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MicroRNAs and the functional β-cell mass: for better or worse

Running title: microRNAs and β-cell mass

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Abstract

Insulin secretion from pancreatic β-cells plays a central role in the control of blood glucose levels. The amount of insulin released by β-cells is precisely adjusted to match the organism requirements. A number of conditions occurring during life, including pregnancy and obesity, can result in a decrease in the sensitivity of insulin target tissues and in the consequent rise in the insulin needs. To preserve glucose homeostasis, the augmented insulin demand requires a compensatory expansion of the mass of pancreatic β-cells and an increase in their secretory activity. This compensatory process is known to be accompanied by modifications in β-cell gene expression but the molecular mechanisms underlying this phenomenon are still poorly understood. Emerging evidence indicate that at least part of these compensatory events may be orchestrated by changes in the level of a novel class of gene regulators, the microRNAs. Indeed, several of these small non-coding RNAs have either positive or negative impacts on β-cell proliferation and survival. The studies reviewed in this paper suggest that the balance between the actions of these two groups of microRNAs with opposing functional effects determines whether β-cells expand sufficiently to maintain blood glucose levels in the normal range or fail to meet the insulin demand with a consequent progression toward diabetes manifestation. A better understanding of the mechanisms governing the changes in the microRNA profile will open the way for the development of new strategies to prevent and/or treat type 2 and gestational diabetes.

Key words: microRNAs, pancreatic islets, diabetes, gene regulation

Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by a diminished sensitivity of insulin target tissues, including liver, skeletal muscles and adipose tissue, and the inability of the pancreatic β-cells to secrete enough insulin to sustain the organism demand. In pre-diabetic conditions, β-cells compensate the insulin resistant state of the target tissues by increasing their number and size and by augmenting the amount of hormone released in response to glucose and fatty acids [1]. The duration of this compensatory state differs between individuals and can eventually last for the entire life. When β-cells can no longer face the elevated insulin demand, they become dysfunctional and their survival is impaired favoring the development of T2DM. Despite intensive efforts, the precise mechanisms underlying compensatory β-cell mass expansion are still not well understood. Changes in the functional β-cell mass are known to necessitate adaptations in gene expression programs but the key factors driving this phenomenon are still not fully elucidated.

In the last few years, microRNAs (miRNAs) have been discovered to form a complex regulatory network governing the activities of β-cells. These small non-coding RNA molecules act as transcriptional repressors by binding to the 3'UTR of target mRNAs (Figure 1) [2]. The current estimations indicate that the human genome encodes for more than 1800 miRNA precursors producing up to 2585 mature miRNAs [3]. Notably, a single miRNA can bind more than hundred different target mRNAs, hence modulating directly or indirectly the expression of numerous genes. Indeed, at least 60% of the protein-coding genes are believed to be under the control of miRNAs [4]. In view of this, miRNAs are now recognized as major regulators of gene expression and key players in the control of several biological and pathological processes, including diabetes mellitus [5]. The potential role of miRNAs in the development of diabetes and its complications has been reviewed extensively in the past years [6-10]. Here, we will focus specifically on the contribution of miRNAs to the control of the functional β-cell mass.

Evidence for the involvement of miRNAs in the control of pancreatic β-cell mass and function

The potential role of miRNAs in β-cell differentiation and in the control of β-cell function was initially investigated by knocking-down Dicer1, the enzyme required for the maturation of most miRNAs (see Figure 1). Whole-body deletion of *Dicer1* in mice results in early embryonic lethality [11] but, surprisingly, *Dicer1*-hypomorphic mice, expressing about 20% of wild type *Dicer1* level, are viable and display histologically normal tissue development throughout fetal and neonatal stages. However, starting from 4 weeks of age, the pancreas showed morphologic abnormalities [12]. Lynn and colleagues generated a pancreatic *Dicer1*-null mice who survived until birth but died at 3 days of age (P3) [13]. These Pdx1-Cre *Dicer1* animals displayed severe defects in pancreas development and in the differentiation of the endocrine lineage, with strong impairment in β-cell formation. In another study, deletion of *Dicer1* specifically in pancreatic endocrine progenitor cells did not affect specialization of hormone-expressing cells during embryogenesis but displayed loss of hormone expression during neonatal phase. [14]. Moreover, during the neonatal period these Ngn3-Cre *Dicer* mice exhibited altered islet organization, reduced β-cell mass and decreased proinsulin mRNA expression with a consequent reduction in insulin content. As expected, these mice developed hyperglycemia and overt diabetes within 2 weeks of age [14].

To investigate more precisely the role of miRNAs in insulin-secreting cells, *Dicer1* was conditionally deleted using *Cre recombinase* system under control of the promoter of the rat insulin 1 (RIP1) or 2 (RIP2) gene. Of note, rodents have 2 nonallelic insulin genes as opposed to human that have a single copy of the insulin gene. Both RIP1 and RIP2 are commonly used to delete a gene specifically in beta-cells. Kalis *et al*. generated a β-cell specific deletion of *Dicer1* [15] under the regulation of RIP1. Fetal and neonatal β-cell development was not affected but RIP1-Cre *Dicer1* null mice became progressively hyperglycemic, leading to diabetes onset in adulthood. These animals display defects in islet number, size and architecture, in β-cell mass and in insulin biosynthesis and secretion. Two other research groups developed conditioned *Dicer1* deletion under the regulation of RIP2. Lynn and colleagues did not observed any morphological defect in pancreatic islets of 8 month-old mice [13]. In opposite, Mandelbaum and colleagues observed reduced beta-cell number and mass and insulin content in pancreatic islets of 4 month-old mice [16]. Moreover, these RIP2-Cre *Dicer1* null mice were glucose intolerant at the age of 1-2 month and the same trend was also observed at the age of 8-10 months. Of note, mutant mice exhibited mosaic islets, meaning that not all beta-cells underwent *Cre* recombination leading to a mix of “mutant” (*Cre*-positive cells in which Dicer1 activity was disrupted) and wild-type (*Cre*-

negative) beta-cells. Interestingly, in early post-natal life, most of the beta-cells were knocked down for *Dicer1* but a gradual loss of mutant beta-cells was than observed. By the age of P30, almost no *Cre*-positive cells could be detected. Therefore, the proliferation of Cre-negative cells progressively compensated for the reduction of beta-cell mass [16]. Finally, Melkman-Zehavi *et al.* engendered tamoxifen-inducible RIP-Cre *Dicer1* mice to explore the involvement of miRNAs in adult β-cell function [17]. Tamoxifen-treated animals developed hyperglycemia in both fasted and fed states and insulin intolerance few weeks after the injections. Interestingly, the 50% decrease in *Dicer1* measured in pancreatic islets of Tamoxifen-treated mice correlated with a reduction of the level of several miRNAs and with a marked decline in *insulin* gene expression, leading to reduced insulin content and impaired glucose-induced insulin secretion. As expected, the β-cell mass was not affected by the loss of *Dicer1* in adult mice [17]. Taken together, these studies underline the key role of Dicer1, and its associated miRNA network, in endocrine pancreas development, β-cell differentiation, preservation of the β-cell mass and proper function of insulin-secreting cells.

In adult mice, similar results were obtained when *Ago2* expression was deregulated. Ago2 is the most abundant member of the argonaute protein family in β-cells and is the catalytic component of the RNA-induced silencing complex (RISC) complex necessary for miRNA action (see Figure 1). Mice overexpressing *Ago2* in β-cells showed normal body weight and plasmatic concentration of glucose and insulin [18]. However, when challenged with glucose, mice overexpressing *Ago2* exhibited an impaired insulin secretion leading to transient elevation in glycaemia. Morphometric analysis revealed an increase in β-cell number, mass and proliferative capacity. In contrast, knockdown of *Ago2* in β-cells resulted in the reduction in the number, mass and proliferative capacity of β-cell and in a lower insulin content [18]. Islet architecture was not affected and no changes in cell-death were detected. Loss of *Ago2* resulted in enhanced insulin secretion in response to glucose, leading to improved glucose tolerance. Of note, *Ago1* expression was increased in pancreatic islets of *Ago2* knockout mice, suggesting a partial compensatory mechanism. Taken together, these results indicate that an appropriate level of Ago2 is necessary to maintain an appropriate functional β-cell mass.

Role of specific miRNAs in the development of β-cells and in the control of their activities

The involvement in β-cell development and function of particular miRNAs highly expressed in pancreatic islets such as let-7, miR-7 and miR-375, was explored in detail both *in vitro* and *in vivo*. Let-7 was the first miRNA discovered in *C. elegans* [19]. Mammalian cells are known to contain a family of closely related let-7 miRNAs sharing a common seed region. Let-7 family members are involved in cell differentiation and have been associated to the development of several forms of cancer [20]. Members of the let-7 family are highly expressed in pancreatic islets [21, 22] but their precise role in regulating the activity of insulin-secreting cells is still not completely understood. Transgenic mice overexpressing let-7a/d/f or let-7g in all tissues have reduced body size and weight, decreased fat mass and impaired glucose homeostasis mainly due to a decrease in insulin release by the endocrine pancreas in response to glucose [23, 24]. Interestingly, overexpression of let-7a/d/f solely in β-cells led also to impaired glucose tolerance, but not when let-7 expression was selectively increased either in muscle, adipose tissue, neurons, or adipocytes, suggesting a preponderant role of β-cells in this metabolic phenotype. In contrast, global knockdown of let-7 using anti-miR oligonucleotides improved glucose homeostasis and was sufficient to prevent impaired glucose intolerance induced by high-fat feeding, but this was mainly the result of improved insulin sensitivity of target tissues [23]. Looking in more details at the mechanisms in liver and muscles, let-7 was found to regulate the expression of the insulin receptor and of insulin receptor substrate 2 (IRS2) [23] and to affect the insulin-PI3K-mTOR axis [24]. The mode of action of let-7 in β-cells remains to be investigated.

Mir-375 was first cloned from an insulin-secreting cell line and was found to be highly enriched in the endocrine pancreas [25]. In their pioneering work, Poy *et al.* demonstrated that blockade of miR-375 expression *in vitro* results in enhanced glucose-induced insulin secretion whereas the overexpression of this non-coding RNA reduces insulin release in response to different secretagogues. This effect was at least in part attributed to silencing of *myotrophin*, one of the direct targets of miR-375 [25]. In addition to its negative effect on insulin exocytosis, overexpression of miR-375 was reported to inhibit insulin gene expression in response to glucose by down-regulating *PDK1* and thus inhibiting the phosphoinositol-3-kinase (PI3K) pathway [26]. The expression of miR-375 was found to be under the control of two transcription factors playing a pivotal role in islet cell differentiation, namely NeuroD1 and Pdx1 [27]. Indeed, *in vivo* studies revealed that miR-375 is involved in the development of the endocrine pancreas [28, 29]. Deletion of *miR-375* in mice resulted in a reduction in the β-cell mass and in an increase in the α-cell number, leading to disrupted

islet architecture [29]. In line with these observations, KO-*miR375* mice exhibited hyperglycemia and elevated plasma glucagon levels. In contrast, plasma insulin levels and glucose-induced insulin secretion were unaffected. When these animals were crossed with leptin deficient *ob/ob* mice, they developed severe diabetes due to the incapacity of β-cells to expand and compensate for obesity-associated insulin resistance [29]. The α-cell number was also increased in islets of KO-*miR375*-*ob/ob* compared to WT-*ob/ob* mice. Taken together, these results point to a key role for miR-375 in regulating islet cell development and β-cell mass expansion. A direct causative link between miR-375 dysregulation and diabetes development remains however to be settled. In fact, an increase in the level of pre-miR-375 has been reported in GK rats [26] but this observation was not confirmed by an independent study [30]. A small elevation of miR-375 was also reported in islets of *ob/ob* and DIO mice [18, 29], but changes in miR-375 expression were neither detected in other studies by microarray profiling of islets of *ob/ob*, *db/db* and DIO mice [31, 32] nor in islets of T2DM human donors [21, 33]. Interestingly, increased levels of miR-375 were observed in the islets of the offspring of rats fed a low-protein diet during pregnancy. The level of miR-375 remained elevated until the adulthood, suggesting that deregulation of this miRNA contributes to defective endocrine pancreas activity and to the increased risk of developing T2DM typically observed upon in utero malnutrition [34].

As explained above, miR-375 plays complex regulatory roles in pancreatic islets being involved not only in the control of β-cell mass expansion and insulin secretion but governing also the activity and proliferation of glucagon-secreting α-cells that have opposing effects on blood glucose levels. All studies carried out so far searched for changes in the expression of miR-375 in whole islets. However, changes in the balance between the levels of miR-375 in α- and β-cells will probably have more relevant impacts on the metabolic control. Detailed analysis of miR-375 expression in FACS-sorted α- and β-cells isolated from diabetes animal models or from T2DM human donors will help addressing this issue.

In human and mouse, miR-7 is highly enriched in the developing endocrine pancreas and in the islets of Langerhans in adults [35-37]. Down-regulation of miR-7 in mice embryos using small oligonucleotides called morpholinos resulted in a decrease in β-cell number and in reduced insulin production, leading to post-natal glucose intolerance [38]. Interestingly, the expression of miR-7 is regulated by the endocrine-specific transcription factors NeuroD/beta2 and Ngn3 [37]. Three different pre-miR-7 precursors (miR-7a1, miR-7a2 and miR-7b) are co-expressed in mammalian pancreatic islets and generate two mature miR-7 isoforms (miR-

7a and miR-7b) sharing the same seed sequence. Of the three precursors, miR-7a2 is the most abundant in mice islets [39]. In fact, deletion of pre-miR-7a1 decreased the level of mature miR-7 by only 20% whereas deletion of pre-miR-7a2 reduced the expression by 80%. RIP-Cre-*miR-7a2* mice exhibited improved glucose tolerance mainly resulting from enhanced insulin release. No differences in islet architecture and β-cell mass were observed in KO-*miR7* mice compared to wild type mice. Augmentation of insulin secretion in response to secretagogues was also detected *ex vivo* in islets isolated from 5 and 35 weeks old KO-*miR7* mice. In agreement with these findings, several genes involved in late stages of insulin granule fusion, such as *SNCA,* were identified as direct targets of miR-7. In the same study, deregulation of miR-7 expression *in vivo* did not impact on cell survival and β-cell proliferation [39]. However, another group reported that blockade of miR-7a in adult mouse islet cells activates the mTOR pathway and promotes β-cell proliferation [40]. Thus, changes in the level of miR-7 can modulate the activities of β-cells and deregulation of its expression can potentially affect the adaptive capacity of β-cells, contributing to the development of diabetes. The expression of miR-7 was reported to be decreased by about half in mice fed a high fat diet, in *ob/ob* mice and in young *db/db* mice [21, 39] but to be increased in islets of older diabetic *db/db* mice [39]. In obese and T2DM human donors, expression of miR-7 was found down-regulated [21]. Other studies reported no significant differences in miR-7 levels upon global miRNA profiling of islets isolated from these and other animal models or from T2DM donors [18, 30-32]. The level of this miRNA seems to vary over the different stages of the disease with an initial decrease in response to insulin resistance followed by an increase during the decompensation phase. Thus, according to the time point selected, changes in miR-7 level may possibly have escaped detection.

Interestingly, a novel class of non-coding RNAs, the circular RNAs, was recently described [41]. One of them, ciRS-7 that is highly expressed in nervous tissues is suggested to modulate the availability of miR-7 since its sequence contains more than 60 conserved binding sites for this specific miRNA [42, 43]. Therefore, the expression of miR-7 seems to be tightly regulated and measurement of ciRS-7 would be of interest in β-cells under physiological and physiopathologial conditions.

Contribution of other miRNAs to compensatory β-cell mass expansion or to β-cell failure

Beside let-7, miR-7 and miR-375, several other miRNAs have been investigated *in vitro* or *ex vivo* in β-cells for their involvement in the regulation of insulin biosynthesis and secretion, proliferation and apoptosis/survival. Here, we will focus exclusively on miRNAs that display expression changes in diabetes animal models or in the islets of T2DM donors. For a more exhaustive list of miRNAs potentially affecting the activities of β-cells we refer the reader to other recent reviews in the field [6-9].

To identify miRNAs potentially contributing to compensatory β-cell mass expansion or, on the contrary, to β-cell dysfunction associated with the manifestation of T2DM, several studies analyzed the miRNA expression profiles in well-characterized diabetes animal models. The Goto-kakizaki (GK) rat is a model of non-obese, insulin resistant and moderately hyperglycaemic animals that spontaneously develop T2DM early in life. Pancreatic islets of GK rats exhibit defective insulin release in response to glucose [44]. Esguerra *et al.* performed a global miRNA profiling of islets of 8 weeks old GK rats and identified 30 miRNAs differentially expressed compared to Wistar control rats [30]. The changes of several upregulated (miR-124, -130a, -132, -142-3p, -142-5p -212, -335, -376a, -409-3p and -433) or down-regulated (miR-708) miRNAs were then confirmed by qPCR. The expression of four of these miRNAs (miR-130a, miR-132, miR-212 and miR-335) were found to be regulated by hyperglycemia. Computational predictions revealed that the putative target of the upregulated miRNAs are enriched for genes involved in the secretory process and were suggested to contribute to the defective insulin secretion observed in GK rats [30]. However, the functional impact of the increase in the level of these miRNA was not experimentally assessed in this study.

To understand the influence of the genetic background and obesity on miRNA regulation, Zhao *et al*. quantified by microarray the expression of these non-coding RNAs in pancreatic islets of diabetes-resistant B6 mice and diabetes-susceptible BTBR mice and compared their expression profiles to their morbidly obese counterpart, the B6-*ob/ob* and the BTBR-*ob/ob* mice, respectively [32]. Obesity affected miRNA expression profiles in both B6 and BTBR strains. Among the observed changes, miR-27b, miR-184, miR-203, miR-210, miR-338-3p and miR-383 were found to be down-regulated in both B6-*ob/ob* and BTBR-*ob/ob* mice compared to their respective lean controls, whereas miR-34a, miR-34b, miR-132, miR-152, miR-199a and miR-212 were upregulated. Interestingly, some of the miRNA changes were observed in only one strain, such as miR-204 and miR-7b that were modified in response to obesity in BTBR but not in B6 mice. The magnitude of deregulation was also

different between the two strains. For example, miR-132 and miR-212 were increased more than 14 times in B6 mice and only 3 times in BTBR mice. Therefore, the genetic background seems to influence the changes in miRNA expression observed in response to obesity. A better understanding of the roles of these miRNAs in the regulation of β-cell function and survival may help understanding the causes underlying resistance (B6) and susceptibility (BTBR) to diabetes.

Nesca *et al.* used two models of T2DM to assess whether miRNA deregulation can contribute to β-cell adaptation to insulin resistance and to β-cell dysfunction associated with the manifestation of the disease [31]. The global miRNA profile of pancreatic islets was investigated in pre-diabetic and diabetic *db/db* mice and in diet-induced obesity (DIO) mice. Compared to the respective controls, more than 60 miRNAs were found to be differentially expressed in these two obesity-associated animal models of T2DM. As expected, most of the changes detected in these two models were consistent with those reported in *ob/ob* mice [32]. Interestingly, miR-132 displayed a striking increase and miR-184, miR-203, miR-210 and miR-338-3p a strong down-regulation already in young (6 weeks old) *db/db* mice that are obese and insulin-resistant but still normoglycemic. The reduction of miR-184 and miR-210 was further decreased in older, overtly diabetic, *db/db* mice while the level of miR-132, miR-203 and miR-338-3p remained approximately constant between pre-diabetic and diabetic animals. The expression of many other miRNAs was only altered in diabetic *db/db* mice. For example, miR-21, miR-34a, miR-146, miR-199a-3p and -5p and miR-802 were increased whereas the level of miR-383 declined [31]. The increase of miR-802 in pancreatic islets as well as in several other tissues of *db/db* mice including liver and white adipocytes was also reported by Kornfeld *et al.* [45]. *Db/db* mice are severely obese and diabetic while DIO mice are moderately overweight after being fed on high-fat diet for 8 weeks and are in a pre-diabetic state (insulin resistant, hyperglycemic and hyperinsulinemic). However, global miRNA profiling of pancreatic islets of these two models led to partially overlapping changes in miRNA expression.

The functional roles of several of these miRNAs were investigated in details by mimicking the up- or the down-regulation observed in *db/db* mice and/or DIO mice in dispersed rat and human islet cells [31]. The alteration of the level of miR-21, miR-34a, miR-146a, miR-199a-3p, miR-203, miR-210 and miR-383 were found to increase the fraction of cells undergoing apoptosis [31, 46]. Moreover, the overexpression of some of these miRNAs was found to perturb insulin secretion. The changes in the level of these miRNAs occur only

in diabetic or glucose-intolerant animals suggesting that they contribute to β-cell dysfunction associated with T2DM. So far the specific role of miR-802 in β-cells has not been investigated. However, transgenic mice overexpressing miR-802 developed glucose intolerance and insulin resistance, suggesting the involvement of this miRNA in the regulation of glucose homeostasis *in vivo* [45]. The expression of miR-802 is particularly elevated in hepatocytes where it regulates insulin sensitivity by targeting *Hfn1b*. This transcription factor plays a role also in β-cells and mutations in the *Hfn1b* gene can cause the development of a rare form of Maturity Onset Diabetes of the Young (MODY5). Thus, an increase in the expression of miR-802 may potentially contribute also to failure of β-cells under insulin-resistant conditions.

Another group of miRNAs, including miR-204 and miR-200 family members, has been shown to be induced by the Thioredoxin-interacting protein (TXNIP), a cellular redox regulator that is up-regulated in diabetes and triggers β-cell apoptosis [47, 48]. The induction of miR-204 triggered by TXNIP was found to reduce MafA expression and to cause an impairment in insulin biosynthesis [48]. In contrast, miR-200 overexpression promotes β-cell apoptosis and inhibits epithelial-mesenchymal transition, a process thought to be involved in compensatory β-cell mass expansion [47].

In contrast to the miRNAs described above, miR-132, miR-184 and miR-338-3p have their expression already modified during the compensatory phase and have a positive impact of β-cell activities. Increase in the level of miR-132 favored cell survival under pro-apoptotic conditions such as prolonged exposure to elevated concentrations of cytokines or free fatty acids [31]. Moreover, overexpression of miR-132 amplified the amount of insulin secreted in response to glucose without affecting insulin content and promoted β-cell proliferation in dispersed rat islet cells. The beneficial effect of miR-132 on insulin release has been suggested to be mediated by direct down-regulation of carnitine acyl-carnitine translocase (CACT), a mitochondrial protein important for β-oxidation [49].

Down-regulation of miR-184 also increased β-cell proliferation [31]. The role of miR-184 in the control of β-cell functions was investigated *in vivo* by Tattikota *et al* [18]. This independent study confirmed the decrease in the expression of miR-184 in *ob/ob*, *db/db* and DIO mice and demonstrated also a reduction of the level of this non-coding RNA in islets of T2DM human donors. Interestingly, constitutive *miR-184* deletion in mice resulted in a reduction of blood glucose levels in fasted state combined with increased plasma insulin

concentrations. In response to a glucose challenge, KO-*miR184* mice displayed improved glucose tolerance and transiently elevated insulin release without any difference in insulin sensitivity in target tissues. Moreover, analysis of pancreatic sections of these mice revealed an increase in β-cell number and mass and an augmentation of the β-cell proliferative rate. As computationally predicted, *Ago2* was confirmed to be a direct target of miR-184 and its expression was inversely correlated with the level of the miRNA in islets of human donors. To investigate the role of miR-184 and *Ago2* in the compensatory phase preceding the manifestation of T2DM, transgenic *miR-184* were crossed with *ob/ob* mice, resulting in sustained expression of miR-184 (as opposed to the diminution of miR-184 observed in WT *ob/ob* mice) and a decrease in *Ago2* expression without affecting the level of abundant islet miRNAs such as miR-375. Transgenic *miR-184 ob/ob* mice exhibited more pronounced hyperglycemia and reduced circulating insulin levels compared to WT *ob/ob* mice due to the incapacity of these animals to compensate the insulin resistant state with an increase in the β-cell mass. Pancreatic insulin content was also blunted in these transgenic *ob/ob* animals. Of note, α-cell mass was not affected by the overexpression of miR-184. These results suggest that the reduction of miR-184 observed in pancreatic islets of pre-diabetic and diabetic rodent models and of human T2DM donors is part of the mechanism enabling the β-cells to compensate for the insulin-resistant state of target tissues.

Another miRNA displaying expression changes during the β-cell compensatory phase of *db/db* mice and DIO mice is miR-338-3p. Interestingly, the level of this non-coding RNA is decreased not only in these diabetes models but also during pregnancy, another condition characterized by compensatory β-cell mass expansion [50]. Down-regulation of miR-338-3p in dispersed rat islet cells favored β-cell proliferation without affecting insulin content and secretion and protected both rat and human islet cells against cytokine- and fatty acid-induced apoptosis [50]. The direct targets of miR-338-3p mediating this effect were not identified. However, the beneficial effects of miR-338-3p down-regulation in β-cells correlated with a reduction in the expression of pro-apoptotic *Bad* and an elevation of genes playing key roles in β-cell survival and proliferation such as *Bcl2*, *Birc5*, *Foxm1*, *CyclinD1*, *Igf1* and *Irs2*. Looking at the signaling mechanisms regulating the expression of miR-338-3p revealed that the level of this miRNA is controlled by hormones capable of activating the cAMP-dependent pathway such as the incretin GLP-1 and, via its unconventional receptor GPR30, estrogen. Indeed, the induction of β-cell proliferation by GLP1 was blunted by

overexpressing miR-338-3p, suggesting that the beneficial effect of GLP1 on β-cell mass is achieved, at least in part, by reducing miR-338-3p.

miRNA changes in islets of human T2DM donors

A limited number of studies have been carried out to detect deregulation in miRNA profiles in human pancreatic islets of T2DM individuals. In non-diabetic donors, a positive association was observed between miR-122, miR-127-3p, miR-184 and miR-375 expression and the level of insulin mRNA, and a negative correlation was detected between miR-127-3p and miR-184 and the amount of insulin released in response to glucose. However, these associations were absent in glucose intolerant donors [51]. In the same study, miR-21 was found to be significantly upregulated and miR-127-3p and miR-375 had a tendency to be more elevated in islets of glucose intolerant donors [51]. In other independent studies, expression of miR-375 was found to be increased in total pancreas [52] but not in isolated pancreatic islets of T2DM donors [33]. Two other miRNAs, miR-187 and miR-124a, were found to be upregulated in islets of individuals suffering from T2DM, whereas miR-7 and miR-184 were down-regulated [18, 33, 39, 53]. Overexpression of both miR-187 and miR-124a was linked in *in vitro* rodent models to a reduction in glucose-induced insulin secretion by directly targeting *HIPK3* or *Rab27A*, *Mtpn* and *Foxa2*, respectively [33, 53-55]. As already mentioned above, the role of miR-7 and miR-184 were investigated in details in rodent animal models [18, 39].

A recently published study led to the identification of several miRNAs differentially expressed in islets from T2DM versus non-diabetic donors [21]. The expression of, miR-369, miR-487a, miR-655 and miR-656 were diminished whereas miR-7, miR-187, miR-187*, miR-224 and miR-589 were augmented in T2DM donors. Interestingly many of the miRNAs deregulated in T2DM donors belong to a cluster that is generated from the maternally imprinted locus DLK1-MEG3. *MEG3* and 8 miRNA members of this cluster displayed reduced levels in islets of diabetic patients. Moreover, the miRNAs of DLK1-MEG3 cluster had, on average, a 16-fold higher expression in human FACS-sorted β-cells compared to α-cells. RNA level of *MEG3* was also found to be highly enriched in β-cells. Interestingly, β-cell-specific expression of this miRNA cluster could be attributed to the methylation status of the *MEG3* promoter, which was impaired in islets from T2DM donors. Finally, *IAPP* and *TP53INP1* were identified as direct targets of this miRNA cluster and an elevation in the

expression of these genes was suggested to contribute to β-cell death observed in T2DM. Taken together, this elegant study suggest that epigenetic modifications resulting in the reduction of the level of the DLK1-MEG3 miRNA cluster can lead to β-cell dysfunction and can predispose the individual to the development of T2DM.

Studies in human islets are obviously highly relevant for the understanding of the causes of T2DM and for identifying new strategies to cure this metabolic disease. As discussed in detail elsewhere [56], human islet studies provide precious information that cannot be obtained with animal models but are subjected to major limitations. In fact, human islets can only be obtained from cadaveric donors, drastically limiting the amount of material available for these experiments. Consequently, in most studies the cohorts are relatively small, inevitably leading to results variability and difficulties in matching control and diabetic donors for confounding factors such as body mass index (BMI), age, gender and ethnicity. Moreover, the interpretation of human islet data can be influenced by the duration of the disease, the medications used to control the glycaemia and possible differences in the α/β cell ratio occurring in T2DM patients [57, 58]. Because of all these limitations, the use of animal models is indispensable to precisely understand the molecular mechanisms underlying the manifestation of the disease and will be required also in the future to complement the studies carried out with human islets.

Conclusion and perspectives

miRNAs are now recognized as important regulators of β-cell survival, proliferation and function. Hence, it was not surprising to find changes in their expression under conditions associated with modifications in the functional β-cell mass. A closer look at the results obtained by independent investigators, reveals that the miRNAs displaying expression changes can be subdivided in two groups displaying opposing functional effects (Figure 2). Under conditions of insulin resistance, the expression of several miRNAs including miR-7, miR-132, miR-184 and miR-338-3p and possibly miR-375 is modified in order to promote compensatory β-cell mass expansion and amplification of insulin secretion [18, 31, 49, 50]. On the other hand, deregulation of the DLK1-MEG3 miRNA cluster and of miR-34, miR-124a, miR-146, miR-187, miR-199a-5p, miR-203, miR-210 and miR-383 have a detrimental impact on β-cell survival and function and can predispose to the development of T2DM [21, 31, 33, 53]. An appropriate balance between the level of the miRNAs with a positive and a

negative impact on proliferation and survival of β-cells is essential to compensate for the rise in the insulin needs under conditions of pregnancy and obesity and to maintain blood glucose homeostasis. In fact, excessive expression of miRNAs with a deleterious impact on β-cell proliferation will promote progression toward glucose intolerance and T2DM onset. On the other side, unbalanced expression of miRNAs triggering compensatory β-cell mass expansion may also be inappropriate. In fact, many of these miRNAs are oncomiRs and are deregulated in several forms of cancers [59]. Thus, in the absence of a counteracting effect, these miRNAs can potentially trigger uncontrolled proliferation of insulin-secreting cells and favor the development of insulinomas. A precise understanding of the functional interplay between these two groups of miRNAs expressed in β-cells will be essential for the design of new therapeutic approaches to prevent and treat different forms of diabetes.

Conflict of interest

The authors have declared no conflict of interest.

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Figure legends

Figure 1: **The canonical miRNA biogenesis pathway.** 1- miRNAs are transcribed by the RNA polymerase II as primary molecules (pri-miRNAs) containing characteristic stem-loop structures. 2- Pri-miRNAs are recognized by the microprocessor complex composed of DGCR8/Pasha proteins and cleaved by the RNase III-type enzyme Drosha to produce hairpin-structured precursors of 70 nucleotides (pre-miRNAs). 3- Thereafter, miRNAs precursors are transported to the cytoplasm by Exportin-5 and 4- further cleaved by the endoribonuclease Dicer to generate an imperfect miRNA/miRNA* duplex of around 22 nucleotides. 5- Mature miRNA strands in association with members of the Argonaute family are incorporated into the RNA-induced silencing complex (RISC). 6- Finally, the RISC complex guided by the mature miRNA binds to miRNA recognition element within the 3'untranslated region (3'UTR) of the target mRNA, leading to translational inhibition and/or transcript degradation.

Figure 2: **Deregulation of particular miRNAs during the development of T2DM.** In healthy non-diabetic state, pancreatic beta-cells secrete insulin in response to the organism demand. However, in genetically susceptible individuals that face environmental factors like overnutrition, inactivity and ageing, beta-cells need to compensate to overcome the insulin resistant state of the target tissues. Augmentation of miR-132 and reduction of miR-7, miR-184 and miR-338-3p levels have been suggested to be part of mechanisms leading to increase beta-cell mass and function. Appropriate amount of miR-375 is also required for proper adaptation of beta-cells to insulin resistant state. T2DM develops when beta-cells are unable anymore to face the organism need in insulin. Downregulation of the miRNA cluster DLK1-MEG3 and miR-203, miR-210, miR-383 as well as upregulation of miR-34a, miR-124a, miR-146, miR-187 and miR-199a favor beta-cell dysfunction and death associated with onset of diabetes.

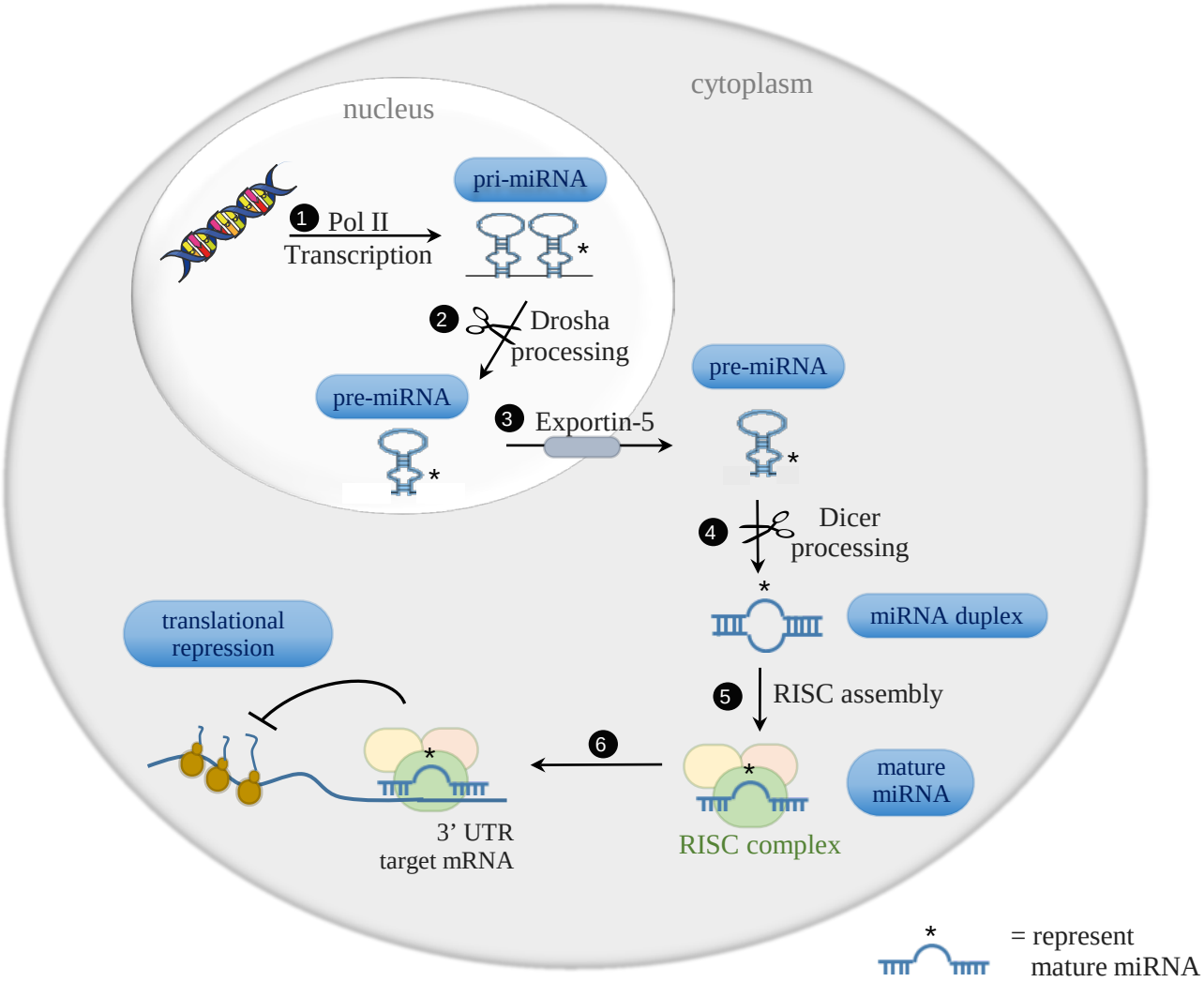

Figure 1: Guay and Regazzi

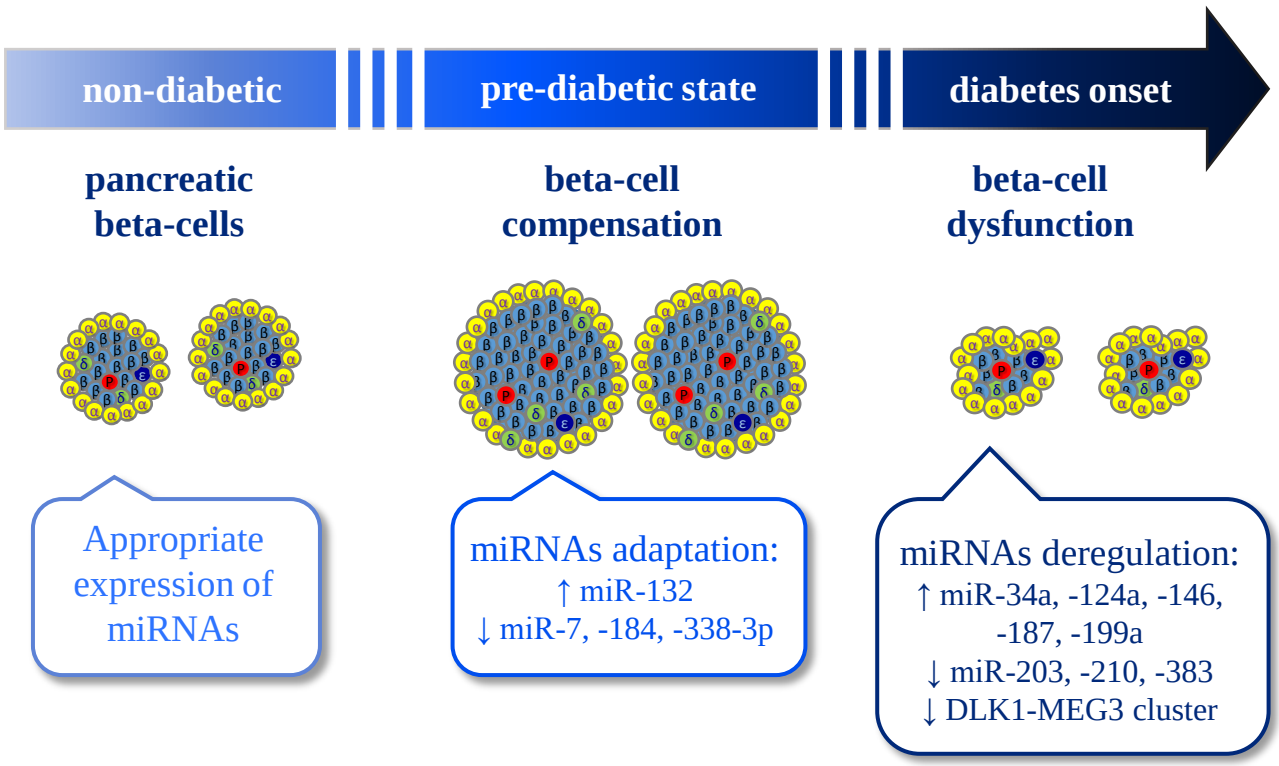

Figure 2: Guay and Regazzi