REVIEW

MicroRNA miR-124a, a negative regulator of insulin secretion, is hyperexpressed in human pancreatic islets of type 2 diabetic patients

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MicroRNAs are a class of negative regulators of gene expression, which have been demonstrated to be involved in the development of endocrine pancreas and in the regulation of insulin secretion. Type 2 Diabetes (T2D) is a metabolic disease characterized by insulin-resistance in target tissues and by the functional alteration of pancreatic insulin-secreting beta-cells. Recently, we characterized the expression levels of microRNAs miR-124a and miR-375, both involved in the control of beta cell function, in human pancreatic islets obtained from T2D and from age-matched non-diabetic organ donors. We observed the hyperexpression of miR-124a in human pancreatic islets obtained from T2D patientsvs non-diabetic subjects, while miR-375 did not result differentially expressed. Moreover, we demonstrated that miR-124a overexpression in MIN6-pseudoislets reduced glucose-stimulated insulin secretion. Among predicted miR-124a target genes we focused on Foxa2 and Mtpn, which are both involved in the regulation of insulin secretion and of glucose sensing. Indeed, using luciferase assay, we validated miR-124a targeting Foxa2 and Mtpn 3'UTR sequences. Accordingly, upon miR-124a inhibition in MIN6 pseudoislets, we detected the upregulation of Foxa2 and Mtpn and of other selected miR-124a predicted target genes such as Akt3, Flot2, Sirt1, and NeuroD1, indicating a possible role for such a microRNA in the control of several beta-cell functions. In conclusion, we uncovered a major hyperexpression of miR-124a in T2D islets, whose silencing resulted in increased expression of target genes of major importance for beta cell function and whose overexpression impaired glucose stimulated insulin secretion, leading to the hypothesis that an altered miR-124a expression may contribute to beta cell dysfunction in type 2 diabetes.

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MicroRNAs are a class of small endogenous RNAs, 19-23 nucleotides long, which negatively regulate gene expression [1]. They specifically bind to the 3'UTR (UnTranslated Region) of mRNA by a sequence-dependent mechanism, and act as suppressors of mRNA translation or by sequestering their targets to specific cytoplasmic loci, leading to mRNA degradation [2].

During the last decade, microRNAs have been widely associated to the regulation of several biological processes such as cell cycle, apoptosis, cell differentiation or embryonic development, through the modulation of pivotal factors, involved in these phenomena [3, 4]. Since microRNAs can control post-transcriptional expression of genes involved in such important biological pathways, alterations of their expression may lead to major defects in several cellular

functions. Indeed, changes in microRNA expression have been reported in many diseases such as cancer, autoimmune and metabolic disorders, including type 1 (T1D) and type 2 diabetes (T2D) [5, 6].

T2D is a metabolic disease characterized by target tissues insulin-resistance and by functional alterations of pancreatic insulin-secreting beta-cells, leading to chronic elevation of blood glucose levels (hyperglycaemia). In insulin-resistant individuals, a reduced beta-cell mass as well as a defective insulin–secretory machinery contribute to the lack of compensatory beta-cell response and, consequently, to diabetes development [7, 8].

Recently, several studies focused on the role of microRNAs as regulators of beta-cell functions both in healthy conditions and in diabetes [9,10]. The major evidence of the role of microRNAs in insulin secreting cells has been uncovered by specifically deleting Dicer1 enzyme in mouse beta-cells, thus rendering these cells incapable of synthesizing microRNAs. Indeed, RIP-CRE Dicer1-KO mice have been reported to become progressively hyperglycemic and finally to develop diabetes. Moreover, these mice display impaired insulin biosynthesis and secretion as well as abnormal number and size of pancreatic islets [11]. These data indicate an essential role of microRNA-mediated regulation of beta-cell mass and functions.

One of the first microRNAs shown to regulate beta-cell functional pathways was reported by the group of Poy M in 2007 [12], with the identification of a microRNA, miR-375, specifically and highly expressed in human and mouse pancreatic islets. In addition, these authors reported a specific role for this microRNA in the regulation of insulin secretion. Indeed, miR-375 was able to specifically inhibit glucose-induced insulin secretion by targeting Myotrophin (Mtpn), a protein involved in remodeling of F-actin filaments and in secretory granules exocytosis. Recent evidence demonstrated that miR-375 is not only involved in insulin secretion but also in the control of beta-cell proliferation and mass. Moreover, it has been shown that miR-375-KO mice are hyperglycemic and exhibit a reduced beta-cell mass, as a result of impaired proliferation. It was demonstrated that these effects were mainly due to the loss of miR-375 in the control of the expression of a cluster of genes, which negatively regulate cell growth and proliferation [13]. Moreover, the activity of miR-375 has also been linked to the control of pancreas development and of beta-cell differentiation as well as beta-cell proliferation and insulin secretion [14, 15].

Together with miR-375, other microRNAs have been reported to have a major role in the regulation pancreatic islet functions. Among them, microRNA miR-124a was identified as a regulator of both insulin secretion and of beta-cell development [16].

MicroRNA miR-124a, together with miR-9, has been firstly characterized as a promoter of neural-lineage differentiation [17]. Such microRNAs were initially identified as brain-specific factors, which mainly regulate neurogenesis and neural functions. Later, miR-124a and miR-9 were also linked to the control of insulin secretion and of beta-cell development both in mouse and in man. In 2007, Baroukh*et al*. reported for the first time a specific role of miR-124a in pancreas development and in beta-cell functional regulation [18], describing an increased expression of miR-124a during mouse embryonic pancreas development, thus indicating a possible role for such microRNA in the control of pancreas organogenesis. Moreover, they analyzed miR-124a potential target genes involved in beta-cell development or in beta-cell function; among several target genes, they focused on Foxa2, a major beta-cell transcription factor which regulates both beta-cell differentiation and insulin secretion. A specific targeting of miR-124a on Foxa2 gene, which in turn regulates insulin secretion, was demonstrated, thus suggesting a role for this microRNA in beta-cell activity.

Although several investigations have focused on the role of microRNAs in T2D animal models or in mechanistic evaluation of their function in murine beta-cell lines, only few studies analyzed differential microRNA expression in human pancreatic islets from T2D patientsvs non-diabetic subjects. This was mainly due to the limited availability of islet preparations, especially from type 2 diabetic patients. Another important issue is the heterogeneity among T2D multiorgan donors and the high variability of microRNA expression due to several factors such as age, body mass index (BMI) or type of anti-hyperglycemic treatment [19].

The paucity of data on microRNAs expression in human pancreatic islets in T2D and the importance of miR-375 and miR-124a in the regulation of several beta-cell functions, prompted us to analyze their expression in T2D vs non-diabetic human pancreatic islets [20]. In this study, we reported the analysis of miR-124a and miR-375 expression in human pancreatic islets from 6 non-diabetic and 5 T2D multiorgan donors using stem-loop RT Real Time PCR. Interestingly, we detected a significant upregulation of miR-124a in T2D islets respect to control donors; in contrast, no differential expression of miR-375 was observed. In addition, the expression levels of miR-124a in non-diabetic pancreatic islets resulted extremely low in comparison with that of several other pancreatic islet microRNAs, suggesting the need to maintain low miR-124a expression levels in adult human pancreatic islet cells for proper islet function.

Consequently, hyperexpression of miR-124a that we reported in T2D islets may contribute to the development and/or to the progression of beta-cell dysfunction (e.g. reduced glucose-stimulated insulin secretion, increased apoptosis, reduced islet neogenesis,).

In our study miR-124a expression levels in T2D pancreatic islets did not correlate with age or BMI nor its expression was increased upon high glucose exposure as evidenced by in-vitro treatment of non-diabetic human pancreatic islets, indicating that factors other than hyperglycemia determine the hyper-expression of this microRNA in T2D islets.

Only few studies were performed on microRNA profiles analysis in T2D, with some reporting differential expression of a series of microRNAs in human pancreatic islets. A recent study published by Bolmeson*et al*. described the differential expression of several microRNAs in human pancreatic islets obtained from glucose intolerant subjects [21]. They analyzed specific microRNAs chosen from an initial selection of those most highly expressed in non-diabetic human pancreatic islets. Among differentially expressed microRNAs, they identified the upregulation of miR-375, while did not select miR-124a for further analyses. In another study, Kameswaran*et al*. described small RNAs analysis profile of T2D pancreatic islets versus non-diabetic controls using RNA sequencing [22]. They did not report any differential expression of miR-124a in T2D donors; however, this might be due to the low expression of miR-124a in non-diabetic islets and to the relatively high stringency of RNA-seq when selecting candidate small RNAs differentially expressed. These discrepancies among different studies might be also attributed to the heterogeneity of patient cohorts analyzed. The data generated by Kameswaran*et al*. seem to be discordant also from those of Tattikota*et al*[23] who reported the down-regulation of miR-184 in T2D islets (measured by RT-PCR), while no differential expression regarding this microRNA was observed in Kameswaran study.

MiR-124a effect(s) on beta-cell functions have been partially characterized by several authors [16]. Indeed, previous studies highlighted a pivotal role for miR-124a in the control of insulin granules exocytosis and of glucose sensing machinery. We confirmed that overexpression of miR-124a in MIN6-pseudoislets inhibits glucose-stimulated insulin secretion (16.7mM glucose) while it does not affect insulin secretion at lower glucose (3.3 mM) levels. This effect appears to be mediated by the combined downregulation of miR-124a target genes, Foxa2 and Mtpn. Foxa2 exerts its effect through the regulation of Kcnj11(potassium channel, inwardly rectifying subfamily J, member 11) and Abcc8(ATP-binding cassette, sub-family C (CFTR/MRP), member 8) genes, the subunits of the ATP-dependent K (*K*ATP) channel, while Mtpn regulates trafficking of insulin granules and their docking to the plasma membrane. Using luciferase-reporter assays, we confirmed the direct binding of miR-124a to the 3'UTR of both Foxa2 and Mtpn genes; specifically, luciferase activity controlled by Foxa2 and by Mtpn 3'UTR was decreased upon miR-124a overexpression. Accordingly, by mutating miR-124a binding sequences within 3'UTR of Foxa2 and Mtpn genes, we observed a restoration of luciferase activity, indicating a specific activity of miR-124a in the 3'UTR of these genes. Moreover, we observed an upregulation of Foxa2 and Mtpn expression following miR-124a inhibition in MIN6-pseudoislets, indicating that miR-124a negatively regulates their expression. In the light of miR-124a increased expression reported in T2D islets, these data suggest a possible role for this microRNA in the downregulation of Foxa2 and of Mtpn expression in T2D pancreatic islets, thus contributing to impaired insulin secretion and defective glucose sensing machinery.

In addition, although miR-124a negatively regulates Foxa2 and Mtpn, we cannot rule out that its effects on pancreatic islets may also be mediated by additional target genes (**Figure 1**). To this end, we looked for any predicted miR-124a target gene, which may be involved in beta-cell functions, besides insulin secretion and glucose-sensing mechanisms. The recently developed microRNA prediction algorithms allowed us to identify additional putative miR-124a target genes with fundamental functions within beta-cell. Namely we detected: Akt3, Sirt1, Flot2 and NeuroD1/Beta2 [24-27]. These miR-124a target genes showed increased expression upon miR-124a inhibition in MIN6-pseudoislets, indicating direct or indirect effect(s) of this microRNA in the regulation of their expression. Luciferase assays on 3'UTR will clarify whether such regulation is directly mediated by miR-124a or not. The upregulation of miR-124a observed in pancreatic islets from T2D organ donors may lead to a decreased expression of its target genes with effects on islet functions depending on the specific gene targeted. For example, decreased expression of NeuroD1, a transcription factor involved in insulin gene transcription, may lead to decreased insulin biosynthesis [28]. NeuroD1 is also involved in the regulation of several genes, which control the identity of beta-cell. Loss of phenotype specification (or dedifferentiation) has been recently proposed as a possible cause of beta-cell dysfunction in T2D [29]. We may speculate that upregulation of miR-124a leads to downregulation of NeuroD1, thus enhancing or favoring dedifferentiation phenomena. Disruption of insulin signaling through down-regulation of Akt3 or of Flot2 may represent other putative mechanisms of beta cell dysfunction: a not

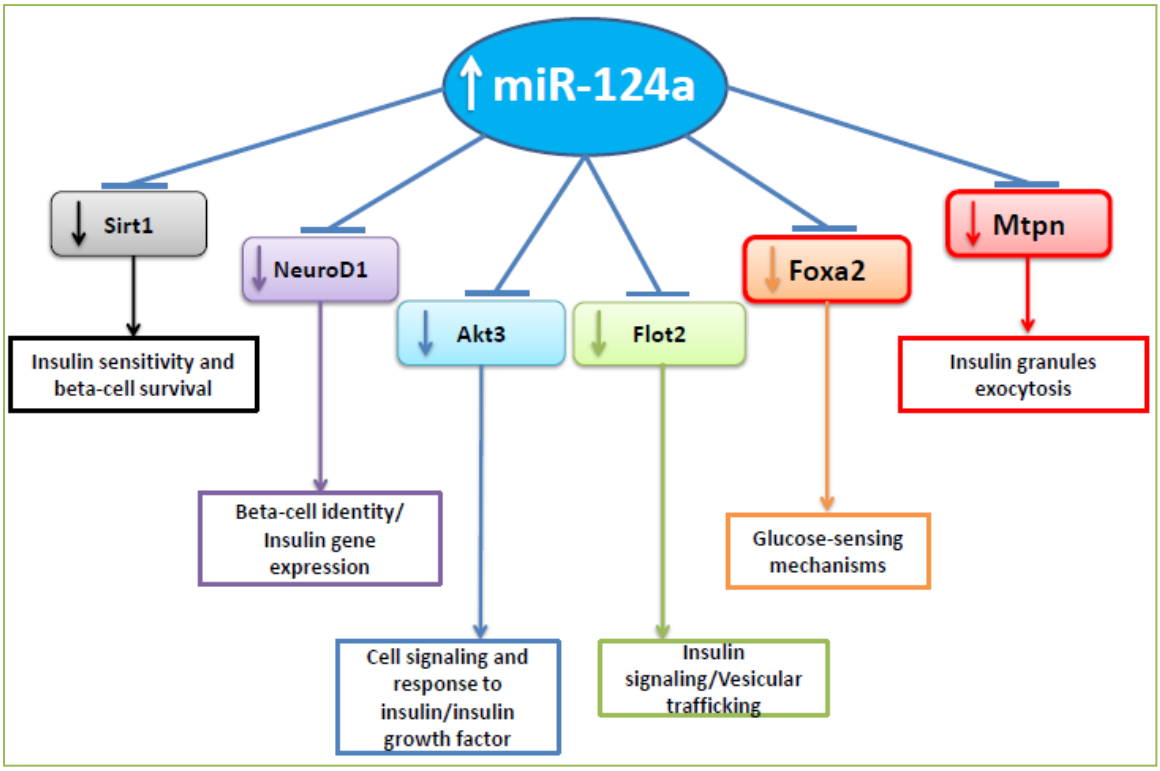

Figure1.MicroRNA miR-124a negatively regulates the expression of multiple genes in insulin-secreting cells. MicroRNA miR-124a is hyperexpressed in T2D human pancreatic islets and targets several genes involved in beta-cell function. Foxa2 and Mtpn genes (highlighted in the red boxes) are experimentally validated targets, while Akt3, Sirt1, Flot2 and Neurod1 are predicted targets. All reported genes showed increased expression levels upon miR-124a inhibition in MIN6 pseudoislets leading us to hypothesize the downregulation of these target genes caused by miR-124a hyperexpression in human pancreatic islets from T2D donors.

fully efficient insulin/insulin growth factor signaling has been reported to induce an impaired beta-cell response in insulin resistance, secondary to a lack of compensatory response in terms of proliferation and growth [31]. Finally, Sirt1, a NAD-dependent deacetylase involved in metabolic control of cell survival and in insulin secretion, may also be downregulated by miR-124a, thus potentially contributing to decreased insulin response to glucose and/or to a reduced beta-cell survival.

Another interesting aspect of miR-124a hyperexpression in T2D human pancreatic islets is represented by the cause(s) of such hyperexpression. In our study we could rule out the contribution of age, sex, BMI or hyperglycemia. Of note, specific polymorphisms related to miR-124a gene sequence and/or flanking gene sequences might contribute to the observed effect. A recent study revealed a specific polymorphism of miR-124a gene (C/G rs531564) in a cohort of T2D patients [30]. This SNP has been detected outside the mature miR-124a seed region DNA sequence, more specifically in the pre-miR sequence. The G allele of rs531564 in hsa-miR-124a gene appears to be a risk allele (OR = 2.15, P = 0.008), which confers a specific detrimental phenotype to beta-cells. Indeed, a previous study reported that the G allele produces a pre-miR-124a secondary structure different from that of the C allele, changing the conformation of the stem-loop shaped structure and thus leading to changes in pre-miR stability [32]. Moreover, it was observed that the G allele is associated to increased expression of miR-124a, which is in line with our findings.

Overall, emerging data suggest that miR-124a is hyperexpressed in pancreatic islets from T2D multiorgan donors respect to non-diabetic subjects and that it may be involved in beta-cell dysfunction. We hypothesize a specific role of miR-124a in the regulation of insulin secretion and of glucose sensing machinery, suggesting a potential involvement of islet miR-124a hyperexpression in beta-cell dysfunction in T2D. Indeed, as previously published, we confirmed miR-124a role in the control of two master gene regulators of insulin secretion (Foxa2 and Mtpn) and shed light on new possible miR-124a target genes (Flot2, NeuroD1, Akt3 and Sirt1) with a pivotal role in beta-cell functional control. Although further studies are required to fully understand the role of miR-124a in human pancreatic islets, accumulating evidence indicates that altered expression of specific microRNAs, including miR-124a, may contribute to the pathogenesis of T2D.

References

1. Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 2005; 6(5):376-85.
2. Wilczynska A, Bushell M. The complexity of miRNA-mediated repression. Cell Death Differ 2015; 22:22-33.
3. Soifer HS1, Rossi JJ, Saetrom P. MicroRNAs in disease and potential therapeutic applications. Mol Ther2007; 15:2070-9.
4. Sebastiani G, Vendrame F, Dotta F. MicroRNAs as new tools for exploring type 1 diabetes: relevance for immunomodulation and transplantation therapy. Transplant Proc2011; 43:330-2.
5. Fernandez-Valverde SL, Taft RJ, Mattick JS. MicroRNAs in β-cell biology, insulin resistance, diabetes and its complications. Diabetes 2011; 60:1825-31.
6. Sebastiani G, Grieco FA, Spagnuolo I, Galleri L, Cataldo D, Dotta F. Increased expression of microRNA miR-326 in type 1 diabetic patients with ongoing islet autoimmunity. Diabetes Metab Res Rev2011; 27:862-6.
7. Weir GC, Bonner-Weir S. Sleeping islets and the relationship between beta-cell mass and function. Diabetes 2011; 60: 2018-9.
8. Kahn SE. The relative contributions of insulin resistance and beta cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia 2003; 46: 3-19.
9. Melkman-Zehavi T, Oren R, Kredo-Russo S, Shapira T, Mandelbaum AD, Rivkin N, *et al*miRNAs control insulin content in pancreatic beta cells via downregulation of transcriptional repressors. EMBO J 2011; 30: 835-845.
10. Karolina DS, Armugam A, Tavintharan S, Wong MT, Lim SC, Sum CF, *et al*. MicroRNA-144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. PLoS One 2011; 6:e22839.
11. Kalis M, Bolmeson C, Esguerra JL, Gupta S, EdlundA, Tormo-Badia N, *et al*. Beta-cell specific deletion of dicer1 leads to defective insulin secretion and diabetes mellitus. PLoS One 2011; 6:e29166.
12. Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, *et al*. A pancreatic islet-specific microRNA regulates insulin secretion. Nature 2004; 432: 226-230
13. Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, *et al*. miR-375 maintains normal pancreatic alpha- and beta-cell mass. Proc NatlAcadSci U S A 2009; 7;106:5813-8.
14. Avnit-Sagi T, Kantorovich L, Kredo-Russo S, Hornstein E, Walker MD. The promoter of the pri-miR-375 gene directs expression selectively to the endocrine pancreas. PLoS One2009; 4: e5033.
15. Kloosterman WP, Lagendijk AK, Ketting RF, Moulton JD, Plasterk RH. Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. PLoSBiol 2007; 5:e203.
16. Baroukh NN, Van Obberghen E. Function of microRNA-375 and microRNA-124a in pancreas and brain. FEBS J 2009; 276: 6509-21.
17. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells 2006;24:857-64.
18. Baroukh N, Ravier MA, Loder MK, Hill EV, Bounacer A, Scharfmann R, *et al*. MicroRNA-124a regulates FoxA2 expression and intracellular signaling in pancreatic beta-cell lines. J.Biol.Chem 2007; 282: 19575-19588.
19. Locke JM, Harries LW. MicroRNA expression profiling of human islets from individuals with and without type 2 diabetes: promises and pitfalls. Biochem Soc Trans 2012; 40:800-3.
20. Sebastiani G, Po A, Miele E, Ventriglia G, Ceccarelli E, Bugliani M, Marselli M, *et al*. MicroRNA-124a is hyperexpressed in type 2 diabetic human pancreatic islets and negatively regulates insulin secretion. ActaDiabetol2014 [Epub ahead of print].
21. Bolmeson C, Esguerra JL, Salehi A, Speidel D, Eliasson L, Cilio CM. Differences in islet-enriched miRNAs in healthy and glucose intolerant human subjects. Biochem. Biophys Res Commun 2011; 404: 16-22.
22. Kameswaran V, Bramswig NC, McKenna LB, Penn M, Schug J, Hand NJ, *et al*. Epigenetic regulation of the DLK1-MEG3 microRNA cluster in human type 2 diabetic islets. Cell Metab 2014; 19: 135-45.
23. Tattikota SG, Rathjen T, McAnulty SJ, Wessels HH, Akerman I, van de Bunt M, *et al*. Argonaute 2 mediates compensatory expansion of the pancreatic β cell. Cell Metab 2014; 19: 122-34.
24. Frick M, Bright NA, Riento K, Bray A, Merrified C, Nichols BJ. Coassembly of flotillins induces formation of membrane microdomains, membrane curvature, and vesicle budding. Curr.Biol 2007; 17: 1151-6.
25. Andrali SS, Sampley ML, Vanderford NL, Ozcan S. Glucose regulation of insulin gene expression in pancreatic beta-cells. Biochem J 2008; 415: 1-10.
26. Schultze SM, Jensen J, Hemmings BA, Tschopp O, Niessen M. Promiscuous affairs of PKB/AKT isoforms in metabolism. Arch.Physiol.Biochem 2011; 117: 70-7.
27. Wojcik M, Mac-Marcjanek K, Wozniak LA. Physiological and pathophysiological functions of SIRT1. Mini Rev Med Chem 2009; 9: 386-94.
28. Andrali SS, Sampley ML, Vanderford NL, Ozcan S. Glucose regulation of insulin gene expression in pancreatic betacells. Biochem J 2008; 415:1–10.
29. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 2012;150:1223-34.
30. Wang Q1, Jin T. The role of insulin signaling in the development of β-cell dysfunction and diabetes. Islets 2009; 1:95-101.
31. Ciccacci C, Di Fusco D, Cacciotti L, Morganti R, D'Amato C, Greco C, *et al*. MicroRNA genetic variations: association with type 2 diabetes. ActaDiabetol 2013; 50: 867-72.
32. Qi L, Hu Y, Zhan Y, Wang J, Wang BB, Xia HF, *et al*. A SNP site in pri-miR-124 changes mature miR-124 expression but no contribution to Alzheimer's disease in a Mongolian population. NeurosciLett 2012; 515: 1-6.