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Review

PAX proteins and their role in pancreas

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ABSTRACT

Gene regulatory factors that govern the expression of heritable information come in an array of flavors, chiefly with transcription factors, the proteins which bind to regions of specific genes and modulate gene transcription, subsequently altering cellular function. PAX transcription factors are sequence-specific DNA-binding proteins exerting its regulatory activity in many tissues. Notably, three members of the PAX family namely PAX2, PAX4 and PAX6 have emerged as crucial players at multiple steps of pancreatic development and differentiation and also play a pivotal role in the regulation of pancreatic islet hormones synthesis and secretion. Providing a comprehensive outline of these transcription factors and their primordial and divergent roles in the pancreas is far-reaching in contemporary diabetes research. Accordingly, this review furnishes an outline of the role of pancreatic specific PAX regulators in the development of the pancreas and its associated disorders.

Contents

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1. Introduction

The development of pancreas encompasses a diverse range of developmental regulators including PAX proteins. Paired box (PAX), a family of transcriptional genes is highly conserved and play a key role in embryonic patterning and organogenesis [1]. All PAX factors comprises of a DNA binding domain namely bipartite paired domain (PD) with 128 amino acids and four of nine PAX factors (PAX3, PAX4, PAX6 AND PAX7) contain additional helix turn helix homeodomain (HD) with 60 amino acids which evinces its role in DNA binding or transcriptional activation via these distinct domains. In mammals, PAX genes are grouped under four subfamilies based on the presence or absence of two domains such as octapeptide motif which is distal to the PD and complete or truncated homeodomain [2]. The octapeptide motif (8 amino acids) serves as a binding region for protein co-factors and well known for its transcriptional inhibitory action of the downstream genes [3]. Members of PAX family comprise of a C-terminal transactivation domain (TD) with a proline, threonine and serine-rich region conciliating transcriptional regulation [4]. Nine PAX genes (PAX1-PAX9) in mammals are evolutionarily conserved, with orthologues in insects, amphibians and birds [5].

Based on the genomic structure, sequence similarity and conserved function, the PAX genes are assorted into four subfamilies [Fig. 1]. Subfamily I (PAX1 and PAX9) consists of a paired domain and octapeptide, subfamily II (PAX2, PAX5 and PAX8) with a paired domain, a partial homeodomain and octapeptide. Subfamily III (PAX3 and PAX7) consists of a paired domain, complete homeodomain and octapeptide, subfamily IV (PAX4 and PAX6) comprises a paired and complete homeodomain [6]. Further, splice variants of PAX members were also identified and said to have different transactivation potentials in regulating target genes by acting either as an enhancer or repressor. The alternative splicing events result in either removal of transactivation and/or inhibitory domains or insertion of novel protein domains at C-terminal or N-terminal of PAX proteins [7,8]. Interestingly, both PAX-2 and PAX-8 in humans encompass a number of alternatively spliced isoforms with significantly distorted protein structure and sequence in their respective C-terminal domains [7]. Similarly, alternative splicing events in the PAX6 N-terminal paired domain engenders several protein isoforms with diverse DNA-binding properties [8]. Expression of mutant PAX genes and the resulting abnormalities emphasizes its importance in the development of eyes, ear, brain, kidney, thyroid gland, immune system, teeth and the pancreas [5,9]. Inactivation of all nine PAX genes by targeted mutagenesis results in embryonal or neonatal death in mice [10].

Thus far, PAX2 and PAX8 (subfamily II), as well as PAX4 and PAX6 (subfamily IV) are said to be expressed in pancreas and chiefly PAX2, PAX4 and PAX6 have been linked with pancreatic development and function [Fig. 2], whereas small nucleotide polymorphism or mutations in PAX4, PAX6 and PAX8 genes results in impaired glucose homeostasis and Diabetes Mellitus (DM) [11]. The above established facts provoke the need for investigating the role of pancreas specific PAX members and this review summarizes the prevalent science of PAX proteins in pancreatic development, differentiation and function.

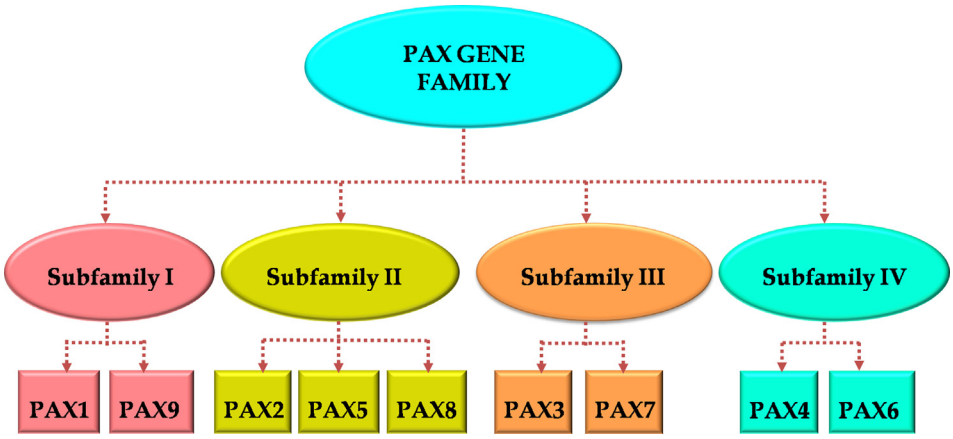

Fig. 1 – Classification of PAX gene family.

2. Subfamily II

2.1. PAX2

The second subgroup member of PAX family, PAX2 is a requisite for the development of central nervous system, eye, ear, kidney and mammary gland [12–14]. The two isoforms of PAX2 (PAX-2A and PAX-2B) were detected in adult rat islets by RT-PCR and binding of these isoforms to the enhancer element (G1 and G3), transactivates the glucagon promoter in a dose-dependent manner [15]. Immunoreactive PAX2 protein was detected from glucagon-producing mouse (alpha TC1) and hamster (InR1-G9) islet cell lines and an *in-vitro* study with transfected fibroblast and islet cell lines validates that PAX2, as the regulator of islet proglucagon gene expression [16]. The maximal level of PAX2 transcripts were expressed as early as embryonic day (E10.5) in mice, remarkably PAX2 mRNA was also seen in human islets. Heterozygous $PAX2^{1Neu}$ and homozygous mutant mice showed two to three-fold increase in pancreatic volume, predominantly occupied by beta cells of islets larger in number and size and not by alpha cells. The role of PAX2 in determining the endocrine-exocrine fate designates it as a critical regulator, the loss of which leads to the expansion of endocrine cells during embryonic development [17]. Hence, to divulge the role of PAX2 in pancreatic development and function and pathophysiology is still uncertain, revealing the need for further studies.

2.2. PAX8

Genome wide analysis unveiled the expression of paired box transcription factor PAX8 in islets of pregnant mice [18]. Under non-pregnancy condition, PAX8 transcripts were found to be absent in adult murine islets whereas low levels of mRNA were seen in adult human islets [19]. The expression of PAX8 was also witnessed in thyroid and urogenital system of mouse and human embryogenesis [20]. Former studies upheld the necessity of PAX8 in the development of thyroid and production of thyroxine (T_4) while, low levels of free T_4 cognates with gestational diabetes, thus evincing the link between altered PAX8 expression and DM under specified metabolic conditions like pregnancy [21]. Also, studies confirmed the association of PAX8 SNPs with type II diabetes in African American families [22]. Still, further research is needed to affirm the role of PAX8 in pancreatic endocrine physiology.

3. Subfamily IV

3.1. PAX4

The paired domain containing transcription factor 4, is crucial for the development of pancreatic beta and delta cell. PAX4 expression was first detected in the murine pancreas at embryonic day E9.5 and the ventral spinal cord during embry-

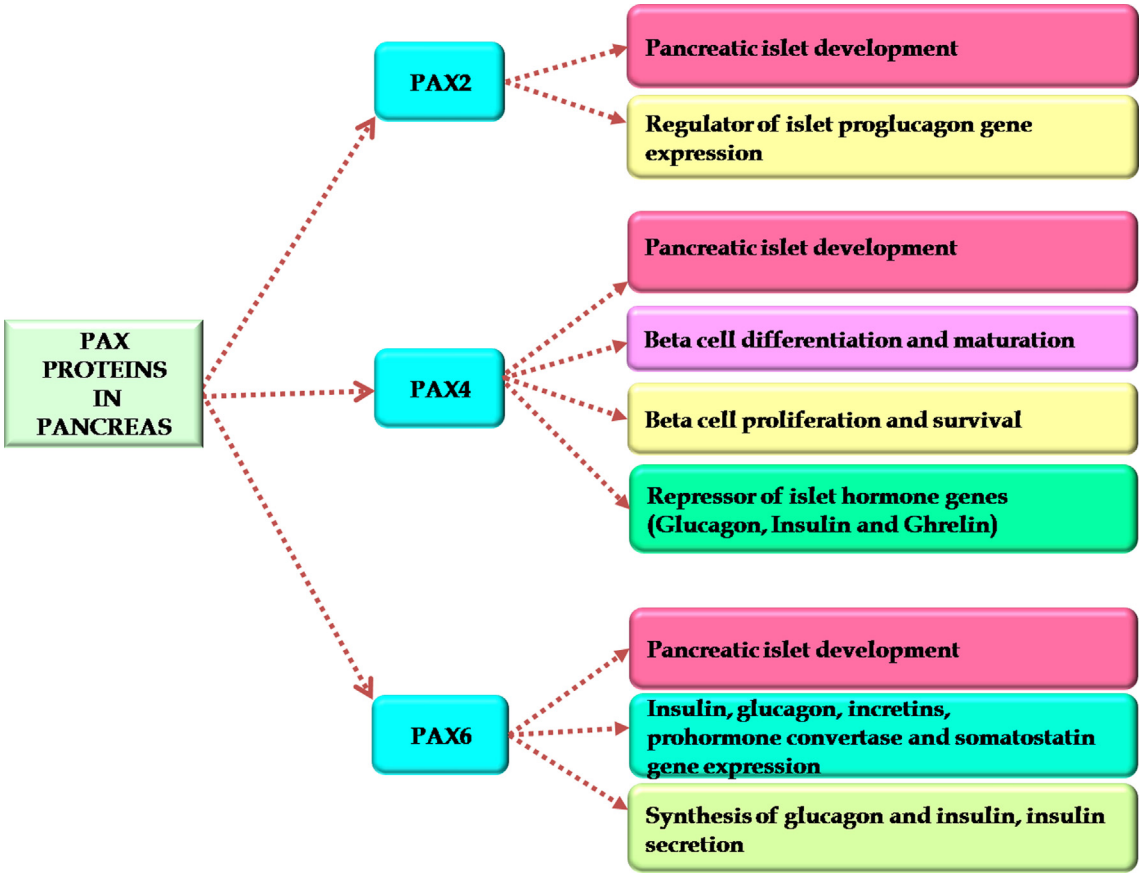

Fig. 2 – Functions of PAX proteins in pancreas.

onic development and maximal expression was seen at E13.5-15.5 [23]. PAX4 expression was downregulated shortly after birth whereas its expression was still detectable in adult islets [24].

3.1.1. *Role of PAX4 in pancreatic islet development*

Lineage analysis of PAX4 positive cells revealed that they are equally essential for all the four types of islets [25]. PAX4 mutant ($PAX4^{-/-}$) mice resulted in complete deprivation of beta and delta cells and died within 3 days after birth due to diabetes. Remarkably, mice harboring a heterozygous mutation of PAX4 ($PAX4^{+/-}$) had beta cells and did not develop diabetes, unfolding the fact that a single copy of PAX4 is necessary for normal beta cell development and function [23].

3.1.2. *Role of PAX4 in beta cell function*

Reports by *in-vivo* studies in mice assorted that over expression of PAX4 stimulated the endocrine precursor cells and matured alpha cells to adopt into beta cells [26]. Likewise, *in-vitro* studies with embryonic glucagon positive cells affirmed that over expression of PAX4 promoted their neogenesis followed by renovation into beta like cells [26,27]. Also, PAX4 mediated downregulation of Arx in glucagon-expressing cells was found to induce the conversion of alpha cells into beta like cells, validating the importance of PAX4 in beta cell differentiation. Studies revealed that PAX4 decreased the expression of glucagon, ghrelin and insulin by acting as a key repressor of islet hormone genes [28] and also inhibit the expression of islet amyloid polypeptide [29] and glucose transporter 2 (GLUT2) [30], thus speculating the importance of PAX4 in beta cell maturation. *In-vitro* studies with overexpression of PAX4 in human and rat islets induced the beta cell proliferation; elucidating its role in beta cell survival [31,32]. Also, *in-vivo* studies confirmed that PAX4 over expression protected the beta cells against streptozotocin (STZ) induced DM in adult mice [32].

3.1.3. *Role of PAX4 in diabetes*

Several studies have shown that various mutations of PAX4 resulted in type I diabetes (T1DM), early onset of type 2 diabetes (T2DM), ketosis prone diabetes mellitus (KPDM) and maturity onset of the diabetes of the young (MODY) in humans [33–36]. Mutations in PAX4 and its associated diabetes over different populations are reviewed in Table 1.The available literature on PAX4 polymorphisms/mutations upholds PAX4 as an important DM susceptibility gene and a valuable target for developing new therapies as an intervention for DM. Despite of all available findings, development of novel approaches towards DM treatment greatly relies upon mechanism based studies of PAX4.

3.2. PAX6

Paired box gene PAX6 plays a vital role in the early development of pancreatic islet cells, nose, eyes and central nervous system. PAX6 was found to be expressed in mouse pancreas at embryonic day 9.0 in both dorsal and ventral pancreatic buds and is restricted to all endocrine cells during development. In the adult murine pancreas, PAX6 expression was seen in all endocrine cells [50–52]. Mutations in the PAX6 gene manifest hereditary irregularities in human, rat and mice. In the knockout mice, Sey/Sey (mutant small eyes) homologous embryo and Sey 1Neu mutation of PAX6 resulted in developmental malformations such as failure in eyes, nasal structure, forebrain patterning abnormalities and modifications in pancreatic islet morphology resulting in neonatal lethality [50,51,53,54].

3.2.1. *Role of PAX6 in islet hormone gene expression*

Studies with homozygous null mutant PAX6 mice revealed that PAX6 is vital for the expression of insulin and glucagon and also for differentiation and functioning of alpha and beta cells of the pancreas [55]. PAX6 deletion study in adult mice by tamoxifen injection analyzed through RNA sequencing and quantitative real-time PCR affirmed the reduction in expression of specific beta cell genes, which signifies PAX6 requirement in the functional identity of adult beta cells and carriers of PAX6 mutants showed impaired insulin secretion [56]. A pancreatic endocrine specific PAX6 ablation in homozygosis exhibits altered glucose regulation which was seen in mice; where immunohistochemical studies reported a reduction in insulin and glucagon expressing cells [52]. In adult islets, the absence of PAX6 resulted in reduced secretion of insulin, glucagon and somatostatin [57]. Functional studies revealed that PAX6 directly mediates the expression of metabolic homeostasis genes namely insulin, glucagon, incretins, prohormone convertase and somatostatin [53,58–59].

In addition, PAX6 which is known to have DNA binding property also upholds protein-protein interaction through its paired domain and thereby regulates the transcription of different genes. Ritz-Laser et al. (2002) have proved that the pancreatic beta cell specific transcription factor PAX4 inhibits glucagon gene expression through PAX6 and have demonstrated that the transcriptional inhibition of glucagon gene expression during pancreatic development is mediated by direct DNA binding competition between PAX4 and PAX6 and further protein-protein interaction of these two transcription factors through PD of PAX6 [28].

3.2.2. *Role of PAX6 in insulin secretion*

PAX6 (R240) stop mutation was found to be linked with a deficiency of prohormone convertase which is essential for the conversion of proinsulin to insulin and human aniridia carriers of such mutation exhibited an increased proinsulin/C-peptide ratio which ends up with abnormal proinsulin processing and impaired glucose challenged insulin secretion [60,61]. Similarly, mice with heterozygous PAX6 (R266) stop mutation displayed prohormone convertase 1/3 deficiency which results in abnormal proinsulin processing and defect in glucose metabolism [60]. Likewise, rat harboring heterozygous PAX6 (rSey/+) mutation showed impairment in glucose-stimulated first-phase insulin secretion [62]. Mice with heterozygous mutation of PAX6 revealed that suppression of circulating GLP1 and its mRNA in the intestine and also they exhibited lower insulin secretion in islets [63]. In PAX6-deficient YFP-labeled cells, loss of expression of glucagon like peptide 1 receptor (GLP1R) plays a significant role in glucose stimulated insulin secretion (GSIS) [64].

Table 1 – PAX4 mutations associated with different types of diabetes mellitus and its consequences in various populations.

Population	Mutation type	Type of DM	Consequences	Authors
Japanese	R121W (Missense mutation)	T2DM	Beta cell dysfunction	Shimajiri et al. [37] Tokuyama et al. [38] Cho et al. [39] Ma et al. [40]
Asian (East Asian and Chinese)	rs6467136 and rs10229583	T2DM	–	Shimajiri et al. [41] Kanatsuka et al. [42]
West African ancestry	R133, R37W	KPDM	Modification of beta cell functionality	Mauvais-Jarvis et al. [36]
Thai, North Indian	PAX4 R192H	MODY	Alteration of transcriptional repression activity of PAX4 under glucotoxic condition, decreased beta cell survival and beta cell dysfunction	Kooptiwut et al. [43] Chavali et al. [44] Sujjitjoon et al. [45]
Thai	PAX4 R164W	MODY	Alteration in transcriptional functionality, glucose induced apoptosis in INS-1 cells	Sujjitjoon et al. [46]
Thai	IVS7-1G > A (Heterozygous)	MODY (Type 9)	Alteration in transcriptional functionality, high glucose induced apoptosis in INS-1 cells	Sujjitjoon et al. [46]
Japanese	c.374-412 del39	MODY	Altered splicing mechanism-non existence of a part of homeodomain, loss in repression functionality.	Jo et al. [47]
Caucasian	rs712701 (P321H)	Correlation with T1DM	–	Hermann et al. [48] Zhang et al. [49]
Finish, Hungarian and Asian	rs712701 (P321H)	No correlation with T1DM	–	Hermann et al. [48] Zhang et al. [49]

3.2.3. *Role of PAX6 in hyperglycemia*

A recent study confirmed that adult mice harboring beta-cell-specific PAX6 knockout developed hyperglycemia [64]. Studies by Balakrishnan et al., 2014 indicated that high glucose concentrations down regulate PAX6 expression through the c-Jun N-terminal kinase (JNK)/p38 mitogen activated protein kinase (p38 MAPK) pathways in INS-1 cells [65]. Concurrently, Tsui et al., 2014 showed that glucose up regulates the CCCTC-binding factor and consequently down regulates PAX6 expression through ERK signaling in β-TC-1-6 cells [66]. In addition, PAX6 expression was reduced in beta cells of hyperglycemic and insulin-resistant mice confirm the significance of appropriate expression levels of PAX6 for the maintenance of beta cell identity and glucose homeostasis [67]. Several studies highlights the expression pattern of ARAP1 regulated by PAX6, which is associated with increased risk of T2DM, suggesting that PAX gene targets might hold promising therapeutic potential [68]. Thus, it is evident that PAX6 plays a prominent role in the regulation of islet function and glucose metabolism.

4. PAX proteins – as therapeutic tool

Recent experimental studies highlight the significance of beta cell failure in the pathogenesis of diabetes. Successful islets and whole pancreas transplantations have already proved that inadequate insulin could be restored by replacement of functional beta cells. Currently, efforts are aiming at identifying novel tiny molecules that are able to stimulate beta cell proliferation, insulin secretion and reprogramming from other cell types in order to treat diabetes. Studies with adenovirus-mediated PAX4 expression stimulated phenotypic conversion of alpha cells to beta cell in both αTC1.9 and isolated human islets [69] and further, administration of intra-bile ductal injection of adenoviruses expressing human PAX4 into insulin deficient diabetic mouse model showed decreased random blood glucose levels [70], thus endorsing PAX4 as a novel target for gene therapeutic approach to treat insulin deficient diabetes. Concurrently, in a model of experimental autoimmune diabetes (RIP-B7.1 mice), beta cells are protected from apoptosis by PAX4 expression, concerning a significant reduction in islet immune cells infiltration (insulitis), spotlighting the association of this transcription factor in immune modulation. Galectin 9 (LGALS9), a potent immunomodulator involved in this immune modulation process is known to be up regulated by PAX4 [32], which is proficient of reducing insulitis and hyperglycemia in NOD (Non Obese Diabetic) mice [71,72] and also prolonging islet grafts survival [73]. A recent study confirmed that beta-cell-specific PAX6 knockout adult mice developed hyperglycemia [64]. Also, high glucose suppression of PAX6 regulates tumor-suppressor phosphatase with tensin homology (PTEN) and cyclin D1, thereby involved in beta cell survival [65]. Hence, it is clear that PAX proteins can be used as a prognostic aid for DM and its treatment by replenishing beta cells.

5. Conclusion

Targeting transcription factors for remedial expand is the focus of zealous research as being able to influence transcriptional expression patterns would offer a novel approach for the treatment of many human diseases. Members of the PAX family of transcription factors orchestrate crucial tasks in pancreatic development and physiology. Modulated expressions of PAX4 and PAX6 have been repeatedly connected with diabetes. Much evidences designates that any alteration in PAX6, both over and restricted expression is linked with altered glucoregulation thereby involved in the development of DM. Targets of PAX4 and PAX6 might be hopeful candidates for the evolution of novel therapies to treat this dreadful disease. Further, insights on pancreatic specific PAX genes would offer a better perspective to extend the mechanism based interventions to impede and cure DM.

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Declaration of interest

None

REFERENCES

[1] Paixão-Côrtes VR, Salzano FM, Bortolini MC. Origins and evolvability of the PAX family. Semin Cell Dev Biol 2015;44:64–74.
[2] Mayran A, Pelletier A, Drouin J. Pax factors in transcription and epigenetic remodelling. Semin Cell Dev Biol 2015;44:135–44.
[3] Lechner MS, Dressler GR. Mapping of Pax-2 transcription activation domains. J Biol Chem 1996;271:21088–93.
[4] Robichaud GA, Nardini M, Laflamme M, Cuperlovic-Culf M, Ouellette RJ. Human Pax-5 C-terminal isoforms possess distinct transactivation properties and are differentially modulated in normal and malignant B cells. J Biol Chem 2004;279:49956–63.
[5] Blake JA, Ziman MR. Pax genes: regulators of lineage specification and progenitor cell maintenance. Development 2014;141:737–51.
[6] Mansouri A, Goudreau G, Gruss P. Pax genes and their role in organogenesis. Cancer Res 1999;59:1707–9.
[7] Kozmik Z, Kurzbauer R, Dorfler P, Busslinger M. Alternative splicing of Pax-8 gene transcripts is developmentally regulated and generates isoforms with different transactivation properties. Mol. Cell. Bio l 1993;13:6024–35.
[8] Kozmik Z, Czerny T, Busslinger M. Alternatively spliced insertions in the paired domain restrict the DNA sequence specificity of Pax6 and Pax8. EMBO J 1997;16:6793–803.

[9] Sharma R, Sanchez-Ferras O, Bouchard M. Pax genes in renal development, disease and regeneration. Semin Cell Dev Biol 2015;44:97–106.
[10] Dohrmann C, Gruss P, Lemaire L. Pax genes and the differentiation of hormone-producing endocrine cells in the pancreas. Mech Dev 2000;92:47–54.
[11] Martin-Montalvo A, Lorenzo PI, López-Noriega L, Gauthier BR. Targeting pancreatic expressed PAX genes for the treatment of diabetes mellitus and pancreatic neuroendocrine tumors. Expert Opin Ther Targets 2017;21:77–89.
[12] Nornes HO, Dressler GR, Knapik EW, Deutsch U, Gruss P. Spatially and temporally restricted expression of Pax2 during murine neurogenesis. Development 1990;109:797–809.
[13] Terzic J, Muller C, Gajovic S, Saraga-Babic M. Expression of PAX2 gene during human development. Int J Dev Biol 1998;42:701–7.
[14] Torres M, Gómez-Pardo E, Dressler GR, Gruss P. Pax-2 controls multiple steps of urogenital development. Development 1995;121:4057–65.
[15] Ritz-Laser B, Estreicher A, Gauthier B, Philippe J. The paired homeodomain transcription factor PAX-2 is expressed in the endocrine pancreas and transactivates the glucagon gene promoter. J Biol Chem 2000;275:32708–15.
[16] Flock G, Drucker DJ. Pax-2 activates the proglucagon gene promoter but is not essential for proglucagon gene expression or development of proglucagon-producing cell lineages in the murine pancreas or intestine. Mol Endocrinol 2002;16:2349–59.
[17] Zaiko M, Estreicher A, Ritz-Laser B, Herrera P, Favor J, Meda P, et al. Pax2 mutant mice display increased number and size of islets of Langerhans but no change in insulin and glucagon content. Eur J Endocrinol 2004;150:389–95.
[18] Rieck S, White P, Schug J, Fox AJ, Smirnova O, Gao N, et al. The transcriptional response of the islet to pregnancy in mice. Mol Endocrinol 2009;23:1702–12.
[19] Lorenzo PI, Jimenez Moreno CM, Delgado I, Cobo-Vuilleumier N, Meier R, Gomez-Izquierdo L, et al. Immunohistochemical assessment of Pax8 expression during pancreatic islet development and in human neuroendocrine tumors. Histochem Cell Biol 2011;136:595–607.
[20] Poleev A, Fickenscher H, Mundlos S, Winterpacht A, Zabel B, Fidler A. PAX8, a human paired box gene: isolation and expression in developing thyroid, kidney and Wilms' tumors. Development 1992;116:611–23.
[21] Velkoska Nakova V, Krstevska B, Dimitrovski Ch, Simeonova S, Hadzi-Lega M, Serafimoski V. Prevalence of thyroid dysfunction and autoimmunity in pregnant women with gestational diabetes and diabetes type 1. Prilozi 2010;31:51–9.
[22] Elbein SC, Das SK, Hallman DM, Hanis CL, Hasstedt SJ. Genome-wide linkage and admixture mapping of Type 2 diabetes in African American families from the american diabetes association GENNID (Genetics of NIDDM) study cohort. Diabetes 2009;58:268–74.
[23] Sosa-Pineda B, Chowdhury K, Torres M, Oliver G, Gruss P. The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. Nature 1997;386:399–402.
[24] Heremans Y, Van De Casteele M, Int Veld P, Gradwohl G, Serup P, Madsen O, et al. Recapitulation of embryonic neuroendocrine differentiation in adult human pancreatic duct cells expressing neurogenin 3. J Cell Biol 2002;159:303–12.
[25] Lorenzo PI, Fuente-Martin E, Brun T, Cobo-Vuilleumier N, Jimenez-Moreno CM, Herrera Gomez IG, et al. PAX4 defines an expandable beta-cell subpopulation in the adult pancreatic islet. Sci Rep 2015;5:15672.
[26] Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 2009;138:449–62.
[27] Al-Hasani K, Pfeifer A, Courtney M, Ben-Othman N, Gjernes E, Vieira A, et al. Adult duct-lining cells can reprogram into ß-like cells able to counter repeated cycles of toxin-induced diabetes. Dev Cell 2013;26:86–100.
[28] Ritz-Laser B, Estreicher A, Gauthier BR, Mamin A, Edlund H, Philippe J. The pancreatic beta-cell-specific transcription factor Pax-4 inhibits glucagon gene expression through Pax-6. Diabetologia 2002;45:97–107.
[29] He KHH, Lorenzo PI, Brun T, Jimenez Moreno CM, Aeberhard D, Ortega JV, et al. In vivo conditional pax4 overexpression in mature islet β-cells prevents stress-induced hyperglycemia in mice. Diabetes 2011;60:1705–15.
[30] Campbell SC, Cragg H, Elrick LJ, Macfarlane WM, Shennan KI, Docherty K. Inhibitory effect of pax4 on the human insulin and islet amyloid polypeptide (IAPP) promoters. FEBS Lett 1999;463:53–7.
[31] Brun T, Franklin I, St-Onge L, Biason-Lauber A, Schoenle EJ, Wollheim CB, et al. The diabetes-linked transcription factor PAX4 promotes {beta}-cell proliferation and survival in rat and human islets. J Cell Biol 2004;167:1123–35.
[32] Mellado-Gil JM, Jiménez-Moreno CM, Martin-Montalvo A, Alvarez-Mercado AI, Fuente-Martin E, Cobo-Vuilleumier N, et al. PAX4 preserves endoplasmic reticulum integrity preventing beta cell degeneration in a mouse model of type 1 diabetes mellitus. Diabetologia 2016;59:755–65.
[33] Surapolchai P, Hongeng S, Mahachoklertwattana P, Pakakasama S, Winaichatsak A, Wisanuyothin N, et al. Impaired glucose tolerance and insulin resistance in survivors of childhood acute lymphoblastic leukemia: prevalence and risk factors. J Pediatr Hematol Oncol 2010;32:383–9.
[34] Biason-Lauber A, Boehm B, Lang-Muritano M, Gauthier BR, Brun T, Wollheim CB, et al. Association of childhood type 1 diabetes mellitus with a variant of PAX4: possible link to beta cell regenerative capacity. Diabetologia 2005;48:900–5.
[35] Plengvidhya N, Kooptiwut S, Songtawee N, Doi A, Furuta H, Nishi M, et al. PAX4 mutations in Thais with maturity onset diabetes of the young. J Clin Endocrinol Metab 2007;92:2821-6.
[36] Mauvais-Jarvis F, Smith SB, Le May C, Leal SM, Gautier JF, Molokhia M, et al. PAX4 gene variations predispose to ketosis-prone diabetes. Hum Mol Genet 2004;13:3151–9.
[37] Shimajiri Y, Sanke T, Furuta H, Hanabusa T, Nakagawa T, Fujitani Y, et al. A missense mutation of Pax4 gene (R121W) is associated with type 2 diabetes in Japanese. Diabetes 2001;50:2864–9.
[38] Tokuyama Y, Matsui K, Ishizuka T, Egashira T, Kanatsuka A. The Arg121Trp variant in PAX4 gene is associated with beta-cell dysfunction in Japanese subjects with type 2 diabetes mellitus. Metabolism 2006;55:213–6.
[39] Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet 2011;44:67–72.
[40] Ma RC, Hu C, Tam CH, Zhang R, Kwan P, Leung TF, et al. Genome-wide association study in a Chinese population identifies a susceptibility locus for type 2 diabetes at 7q32 near PAX4. Diabetologia 2013;56:1291–305.
[41] Shimajiri Y, Shimabukuro M, Tomoyose T, Yogi H, Komiya I, Takasu N, et al. PAX4 mutation (R121W) as a prodiabetic variant in Okinawans. Biochem Biophys Res Commun 2003;302:342–4.
[42] Kanatsuka A, Tokuyama Y, Nozaki O, Matsui K, Egashira T. [beta]-cell dysfunction in late-onset diabetic subjects

carrying homozygous mutation in transcription factors NeuroD1 and Pax4. Metabolism 2002;51:1161–5.
[43] Kooptiwut S, Plengvidhya N, Chukijrungroat T, Sujjitjoon J, Semprasert N, Furuta H, et al. Defective PAX4 R192H transcriptional repressor activities associated with maturity onset diabetes of the young and early onset-age of type 2 diabetes. J Diabetes Complications 2012;26:343–7.
[44] Chavali S, Mahajan A, Tabassum R, Dwivedi OP, Chauhan G, Ghosh S, et al. Association of variants in genes involved in pancreatic β-cell development and function with type 2 diabetes in North Indians. J Hum Genet 2011;56:695–700.
[45] Sujjitjoon J, Kooptiwut S, Chongjaroen N, Semprasert N, Hanchang W, Chanprasert K, et al. PAX4 R192H and P321H polymorphisms in type 2 diabetes and their functional defects. J Hum Genet 2016;61:943–9.
[46] Sujjitjoon J, Kooptiwut S, Chongjaroen N, Tangjittipokin W, Plengvidhya N, Yenchitsomanus PT. Aberrant mRNA splicing of paired box 4 (PAX4) IVS7-1G>A mutation causing maturity-onset diabetes of the young, type 9. Acta Diabetol 2016;53:205–16.
[47] Jo W, Endo M, Ishizu K, Nakamura A, Tajima T. A novel PAX4 mutation in a Japanese patient with maturity-onset diabetes of the young. Tohoku J Exp Med 2011;223:113–8.
[48] Hermann R, Mantere J, Lipponen K, Veijola R, Soltesz G, Otonkoski T, et al. Lack of association of PAX4 gene with type 1 diabetes in the Finnish and Hungarian populations. Diabetes 2005;54:2816–9.
[49] Zhang Y, Xiao X, Liu Y, Zhu X, Wenhui L, Li N, et al. The association of the PAX4 gene with type 1 diabetes in Han Chinese. Diabetes Res Clin Pract 2008;81:365–9.
[50] Sander M, Neubüser A, Kalamaras J, Ee HC, Martin GR, German MS. Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. Genes Dev 1997;11:1662–73.
[51] St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A, Gruss P. Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. Nature 1997;387:406–9.
[52] Ashery-Padan R, Zhou X, Marquardt T, Herrera P, Toube L, Berry A, et al. Conditional inactivation of Pax6 in the pancreas causes early onset of diabetes. Dev Biol 2004;269:479–88.
[53] Hill RE, Favor J, Hogan BL, Ton CC, Saunders GF, Hanson IM, et al. Mouse small eye results from mutations in a paired-like homeobox-containing gene. Nature 1991;354:522–5.
[54] Stoykova A, Fritsch R, Walther C, Gruss P. Forebrain patterning defects in Small eye mutant mice. Development 1996;122:3453–65.
[55] Heller RS, Stoffers DA, Liu A, Schedl A, Crenshaw 3rd EB, Madsen OD, et al. The role of Brn4/Pou3f4 and Pax6 in forming the pancreatic glucagon cell identity. Dev Biol 2004;268:123–34.
[56] Mitchell RK, Nguyen-Tu MS, Chabosseau P, Callingham RM, Pullen TJ, Cheung R, et al. The transcription factor Pax6 is required for pancreatic β cell identity, glucose-regulated ATP synthesis, and Ca2+ dynamics in adult mice. J Biol Chem 2017;292:8892–906.
[57] Hart AW, Mella S, Mendrychowski J, van Heyningen V, Kleinjan DA. The developmental regulator Pax6 is essential for maintenance of islet cell function in the adult mouse pancreas. PLoS ONE 2013;8 e54173.
[58] Dames P, Puff R, Weise M, Parhofer KG, Goke B, Gotz M, et al. Relative roles of the different Pax6 domains for pancreatic alpha cell development. BMC Dev Biol 2010;10:39.
[59] Ritz-Laser B, Estreicher A, Klages N, Saule S, Philippe J. Pax-6 and Cdx-2/3 interact to activate glucagon gene expression on the G1 control element. J Biol Chem 1999;274:4124–32.
[60] Wen JH, Chen YY, Song SJ, Ding J, Gao Y, Hu QK, et al. Paired box 6 (PAX6) regulates glucose metabolism via proinsulin processing mediated by prohormone convertase 1/3 (PC1/3). Diabetologia 2009;52:504–13.
[61] Yasuda T, Kajimoto Y, Fujitani Y, Watada H, Yamamoto S, Watarai T, et al. PAX6 mutation as a genetic factor common to aniridia and glucose intolerance. Diabetes 2002;51:224–30.
[62] Kuroda A, Kaneto H, Fujitani Y, Watada H, Nakatani Y, Kajimoto Y, et al. Mutation of the Pax6 gene causes impaired glucose-stimulated insulin secretion. Diabetologia 2004;47:2039–41.
[63] Ding J, Gao Y, Zhao J, Yan H, Guo SY, Zhang QX, et al. Pax6 haploinsufficiency causes abnormal metabolic homeostasis by down-regulating glucagon-like peptide 1 in mice. Endocrinology 2009;150:2136–44.
[64] Ahmad Z, Rafeeq M, Collombat P, Mansouri A. Pax6 inactivation in the adult pancreas reveals ghrelin as endocrine cell maturation marker. PLoS ONE 2015;10 e0144597.
[65] Balakrishnan S, Sadasivam M, Kannan A, Panneerselvam A, Prahalathan C. Glucose modulates Pax6 expression through the JNK/p38 MAP kinase pathway in pancreatic beta-cells. Life Sci 2014;109:1–7.
[66] Tsui S, Dai W, Lu L. CCCTC-binding factor mediates effects of glucose on beta cell survival. Cell Prolif 2014;47:28–37.
[67] Swisa A, Avrahami D, Eden N, Zhang J, Feleke E, Dahan T, et al. PAX6 maintains β cell identity by repressing genes of alternative islet cell types. J Clin Invest 2017;127:230–43.
[68] Kulzer JR, Stitzel ML, Morken MA, Huyghe JR, Fuchsberger C, Kuusisto J, et al. A common functional regulatory variant at a type 2 diabetes locus upregulates ARAP1 expression in the pancreatic beta cell. Am J Hum Genet 2014;94:186–97.
[69] Courtney M, Gjernes E, Druelle N, Ravaud C, Vieira A, Ben-Othman N, et al. The inactivation of Arx in pancreatic alpha-cells triggers their neogenesis and conversion into functional beta-like cells. PLoS Genet 2013;9 e1003934.
[70] Zhang Y, Fava GE, Wang H, Mauvais-Jarvis F, Fonseca VA, Wu H. PAX4 gene transfer induces α-to-β cell phenotypic conversion and confers therapeutic benefits for diabetes treatment. Mol Ther 2016;24:251–60.
[71] Chou FC, Shieh SJ, Sytwu HK. Attenuation of Th1 response through galectin-9 and T-cell Ig mucin 3 interaction inhibits autoimmune diabetes in NOD mice. Eur J Immunol 2009;39:2403–11.
[72] Kanzaki M, Wada J, Sugiyama K, Nakatsuka A, Teshigawara S, Murakami K, et al. Galectin-9 and T cell immunoglobulin mucin-3 pathway is a therapeutic target for type 1 diabetes. Endocrinology 2012;15:612–20.
[73] Chou FC, Kuo CC, Wang YL, Lin MH, Linju Yen B, Chang DM, et al. Overexpression of galectin-9 in islets prolongs grafts survival via downregulation of Th1 responses. Cell Transplant 2013;22:2135–45.