

Original Article

MicroRNA-223 inhibits hepatic gluconeogenesis by targeting forkhead box O1

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Abstract: The gluconeogenesis pathway was considered as a potential therapeutic target for preventing type 2 diabetes (T2D). Recently, the emergence of (miRNAs, miRs) as important regulators of metabolism has garnered much interest. In this study, we explored the physiological functions of miR-223 in hepatic gluconeogenesis *in vitro*. Quantitative real-time RT-PCR (qRT-PCR) and Western blot were used to measure the expression levels of mRNA and protein, respectively. Bioinformatics-based prediction and luciferase reporter assay were carried out to determine the potential target of miR-223. Glucose output assay was performed to detect the glucose production in primary hepatocytes. The results showed that glucagon inhibited the miR-223 expression through protein kinase A (PKA) signaling in primary hepatocytes. Biological function analysis showed that upregulation of miR-223 restrained phosphoenolpyruvate carboxykinase (PCK) and glucose-6-phosphatase (G6P) expression and glucose production, and knockdown of miR-223 demonstrated an opposite effect in primary hepatocytes. Furthermore, we found Forkhead Box O1 (FOXO1) was a target of miR-223 and the subsequent functional analysis of FOXO1 verified the regulatory effect of miR-223 on FOXO1. Taken together, our present work highlighted the key role of miR-223 in control of hepatic gluconeogenesis, and, notably, mechanistic analysis revealed that FOXO1 was a direct functional target of miR-223, and miR-223 inhibited PCK and G6P expression, as well as glucose production at least in part by targeting FOXO1. These findings probe the feasibility of miRNA-directed therapy for the treatment of hyperglycemia in T2D.

Keywords: Hepatic gluconeogenesis, microRNA-223, Forkhead Box O1, type 2 diabetes

Introduction

Glucose is a primary fuel of the organisms. Thus, the maintenance of the glucose homeostasis exerts a key role in maintaining the survival of the organisms. The glucose homeostasis is controlled by the intake and absorption of dietary carbohydrates, the glucose utilization in skeletal muscles or adipocytes and the hepatic glucose output [1]. The liver has the unique capacity in sustaining blood glucose levels, which regulates an equilibrium between plasma and hepatic glucose concentrations by regulating glucose uptake and storage [1, 2]. Under feeding state, the liver elevates glucose intake, which is converted directly to glycogen or triglycerides for energy production and storage [3]. In the fasted condition, the increased glucagon levels that link to the decreased insulin content are responsible for the glucose consumption by promoting glycogen breakdown in liver [4]. Prolonged starvation leads to hepatic gluconeogenesis that provides an important

event to maintain blood glucose balance [5]. The rate-limiting enzymes, phosphoenolpyruvate carboxykinase (PCK) and glucose-6-phosphatase (G6P), are the main regulators in the process of hepatic gluconeogenesis [6]. Glucagon induces glucose production through the cyclic AMP (cAMP)/protein kinase A (PKA) signaling cascade to form transcriptional machinery that consists of cAMP response element-binding protein 1 (CREB) and CREB-regulated transcription coactivator 2 (CRTC2), thereby promoting PCK and G6P expression [7]. Inversely, it is reported that insulin suppresses hepatic gluconeogenesis via phosphorylation of CREB binding protein and FOXO1/PGC-1 α pathway [8, 9]. Abnormally high levels of hepatic glycogenolysis contribute to the dysregulation and over-production of glucose in people suffering from type 2 diabetes (T2D) [10, 11]. In the recent past, key gluconeogenic enzymes and transcription factor involved in glucose metabolism have been attracted more attention as therapeutic targets for treatment

miRNA inhibits hepatic gluconeogenesis

of diabetes [12, 13]. Therefore, finding novel therapeutic targets to suppress gluconeogenesis pathway may facilitate the therapy for T2D-induced hyperglycemia.

MicroRNAs (miRNAs, miRs) are a class of non-protein-coding RNAs containing approximately 19-25 nucleotides, and they regulate the expression of target genes by predominantly binding to 3'untranslated regions (3'UTR) of specific target genes, leading to either translational repression or mRNA degradation. Bioinformatic tools reveal that one miRNA may potentially regulate multiple target genes, suggesting the potential function of miRNAs on almost every aspect of cell physiology. Currently, miRNAs are used as important regulators and potential therapeutic targets for treating metabolic diseases [14]. For example, recent studies revealed that miR-29a-c and miR-23a could be linked to the glucose metabolism [15, 16]. Moreover, miR-375 and miR-214 presented important roles in the gluconeogenesis [17-19]. Among all known miRNAs, miR-223 functions as a potential indicator of some immune disorders, and dysregulation of miR-223 has been involved in several inflammatory responses, such as rheumatoid arthritis and type 2 diabetes mellitus. Additionally, miR-223 has been reported have regulatory effects on glucose metabolism. However, the role and molecular mechanism of miR-223 in hepatic gluconeogenesis remain unclear.

In the present study, we used online softwares including TargetScan and miRanda to perform target prediction analysis, and we observed that FOXO1 is a functional target of miR-223. Previous study reported that FOXO1 is an essential element in controls of hepatic gluconeogenesis. Hence, we speculated that miR-223 control hepatic gluconeogenesis partly through regulating FOXO1 and further explored whether miR-223 targets FOXO1 to regulate gluconeogenesis causing the alteration of PCK and G6P expression and glucose generation in the human primary hepatocytes.

Materials and methods

Cell culture and treatments

Primary human hepatocytes (PHHs) were obtained from Clonetics (BioWhittaker Inc., Walkersville, MD, USA) and cultured according to the vendor's instructions. The PHHs were

treated with PKA pathway inhibitor, N-[2-p-bromocinnamylamino ethyl]-5-isoquinolinesulfonamide (H89) at the level of 10 μ M, to measure whether glucagon regulates miR-223 expression through the cAMP/PKA pathway. Moreover, the PHHs were transfected with miR-223 mimics, miR-223 inhibitor, small interfering RNA against FOXO1 (si-FOXO1), pCMV-HA vector containing FOXO1 (HA-FOXO1), and the responding controls by Lipofectamine 2000 (Invitrogen) according to the manufacturer's suggestions.

Glucose output assay

After three washes with PBS, cells were incubated in Krebs-Ringer buffer (pH 7.4) containing 20 mM sodium lactate and 2 mM sodium pyruvate for 5 h. Then the glucose concentration in the culture medium was detected using a glucose production assay kit purchased from Sigma-Aldrich (St Louis, MO, USA). The readings from each sample were normalized to the content of total protein measured from the whole-cell lysates.

Quantitative real-time PCR (qRT-PCR)

Total RNA was obtained from the primary hepatocytes by Trizol reagent (Invitrogen) in accordance with the manufacturer's suggestions. To measure the level of miR-223, the reverse transcription was performed using One Step Prime Script miRNA cDNA Synthesis Kit purchased from Takara (Dalian, China) and qRT-PCR was carried out by TaqMan microRNA assays (Applied Biosystems, Foster City, CA, USA). The endogenous control was U6 snRNA. To quantify mRNA expression of FOXO1, PCK and G6P, the RevertAid first strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania) was used to synthesize cDNAs. The qRT-PCR was carried out by SYBR Green assays (Takara). The indicated gene expression was normalized to β -actin mRNA expression.

Western blot assay

Protein was extracted from the primary hepatocytes by lysis buffer. And then the extraction was measured by the BCA protein assay. Equal amounts of protein were electrophoresed on SDS-polyacrylamide gel electrophoresis SDS-PAGE and then transferred onto nitrocellulose membranes. Next, the sheets were incubated with primary antibodies against FOXO1 (Cell

miRNA inhibits hepatic gluconeogenesis

Signaling Technology, Danvers, MA, USA), phosphorylated PKA (p-PKA) substrate (Cell Signaling Technology), PCK (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and G6P (Abcam, Cambridge, UK). After three washes, the secondary antibodies (Sigma-Aldrich) were added. The blots were detected using enhanced chemiluminescence detection kit (Thermo scientific, Rockford, IL, USA).

Luciferase reporter assay

The 3'UTR sequences of the human FOXO1 mRNA containing putative target sites of miR-223 were designed and provided by GenePharma (Shanghai, China). Then the fragments were inserted into downstream of the firefly luciferase gene in pmirGLO vector containing both firefly luciferase gene and Renilla luciferase (Promega, Madison, WI, USA), which were designated as wild-type FOXO1 3'UTR (WT). The mutated oligonucleotides were also cloned into the same region of the vector, resulting in mutant FOXO1 3'UTR (MUT). Vectors containing 3'UTR of FOXO1 or the mutant one were co-transfected with miR-223 mimics or negative control into the PHHs using Lipofectamine 2000 (Invitrogen). Forty-eight hours later, we collected the cells, and the Dual-Luciferase Reporter Assay System (Promega) was used to detect both firefly and renilla luciferase activities. Firefly/Renilla luminescence represented the activity of firefly luciferase of each sample well.

Statistical analysis

The results were presented as mean \pm standard deviation (SD). Significant difference was assessed by Student's *t*-test or one-way analysis of variance (ANOVA). All statistical analyses were carried out by SPSS 15.0 software (SPSS, Chicago, IL, USA). A *P* value <0.05 was considered statistically significant.

Results

Glucagon suppressed miR-223 expression in primary hepatocytes

To explore the role of miR-223 among the process of gluconeogenesis, we measured the expression of miR-223 in primary hepatocytes treated with glucagon. As illustrated in **Figure 1A**, the glucagon exposure in primary hepato-

cytes time-dependently reduced miR-223 level. Furthermore, the hepatocytes treated with glucagon in the range from 0 to 100 nM showed a concentration-dependent decrease of miR-223 expression (**Figure 1B**). The PCK and G6P mRNAs were also decreased in primary hepatocytes treated with 100 nM glucagon (**Figure 1C** and **1D**). Additionally, to explore the mechanism by which glucagon modulated miR-223 expression in primary hepatocytes, we measured the activation of PKA, a critical component of the PKA signaling. As expected, glucagon obviously reduced the miR-223 level and promoted the level of phosphorylated PKA (p-PKA) substrate, however, 10 μ M of H89 reversed these effects (**Figure 1E** and **1F**).

miR-223 inhibited glucose production in primary hepatocytes

As described above, glucagon could suppress miR-223 expression in primary hepatocytes. Then we explored the effect of miR-223 on gluconeogenesis by loss-and gain-of-function experiments. As demonstrated in **Figure 2A**, the miR-223 mimics significantly increased miR-223 expression in the hepatocytes. Moreover, under the conditions of either basic or glucagon (Glu)+dexamethasone (Dex), miR-223 overexpression markedly restrained mRNA and protein levels of PCK and G6P, as well as glucose production (**Figure 2B-F**). Inversely, the miR-223 inhibitor led to decreased expression of miR-223, in turn enhanced the PCK and G6P expression at both mRNA and protein levels, and promoted glucose generation, under the basic or glucagon+dexamethasone circumstance (**Figure 2G-L**).

FOXO1 was a functional target of miR-223 in primary hepatocytes

To understand the mechanism involved in miR-223-induced impediment of gluconeogenesis, we searched the targets of miR-223. Using the online softwares TargetScan and miRanda, we found 3'UTR of FOXO1 mRNA contained one conserved target site of miR-223 (**Figure 3A**). The possibility of the bioinformatical prediction was evaluated by dual-luciferase reporter assay. The data indicated that the miR-223 markedly inhibited the luciferase activity when the reporter contained the wild-type 3'UTR of FOXO1 (**Figure 3B**). The data confirmed our

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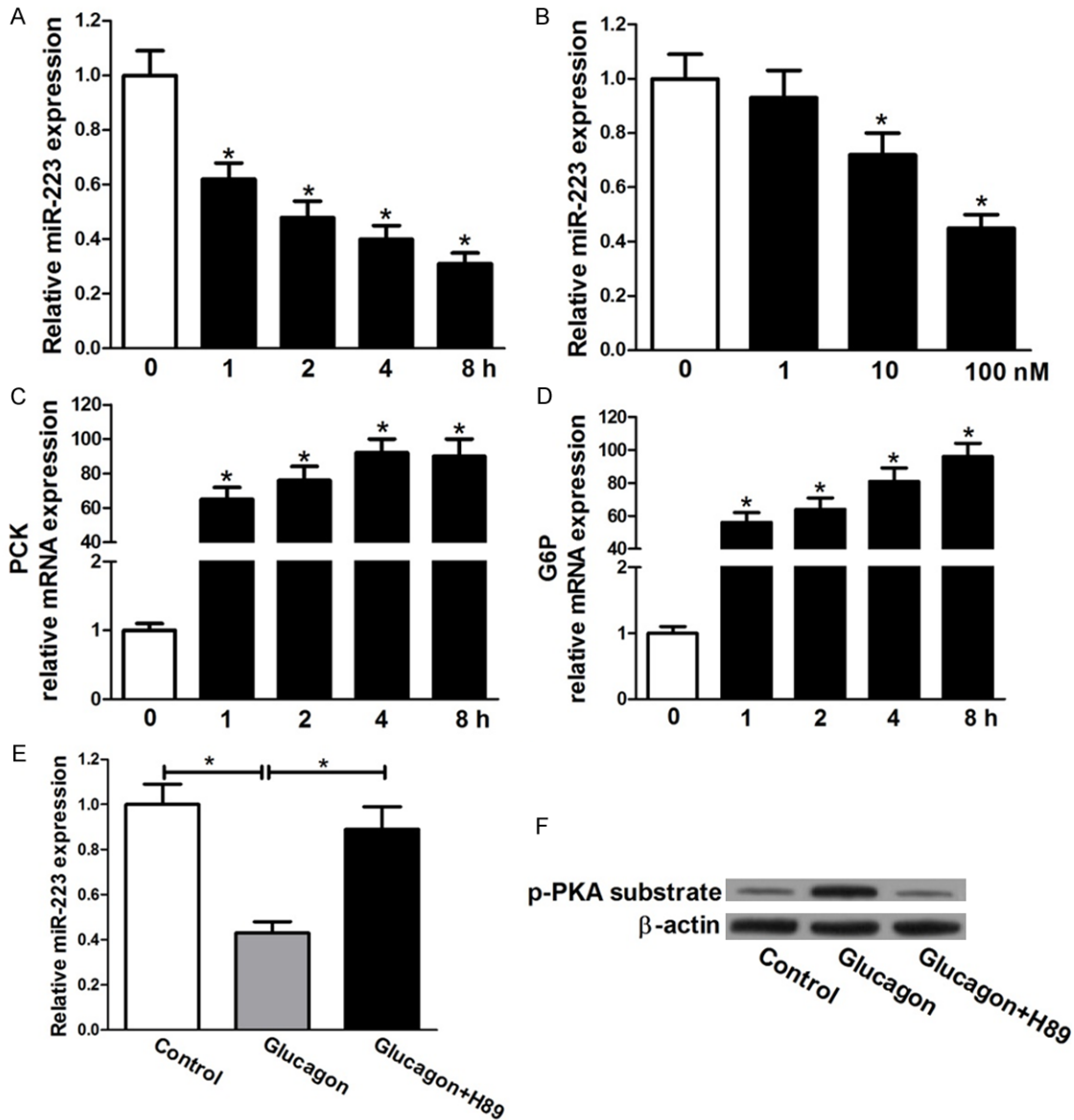


Figure 1. Glucagon suppressed miR-223 expression through protein kinase A (PKA) pathway in primary human hepatocytes (PHHs). A and B. Exposure of PHHs to 100 nM glucagon for 0 to 8 h or 0 to 100 nM glucagon for 8 h significantly reduced miR-223 expression in a dose- and time-dependent manner. C and D. The expression levels of PCK and G6P mRNAs were decreased in primary hepatocytes treated with 100 nM glucagon. E and F. Glucagon obviously restrained miR-223 expression and elevated levels of phosphorylated PKA (p-PKA) substrate, however, these effects were reversed by H89 at a concentration of 10 μ M for 8 h. * $P < 0.05$.

speculation that FOXO1 was a target of miR-223. Then we detected FOXO1 expression at both mRNA and protein levels when the hepatocytes were treated with miR-223 mimics or inhibitors. As exhibited in **Figure 3C** and **3D**, forced expression of miR-223 obviously decreased FOXO1 mRNA and protein expression. Conversely, downregulation of miR-223 obviously increased the FOXO1 expression (**Figure 3E** and **3F**).

FOXO1 mediated the suppressive effects of miR-223 on gluconeogenesis

Given that FOXO1 was one of the targets of miR-223 and FOXO1 expression was controlled by miR-223 in primary hepatocytes, we speculated that miR-223 mediated gluconeogenesis by regulating FOXO1 expression. We transfected PHHs with si-FOXO1, HA-FOXO1 and the responding controls, and detected the mRNA

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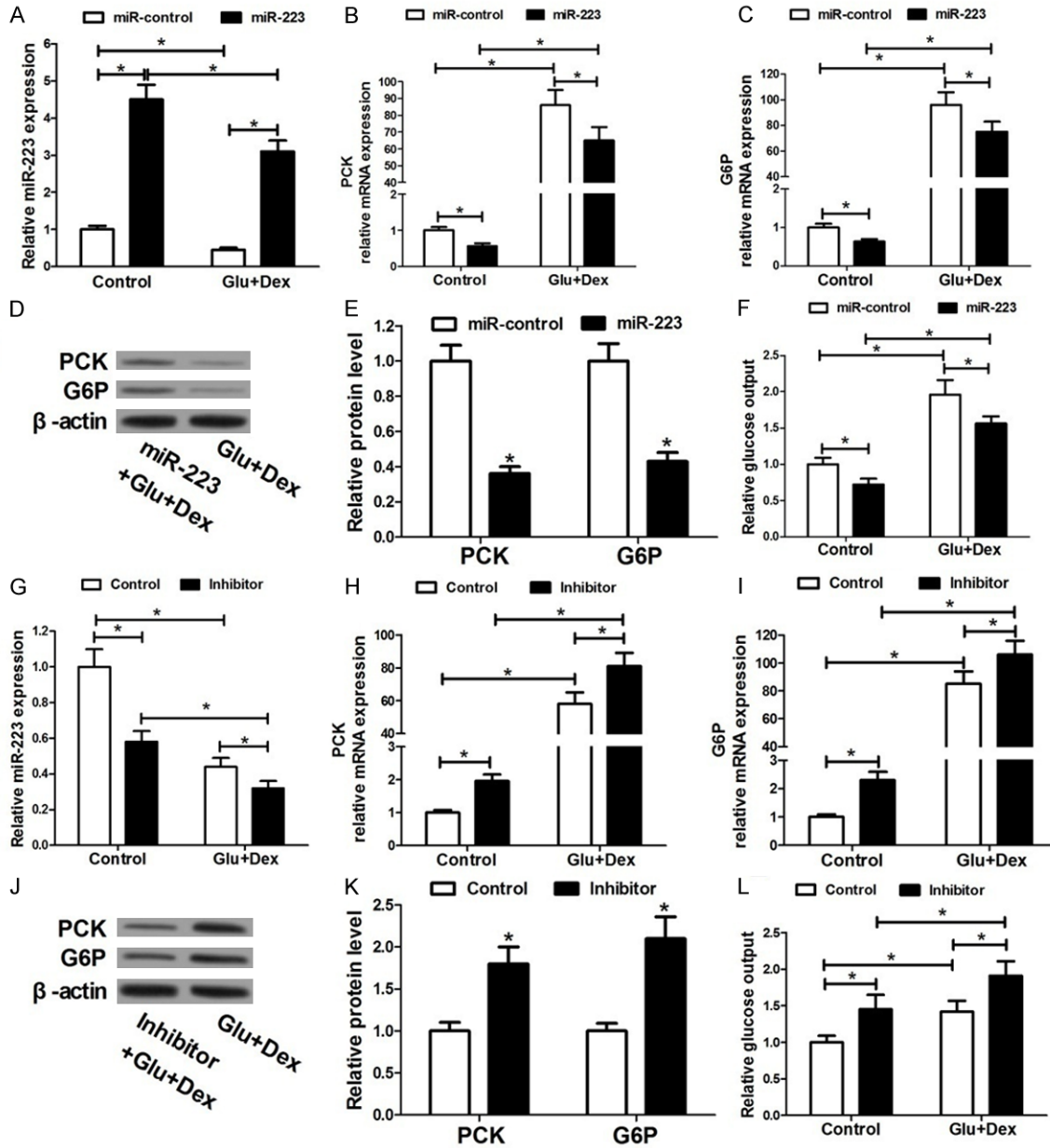


Figure 2. miR-223 inhibited hepatic gluconeogenesis *in vitro*. A. The expression of miR-223 in primary hepatocytes treated with miR-223 mimics for 48 h or/and glucagon (Glu, 100 nM)+dexamethasone (Dex, 1 μ M) for 8 h. B-F. Under the conditions of either basic or glucagon+dexamethasone, miR-223 overexpression markedly restrained mRNA and protein levels of PCK and G6P, as well as glucose production. G-L. The miR-223 inhibitor led to decreased expression of miR-223 and increased expression levels of PCK and G6P mRNA and protein, as well as glucose production, under either basic or glucagon+dexamethasone-stimulated conditions. * $P < 0.05$.

and protein levels of PCK and G6P, as well as glucose production. As predicted, gene silencing of FOXO1 reduced mRNA and protein levels of PCK and G6P, and the glucose production (Figure 4A-C). Furthermore, overexpression of FOXO1, on the contrary, induced higher levels of PCK and G6P expression, and glucose generation in the hepatocytes (Figure 4D-F). To fur-

ther confirm whether miR-223 inhibited gluconeogenesis by directly regulating FOXO1, the primary hepatocytes were co-treated with miR-223 mimics and HA-FOXO1. The results showed that increased expression of FOXO1 significantly overturned the inhibition of PCK and G6P expression and glucose production by miR-223 (Figure 4G-I). Taken together, these data

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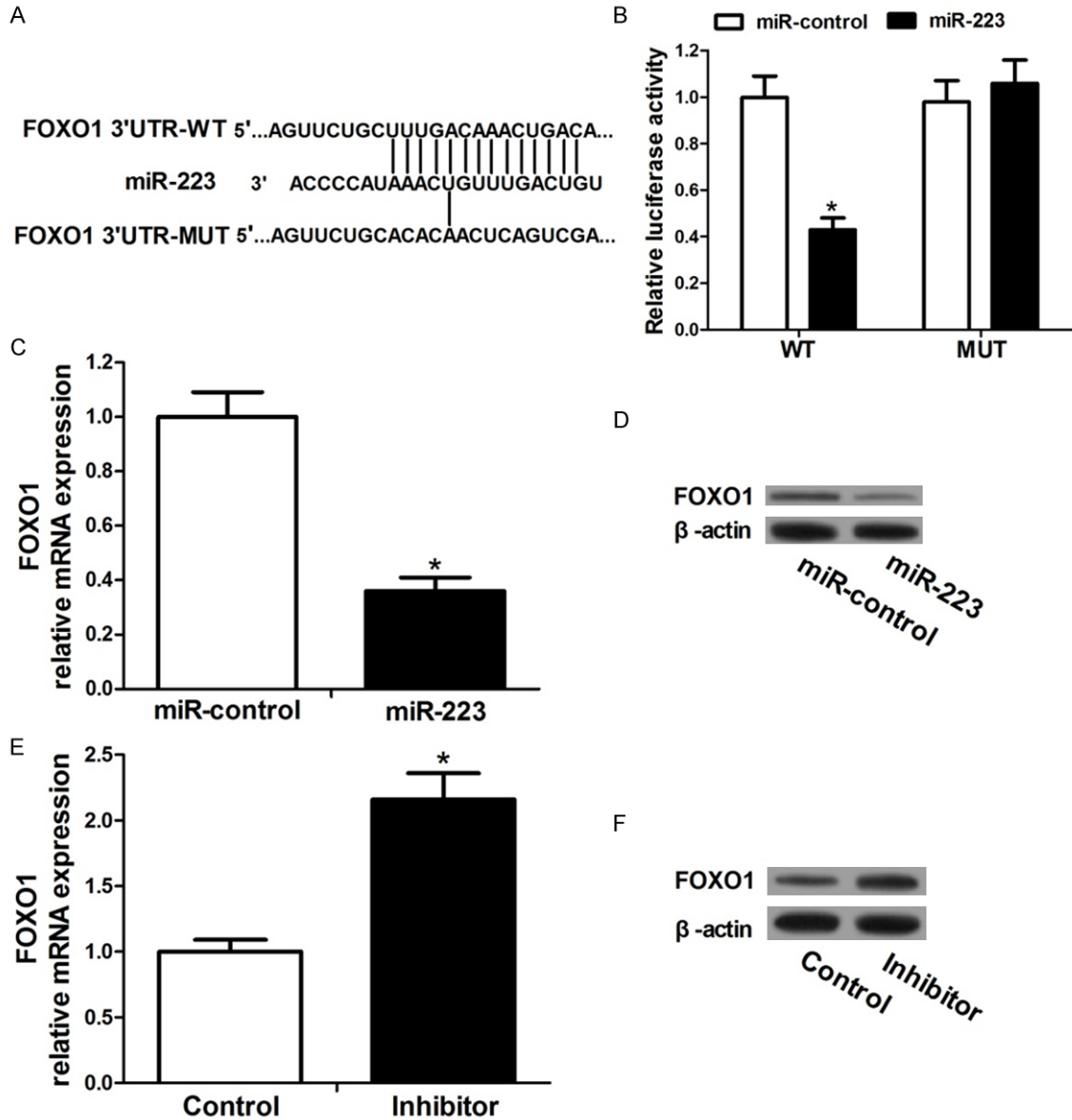


Figure 3. FOXO1 was a target of miR-223 in PHHs. A. Prediction analysis showed that FOXO1 was a target of miR-223 and the putative binding sequence was in the 3'UTR of FOXO1. B. miR-223 significantly inhibited the luciferase activity of the reporter when the reporter contained wild-type 3'UTR of FOXO1 (WT). C and D. Forced expression of miR-223 obviously decreased FOXO1 expression at both mRNA and protein levels. E and F. Downregulation of miR-223 markedly increased the FOXO1 mRNA and protein levels. * $P < 0.05$.

were consistent with our hypothesis and indicated that miR-223 might inhibit gluconeogenesis through targeting FOXO1 in primary hepatocytes.

Discussion

Recently, the transcription regulators involved in glucose metabolism are considered as therapeutic targets to treat T2D [13]. The newly

emerged miRNAs as important regulators of metabolism has garnered much interest [20]. For example, miR-29a-c, miR-214, miR-23a as well as miR-33 play a crucial role in controlling glucose metabolism and are used as important regulators and potential therapeutic targets for treating metabolic diseases [19, 21, 22]. In this work, we observed that miR-223 level was suppressed by glucagon in PHHs. Furthermore, the primary hepatocytes treated with glucagon

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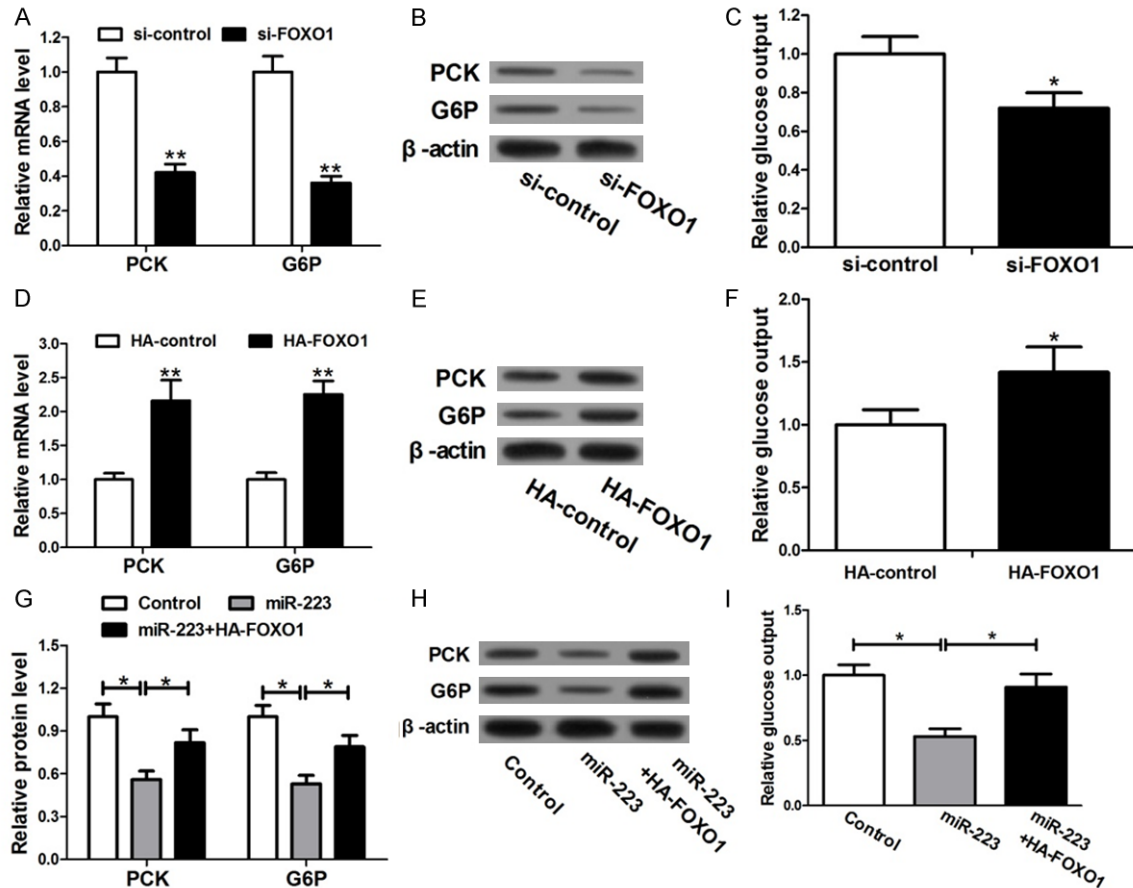


Figure 4. FOXO1 mediated the inhibitory effect of miR-223 on hepatic gluconeogenesis *in vitro*. A-C. The PHHs were transfected with negative control (si-control) or si-FOXO1 for 48 h. Gene silencing of FOXO1 reduced mRNA and protein levels of PCK and G6P, as well as the glucose production in primary hepatocytes. D-F. The PHHs were transfected with negative control (HA-control) or HA-FOXO1 for 48 h. Overexpression of FOXO1 induced higher levels of PCK and G6P mRNA and protein expression, as well as glucose production in primary hepatocytes. G-I. The PHHs were transfected with negative control (Control) or miR-223 mimics (miR-223), or co-transfected with miR-223 mimics and HA-FOXO1 (miR-223+HA-FOXO1) for 48 h. Increased expression of FOXO1 significantly overturned the suppressive effect of miR-223 on PCK and G6P expression, and the glucose production in primary hepatocytes. * $P < 0.05$.

exhibited increased expression of PCK and G6P. These findings suggest that miR-223 may serve as a key regulator of gluconeogenesis in hepatocytes.

Besides, glucagon induced decreased expression of miR-223 and increased expression of phosphorylated PKA (p-PKA) substrate, however, these effects were overturned by H89, a widely used PKA inhibitor [23]. It is reported that glucagon elevates glucose generation through the cAMP/PKA signaling pathway [7]. The interaction between glucagon and its receptor on the hepatocyte plasma membrane results in stimulation of adenylyl cyclase (AC), generation of the cAMP, and activation of PKA, thereby inducing the hepatic glucose output

[24]. Our result indicates that miR-223 expression was, at least partly, regulated by glucagon-PKA signaling pathway.

We next identified the function of miR-223 in hepatic gluconeogenesis by applying gain- and loss-of-function approaches in primary hepatocytes. The results indicated that the forced expression of miR-223 hampered PCK and G6P expression, and glucose generation in hepatocytes, whereas, glucagon reversed this negative effects of miR-223 on hepatic gluconeogenesis. In addition, we observed that reduced miR-223 expression resulted in increased expression of PCK and G6P, and the glucose production in primary hepatocytes. These results revealed the pivotal role of miR-223 in

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controlling hepatic gluconeogenesis in primary hepatocytes.

Furthermore, we explored the mechanisms by which miR-223 regulated the hepatic gluconeogenesis. The prediction analysis from TargetScan and miRanda tools showed that FOXO1 was a potential target of miR-223. FOXO1 belongs to the FOXO family of forkhead transcription factors, which is crucial in regulating the effects of insulin on G6Pase gene expression [25]. The activity of FOXO1 needs its accumulation in the nucleus, which is crucial in regulating the downstream molecule genes. The unphosphorylated form of FOXO1 can identify the insulin response element (IRE) in the promoter regions of multiple target genes, including the PCK and G6Pase genes, and activates their transcription [26, 27]. Moreover, miR-223 could downregulate the expression of FOXO1 to inhibit cell proliferation in various cancer cell lines [28]. Based on the above results, we hypothesized that miR-223 might target FOXO1 to regulate hepatic gluconeogenesis. Our experimental results presented that the change of miR-223 level could regulate the expression of FOXO1 and the effect of FOXO1 on PCK and G6P expression, and the glucose production was consistent with the role of miR-223 mimics or miR-223 inhibitor in hepatic gluconeogenesis. Moreover, the inhibitory effect of miR-223 on hepatic gluconeogenesis was relieved by addition of FOXO1. These findings suggested that miR-223 targeted FOXO1 to inhibit PCK and G6P expression, and the glucose production in hepatocytes. And the data supported our hypothesis that miR-223 partly regulated FOXO1 expression to inhibit hepatic gluconeogenesis.

In all, our work suggested a key role of miR-223 in controlling gluconeogenesis by regulating FOXO1 expression in PHHs. We provided evidence to verify that FOXO1 was a functional target of miR-223, and miR-223 inhibits PCK and G6P expression, as well as glucose production at least in part by targeting FOXO1. These findings offer a therapeutic target to treat hyperglycemia in T2D.

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Disclosure of conflict of interest

None.

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References

- [1] Nordlie RC, Foster JD and Lange AJ. Regulation of glucose production by the liver. *Annu Rev Nutr* 1999; 19: 379-406.
- [2] Pilkis S and Claus T. Hepatic gluconeogenesis/glycolysis: regulation and structure/function relationships of substrate cycle enzymes. *Annu Rev Nutr* 1991; 11: 465-515.
- [3] Zhang Y, Xu D, Huang H, Chen S, Wang L, Zhu L, Jiang X, Ruan X, Luo X, Cao P, Liu W, Pan Y, Wang Z and Chen Y. Regulation of glucose homeostasis and lipid metabolism by PPP1R3G-mediated hepatic glycogenesis. *Mol Endocrinol* 2014; 28: 116-126.
- [4] Kolnes AJ, Birk JB, Eilertsen E, Stuenkel JT, Wojtaszewski JF and Jensen J. Epinephrine-stimulated glycogen breakdown activates glycogen synthase and increases insulin-stimulated glucose uptake in epitrochlearis muscles. *Am J Physiol Endocrinol Metab* 2015; 308: E231-240.
- [5] Marcelo KL, Means AR and York B. The Ca²⁺/Calmodulin/CaMKK2 Axis: Nature's Metabolic CaMshaft. *Trends Endocrinol Metab* 2016; 27: 706-18.
- [6] Mosseri R, Waner T, Shefi M, Shafir E and Meyerovitch J. Gluconeogenesis in non-obese diabetic (NOD) mice: in vivo effects of vanadate treatment on hepatic glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. *Metabolism* 2000; 49: 321-325.
- [7] Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C and Puigserver P. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 2001; 413: 179-183.
- [8] Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H and Accili D. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1 α interaction. *Nature* 2003; 423: 550-555.
- [9] He L, Sabet A, Djedjos S, Miller R, Sun X, Husain MA, Radovick S and Wondisford FE. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell* 2009; 137: 635-646.
- [10] Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J and Gerich J. Role of reduced suppression of glucose production and

miRNA inhibits hepatic gluconeogenesis

- diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 1992; 326: 22-29.
- [11] Wajngot A, Chandramouli V, Schumann WC, Ekberg K, Jones PK, Efendic S and Landau BR. Quantitative contributions of gluconeogenesis to glucose production during fasting in type 2 diabetes mellitus. *Metabolism* 2001; 50: 47-52.
- [12] Pei L, Waki H, Vaitheesvaran B, Wilpitz DC, Kurland IJ and Tontonoz P. NR4A orphan nuclear receptors are transcriptional regulators of hepatic glucose metabolism. *Nat Med* 2006; 12: 1048-1055.
- [13] Oh KJ, Han HS, Kim MJ and Koo SH. Transcriptional regulators of hepatic gluconeogenesis. *Arch Pharm Res* 2013; 36: 189-200.
- [14] Lynn FC. Meta-regulation: microRNA regulation of glucose and lipid metabolism. *Trends Endocrinol Metab* 2009; 20: 452-459.
- [15] Wang B, Hsu SH, Frankel W, Ghoshal K and Jacob ST. Stat3-mediated activation of microRNA-23a suppresses gluconeogenesis in hepatocellular carcinoma by down-regulating Glucose-6-phosphatase and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha. *Hepatology* 2012; 56: 186-197.
- [16] Liang J, Liu C, Qiao A, Cui Y, Zhang H, Cui A, Zhang S, Yang Y, Xiao X and Chen Y. MicroRNA-29a-c decrease fasting blood glucose levels by negatively regulating hepatic gluconeogenesis. *J Hepatol* 2013; 58: 535-542.
- [17] Li X. MiR-375, a microRNA related to diabetes. *Gene* 2014; 533: 1-4.
- [18] El Ouaamari A, Baroukh N, Martens GA, Lebrun P, Pipeleers D and Van Obberghen E. miR-375 targets 3'-phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biological responses in pancreatic β -cells. *Diabetes* 2008; 57: 2708-2717.
- [19] Li K, Zhang J, Yu J, Liu B, Guo Y, Deng J, Chen S, Wang C and Guo F. MicroRNA-214 suppresses gluconeogenesis by targeting activating transcriptional factor 4. *J Biol Chem* 2015; 290: 185-195.
- [20] Rottiers V and Näär AM. MicroRNAs in metabolism and metabolic disorders. *Nat Rev Mol Cell Biol* 2012; 13: 239-250.
- [21] Tomasetti M, Amati M, Santarelli L and Neuzil J. MicroRNA in Metabolic Re-Programming and Their Role in Tumorigenesis. *Int J Mol Sci* 2016; 17: 754.
- [22] Karolina DS, Armugam A, Sepramaniam S and Jeyaseelan K. miRNAs and diabetes mellitus. *Expert Rev Endocrinol Metab* 2014; 7: 281-300.
- [23] He J, Wang C, Sun Y, Lu B, Cui J, Dong N, Zhang M, Liu Y and Yu B. Exendin-4 protects bone marrow-derived mesenchymal stem cells against oxygen/glucose and serum deprivation-induced apoptosis through the activation of the cAMP/PKA signaling pathway and the attenuation of ER stress. *Int J Mol Med* 2016; 37: 889-900.
- [24] Miller RA, Chu Q, Xie J, Foretz M, Viollet B and Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature* 2013; 494: 256-260.
- [25] Hirota K, Sakamaki JI, Ishida J, Shimamoto Y, Nishihara S, Kodama N, Ohta K, Yamamoto M, Tanimoto K and Fukamizu A. A combination of HNF-4 and Foxo1 is required for reciprocal transcriptional regulation of glucokinase and glucose-6-phosphatase genes in response to fasting and feeding. *J Biol Chem* 2008; 283: 32432-32441.
- [26] Schmoll D, Walker KS, Alessi DR, Grempler R, Burchell A, Guo S, Walther R and Unterman TG. Regulation of Glucose-6-phosphatase Gene expression by protein kinase B α and the forkhead transcription factor FKHR. Evidence for insulin response unit-dependent and-independent effects of insulin on promoter activity. *J Biol Chem* 2000; 275: 36324-36333.
- [27] Hall RK, Yamasaki T, Kucera T, Waltner-Law M, O'Brien R and Granner DK. Regulation of Phosphoenolpyruvate Carboxykinase and Insulin-like Growth Factor-binding Protein-1 Gene Expression by Insulin THE ROLE OF WINGED HELIX/FORKHEAD PROTEINS. *J Biol Chem* 2000; 275: 30169-30175.
- [28] Wu L, Li H, Jia CY, Cheng W, Yu M, Peng M, Zhu Y, Zhao Q, Dong YW and Shao K. MicroRNA-223 regulates FOXO1 expression and cell proliferation. *FEBS Lett* 2012; 586: 1038-1043.