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Micro-RNA-124-5p promotes insulin producing cell differentiation through regulating transcriptional factor NKX6.1

Xianjun Zhang [a,1], Shunzi Shao [c,1], Xijiang Zhao [a], Meng Zhang [a,**], Jingbo Wang [b,*]

[a] Department of Orthopedics, The Affiliated Hospital of Jiangnan University, Wuxi, Jiangsu, 214122, China
[b] College of Pharmacy, Shenzhen Technology University, Shenzhen, 518000, China
[c] Department of Gastroenterology, The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, Jiangsu, 211100, China

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ABSTRACT

Aims: Differentiating human embryonic stem cells into pancreatic β cells has been proposed as a practical approach to managing diabetes. There have been several protocols attempting to generate β-like cells or insulin-producing cells (IPCs), but their low efficiency is a common issue. The expression level of Nkx6.1 is crucial for maintaining pancreatic β cell identity, while the proportion of PDX1 and Nkx6.1 double positive cells were not satisfied in the present protocols, leading to relative low efficiency in the differentiation into IPCs. This study aims to identify the mechanism underlying the regulation of Nkx6.1 during IPC differentiation and provide new insights for diabetes therapy.

Methods: In the current study, human embryonic stem cell (hESC) line H1 was used to perform IPC specifications. Immunofluorescence, flow cytometry, and qPCR were conducted to analyze gene expression. In addition, insulin and C-peptide were measured through glucose-stimulated insulin secretion (GSIS) assays and ELISA.

Results: We found that the transcription factor NKX6.1, a crucial inducer of early pancreatic development and IPC generation, was downregulated by micro-RNA-124-5p (miR-124-5p) in hESCs during IPC differentiation. Also, we observed that miR-124-5p was upregulated and bound to the 3' untranslated region (3' UTR) of NKX6.1 in pancreatic progenitor (PP), which subsequently suppressed PP differentiation. Moreover, inhibiting miR-124-5p induced the generation of IPCs.

Conclusion: The current study results demonstrated an important role for miR-124-5p in regulating NKX6.1 expression, which appears to be a practical strategy for producing IPCs.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease caused by the failure of blood glucose regulation, which mainly causes severe hyperglycemia, leading to tissue or organ damage and even death [1,2]. Insulin therapy is one of the main clinical strategies to maintain blood glucose homeostasis. However, insulin therapy cannot delay diabetes progression [3,4]. Islet transplantation based on Edmonton protocol has benefited many diabetic patients [5,6]. However, due to the shortage of islet donors, progress in islet transplantation has recently been extremely limited. Xenotransplantation of islets is an alternative strategy to investigate new sources of islets [7]. However, strong xenograft rejection and the risk of xenoantigens would lead to various potential safety problems. Therefore, it is fundamental to find alternative resources of pancreatic β cells for diabetes treatment.

Human embryonic stem cells (hESCs) derived from human blastocysts can proliferate indefinitely with the capacity to differentiate into any other cell types in the body, including pancreatic β cells [8,9]. After definitive endoderm (DE) induction, hESCs could further differentiate into a primitive gut tube, postforegut endoderm (PE), pancreatic progenitor (PP), and then insulin-producing cells (IPCs) in vitro [10]. Although a series of optimized protocols for preparing IPCs or β-like cells have been proposed [10–13], the low differentiation efficiency of hESCs into IPCs has not been significantly improved. One of the key difficulties is the inability to produce a sufficient proportion of PP during hESCs differentiation. In addition to transcription factors PDX1 and Ngn3,

* Corresponding author.
** Corresponding author. The Affiliated Hospital of Jiangnan University, Wuxi, Jiangsu, 214122, China.
E-mail addresses: bh4tat@163.com (M. Zhang), wangjingbo@sztu.edu.cn (J. Wang).
[1] These authors contribute equally to this work.

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NKX6.1 plays a critical function in early pancreatic development [14]. NKX6.1 is expressed after PDX1 induction in PP stage, and its expression in PDX1 positive-expressing cells determines islet cell fate [15–17]. Additionally, NKX6.1 is crucial for maintaining pancreatic β cell identity in vivo, and enhanced NKX6.1 expression levels result in increased glucose-stimulated insulin secretion (GSIS) [18]. However, under the present induction protocols, the proportion of PDX1 and NKX6.1 double-positive PP cells is very low, resulting in relatively low differentiation efficiency into IPCs.

MicroRNAs (miRNAs) are 18–24 nt RNAs, which are post-transcriptional factors via regulating target mRNA stability or translation [19–21]. miRNAs play important roles in numerous physiological processes, including pancreatic development [22]. miR-375, a highly expressed miRNA in islets, is essential for normal glucose homeostasis [23]. Induction of miR-375 expression exogenously in β cells of miR-375 knockout mice maintains β cell phenotypes [24]. Besides, miR-26a can regulate β cell insulin secretion and affect peripheral insulin sensitivity in a non-cell-autonomous manner through exosomes [25]. Although NKX6.1 was identified as a crucial transcription factor during pancreatic development and β cell function, NKX6.1 regulation by miRNAs has received little attention.

This study aimed to identify miRNAs that regulate NKX6.1 expression. We found miR-142-5p was upregulated during the differentiation of hESCs into IPCs. It has been reported that miR-124-5p participate in inflammatory pathways and regulate macrophage phagocytosis as well as angiogenesis [26]. MiR-124-5p could inhibit the growth of high-grade gliomas through posttranscriptional regulation of related genes [27]. However, the role and molecular mechanisms of miR-124-5p during IPC differentiation is poorly confirmed and its regulatory effect on NKX6.1 has not been studied. But previous research confirmed that miR-124-5p can decrease the expression of α-ENaC in alveolar epithelial cells by targeting the 3'UTR [28]. In the present study, we found that miR-124-5p regulates Nkx6.1 expression through targeting its 3'UTR. And remarkably, miR-142-5p inhibition significantly promoted IPC production and insulin secretion, indicating that miR-142-5p would be a target for efficient β-cell generation and diabetes treatment.

2. Materials and methods

2.1. hESCs culture and IPC specification

Human embryonic stem cell (hESC) line H1 and H9 was purchased from the Cell Bank of the Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences. hESCs were cultured in f mTeSR™1 (STEMCELL Technologies, #85850) and passaged every 5–6 days using ReLeSR (STEMCELL Technologies, #05873).

IPC specification was performed using a previously mentioned protocol [10]. Briefly, H1 and H9 cells were cultured at 6×10^5 cells/mL in mTeSR1 media with 10 μM Y27632 (Selleck, S1049). The induction of IPC specification was started on the third day after seeding, and the media were changed daily, as illustrated in Supplementary Table 1.

2.2. Luciferase assay

To construct the luciferase expression system, NKX6.1 3'UTR (containing miR-124-5p binding site or mutant [miR-124-5p binding site ACUUUAU to ACAAAUU or UGAAAUA]) was cloned after the CDS of the luciferase gene. HEK293 cells in 12-well plate were transfected with the luciferase expression plasmid (0.5–1 μg) and/or miR-124-5p mimics (100–200 nM) by using Lipofectamine 3000 (Thermo, L3000015) according to the manufacturer's instructions for 24 h. The next day, HEK293 cells were harvested to determine luciferase activity. Luciferase activity was analyzed by Dual-Luciferase Reporter Assay System (Promega, E1910). NKX6.1 3'UTR sequence is listed in Supplementary Table 1.

The miR-7116-5p mimics (0.2–1 nM in BV2 cells and 100 nM for luciferase assays in HEK293 cells) and siRNAs (100 nM) were introduced into the cells by using siIMPORTER (Invitrogen, 64–101) according to the manufacturer's instructions.

2.3. Immunofluorescence

We followed the methods of Qian He et al. [29]. Briefly, the cells were crosslinked by 4–8% paraformaldehyde for 15 min after washing twice with 0.1 mM phosphate-buffered saline (PBS). The cells were then incubated with 5% BSA and 0.1% Triton X-100 in PBS for 1 h. Subsequently, cells were incubated with primary antibodies (anti-SOX17 Abcam ab84990 1:1000, anti-FOXA2 R&D AF2400 1:500, anti-NKX6.1 Abcam ab221549 1:1000, anti-insulin Abcam ab181547 1:500, and anti-PDX1 ab84987 Abcam 1:1000) at 4 °C overnight. The next day, the slices were incubated with the secondary antibody (Thermo A32731 A32728 1:1000) conjugated with a fluorophore at room temperature for 2 h after washing with PBS. The nucleus was then stained with 4,6-diamidino-2-phenylindole (DAPI, Thermo, 62248).

2.4. Flow cytometry

We followed the methods of Qian He et al. [29]. Briefly, the cells were harvested and dissociated into single cells. After centrifuging at 400 g for 3 min, cells were incubated with 4–8% paraformaldehyde for 15 min at room temperature. Sequentially, the diluted BD Perm/Wash™ Buffer was used to wash the cells twice. And then the cells were incubated with 5% BSA and 0.1% Triton X-100 in PBS for half an hour. After washing twice with PBS, antibodies of protein (SOX17-APC IC1924A R&D, FOXA2-488 R&D IC2400G, NKX6.1-647 BD pharmingen 563338, insulin-APC R&D IC1417A, or PDX1-488 R&D IC2419G) or isotypes (R&D IC108A IC108G IC006A IC0041G IC002R) were then added for staining. The flow cytometry was then performed to analyze the 488 and APC/647 population.

2.5. RNA isolation, reverse transcription and qPCR

Total RNA was extracted from harvested cells using Trizol (Thermo 15596026). cDNA was then synthesized with TIANScript II cDNA by manufacturer's protocol (TIANGEN, KR104). The expression levels of genes were normalized by β-actin and quantified by quantitative real-time PCR (qPCR) using SuperReal PreMix (TIANGEN, FP204). Expression levels of miR-124-5p and its precursor were conducted using TaqMan miRNA assays (Thermo, 4440888). Primers are listed in Supplementary Table 1.

2.6. Glucose stimulated insulin secretion (GSIS) assays

IPCs were harvested and washed twice with Krebs buffer (Sigma-Aldrich, K4002). Following that, cells were incubated at 2.8 mM glucose for 1 h and subsequently in 16.7 mM glucose. The supernatant was collected and centrifuged to remove cell pellet. Insulin and C-peptide in the supernatant were analyzed using an ELISA kit (R&D, DINS00 DICP00).

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 to evaluate differences between different groups. All data were presented as mean ± SEM. Comparisons between groups for statistical significance were performed with Student's t-test or analysis of variance (ANOVA) with Tukey (one-way ANOVA) or Bonferroni (two-way ANOVA) post hoc test in multiple groups. The results were considered significantly different at $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $\#P < 0.05$; $\#\#P < 0.01$; $\#\#\#P < 0.001$, respectively.

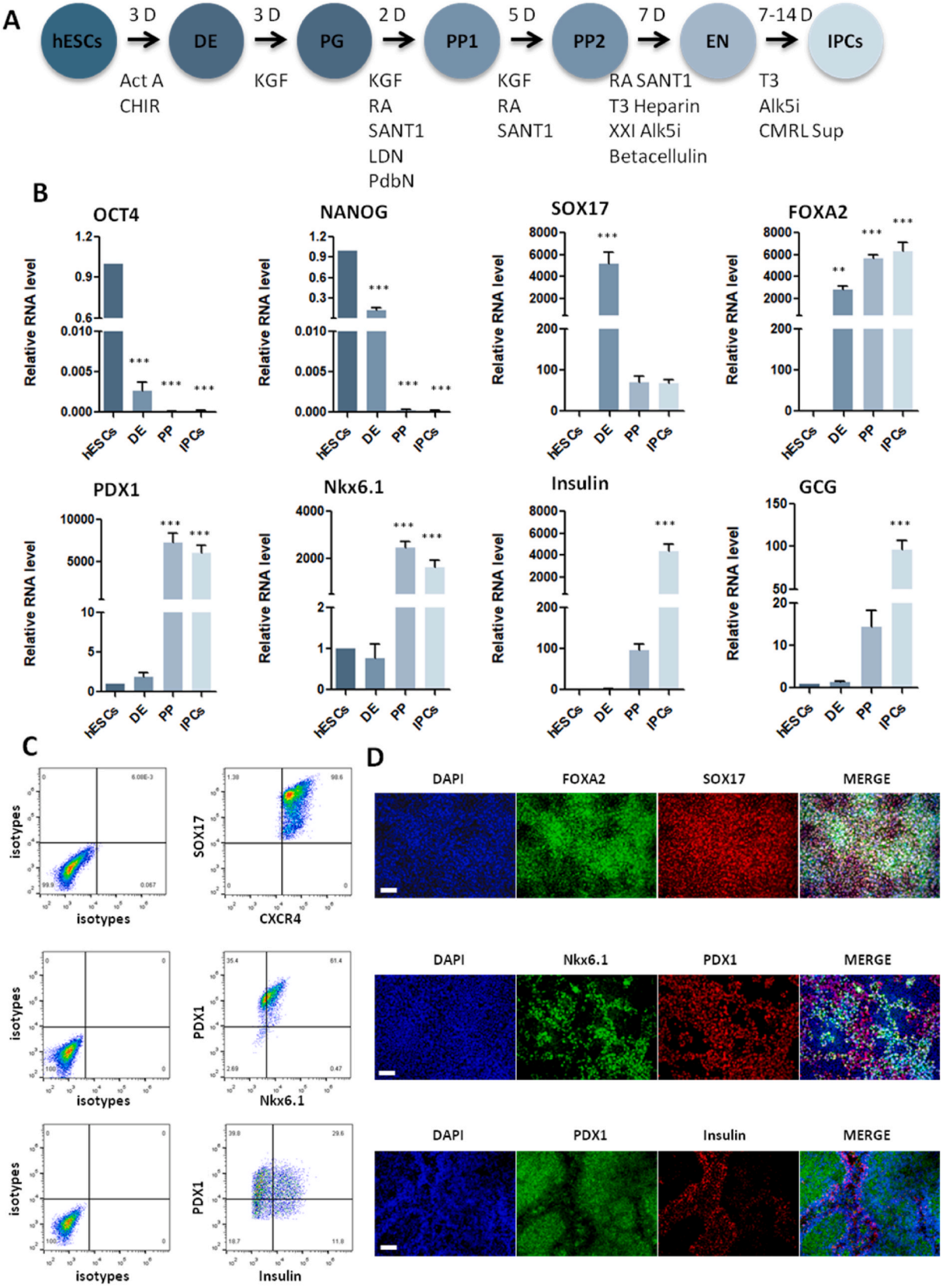

Fig. 1. Differentiation of IPC from hESCs. A. Differentiation protocol for IPC specification from hESCs. DE, definitive endoderm; PG, primitive gut tube; PP, pancreatic progenitor; EN, endocrine cells; IPCs, insulin-producing cells. B, qPCR analysis of the marker gene expression at hESCs, DE, PP, and IPCs stages. C, flow cytometry analysis of SOX17+/CXCR4+, PDX1+/NKX6.1+, and PDX1+/insulin+ populations at DE, PP and IPCs stages. D, representative immunofluorescence images staining with antibodies against SOX17, FOXA2, PDX1, NKX6.1, and insulin. DAPI serves as a nucleus indicator. Scale bar, 200 μm. All data were presented as mean ± SEM. Comparisons between groups for statistical significance were performed using one-way ANOVA with Tukey's post hoc test. **P < 0.01, ***P < 0.001 versus hESCs.

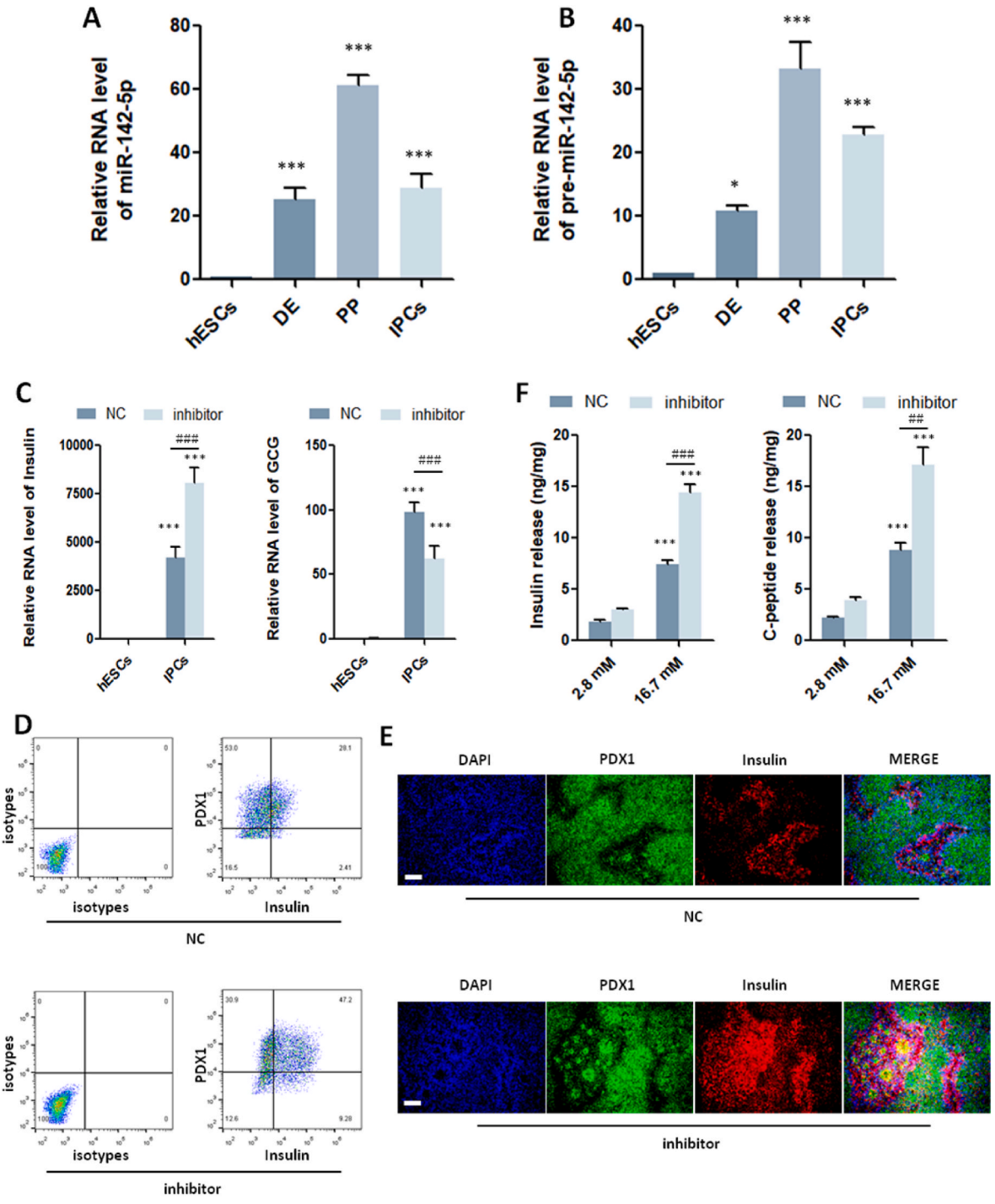

Fig. 2. miR-124-5p regulates the specification of IPCs from hESCs. A. qPCR analysis of miR-124-5p expression at hESCs, DE, PP, and IPCs stages. B, qPCR analysis of pre-miR-124-5p expression at hESCs, DE, PP, and IPCs stages. C. qPCR analysis of insulin and GCG expression at IPCs stage after administration of miR-124-5p inhibitor. D, flow cytometry analysis of PDX1+/insulin+ population at IPCs stage after administration of miR-124-5p inhibitor. E, representative immunofluorescence images staining with antibodies against PDX1 and insulin. DAPI serves as a nucleus indicator. Scale bar, 200 μm. F, glucose-stimulated insulin secretion assays showed insulin and c-peptide release at IPCs stage after administration of miR-124-5p inhibitor. DE, definitive endoderm; PP, pancreatic progenitor; IPCs, insulin-producing cells. All data were presented as mean ± SEM. Comparisons between groups for statistical significance were performed using one-way ANOVA with Tukey's post hoc test. *P < 0.05,**P < 0.01, ***P < 0.001 versus hESCs or hESCs-NC. ##P < 0.01, ###P < 0.001 versus IPCs-NC.

3. Results

3.1. Generation of IPCs from hESCs

To investigate the potential regulator of NKX6.1 during differentiation of human embryonic stem cells (hESCs) to insulin-producing cells (IPCs), we followed the established protocol for IPC generation from definitive endoderm (DE), primitive gut (PG), pancreatic progenitor (PP) and then to IPCs [10] (Fig. 1A). As illustrated in Fig. 1B, quantitative PCR (qPCR) analysis demonstrated that self-renewal transcriptional factors, OCT4 and NANOG, were highly expressed in hESCs, whereas the level of SOX17, as well as FOXA2, was substantially

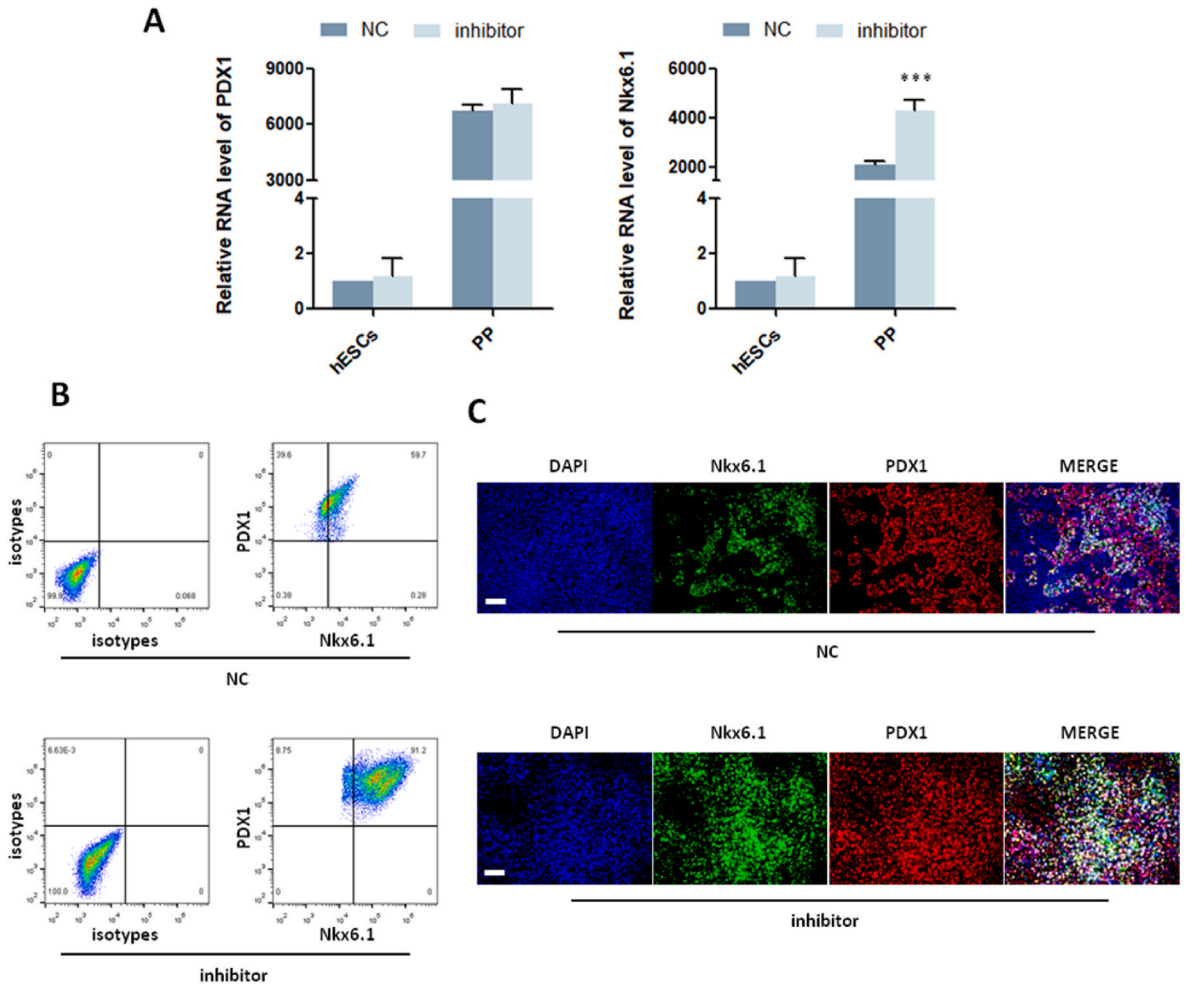

Fig. 3. The effects of miR-124-5p on the regulation of PP differentiation. A. qPCR analysis of PDX1 and NKX6.1 expression at PP stage after administration of miR-124-5p inhibitor. B, flow cytometry analysis of the PDX1+/NKX6.1+ population at PP stages after administration of miR-124-5p inhibitor. C, representative immunofluorescence images staining with antibodies against PDX1 and NKX6.1. DAPI serves as a nucleus indicator. Scale bar, 200 μm. PP, pancreatic progenitor. All data were presented as mean ± SEM. Comparisons between groups for statistical significance were performed using two-way ANOVA with Bonferroni post hoc test. ***P < 0.001 versus PP-NC.

upregulated in DE. Additionally, PDX1 and NKX6.1 expression was strongly enhanced during PP differentiation. Furthermore, insulin was significantly expressed in IPCs stage, whereas α cell marker, glucagon (GCG) was slightly expressed (Fig. 1B). To further assess the protocol for IPC generation, we conducted flow cytometry and immunofluorescence. $SOX17^+/CXCR4^+$ population was denoted as DE cells, and $PDX1^+/NKX6.1^+$, $PDX1^+/insulin^+$ were referred to as PP and IPC cells, respectively. DE, PP and IPC populations reached up to 99.6%, 61.4% and 29.6%, respectively (Fig. 1C). Consistent with that, the fluorescence intensities of SOX17/FOXA2, PDX1/NKX6.1, and PDX1/insulin were evident in DE, PP, and IPC cells, respectively (Fig. 1D). Consistent with qPCR results, these results indicate that IPC generation protocol in the current study recapitulates crucial developmental stages in pancreatic β cell differentiation.

3.2. *miR-124-5p is upregulated during the differentiation of IPCs from hESCs*

We subsequently measured the expression level of miRNAs during differentiation of IPCs from hESCs. As displayed in Fig. 2A and Fig. S1A, the mature miR-124-5p level was gradually upregulated. miR-124-5p expression was found to be low in hESCs, but greatly increased in DE and peaked in PP stage. Although miR-124-5p expression was down-regulated in IPCs compared to PP, it remained significantly greater than in hESCs (Fig. 2A). To further validate the expression level of miR-124-5p, we assess the pre-form of miR-124-5p in different differentiation stages. Consistently, the expression pattern of pre-miR-124-5p was similar to that of the mature form (Fig. 2B, Fig. S1B).

3.3. *Inhibition of miR-124-5p promotes the specification of IPCs from hESCs*

To investigate the function of miR-124-5p, hESCs were treated with miR-124-5p inhibitor during differentiation into IPCs. qPCR analysis suggested that the level of insulin mRNA expression was remarkably upregulated after miR-124-5p inhibitor administration, while the α cell marker, GCG, was significantly decreased (Fig. 2C). While in H9 cell line, ELISA results demonstrated that inhibition of miR-124-5p significantly enhance the secretion of insulin and C-peptide in IPCs (Fig. S1C). These results indicate that inhibiting miR-124-5p probably promoted the specification of IPCs; nevertheless, it suppressed the specification of α like cells. To further validate miR-124-5p function, we performed flow cytometry and immunofluorescence. As illustrated in Fig. 2D, $PDX1^+/insulin^+$ population was increased from 28.1% to 47.2% after miR-124-5p inhibitor administration. Additionally, the fluorescence intensity of PDX1/insulin was substantially enhanced (Fig. 2E). Moreover, cells treated with miR-124-5p inhibitor were subjected to glucose-stimulated insulin secretion (GSIS) experiments to assess their ability to respond to

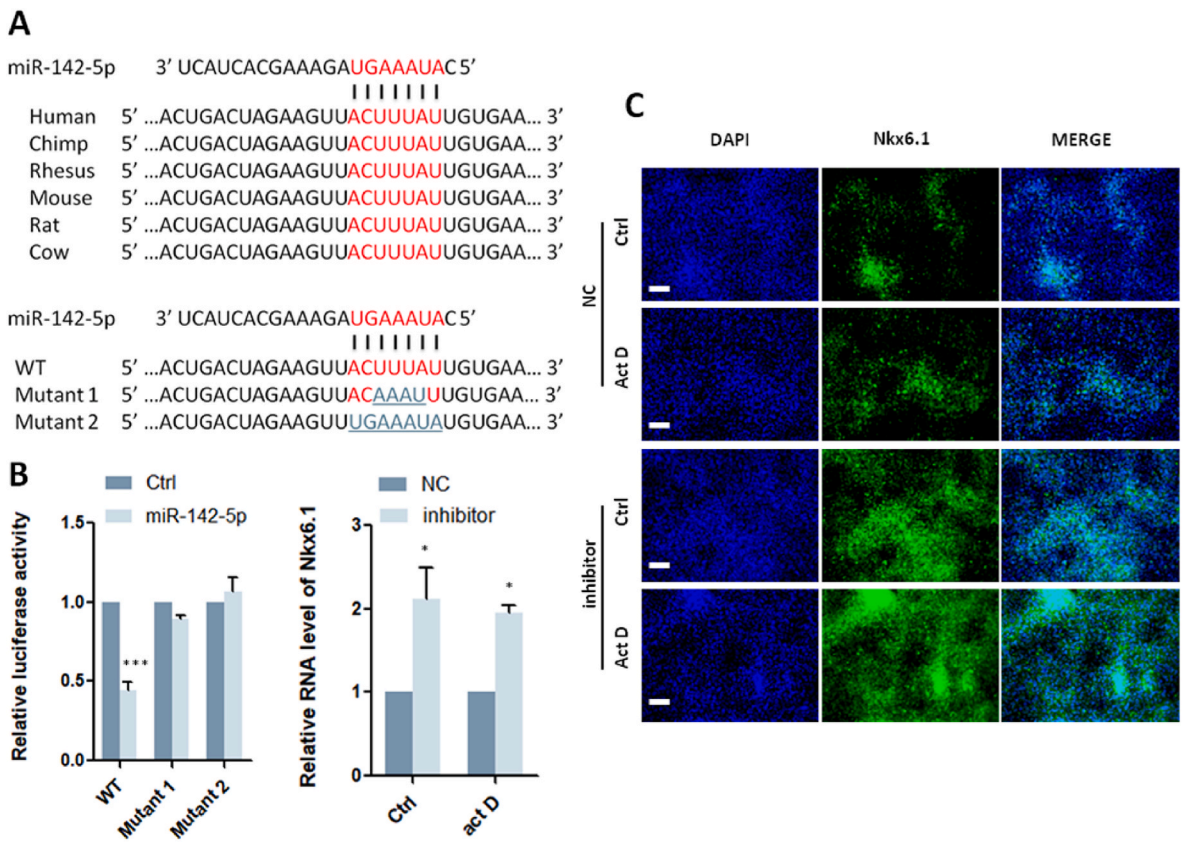

Fig. 4. miR-124-5p regulates NKX6.1 expression. A. the predicted binding region of miR-124-5p in the 3'UTR of NKX6.1 of different species (up) and the design of mutants of NKX6.1 for luciferase assays (bottom). B, luciferase activity analysis in HEK293 cells transfected with miR-124-5p and wild type/mutant 3'UTR of NKX6.1. C, representative immunofluorescence images staining with antibodies against NKX6.1. DAPI serves as a nucleus indicator. Scale bar, 200 μm. PP, pancreatic progenitor; WT, wild type; ActD, Dactinomycin. All data were presented as mean ± SEM. Comparisons between groups for statistical significance were performed using one-way ANOVA with Tukey's post hoc test. ***P < 0.001 versus WT-Ctrl.

different glucose concentrations. After administration of miR-124-5p inhibitor, a significant increase of both insulin and C-peptide release was observed in IPCs challenged with 16.7 mM glucose (Fig. 2F). Together, the results above demonstrate that miR-124-5p inhibition promotes the specification of IPCs from hESCs.

3.4. Inhibition of miR-124-5p does not affect DE differentiation

To elucidate the mechanism by which miR-124-5p regulates the specification of IPCs from hESCs, DE differentiation efficiency was detected after treatment with miR-124-5p inhibitor, which was 2 '-methoxy modified RNA oligonucleic acid that targeting miR-124 sequence (GenePharma). As indicated in Fig. S2A, mRNA levels of SOX17 and FOXA2 were not affected by miR-124-5p inhibitor in DE stage, although miR-124-5p level was upregulated (Fig. 2A). Additionally, the SOX17+/CXCR4+ population was not significantly changed either (Fig. S2B). Consistently, no differences were observed in the fluorescence intensity of SOX17/FOXA2 between NC and miR-124-5p inhibitor group (Fig. S2C). Indeed, these findings indicate that miR-124-5p inhibition does not affect DE differentiation.

3.5. Inhibition of miR-124-5p enhances NKX6.1 during PP differentiation

Following that, we examined whether miR-124-5p would regulate PP differentiation. As displayed in Fig. 3A, the mRNA level of PDX1 was not affected by miR-124-5p inhibitor. Meanwhile, NKX6.1 mRNA level was significantly upregulated during PP differentiation. Moreover, FACS analysis revealed that $PDX1^{+}/NKX6.1^{+}$ population was increased from 59.7% to 91.2% after administration of miR-124-5p inhibitor (Fig. 3B). Similarly, the fluorescence intensity of PDX1 was not significantly changed, while the intensity of NKX6.1 was remarkably enhanced in PP stage (Fig. 3C). Therefore, the results imply that miR-124-5p probably directly affects NKX6.1 expression and further regulates the specification of PP and IPCs.

3.6. miR-124-5p regulates NKX6.1 expression through targeting its 3'UTR

To elucidate the mechanism by which miR-124-5p regulates NKX6.1 expression, we measured whether miR-124-5p would directly target the 3' untranslated region (3'UTR) of NKX6.1. After analysis by TargetScan (http://www.targetscan.org/mamm_31/), we found that the seed sequence of miR-124-5p could match 3'UTR of NKX6.1 (Fig. 4A). Moreover, the matched region of NKX6.1 was conservative among different species (Fig. 4A). To validate that seed sequence regulates NKX6.1 expression, we conducted luciferase assays. Two mutant 3'UTR at miR-124-5p matched region were designed to construct luciferase plasmids and co-introduced into HEK293 cells with miR-124-5p mimics (Fig. 4A). As presented in Fig. 4B, the luciferase activity was significantly suppressed in HEK293 cells transfected with wild type 3'UTR and miR-124-5p mimics. Meanwhile, the luciferase activity of the two mutant groups did not significantly change compared to the control group. To further demonstrate that miR-124-5p can regulate NKX6.1 expression at post-transcriptional level, we used Dactinomycin (ActD) to inhibit RNA synthesis. Administration of ActD alone for 4 h did not significantly change the fluorescence intensity of NKX6.1 (Fig. 4C). However, similar to the above results, miR-124-5p inhibitor enhanced the fluorescence expression of NKX6.1. Moreover, treatment with miR-

124-5p inhibitor and ActD did not affect NKX6.1 regulation by a miR-124-5p inhibitor (Fig. 4C). CCK-8 assay also proved that miR-124-5p inhibitor could not influence beta-cell proliferation (Fig. S3). Collectively, miR-124-5p regulates NKX6.1 expression through targeting its 3'UTR and further contributes to the specification of IPCs from hESCs.

4. Discussion

In the present study, we propose a novel mechanism by which miR-124-5p regulates NKX6.1 expression that affects the specification of IPCs from hESCs, which is supported by a wide range of evidences. First, miR-124-5p is upregulated during differentiation of IPCs from hESCs. Second, miR-124-5p suppression enhances NKX6.1 expression during PP differentiation. Third, miR-124-5p regulates NKX6.1 expression through targeting its 3'UTR. Eventually, inhibition of miR-124-5p promotes the specification of IPCs from hESCs. Therefore, miR-124-5p could be a potential target to enhance differentiation efficiency of β cells.

An important finding of the current study is that miR-124-5p directly regulates NKX6.1 expression by targeting its 3'UTR and further contributes to PP differentiation. However, miR-124-5p and its precursor form were upregulated from DE stage, and DE differentiation was not affected by miR-124-5p inhibition. This result indicates that some inhibitory signaling pathways or factors were probably involved miR-124-5p function. Competing endogenous RNAs (ceRNAs) are a group of long noncoding RNA (lncRNA) with miRNA binding regions and act as miRNA sponges to antagonize miRNA function. Therefore, identifying a potential ceRNA of miR-124-5p in DE stage would explain the current finding and expand our understanding of the mechanism underlying the regulation of miR-124-5p function.

Since miRNAs are characterized by relatively high stability in the serum, previous studies have attributed several specific serum miRNA signatures to different pathological disorders. Additionally, circulating miR-124-5p is highly upregulated in dilated cardiomyopathy and proposed as a biomarker for diastolic dysfunction. In the current study, we also found that miR-124-5p was upregulated during the specification of IPCs from hESCs, and miR-124-5p inhibition could enhance NKX6.1 expression and further contribute to IPCs differentiation. Therefore, it is promising to measure miR-124-5p level in the cell supernatant and its relevance to the efficiency of IPC differentiation, which can be satisfied with the requirement of a cell-nondestructive index of IPC specification monitor.

Author contributions

XJ. Zhang performed almost all the experiments and wrote the paper. M.Z. did flow cytometry and statistical analysis. SZ. S. and XJ. Zhao did qPCR work. JB.W. supervised the study and wrote the paper.

Ethics approval

Not applicable.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2022.101273.

References

[1] K. Kaul, et al., Introduction to diabetes mellitus, Adv. Exp. Med. Biol. 771 (2012) 1–11.
[2] J.M. Forbes, M.E. Cooper, Mechanisms of diabetic complications, Physiol. Rev. 93 (1) (2013) 137–188.
[3] B. Freeland, M.S. Farber, A review of insulin for the treatment of diabetes mellitus, Home Healthc. Nurse 34 (8) (2016) 416–423.
[4] E.M. Vivian, S.V. Olarte, A.M. Gutierrez, Insulin strategies for type 2 diabetes mellitus, Ann. Pharmacother. 38 (11) (2004) 1916–1923.
[5] M.R. Rickels, R.P. Robertson, Pancreatic islet transplantation in humans: recent progress and future directions, Endocr. Rev. 40 (2) (2019) 631–668.
[6] A.M. Shapiro, M. Pokrywczynska, C. Ricordi, Clinical pancreatic islet transplantation, Nat. Rev. Endocrinol. 13 (5) (2017) 268–277.
[7] H. Zhu, et al., Microencapsulated pig islet xenotransplantation as an alternative treatment of diabetes, Tissue Eng. B Rev. 21 (5) (2015) 474–489.
[8] P. Damdimopoulou, et al., Human embryonic stem cells, Best Pract. Res. Clin. Obstet. Gynaecol. 31 (2016) 2–12.
[9] A.C. Chen, et al., Human embryonic stem cells as an in vitro model for studying developmental origins of type 2 diabetes, World J. Stem Cell. 12 (8) (2020) 761–775.
[10] F.W. Pagliuca, et al., Generation of functional human pancreatic beta cells in vitro, Cell 159 (2) (2014) 428–439.
[11] A. Rezania, et al., Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells, Nat. Biotechnol. 32 (11) (2014) 1121–1133.
[12] H.A. Russ, et al., Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro, EMBO J. 34 (13) (2015) 1759–1772.
[13] S. Hrvatin, et al., Differentiated human stem cells resemble fetal, not adult, beta cells, Proc. Natl. Acad. Sci. U. S. A. 111 (8) (2014) 3038–3043.
[14] Aigha II, E.M. Abdelalim, NKX6.1 transcription factor: a crucial regulator of pancreatic beta cell development, identity, and proliferation, Stem Cell Res. Ther. 11 (1) (2020) 459.
[15] J.K. Pedersen, et al., Endodermal expression of Nkx6 genes depends differentially on Pdx1, Dev. Biol. 288 (2) (2005) 487–501.
[16] S. Al-Khawaga, et al., Pathways governing development of stem cell-derived pancreatic beta cells: lessons from embryogenesis, Biol. Rev. Camb. Phil. Soc. 93 (1) (2018) 364–389.
[17] B. Memon, E.M. Abdelalim, Stem cell therapy for diabetes: beta cells versus pancreatic progenitors, Cells 9 (2) (2020).
[18] A.E. Schaffer, et al., Nkx6.1 controls a gene regulatory network required for establishing and maintaining pancreatic Beta cell identity, PLoS Genet. 9 (1) (2013), e1003274.
[19] J.R. Neilson, P.A. Sharp, Small RNA regulators of gene expression, Cell 134 (6) (2008) 899–902.
[20] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2) (2004) 281–297.
[21] L. He, G.J. Hannon, MicroRNAs: small RNAs with a big role in gene regulation, Nat. Rev. Genet. 5 (7) (2004) 522–531.
[22] M.P. LaPierre, M. Stoffel, MicroRNAs as stress regulators in pancreatic beta cells and diabetes, Mol. Metabol. 6 (9) (2017) 1010–1023.
[23] M.N. Poy, et al., miR-375 maintains normal pancreatic alpha- and beta-cell mass, Proc. Natl. Acad. Sci. U. S. A. 106 (14) (2009) 5813–5818.
[24] M. Latreille, et al., miR-375 gene dosage in pancreatic beta-cells: implications for regulation of beta-cell mass and biomarker development, J. Mol. Med. (Berl.) 93 (10) (2015) 1159–1169.
[25] M.N. Poy, et al., A pancreatic islet-specific microRNA regulates insulin secretion, Nature 432 (7014) (2004) 226–230.
[26] R.K. Wang, et al., MicroRNA-124-5p delays the progression of cerebral aneurysm by regulating FoxO1, Exp. Ther. Med. 22 (4) (2021) 1172.
[27] Q. Chen, G. Lu, Y. Cai, Y. Li, R. Xu, Y. Ke, S. Zhang, miR-124-5p inhibits the growth of high-grade gliomas through posttranscriptional regulation of LAMB1, Neuro Oncol. 16 (2014) 637–651, https://doi.org/10.1093/neuonc/not300.
[28] Y. Ding, et al., Lipopolysaccharide inhibits alpha epithelial sodium channel expression via MiR-124-5p in alveolar type 2 epithelial cells, BioMed Res. Int. 2020 (2020) 8150780.
[29] Q. He, et al., IFI16 promotes human embryonic stem cell trilineage specification through interaction with p53, NPJ Regen. Med. 5 (1) (2020) 18.