MicroRNAs: markers of β-cell stress and autoimmunity

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Purpose of review
We discuss current knowledge about microRNAs (miRNAs) in type 1 diabetes (T1D), an autoimmune disease leading to severe loss of pancreatic β-cells. We describe: the role of cellular miRNAs in regulating immune functions and pathways impacting insulin secretion and β-cell survival; circulating miRNAs as disease biomarkers.

Recent findings
Studies examined miRNAs in experimental models and patients, including analysis of tissues from organ donors, peripheral blood cells, and circulating miRNAs in serum, plasma, and exosomes. Studies employed diverse designs and methodologies to detect miRNAs and measure their levels. Selected miRNAs have been linked to the regulation of key biological pathways and disease pathogenesis; several circulating miRNAs are associated with having T1D, islet autoimmunity, disease progression, and immune and metabolic functions, for example, C-peptide secretion, in multiple studies.

Summary
A growing literature reveals multiple roles of miRNAs in T1D, provide new clues into the regulation of disease mechanisms, and identify reproducible associations. Yet challenges remain, and the field will benefit from joint efforts to analyze results, compare methodologies, formally test the robustness of miRNA associations, and ultimately move towards validating robust miRNA biomarkers.

Keywords
biomarkers, islet autoimmunity, microRNAs, prevention, type 1 diabetes

INTRODUCTION

Type 1 diabetes (T1D) is a chronic autoimmune disease leading to severe loss of pancreatic β-cells. Cells of the innate and adaptive immune systems invade pancreatic islets giving rise to the inflammatory lesion termed insulitis [1] leading to impaired β-cell function and survival; ultimately, autoreactive lymphocytes cause immune-mediated β-cell destruction [2]. Autoantibodies to islet autoantigens are robust diagnostic and predictive biomarkers [3]. In prospective studies of nondiabetic relatives, positivity for multiple autoantibodies confers high risk of T1D but do not predict time to onset [4–7]. Other biomarkers include risk alleles, mRNA profiles [8,9], immune cellular markers (autoreactive T cells, regulatory T cells, phenotypes, etc.), markers of β-cell destruction (for example, levels of de-methylated insulin gene DNA) [10,11], and metabolic testing [oral glucose tolerance test (OGTT)] [6,7,12]. Yet there is an unmet need for additional biomarkers that, by themselves or in combination, can improve prediction [13]. This review will discuss emerging data about the role of cellular microRNAs (miRNAs) in regulating immune functions and pathways impacting insulin secretion and β-cell survival, and emerging knowledge about circulating miRNAs as disease biomarkers.

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KEY POINTS

- MiRNAs regulate genes involved in pancreas development, β-cell function, insulin secretion, inflammation, and immunity, which are pathways relevant to T1D.
- Multiple studies of circulating miRNAs report associations with overlapping miRNAs, indicating some level of replication, and that these miRNAs have potential as biomarkers.
- Collaborative approaches for study design, sample exchange, and standardization, and further replication of results in multiple cohorts are needed for the validation of robust biomarkers.
- Certain miRNAs may be enriched in secretory vesicles, such as exosomes; the identification of β-cell-derived exosomes would empower noninvasive studies (liquid biopsy) of circulating miRNAs to directly monitor β-cell dysfunction and possibly death, which would be critical for assessing progression of islet autoimmunity and disease prevention.
- The identification of β-cell-derived exosomes is a research priority, and could be transformative, as it would empower noninvasive studies (liquid biopsy) of circulating miRNAs to directly monitor β-cell dysfunction and possibly death, and as such become critical for assessing progression of islet autoimmunity and disease prevention.

MICRORNAS AS REGULATORS OF GENE EXPRESSION AND BIOMARKERS

miRNAs are small, noncoding RNAs that regulate gene expression [14]. At present, 2,717 human, validated miRNAs are listed on miRBase.org. miRNA regulation involves degradation and suppression of a target mRNA, upon miRNA binding to complementary sequences in the 3′ UTR of its target mRNA [15]. miRNA expression profiles vary in different cells, are modulated by various signals, and each miRNA may have several hundred mRNA targets [16]; genes encoding for miRNAs undergo epigenetic regulation [17]. Although miRNAs play a fundamental role in physiology by regulating virtually every biological pathway, growing literature has associated miRNAs with human diseases. miRNAs exist in biological fluids, including serum and plasma, where they are complexed with ribonucleoproteins and resistant to degradation [18,19]. miRNAs are also packaged in secretory microvesicles, such as exosomes [20,21], which mediate genetic exchange among cells [22]. Circulating miRNAs might reflect ongoing biological responses and could be exploited as disease biomarkers, informing about disease state, progression, prognosis, and response to therapy [23,24].

ROLE OF CELLULAR MICRORNAS IN REGULATING IMMUNE AND B-CELL FUNCTIONS IN TYPE 1 DIABETES

miRNAs regulate several β-cell functions, including insulin secretion, responses to environmental, inflammatory, and immune stressors, as well as development, differentiation, and survival [25–28]. Mice with β-cell-specific knockout of Dicer, the enzyme that is critical to miRNA biosynthesis, exhibit altered islet morphology, decreased β-cell mass, defective insulin production and secretion [29,30]. miRNA levels in pancreatic islets change during physiologic conditions [31] because of increased metabolic demand [32] or as consequence of a pathologic process.

Roggli *et al.* [33] investigated miRNAs in β-cell function and survival under inflammatory conditions. Unbiased microarray profiling of an insulin-secreting cell line treated with proinflammatory cytokines revealed increased levels of miR-21-5p, miR-34a, and miR-146a-5p. These miRNAs were also upregulated in freshly isolated human islets treated with IL-1β and in islets from prediabetic non-obese diabetic (NOD) mice (8–13 weeks of age), a model of autoimmune diabetes [34]. Inhibition of miR-34a and miR-146a-5p protected β-cells from cytokine-induced death [33]. MiR-21-5p was associated with β-cell failure [33,35] and death via the inhibition of the anti-apoptotic BCL2 [36]; miR-21-5p also targets PDCD4, which promotes cells death via proapoptotic Bax proteins; thus, miR-21-5p may also promote β-cell resistance to death and protect from autoimmune diabetes by suppressing PDCD4 [37]. MiR-21-5p is linked to multiple autoimmune diseases [38–44]; it modulates T-cell activation [45] and differentiation, including Th17 differentiation [46–51], and miR-21-5p levels were higher in effector and memory T-cells compared with naïve T-cells [52]. MiR-21 has been shown to function as positive, indirect regulator of FOXP3 in Treg cells [53]. Thus, miR-21-5p may also play a role in modulating T1D-associated immune responses.

The miR-29a/b/c cluster was also upregulated during progression of islet autoimmunity in NOD mice. MiR-29a, as well as several others, were upregulated in human islets exposed to Coxsackie B virus [54], noting that enteroviruses are considered environmental triggers in T1D [55–57]. Increased levels of miR-29 family members in β-cells decreased insulin mRNA content, decreased glucose-stimulated insulin secretion (GSIS) and contributed to

cytokine-induced apoptosis by downregulating the anti-apoptotic protein Mcl1 [58].

miRNA expression profiling of human islets reported 22 downregulated and 35 upregulated miRNAs following exposure to IL-1β and IFNγ. Three downregulated miRNAs: miR-23a-3p, miR-23b-3p, and miR-149-5p, modulate β-cell apoptosis and the Bcl-2 pro-apoptotic proteins DP-5 and PUMA [59▪]. Collectively, these studies show that inflammatory signals observed in T1D can lead to β-cell dysfunction and death through the modulation of certain miRNAs.

Not all miRNA expression profiles, however, observed in freshly isolated NOD prediabetic islets could be reproduced *in vitro* by cytokines exposure, suggesting that ex-vivo profiles include the contribution of infiltrating immune cells; moreover, interactions between endocrine and immune cells may modulate miRNA expression. Consistent with this notion, Guay *et al.* [60] reported that exposure of islet cells to T-cell exosomes led to increased levels of miR-142-3p, miR-142-5p, miR-155, and to β-cell apoptosis; *in vivo* blocking of these miRNAs in β-cells of pre-diabetic NOD mice decreased T1D incidence and was accompanied by reduced islet inflammation and T-cell recruitment [60]. This observation potentially uncovers a key role for infiltrating T-cell exosomes in delivering miRNAs to β-cells, which may trigger apoptosis.

miRNAs also regulate key functions of the immune system in multiple cell types [61–64], including lymphocytes and regulatory T (Treg) cells [62,65,66]. For example, miR-155 is linked to key immunological functions [52,62,67,68], and its expression in Tregs depends on the Treg-specific transcription factor Foxp3 [69]. MiR-155 down-regulates SOCS1, relieving inhibition on the IL-2-dependent Stat5 activation. Thus, miRNAs play a key role in Treg function and IL-2/IL-2R signalling, which are both linked to T1D [70,71]. MiR-146a-5p is required for the suppressive function of Treg cells [72]. Researchers investigated the function of miRNA expression levels in Treg and conventional T-cells in patients' peripheral blood and, since the advent of the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD) [73], in pancreas tissue and pancreatic lymph nodes (PLNs) from organ donors with T1D. Treg cells from the PLNs of T1D patients, but not peripheral blood, had impaired in-vitro suppressive capability compared with nondiabetic donors [74]. This defect was associated with miR-125a-5p upregulation in PLN Treg cells from T1D donors and a reduction in the chemokine receptor CCR2, suggesting that Treg migration to infiltrated islets may be impaired in patients [75▪]. In contrast, islet-reactive effector T-cells circulate in both patients and healthy individuals but only home to the pancreas in patients [76]. These studies highlight the importance of assessing miRNAs in immune cells, in the context of the target organ.

Studies examined miRNAs in circulating T-cells from T1D patients and in at-risk, autoantibody-positive relatives. Naïve Tregs from autoantibody-positive individuals displayed two differentially expressed miRNAs, let-7c, and miR-15a; 32 miRNAs were dysregulated in naïve $CD4^+$ T-cells, potentially paving the way for altered miRNA expression in effector memory T-cells in T1D [77]. Serr *et al.* reported upregulation of the miR-181a/NFAT axis in $CD4^+$ T-cells from children at an early stage of islet autoimmunity; elevated miR-181a activity increased TCR signal strength and co-stimulation expression links NFAT expression to decreased Treg cell function *in vitro* [78▪]; these alterations may be associated with impaired immune tolerance and activation of autoimmunity. The same group reported an enrichment of insulin-specific $CXCR5^+CD4^+$ T-follicular helper (TFH) precursors (a subset essential for induction of high-affinity antibodies), which was correlated with high miR-92a abundance during onset of autoimmunity; kruppel-like factor 2 (KLF2) was a target of miR-92a in TFH precursors. Moreover, miR-92a inhibition blocked TFH induction and reduced islet autoimmunity in NOD mice [79]. Autoreactive T-cells from patients expressed increased levels of miR-98, miR-23b, and miR-590-5p and conversely had reduced expression of their target genes TRAIL, TRAIL-R2, FAS, and FASLG; as these are members of the extrinsic apoptosis pathway, these findings support a role for miRNAs in repressing pro-apoptotic pathways, which may in turn promote unrestricted expansion of diabetogenic cytotoxic T-cells [80]. Thus, studies of peripheral blood immune subsets can help understanding the impact of miRNAs on the regulation of islet autoimmunity and disease progression, a heterogeneous process proceeding at different rates in individuals.

miRNAs have been linked to the regulation of the expression of β-cell autoantigens, such as islet antigen (IA)-2, IA-2β, and glutamate decarboxylase (GAD65); these are modulated by the imprinted 14q32 miRNA cluster in MIN6 cells and mouse islets [81]. Levels of miRNAs involved in autoantigen regulation increased in high glucose conditions [81,82].

Collectively, these findings identify multiple miRNA-regulated pathways in immune cells and β cells, and that miRNA regulation may also involve the transfer of miRNAs via exosomes. Thus, miRNAs are involved in the cross-talk between islet cells and immune cells during the development of T1D [83].

CIRCULATING MICORNAS AS TYPE 1 DIABETES BIOMARKERS

Circulating miRNAs may reflect ongoing disease processes (autoimmunity, β-cell death and dysfunction, viral infections) and could improve prediction. Investigators are seeking: miRNAs or miRNA profiles, which predict the triggering of islet autoimmunity, before autoantibody conversion, for primary prevention; miRNAs associated with disease progression, to enhance stratification by risk categories and predicted time to diagnosis in prevention trials; miRNAs predicting C-peptide decline after diagnosis [84], to advance stratification and efficiency of clinical trial design.

Several studies explored associations of circulating miRNAs in T1D patients; we discuss 11 published studies relevant to disease pathogenesis, disease risk, and C-peptide decline. Seven studies examined cohorts of newly diagnosed patients and two examined patients with long disease duration yet claimed a link to pathogenesis; three studies examined autoantibody-positive relatives at increased T1D risk. These investigations differed in sample types examined (serum, plasma, serum-derived exosomes), molecular methods used to assay miRNAs (PCR-based, sequencing, etc.), number and which miRNAs were assayed (ranging from 1 to 2,083), data normalization, and statistical analysis approaches. Details of these studies and a list of 31 miRNAs identified in at least two studies are reported in Table 1. Three miRNAs were associated with T1D in five studies (miR-24-3p, miR-375, miR-25-3p), one in four studies (miR-148a-3p), five in three studies (miR-21-5p, miR-93-5p, miR-146a-5p, miR-29a-3p, miR-21-3p), and twenty-two in two studies (Table 1). We discuss here nine miRNAs associated with T1D in at least three studies. Table 2 lists major pathways impacted by these miRNAs.

MiR-375 regulates insulin secretion [102], exocytosis [101], pancreas development [104,111], and in the maintenance of α-cell and β-cell phenotypes [105]. Although not exclusively expressed in β-cells, miR-375 levels are increased following acute β-cell loss in experimental animals [112]. It is not clear whether miR-375 is a robust biomarker of chronic, low-level β-cell destruction as seen during the course of islet autoimmunity; so far miR-375 was not detected at increased levels in three studies of prediabetic individuals [90,93▪,94▪], yet it was increased in three of seven studies of patients with recent onset T1D [88,92▪▪,95▪▪] and two studies of patients with longstanding diabetes [86,89].

MiR-24-3p levels were higher in patients than controls in three studies (two of new onset patients) and reduced in a small study of eight patients with recently diagnosed T1D [94▪]; miR-24-3p was also a predictor of future C-peptide decline after diagnosis [92▪▪]. MiR-148a-3p was upregulated in the serum of T1D patients in three studies [85,87,89] and in autoantibody-positive relatives carrying high-risk HLA types [94▪]. Both miR-24-3p and miR-148a-3p modulate insulin biosynthesis [98].

MiR-25-3p was upregulated in patients in three studies [68,74,75▪] and downregulated in one [94▪], but it was also upregulated in autoantibody-positive prediabetic individuals compared with their autoantibody-negative siblings [93▪]; it correlated with C-peptide levels and residual β-cell function at diagnosis [69]; miR-25-3p reportedly suppresses the translation of the insulin gene mRNA [98] and modulates apoptosis [100].

Other miRNAs consistently upregulated in patients with T1D and/or prediabetic individuals were miR-21-3p, miR-21-5p, and 29a-3p. MiR-21-3p serum levels were increased in relatives with multiple autoantibodies who progressed to diabetes compared with those who had not yet been diagnosed [93▪], and in recent onset patients [95▪▪]; a well-defined target of miR-21-3p is the histone deacetylase-8 mRNA [105], belonging to a class of deacetylases with allelic variants linked to T1D risk [112]; deacetylases are associated with inflammatory responses, insulin resistance, and β-cell failure, especially in response to IL-1ß [113].

The molecular associations of miR-21-5p with T1D were already discussed; miR-21-5p levels are reduced in PBMCs from T1D patients [114] but elevated in serum [89,85]. Lakhter *et al.* [95▪▪] reported elevated levels of miR-21-5p in serum-derived exosomes but not in serum from T1D patients, and elevated circulating levels of miR-21-3p. The enrichment of miR-21-5p in exosomes suggests that for some miRNAs, exosome purification may be required to detect differences; examining exosomes may also inform about the biology of circulating miRNAs.

Another miRNA of interest is miR-29a-3p: its levels were increased in relation to diabetes progression in relatives with autoantibodies [93▪]. The expression of miR-29a-3p is three-fold higher in human β cells compared with α cells [115]; diabetes develops in miR-29a-3p-deficient mice following unfolded protein stress responses [109]. Elevated glucose increases miR-29a-3p levels in rat and human islets [116]; overexpression of miR-29 miRNAs in islet cells impairs glucose-stimulated insulin secretion, via decreased expression of the transcription factor Onecut2 and elevated granuphilin, an inhibitor of β-cell exocytosis [58], through the targeting of Syntaxyn-1a, one of the two t-SNAREs

Table 1. Circulating microRNAs associated with type-1 diabetes by at least two independent studies

A

First author [Ref]	Publication year	Population examined: T1D: New onset (Y/N)	T1D: *n* (mean age)	T1D: *n* (mean age)	Control: *n* (mean age)	At-risk prediabetes: Sample type	Assay type	Number of miRNAs studied
Nielsen *et al.* [85]	2012	Y	275 (12)	151	n/a	Serum	Sequencing/qPCR	240/47
Latreille *et al.* [86]	2015	N	38 (43.6)	51 (40.8)	n/a	Plasma	TLDA qPCR	1
Assmann *et al.* [87]	2015	Y[a]	13 (n/a)	20 (n/a)	n/a	Plasma	TLDA qPCR	48
Marchand *et al.* [88]	2016	Y	22 (9.8)	10 (9.9)	n/a	Serum	TLDA qPCR	1
Seyhan *et al.* [89]	2016	N	16 (25.9)	27 (25.3)	n/a	Plasma	TLDA qPCR	28
Yin *et al.* [90]	2016	n/a	n/a	n/a	35 (n/a)[b]	Serum	TLDA qPCR	754
Erener *et al.* [91]	2017	Y	38 (8.9)[c]	32 (8.8)	n/a	Plasma	Exiqon LNA qPCR	745
Samandari *et al.* [92▪▪]	2017	Y	40 (8.7)	n/a	n/a	Plasma	Exiqon LNA qPCR	745
Snowhite *et al.* [93▪]	2017	n/a	n/a	n/a	150 (11)[d]	Serum	Exiqon LNA qPCR	93
Åkerman *et al.* [94▪]	2018	Y	8 (11.7)	17 (11.8)	21 (10.2)	Serum	Exiqon LNA qPCR	179
Lakhter *et al.* [95▪▪]	2018	Y	19 (10.5)	16 (10.5)	n/a	Serum/exosomes	Digital droplet PCR	1

B

	miR-24-3p	miR-25-3p	miR-375	miR-148a-3p	miR-146a-5p	miR-21-3p	miR-21-5p	miR-29a-3p	miR-93-5p	let-7g-5p	miR-103a-3p	miR-106a-5p	miR-126-3p	miR-139-5p	miR-140-5p	miR-144-5p	miR-148b-3p	miR-152-3p	miR-16-5p	miR-181a-5p	miR-19a-3p	miR-200a-3p	miR-20a-5p	miR-210-3p	miR-222-3p	miR-26b-5p	miR-27b-3p	miR-30c-5p	miR-30e-5p	miR-34a-5p	miR-451a
Nielsen *et al.* [85]	X	X		X			X	X			X							X		X		X		X		X	X				
Latreille *et al.* [86]			X																												
Assmann *et al.* [87]				X	X	X			X		X		X				X					X		X							
Marchand *et al.* [88]			X																												
Seyhan *et al.* [89]	X		X	X			X																							X	
Yin *et al.* [90]					X																							X			
Erener *et al.* [91]	X	X							X	X					X	X								X					X		
Samandari *et al.* [92▪▪]	X	X	X		X																										
Snowhite *et al.* [93▪]		X				X		X				X							X		X		X								X
Åkerman *et al.* [94▪]	X	X		X				X	X	X		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X
Lakhter *et al.* [95▪▪]			X			X	X							X																	
Total studies reporting	5	5	5	4	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

[a]Less than 5 years type-1 diabetes (T1D) duration.
[b]Included 40 autoantibody-negative relatives as control group.
[c]This study included three cohorts of patients with recent onset and included extended follow-up for some patients.
[d]This study included 150 family-matched, autoantibody-negative siblings.

Table 2. Biological pathways and experimentally validated gene targets of nine microRNAs most reproducibly associated with type-1 diabetes

	Biological pathways	Validated targets	References
miR-24-3p	Cell proliferation, insulin synthesis, lipotoxicity	Men1, Sox6, NeuroD1, HNFa	[96–98]
miR-25-3p	Suppression of INS mRNA translation, activation of apoptosis related to oxidative stress pathways through suppression of the mitochondrial calcium uniport (MCU)	INS, MCU	[99,100]
miR-375	Insulin synthesis, exocytosis, cellular growth and proliferation, pancreas development, maintenance of α-cell and β-cell phenotypes	Mtpn1, Cadmn1, Rasd1, Eafle1, Rgd16, Cav1, Id3, HuD	[101–105]
miR-148a-3p	Insulin synthesis	Sox6	[98]
miR-146a-5p	Cytokine-induced cell death, suppressive function of Treg cells		[33]
miR-21-5p	β-cell apoptosis, T-cell activation and differentiation, modulation of Foxp3 in Treg cells	BCL2, Pdcd4	[33,37]
miR-21-3p	Insulin resistance, inflammation, β-cell failure in response to cytokines	Hdac8	[106]
miR-29a-3p	Glucose-stimulated insulin secretion, exocytosis, apoptosis	Onecut2, STX1a, Mct1, Mcl1	[33,107–109]
miR-93-5p	Insulin resistance throguh modulation of Glut4	Glut4	[110]

receptors [soluble NSF (*N*-ethylmaleimide sensitive fusion proteins) Attachment Protein Receptor] involved in insulin exocytosis [107]. MiR-29a-3p (and miR-29b-3p) also modulate insulin secretion by targeting the SLC16A1 mRNA in β cells, encoding for the monocarboxylate transporter-1 (MCT1) [108]. Mir-29a-3p also promotes apoptosis via suppression of the anti-apoptotic Mcl1 (myeloid cell leukemia sequence 1) [58]. MiR-29a-3p (miR-29b-3p, miR-29c-3p) levels were elevated in NOD islets during diabetes progression and are upregulated in isolated mouse and human islets following exposure to inflammatory cytokines [58,35]. Thus, the elevated levels of miR-29a-3p in autoantibody-positive relatives may reflect islet inflammation and impaired insulin secretion as disease progresses; this is also as suggested by the reported inverse correlation with OGTT C-peptide values [93▪]. Moreover, both miR-21-3p and miR-29a-3p levels increase in human islet cells following infection with the enterovirus Coxsackie B5 strain [54]; future studies should investigate whether the increased levels of circulating miR-21-3p and miR-29a-3p in autoantibody-positive relatives may potentially reflect viral infections in the pancreas. Finally, miR-93-5p was differentially expressed in three studies in comparison of T1D patients and controls; it was upregulated in two studies [91,87] and downregulated in the other study [94▪]. Not only MiR-93-5p but also miR-21a-5p and miR-29a-3p, reportedly regulate the levels of the glucose transporter GLUT4 in muscle and adipose tissue [110,117], and could be involved in insulin resistance.

Overall, studies are independently identifying associations with selected circulating miRNAs; importantly, several have been linked to biological pathways involved in β-cell function, inflammation, and T1D.

CONCLUSION

miRNAs regulate genes involved in pancreas development, β-cell function, insulin secretion, inflammation, and immunity, and several miRNAs are dysregulated in T1D. Studies have identified associations with overlapping, circulating miRNAs; these are potential biomarkers, despite different study populations, study design, biological fluids examined, and molecular methods used for purification and detection. Future collaborative studies should involve sample exchange and assay standardization, and achieve further replication in multiple cohorts, which is critical for the validation of robust biomarkers.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Campbell-Thompson M, Fu A, Kaddis JS, *et al.* Insulitis and β-cell mass in the natural history of type 1 diabetes. Diabetes 2015; 65:719–731.
2. Pugliese A. Autoreactive T cells in type 1 diabetes. J Clin Invest 2017; 127:2881–2891.
3. Ziegler AG, Rewers M, Simell O, *et al.* Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013; 309:2473–2479.
4. Skyler JS, Sosenko JM. The evolution of type 1 diabetes. JAMA 2013; 309:2491–2492.
5. Insel RA, Dunne JL, Atkinson MA, *et al.* Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015; 38: 1964–1974.
6. Ismail HM, Xu P, Libman IM, *et al.*, Type 1 Diabetes TrialNet Study Group. The shape of the glucose concentration curve during an oral glucose tolerance test predicts risk for type 1 diabetes. Diabetologia 2017; 61:84–92.
7. Sosenko JM, Skyler JS, Herold KC, *et al.*, Type 1 Diabetes TrialNet and Diabetes Prevention Trial–Type 1 Study Groups. The metabolic progression to type 1 diabetes as indicated by serial oral glucose tolerance testing in the Diabetes Prevention Trial-type 1. Diabetes 2012; 61:1331–1337.
8. Cabrera SM, Chen YG, Hagopian WA, *et al.* Blood-based signatures in type 1 diabetes. Diabetologia 2016; 59:414–425.
9. Yip L, Fathman CG. Type 1 diabetes in mice and men: gene expression profiling to investigate disease pathogenesis. Immunol Res 2014; 58: 340–350.
10. Akirav EM, Lebastchi J, Galvan EM, *et al.* Detection of β cell death in diabetes using differentially methylated circulating DNA. Proc Natl Acad Sci U S A 2011; 108:19018–19023.
11. Fisher MM, Perez Chumbiauca CN, Mather KJ, *et al.* Detection of islet β-cell death in vivo by multiplex PCR analysis of differentially methylated DNA 2013. Endocrinology 2013; 154:3476–3481.
12. Sosenko JM, Skyler JS, Beam CA, *et al.* The development and utility of a novel scale that quantifies the glycemic progression toward type 1 diabetes over 6 months. Diabetes Care 2015; 38:940–942.
13. Maecker HT, Lindstrom TM, Robinson WH, *et al.* New tools for classification and monitoring of autoimmune diseases. Nat Rev Rheumatol 2012; 8:317–328.
14. Yates LA, Norbury CJ, Gilbert RJC. The long and short of microRNA. Cell 2013; 153:516–519.
15. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116:281–297.
16. Zhdanov VP. Conditions of appreciable influence of microRNA on a large number of target mRNAs. Mol Biosyst 2009; 5:638–643.
17. Sato F, Tsuchiya S, Meltzer SJ, Shimizu K. MicroRNAs and epigenetics. FEBS J 2011; 278:1598–1609.
18. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011; 39: 7223–7233.
19. Li L, Zhu D, Huang L, *et al.* Argonaute 2 complexes selectively protect the circulating microRNAs in cell-secreted microvesicles. PloS One 2012; 7:e46957.
20. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014; 30:255–289.
21. Conigliaro A, Fontana S, Raimondo S, Alessandro R. Exosomes: nanocarriers of biological messages. Adv Exp Med Biol 2017; 998:23–43.
22. Valadi H, Ekström K, Bossios A, *et al.* Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9:654–659.
23. Backes C, Meese E, Keller A. Specific miRNA disease biomarkers in blood, serum and plasma: challenges and prospects. Mol Diagn Ther 2016; 20:509–518.
24. Wang J, Chen J, Sen S. Microrna as biomarkers and diagnostics. J Cell Physiol 2016; 231:25–30.
25. Filios SR, Shalev A. β-cell microRNAs: small but powerful. Diabetes 2015; 64:3631–3644.
26. Calderari S, Diawara M, Garaud A, Gauguier D. Biological roles of microRNAs in the control of insulin secretion and action. Physiol Genomics 2017; 49:1–10.
27. Motterle A, Sanchez-Parra C, Regazzi R. Role of long non-coding RNAs in the determination of β-cell identity. Diabetes Obes Metab 2016; 18(Suppl 1):41–50.
28. Singer RA, Arnes L, Sussel L. Noncoding RNAs in β cell biology. Curr Opin Endocrinol Diabetes Obes 2015; 22:77–85.
29. Kalis M, Bolmeson C, Esguerra JLS, *et al.* Beta-cell specific deletion of Dicer1 leads to defective insulin secretion and diabetes mellitus. PLoS One 2011; 6:e29166.
30. Mandelbaum AD, Melkman-Zehavi T, Oren R, *et al.* Dysregulation of Dicer1 in beta cells impairs islet architecture and glucose metabolism. Exp Diabetes Res 2012; 6:470302.
31. Tugay K, Guay C, Marques AC, *et al.* Role of microRNAs in the age-associated decline of pancreatic beta cell function in rat islets. Diabetologia 2016; 59:161–169.
32. Jacovetti C, Abderrahmani A, Parnaud G, *et al.* MicroRNAs contribute to compensatory β cell expansion during pregnancy and obesity. J Clin Invest 2012; 122:3541–3551.
33. Roggli E, Britan A, Gattesco S, *et al.* Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic beta-cells. Diabetes 2010; 59:978–986.
34. Chaparro RJ, Dilorenzo TP. An update on the use of NOD mice to study autoimmune (Type 1) diabetes. Expert Rev Clin Immunol 2010; 6:939–955.
35. Bravo-Egana V, Rosero S, Klein D, *et al.* Inflammation-mediated regulation of MicroRNA expression in transplanted pancreatic islets. J Transplant 2012; (2012):723614.
36. Sims EK, Lakhter AJ, Anderson-Baucum E, *et al.* MicroRNA 21 targets BCL2 mRNA to increase apoptosis in rat and human beta cells. Diabetologia 2017; 60:1057–1065.
37. Ruan Q, Wang T, Kameswaran V, *et al.* The microRNA-21-PDCD4 axis prevents type 1 diabetes by blocking pancreatic beta cell death. Proc Natl Acad Sci U S A 2011; 108:12030–12035.
38. Wang S, Wan X, Ruan Q. The microRNA-21 in autoimmune diseases. Int J Mol Sci 2016; 17:864.
39. Pan W, Zhu S, Yuan M, *et al.* MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. J Immunol 2010; 184:6773–6781.
40. Stagakis E, Bertsias G, Verginis P, *et al.* Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: miR-21 regulates aberrant T cell responses through regulation of PDCD4 expression. Ann Rheum Dis 2011; 70:1496–1506.
41. Fenoglio C, Cantoni C, De Riz M, *et al.* Expression and genetic analysis of miRNAs involved in CD4+ cell activation in patients with multiple sclerosis. Neurosci Lett 2011; 504:9–12.
42. Meisgen F, Xu N, Wei T, *et al.* MiR-21 is up-regulated in psoriasis and suppresses T cell apoptosis. Exp Dermatol 2012; 21:312–314.
43. Killeen ME, Ferris L, Kupetsky EA, *et al.* Signaling through purinergic receptors for ATP induces human cutaneous innate and adaptive Th17 responses: implications in the pathogenesis of psoriasis. J Immunol 2013; 190:4324–4336.
44. Dong L, Wang X, Tan J, *et al.* Decreased expression of microRNA-21 correlates with the imbalance of Th17 and Treg cells in patients with rheumatoid arthritis. J Cell Mol Med 2014; 18:2213–2224.
45. Wang L, He L, Zhang R, *et al.* Regulation of T lymphocyte activation by microRNA-21. Mol Immunol 2014; 59:163–171.
46. Salaun B, Yamamoto T, Badran B, *et al.* Differentiation associated regulation of microRNA expression in vivo in human CD8+ T cell subsets. J Transl Med 2011; 9:44.
47. Smigielska-Czepiel K, van den Berg A, Jellema P, *et al.* Dual role of miR-21 in CD4+ T-cells: activation-induced miR-21 supports survival of memory T-cells and regulates CCR7 expression in naive T-cells. PLoS One 2013; 8:e76217.

48. Kroesen BJ, Teteloshvili N, Smigielska-Czepiel K, *et al.* Immuno-miRs: critical regulators of T-cell development, function and ageing. Immunology 2015; 144:1–10.
49. Murugaiyan G, da Cunha AP, Ajay AK, *et al.* MicroRNA-21 promotes Th17 differentiation and mediates experimental autoimmune encephalomyelitis. J Clin Invest 2015; 125:1069–1080.
50. Honardoost MA, Naghavian R, Ahmadinejad F, *et al.* Integrative computational mRNA-miRNA interaction analyses of the autoimmune-deregulated miRNAs and well-known Th17 differentiation regulators: an attempt to discover new potential miRNAs involved in Th17 differentiation. Gene 2015; 572:153–162.
51. Teteloshvili N, Kluiver J, van der Geest KSM, *et al.* Age-associated differences in MiRNA signatures are restricted to CD45RO negative T cells and are associated with changes in the cellular composition, activation and cellular ageing. PLoS One 2015; 10:e0137556.
52. Wu H, Neilson JR, Kumar P, *et al.* miRNA profiling of naïve, effector and memory CD8 T cells. PLoS One 2007; 2:e1020.
53. Rouas R, Fayyad-Kazan H, El Zein N, *et al.* Human natural Treg microRNA signature: role of microRNA-31 and microRNA-21 in FOXP3 expression. Eur J Immunol 2009; 39:1608–1618.
54. Kim KW, Ho A, Alshabee-Akil A, *et al.* Coxsackievirus B5 infection induces dysregulation of microRNAs predicted to target known type 1 diabetes risk genes in human pancreatic islets. Diabetes 2016; 65:996–1003.
55. Dotta F, Censini S, van Halteren AGS, *et al.* Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci U S A 2007; 104:5115–5120.
56. Hyöty H. Viruses in type 1 diabetes. Pediatr Diabetes 2016; 17(Suppl 22): 56–64.
57. Sioofy-Khojine AB, Lehtonen J, Nurminen N, *et al.* Coxsackievirus B1 infections are associated with the initiation of insulin-driven autoimmunity that progresses to type 1 diabetes. Diabetologia 2018; 61:1193–1202.
58. Roggli E, Gattesco S, Caille D, *et al.* Changes in microRNA expression contribute to pancreatic β-cell dysfunction in prediabetic NOD mice. Diabetes 2012; 61:1742–1751.
59. ■ Grieco FA, Sebastiani G, Juan-Mateu J, *et al.* MicroRNAs miR-23a-3p, miR-23b-3p, and miR-149-5p Regulate the Expression of Proapoptotic BH3-Only Proteins DP5 and PUMA in Human Pancreatic β-Cells. Diabetes 2017; 66:100–112.

This is the first study perfoming miRNA profiling from human islets treated with proinflammatory cytokines. Authors found a novel cross-talk between a family of miRNA and key apoptotic Bcl-2 proteins in β-cell.

60. Guay C, Kruit J, Rome S, *et al.* MiRNAs transferred by exosomes from T lymphocytes to beta cells contribute to type 1 diabetes development. Diabetologia 2017; 60:S65.
61. Cobb BS, Nesterova TB, Thompson E, *et al.* T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer. J Exp Med 2005; 201:1367–1373.
62. Cobb BS, Hertweck A, Smith J, *et al.* A role for Dicer in immune regulation. J Exp Med 2006; 203:2519–2527.
63. Koralov SB, Muljo SA, Galler GR, *et al.* Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. Cell 2008; 132:860–874.
64. Muljo SA, Ansel KM, Kanellopoulou C, *et al.* Aberrant T cell differentiation in the absence of Dicer. J Exp Med 2005; 202:261–269.
65. Zhou X, Jeker LT, Fife BT, *et al.* Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. J Exp Med 2008; 205:1983–1991.
66. Liston A, Lu LF, O'Carroll D, *et al.* Dicer-dependent microRNA pathway safeguards regulatory T cell function. J Exp Med 2008; 205:1993–2004.
67. Rodriguez A, Vigorito E, Clare S, *et al.* Requirement of bic/microRNA-155 for normal immune function. Science 2007; 316:608–611.
68. Thai TH, Calado DP, Casola S, *et al.* Regulation of the germinal center response by microRNA-155. Science 2007; 316:604–608.
69. Lu LF, Thai TH, Calado DP, *et al.* Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity 2009; 30:80–91.
70. Lowe CE, Cooper JD, Brusko T, *et al.* Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. Nat Genet 2007; 39:1074–1082.
71. Hull CM, Peakman M, Tree T. Regulatory T cell dysfunction in type 1 diabetes: what's broken and how can we fix it? Diabetologia 2017; 60:1839–1850.
72. Lu LF, Boldin MP, Chaudhry A, *et al.* Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell 2010; 142:914–929.
73. Pugliese A, Yang M, Kusmarteva I, *et al.* The Juvenile Diabetes Research Foundation Network for Pancreatic Organ Donors with Diabetes (nPOD) Program: goals, operational model and emerging findings. Pediatr Diabetes 2014; 15:1–9.
74. Ferraro A, Socci C, Stabilini A, *et al.* Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. Diabetes 2011; 60:2903–2913.
75. ■ Sebastiani G, Ventriglia G, Stabilini A, *et al.* Regulatory T-cells from pancreatic lymphnodes of patients with type-1 diabetes express increased levels of microRNA miR-125a-5p that limits CCR2 expression. Sci Rep 2017; 7:6897.

This is the first study reporting miRNAs signature in Treg cells deriving from PLN of T1D patients. These cells showed a specific increase in miR-125a-5p, thus decreasing its target gene CCR2 of potential importance for Treg migration.

76. Culina S, Lalanne AI, Afonso G, *et al.* Islet-reactive CD8+T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. Sci Immunol 2018; 3:; pii: eaao4013.
77. Zhang Y, Feng ZP, Naselli G, *et al.* MicroRNAs in CD4(+) T cell subsets are markers of disease risk and T cell dysfunction in individuals at risk for type 1 diabetes. J Autoimmun 2016; 68C:52–61.
78. ■ Serr I, Scherm MG, Zahm AM, *et al.* A miRNA181a/NFAT5 axis links impaired T cell tolerance induction with autoimmune type 1 diabetes. Sci Transl Med 2018; 10; pii: eaag1782.

In this article, Serr *et al.* found enhanced miR-181a expression in $CD4^+$ T cells from children with ongoing islet autoimmunity. MiR-181a increase caused an increase in signal strength of stimulatory and costimulatory signals, thus decreasing Treg cell induction.

79. Serr I, Fürst RW, Ott VB, *et al.* miRNA92a targets KLF2 and the phosphatase PTEN signaling to promote human T follicular helper precursors in T1D islet autoimmunity. Proc Natl Acad Sci U S A 2016; 113:6659–6668.
80. de Jong VM, van der Slik AR, Laban S, *et al.* Survival of autoreactive T lymphocytes by microRNA-mediated regulation of apoptosis through TRAIL and Fas in type 1 diabetes. Genes Immun 2016; 17:342–348.
81. Abuhatzira L, Xu H, Tahhan G, *et al.* Multiple microRNAs within the 14q32 cluster target the mRNAs of major type 1 diabetes autoantigens IA-2, IA-2β, and GAD65. FASEB J 2015; 29:4374–4383.
82. Mandemakers W, Abuhatzira L, Xu H, *et al.* Co-regulation of intragenic microRNA miR-153 and its host gene Ia-2 β: identification of miR-153 target genes with functions related to IA-2β in pancreas and brain. Diabetologia 2013; 56:1547–1556.
83. Ventriglia G, Nigi L, Sebastiani G, Dotta F. MicroRNAs: novel players in the dialogue between pancreatic islets and immune system in autoimmune diabetes. Biomed Res Int 2015; 2015:749734.
84. Greenbaum CJ, Beam CA, Boulware D, *et al.,* Type 1 Diabetes TrialNet Study Group. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. Diabetes 2012; 61:2066–2073.
85. Nielsen LB, Wang C, Sørensen K, *et al.* Circulating levels of microRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that miR-25 associates to residual beta-cell function and glycaemic control during disease progression. Exp Diabetes Res 2012; 2012:896362.
86. Latreille M, Herrmanns K, Renwick N, *et al.* miR-375 gene dosage in pancreatic β-cells: implications for regulation of β-cell mass and biomarker development. J Mol Med 2015; 93:1159–1169.
87. Assmann TS, Coutinho MKP, Tschiedel B, *et al.* Circulating microRNAs as biomarkers for type 1 diabetes mellitus. Diabetol Metab Syndr 2015; 7(Suppl 1):A206.
88. Marchand L, Jalabert A, Meugnier E, *et al.* miRNA-375 a sensor of glucotoxicity is altered in the serum of children with newly diagnosed type 1 diabetes. J Diabetes Res 2016; 2016:1869082.
89. Seyhan AA, Nunez Lopez YO, Xie H, *et al.* Pancreas-enriched miRNAs are altered in the circulation of subjects with diabetes: a pilot cross-sectional study. Sci Rep 2016; 6:31479.
90. Yin C, Weiland M, Li J, *et al.* Serum miRNAs as potential biomarkers for early prediction of type 1 diabetes. FASEB J 2016; 30(Suppl 1).
91. Erener S, Marwaha A, Tan R, *et al.* Profiling of circulating microRNAs in children with recent onset of type 1 diabetes. JCI Insight 2017; 2:e89656.
92. ■■ Samandari N, Mirza AH, Nielsen LB, *et al.* Circulating microRNA levels predict residual beta cell function and glycaemic control in children with type 1 diabetes mellitus. Diabetologia 2017; 60:354–363.

This study demonstrated that the assessment of circulating miR-193-3p at 3 months could predict residual β-cell function 1 year after diagnosis in children with T1D. Taking into account T1D heterogeneity, this study underlines the potential role of circulating miRNAs as predictive biomarkers for patient's stratifications.

93. ■ Snowhite IV, Allende G, Sosenko J, *et al.* Association of serum microRNAs with islet autoimmunity, disease progression and metabolic impairment in relatives at risk of type 1 diabetes. Diabetologia 2017; 60:1409–1422.

This article revealed that increased levels of selected circulating miRNAs were associated with disease progression in autoantibody-positive individuals. Moreover, some of the miRNAs identified were associated with β-cell function. This work suggests the possibility to combine miRNAs to other biomarkers in order to improve T1D prediction.

94. ■ Åkerman L, Casas R, Ludvigsson J, *et al.* Serum miRNA levels are related to glucose homeostasis and islet autoantibodies in children with high risk for type 1 diabetes. PLoS One 2018; 13:e0191067.

In this article, Akerman *et al.* assessed serum miRNAs in high-risk individuals positive for multiple islet autoantibodies, healthy children and recent onset T1D patients. Although no specific miRNA profile was identified for the high-risk group, same miRNAs appeared to reflect glycemic status and ongoing autoimmunity.

95. ■■ Lakhter AJ, Pratt RE, Moore RE, *et al.* Beta cell extracellular vesicle miR-21-5p cargo is increased in response to inflammatory cytokines and serves as a biomarker of type 1 diabetes. Diabetologia 2018; 61:1124–1134.

This article revealed for the first time that the expression of total circulating miRNAs can differ from serum-derived extracellular vescicles, thus encouraging miRNA analysis in secreted subcellular compartments in addition to total biofluids in order to better uncover potential T1D-related biomarkers.

96. Zhu Y, You W, Wang H, *et al.* MicroRNA-24/MODY gene regulatory pathway mediates pancreatic β-cell dysfunction. Diabetes 2013; 62:3194–3206.
97. Iguchi H, Ikeda Y, Okamura M, *et al.* SOX6 attenuates glucose-stimulated insulin secretion by repressing PDX1 transcriptional activity and is down-regulated in hyperinsulinemic obese mice. J Biol Chem 2005; 280:37669–37680.
98. Melkman-Zehavi T, Oren R, Kredo-Russo S, *et al.* miRNAs control insulin content in pancreatic β-cells via downregulation of transcriptional repressors. EMBO J 2011; 30:835–845.
99. Setyowati Karolina D, Sepramaniam S, Tan HZ, *et al.* MiR-25 and miR-92a regulate insulin I biosynthesis in rats. RNA Biol 2013; 10:1365–1378.
100. Pan L, Huang B-J, Ma X-E, *et al.* MiR-25 protects cardiomyocytes against oxidative damage by targeting the mitochondrial calcium uniporter. Int J Mol Sci 2015; 16:5420–5433.
101. Salunkhe VA, Esguerra JL, Ofori JK, *et al.* Modulation of microRNA-375 expression alters voltage-gated Na(+) channel properties and exocytosis in insulin-secreting cells. Acta Physiol (Oxf) 2015; 312:882–892.
102. Poy MN, Eliasson L, Krutzfeldt J, *et al.* A pancreatic islet-specific microRNA regulates insulin secretion. Nature 2004; 432:226–230.
103. Kloosterman WP, Lagendijk AK, Ketting RF, *et al.* Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. PLoS Biol 2007; 5:e203.
104. Joglekar MV, Joglekar VM, Hardikar AA. Expression of islet-specific microRNAs during human pancreatic development. Gene Expr Patterns 2009; 9:109–113.
105. Poy MN, Hausser J, Trajkovski M, *et al.* miR-375 maintains normal pancreatic alpha- and beta-cell mass. Proc Natl Acad Sci U S A 2009; 106: 5813–5818.
106. Kim HS, Shen Q, Nam SW. Histone deacetylases and their regulatory micrornas in hepatocarcinogenesis. J Korean Med Sci 2015; 30: 1375–1380.
107. Bagge A, Dahmcke CM, Dalgaard LT. Syntaxin-1a is a direct target of miR-29a in insulin-producing β-cells. Horm Metab Res 2013; 45:463–466.
108. Pullen TJ, da Silva Xavier G, Kelsey G, Rutter GA. miR-29a and miR-29b contribute to pancreatic beta-cell-specific silencing of monocarboxylate transporter 1 (Mct1). Mol Cell Biol 2011; 31:3182–3194.
109. Dooley J, Garcia-Perez JE, Sreenivasan J, *et al.* The microRNA-29 family dictates the balance between homeostatic and pathological glucose handling in diabetes and obesity. Diabetes 2016; 65:53–61.
110. Chen YH, Heneidi S, Lee JM, *et al.* miRNA-93 inhibits GLUT4 and is overexpressed in adipose tissue of polycystic ovary syndrome patients and women with insulin resistance. Diabetes 2013; 62:2278–2286.
111. Erener S, Mojibian M, Fox JK, *et al.* Circulating miR-375 as a biomarker of β-cell death and diabetes in mice. Endocrinology 2013; 154:603–608.
112. Nerup J, Pociot F; European Consortium for IDDM Studies. A genomewide scan for type 1-diabetes susceptibility in Scandinavian families: identification of new loci with evidence of interactions. Am J Hum Genet 2001; 69: 1301–1313.
113. Christensen DP, Dahllöf M, Lundh M, *et al.* Histone deacetylase (HDAC) inhibition as a novel treatment for diabetes mellitus. Mol Med 2011; 17: 378–390.
114. Salas-Pérez F, Codner E, Valencia E, *et al.* MicroRNAs miR-21a and miR-93 are down regulated in peripheral blood mononuclear cells (PBMCs) from patients with type 1 diabetes. Immunobiology 2013; 218:733–737.
115. Klein D, Misawa R, Bravo-Egana V, *et al.* MicroRNA expression in alpha and beta cells of human pancreatic islets. PLoS One 2013; 8:e55064.
116. Bagge A, Clausen TR, Larsen S, *et al.* MicroRNA-29a is up-regulated in beta-cells by glucose and decreases glucose-stimulated insulin secretion. Biochem Biophys Res Commun 2012; 426:266–272.
117. Esteves JV, Enguita FJ, Machado UF. MicroRNAs-mediated regulation of skeletal muscle GLUT4 expression and translocation in insulin resistance. J Diabetes Res 2017; 2017:7267910.