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Original Article

miR-124a targets PDK1/Akt/GSK-3β signaling in pancreatic β-Cells and regulates biological responses to glucose

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Abstract: MicroRNAs (miRNAs) are short non-coding RNAs that have been implicated in fine-tuning gene regulation. Here, we focused on the pancreas-specific miR-124a as a potential regulator of its predicted target 3'-phosphoinositide-dependent protein kinase-1 (PDK1), and we analyzed its implication in the response of insulin-producing cells to elevation of glucose levels. We used insulinoma-1E cells to analyze the effects of miR-124a on PDK1 protein level and downstream protein kinase B/glycogen synthesis kinase-3β (Akt/GSK-3β) signaling, glucose-induced insulin gene expression, and insulinoma-1E cells proliferation. Moreover, we analyzed the effect of glucose on miR-124a expression in both INS-1E cells and primary rat islets. Finally, miR-124a expression in isolated islets was analyzed in diabetic Goto-Kakizaki (GK) rats. Our findings provide evidence for a role of miR-124a in the regulation of PDK1, a key molecule in Akt/GSK-3β signaling in pancreatic β-cells. The effects of glucose on miR-124a are compatible with the idea that miR-124a is involved in glucose regulation of insulin gene expression and β-cell growth.

Keywords: miR-124a, PDK1, β-cells, insulin

Introduction

Type 2 diabetes are characterized by an inability of the functional β-cell population to meet chronically increased metabolic demands for insulin, as occurring under variable states of insulin resistance [1]. The normal β-cell mass can adapt to a sustained stimulation by recruiting β-cells into a higher translational and insulin synthetic activity and possibly by an expansion of its total cell counts. The molecular mechanisms involved in this chronic adaptation of the β-cell population are not completely identified. Several reports have highlighted the importance of protein kinase B (Akt) signaling in β-cell physiology [2]. For example, glucose stimulates the insulin gene promoter activity via a cascade involving Akt kinase. The 3-phosphoinositide-dependent protein kinase 1 (PDK1) is important in regulating glucose and energy homeostasis [3]. PDK1 was initially recognized by its ability to phosphorylate in the presence of lipid products generated by Akt, the activation loop of Akt on Ser-473. Indeed, glucose triggers PDK1 via the Akt pathway, which induces nuclear translocation of pancreatic duodenal ho-meobox-1 (PDX-1), and the latter then increases insulin gene transcription [4].

Furthermore, glucose promotes β-cell survival through the Akt/glycogen synthase kinase-3β (GSK-3β) cascade [5]. Different actors of the Akt cascade have been identified as critical control points in insulin signaling, among them transcription factor, GSK-3β. GSK-3β is involved in the storage of glucose into glycogen. In vivo, increased GSK-3β activity is an early event in the development of insulin resistance where glycogen synthesis is impaired in type 2 diabetes and inhibition of GSK-3β in Zucker diabetic fatty rats lead to an improvement in both insulin action and glucose uptake [6]. The role of PDK1 in vivo has been addressed in different organisms by genetic deletion of PDK1 homologs. In short, knockout studies revealed a central role of PDK1 in regulation of cell growth and organ development. Importantly, PDK1 ablation in

β-cells induces diabetes consecutively to a reduction in β-cell mass [7].

MicroRNAs (miRNAs) are endogenous small noncoding RNAs of 21-25 nucleotides that could bind to 3' untranslated region of the mRNAs of protein-coding genes to down-regulate their expression. The discovery of microRNAs has opened an entirely new line of thoughts regarding the regulation of signaling by growth factors and hormones and its perturbations in situations associated to disease processes [8]. Several studies have been conducted to evaluate specifically expressed miRNAs in the diverse types of diabetes, particularly associated with the regulation of insulin production and secretion, differentiation of human preadipocytes, and association with Type 2 diabetes pathogenesis. miR-124a is preferentially expressed in brain and pancreas, which is another one of important miRNAs in regulating pancreas development. miR-124a expression is strikingly increased at e18.5 compared with e14.5, two key stages of mouse embryonic pancreas development [9]. miR-124a targets gene encoding forkhead box protein A2 (Foxa2), which is essential in β-cell differentiation and pancreas development. In pancreatic β-cell line INS-1E cells, miR-124a is a glucose-induced miRNA [10].

Here, we identify miR-124a involving in insulin signaling in pancreatic β-cells. Using computational analysis, we found that miR-124a targets PDK1, a key player in the Akt-kinase cascade. By gain- and loss-of-function experiments, we found that miR-124a regulates PDK1 protein level, resulting in modulation of glucose-stimulatory action on insulin gene expression, DNA synthesis and cell proliferation. Furthermore, exposure of either INS-1E cells or freshly isolated rat islets to glucose modulates endogenous miR-124a levels, suggesting its involvement in regulation of glucose responsiveness of β-cells. Finally, miR-124a expression is found to be decreased in diabetic Goto-Kakizaki (GK) rats compared with Wistar rats.

Materials and methods

Cell culture and transfections

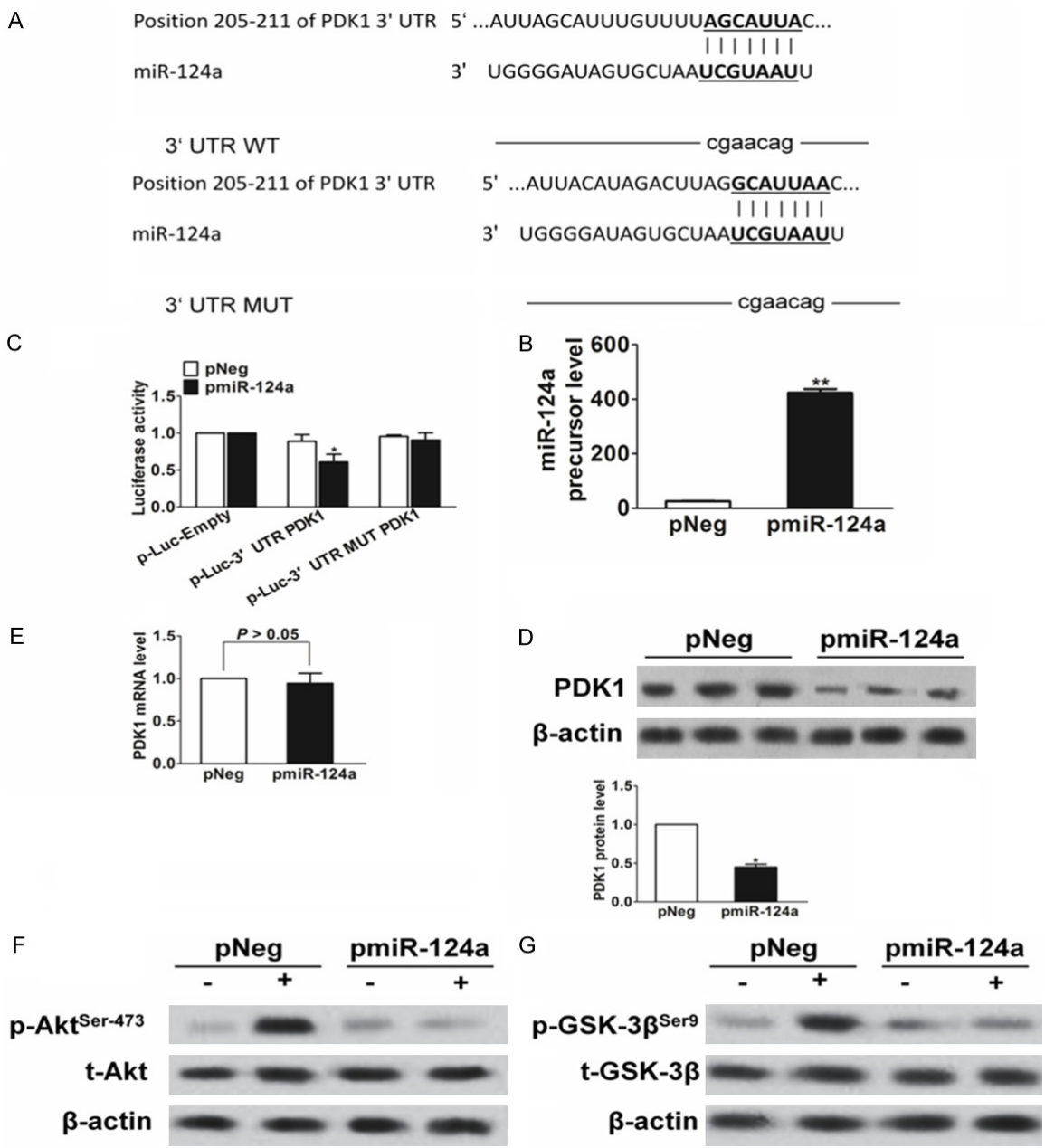
INS-1E β-cells were maintained in RPMI-1640 containing 11 mM glucose supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 10 mM HEPES, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol in humidified 5% CO_2, 95% air at 37°C. LipofectAMINE 2000 transfection reagent (Invitrogen, Life Technologies) was used to transfect INS-1E cells. A total of 3 μg pmiR-124a or pNeg and the indicated amounts of 2'-O-methyl-miR-124a or 2'-O-methyl-GFP antisense oligonucleotides and 2 μl Lipofectamine® 2000 were used per well with each containing 5×10^5 cells.

Islet preparation

Islets were isolated by collagenase digestion, elutriation, and manual handpicking from adult male Wistar rats (150-250 g; Shanghai Slack laboratory animal co., LTD). Animals were bred according to regulations of animal welfare and used in experiments that were approved by the local ethical committee of Affiliated Hospital of Qingdao University. Islets were cultured in Ham's F10 nutrient mixture (Gibco) supplemented with 0.5% BSA, 2 mM glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 2% FBS, and the indicated glucose concentration [11].

DNA constructs

Expression vector driving expression of miR-124a was prepared by introducing oligonucleotides corresponding to the murine precursor sequence of miR-124a into pcDNA6.2 (pmiR-124a) (Invitrogen). The oligonucleotide sequences were as follows: sense, 5'-TGCTGCCCCGCGACGAGCCCC-TCGCACAAACCGGACCTGAGCGTTTTGTTCGTTCGGCTCGCGTGAGGC-3'; and antisense, 5'-CCTGGCCTCACGCGAGCCGAACGAACAAAACGCTCAGGTCCGGTTTGTG CGAGGGGCTCGTCGCGGGGC-3'. As negative control, we used pNeg driving the expression of an unrelated known microRNA precursor (Invitrogen). The rat PDK1 3'UTR target site was cloned using the following oligonucleotides: sense, 5'-ACCCAACCACACAAAGAACAAAA-3'; and antisense, 5'-TTTTGTTCTTTGTGTGGTTGGGT-3' in the 3'UTR of the Renilla luciferase reporter vector, pmiR-Report luciferase (Ambion), as described previously. As a negative control response element, we used a mutated sequence by inserting the following oligonucleotides: sense, 5'-ACCCAACCACACCCCTCCTGGGG-3'; and antisense, 5'-CCCCAGGAGGGGTGTGGTT-GGGT-3'. Loss of function



Figrue 1. PDK1 is a target of miR-124a. A. Sequence of the miR-124a binding sites within the PDK1 3'-UTR and a schematic of the reporter construct showing the PDK1 3'-UTR sequence and the mutated PDK1 3'-UTR sequence. B. INS-1E cells were transfected with pmiR-124a or pNeg. miR-124a precursor was quantified by quantitative RT-PCR. Data were collected from three independent experiments and were average ± SD. values. $^{**}P < 0.05$ compared to pNeg. C. Luciferase activity of the PDK1 reporter in the presence of pmiR-124a or pNeg. Data were collected from three independent experiments and were average ± SD. values. $^{*}P < 0.01$ compared to p-Luc-Empty. D. INS-1E cells were transfected pmiR-124a or pNeg, PDK1 or to β-actin were analyzed by Western blot. Data represent three independent transfections done in triplicate, ± SD, with $n = 3$. $^{*}P < 0.05$ compared to pNeg. E. NS-1E cells were transfected pmiR-124a or pNeg. PDK1 mRNA levels were quantified by RT-PCR analysis. Data represent three independent transfections done in triplicate, ± SD, with $n = 3$. F. INS-1E cells were transfected with pNeg or pmiR-375. Cells were stimulated with insulin. Protein extracts were analyzed by western blot using antibody to phosphor-Ser-473 Alt and total Akt. G. Analysis of GSK-3β phosphorylation. Protein extracts were analyzed by western blot using antibody to phosphor-GSK3β and total GSK-3β.

experiments were carried out using the following: 2'-O-methyl-124a, UGCAUCACGCGAGCCGAACGAACAAAUAAGL, and 2'-O-methyl-eGFP, AAGGCAAGCUGACCCUGAAGUL.

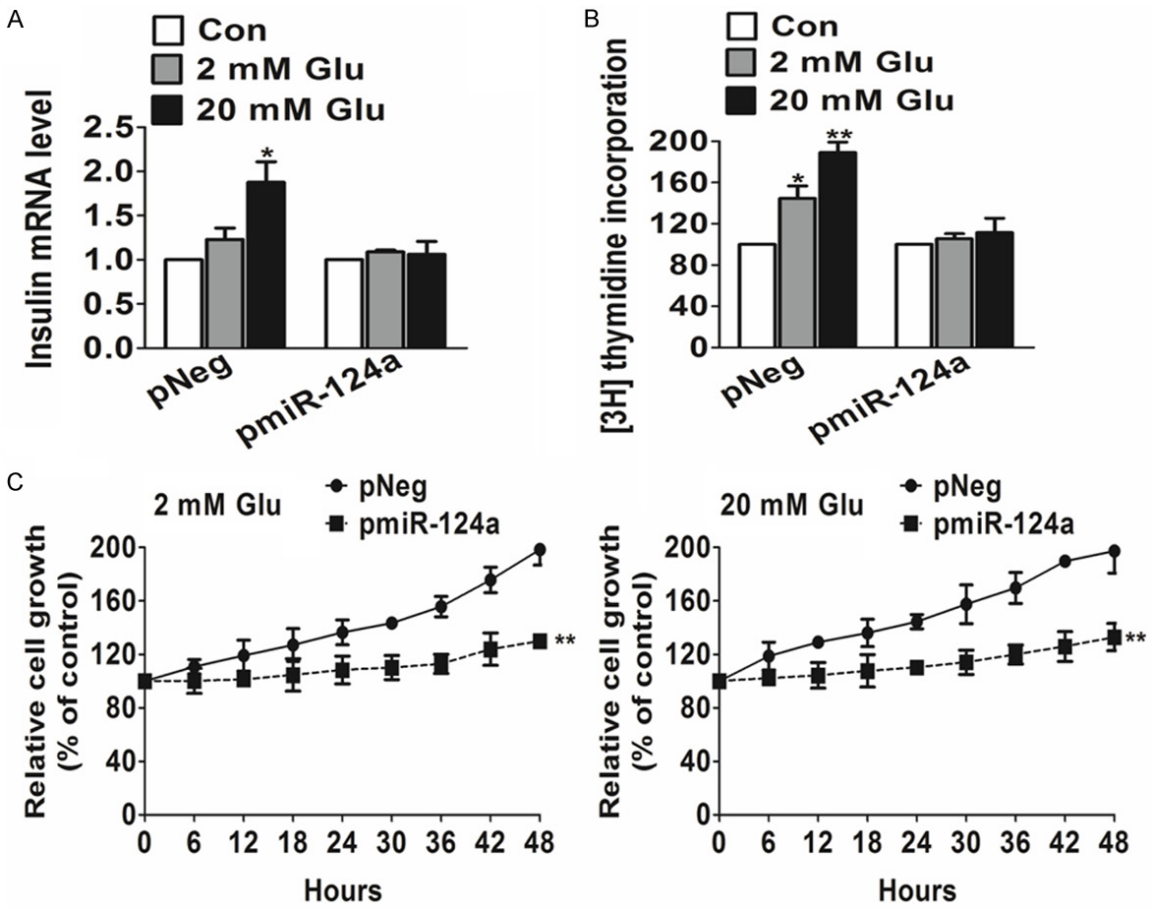


Figure 2. Effect of miR-124a on glucose-enhanced insulin gene expression and cell proliferation. A. Quantification of insulin mRNA. INS-1E cells were transfected pmiR-124a or pNeg and stimulated with glucose. Insulin mRNA expression was analyzed by quantitative RT-PCR. Data represent five independent experiments carried out in triplicate, ± SD, with n = 3. $^{*}P < 0.05$ compared with control. B. Measurement of [methyl-^{3}H] thymidine incorporation. INS-1E cells were transfected pmiR-124a or pNeg and stimulated with glucose. Cell proliferation was assessed by measuring [methyl-^{3}H] thymidine incorporation. Data represent three independent experiments done in triplicate, ± SD, with n = 3. $^{*}P < 0.05$ compared with control. C. INS-1E cells were transfected pmiR-124a or pNeg and stimulated with glucose. Cell proliferation was assessed by MTT assay. Data represent three independent experiments done in triplicate, ± SD, with n = 3. $^{**}P < 0.01$ compared with pNeg.

Luciferase assay

INS-1E cells were cultured in six-well plates and transfected with different reporter vectors (p-Luc-Empty, p-Luc 3'UTR PDK1 or p-Luc-3'UTR MUT PDK1) and cotransfected with pNeg or pmiR-124a. Cells were assayed 48 h after transfection with the dual-luciferase reporter assay system (Promega). Luciferase activity was normalized by β-galactosidase activity [12].

RNA RT-PCR and real-time PCR

RNA from transfected β-cells and rat islets was isolated using TRIzol reagent (Invitrogen). For each transfected well, 1 μg total RNA was reverse-transcribed. For microRNAs precursor detection, RT was performed as described. The following forward and reverse primers were used for amplification: PDK1, 5'-CCCACGTGATGGACTCAAAGA-3' (reverse) and 5'-AAGGGTACGGGCCTCTCAAA-3' (forward); Insulin, 5'-GTGCACCAACAGGGCCAT-3' (reverse) and 5'-CAGAGACCATCAGCAAGCAGG-3' (forward); U6, 5'-AACGCTTCACGAATTTGCGT-3' (reverse) and 5'-CTCGCTTCGGCAGCACA-3' (forward); and 36B4, 5'-ATGATCAGCCCGAAGGAGAAGG-3' (reverse) and 5'-CCACGAAAATCTCCAGAGGCAC-3' (forward).

Western blotting assay

Two days after transfection, INS-1E cells were washed with ice-cold PBS and processed for protein isolation. For Western blotting, total proteins were separated by electrophoresis and transferred to polyvinylidene difluoride

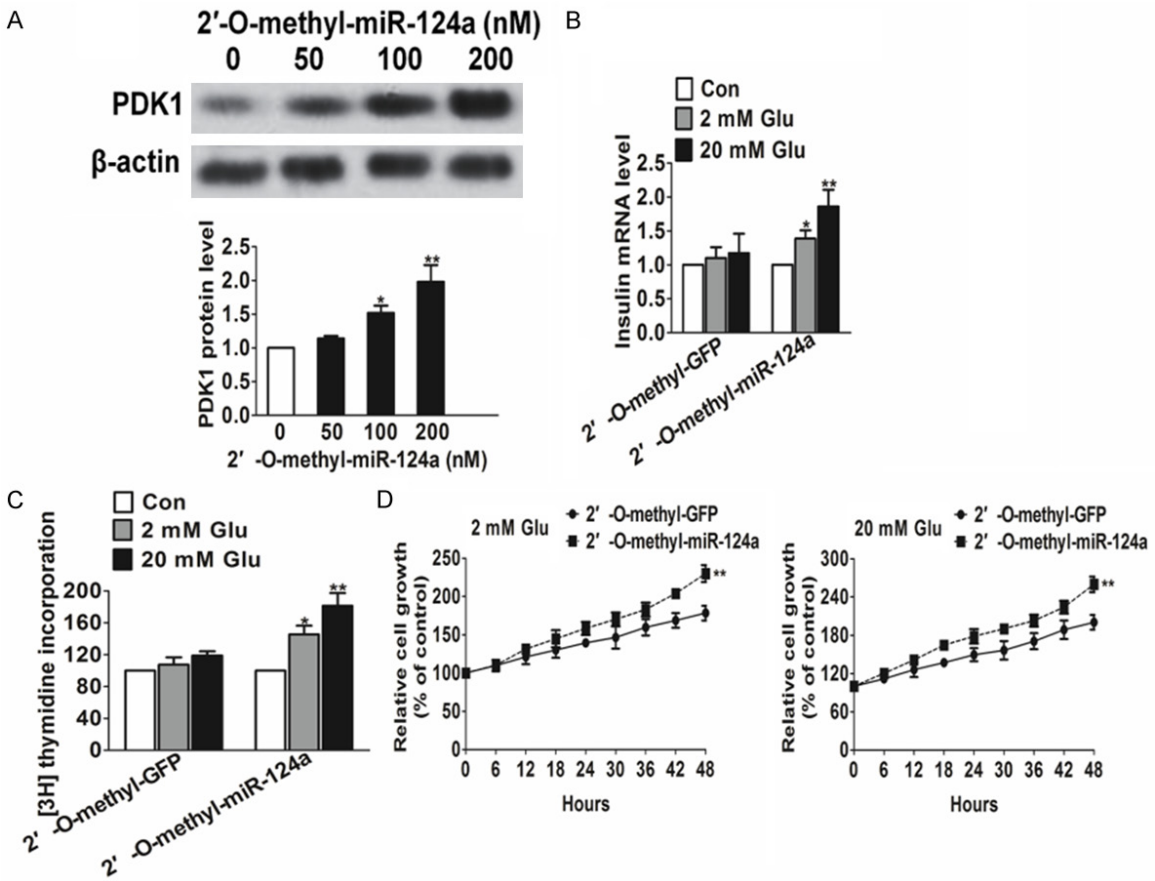


Figure 3. Effect of 2'-O-methyl-miR-124a antisense oligonucleotides on PDK1, glucose-enhanced insulin mRNA, and cell proliferation. A. Analysis of PDK1 protein. INS-1E cells were transfected with 2'-O-methyl-miR-124a. Protein extracts were analyzed by Western blot using antibody to PDK1 or to β-actin. Quantification of PDK1 protein. Data represent three independent transfections, ± SD, with n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with 0 nM. B. Quantification of insulin mRNA level. INS-1E cells were transfected with either 2'-O-methyl-GFP or 2'-O-methyl-miR-124a. Cells were treated with glucose and RNA extracts were analyzed by RT-PCR for the expression of insulin transcript level. Data represent three independent experiments done in triplicate, ± SD, with n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control. C. INS-1E cells were transfected 2'-O-methyl-miR-124a or 2'-O-methyl-GFP and were treated with glucose. [methyl-^{3}H] thymidine incorporation was measured. Data represent four independent experiments, done in triplicate, ± SD, with n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control. D. INS-1E cells were transfected 2'-O-methyl-miR-124a or 2'-O-methyl-GFP and were assayed by MTT. Data represent four independent experiments, done in triplicate, ± SD, with n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with 2'-O-methyl-GFP.

membranes (Immobilon-P; Millipore, Bedford, MA) followed by blotting. Immunodetection was performed using affinity-purified polyclonal antibodies to PDK1, and phosphor-GSK-3β Ser9, total GSK-3β, phosphor-Akt-Ser-473, total Akt (Santa Cruz Biotechnology, Santa Cruz, CA). To assess the total protein amount, membranes were stripped and reprobed with antibody to β-actin (Sigma-Aldrich, St. Quentin-Fallavier, France).

Cell viability assay

2×10^5 cells per well were seeded in 12-well plates and were transfected with pmiR-124a or pNeg. After 48 h, cell viability was assessed by the ability of metabolically active cells to reduce tetrazolium salt to orange-colored formazan compounds. The absorbance of the samples was measured with a spectrophotometer reader (450 nm). Data shown correspond to mean values from three independent experiments.

[methyl-^{3}H] thymidine incorporation assay

Cells were plated in six-well plates at a density of 5×10^5 cells per well. Cells were transfected with pmiR-124a or pNeg. 24 hours later, cells were starved in RPMI containing 0.5% FBS for 24 h and then replaced in RPMI 10% FBS. After

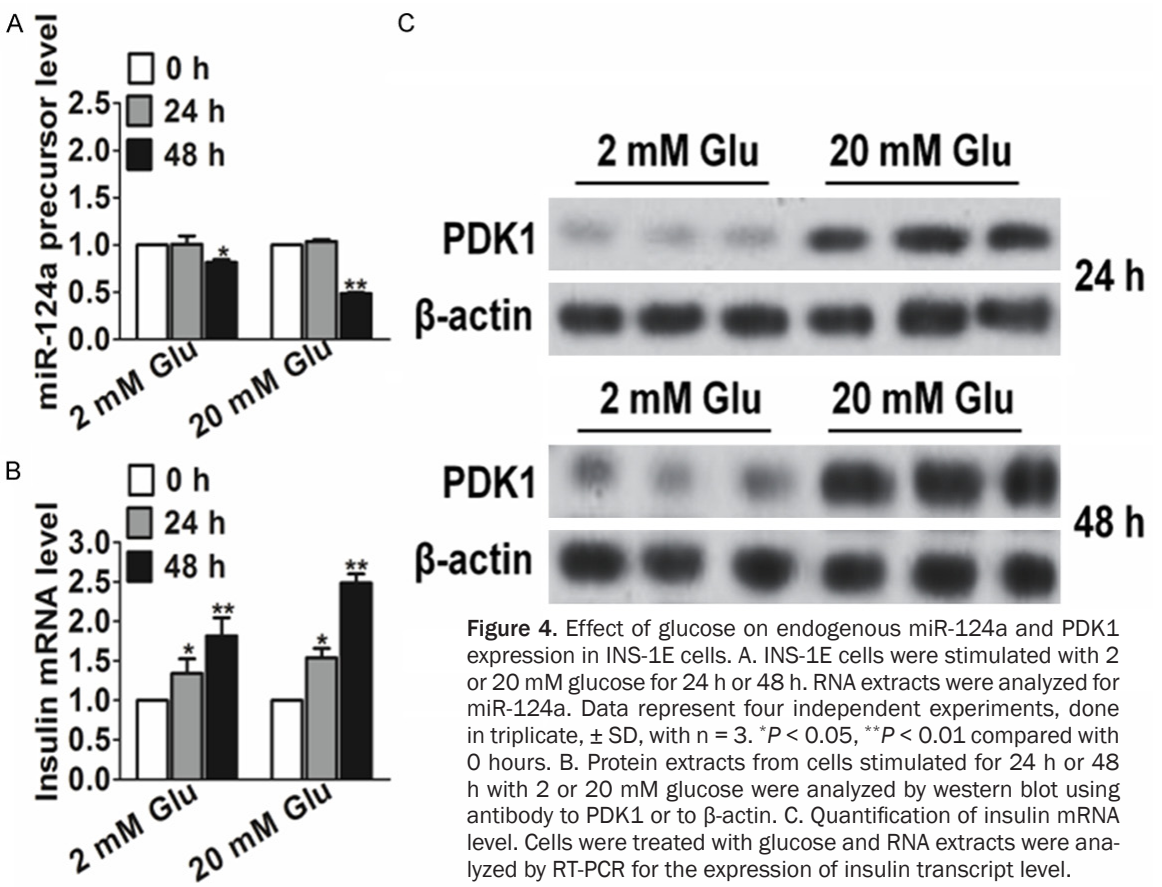


Figure 4. Effect of glucose on endogenous miR-124a and PDK1 expression in INS-1E cells. A. INS-1E cells were stimulated with 2 or 20 mM glucose for 24 h or 48 h. RNA extracts were analyzed for miR-124a. Data represent four independent experiments, done in triplicate, ± SD, with n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with 0 hours. B. Protein extracts from cells stimulated for 24 h or 48 h with 2 or 20 mM glucose were analyzed by western blot using antibody to PDK1 or to β-actin. C. Quantification of insulin mRNA level. Cells were treated with glucose and RNA extracts were analyzed by RT-PCR for the expression of insulin transcript level.

24 h, DNA synthesis was assayed by adding 1 μCi [methyl-^{3}H] thymidine/well and by incubating the cells for another 2 h. Then cells were washed twice with PBS, fixed with 10% trichloroacetic acid for 30 min, and solubilized by adding 300 μl 0.2 N NaOH to each well. Radioactivity, reflecting incorporation of [methyl-^{3}H] thymidine into DNA, was measured by adding scintillation liquid and counting [13].

Statistical analysis

Numerical results were analyzed using independent mean T-test and expressed in mean ± standard deviation (SD). Statistical analysis was performed using post hoc testing using Bonferroni's method. Differences were considered statistically significant at $P < 0.05$.

Results

miR-124a regulates PDK1 protein level and decreases insulin signaling downstream of PDK1

To identify potential targets of miR-124a, we applied several algorithms that predict the mRNA targets of miRNAs-TargetScan, PicTar and miRanda-mirSVR [14]. The candidate target genes were predicted based on the representation of miR-124a recognition sites in their 3'-UTRs. As predicted, complementarity can be found between miR-124a and PDK1 3'-UTR (**Figure 1A**). To investigate whether miR-124a affects PDK1, plasmids driving the expression of miR-124a precursor (pmiR-124a) or control (pNeg) were transfected in INS-1E cells. pmiR-124a significantly increased miR-124a precursor levels, as assayed by real-time PCR (**Figure 1B**). To test whether or not PDK1 is a target of miR-124a, we cloned the putative 3'UTR target site downstream of a luciferase reporter gene and cotransfected this p-Luc-3'UTR PDK1 construct into INS-1E cells with pmiR-124a or pNeg. Luciferase activity of cells transfected with pmiR-124a and p-Luc-3'UTR PDK1 was markedly decreased compared with cells cotransfected with control pNeg and p-Luc-3'UTR PDK1 (**Figure 1C**). The same assay was performed for another reporter plasmid containing mutated PDK1 3'-UTR in miR-124a binding sites. With negative control constructs

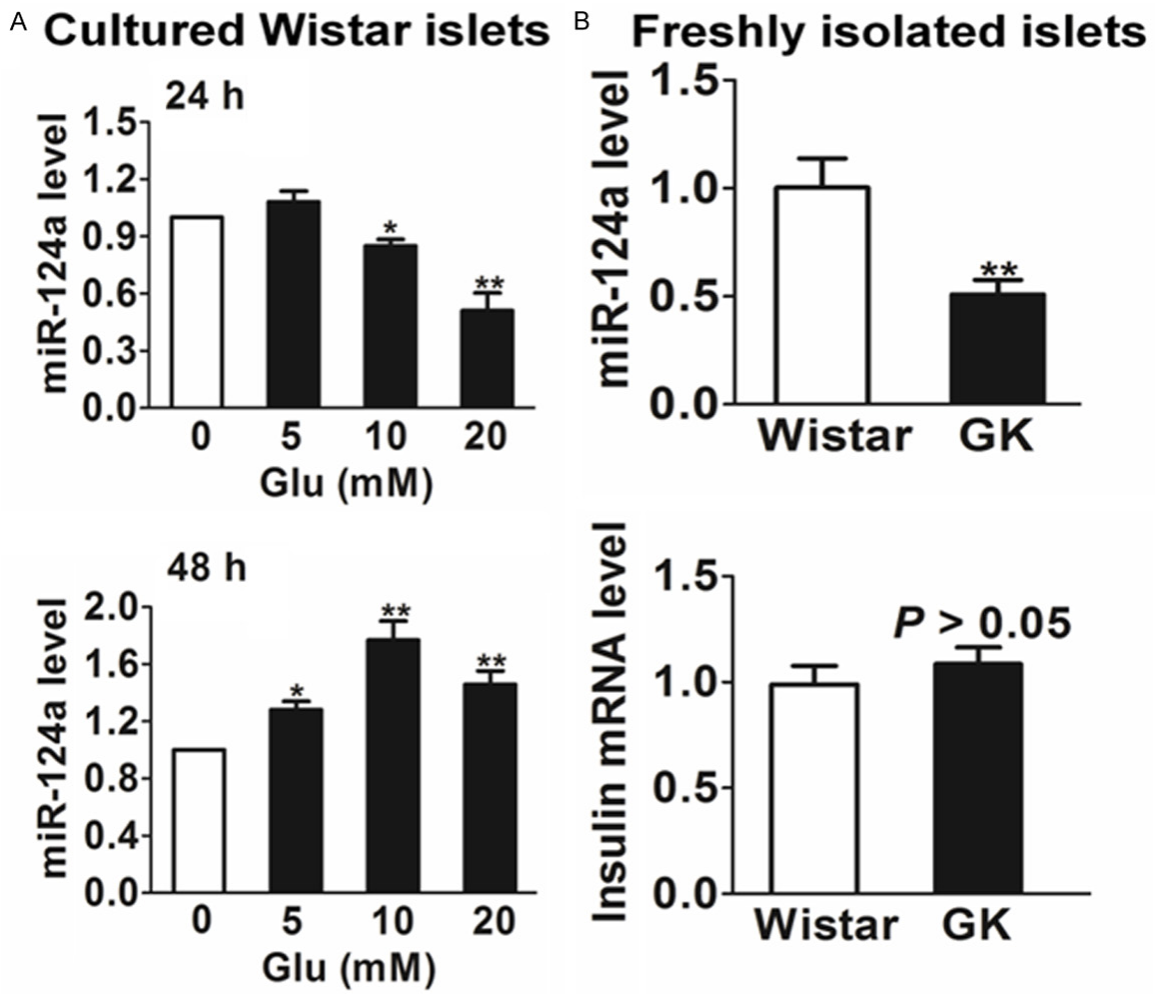


Figure 5. Study of endogenous miR-375 expression in freshly isolated rat islets. A. Rat islets were maintained as described in research design and methods. Thereafter, islets were treated with either 5, 10, or 20 mM glucose for 24 h or 48 h. RNA extracts were reverse-transcribed and analyzed by quantitative RT-PCR for miR-375. Results are means ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control. B. Freshly isolated islets from Wistar (n = 6) and GK rats (n = 6) were prepared as previously described. RNA extracts were analyzed by quantitative RT-PCR for pre-miR-124a and insulin mRNA. Results are means ± SD, $^{**}P < 0.01$ compared with Wistar rats.

p-Luc-Empty and p-Luc-3'UTR MUT PDK1, no reduced luciferase activity was observed when cells were cotransfected with pmiR-124a compared with pNeg (**Figure 1C**). These results indicate that miR-124a represses the 3'-UTR of PDK1 and that the expression of PDK1 is directly regulated by miR-124a. Functional analysis shown that miR-124a precursor over-expression in INS-1E cells results in the reduction of PDK1 protein (**Figure 1D**) without affecting PDK1 mRNA level (**Figure 1E**), which future indicating that miR-124a acts as a translational repressor.

In pancreatic β-cells, the PDK1/Akt signaling pathway is used by insulin to elicit several actions of hormones [15]. It is generally believed that after insulin stimulation, PDK1 is recruited to the plasma membrane and phosphorylates Akt kinase on Ser-3473, which becomes activated and phosphorylates a series of substrates, including transcription factor GSK-3β. Down-regulation of PDK1 in β-cells is expected to cause a decrease in insulin-induced signaling dependent on this particular kinase. To study the effect of miR-124a on insulin signaling, we examined the phosphorylation state of Akt. Immunoblot analysis showed that in response to insulin, Akt phosphorylation on Ser-473 was less abundant in cells over-expressing miR-124a precursor compared with control cells (**Figure 1F**). Consistently, miR-

124a precursor also reduced insulin-induced phosphorylation of GSK-3β (**Figure 1G**).

miR-124a decreases glucose-induced insulin gene expression and DNA synthesis

Because PDK1/Akt kinase signaling was associated with the up-regulation of insulin gene expression induced by glucose, we examined the effect of miR-124a on insulin gene expression in response to glucose. As expected, glucose successfully induced insulin gene expression in control INS-1E cells, but this effect was lost in cells transfected pmiR-124a (**Figure 2A**). To test whether PDK1 was involved in the glucose-induced increase in INS-1E cell proliferation, we analyzed the impact of miR-124a on cellular [methyl-^{3}H] thymidine incorporation and on INS-1E cells proliferation by (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) MTT assay. As illustrated in **Figure 2B**, the stimulatory effect of glucose on DNA synthesis was reduced when miR-124a precursor was over-expressed in INS-1E cells. The proliferation of cells over-expressing pre-miR-124a was inhibited in response to glucose, compared to control INS-1E cells (**Figure 2C**).

2'-O-methyl-miR-124a increases PDK1 protein level and glucose-stimulatory action on insulin mRNA and DNA synthesis

Because miR-124a targets PDK1 and impairs glucose-stimulated insulin gene expression, as wells NS-1E cell proliferation, we investigated whether anti-sense oligonucleotides of miR-124a induce opposite effects. Using 2'-O-methyl-miR-124a anti-sense oligonucleotides, we found that blocking miR-124a augment PDK1 protein (**Figure 3A**). Importantly, we found that 2'-O-methyl-miR-124a-induced miR-124a depletion increased glucose-enhanced insulin mRNA (**Figure 3B**). Finally, 2'-O-methyl-miR-124a increases the glucose stimulatory action on [^{3}H] thymidine incorporation compared with 2'-O-methyl-GFP (**Figure 3C**) and promoted NS-1E cell proliferation in response to glucose (**Figure 3D**).

miR-124a and PDK1 are inversely correlated in glucose-stimulated INS-1E cells

To further document the role of miR-124a, we analyzed the expression of miR-124a in INS-1E cells treated for 24 or 48 h with 2 or 20 mM glucose. We found that glucose induced a robust decrease in miR-124a (**Figure 4A**). Furthermore, western blotting analyses shown that PDK1 protein level were increased within 24 h of glucose treatment, especially in 48 h of glucose stimulation (**Figure 4B**). Finally, decreased miR-124a with increase in PDK1 level seen after glucose treatment occurred with enhanced insulin gene expression (**Figure 4C**).

Glucose regulates miR-124a expression in freshly isolated rat pancreatic islets

To characterize the glucose-mediated miR-124a regulation, we analyzed the expression level of miR-124a precursor in isolated rat islets that had been exposed to 5, 10, or 20 mM glucose for 24 or 48 h, which represent conditions in which the acute and chronic influences, respectively, of glucose can be studied. After 24 h at stimulatory glucose concentrations (10 or 20 mM), miR-124a expression was lower than in the basal 5 mM condition (**Figure 5A**). After 48 h, a lower expression level was measured at 5 mM than at 10 and 20 mM, whereas the level at 20 mM tended to be lower than at 10 mM (**Figure 5A**).

To study miR-124a expression under in vivo conditions of hyperglycemia, we analyzed islets from diabetic GK rats. We found that miR-124a was down-regulated in diabetic GK rats compared with control Wistar rats (**Figure 5B**). Furthermore, in GK rats, this decrease in miR-124a expression was associated with a modest, albeit not significant, increased in insulin mRNA level (**Figure 5B**). Finally, the expression of miR-124a, which had been involved in insulin mRNA expression, was up-regulated in GK rats (**Figure 5B**).

Discussion

MicroRNAs (miRNAs) are endogenously produced short non-coding RNAs that have been shown to play a key role in mammalian post-transcriptional gene expression by repressing translation or inducing target degradation, ultimately resulting in gene silencing. miRNAs are ubiquitously expressed throughout the mammalian system and therefore capable of regulating several key biological pathways and cellular functions [16-18]. Although several mic-

roRNAs have been implicated in a variety of disease processes, only a few have been linked to insulin signaling and type 2 diabetes. Using either a gain- or loss-of-function approach, we show here in a pancreatic β-cell line that miR-124a is able to down-regulate PDK1 protein by interfering directly with its mRNA. By targeting PDK1, a key player in the Akt-GSK-3β signaling cascade, miR-124a decreases insulin-induced phosphorylation of Akt and GSK-3β, both acting downstream of PDK1. Previous studies have shown that inhibition of the Akt signaling cascade dampens glucose-induced insulin gene expression [19]. To document the biological role of miR-124a as an inhibitor of Akt signaling downstream of PDK1, we analyzed the effect of miR-124a over-expression or depletion on glucose-induced insulin gene expression. Our findings that miR-124a controls insulin gene expression stimulated by glucose. A chief observation of our study is that miR-124a expression seems to be glucose sensitive. Importantly, the increased insulin gene expression seen in INS-1E cells exposed to high glucose is associated with reduced expression of miR-124a.

Although glucose decreased miR-124a expression within 24 h in INS-1E cells, a longer exposure time (48 h) was needed to observe the downstream stimulatory effect on PDK1 protein expression. This suggests that miR-124a suppresses synthesis of PDK1 protein. Interestingly, prolonged glucose exposure of INS-1E cells has been shown to increase insulin receptor substrate 2 gene expression, protein localization to the plasma membrane, and Akt phosphorylation. These observations together with ours indicate that in INS-1E cells, glucose induces a decrease in miR-124a followed by an increase in PDK1 protein and an activation of the Akt cascade and, hence, β-cell proliferation. Glucose was also found to regulate miR-124a expression in primary islet tissue. An incubation assay was used to measure levels in freshly isolated rat islets at glucose concentrations known to have exerted dose-dependent stimulations of their metabolic, secretory, and protein biosynthetic activities. We found miR-124a expression to be lower at 10 and 20 mmol/l than at 5 mmol/l glucose, which is similar to the effect seen in INS-1E cells. The 48 h culture condition compares expression in islets that have maintained this functional responsiveness. Islets isolated from diabetic GK rats exhibited a lower miR-124a expression than islets from Wistar rats, which can be considered as further evidence for the suppressing effect of supraphysiological glucose concentrations.

Decreased PDK1 in human glioblastoma cells obtained with antisense oligonucleotides or with RNA interference blocks cell proliferation [20]. Here, we find that miR-124a down-regulates PDK1 and may therefore impact on cell proliferation given its key role in the Akt/GSK-3β cascade. Our results point to a similar antiproliferative action of miR-124a, because we found that miR-124a attenuates cell viability and proliferation. A first key finding of our work is that miR-124a is regulated by glucose, which is the central molecule in islet metabolism and physiology. A second important observation is that miR-124a inhibits glucose-induced INS-1E cell proliferation. This result is particularly interesting in the context of diabetes. Taking into account the fact that the miR-124a, as an important regulator of glucose-stimulated insulin gene expression and proliferation of pancreatic β-cells, miR-124a emerges as a target that should be prioritized to enhance islet function and to combat β-cell failure.

Disclosure of conflict of interest

None.

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