MicroRNAs in Diabetes and Obesity
Research Article • DOI: 10.2478/micrnado-2013-0002 • MICRNADO • 2013 • 3–8

VERSITA

MicroRNAs: emerging regulators for development of pancreatic islet lineages

Abstract
MicroRNAs belong to a family of small (~23 nt) non-coding RNAs that mediate posttranscriptional gene silencing. They are emerging as important new regulators of differentiation and development. Knowledge of their role in pancreas and islet development, may help in developing a regenerative therapy for diabetes mellitus, a metabolic disorder affecting hundreds of millions of people worldwide. In this minireview, we summarize the latest evidence of the role these new regulators play in islet lineage development, aiming to attract more research into this important developmental regulators.

Keywords
MicroRNAs • Islet progenitors • Differentiation

Fang-Xu Jiang*,
Grant Morahan

Centre for Diabetes Research,
Western Australian Institute for Medical Research,
and Centre for Medical Research,
The University of Western Australia,
Perth 6000, Australia

Received 05 November 2012
Accepted 28 November 2012

Introduction

Various families of transcription factors (TFs) are known to be essential regulators of differentiation and development [1]. In addition, microRNAs (miRs), a family of non-coding small RNAs, have recently emerged as important regulatory molecules [2,3]. MiRs are small (~23 nt) RNAs [4] that can mediate posttranscriptional gene silencing by binding perfectly or imperfectly to the 3' untranslated region of their cognate mRNA sequences and forming an RNA-induced silencing complex (RISC) [4].

Studies in recent years have begun to shed light on the importance of miR species on the development of islet cells. The latter play a key role in maintaining glucose homeostasis, dysregulation of which may result in diabetes mellitus, a metabolic disorder currently affecting approximately 346 million people worldwide [5]. To better understand the roles of miRs on pancreas and islet lineage development, a brief introduction of normal development of the pancreas will be provided. In this minireview, we will briefly introduce the biogenesis of miR species, summarize the current knowledge of these species on islet lineage development that may stimulate further research in this area. Readers interested in effects of miRs on other traits relevant to diabetes, such as β-cell function, glucose homeostasis, and pancreas regeneration are referred to recent reviews [6-10].

Development of islet cells

The islets of Langerhans are embedded within the pancreas, an endoderm-derived organ. The endoderm is one of the three primitive germ layers formed during the early embryonic stage known as gastrulation. Taking the mouse as an example, the pancreas originates from the thickened definite endoderm epithelium along the dorsal and ventral surfaces of the posterior foregut. These thickenings can be identified histologically at embryonic day (E) 9.0-9.5 [11], and are marked by the expression of Pdx1 (pancreas and duodenum transcription factor 1), a TF essential for pancreas outgrowth in early development and β-cell function in adults [12,13]. Shortly afterwards, a small group of cells emerges, marked by expression of neurogenin 3 (Ngn3), a basic helix-loop-helix transcription factor [12,14,15]. These are islet progenitors and can give rise to all mature cell types of the islet. Subsequently, these epithelia evaginate into the surrounding mesoderm-derived mesenchymal tissue and form dorsal and ventral pancreatic buds. These buds continue to expand, branch and fuse as a result of gut rotation bringing the buds together. The fused developing pancreas continues to proliferate, differentiate and, ultimately, develop into the mature pancreas. Concomitant with pancreas organogenesis, $Ngn3^+$ progenitors differentiate into five types of islet cells that work collectively to control glucose homeostasis. The biological process and its regulation by key transcription factors are simplified in Figure 1 and also discussed in more detail in recent reviews [16-18].

Biogenesis of miR species

There are at least two biochemical pathways for synthesis of miRs. Most are formed from the canonical pathway, which

* *E-mail: fangxu.jiang@uwa.edu.au*

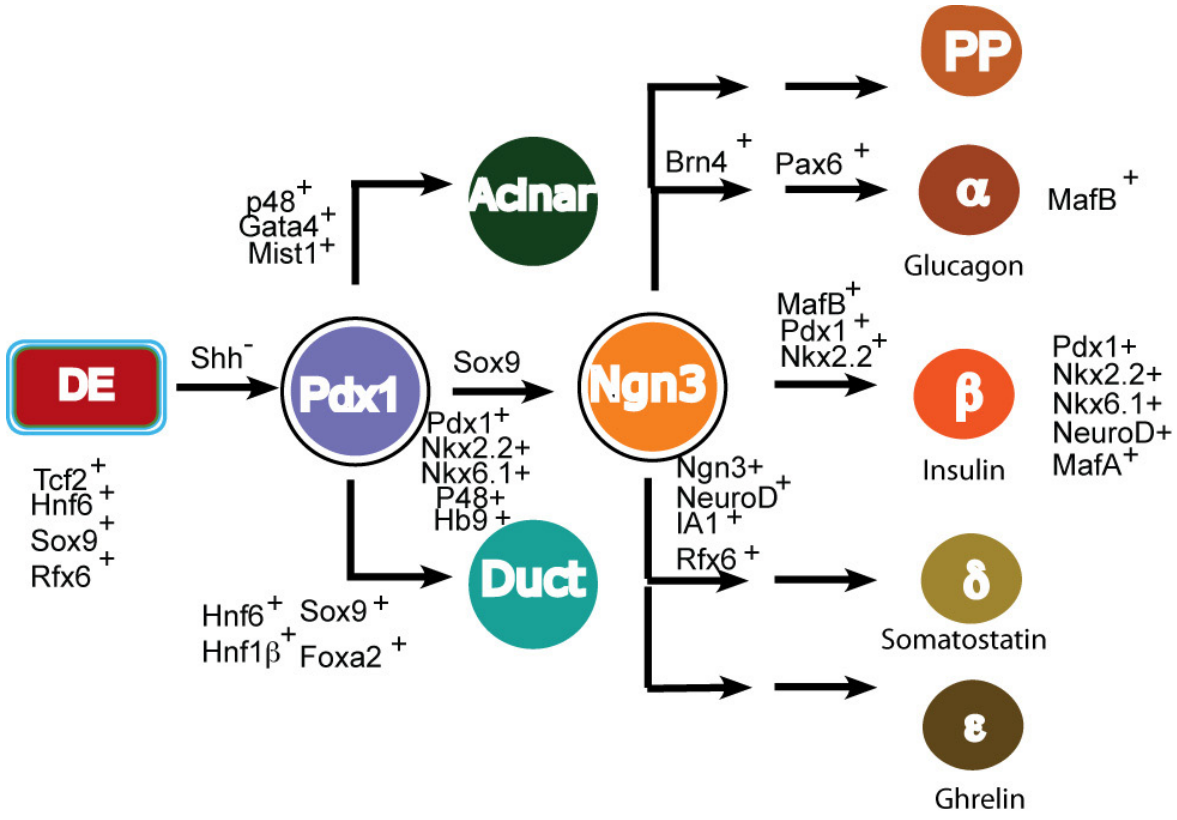

Figure 1. Diagram depicted to show a normal developmental pathway that gives rise to functional β cells. When activated Tcf2 (T cell factor 2, also known as hepatocyte nuclear factor 1b, Hnf1b), Hnf6 (also known as Onecut 1), Sox9 (the Sry-related HMG box transcription factor 9) and Rfx6 (regulatory factor X-box binding 6), a defined domain of definitive endoderm (DE) commits to the foregut endoderm. Suppression of sonic hedgehog signalling in the latter region leads to the development of pancreatic progenitors (Pdx1), which are marked by expression of a number of transcription factors, especially Pdx1 (pancreas and duodenum transcription factor 1, also known as Ipf1), pancreas transcription factor 1a (Ptf1a), Nkx2.2 (Nk family homeobox factor 2.2), Nkx6.1 and Sox9. The expression of Sox9 and suppression of the Notch signalling activate neurogenin-3 (Ngn3), which allows these pancreatic progenitors to commit to precursors of the endocrine islet lineages, the endocrine Ngn3+ progenitors that also express IA1 (insulinoma associated 1), NeuroD (neural differentiation 1), Isl1 (Islet 1), Pax6 (paired box factor 6) and Rfx6. The endocrine progenitors then may differentiate into five types of islet cells [α, β, δ (somatostatin), PP (pancreatic polypeptide) and epsilon (ghrelin)]. For example, a group of MafB- (musculoaponeurotic fibrosarcoma oncogene family protein B), Pdx1-, Pax4- and Nkx2.2-expressing cells will become to mature insulin-secreting β cells.

involves four steps. First, in the nucleus, the RNA polymerase II synthesises a 5′-capped, spliced, and polyadenylated primary RNA precursor (pri-miR), consisting of a stem (containing the miR) and a terminal loop. Second, the enzyme Drosha in the nuclear microprocessor complex catalyzes the removal of the flanking sequences from the pri-miR and releases an intermediate stem–loop structure (known as pre-miR, ~70 nt). Third, the pre-miR is then transported to the cytoplasm by exportin-5. Fourth, the cytoplasmic RNase III Dicer1 mediates the generation of a mature miR duplex which is finally incorporated into the RISC to recognize and bind the target mRNA and perform its biological function. In the non-canonical pathway, miRs are transcribed directly as endogenous short hairpin RNAs or produced directly through splicing from introns that refold into hairpins; the last two steps are the same as in the canonical pathway. Readers are referred to recent excellent reviews for detailed description of miR biogenesis [19-21] and roles in general differentiation and cell fate decisions [3].

Direct evidence that miRs modulate pancreas and islet development

As it encodes the RNase III enzyme required for generation of mature miR, *Dicer1* is critical for global production of major, if not all, miR species. Hence, genetic deletion of this gene should provide an ideal model to understand the function of this new regulatory species in pancreatic endocrine development. Indeed, when *Dicer1* is deleted in *Pdx1*-expressing cells, the resulting animals exhibit gross, dramatic defects in all three pancreatic lineages including exocrine, ductal and endocrine tissues. In particular, the number of glucagon-secreting α, insulin-secreting β, somatostatin-secreting γ and pancreatic polypeptide-secreting PP cells was reduced at E18.5 by 79%, 94%, 95% and 86% respectively [22] (Figure 2). These data convincingly indicate that miR species as a whole play key roles in islet cell development.

This is a remarkable achievement and sets the stage for identifying which particular miR or cluster of miRs is critical for

normal development of all islet cell types and at what stage of development. As *Dicer1* is highly up-regulated from definitive endoderm to pancreatic progenitor stages (approximately 3.2-fold) (F.X. Jiang, unpublished observation), we speculate that the deletion of miR production mainly affects specification of Pdx1$^+$ progenitors. In other words, the gross phenotypes in *Dicer*-null mice are caused by the loss of a combination of several miRs around the Pdx1$^+$ progenitor stage. Nevertheless, miR species may not have a critical role in late islet differentiation as deletion of *Dicer 1* directed by either Ngn3 or insulin gene promoters does not obviously affect differentiation of islet lineages (Figure 2).

Interestingly, a recent study showed that when Ngn3$^+$ cells differentiate, miRs-294, -295 and -302d that normally promote the cell cycle G_1-S transition were highly upregulated [23], consistent with the fact that differentiated β cells regain ability to proliferate. MiR profiling reveals that miRs-15a, -15b, -16 and -195 are associated with Ngn3 expression; overexpression of these miRs in the developing pancreas *in vitro* inhibits the differentiation of both α and β cells [24]. How these miR species regulate differentiation of Ngn3$^+$ progenitors remain to be delineated.

In humans, miR profiling also reveals that miRs-7, -9, -375 are highly expressed during pancreatic islet development [25]. Real time quantitative RT-PCR analysis showed that miR-7 is expressed in the developing human pancreas from around 9 week gestational age (wga) and peaks between 14 and 18 wga, a period in which the number of endocrine cells increase exponentially [26]. Consistent with this, *in situ* hybridization analysis confirms that miR-7 is mainly expressed in developing islet cells that express Pdx1, Ngn3 and Isl1 [27].

Deletion of *dicer1* provides direct evidence that miRs modulate islet function

As *Dicer1* is up-regulated (2.6-fold) again from E18.5 to day 1 postnatally (F.X. Jiang, unpublished observation), β-specific *dicer1*-null mice may have defects in gene expression and in function of postnatal islet cells. Indeed, a recent study demonstrated that β-specific deletion of *Dicer1* led to impaired glucose-stimulated insulin secretion at 8 weeks and diabetes at later ages (80% at 12 weeks and 100% at 25 weeks) due to a progressive decrease of β-cell number and mass [28]. Further studies showed that deletion of *Dicer1* in β cells halved production of miR-7 and miR-375, and dramatically reduced insulin mRNA and protein [28,29] (Figure 3). With an inducible model, removal of *Dicer1* production in β cells causes diabetes in 2 weeks, probably due to up-regulation of insulin transcriptional repressor genes such as *Sox6* and *Bhlhe22* [29]. This model may also be useful in addressing what other miR species have been reduced, how the affected miRs have caused the up-regulation of insulin repressor genes and other genes.

MiR-375 modulates islet cell fate

In addition to regulating insulin secretion [30], miR-375 plays multiple roles in the development of pancreatic endocrine cells. It is highly expressed during specification of definitive endoderm [31] though its function at this stage remains to be identified. As miR-375 colocalizes with Pdx1 in the E14.5 pancreas [22], it may play a role in affecting lineage specifications during pancreas and islet development. Indeed, mice lacking miR-375 exhibit an increased number of α cells with a decreased number of β cells, and develop postnatal hyperglycemia [32]. However, targeted inhibition of miR-375 by morphino (a synthetic oligonucleotide which specifically targets mature miR or its precursor and temporally knocks down the miR) in zebrafish only disturbs the architecture, but not the cellular development of the islets [33]. Whether this discrepancy is due to a species difference needs to be addressed.

MiR-7 modulates islet cell fate

MiR-7 is an evolutionarily conserved miR species encoded by three different genomic loci in humans and mice (for example in mice: mmu-mir-7a-1, mmu-mir-7a-2 and mmu-mir-7b). Immunostaining showed that miR-7 colocalizes with islet progenitors [34]. Knockdown of miR-7 by intrauterine fetal heart injection of antisense morphino decreased β-cell number and insulin production and, induced postnatal glucose intolerance [34], suggesting that miR-7 plays a critical role in islet cell fate allocation and β-cell function. Indeed, recent overexpression or deletion of miR-7 by transgenic or knockout routes led to

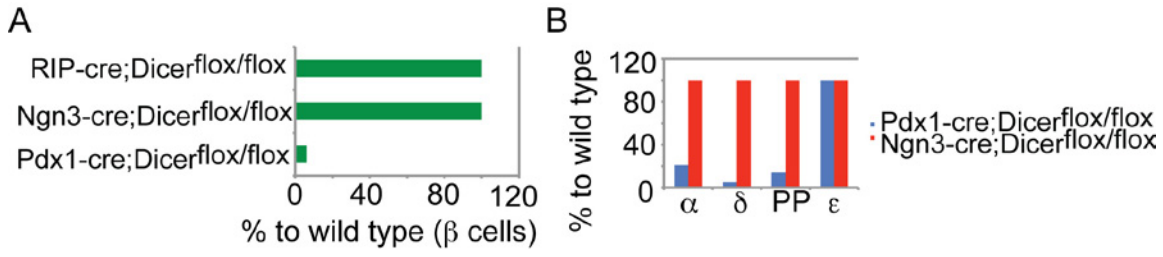

Figure 2. Deletion of miR production in embryonic Pdx1-expressing progenitors blocked islet cell development. Two loxp sites are inserted around an exon that encodes most of the second RNase III domain of the single copy Dicer1 in the genome. Up crossing with the Pdx1-Cre mouse line, the targeted deletion of Dicer1 in principle yields deficient of most mature miRs in Pdx1+ progenitors and their progeny. (A) % of β cells determined at E18.5 after conditional deletion of miR production. (B) % of other islet cells determined at E18.5 after conditional deletion of miR production. (A) and (B) were plotted from the data (Lynn et al., 2007).

respective suppression or promotion of differentiation of α and β cells. This was apparently mediated by affecting expression of *Pax6,* a key transcription factor gene [35].

Other miR species might modulate pancreas development

There are several pieces of indirect evidence suggest that other miR species also modulate development of islet lineages and their progenitors. Bioinformatic algorithms indicate that one conserved miR-495 target site is present in the 3′UTR of *Hnf6*, which encodes a TF critical for specification of pancreatic progenitors from posterior foregut (Figure 1). Luciferase assays demonstrated that transient expression of miR-495 suppresses Hnf6 promoter activity [36]. In contrast, repressing Ptf1a activity in the multipotent AR42J cell line leads to overexpression of miR-18a and reduces levels of amylase 2 and carboxypeptidase 1 mRNAs (markers of acinar cells) [37]. However, whether this suggests that miR-18a has a role in specification of pancreatic progenitors requires further investigation (Figure 3). Finally, the *Let7* family is the first discovered, highly conserved miR species. Mice with *Pdx1*-controled overexpression of *Let7* do not exhibit glucose-induced intolerance unlike with systematic overexpression which reduces GSIS [38,39], suggesting that this family may not modulate islet development and function. A high level expression of miR-30 family is essential for maintenance of the epithelial phenotype of hormone-secreting cells [40]. Furthermore, miR-30d is capable of inducing insulin gene expression [41] via a MafA-associated mechanism [42].

Concluding remarks

Compared to the knowledge of miRNA species on ectoderm- and mesoderm-derived lineage development [3], our understanding of their roles on endoderm-derived lineages including islet cells remains at an early stage. As there is biphasic expression of *Dicer1* during the transition from definitive endoderm to pancreatic progenitors (approximately 3.2-fold), and from E18.5 to day 1 postnatally (2.6-fold) (F.X. Jiang, unpublished observation), miR species may have biphasic roles in islet development and function. However, how many miRs are specific and how they regulate the development and function of endocrine lineages remain largely unknown. This is partially due to the absence of suitable systems for islet cell development and simple readout assays. As TFs are key regulators for cell fate and lineage differentiation, it is reasonable to believe that miRs act by degrading or inhibiting cognate transcription factor mRNAs. We believe that genetic loss and gain functional studies (including tissue-specific and temporal manipulations) on miR species need to be strengthened. Now

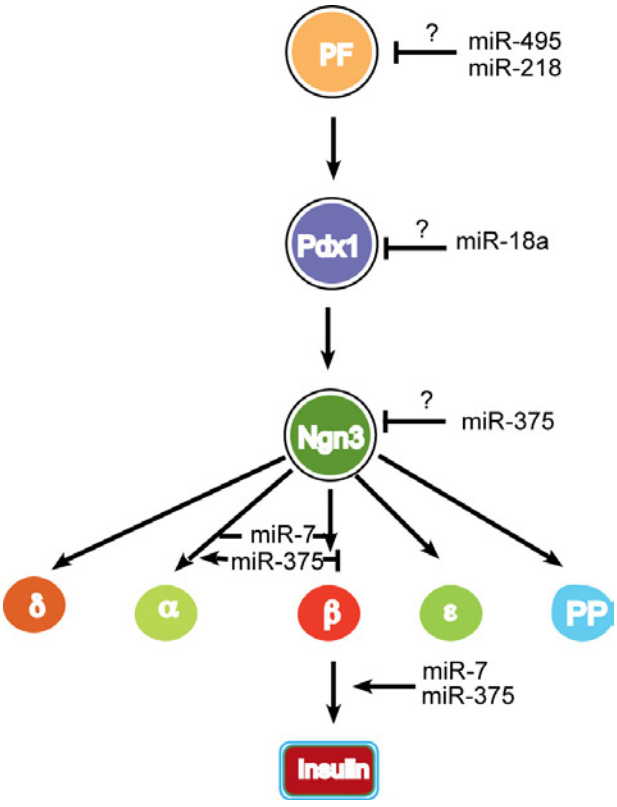

Figure 3. Diagram showing the targets of miR actions during islet development and function. As posterior foregut (PF) cells commit to pancreatic progenitors (Pdx1) that subsequently give rise to islet progenitors (Ngn3), several miR species may affect the differentiation processes. When Ngn3+ progenitors differentiate into functional islet cells, miR-375 and miR-7 regulate cell-fate and insulin secretion.

miR expression can be dynamically profiled by real time RT-PCR arrays, which may attract more researchers into this area. More encouragingly, the recent creation of a resource of vectors and mutant embryonic stem cells for targeted deletion of miRs in mice [43] will allow common reagents, assays and technologies to be used by the research community worldwide, which will undoubtedly facilitate further discoveries in this vital research in the years to come.

Acknowledgements

The authors are supported by grants from Juvenile Diabetes Research Foundational International (4-2006-1025), the Diabetes Research Foundation of Western Australia, the University of Western Australia, National Health and Medical Research Council Program Grant (53000400) and the Medical Research Foundation of Royal Perth Hospital.

References

[1] Leeb M, Wutz A (2012) Establishment of epigenetic patterns in development. Chromosoma 121: 251-262.
[2] Pauli A, Rinn JL, Schier AF (2011) Non-coding RNAs as regulators of embryogenesis. Nat Rev Genet 12: 136-149.
[3] Ivey KN, Srivastava D (2010) MicroRNAs as regulators of differentiation and cell fate decisions. Cell Stem Cell 7: 36-41.
[4] Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136: 215-233.
[5] Scully T (2012) Diabetes in numbers. Nature 485: S2-3.
[6] Lynn FC (2009) Meta-regulation: microRNA regulation of glucose and lipid metabolism. Trends Endocrinol Metab 20: 452-459.
[7] Joglekar MV, Parekh VS, Hardikar AA (2007) New pancreas from old: microregulators of pancreas regeneration. Trends Endocrinol Metab 18: 393-400.
[8] Fernandez-Valverde SL, Taft RJ, Mattick JS (2011) MicroRNAs in beta-cell biology, insulin resistance, diabetes and its complications. Diabetes 60: 1825-1831.
[9] Kolfschoten IG, Roggli E, Nesca V, Regazzi R (2009) Role and therapeutic potential of microRNAs in diabetes. Diabetes Obes Metab 11 Suppl 4: 118-129.
[10] Dumortier O, Van Obberghen E (2012) MicroRNAs in pancreas development. Diabetes Obes Metab 14 Suppl 3: 22-28.
[11] Pictet RL, Clark WR, Williams RH, Rutter WJ (1972) An ultrastructural analysis of the developing embryonic pancreas. Dev Biol 29: 436-467.
[12] Gu G, Dubauskaite J, Melton DA (2002) Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development 129: 2447-2457.
[13] Ohneda K, Mirmira RG, Wang J, Johnson JD, German MS (2000) The homeodomain of PDX-1 mediates multiple protein-protein interactions in the formation of a transcriptional activation complex on the insulin promoter. Mol Cell Biol 20: 900-911.
[14] Gradwohl G, Dierich A, LeMeur M, Guillemot F (2000) neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci U S A 97: 1607-1611.
[15] Xu X, D'Hoker J, Stange G, Bonne S, De Leu N, et al. (2008) Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132: 197-207.
[16] Jiang FX, Morahan G (2012) Pancreatic stem cells: from possible to probable. Stem Cell Rev 8: 647-657.
[17] Pan FC, Wright C (2011) Pancreas organogenesis: from bud to plexus to gland. Dev Dyn 240: 530-565.
[18] Oliver-Krasinski JM, Stoffers DA (2008) On the origin of the beta cell. Genes Dev 22: 1998-2021.
[19] Gangaraju VK, Lin H (2009) MicroRNAs: key regulators of stem cells. Nat Rev Mol Cell Biol 10: 116-125.
[20] Inui M, Martello G, Piccolo S (2010) MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 11: 252-263.
[21] Yi R, Fuchs E (2011) MicroRNAs and their roles in mammalian stem cells. J Cell Sci 124: 1775-1783.
[22] Lynn FC, Skewes-Cox P, Kosaka Y, McManus MT, Harfe BD, et al. (2007) MicroRNA expression is required for pancreatic islet cell genesis in the mouse. Diabetes 56: 2938-2945.
[23] Miyatsuka T, Kosaka Y, Kim H, German MS (2011) Neurogenin3 inhibits proliferation in endocrine progenitors by inducing Cdkn1a. Proc Natl Acad Sci U S A 108: 185-190.
[24] Joglekar MV, Parekh VS, Mehta S, Bhonde RR, Hardikar AA (2007) MicroRNA profiling of developing and regenerating pancreas reveal post-transcriptional regulation of neurogenin3. Dev Biol 311: 603-612.
[25] Joglekar MV, Joglekar VM, Hardikar AA (2009) Expression of islet-specific microRNAs during human pancreatic development. Gene Expr Patterns 9: 109-113.
[26] Jeon J, Correa-Medina M, Ricordi C, Edlund H, Diez JA (2009) Endocrine cell clustering during human pancreas development. J Histochem Cytochem 57: 811-824.
[27] Correa-Medina M, Bravo-Egana V, Rosero S, Ricordi C, Edlund H, et al. (2009) MicroRNA miR-7 is preferentially expressed in endocrine cells of the developing and adult human pancreas. Gene Expr Patterns 9: 193-199.
[28] Kalis M, Bolmeson C, Esguerra JL, Gupta S, Edlund A, et al. (2011) Beta-cell specific deletion of Dicer1 leads to defective insulin secretion and diabetes mellitus. PLoS One 6: e29166.
[29] Melkman-Zehavi T, Oren R, Kredo-Russo S, Shapira T, Mandelbaum AD, et al. (2011) miRNAs control insulin content in pancreatic beta-cells via downregulation of transcriptional repressors. EMBO J 30: 835-845.
[30] Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, et al. (2004) A pancreatic islet-specific microRNA regulates insulin secretion. Nature 432: 226-230.
[31] Hinton A, Afrikanova I, Wilson M, King CC, Maurer B, et al. (2010) A distinct microRNA signature for definitive endoderm derived from human embryonic stem cells. Stem Cells Dev 19: 797-807.
[32] Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, et al. (2009) miR-375 maintains normal pancreatic alpha- and beta-cell mass. Proc Natl Acad Sci U S A 106: 5813-5818.
[33] Kloosterman WP, Lagendijk AK, Ketting RF, Moulton JD, Plasterk RH (2007) Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. PLoS Biol 5: e203.
[34] Nieto M, Hevia P, Garcia E, Klein D, Alvarez-Cubela S, et al. (2011) Anti sense miR-7 impairs insulin expression in developing pancreas and in cultured pancreatic buds. Cell Transplant.
[35] Kredo-Russo S, Ness A, Mandelbaum AD, Walker MD, Hornstein E (2012) Regulation of pancreatic microRNA-7 expression. Exp Diabetes Res 2012: 695214.

[36] Simion A, Laudadio I, Prevot PP, Raynaud P, Lemaigre FP, et al. (2010) MiR-495 and miR-218 regulate the expression of the Onecut transcription factors HNF-6 and OC-2. Biochem Biophys Res Commun 391: 293-298.

[37] Yang Y, Ding L, An Y, Zhang ZW, Lang Y, et al. (2012) MiR-18a regulates expression of the pancreatic transcription factor Ptf1a in pancreatic progenitor and acinar cells. FEBS Lett.

[38] Zhu H, Shyh-Chang N, Segre AV, Shinoda G, Shah SP, et al. (2011) The Lin28/let-7 axis regulates glucose metabolism. Cell 147: 81-94.

[39] Frost RJ, Olson EN (2011) Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. Proc Natl Acad Sci U S A 108: 21075-21080.

[40] Joglekar MV, Patil D, Joglekar VM, Rao GV, Reddy DN, et al. (2009) The miR-30 family microRNAs confer epithelial phenotype to human pancreatic cells. Islets 1: 137-147.

[41] Tang X, Muniappan L, Tang G, Ozcan S (2009) Identification of glucose-regulated miRNAs from pancreatic {beta} cells reveals a role for miR-30d in insulin transcription. RNA 15: 287-293.

[42] Zhao X, Mohan R, Ozcan S, Tang X (2012) MicroRNA-30d induces insulin transcription factor MafA and insulin production by targeting mitogen-activated protein 4 kinase 4 (MAP4K4) in pancreatic beta-cells. J Biol Chem 287: 31155-31164.

[43] Prosser HM, Koike-Yusa H, Cooper JD, Law FC, Bradley A (2011) A resource of vectors and ES cells for targeted deletion of microRNAs in mice. Nat Biotechnol 29: 840-845.