Hepatocyte Nuclear Factor-1α Modulates Pancreatic β-Cell Growth by Regulating the Expression of Insulin-Like Growth Factor-1 in INS-1 Cells

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Maturity-onset diabetes of the young type 3 (MODY3) is characterized by impaired insulin secretion. Heterozygous mutations in the gene encoding hepatocyte nuclear factor (HNF)-1α are the cause of MODY3. Transgenic mice overexpressing dominant-negative HNF-1α mutant in pancreatic β-cells and HNF-1α knockout mice are animal models of MODY3. These mice exhibit defective glucose-stimulated insulin secretion and have reduced β-cell mass and β-cell proliferation rate. Here we examined the effect of HNF-1α on β-cell proliferation by overexpressing a human naturally occurring dominant-negative mutation P291fsinsC in INS-1 cells under the control of doxycycline-induction system. INS-1 cells overexpressing P291fsinsC showed apparent growth impairment. The proliferation rate estimated by [^{3}H]thymidine incorporation was significantly reduced in P291fsinsC-expressing INS-1 cells compared with non-induced or wild-type HNF-1α-overexpressing INS-1 cells. Growth inhibition occurred at the transition from G1 to S cell cycle phase, with reduced expression of cyclin E and upregulation of p27. cDNA array analysis revealed that the expression levels of IGF-1, a major growth factor for β-cells, and macrophage migration inhibitory factor (MIF), a cytokine expressed in pancreatic β-cells, were reduced in P291fsinsC-HNF-1α–expressing INS-1 cells. Although MIF seemed to have proliferative function, blockade of MIF action by anti-MIF antibody stimulated INS-1 cell proliferation, excluding its direct role in the growth impairment. However, addition of IGF-1 to P291fsinsC–expressing INS-1 cells rescued the growth inhibition. Our data suggest that HNF-1α is critical for modulating pancreatic β-cell growth by regulating IGF-1 expression. IGF-1 might be a potential therapeutic target for the treatment of MODY3. ***Diabetes*** **51:1785–1792, 2002**

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CDK, cyclin-dependent kinase; FACS, fluorescence-activated cell sorter; HGF, hepatocyte growth factor; HNF, hepatocyte nuclear factor; IGFBP, IGF binding protein; MIF, migration inhibitory factor; MODY, maturity-onset diabetes of the young; PI, phosphatidylinositol; PL, placental lactogen; WT, wild-type.

Maturity-onset diabetes of the young (MODY) is a group of disorders characterized by early onset of diabetes, pancreatic β-cell dysfunction, and autosomal dominant inheritance (1–3). MODY type 3 (MODY3) is caused by heterozygous mutations in the gene encoding a homeodomain-containing transcription factor hepatocyte nuclear factor (HNF)-1α (4). Human HNF-1α has 631 amino acids and comprises three functional domains, a dimerization domain (amino acids 1–32), a DNA-binding domain (amino acids 150–280), and a transactivation domain (amino acids 281–631). P291fsinsC is the most common mutation in HNF-1α gene, and this frameshift mutation leads to the synthesis of a protein of 315 amino acids lacking most of the transactivation domain. We have shown that P291fsinsC-HNF-1α acts in a dominant-negative manner (5,6). To understand the molecular mechanisms by which mutations in the HNF-1α gene lead to diabetes, we generated β-cell–targeted transgenic mice overexpressing dominant-negative mutant HNF-1α (7,8). The transgenic mice developed diabetes with impaired glucose-stimulated insulin secretion, as seen in patients with MODY3. In addition, a significant reduction of β-cell number and proliferation rate was observed in these transgenic mice. A reduction in β-cell mass and a lack of accelerated proliferation rate in the presence of hyperglycemia have been reported in HNF-1α (−/−) knockout mice (9). These observations suggest that suppression of HNF-1α function might lead to reduced β-cell proliferation and β-cell number. Adult pancreatic β-cells have a relatively low mitotic index, but they can still proliferate after stimulation by several factors, including glucose itself, IGF-1, placental lactogen (PL), gastrin, hepatocyte growth factor (HGF), and growth hormone (10–15). The regulation of β-cell growth by HNF-1α, however, is not clear.

P291fsinsC–HNF-1α expression in INS-1 cells by a doxycycline-inducible system results in a defect in glucose transport, glycolysis, and mitochondrial function (6). Using this system, we report that dominant-negative suppression of HNF-1α in INS-1 cells strongly inhibited cell growth at G1 to S phase. cDNA array studies showed changes in the expression levels of several genes involved in cell growth and cell cycle. We further showed that addition of IGF-1 rescued the proliferation impairment of these cells.

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Our results suggest that HNF-1α is critical for the regulation of pancreatic β-cell growth by regulating IGF-1 gene expression and that IGF-1 might be a potential therapeutic target for the treatment of MODY3.

RESEARCH DESIGN AND METHODS

Cell culture and cell count. The establishment of INS-1 cells that overexpress wild-type (WT) HNF-1α (HNF1α#15 cells) and P291fsinsC-HNF-1α (HNF1α-P291fsinsC#32 cells) under the control of the reverse tetracycline-dependent transactivator has been described previously (6). The INS-r3 cell line, which expresses the reverse tetracycline-dependent transactivator, was used as control. Unless otherwise indicated, the cells were maintained in RPMI-1640 medium containing 11 mmol/l glucose, 150 μg/ml G418, and 100 μg/ml hygromycin. Doxycycline (500 ng/ml) was used to induce the expression of WT and P291fsinsC-HNF-1α. INS-1 cells (1×10^5 cells/10-cm dish) were cultured in medium with or without doxycycline for 24, 48, and 96 h, and cell numbers were counted.

[^{3}H]thymidine incorporation. Incorporation of [^{3}H]thymidine was used as an indicator of INS-1 cell proliferation, as described previously (11). HNF1α#15 cells and HNF1α-P291fsinsC#32 cells (5×10^4 cells/well) were cultured on 96-well plates, and HNF-1α was induced with doxycycline for 48 and 96 h. In the last 4 h, 5 μCi/ml [^{3}H]thymidine was added to monitor the degree of DNA synthesis. The cells were subsequently washed, harvested, and transferred to glassfiber filters. The incorporation of [^{3}H]thymidine into DNA was measured by liquid scintillation counting.

Protein immunoblotting. Immunoblotting procedures were performed as described previously (5) using enhanced chemiluminescence. Anti–HNF-1α monoclonal antibody was purchased from BD Transduction Laboratories (San Diego, CA).

Fluorescence-activated cell sorter analysis. Fluorescence-activated cell sorter (FACS) analysis was carried out as described before (16). In brief, HNF1α-P291fsinsC#32 cells, which had been cultured in the presence or absence of doxycycline for 48 and 96 h, were trypsinized and suspended in 1 ml of hypotonic fluorochrome solution (50 μg/ml propidium iodide in 0.1% sodium citrate containing 0.1% Triton X-100). The solution was incubated at 4°C for 1 h and analyzed with a FACSCalibur laser-based flow cytometer (Becton Dickinson, Immunocytometry Systems, Mountain View, CA). The cell cycle-phase distribution was analyzed with a software program Modfit (Becton Dickinson).

Screening for cDNA arrays. The Atlas rat 1.2 array (Clontech Laboratories, Palo Alto, CA), which contains 1,176 different clones, was used to test the gene expression patterns of P291fsinsC-HNF-1α induced/noninduced INS-1 cells. The screening of the array was performed according to the protocol described by the manufacturer. Briefly, total RNA was isolated from HNF1α-P291fsinsC#32 cells, cultured with or without doxycycline for 48 h, using Atlas Pure Total RNA Labeling kit (Clontech). cDNA was synthesized using 50 μg of total RNA in a 10-μl volume containing 50 mmol/l Tris-HCl (pH 8.3), 75 mmol/l KCl, 3 mmol/l $MgCl_2$, 0.5 mmol/l dNTP mixture except dATP, 5 mmol/l dithiothreitol, gene-specific cDNA synthesis primer mix (Clontech), and Moloney murine leukemia virus reverse transcriptase in the presence of 50 μCi [^{32}P]dATP (3,000 Ci/mmol; Amersham, Piscataway, NJ). The membrane was hybridized, washed, and exposed to a phosphoimager screen. The experiment was repeated twice, and averaged images were analyzed with AtlasImage 1.5 software (Clontech) using the tubulin gene as an internal control.

Northern blotting. Total RNA from doxycycline-induced and noninduced HNF1α-P291fsinsC#32 cells was extracted with TRIzol reagent (Gibco BRL, Rockville, MD). mRNA was prepared using Oligotex-dT30(super) mRNA purification kit (TaKaRa, Shiga, Japan). One microgram of mRNA was separated on 1% agarose gel and blotted onto Hybond N^+ membrane (Amersham). The membranes were prehybridized and then hybridized to ^{32}P-labeled probes in QuikHyb hybridization solution (Stratagene, La Jolla, CA). The following cDNAs were provided by the indicated investigators: murine cyclin D2 (Dr. H. Matsusima, Nippon Roche Research Institute, Tokyo), murine cyclin A and human p27 (Dr. H. Kiyokawa, University of Illinois, Chicago, IL), murine p19 (Dr. C. Sherr, Howard Hughes Medical Institute, Memphis, TN), and murine p21 (Dr. B. Vogelstein, Johns Hopkins Oncology Center, Baltimore, MD). cDNA probes for cyclin E, cdk4, IGF-1, migration inhibitory factor (MIF), and glyceraldehyde-3-phosphate dehydrogenase were prepared by RT-PCR and confirmed by sequencing.

Blockade of MIF activity by anti-MIF neutralizing antibody. INS-1 cells (1×10^5) were plated on each well of 96-well plates for 24 h then cultured in RPMI-1640 medium with 11 mmol/l glucose, 0.1% BSA, 5 μCi/ml [^{3}H]thymidine, together with a neutralizing anti-murine MIF monoclonal antibody (14.15.5, IgG1 subclass) (17,18) or an isotype IgG1 control (clone 11711.11, R&D Systems, Minneapolis, MN) for 24 h. The cells were harvested, and [^{3}H]thymidine incorporation was quantified.

Effect of IGF-1 on INS-1 cell growth. HNF1α-P291fsinsC#32 cells (1×10^5) were synchronized in 2.5 mmol/l glucose in 0.1% BSA for 12 h. Then the cells were cultured in RPMI-1640 with the indicated concentration of glucose and IGF-1 in the presence of 5 μCi/ml [^{3}H]thymidine for 24 h. After the culture, [^{3}H]thymidine incorporation was determined as described above.

Statistical analysis. All data were expressed as means ± SE. Differences between groups were examined for statistical significance using the Student's *t* test. $P < 0.05$ denoted the presence of a statistically significant difference.

RESULTS

Dominant suppression of HNF-1α in INS-1 cells results in cell growth inhibition. We first examined the effect of P291fsinsC-HNF-1α on INS-1 growth. HNF1α-P291fsinsC#32 cells (1×10^5) were cultured in the presence or absence of doxycycline for 24–96 h. The cell number did not differ between noninduced and induced cells at 24 and 48 h (Fig. 1*A*). However, the number of P291fsinsC-HNF-1α induced cells was significantly reduced compared with noninduced cells at 96 h (5.5×10^5 cells vs. 15×10^5 cells; $P < 0.001$). INS-1 cell proliferation was further determined by [^{3}H]thymidine incorporation. The proliferation rate of doxycycline-induced HNF1α-P291fsinsC#32 cells at 48 and 96 h was reduced to 63.2% ($P < 0.001$) and 41.9% ($P < 0.001$), respectively, relative to noninduced cells (Fig. 1*B*). The growth of INS-r3 cells, which express the reverse tetracycline-dependent transactivator alone, was not affected by addition of doxycycline, suggesting that doxycycline itself does not alter INS-1 cell proliferation (Fig. 1*B*). HNF1α-P291fsinsC#32 cells and HNF1α#15 cells expressed >50-fold of HNF-1α over the endogenous protein (6). However, induction of WT-HNF-1α did not reduce INS-1 cell proliferation. These results strongly suggest that the impaired growth of P291fsinsC#32 cells is a specific effect of dominant-negative HNF-1α. Doxycycline at 10 ng/ml induced a small amount of P291fsinsC-HNF-1α, the expression level of which was similar to that of endogenous HNF-1α (Fig. 1*C*). At that concentration, [^{3}H]thymidine incorporation of P291fsinsC-HNF-1α INS-1 cells was significantly reduced to 84.0% ($P < 0.05$) of that of noninduced cells (Fig. 1*D*), suggesting that even a small increase of mutant HNF-1α might impair β-cell proliferation.

Growth impairment is at the transition of G1 to S phase of the cell cycle. FACS analysis was carried out to determine the growth arrest phase of the cell cycle. The cell cycle is divided into five different phases: G0 (reversible quiescence), G1 (first gap phase), S (DNA synthesis), G2 (second gap phase), and M (mitosis phase) (19). Overexpression of P291fsinsC-HNF-1α for 48 h significantly increased INS-1 cells at G0/G1 phase (induced, 72.4%; noninduced, 65.2%; $P < 0.01$) (Fig. 2*A* and *B*), and cells in this phase increased at 96 h (induced, 77.1%; noninduced, 67.3%; $P < 0.01$) (Fig. 2*C* and *D*). In contrast, cells at the S phase were significantly decreased (48 h: induced, 19.2%; noninduced, 25.4%; $P < 0.01$; 96 h: induced, 16.5%; noninduced, 23.2%; $P < 0.01$). These data suggest that the growth inhibition by P291fsinsC-HNF-1α occurred at G1 phase or at the transition phase from G1 to S phase of the cell cycle.

Identification of genes responsible for cell growth impairment. To identify the genes that could be responsible for the cell growth impairment, we studied the gene expression profile of induced and noninduced HNF1α-

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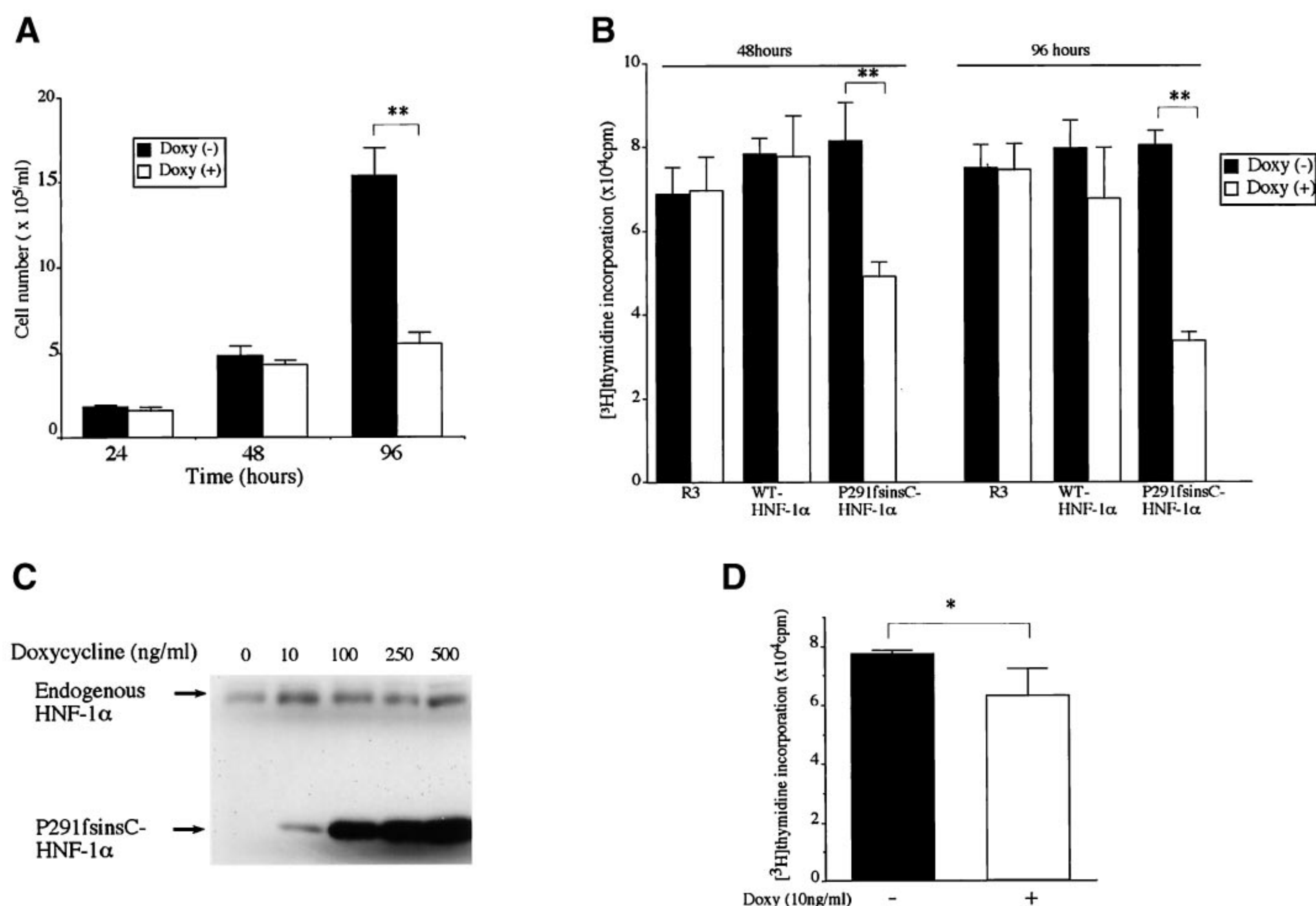

FIG. 1. Effects of P291fsinsC-HNF-1α on cell growth. *A*: Number of HNF-1α-P291fsinsC#32 INS-1 cells cultured with or without 500 ng/ml doxycycline (Doxy) for 24, 48, or 96 h. Data are means ± SE of five independent counts. **$P < 0.001$. *B*: [^{3}H]thymidine incorporation in induced and noninduced INS-r3, HNF-1α#15, and HNF-1α-P291fsinsC#32 cells for 48 or 96 h. Data are means ± SE of three independent experiments performed in triplicate. **$P < 0.001$. *C*: Expression of endogenous HNF-1α and P291fsinsC-HNF-1α in HNF-1α-P291fsinsC#32 cells cultured with different concentrations of doxycycline for 48 h. *D*: [^{3}H]thymidine incorporation of HNF-1α-P291fsinsC#32 cells cultured with or without 10 ng/ml doxycycline for 48 h. Data are means ± SE of three experiments performed in triplicate. *$P < 0.01$.

P291fsinsC#32 cells with cDNA array. The expression of a majority of the genes analyzed was not changed. Of the 1,176 genes surveyed, 31 genes (2.6%) were reduced and 9 gene transcripts were increased (more than twofold) in P291fsinsC-HNF-1α–induced cells compared with noninduced cells. Because HNF-1α is a transcriptional activator, the upregulation of genes in HNF1α-P291fsinsC#32 cells is probably a secondary effect. The genes with altered expression levels after induction are listed in Table 1. Aldolase B gene expression was reduced in HNF1α-P291fsinsC#32 cells as shown previously (6). Cyclin D and E are cell cycle regulatory molecules at G1/S phase (19). Cyclins assemble with cyclin-dependent kinases (CDKs), such as cdk4. CDK inhibitors also play important roles in cell cycle control. The expression of cyclin D2, which is a major form of cyclin D in INS-1 cells (20), was increased in HNF1α-P291fsinsC#32 cells (Table 1, Fig. 3). Cyclin E is induced after cyclin D during the progression through G1 and makes a transition from G1 to S phase. The expression of cyclin E was reduced in HNF1α-P291fsinsC#32 cells. p27 is a CDK inhibitor and is implicated in mediating growth inhibitory signals. The level of p27 was increased in P291fsinsC-HNF-1α–induced cells. Cyclin A is required for S phase transition. Cyclin A RNA level was similar between noninduced and induced cells. The expression level of other CDK inhibitors (p21 and p19) and cdk4 was not affected (Fig. 3).

The cell cycle is controlled by growth factors, and several growth factors have been implicated in the regulation of pancreatic β-cell proliferation. For example, IGF-1 (11), IGF-2 (21), PL (12), gastrin (13), HGF (14), and leukemia inhibitory factor (22) have been shown to increase the number of β-cells in vivo. The expression levels of IGF-2, PL, and HGF were not changed by induction of P291fsinsC-HNF-1α (data not shown). Despite the impaired growth of HNF1α-P291fsinsC#32 cells, expression levels of gastrin (twofold) and leukemia inhibitory factor (twofold) were increased (Table 1). Upregulation of these growth factors may be a compensatory effect and the cause of increased expression of cyclin D2. In contrast, IGF-1 mRNA was downregulated in HNF1α-P291fsinsC#32 cells (Table 1, Fig. 3). HNF-1α is known to activate the transcription of the IGF-1 gene (23,24). IGF binding proteins (IGFBPs) are also involved in the regulation of cell proliferation by binding IGFs (25). The expression levels of IGFBP-1, -2, and -3 were similar in induced and noninduced HNF1α-P291fsinsC#32 cells (data not shown). It has been indicated that ribosomal protein levels are decreased in quiescent cells in comparison with

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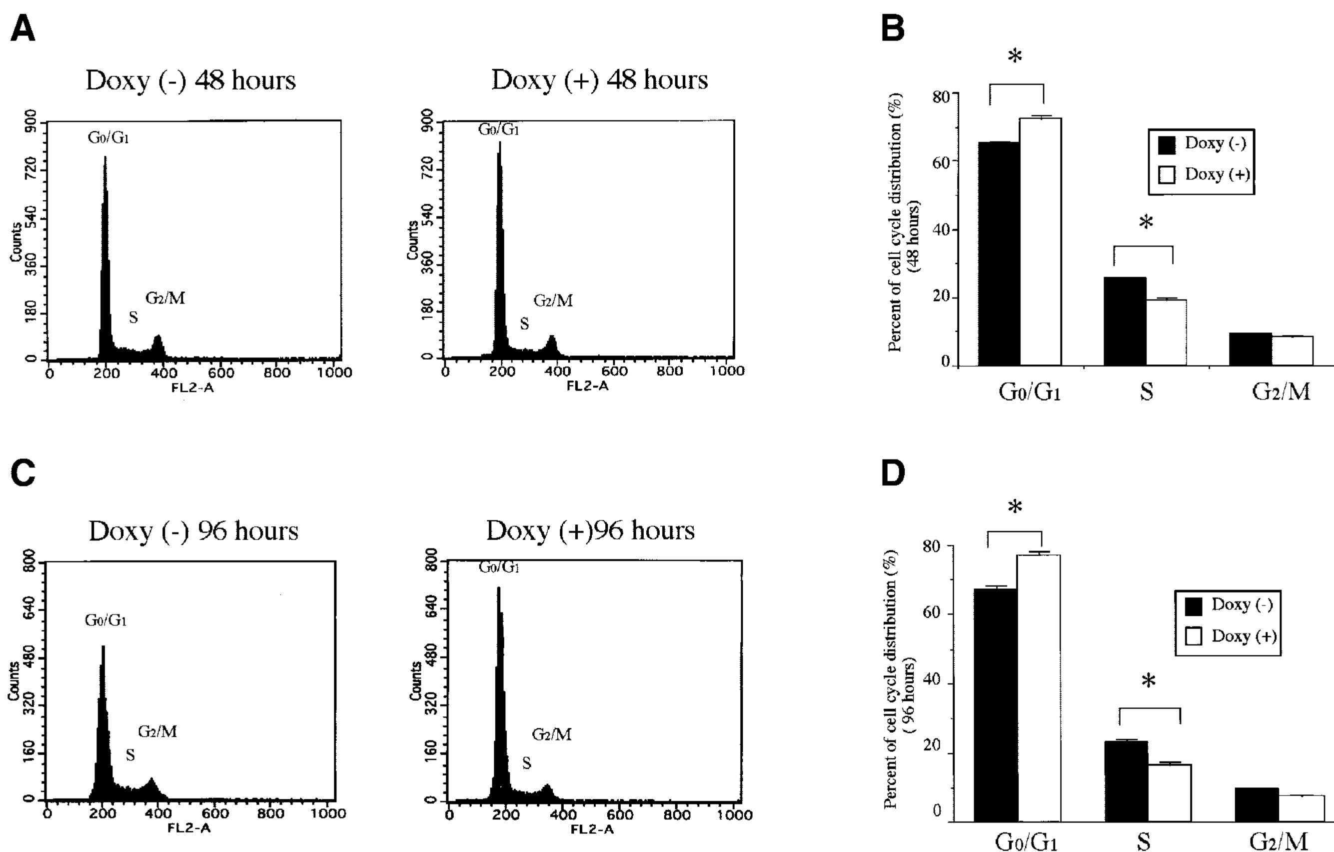

FIG. 2. Cell cycle distribution of induced and noninduced INS-1 cells. HNF-1α-P291fsinsC#32 cells cultured with or without doxycycline for 48 or 96 h were assessed for cell cycle distribution by FACS (*A* and *C*), and the cell cycle phase distribution was analyzed by Modfit software program (*B* and *D*). Data are means ± SE of three experiments performed in triplicate. *$P < 0.01$.

actively proliferating cells (26,27). The expression levels of several ribosomal proteins were decreased in HNF1α-P291fsinsC#32 cells. Macrophage MIF is a proinflammatory cytokine, but it is also associated with cell proliferation (17,28). MIF expression was reduced in HNF1α-P291fsinsC#32 cells. These results suggest that reduced expression of MIF and IGF-1 genes might be associated with the impaired proliferation of HNF1α-P291fsinsC#32 cells.

MIF is not involved in growth impairment of INS-1 cells. Previous studies have shown that MIF has cell growth–promoting (17,28) or growth-inhibitory effects (29). We examined the role of MIF in INS-1 cell proliferation by using a neutralizing antibody to block endogenous MIF action (17,18). [^{3}H]thymidine incorporation was fourfold higher in INS-1 cells treated with 11 mmol/l glucose than in those treated by 2.5 mmol/l glucose ($P < 0.001$; Fig. 4). Normal IgG1 had no effect on cell proliferation, but addition of anti-MIF monoclonal antibody further enhanced the proliferation of glucose-stimulated INS-1 cells by twofold ($P < 0.001$). It has been reported that MIF inhibits cell growth by interacting with Jun activation domain-binding protein (29). The mechanisms by which blockade of MIF action in INS-1 cells promotes growth are unknown. However, these data suggest that downregulation of the MIF gene is not involved in the growth impairment in HNF1α-P291fsinsC#32 cells.

IGF-1 rescues the proliferation impairment of HNF1α-P291fsinsC#32 cells. We examined whether IGF-1 can rescue impaired cell proliferation of HNF1α-P291fsinsC#32 cells. Cells were starved at 2.5 mmol/l glucose and then stimulated with 6 or 11 mmol/l glucose. At these concentrations, glucose effectively increased the cell proliferation rate in the absence of doxycycline (6 mmol/l: 2.6-fold, $P < 0.001$; 11 mmol/l: 4.5-fold, $P < 0.001$, compared with 2.5 mmol/l glucose; Fig. 5). In the presence of 6 mmol/l glucose, IGF-1 at 0.1–100 nmol/l increased [^{3}H]thymidine incorporation into noninduced HNF1α-P291fsinsC#32 cells (2.7- to 3.8-fold greater than 6 mmol/l glucose alone; $P < 0.001$; Fig. 5*A*). Similar results were obtained when cells were cultured at 11 mmol/l glucose (Fig. 5*B*). In the presence of doxycycline, no stimulatory effect was noted for 6 mmol/l glucose compared with 2.5 mmol/l glucose, and [^{3}H]thymidine incorporation in HNF1α-P291fsinsC#32 cells was significantly decreased ($P < 0.001$, compared with noninduced HNF1α-P291fsinsC#32 cells). However, IGF-1 at 0.1–100 nmol/l significantly increased [^{3}H]thymidine incorporation in P291fsinsC-HNF-1α–induced INS-1 cells ($P < 0.001$). The addition of IGF-1 in the presence of 11 mmol/l glucose almost completely rescued the reduced [^{3}H]thymidine incorporation of HNF1α-P291fsinsC#32 cells. These results strongly suggest that IGF-1 restores β-cell proliferation caused by dominant suppression of HNF-1α in INS-1 cells.

DISCUSSION

Previous studies reported that pancreatic β-cell mass and β-cell proliferation rate were reduced in several animal models of HNF-1α diabetes (7–9). To clarify the mecha-

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TABLE 1
Differentially regulated genes in doxycycline-induced and noninduced INS-1 cells

GenBank accession no.	Gene/protein	Ratio of Doxy (+) to Doxy (−)
Cell cycle–related proteins		
U20283	G1/S-specific cyclin E (CCNE)	0.5
D16308	G1/S-specific cyclin D2 (CCND2)	2.0
D83792	cyclin-dependent kinase inhibitor p27(p27KIP1)	2.0
Hormone and growth factors		
U62326	macrophage MIF	0.4
M15480	IGF-1B, IGF-1	0.7
M32748	leukemia inhibitory/cholinergic neuronal differentiation factor (LIF/DIF)	2.0
M31176	gastrin-releasing peptide precursor (GRP); neuromedin C	2.0
M38653	gastrin	2.0
Metablism and enzymes		
M10149	fructose-bisphosphate aldolase B (ALDOB); liver-type aldolase	0.3
M91597	NDK-B; nucleoside diphosphate kinase B; metastasis-reducing protein	0.4
U73174	glutathione reductase	0.4
U60882	protein arginine *N*-methyltransferase 1	0.5
X15030	cytochrome c oxidase, subunit Va, mitochondrial	0.5
S68245	carbonic anhydrase 4	0.5
J03752	microsomal glutathione S-transferase (GST12; MGST1)	0.5
Y00404	copper-zinc–containing superoxide dismutase 1 (Cu-Zn SOD1)	0.6
Translation		
X51707	S19; 40S ribosomal protein S19	0.3
K02933	40S ribosomal protein S17 (RPS 17)	0.3
X78327	ribosomal protein L13	0.4
X53504	ribosomal protein L12	0.4
X87106	ribosomal protein L10	0.5
M27905	60S ribosomal protein L21	0.5
M18547	40S ribosomal protein S12	0.5
X14210	ribosomal protein S4	0.6
X62146	ribosomal protein L11	0.6
DNA binding and repair		
U23769	LIM domain protein CLP36, homologous to rat RIL	0.3
M64986	high-mobility group protein 1	0.6
M57299	Y box–binding protein 1 (YBX1; YB1)	0.6
Ion and other channels		
M91597	CCHB3; calcium channel	0.3
D13985	chloride channel RCL1	0.7
AF007775	aquaporin (pancreas and liver; AQP 8)	2.2
Signal pathway–related proteins		
M17528	guanine nucleotide-binding protein G(I) alpha 2 subunit (GNAI2)	0.4
M35862	Mak; male germ cell-associated kinase	0.7
M16800	myelin-associated glycoprotein precursor (L-MAG/S-MAG)	2.0
Oncogenes and tumor suppressors		
X67788	ezrin; cytovillin 2; villin 2 (VIL2)	0.3
U49729	BAX-alpha	0.5
Receptors and receptor-associated proteins		
U60882	protein arginine *N*-methyltransferase 1	0.5
U41744	PDGF-associated protein	0.5
L31771	α-1D adrenergic receptor (ADRA1D)	2.0
L19660	gastric inhibitory polypeptide receptor precursor (GIP-R)	3.0

nism(s) underlying the reduction, we analyzed in the present study the cell growth of HNF1α-P291fsinsC#32 cells. Induction of P291fsinsC-HNF-1α but not WT-HNF-1α resulted in impairment of cell growth at the G1/S phase. The results are in accordance with reduced BrdU incorporation in pancreatic β-cells of transgenic mice expressing the same mutant HNF-1α (7). Interestingly, there was a reduction in thymidine incorporation in HNF1α-P291fsinsC#32 cells with only 10 ng/ml doxycycline (Fig. 1*D*), the dose inducing HNF-1α mutant to a level equivalent to that of endogenous WT-HNF-1α. This is the same situation as seen in patients with heterozygous HNF-1α mutations.

By screening mRNA levels of 1,176 genes, we identified downregulation of IGF-1 and cyclin E genes and upregulation of p27 gene in HNF1α-P291fsinsC#32 cells. Reduced expression of cyclin E and increased expression of p27 confirm our results that impairment of cell growth in HNF1α-P291fsinsC#32 cells occurs at the G1/S phase. Upregulation of p27 was also seen in INS-1 cells overexpressing an artificial dominant-negative mutant HNF-1α

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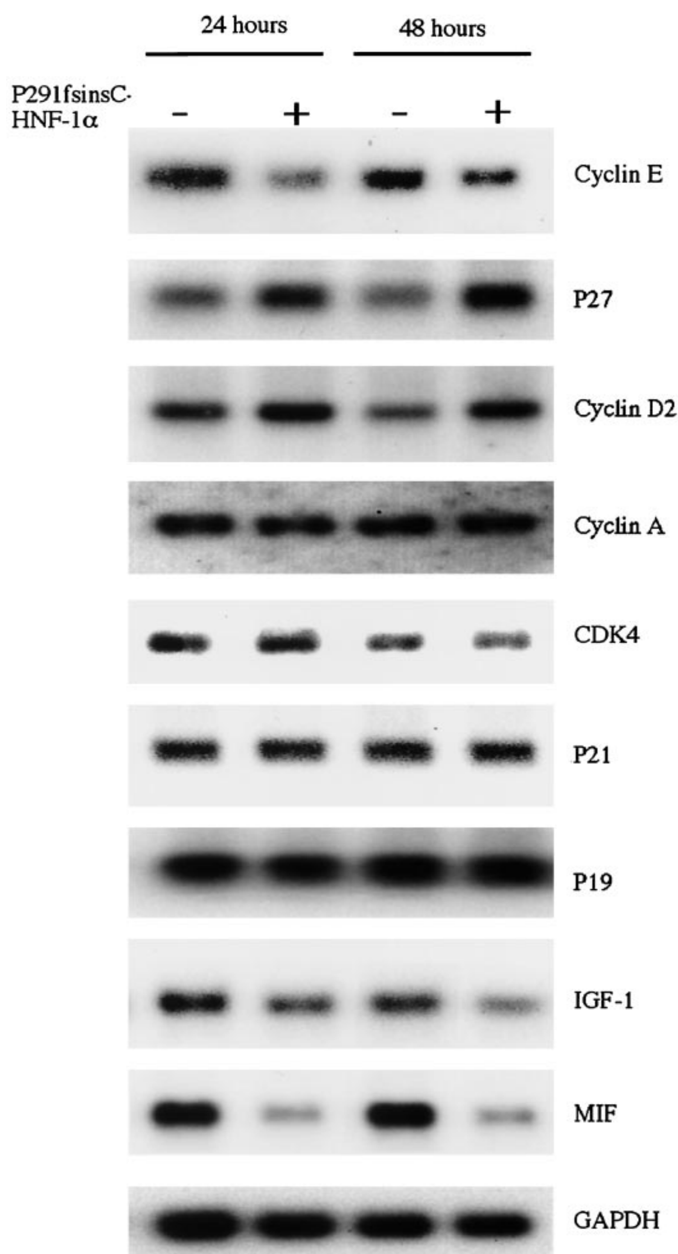

FIG. 3. Effects of P291fsinsC-HNF-1α on cell growth–related genes were examined by Northern blotting. mRNA (1 μg) was obtained from noninduced HNF-1α-P291fsinsC#32 cells or same cells induced by treatment with 500 ng/ml doxycycline for 24 or 48 h. mRNA was then transferred to membranes, and the expression of the genes was analyzed by hybridization with indicated cDNA probes. −, noninduced; +, induced.

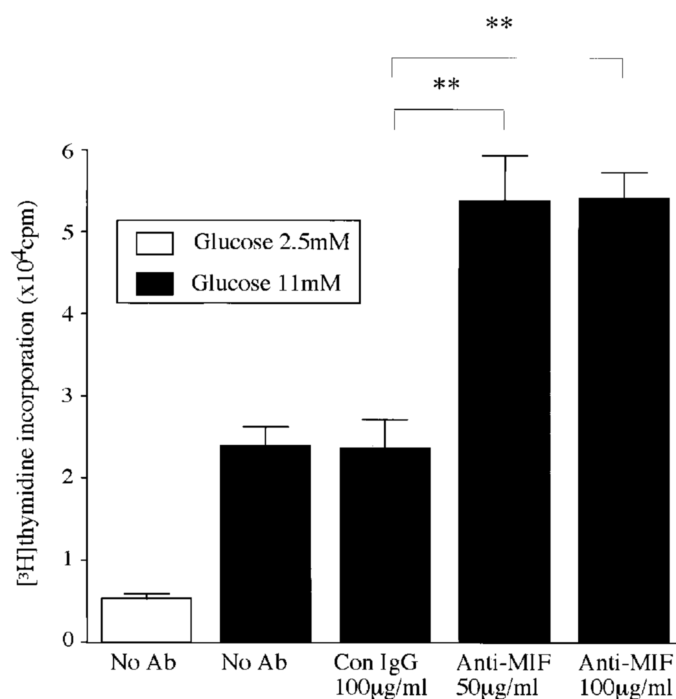

FIG. 4. Neutralization of MIF stimulates INS-1 cell proliferation. INS-1 cells (1×10^5) were synchronized by overnight incubation in serum-free RPMI-1640 medium with 2.5 mmol/l glucose and 0.1% BSA. Glucose (11 mmol/l) was added to synchronized cells with anti-MIF monoclonal antibody or an isotype control monoclonal antibody (Con IgG). Cell proliferation rate was assessed by [^{3}H]thymidine incorporation. Data are means ± SE of triplicate assays from three separate experiments. **P < 0.001. Ab, antibody.

(30). IGF-1 is a major growth factor for β-cell proliferation and is expressed in pancreatic islets (31,32). The expression of IGF-1 gene is regulated by HNF-1α (11,23,24,33). The IGF-1/IGF-1 receptor/insulin receptor substrate-2 axis has been shown to be important in the regulation of β-cell mass/compensation (34). We believe that reduced expression of IGF-1 could explain, at least in part, the impaired cell proliferation in HNF1α-P291fsinsC#32 cells. Previous studies suggested that p27 is a downstream target of the phosphatidylinositol (PI) 3-kinase/Akt pathway and IGF-1–mediated activation of PI 3-kinase results in downregulation of p27, increased cyclin E activity, and enhanced cell proliferation at G1/S phase in skeletal muscle satellite cells (35). Forkhead transcription factor FKHR-L1 is a downstream molecule of the PI 3-kinase/Akt signaling pathway. Activation of PI 3-kinase represses the transcription of p27 through inhibition of FKHR-L1 in Ba/F3 cells (36). Downregulation of IGF-1 gene expression might cause upregulation of p27 and cell growth arrest via the IGF-1/insulin receptor substrate/PI 3-kinase/Akt/FKHR signaling pathway in HNF1α-P291fsinsC#32 cells. We found that addition of IGF-1 can partially (with 6 mmol/l glucose) or completely (with 11 mmol/l glucose) rescue the proliferation defect caused by suppression of HNF-1α activity. In contrast, addition of gastrin (0.1–10 nmol/l) did not promote cell proliferation of INS-1 cells (data not shown). Twofold increase of gastrin expression in INS-1 cells might have only slight effect on cell proliferation. It remains to be established whether administration of IGF-1 can prevent the reduced β-cell proliferation or diabetes in P291fsinsC-HNF-1α transgenic mice.

MODY3 patients display progressive loss of β-cell function, as shown by age-related deteriorating glucose tolerance and increasing treatment requirements (1,37). It is not clear whether reduced β-cell growth and β-cell mass are implicated in human MODY3, but the failure of β-cell proliferation in patients with HNF-1α mutation may lead to progressive β-cell dysfunction and insulin deficiency. If this is the case, then IGF-1 could have therapeutic benefit in the treatment of MODY3.

We also found that the expression level of MIF is decreased in HNF1α-P291fsinsC#32 cells, suggesting that MIF is a novel target of HNF-1α. Downregulation of MIF expression might be involved in impaired insulin secretion

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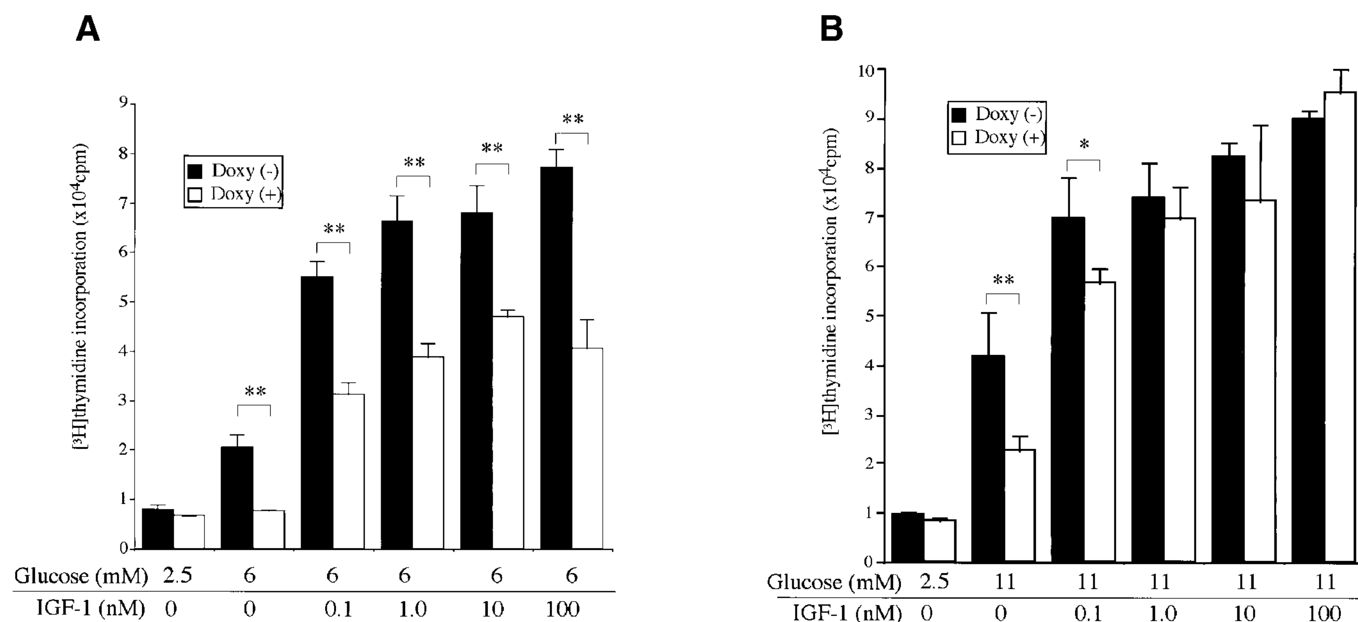

FIG. 5. Effects of IGF-1 on HNF-1α-P291fsinsC#32 cell proliferation. HNF-1α-P291fsinsC#32 cells (1×10^5) were cultured overnight in RPMI-1640 medium with 2.5 mmol/l glucose and 0.1% BSA, with or without 500 ng/ml doxycycline. Glucose (11 mmol/l) with different concentrations of IGF-1 was then added to the synchronized cells and incubated for 24 h. Cell proliferation rate was assessed by [^{3}H]thymidine incorporation. Data are means ± SE of triplicate assays from three independent experiments. *$P < 0.01$; **$P < 0.001$. Doxy, doxycycline.

in P291fsinsC-HNF-1α–expressing INS-1 cells. MIF and insulin co-localize within the secretory granules of pancreatic β-cells, and MIF regulates insulin release in an autocrine manner (18). Immunoneutralization of MIF reduced both the first and second phases of glucose-stimulated insulin secretion in rat islets (18). However, addition of MIF alone may not be sufficient for restoring the impaired insulin secretion in HNF1α-P291fsinsC#32 cells because suppression of HNF-1α has multiple consequences for β-cells, including glucose transport, glycolysis, and mitochondrial oxidation (6).

In summary, our results indicate that HNF-1α is critical for β-cell growth by regulating IGF-1 actions. Additional studies are necessary to elucidate the downstream signaling pathway of IGF-1. IGF-1 might be beneficial for the treatment of MODY3 or perhaps even other forms of diabetes.

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