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Identification of hepatocyte growth factor activator (Hgfac) gene as a target of HNF1α in mouse β-cells

Tsuyoshi Ohki [a,b], Yoshifumi Sato [a], Tatsuya Yoshizawa [a], Ken-ichi Yamamura [c], Kentaro Yamada [b], Kazuya Yamagata [a,*]

[a] Department of Medical Biochemistry, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan
[b] Division of Endocrinology and Metabolism, Kurume University School of Medicine, Kurume, Japan
[c] Division of Developmental Genetics, Center for Animal Resources and Development, Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan

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ABSTRACT

HNF1α is a transcription factor that is expressed in pancreatic β-cells and mutations of the HNF1α gene cause a form of monogenic diabetes. To understand the role of HNF1α in pancreatic β-cells, we established the MIN6 β-cell line that stably expressed HNF1α-specific shRNA. Expression of the gene encoding hepatocyte growth factor (HGF) activator (Hgfac), a serine protease that efficiently activates HGF, was decreased in HNF1α KD-MIN6 cells. Down-regulation of *Hgfac* expression was also found in the islets of HNF1α (+/−) mice. Reporter gene analysis and the chromatin immunoprecipitation assay indicated that HNF1α directly regulates the expression of *Hgfac* in β-cells. It has been reported that HGF has an important influence on β-cell mass and β-cell function. Thus, HNF1α might regulate β-cell mass or function at least partly by modulating Hgfac expression.

1. Introduction

HNF1α is a transcription factor that belongs to a subclass of the homeodomain family, and it is expressed in the liver, pancreas, kidney, and intestine [1,2]. HNF1α has an N-terminal dimerization domain, a DNA-binding domain with POU-like and homeodomain-like motifs, and a C-terminal transactivation domain [3]. We previously reported that heterozygous mutations of the HNF1α gene cause a form of monogenic diabetes known as maturity-onset diabetes of the young type 3 (MODY3) [4]. Clinical studies have shown that the primary cause of MODY3 is impairment of insulin secretion in response to a glucose load [5]. Mutant mice with loss of HNF1α function also develop diabetes due to impaired insulin secretion [6,7], indicating an important role of HNF1α in pancreatic β-cells. Interestingly, these mutant mice exhibit progressive reduction of β-cell numbers, suggesting that some target genes of HNF1α are also required for the maintenance of a normal β-cell mass.

To better understand the role of HNF1α in pancreatic β-cells and in the molecular mechanisms of MODY3, identification of the full spectrum of genes regulated by this factor in β-cells is necessary. Previous studies have demonstrated that *Slc2a2* (encoding glucose transporter 2 (GLUT2)), *Pklr* (encoding liver pyruvate kinase), *Tmem27* (encoding collectrin), *Hnf4a* (encoding HNF4α), and *Foxa3* (encoding HNF3γ) are direct targets of HNF1α in β-cells [8–12]. Genome-wide expression profiling has also been performed to identify additional targets of HNF1α using pancreatic islets obtained from control and HNF1α (−/−) knockout (KO) mice [13]. Although this approach revealed that expression of 5.6% of all genes was down-regulated in HNF1α KO islets, these changes might have been secondary to the onset of hyperglycemia or other effects of the diabetic state in HNF1α KO mice.

To identify the direct target genes of HNF1α in β-cells by another approach, we established the MIN6 β-cell line that stably expressed HNF1α-specific shRNA (HNF1α KD-MIN6 cells) and then compared the gene expression profile between control MIN6 cells and HNF1α KD-MIN6 cells. As a result, we demonstrated the down-regulation of several genes, including *Slc2a2*, *Tmeme27*, and *Hnf4a*, in HNF1α KD-MIN6 cells. We also found that expression of the gene encoding hepatocyte growth factor (HGF) activator (Hgfac), a serine protease that efficiently activates HGF [14], was decreased in HNF1α KD-MIN6 cells. Down-regulation of *Hgfac* expression was also found in the islets of HNF1α (+/−) mice. Reporter gene analysis and the chromatin immunoprecipitation assay confirmed that HNF1α directly regulates the expression of *Hgfac* in β-cells.

* Corresponding author. Address: Department of Medical Biochemistry, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, Kumamoto 860-8556, Japan. Fax: +81 96 364 6940.
E-mail address: k-yamaga@kumamoto-u.ac.jp (K. Yamagata).

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2. Material and methods

2.1. Cell culture

The MIN6 pancreatic β-cell line was maintained in Dulbecco's modified Eagles' medium (DMEM) (25 mM glucose) containing 10% (v/v) fetal bovine serum, 50 μM β-mercaptoethanol (β-ME), 50 U/ml penicillin, and 50 μg/ml streptomycin at 37 °C under 5% CO_2 [15]. Hela cells and Plat-E retrovirus packaging cells [16] were maintained in DMEM containing 10% (v/v) fetal bovine serum.

2.2. Retroviral infection

A specific shRNA sequence for mouse HNF1α (5′-CGAAGATGGTCAAGTCGTA-3′) was designed using the Clontech RNAi target sequence (http://bioinfo.clontech.com/). Oligonucleotides encoding shRNA were synthesized and cloned into the pSIREN-RetroQ retroviral shRNA expression vector (Clontech/Takara, Japan). Then the pSIREN-RetroQ-HNF1α vector and the negative control pSIREN-RetroQ vector were transfected into Plat-E cells using FuGENE6 (Roch, Germany). MIN6 cells were infected with either retrovirus and then selected by incubation with puromycin (5 μg/ml) to generate MIN6 cells stably expressing HNF1α shRNA (HNF1α KD-MIN6 cells) or negative control shRNA (control MIN6 cells), as described previously [17].

2.3. Quantitative RT-PCR

Total RNA was extracted by using Sepasol RNA I super reagent (Nacalai Tesque, Japan) or an RNeasy micro kit (Qiagen, CA). Quantitative real-time PCR was performed with SYBR Premix Ex Taq II (RR820A, TaKaRa) in an ABI 7300 thermal cycler (Applied Biosystems, CA). The specific primers were as follows: *Hnf1α* (5′-AAGAGCCCACAGGCGATGAG-3′ and 5′-TGGATGCACTCCGCCCTATT-3′), *Hgfac* (5′-GCACCTGCCACCTGATTGTG-3′ and 5′-GCCACGCCTCGGTACTCTGT-3′), *Slc2a2* (5′-CGTCCTACGGCTCTGGCACT-3′ and 5′-CACCCCAGCGAAGAGGAAGA-3′), *Tmem27* (5′-ATTCGGTGTGATATTTTGCATTGT-3′ and 5′-TCCAGGTGGTCCTTTGTTGTT-3′), and TATA-binding protein (*Tbp*) (5′-CCCCTTGTACCCTTCACCAAT-3′ and 5′-GAAGCTGCGGTACAATTCCAG-3′). Relative expression of each gene was normalized for that of *Tbp*.

2.4. Western blotting

Cells were lysed in RIPA buffer (50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 0.1% SDS, 1% NP-40, 5 mM EDTA, 0.5% sodium deoxycholate), and 1/100 (v/v) protease inhibitor cocktail (Nacalai Tesque). Total protein was separated by SDS polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride (PVDF) membrane (ImmobilonP; Millipore, MA), which was probed with the primary antibodies. After incubation with the secondary antibodies, proteins were visualized using Chemi-Lumi One Super (Nacalai Tesque) and a LAS-1000 imaging system (Fuji Film, Japan). The primary antibodies used in this study were anti-HNF1α (1:1000) (610902; Becton, Dickinson and Company, NJ) and anti-β-actin (1:5000) (A5441; Sigma–Aldrich, MO).

2.5. Insulin secretion

After reaching 80% confluence, MIN6 cells were plated in 24-well plates at a density of 3×10^5 cells per well. After culture for 72 h, cells were preincubated at 37 °C for 60 min in Hepes–Krebs buffer (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20 mM $NaHCO_3$, 2.2 mM glucose, and 10 mM Hepes) containing 0.5% (w/v) bovine serum albumin (BSA) [17]. Then the cells were incubated for 60 min in Hepes–Krebs buffer containing 22 mM glucose and insulin production was measured by using a mouse insulin ELISA kit (AKRIN-011T; Shibayagi, Japan).

2.6. Microarray analysis

Total RNA was extracted from control MIN6 cells and HNF1α KD-MIN6 cells using a miRCURY RNA isolation kit (Exiqon, Denmark) according to the manufacturer's instructions. The quality of the RNA was checked by gel electrophoresis and analysis with an Agilent 2100 Bioanalyzer (Agilent Technologies, CA). DNA microarray analysis was performed by the Toray Custom Analysis Service using the 3D-Gene™ DNA chip (Mouse Oligo chip 24K).

2.7. Isolation of pancreatic islets

HNF1α knockout mice were generated that lacked exon 1, which contains the translation start codon [K. Yamamura, and K. Yamagata, unpublished data]. HNF1α (+/−) mice showed normal glucose tolerance, as reported previously [6, and our unpublished data]. Mice were maintained with a 12 h light–12 h dark cycle and were allowed free access to food and water. These experiments were conducted according to the guidelines of the Institutional Animal Committee of Kumamoto University. Islets were isolated from the harvested pancreata of 20-week-old male HNF1α (+/−) mice ($n = 4$) and control HNF1α (+/+) littermates ($n = 4$) by collagenase digestion, as described previously [7].

2.8. Transient transfection and luciferase reporter assay

A 135 bp fragment of the mouse Hgfac gene promoter containing the putative HNF1α-binding site (−135 to −1 relative to the translation start codon when A is numbered as +1) was amplified by PCR using the primers 5′-GCTAGCGGCTGTGGAGGAGCCTAACAGGAT-3′ (underlined nucleotides indicate the cloned NheI site) and 5′-AAGCTTGGCTCCTCCTGAGCTGGCGTGAGG-3′ (underlined nucleotides indicate the cloned HindIII site), and then was subcloned into the pGL3 basic reporter (Promega, WI) to generate pGL3-Hgfac. The HNF1α-binding site was altered to 5′-GGTCGGCCCTTATCA-3′ by PCR-based mutagenesis. The pcDNA3.1-wild-type (WT)-HNF1α and pcDNA3.1-P291fsinsC-HNF1α expression plasmids have been described previously [8]. Hela cells (1.5×10^5 cells/well) or MIN6 cells (3×10^5 cells/well) were seeded into 12-well plates at 18 h before transient transfection was performed using X-treme GENE (Roche) according to the manufacturer's instructions. At 48 h after transfection, luciferase activity was measured by using a Dual-Luciferase Reporter assay system (Promega).

2.9. Chromatin immunoprecipitation (ChIP) assay

MIN6 cells were cross-linked with 1% formaldehyde for 10 min at room temperature. Then the ChIP assay was performed as described previously [17] using an anti-HNF1 antibody (sc-8986, Santa Cruz Biotechnology, CA). Immunoprecipitated DNA was amplified by real-time PCR with specific primers for the promoter region of *Hgfac* containing the HNF1α-binding motif (5′-GGCTGTGGAGGAGCCTAACAGGAT-3′ and 5′-GGCTCCTCCTGAGCTGGCGTGAGG-3′) (P1), as well as primers for the 10 kb upstream region from the *Hgfac* translation start codon (5′-GGGCTGGGGT GC TTCGGGTA-3′ and 5′-GACCCTCCAGCGGATGGC TCA-3′) (P2), the 5.3 kb downstream region from the *Hgfac* translation start (5′-GCTGTGCTGTCCGCTCCCAG-3′ and 5′-CATGTGGCCCCAGCCTGCAA-3′) (P3), and the promoter region of *Tbp* (5′-ATCAGATGTGCGTCAGGCGTT-3′ and 5′-TGCGGAGAAAATGACGCGA-3′) (P4). All PCR

reactions were done by using SYBR Premix Ex Taq II (TaKaRa) in an ABI 7300 thermal cycler (Applied Biosystems).

2.10. Statistical analysis

The significance of differences was assessed with the unpaired *t*-test, and $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Establishment of HNF1α KD-MIN6 cells and microarray analysis

In order to identify novel target genes for HNF1α in pancreatic β-cells, we established MIN6 β-cells that stably expressed HNF1α-specific shRNA (HNF1α KD-MIN6) by retroviral infection. Suppression of endogenous HNF1α expression by shRNA was confirmed at both the mRNA level (39.8% of control, $p = 0.019$) and the protein level (30.6% of control, $p = 0.007$) (Fig. 1A and B). *Slc2a2* and *Tmem27* are direct targets of HNF1α in β-cells [10,11], and expression of both these genes was significantly decreased in HNF1α KD-MIN6 cells (Fig. 1C). Loss of the function of HNF1α leads to impairment of glucose-stimulated insulin secretion by pancreatic β-cells [6,7]. Therefore, we examined insulin secretion by HNF1α KD-MIN6 cells and control MIN6 cells. Insulin secretion by HNF1α KD-MIN6 cells subjected to stimulation with 22 mM glucose was significantly decreased ($p < 0.001$) to 39.2% of that for control cells (Fig. 1D). Suppression of HNF1α expression also reduced insulin secretion by HNF1α KD-MIN6 cells in response to a low glucose concentration (decreased by 41.4%, $p < 0.001$). These results indicate that HNF1α KD-MIN6 cells can be used as a novel cellular model of MODY3.

In order to identify novel target genes of HNF1α in pancreatic β-cells, DNA microarray analysis was performed using control cells and HNF1α KD-MIN6 cells. Microarray analysis identified the down-regulation of 53 genes (0.22% of all expressed genes), which was defined as a signal log ratio $\leqslant -1.5$, in HNF1α KD-MIN6 cells and up-regulation of 38 genes (0.15% of all expressed genes), which was defined as a signal log ratio $\geqslant 2$ (Table 1 and Supplementary Table 1). Several known targets of HNF1α (*Slc2a2*, *Tmem27*, and *Hnf4α* were found in the group of down-regulated genes.

3.2. Hgfac expression in β-cells is regulated by HNF1α

Microarray analysis revealed that expression of the gene encoding hepatocyte growth factor activator (HGFA) was reduced to 25.2% of the control level in HNF1α KD-MIN6 cells (Table 1). Down-regulation of *Hgfac* expression in HNF1α KD-MIN6 cells to 18.7% of the control level ($p < 0.001$) was confirmed by quantitative RT-PCR (Fig. 2A). HNF1α (+/−) mice were reported to be useful for investigating HNF1α-dependent transcription in pancreatic islets [18]. As shown in Fig. 2B, Hgfac mRNA expression was significantly decreased in the islets of HNF1α (+/−) mice to 45.8% of the control level ($p < 0.001$), indicating that *Hgfac* gene transcription is regulated by HNF1α *in vivo* as well as *in vitro*.

Screening of the promoter region of the mouse *Hgfac* gene by using a genomic databank revealed an HNF1α-binding site (nucleotides −83 to −97 relative to the translation start codon when *A* is designated as +1), and this binding site was also confirmed to exist in the human HGFAC gene (Fig. 2C). We cloned a 135 bp fragment

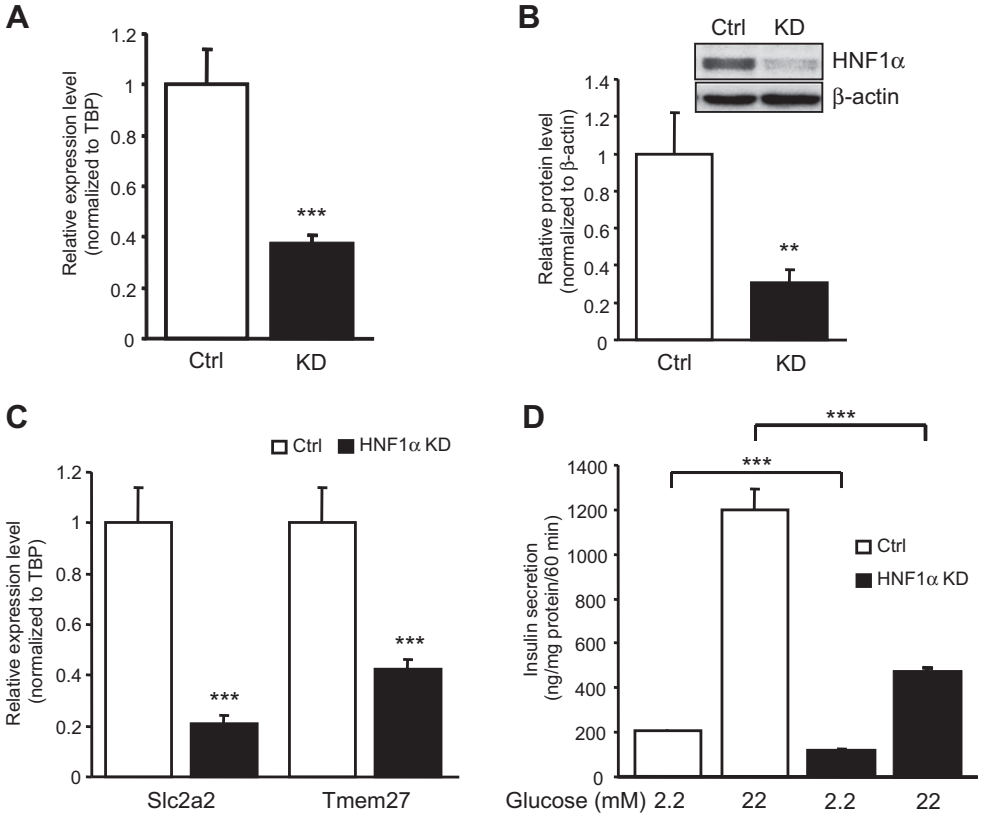

Fig. 1. Gene expression and insulin secretion by HNF1α KD-MIN6 cells. (A) Expression of HNF1α mRNA by control cells (Ctrl) ($n = 3$) and HNF1α KD-MIN6 cells (KD) ($n = 3$). Expression is normalized for that of Tbp. (B) HNF1α protein expression in HNF1α KD-MIN6 cells evaluated by Western blotting ($n = 3$). β-actin was used as the loading control. (C) Expression of Slc2a2 and Tmem27 mRNA was significantly decreased in HNF1α KD-MIN6 cells ($n = 3$). (D) Insulin secretion after exposure to glucose was decreased in HNF1α KD-MIN6 cells ($n = 4$). Data are the mean ± SD ($^{**}p < 0.01$, $^{***}p < 0.001$).

Table 1
Gene list of the down-regulated genes in HNF1α KD-MIN6 cells.

Gene symbol	Gene title	Log ratio	RefSeq transcript ID
Gc	Group specific component	−3.76	NM_008096
Ugt1a6a	UDP glucuronosyltransferase 1 family, polypeptide A7C	−3.68	NM_145079
Slc2a2	Solute carrier family 2 (facilitated glucose transporter), member 2	−3.61	NM_031197
Crp	C-reactive protein, pentraxin-related	−3.48	NM_007768
Ttr	Transthyretin	−3.23	NM_013697
Tmed6	Transmembrane emp24 protein transport domain containing 6	−3.02	NM_025458
Serpina1c	Serine (or cysteine) peptidase inhibitor, clade A, member 1a	−2.90	NM_009245
Iyd	Iodotyrosine deiodinase	−2.80	NM_027391
Slc40a1	Solute carrier family 40 (iron-regulated transporter), member 1	−2.70	NM_016917
Ang1	Angiogenin, ribonuclease A family, member 1	−2.70	NM_007447
Spon2	Spondin 2, extracellular matrix protein	−2.64	NM_133903
Rnase4	Ribonuclease, RNase A family 4	−2.62	NM_021472
Golt1a	Golgi transport 1 homolog A (S. cerevisiae)	−2.53	NM_026680
Serpina1c	Serine (or cysteine) peptidase inhibitor, clade A, member 1a	−2.50	NM_009243
Serpina1d	Serine (or cysteine) peptidase inhibitor, clade A, member 1d	−2.44	NM_009246
Serpina1e	Serine (or cysteine) peptidase inhibitor, clade A, member 1e	−2.33	NM_009247
Guca1a	Guanylate cyclase activator 1a (retina)	−2.29	NM_008189
Nmbr	Neuromedin B receptor	−2.29	NM_008703
Tmem27	Transmembrane protein 27	−2.26	NM_020626
Ang4	Angiogenin, ribonuclease A family, member 4	−2.24	NM_177544
Ang5	Angiogenin, ribonuclease A family, member 5	−2.21	NM_007448
Dpp4	Dipeptidylpeptidase 4	−2.20	NM_010074
Ldha	Lactate dehydrogenase A	−2.11	NM_010699
St6gal1	Beta galactoside alpha 2,6 sialyltransferase 1	−2.05	NM_145933
Hgfac	Hepatocyte growth factor activator	−1.99	NM_019447
Pcsk9	Proprotein convertase subtilisin/kexin type 9	−1.98	NM_153565
Ang5	Angiogenin, ribonuclease A family, member 5	−1.97	NM_007448
Abcg2	ATP-binding cassette, sub-family G (WHITE), member 2	−1.96	NM_011920
Ins1	Insulin I	−1.92	NM_008386
Kif12	Kinesin family member 12	−1.89	NM_010616
Ppp1r1a	Protein phosphatase 1, regulatory (inhibitor) subunit 1A	−1.87	NM_021391
Itga6	Integrin alpha 6	−1.84	NM_008397
Myo15b	Myosin XVB	−1.84	XM_203357
Il20rb	Interleukin 20 receptor beta	−1.83	XM_358706
Degs2	Degenerative spermatocyte homolog 2 (Drosophila), lipid desaturase	−1.81	NM_027299
Tff3	Trefoil factor 3, intestinal	−1.75	NM_011575
Cpn1	Carboxypeptidase N, polypeptide 1	−1.71	NM_030703
Cbs	Cystathionine beta-synthase	−1.71	NM_144855
Insl5	Insulin-like 5	−1.70	NM_011831
Slc16a3	Solute carrier family 16 (monocarboxylic acid transporters), member 3	−1.70	NM_030696
Mbl2	Mannose binding lectin (protein C)	−1.68	NM_010776
Tm4sf4	Transmembrane 4 superfamily member 4	−1.67	NM_145539
Dact2	Dapper homolog 2, antagonist of beta-catenin (xenopus)	−1.63	NM_172826
Dscr1l1	Down syndrome critical region gene 1-like 1	−1.63	NM_030598
Anks4b	Ankyrin repeat and sterile alpha motif domain containing 4B	−1.61	NM_028085
Cacna1 h	Calcium channel, voltage-dependent, T type, alpha 1H subunit	−1.60	NM_021415
Il6ra	Interleukin 6 receptor, alpha	−1.59	NM_010559
Hnf4a	Hepatic nuclear factor 4, alpha	−1.59	NM_008261
Defb1	Defensin beta 1	−1.58	NM_007843
Sgk2	Serum/glucocorticoid regulated kinase 2	−1.56	NM_013731
Sct	Secretin	−1.52	NM_011328
Lgals2	Lectin, galactose-binding, soluble 2	−1.52	NM_025622
Nek6	NIMA (never in mitosis gene a)-related expressed kinase 6	−1.51	NM_021606

of the promoter region upstream of a luciferase reporter gene, and co-expressed it with the HNF1α expression vector in HeLa cells. We found that induction of HNF1α increased *Hgfac* promoter activity to 23.6 times the control level ($p < 0.001$) (Fig. 2D). HNF1α also activated the reporter gene by 3.1-fold ($p < 0.001$) in MIN6 cells, while dominant negative P291fsinsC-HNF1α (a frameshift mutation in the transactivation domain) [8] decreased reporter gene activity to 41.0% of the control level ($p < 0.001$) (Fig. 2E). Mutation of the putative HNF1α-binding site in the reporter gene significantly reduced transcriptional activation by HNF1α (87.3% decrease, $p < 0.001$) (Fig. 2F).

In order to investigate binding of HNF1α to the Hgfac promoter, the chromatin immunoprecipitation (ChIP) assay was performed. Cross-linked chromatin was precipitated with HNF1 antibody, after which the precipitated DNA was analyzed by PCR using primer sets that amplified the promoter region of *Hgfac* containing the putative HNF1α-binding site (P1), the 10 kb upstream region from P1 (P2), the 5.3 kb downstream region from P1 (exon 12) (P3), and the promoter region of *Tbp* (P4). As shown in Fig. 2G, specific binding of HNF1α to the promoter region (P1) was identified in MIN6 cells. These findings indicated that the *Hgfac* gene is directly regulated by HNF1α.

4. Discussion

HGF was originally identified as a potent mitogen for hepatocytes [19], but subsequent studies have shown that it has mitogenic and prosurvival effects on various cells including β-cells. Transgenic overexpression of HGF in pancreatic β-cells increases β-cell replication, mass, and function [20]. Loss of HGF signaling in β-cells during gestation leads to decreased replication and a

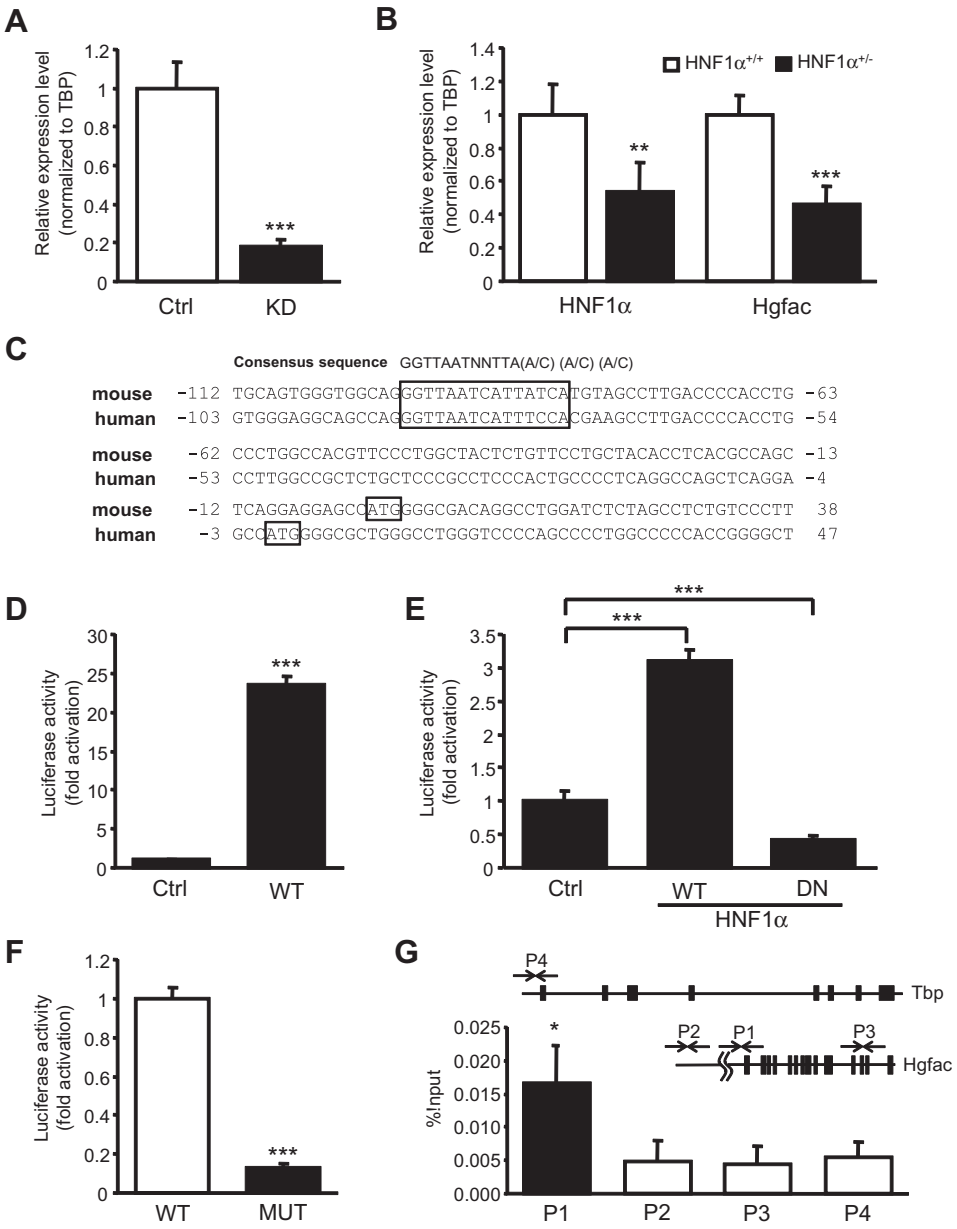

Fig. 2. Transcriptional regulation of *Hgfac* by HNF1α. (A) Expression of Hgfac mRNA by control (Ctrl) cells ($n = 3$) and HNF1α KD-MIN6 cells (KD) ($n = 3$). Expression is normalized for that of Tbp. (B) Expression of HNF1α and Hgfac mRNA in HNF1α (+/+) ($n = 4$) and HNF1α (+/−) islets ($n = 4$). (C) DNA sequences of the mouse and human Hgfac genes. The putative HNF1α-binding site is shown by a box. (D) HeLa cells were cotransfected with 800 ng of the pcDNA3.1-HNF1α expression vector as well as 200 ng of the pGL3-Hgfac reporter vector and 1 ng of pRL-TK. (E) MIN6 cells were cotransfected with 800 ng of the pcDNA3.1-HNF1α (WT) or the pcDNA3.1-P291fsinsC-HNF1α (DN) expression vector as well as 200 ng of the pGL3-Hgfac reporter vector and 0.4 ng of pRL-TK. (F) MIN6 cells were cotransfected with 800 ng of the pcDNA3.1-HNF1α (WT) expression vector as well as 200 ng of the pGL3-Hgfac (WT) or pGL3-Hgfac-mutant (MUT) reporter vector and 0.4 ng of pRL-TK. (G) Chromatin immunoprecipitation assay with MIN6 cells. PCR was performed using 4 different primer sets (P1–P4). The P2–4 regions lack the HNF1α-binding motif. Interaction of HNF1α with the P1 region of *Hgfac* was observed. Data are the mean ± SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

decline of β-cell mass, and loss of HGF signaling also accelerates the onset of diabetes in response to multiple low-dose injections of streptozotocin [21,22]. These data strongly suggest that HGF has an important influence on β-cell mass and β-cell function.

HGF is secreted in a latent form, and proteolytic conversion by the serine protease HGF activator (Hgfac) is required for its activation [14]. In this study, we found that Hgfac expression was markedly decreased in both HNF1α KD-MIN6 cells and islets from HNF1α (+/−) mice. The reporter and ChIP assays demonstrated that HNF1α bound to the conserved binding site of the *Hgfac* promoter and that it activated transcription of this gene, indicating that *Hgfac* is a direct target of HNF1α in β-cells. Although the molecular mechanisms underlying MODY3 are still unknown, HNF1α (−/−) mice and transgenic mice expressing the naturally occurring dominant negative form of human HNF1α (P291fsinsC) in their β-cells exhibit progressive reduction of β-cell mass and β-cell proliferation, indicating that HNF1α is required to maintain the β-cell mass [6,7]. Reduction of *Hgfac* gene expression and a consequent decrease of HGF signaling in β-cells might occur in HNF1α mutant mice as well as in patients with MODY3. It is possible that HNF1α controls β-cells mass at least partly by regulating cellular Hgfac expression. Investigation of β-cell specific Hgfac knockout mice could improve our understanding of how a reduction of HNF1α activity leads to a decline of β-cell mass and the onset of diabetes.

In conclusion, we established a novel cellular model of MODY3, HNF1α KD-MIN6 cells, and we identified many genes that were down-regulated in these cells. Further investigation of HNF1α KD-MIN6 cells could be useful to identify novel target genes of HNF1α in β-cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.134.

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