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Role of islet microRNAs in diabetes: which model for which question

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Abstract

MicroRNAs are important regulators of gene expression. The vast majority of the cells in our body rely on hundreds of these tiny non-coding RNA molecules to precisely adjust their protein repertoire and faithfully accomplish their tasks. Indeed, alterations in the microRNA profile can lead to cellular dysfunction favoring the appearance of several diseases. A specific set of microRNAs plays a crucial role also in pancreatic beta-cell differentiation and is essential for the fine-tuning of insulin secretion and for compensatory beta-cell mass expansion in response to insulin resistance. Recently, several independent studies reported alterations in microRNA levels in the islets of animal models of diabetes and in islets isolated from diabetic patients. Surprisingly, many of the changes in microRNA expression observed in diabetes animal models were not detected in the islets of diabetic patients and vice-versa. These findings are unlikely to merely reflect species differences because microRNAs are highly conserved in mammals. These puzzling results are most probably explained by fundamental differences in the experimental approaches that selectively highlight the microRNAs directly contributing to diabetes development, the microRNAs predisposing individuals to the disease or the microRNAs displaying expression changes subsequent to the instauration of diabetes conditions. Here we will highlight the pertinence of the different models in addressing each of these questions and propose future strategies that should permit to obtain a better understanding of the contribution of microRNAs to the development of diabetes mellitus in human.

MicroRNAs as regulators of beta-cell differentiation and function

Type 2 diabetes is a chronic metabolic disorder characterized by major alterations in gene expression affecting several organs, including the islets of Langerhans. A growing number of studies demonstrate that these changes are not only caused by deregulation of key transcription factors such as MafA or Pdx1 but are also driven by modifications in the level of another group of molecules regulating gene expression, the microRNAs [1-4]. MicroRNAs are small non-coding RNAs (typically 21-23 nucleotide long) that pair to the 3' untranslated region of target mRNAs leading to translational repression and/or a decrease in messenger stability [5].

The importance of the microRNA regulatory network for proper differentiation and function of beta-cells is highlighted by the phenotypic traits of mice lacking Dicer1, an enzyme essential for the generation of most microRNAs [5]. Deletion of *Dicer1* at different stages of pancreas development or of the pancreatic endocrine lineage results in a dramatic loss of microRNAs accompanied by severe defects in pancreas morphology, islet organization, beta-cell formation and insulin biosynthesis [6-8]. The precise role of microRNAs in insulin-secreting cells has been investigated by deleting *Dicer1* specifically in beta-cells. *RIP-Cre-Dicer1* mice exhibit normal beta-cell formation during fetal and neonatal life, but become progressively hyperglycemic and finally develop overt diabetes in the adulthood. These mice display defects in islet number, size and architecture, in beta-cell mass, and in insulin biosynthesis and secretion [9, 10]. Loss of *Dicer1* in the adult does not impact on the total beta-cell mass but results in insufficient insulin biosynthesis and release in response to glucose, causing hyperglycemia in both fed and fasted states [11]. Taken together, these observations point to an essential role for the microRNA network in beta-cell differentiation and function.

Pancreatic beta-cells express a specific set of microRNAs that are present in the cells at very different levels. miR-7, miR-375 and let-7 family members are among the most abundant microRNAs expressed in human and rodent islets and miR-7, miR-184 and miR-375 are highly enriched in islets compared to other tissues [12-15]. The specific role of each of these microRNAs in the regulation of beta-cell activities has been investigated in different *in vivo* and *in vitro* models. Deletion of *MiR-375* in mice results in an altered cellular composition of the pancreatic islets that contain less beta-cells and more alpha-cells than in wild type animals [16]. These mice display hyperglycemia and hyperglucagonemia and if crossed with *ob/ob* mice, a model of obesity and insulin resistance, they develop a severe diabetic state because of the inability of beta-cells to expand and compensate for the increased insulin needs. These together with other studies [16-19]

highlight the central role played by miR-375 in endocrine pancreas development and in the regulation of insulin gene expression and release.

Opposite to the absence of miR-375, the knockout of *MiR-184* in insulin-positive cells results in a rise in beta-cell proliferation and an increase in their number. This is associated with improved insulin release in response to a glucose challenge [20]. The proliferative effect elicited by down-regulation of miR-184 was also observed in dispersed islet cells in an independent *in vitro* study [21]. Moreover, blockade of this microRNA in rat and human islets protected the beta-cells against apoptosis elicited by chronic exposure to pro-inflammatory cytokines or fatty acids (conditions typically associated with the diabetes state) [21]. Therefore, reduction of miR-184 levels favors the replication and survival of insulin-secreting cells and an expansion of the beta-cell mass.

The role of miR-7 in beta-cells has also been the focus of numerous studies. The expression of this microRNA was positively correlated with pancreatic development and beta-cell differentiation in human fetus [22]. Down-regulation of miR-7 in mouse embryos results in a reduction in the number of beta-cells and diminished insulin production, leading to glucose intolerance in the post-natal period [23]. Inhibition of miR-7 using antisense oligonucleotides in isolated adult mouse islet cells was found to activate the mTOR pathway and to promote beta-cell proliferation [24]. However, beta-cell-specific *MiR-7* knockout mice did not show significant differences in beta-cell survival or proliferation, but display enhanced insulin release due to increased expression of key components of the exocytotic machinery permitting an improved response to a glucose challenge [25].

Let-7 was the first microRNA discovered in *C. elegans* [26] and includes a family of 12 closely related microRNAs sharing a common seed region (a sequence spanning from nucleotide 2 to 8 that is important for target recognition). The members of this family are key regulators of embryonic development and important tumor suppressors in adult cells [27]. The role of let-7 family members in the regulation of glucose homeostasis has been investigated in an *in vivo* study using strategies permitting to overexpress or block these microRNAs [28]. Overexpression of let-7 either in all tissues or restricted to beta-cells resulted in impaired glucose tolerance and attenuation of glucose-induced insulin release. Moreover, general down-regulation of let-7 in adult mice obtained by injection of antisense oligonucleotides prevented impaired glucose tolerance in mice fed on a high-fat diet [28].

Overall, these and many other studies (reviewed extensively elsewhere [1-3]) have identified the microRNAs as essential players in beta-cell development and function and in the regulation of whole-body glucose homeostasis.

Inappropriate islet microRNA expression as potential cause of type 2 diabetes

Several research teams have used comparative profiling to identify changes in microRNA expression preceding or coinciding with the manifestation of diabetes and, thus, potentially contributing to the development of the disease. Different rodent models of type 2 diabetes (summarized in Table 1) were analyzed covering various aspects of this complex and multifactorial metabolic disease. An exhaustive list of the microRNAs identified in these studies is provided in Table 2. Interestingly, a group of microRNAs including miR-34a, miR-132, miR-184, miR-199a-5p, miR-210, miR-212, miR-338-3p and miR-383 were found to be deregulated in different type 2 diabetes animal models by independent research groups and their expression changes were confirmed by real-time PCR quantification [20, 21, 29-31]. The functional role of some of these microRNAs in the regulation of beta-cell functions has been investigated in detail. For this purpose, the expression changes observed in pre-diabetic or diabetic animals was mimicked in normal beta-cells. This revealed that the induction of miR-34a, miR-210 and miR-383 promotes apoptosis of beta-cells and/or inhibits glucose-induced insulin secretion [21, 32], indicating a potential involvement of these microRNAs in beta-cell dysfunction and in the development of diabetes. However, not all the changes in microRNA levels detected in the islets of diabetic animals have deleterious impacts on beta-cell activities. Indeed, reduction of miR-184 and miR-338-3p or a rise of miR-132 were found to trigger beta-cell proliferation, improve survival and/or insulin release [20, 21, 31, 33]. This suggests that the modifications in the level of these microRNAs contribute to physiological processes that attempt to compensate for insulin resistance rather than to pathological events causing the appearance of diabetes.

Can findings obtained in diabetes animal models be extrapolated to humans?

Several groups have now extended this type of studies to humans and compared the level of microRNAs in islets obtained from healthy and type 2 diabetic donors, a type of analysis that is expected to become more popular in the coming years. Surprisingly, only a minor fraction of the microRNAs differentially expressed in animal models were also found to be modified in samples collected from type 2 diabetic patients. Conversely, several microRNA changes revealed by the screening of human islets were not previously highlighted by the systematic analysis of islets

isolated from diabetes animal models. Indeed, the islets of type 2 diabetic donors were found to express higher levels of miR-187, miR-187*, miR-224, miR-589 and decreased levels miR-7, miR-369, miR-487a, miR-655 and miR-656 (see Table 3) [13] . Similar changes in the expression of miR-187 and miR-7a were confirmed by independent research groups [25, 34]. These data will need to be reproduced in additional laboratories but if these findings are confirmed what will then be the value of experiments carried out in rodents? Should they be abandoned in favor of experimental approaches focusing exclusively on the analysis of human samples? If not, would it be possible to design experiments in animal models and human islets permitting to reconcile these apparently discrepant findings? In the following paragraphs we will attempt to answer these important and legitimate questions by scrutinizing the advantages and limitations of the experimental models currently available to study the involvement of islet microRNAs in the development of diabetes.

There are several factors that should be considered to explain the differences between the results obtained in human and rodent samples. Human and rodent islets are known to display genetic, morphological and functional specificities but species differences are unlikely to be the major cause of the discrepant findings. Indeed, the sequence, the genomic organization and the signals regulating the expression of almost all microRNAs are highly conserved between mammals. Several experiments carried out with rodent islets have been performed with microarray or quantitative PCR approaches. The use of these highly sensitive techniques may have allowed the detection also of differences in microRNAs expressed at very low level in the cells that may not be functionally relevant. However, the use of different profiling methodologies cannot explain the observed discrepancies. In fact, at least part of the rodent studies were performed with the same approaches applied for the analysis of the microRNAs in human samples. Moreover, differential microRNA expression in rat islet samples determined by small RNA sequencing and with the Agilent microarray platform yielded highly concordant results (Jacovetti, Matkovich and Regazzi, unpublished observation).

We believe the explanations for the differences between the results obtained in rodents and humans should rather be searched in the peculiarities of each experimental model (summarized in Table 4). Animal studies offer the possibility to correlate the kinetics of the microRNA changes with the development of type 2 diabetes. Infact,theonsetofthediseaseoccursatwell-definedtimepoints permitting to focus on microRNA changes immediately preceding the failure of beta-cells and thus most likely to contribute to the development of diabetes. The precise role of the identified microRNAs in the manifestation of the disease can then potentially be assessed by modulating the level of the microRNAs *in vivo*, for instance by transgenesis or by injection of oligonucleotide

derivatives that mimic or sequester the microRNAs. This is an important issue because it is often difficult to determine whether microRNAs differentially expressed in the islets of overtly diabetic individuals are a direct cause of the disease or are the consequence of the chronic exposure of islet cells to elevated levels of glucose, lipids and inflammatory mediators typically occurring under diabetes conditions. As mentioned above, certain modifications in islet microRNA expression may even have a positive impact on islet function [20, 21, 31] and be part of the physiological mechanisms permitting to balance the rise in insulin needs caused by insulin resistance in peripheral tissues encountered in obese and aging individuals.

A unique characteristic of the studies carried out in animal models is the use of congenic strains. This, combined with the possibility to standardize and precisely control the islet isolation procedure minimizes the variability between the biological replicates and allows the generation of highly reproducible data. The reproducibility of the results permits the detection of tiny differences in microRNA expression even when a small group of individuals is compared. Measurements of islet microRNA levels in human islet preparations are usually characterized by much larger inter-individual variations. These can be attributed to a combination of factors potentially modifying the microRNA profile including differences in age, sex, ethnicity, body mass index, the duration of diabetes and the treatment (or not) with different diabetic drugs of the islet donors [35]. In view of the strong inter-individual variability, relatively small changes in microRNA expression will go undetected unless a large number of islet preparations are analyzed. This is a major obstacle because, at present, the availability of human islets is a limiting factor, in particular for samples obtained from type 2 diabetic donors. It is possible that changes in microRNA expression so far observed only in animal models will later be confirmed also in humans when the data of a larger number of diabetic individuals will become available. A representative example is the decrease of islet miR-184 expression observed in several independent animal studies [20, 21, 29, 30]. Changes in the level of this particular non-coding RNA were not detected upon global profiling of a small number of human samples [13, 34] but were readily confirmed by a study focusing specifically on miR-184 in which a large number of islet preparations were compared [20].

A major concern of diabetes animal models is that they may not faithfully recapitulate the conditions of the human disease. For instance, the degree of obesity associated with many traditional type 2 diabetes models like *ob/ob* and *db/db* mice, is exceedingly high and is not representative of the obesity observed in most type 2 diabetes populations [36, 37]. Diet-induced obese mice are probably more representative of human obesity. However, this model is characterized by large inter-individual differences in the response to high-fat diet and the animals

become glucose intolerant but usually do not develop overt type 2 diabetes. For other popular rodent strains such as for instance the GK rats the precise causes of the disease remain unclear [38] and the form of diabetes developed by these animals may be representative only of a very particular subgroup of type 2 diabetes cases in humans. Consequently, changes in the level of certain islet microRNAs identified in animal models may be observed in humans only if specific subpopulations of type 2 diabetic patients are selected.

Finally, an important point to consider is that the differential expression of several microRNAs detected in the islets of type 2 diabetic donors may not be the result of changes occurring during the pre-diabetic phases preceding the development of the disease but may rather reflect pre-existing individual characteristics that predispose to the development of the disease. Indeed, most of the microRNAs that were found to be differentially expressed in the islets of type 2 diabetic donors belong to a large epigenetically controlled cluster generated from an imprinted locus on chromosome 14q32 [13]. As already mentioned above, the studies carried out in animal models are usually performed in congenic individuals. Since in this case all individuals share the same genetic background, the analysis of microRNA expression in the islets isolated from these experimental models will obviously fail in identifying phenotypic differences favoring the occurrence of diabetes.

Future perspectives

As discussed, there are fundamental differences between the studies focusing on diabetes animal models and on the analysis of islets collected from human donors. These two approaches are more likely to highlight either candidate microRNAs showing expression changes that coincide with the development of diabetes or pre-existing microRNA differences that predispose to the manifestation of the disease. Therefore, it is not too surprising that human and rodent studies led so far to the identification of distinct sets of microRNAs. Animal studies are more appropriate to discover a causal link between changes in microRNA expression and the manifestation of diabetes. Thus, these experiments should continue to guide the quest for the microRNAs contributing to beta-cell dysfunction and failure. Confirmation of the relevance of the findings obtained in rodents for the determination of the causes of diabetes in humans will be essential and will be facilitated by a better understanding of the impact on islet microRNA profile of confounding factors such as age, sex and treatment with anti-diabetic drugs. In principle, it should not be too difficult to evaluate the effect of these parameters on islet microRNA expression in animal models. In view of the increasing number of groups entering the field and of the rapid dissemination of platforms offering solutions for the

global assessment of microRNA expression, we are confident that this information will soon become available.

The analysis of the microRNA profile in human islets obtained from healthy and type 2 diabetic donors offers the unique possibility to identify inter-individual characteristics that predispose to the manifestation of the disease. This important information cannot be obtained with commonly used animal models and will complement the knowledge about the role of specific microRNAs acquired in rodents. So far, a major obstacle to the studies performed with human islets from cadaveric donors is represented by the fact that it is not possible to correlate the appearance of the alterations in microRNA expression with the manifestation and progression of diabetes. An interesting approach to partially overcome this limitation would be the generation of so-called “humanized” animal models. This strategy consists in getting rid of the endogenous beta-cells by injecting the animals with streptozotocin [39]. The rodent insulin-secreting cells are then replaced by human islets that are transplanted under the renal capsule and insure the metabolic control. By carefully selecting the recipient animal model, it would be possible to expose the transplanted human islets to diabetogenic conditions such as, for example, obesity or high fat diet feeding and to analyze then the impact on microRNA expression. This approach would permit to directly assess whether human islets exposed *in vivo* to adverse environmental conditions display the same changes in the microRNA profile observed in the islets of the corresponding animal model.

Conclusion

The discovery of microRNAs has opened new perspectives in the understanding of the mechanisms responsible for the failure of beta-cells and the development of type 2 diabetes. This has attracted a lot of interest on these small non-coding RNA molecules and has promoted an exponential increase in the number of studies aiming at identifying the microRNAs involved in this disease. The determination of the relevance for human diabetes of candidate microRNAs identified through experiments carried out in animal models still needs to be demonstrated and will occupy scientists in the coming years. We are only beginning to appreciate the importance of these tiny RNA molecules in islet physiology but their discovery has provided new hope to elucidate the causes of beta-cell dysfunction and of the development of diabetes. MicroRNAs are now entered the limelight and, no matter which experimental model will be used to study them, they will probably not leave the front stage of diabetes research for a while.

Contribution statement

All authors were responsible for drafting the article and revising it critically for important intellectual content. All authors approved the final version of the article.

Conflict of interest

The authors have no duality of interest.

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Table1 Main characteristics of common rodent models of type2 diabetes (T2D)

Species	Models	Main characteristics	Ref.
Rat	Goto-Kakizaki (GK)	- Spontaneously develop T2D unrelated to obesity. - Animals are insulin resistant, moderately hyperglycemic and have impaired insulin secretion in response to glucose. Limitations: The precise cause of the development of T2D remains unclear. Poorly representative of most human T2D populations.	[40]
Mouse	Diet-Induced Obesity (DIO)	- Mice are fed a high-fat diet to induce obesity and insulin resistance. - Model of pre-diabetes or early phases of T2D. Limitations: Large inter-individual differences. Most of the animals become glucose intolerant but never develop overt T2D.	[41]
	db/db	- Leptin receptor deficiency leading to T2D. - Animals are severely obese, insulin resistant, hyperglycemic and hyperinsulinemic. Limitations: Model of extreme obesity that is poorly representative of most human T2D populations.	[37]
	ob/ob	- Leptin deficiency. - Severely obese, insulin resistant and hyperinsulinemic - Beta-cell hyperplasia and compensation for insulin resistance. Limitations: Model poorly representative of human obesity. The animals do not develop T2D.	[36]
	B6 strain	- Resistant to obesity-induced diabetes. - B6-*ob/ob* are hyperinsulinemic and transiently hyperglycemic. - Beta-cell hyperplasia and increase in insulin secretion. Limitations: Need to be crossed with *ob/ob* mice to obtain a phenotype. Not well characterized.	[30, 42]
	BTBR strain	- Susceptible to obesity-induced diabetes - BTBR-*ob/ob* display severe hyperglycemia. - Failure of beta-cells to proliferate and to increase insulin secretion. Limitations: Need to be crossed with *ob/ob* mice to obtain a phenotype. Not well characterized.	[30, 42]

Table 2 Overview of microRNAs reported to be differently expressed in pancreatic islets of different type 2 diabetes animal models.

Animal models	Detection method		microRNA changes	Ref.
GK rats	microarray	Up	let-7i*, miR-7b, -124, -127, -130a, -132, -136*, -142-3p, -142-5p, -152, -199a*-3p, -199a-5p, -212, -335, -369-3p, -376a, -376a*, -376b-3p, -376c, -409-3p, -410, -411, -433, -434	[29]
		Down	*miR28*, -216, -217, -493, -503, -708*	
DIO mice	microarray	Up	let-7d*, miR-7a-1*, -34c, -101b, -125a-3p, -130b*, -132, -152, -182, -193, -200c*, -205, -211, -216b, -221, -322, -323-3p, -337-3p, -362-5p, -380-3p, -433, -455*, -484, -485*, -494, -540-3p, -615-3p, -670, -671-5p, -680, -702, -705, -714, -770-3p, -802, -1224, -1894-5p, -1897-5p, -1904, -1906	[21]
		Down	*let-7b*, miR-10a, -24-1*, -28, -29a*, -30b*, -30c-1*, -31*, -32, -33, -100, -148a*, -181d, -184, -199a-3p, -202-3p, -203, -210, -215, -218, -223, -301b, -328, -335-5p, -344b, -378, -383, -384-5p, -539-5p, -541, -543, -676, -690, -697, -700, -1187, -1198-5p, -1892*	
	qPCR	Up	miR-132, -375	[20, 21, 25]
		Down	*miR-7a, 184, -203, -210, -383*	
db/db mice Young (6 wks)	microarray	Up	miR-22, -132, -139-5p, -141*, -142-3p, -146a , -146b, -150, -152, -182, -193, -212, -301b, -337-3p, -337-5p, -433, -452, -455, -455*, -483, -582-5p, -676, -721	[21]
		Down	*miR-23b, -24-1*, -27b, -31*, -100, -184, -194, -201, -203, -216a, -218, -338-3p, -378, -671-5p, -762, -802*	
	qPCR	Up	miR-132	[21]
		Down	*miR-184, -203, -210, -383*	
Adult (16-20 wks)	microarray	Up	miR-10a, -10b, -21, -22*, -34a, -34b-5p, -34c, -99a, -100, -126-3p, -132, -139-5p, -143, -146a, -146b, -152, -181c, -195, -199a-3p, -199a-5p, -199b*, -212, -320, -322, -337-5p, -365, -455*, -497, -676, -721, -802, -1224	[21]
		Down	*miR-23b, -26a, -27b, -30e, -30e*, -30d, -31, -103, -129-3p, -129-5p, -184, -203, -204, -210, -301a,-324-3p,-324-5p,-325,-328,-331-3p,*	

			-338-3p, -341, -374, -378, -381, -383, -384-5p, -434-3p, -652, -872	
	qPCR	Up	miR-21, -34a, -132, -146, -199a-3p, -199a-5p, -802	[20, 21, 25, 31,
		Down	*miR-7a, -184, -203, -210, -338-3p, -383*	43]
	in situ hybridization	Up	let-7b	[44]
		Down	*miR-30d*	
ob/ob mice B6 strain	microarray	Up	miR-132, -133a, -152, -185, -199a-5p, -199b, -206, -202, -302b, -422a	[30]
		Down	*miR-184, -383*	
	RNA seq	Up	miR-22, -99b, -132, -152, -181d, -183, -212, -337, -433, -455, -494, -574, -666, -671, -708, -1957, -5115	[20]
		Down	*miR-1a, -23b, -27b, -92b, -99a, -100, -125b, -137, -149, -181a, -181b, -184, -203, -210, -215, -221, -222, -335, -338, -378, -383, -672*	
	qPCR	Up	miR-204, -375	[16, 20,
		Down	*miR-7a, -184*	25, 45]
BTBR strain	microarray	Up	miR-34a, -34b, -132, -199a-5p, -212, -379	[30]
		Down	*miR-1, -7b, -17-3p, -27b, -31, -124a, -133a, -147, -184, -187, -198, -203, -204, -207, -210, -211, -294, -302a*, -302b, -302c, -324-3p, -338, -371, -378, -383, -384, -422b*	
	qPCR	Up	miR-204	[45]

Table 3 Overview of microRNAs reported to be differentially expressed in pancreatic islets of human diabetic vs non-diabetic donors.

Human models	Detection method		microRNA changes	Ref.
Type 2 diabetes	Global profiling + qPCR	Up *Down*	miR-187, -187*, -224 and -589 *miR-7, -369, -487a, -655 and -656*	[13, 34]
	qPCR only	*Down*	*miR-7a, miR-184*	[20, 25]

Table 4 Advantages and disadvantages of using diabetes rodent models versus human islet preparations to study the involvement of microRNAs in pancreatic beta-cell dysfunction and in type 2 diabetes (T2D) development.

Diabetes animal models

Advantages:

- Possibility to correlate the kinetics of the microRNA changes with the development of T2D
- Possibility to study the role of microRNAs *in vivo*
- No restriction in the number of samples
- Inter-individual differences can be minimized by the use of congenic strains
- Islet isolation is standardized and highly reproducible

Disadvantages

- Potential differences between humans and rodents (microRNAs levels, cell composition)
- The available animal models match only in part the phenotype of human patients
- Difficult to estimate the influence of the genetic background to diabetes susceptibility

Human islets from T2D patients

Advantages:

- The detected differences in microRNA levels are reflecting the situation in human patients
- Possibility to correlate the level of islet microRNAs and genetic predisposition to diabetes

Disadvantages:

- The number of available islet preparations is limited (in particular for preparations from T2D donors)
- microRNA levels are likely to be influenced by age, sex, ethnicity, treatment etc. of the donor
- Major inter-individual differences
- Islet preparations are difficult to standardize, resulting in important variability in purity, cell viability etc.
- Difficult to correlate the changes in microRNA levels with the development of diabetes
- Difficult to distinguish between causes and consequences of diabetes