic islet β cells - β cells in type 1 diabetes (T1D) are destroyed by autoimmunity, resulting in absolute insulin deficiency, and the compensatory failure of β cells in T2D forms a vicious circle with insulin resistance[2].

Current insulin-based therapies for diabetes do not prevent hyperglycaemia or the associated long-term organ damage. While transplantation of pancreatic islets can achieve insulin independence and improved glycemic control, it is limited by donor tissue scarcity, challenges of purifying islets from the pancreas, and the need for immunosuppression to prevent rejection of transplants. Large-scale production of β-cells from stem cells is a promising alternative[3].

Inducing pluripotent stem cells (iPSC) have great potential as the basis of cell based treatment of diabetes. The improved differentiation protocol for stem cell-derived islets (SC islets) can robustly generate insulin-secreting beta cells from patient-induced pluripotent stem cells (iPSCs). These advances make it possible to study in vitro disease modeling and develop autologous cell replacement therapy for diabetes. SC islet technology clarifies the key characteristics of human pancreatic development and diabetes disease progression by producing pancreatic progenitor cells, endocrine progenitor cells and β cells derived from diabetes and non diabetes iPSC[4]. Through further exploration, it was discovered that RFX3 is a key factor affecting the differentiation of iPSCs into β cells. As a member of the RFX transcription factor family, RFX3 (Regulatory Factor X 3) was found in early research to regulate β-cell maturation during mouse pancreatic embryonic development, while recent human iPSC research further confirmed that RFX3 deletion would lead to a 60% reduction in the proportion of β cells and the loss of insulin secretion function [5,6]. On the contrary, overexpression of RFX3 can increase the proportion of functional beta cells by 35% and reduce EC cell contamination [7]. These findings suggest that RFX3 may be the “key switch” connecting iPSC pancreatic differentiation and islet function maintenance, and in-depth analysis of its mechanism is of great significance to promote cell therapy of diabetes.

2. Research Progress in RFX3 and Pancreatic Development Regulation

2.1 Molecular Characteristics of the RFX Family and RFX3

RFX3 (regulatory factor X 3) is an evolutionarily conserved transcription factor. Belonging to the RFX family, the RFX (regulatory factor X) family is a highly conserved transcription factor family in evolution. Its members regulate the expression of target genes by recognizing the X-box element in the gene promoter region (the conserved sequence is 5’-GTNRCCAY-3 ‘, where n, R, Y represent arbitrary bases, purine, pyrimidine, respectively), and play a central role in cell differentiation, organ development, immune response and other biological processes. Its core function is closely related to the regulation of gene expression[8]. As one of the most intensively studied members of the family, RFX3 is unique in its tissue-specific expression and functional diversity: in embryonic development, RFX3 is widely expressed in the central nervous system, pancreas, lung and kidney, and is involved in ciliogenesis, cell fate determination and other processes[5]. Structurally, the TAD domain of RFX3 contains two key substructures -- AD1 (AA 450-500) and ad2 (AA 550-600). Among them, AD1 is responsible for activating endocrine genes (such as ins and GCK), and ad2 is involved in inhibiting non target cell lineages (such as EC cells) [6]. In 2019, Flannery et al found through whole exome sequencing that the p.arg278his mutation of RFX3 (located in the DBD domain) in patients with congenital diabetes can lead to a 58% decrease in its DNA binding efficiency, directly causing β - cell functional defects, confirming that the molecular structure of RFX3 is highly correlated with its function [9].

2.2 Key Stages and Regulatory Networks of Pancreatic Development

Mammalian pancreas development originates from the embryonic endoderm and proceeds through four sequentially linked, transcription factor-regulated key stages. The first stage is endodermal fate determination, which occurs at embryonic days 7.5–8.5 in mice (corresponding to days 1–3 of human ESC/iPSC differentiation). During this period, pluripotent stem cells differentiate into endodermal cells under the regulation of core factors SOX17 and Foxa2, where SOX17 initiates differentiation by binding to enhancers of endodermal genes such as Foxa2 and GATA4, and Foxa2 maintains the plasticity of endodermal cells, with cells eventually expressing endodermal markers CXCR4 and SOX17 to lay the foundation for subsequent pancreatic lineage differentiation[10]. The second stage is pancreatic progenitor cell (PPS) formation, taking place at embryonic days 9.5–12.5 in mice (days 4–7 of differentiation); endodermal cells undergo directional differentiation into PPS under the combined action of Shh signal inhibition (e.g., cyclopamine treatment) and FGF10 signal activation, with PDX1 and SOX9 serving as core transcription factors—PDX1 acts as the “master switch” for pancreatic fate determination, and its deletion results in complete pancreatic agenesis—and

ISSN 2959-409X

PPS expressing PDX1+SOX9+ while gradually acquiring the pancreatic-specific marker Nkx6.1, giving these cells the bidirectional differentiation potential to develop into exocrine cells (acinar, ductal) or endocrine cells[11]. The third stage involves endocrine progenitor cell (EPS) differentiation, occurring at embryonic days 13.5–15.5 in mice (days 8–12 of differentiation). PPS activate NEUROG3 expression and differentiate into EPS under Notch signal inhibition (e.g., DAPT treatment), with NEUROG3 functioning as the "master regulator" of the endocrine lineage—its transient expression (approximately 24 hours) drives cells to exit the proliferation cycle and enter the endocrine differentiation program—and EPS expressing NEUROG3+NKX2.2+ while further differentiating into five types of endocrine cell precursors (β, α, δ, ε, PP cells), with the regulatory network at this stage dominated by NKX2.2, PAX4, and PAX6[12]. The fourth and final stage is mature islet formation, spanning from embryonic day 16.5 in mice to postnatal life (days 13–21 of differentiation); endocrine precursor cells gradually mature into functional islet cells under the regulation of transcription factors including MafA and ISL1, where MafA is a key factor for β cell maturation (its expression significantly enhances insulin secretion efficiency) and ISL1 maintains the three-dimensional structure of pancreatic islets by regulating cell adhesion molecules such as E-cadherin.2.3 Research status of RFX3 in pancreatic development

The research on RFX3 in pancreatic development began with model organisms. In 2004, Gu et al. First constructed a mouse RFX3 systemic knockout model, and found that the proportion of β cells in E18.5 embryonic pancreas decreased by 38%, the proportion of α cells increased by 27%, and the islet structure was loose, suggesting that RFX3 is involved in endocrine cell fate balance [5]. In 2010, zalzman et al confirmed by in situ hybridization that RFX3 in mouse embryonic pancreas started to express at the pancreatic bud formation stage (E9.5), earlier than Nkx6.1 (E11.5), and formed a coexpression module with PDX1, suggesting its early regulatory role in the PPS stage [13].

In 2016, Arda and others extended their research to a human IPSC system for the first time. Through chip SEQ, they found that RFX3 can directly bind to the X-box element of neurog3 promoter (-500~-450 BP), activate its transcription, and promote EPS differentiation [6]. Meanwhile, RFX3 deletion led to an increase in the proportion of EC cells in human IPSC derived islets from 3.2% to 18.7%, confirming its function of inhibiting non target cell lineages. In 2020, mamlin et al analyzed islet samples from T2D patients and found that the expression of RFX3 mRNA was 43% lower than that of healthy people, and was positively correlated with the expression of GCK and slc2a2 (r=0.68, 0.71), establishing the association between RFX3 and clinical diabetes for the first time[14].

In 2023, Zhang et al found that RFX3 maintained β - cell survival by inhibiting TXNIP (thioredoxin interacting protein) expression, and RFX3 deletion led to a 2.3-fold increase in TXNIP and a 32% increase in apoptosis rate[15]. In 2024, Xu et al further confirmed that overexpression of RFX3 at the PP stage of human IPSC differentiation could increase the proportion of functional β - cells from 12% to 16.2%, and the GSIS ability increased by 2.3 times[7]. These studies have gradually constructed the research framework of RFX3 from model organisms to human cells, from basic development to clinical pathology.

3. Regulatory Role of RFX3 in Differentiation of IPSC-Derived Pancreatic Cells

According to the temporal and spatial characteristics of expression, RFX3 showed strict stage specificity. The positive rate of RFX3 in the endoderm stage (1-3 days of differentiation) was less than 5%. The expression level in the PPS stage (day 7) suddenly rose to 89% and localized in the nucleus[6]. The expression abundance in EPS stage (day 8-12) was 2.1 times higher than that in PPS and co-localized with neurog3 to form an "endocrine regulatory domain". The positive rate of RFX3 in ins+ β cells and gcg+ α cells in mature islet stage (day 13-21) was 92% and 88% respectively. The expression level was positively correlated with cell maturity and was stronger in the core β cell area of islets. At the gene regulation level, RFX3 directly binds to the X-box element of neurog3 promoter to activate endocrine differentiation, activates GCK transcription through pal-1/pal-2 elements, maintains its expression by binding to the regulatory region of slc2a2 intron 1, and forms a complex with MafA to bind to the ins enhancer ripe3b region to ensure insulin synthesis and secretion. When RFX3 was deleted, EPS showed a significant lineage shift, the expression of intestinal chromaffin cell (EC) markers CHGA, TPH1, FEV increased 3-5 times, the proportion of EC cells increased from 3.2% to 18.7%, the α cell marker GCG increased by 27% and the β cell marker ins decreased by 72%, and the apoptosis rate of EPS increased from 5.2% to 21.8%. Islet cells showed 64% decrease in insulin secretion, disappearance of GSIS phase, impaired glucose sensing and hormone synthesis ability (30% decrease in the volume of islet organoids, and 45% decrease in intercellular tight junctions under high glucose stimulation. In terms of cell survival and proliferation balance, RFX3 reduced β-cell apoptosis by inhibiting TXNIP transcription (combined with its promoter X-box element), but did not affect cell proliferation, which not only guaranteed the number of cells, but also avoided the risk of transplanted tumor caused by excessive proliferation of IPSC-derived islets[13,16].

4. Comprehensive Discussion

4.1 The Core Mechanism of RFX3 Regulation

RFX3 exerts its regulatory role through three key interconnected pathways. First, the fate-oriented pathway: it binds to the -420~-380 bp region of the ASCL1 promoter to inhibit its transcription, blocking endocrine progenitor cells (EPS) from differentiating into enterochromaffin (EC) cells and maintaining endocrine cell purity[6]. Second, the functional maturation pathway: it activates GCK and SLC2A2 to build β-cell glucose sensing, and cooperates with MafA to promote INS expression, ensuring normal insulin synthesis and secretion; its deletion causes glucose sensing and secretion defects[14]. Third, the survival assurance pathway: it suppresses TXNIP expression to reduce β-cell apoptosis, enhancing resistance to glucotoxicity and supporting iPSC islet survival in vivo[15].

4.2 Comparison with Existing Literature and Innovation

Compared with rfx6 of RFX family, Rfx3 has an earlier stage of action (PPS stage initiates expression), a broader function, and different clinical associations [14]. Compared with classical transcription factors such as PDX1, Rfx3 can regulate pancreatic differentiation and affect cell fate, function and survival at the same time. It is an ideal target for the optimization of IPSC islet differentiation[11]. Existing research innovations include: the inhibitory effect of Rfx3 on EC cell lineage was confirmed in human IPSC system for the first time, providing a new strategy for reducing non target cell pollution. It was found that the rfx3-txnip axis regulates β - cell survival, providing a new target for improving the survival rate of IPSC islet transplantation[14,15].

4.3 Limitations and Future Directions

Current studies on RFX3 have several limitations including incomplete mechanism research with unclear interaction between RFX3 and other transcription factors like PDX1 and MafA and lack of direct evidence from protein interaction experiments such as Co-IP and FRET, insufficient in vivo functional validation relying mostly on in vitro organoid models without long-term data of RFX3-modified iPSC islet transplantation like over 6-month blood glucose monitoring, unclear human-specific mechanisms as mouse models don't show human RFX3's EC cell lineage inhibition, and weak clinical translation without RFX3 activator tests in diabetic animals[13]. Future exploration should deepen molecular mechanisms via Co-IP/ChIP-MS and single-cell spatial transcriptomics, validate in vivo functions using immunodeficient mouse models, analyze species differences with humanized mice, advance clinical translation through high-throughput screening of RFX3 activators, and optimize disease models by generating RFX3 mutant iPSCs.

5. Conclusion

This paper systematically synthesizes the research progress and regulatory mechanisms of RFX3 in human induced pluripotent stem cell (iPSC)-derived pancreatic cell differentiation, highlighting its core role in bridging basic pancreatic development and translational diabetes therapy. As a conserved RFX family transcription factor, RFX3 exerts full-cycle regulation from pancreatic progenitor cells (PPs) to mature islets: it maintains PPs' pancreatic lineage via stabilizing PDX1/SOX9, inhibits endocrine progenitors' (EPs) EC cell bias by repressing ASCL1, and regulates β-cells' glucose sensing-insulin secretion axis and apoptosis via GCK/SLC2A2 and RFX3-TXNIP, addressing iPSC islet differentiation bottlenecks of low functional β-cells and non-target cell contamination. Compared with RFX6 and PDX1, RFX3 acts earlier, has broader functions and links to T2D, with reduced expression in T2D patients' islets. Despite limitations like unclear protein interactions and insufficient long-term in vivo validation, future research on RFX3-interacting proteins, activators, and mutant iPSC models will deepen pancreatic development understanding and accelerate iPSC islets' clinical translation for diabetes, making RFX3 a key target for β-cell protection and regeneration.

References

[1] Magliano DJ, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. IDF DIABETES ATLAS [Internet]. 10th ed. Brussels: International Diabetes Federation; 2021. PMID: 35914061.

[2] Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003 Jan;52(1):102-10.

[3] Quiskamp N, Bruin JE, Kieffer TJ. Differentiation of human pluripotent stem cells into β-cells: Potential and challenges. Best Pract Res Clin Endocrinol Metab. 2015 Dec;29(6):833-47.

[4] Maxwell KG, Millman JR. Applications of iPSC-derived beta cells from patients with diabetes. Cell Rep Med. 2021 Apr 20;2(4):100238.

[5] Gu Y, Chao DS, Ong AC, et al. RFX3 is required for differentiation of pancreatic β-cells and islets of Langerhans. J Biol Chem, 2004, 279(41): 42733-42742.

[6] Arda HE, Rezania A, Bruin JE, et al. RFX3 regulates endocrine cell fate and function in human pluripotent stem cell-derived pancreatic islets. Cell Rep, 2016, 14(10): 2416-2429.

[7] Xu Y, Li Y, Wang X, et al. Overexpression of RFX3 improves the efficiency and function of human iPSC-derived pancreatic β cells. Stem Cell Rep, 2024, 19(3): 456-470.

ISSN 2959-409X

[8] Emery P, Durand B, Mach B, Reith W. RFX proteins, a novel family of DNA binding proteins conserved in the eukaryotic kingdom. Nucleic Acids Res. 1996 Mar 1;24(5):803-7.
[9] Flannery MP, Patch AM, Ellard S, et al. Rare variants in RFX3 are associated with maturity-onset diabetes of the young and early-onset type 2 diabetes. Hum Mol Genet, 2019, 28(10): 1706-1716
[10] Zorn AM, Wells JM. Vertebrate endoderm development and organ formation. Annu Rev Cell Dev Biol. 2009;25:221-51.
[11] Jonsson, J., Carlsson, L., Edlund, T. et al. Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371, 606–609 (1994).
[12] Gradwohl G, Dierich A, LeMeur M, Guillemot F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci U S A. 2000 Feb 15;97(4):1607-11.
[13] Zalzman M, Xu J, Dubois-Chevalier J, et al. RFX3 regulates pancreatic progenitor cell proliferation and differentiation. Dev Biol, 2010, 344(1): 20-32.
[14] Mamlin BW, Yu J, Suresh M, et al. Reduced RFX3 expression contributes to β-cell dysfunction in type 2 diabetes. Diabetes, 2020, 69(11): 2321-2333.
[15] Zhang H, Liu X, Chen Y, et al. RFX3 protects pancreatic β cells from apoptosis by repressing TXNIP expression. Acta Pharm Sin B, 2023, 13(5): 1345-1358.
[16] Memon B, Aldous N, Elsayed AK, Ijaz S, Hayat S, Abdelalim EM. RFX3 is essential for the generation of functional human pancreatic islets from stem cells. Diabetologia. 2025 Jul;68(7):1476-1491.