suppress target mRNAs [14, 15]; however, it has been suggested that miRNAs may also enhance gene expression [16]. Single miRNA can suppress several mRNA targets, and multiple miRNAs may have influence on a specific pathway [15, 17]. Previous studies reported that several miRNAs play crucial roles in regulating the development and function of pancreatic β-cells [12, 13, 18] and glucose homeostasis [19]. Examples of those miRNAs are miR-26, miR-24, and miR-148 [20], miR-375 [13], miR-21 [21], miR-30d [22, 23], let-7 [24], miR-34a and miR-34c [25], miR-9 [26], and miR-7 [27]. Furthermore, miRNAs have been found to be involved in maintaining β-cell identity [28, 29]. Dysregulated expression of several miRNAs has been reported to be associated with diabetes development [28, 30]. In mouse pancreatic progenitors (PPs), a specific deletion of Dicer1 enzyme, which is universally required for the functional miRNA maturation, led to decreased pancreatic endocrine cell numbers [31]. Furthermore, disruption of Dicer1 in pancreatic β-cells impairs insulin biosynthesis [20].

Recent progress in human induced PSC (hiPSC) technology has paved the way for many essential applications that could be used for disease modeling, targeted therapy, drug screening, and precision medicine. Therefore, here, we take advantage of our recently established FOXA2 knockout hiPSC (*FOXA2*$^{-/-}$iPSC) model to identify the alterations in the miRNA and mRNA profiles in PPs lacking *FOXA2* to understand the miRNA-mRNA regulatory networks regulating pancreatic development. Our results showed that loss of *FOXA2* leads to the upregulation of numerous miRNAs targeting key PP genes involved in pancreatic exocrine and endocrine development.

Materials and Methods

Culture and Differentiation of iPSCs Into Pancreatic Progenitors

iPSC lines (Ctr1-iPSCs and Ctr2-iPSCs) generated and fully characterized in our laboratory were used as we previously reported [32]. *FOXA2* knockout iPSCs from Ctr1-and Ctr2-iPSCs were generated using CRISPR/Cas9 as we recently reported [5]. Both wild-type (WT) and *FOXA2*$^{-/-}$ iPSCs were cultured and maintained using Stemflex media (ThermoFisher Scientific) on Matrigel-coated plates (Corning). iPSC lines were differentiated into PPs using our established protocol (Supplementary Table 1) [33–35].

Immunocytochemistry

Immunostaining was performed on differentiated iPSCs as previously reported [32, 36]. Cells were washed once with PBS then 4% paraformaldehyde (PFA) was added on the cells for 20 min and placed on a shaker at room temperature. The cells were then washed with tris-buffered saline + 0.5% Tween 20 (TBST) thrice in a 10-minute interval on a shaker. Cells were then permeabilized for 15 min at room temperature using phosphate buffered saline (PBS) + 0.5% Triton X-100 (PBST) twice, later blocked overnight with 6% Bovine Serum Albumin (BSA) in PBST at 4°C. Afterwards, guinea pig anti-PDX1 (1:500, ab47308, Abcam) and mouse anti-NKX6.1 (1:2000, F55A12-C, DSHB) primary antibodies diluted in 3% BSA in PBST were added to the cells and incubated overnight at 4°C. Cells were washed three times with TBST and then Alexa Fluor secondary antibodies (ThermoFisher Scientific) diluted in PBS (1:500) were added for 1 h at room temperature then washed again three times using TBST. Cell nuclei were stained for two minutes with Hoechst 33,258 (DAPI) diluted 1:5000 in PBS (Life Technologies, USA). After washing three times with PBS, images were captured using inverted fluorescence microscope (Olympus).

Western Blotting

Total protein was extracted from one well of a 6-well plate using RIPA lysis buffer with protease inhibitor (ThermoFisher Scientific). Measurement of protein concentration was done using Pierce BCA kit (ThermoFisher Scientific). 20 µg of total protein were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with 10% skimmed milk in TBST then incubated with rabbit anti-FOXA2 (1:4000, #3143, Cell Signaling) and mouse anti-β-actin (1:10,000, sc-47,778, Santa Cruz) primary antibodies overnight at 4°C. Membranes were washed with TBST then horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch) diluted in TBST (1:10,000) were added for 1 h at room temperature then washed again using TBST. Membranes were developed using SuperSignal West Pico Chemiluminescent substrate (Pierce, Loughborough, UK) then visualized using iBright™ CL 1000 Imaging System (Invitrogen).

RNA Extraction and RT-qPCR Analysis

1×10^6 cells were collected using 700 µL of TRIzol Reagent (Life Technologies) then total RNA extraction was performed using Direct-zol™ RNA Miniprep (Zymo Research, USA). For mRNA, cDNA was synthesized from 1 µg of RNA using SuperScript™ IV First-Strand Synthesis System following manufacturer protocol (ThermoFisher Scientific, USA). RT-qPCR was performed using GoTaq qPCR SYBR Green Master Mix (Promega, USA) run