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Prevention of Reg I-induced β-cell apoptosis by IL-6/dexamethasone through activation of *HGF* gene regulation

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ABSTRACT

Reg (regenerating gene) product, Reg protein, is induced in pancreatic β-cells and acts as autocrine/paracrine growth factor for regeneration via the cell surface Reg receptor. However, high concentrations of Reg I protein induced β-cell apoptosis. In the present study, we found that hepatocyte growth factor (HGF) attenuated the β-cell apoptosis induced by the high concentrations of Reg I protein and that the combined stimulation of interleukin-6 (IL-6) and dexamethasone (Dx) induced the accumulation of *HGF* mRNA as well as *Reg I* mRNA in β-cells. The accumulation of the *HGF* mRNA was caused by the activation of the *HGF* promoter. Deletion analysis revealed that the region of −96 to −92 of the *HGF* gene was responsible for the promoter activation by IL-6 + Dx. The promoters contain a consensus transcription factor binding sequence for signal transducer and activator of transcription (STAT). Site-directed mutations of STAT-binding motif in the region markedly attenuated the *HGF* promoter activity. Chromatin immunoprecipitation assay showed that STAT3 is located at the active *HGF* promoter in response to IL-6 + Dx stimulation. These results strongly suggest that the combined stimulation of IL-6 and glucocorticoids induces the activation of both *Reg* and *HGF* genes and that the anti-apoptotic effects of HGF against the Reg I-induced apoptosis may help β-cell regeneration by Reg I protein.

1. Introduction

A limited capacity for regeneration in adult β cells is thought to be a predisposing factor for the development of diabetes [1–3]. Thus, strategies for introducing the replication and growth of the β cells mass are important for the prevention and/or treatment of diabetes [2]. We established a model for islet regeneration in 90% depancreatized rats by the administration of poly(ADP-ribose) polymerase (PARP) inhibitors such as nicotinamide (NA) and 3-aminobenzamide (3AB) [4]. By screening a regenerating islet-derived cDNA library, we isolated a novel gene, *Reg* (regenerating gene), specifically expressed in regenerating islets [5] and found that both rat and human Reg protein (rat Reg I and human REG Iα [6,7]) induced the proliferation of β cells to ameliorate the diabetes of 90% depancreatized rats [8] and of non-obese diabetes (NOD) mice [9,10]. Recently, we revealed that the *Reg I* gene transcription was activated by the combined addition of interleukin-6 (IL-6) and dexamethasone (Dx) in β cells and that PARP inhibitors such as NA and 3AB further enhanced the expression [11]. We also found that high concentrations of Reg I protein (300–1000 nM) induced β cell apoptosis via Reg receptor [12]. Thus, we assumed that the Reg–Reg receptor system could regulate both the proliferation and apoptosis of pancreatic β cells to maintain the insulin-producing cell mass by controlling the concentrations of Reg I protein [2].

Accumulating evidence indicates that hepatocyte growth factor (HGF) is an anti-apoptotic as well as a mitogenic and insulinotropic factor for pancreatic β cells [13–16]: HGF was reported to protect insulin-producing rat RINm5F β cells against free fatty acid-induced apoptosis

Abbreviations: 3AB, 3-aminobenzamide; ChIP, Chromatin immunoprecipitation; Dx, dexamethasone; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GATA, transcription factor; HGF, hepatocyte growth factor; HSF-2, heart shock factor 2; IL-6, interleukin-6; NA, nicotinamide; NOD mouse, non-obese diabetic mouse; Nrf-2, nuclear respiratory factor 2; PARP, poly(ADP-ribose) polymerase; Reg, regenerating gene; RT-PCR, reverse transcription polymerase chain reaction; STAT, signal transducer and activator of transcription; WST-1, 4[-3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate

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by counteracting oxidative stress [13]. From the findings, we conceived a possibility that HGF inhibits the high concentration of Reg I protein-induced apoptosis. In the present study, we tested the possibility and found that HGF inhibited the pancreatic β cell apoptosis induced by the high concentrations of Reg I protein and that the combined stimulation of IL-6 and Dx induced the *HGF* transcription via signal transducer and activator of transcription (STAT) binding sequence in pancreatic β cells.

2. Materials and methods

2.1. Cell culture

RINm5F cells, a rat β cell line, were maintained as described [11,12,17–19]. For the stimulation experiments, cells were treated with 20 ng/ml human IL-6 (Genzyme, Cambridge, MA), 100 nM Dx (Sigma-Aldrich, St. Louis, MO), 10 mM NA, 1 mM 3AB, 25 ng/ml recombinant human HGF (Genzyme), 1000 nM rat Reg I protein or combinations thereof [11]. Pancreatic islets were isolated by collagenase digestion method [20,21] from male Wistar rats (200–300 g), transferred to 6-well culture dishes in groups of 200 islets, and stimulated by the combined addition of IL-6 + Dx. 1.1B4 cells, a human pancreatic β cell line, were purchased from European Collection of Cell Culture (Salisbury, UK) and were maintained as described [22].

2.2. Measurement of apoptosis

Rat Reg I protein was expressed in *Pichia pastoris* and purified to homogeneity using cation exchange column chromatography as described [11,12,17]. RINm5F cells were cultured in RPMI 1640 with 1% FCS in the presence of 1000 nM rat Reg I protein with other stimulants for 24 h. Apoptosis was detected by the TUNEL method using an apoptosis screening kit (Wako Pure Chemical, Osaka, Japan) as described [12,19].

2.3. Measurement of viable cell numbers by tetrazolium salt cleavage

After a 24-h incubation of the RINm5F cells (5×10^4 cells/well) in RPMI 1640 with 1% FCS in the presence of stimulants, a solution containing 4[-3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1, F. Hoffmann-La Roche Ltd, Basel, Switzerland) was added to the medium, and the cells were incubated for another 30 min as described [11,12,23].

2.4. Induction of HGF messenger RNA

After a 24-h incubation with various stimulants, cells were harvested, and total RNA was prepared as described [19]. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using primers corresponding to nucleotides 1556–1575 and 2208–2227 of rat *HGF* mRNA [24], and 135–155 and 951–971 for rat glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA as described [18]. Real-time RT-PCR was performed as described [19,25]. In brief, the cDNA was synthesized from total RNA as template using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) for template of real-time PCR and real-time PCR was performed using Fast SYBR® Green Master Mix (Applied Biosystems) and Thermal Cycler Dice® Real Time System (Takara Bio Inc., Otsu, Japan). PCR primers corresponding to nucleotides 2145–2167 and 2235–2254 of human *HGF* mRNA (NM_000601), and 420–437 and 492–509 for human *β-actin* mRNA (NM_001101) were synthesized by NGRL (Sendai, Japan). Target cDNAs were cloned into pBluescript SK(-) plasmid (Stratagene, La Jolla, CA) and sequential 10-fold dilutions from 10^2–10^7 copies/μL were prepared. The serial dilutions were run to verify the specificity and to test the sensitivity of the SYBR Green-based real-time RT-PCR. *HGF* mRNA value was normalized to that of *β-actin* mRNA, which was used to account for differences in the efficiency of reverse transcription between samples.

2.5. Measurement of HGF protein in culture medium

RINm5F cells were cultured in RPMI 1640 with 1% FCS in the presence of stimulants for 24 h. The concentration of HGF protein in the medium was measured by using a rat HGF EIA kit (Institute of Immunology, Tokyo, Japan) according to the instructions of the supplier.

2.6. Construction of reporter plasmid and luciferase assay

A 1395-bp genomic fragment containing the 5′-flanking region of the rat *HGF* gene (−1336 to +59) [26] was inserted into pGL3-Basic vector (Promega, Madison, WI). Unidirectional deletions were made using a Deletion Kit for Kilo-Sequencing (Takara Bio Inc.). Mutants of potential binding sites for Stat and GATA factors (STAT-M1, STAT-M2, GATA-M) were constructed on pGL3 vector containing the *HGF* promoter by PCR. Promoter plasmid was transfected into RINm5F cells by using DMRIE-C (Invitrogen Corp., Carlsbad, CA) as described [11,18]. In brief, RINm5F cells were seeded at 1×10^5 cells per well in a 24-well dish in RPMI1640 supplemented with 10% FCS. After 24 h, the medium of each well was replaced with fresh medium containing stimulants and incubated further for 24 h. Cells were harvested in 1 ml of ice-cold PBS, washed twice with PBS, and extracts were prepared in extraction buffer (0.1 M potassium phosphate, pH 6.8/1 mM DTT). To monitor transfection efficiency, pCMV-SPORT- βgal plasmid (Invitrogen Corp.) was co-transfected in all experiments at a 1:15 dilution. Luciferase activity was measured using a PicaGene Luciferase assay system (Toyo-ink, Tokyo, Japan) and was normalized by the β-galactosidase activity as described [11,18].

2.7. Chromatin immunoprecipitation (ChIP) assays

After 24 h incubation with stimulants, RINm5F and 1.1B4 cells were processed for the ChIP assay as per the protocol described previously [18] using a ChIP-IT kit (Active Motif, Carlsbad, CA) following the manufacturer's instructions. The antibody used for the ChIP was STAT3 antibody (SantaCruz). The sequences for rat and human *HGF* gene promoters were as follows: sense primers, 5′-AGCTGGGATCT GTTGCTTGT-3′ (for rat) and 5′-GGGATCTGTTTGGTGCTGTT-3′ (for human) and antisense primers, 5′-ATGCCGGGCTGAAAGAATCC-3′ (for rat) and 5′-AGTTTGGTCACCCACATGGT-3′ (for human). The PCR products were analyzed on 2.5% agarose gel electrophoresis and documented.

3. Results

3.1. HGF attenuates the apoptosis induced by the high concentrations of Reg I protein

We previously reported that high concentrations of Reg I protein (300–1000 nM) induced the apoptosis of RINm5F β cells [12]. More higher serum concentrations of Reg family protein (over 10 μM) were reported in pathological conditions such as pancreatitis [27]. As shown in Fig. 1A, a single addition of IL-6, Dx, NA, nor 3AB alone did not inhibit the apoptosis induced by 1000 nM Reg I protein. On the other hand, the combined addition of IL-6 and Dx significantly inhibited the apoptosis. The addition of PARP inhibitors, NA or 3AB, to IL-6 + Dx was ineffective. Moreover, the addition of 2.5 and 25 ng/ml HGF showed an anti-apoptotic activity against the Reg I-induced apoptosis as well as the combined addition of IL-6 + Dx. In the presence of 1000 nM Reg I protein, the viable cell numbers were increased by the addition of IL-6 + Dx as well as by the addition of HGF (Fig. 1B). The combined addition of IL-6 + Dx and HGF did not increase significantly more WST-1 cleavage than the addition of IL-6 + Dx or HGF alone. These results

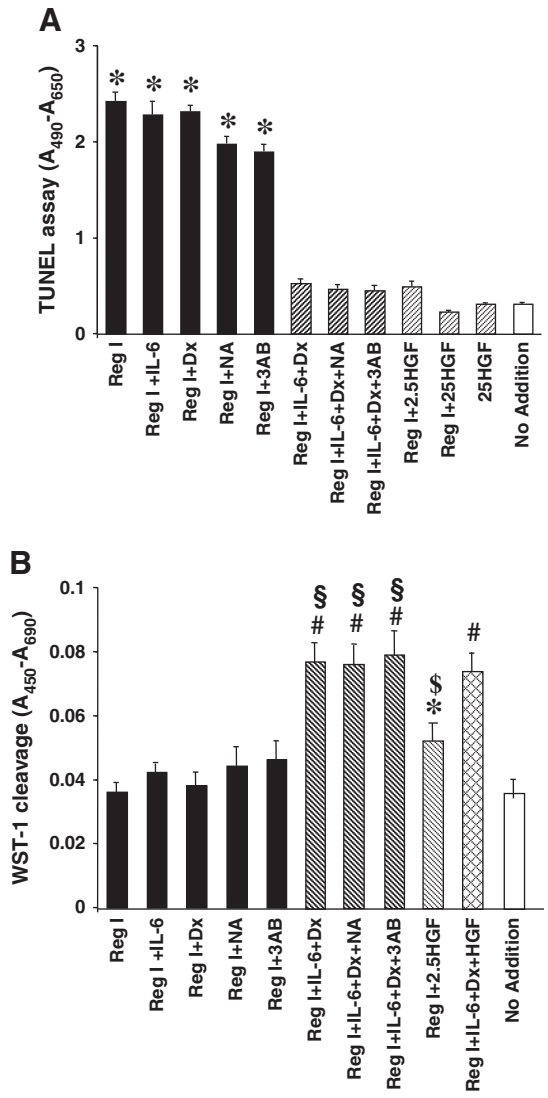

Fig. 1. HGF attenuates the apoptosis induced by the high concentrations of Reg I protein in RINm5F cells. Cells were incubated for 24 h with rat Reg I (1000 nM), IL-6 (20 ng/ml), Dx (100 nM), NA (10 mM), 3AB (1 mM), and HGF (2.5HGF; 2.5 ng/ml, 25HGF; 25 ng/ml). Values are mean ± S.E. of 7 experiments. (A) Apoptosis of RINm5F cells was quantitated by the TUNEL method. Statistical analysis was performed using student's *t* test. Asterisks indicate significant difference from the value with no addition at $P < 0.001$. (B) Cleavage of WST-1 by viable cells was measured. * and #, $P < 0.05$ and $P < 0.001$ when compared with Reg I, respectively. $ and §, $P < 0.05$ and $P < 0.001$ when compared with no addition, respectively.

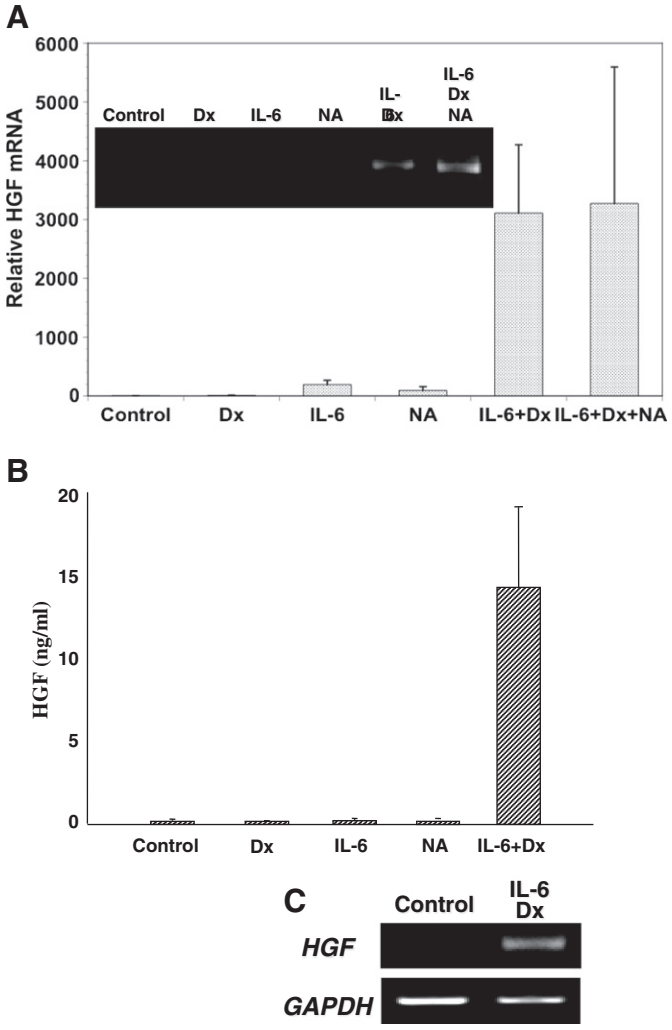

Fig. 2. Induction of *HGF* mRNA and protein by the combined addition of IL-6 + Dx. (A) Real-time RT-PCR of *HGF* mRNA in RINm5F cells. RINm5F cells were treated for 24 h with Dx, IL-6, NA or combinations thereof. All expression data were determined and shown as means ± S.E. (n = 3–6). A representative gel image of RT-PCR of *HGF* is shown as an *inset* figure. (B) Concentrations of HGF protein in the RINm5F medium were measured. The HGF concentrations of the RINm5F cell culture medium in no addition control, IL-6, and Dx were less than 0.2 ng/ml. Values are mean ± S.E. of triplicate experiments. (C) Induction of *HGF* mRNA in primary cultured islets by RT-PCR. Rat islets were incubated in the presence or absence of the mixture of IL-6 + Dx for 24 h. Relative *HGF* mRNA was increased to 4.59 ± 0.84 folds in IL-6 + Dx treated islets compared to that of untreated (control) islets (n = 3; P = 0.0276).

suggest that HGF is induced and secreted from pancreatic β cells by the combined addition of IL-6 + Dx to protect the Reg I-induced apoptosis.

3.2. Induction of HGF by the combined addition of IL-6 + Dx

We then analyzed the *HGF* gene expression in RINm5F cells by RT-PCR. The combined addition of IL-6 + Dx induced the expression of *HGF* mRNA (Fig. 2A), whereas the addition of IL-6, Dx, nor NA alone did not induce the *HGF* mRNA expression. A little increase of the mRNA was observed by the combined addition of IL-6 + Dx + NA. In the RINm5F culture medium, significantly higher concentrations of HGF were detected with the combined addition of IL-6 + Dx (Fig. 2B). We also performed RT-PCR analysis with isolated rat islets. The combined addition of IL-6 + Dx induced the *HGF* mRNA expression in islets and a representative photograph of RT-PCR was shown in Fig. 2C. These results indicate that pancreatic β cells express *HGF* mRNA and secrete HGF protein in response to the inflammatory stimuli such as the combined stimulation of IL-6 and glucocorticoids.

3.3. Activation of the HGF promoter by IL-6 + Dx

To determine whether the increase of *HGF* mRNA was caused by the activation of transcription, a 1395-bp fragment containing 1336 bp of the promoter region of the rat *HGF* gene was fused to the luciferase gene and transfected it into RIN5mF cells. The combined addition of IL-6 + Dx markedly increased the relative luciferase activity, whereas IL-6, Dx, NA, 3AB alone, IL-6 + NA, IL-6 + 3AB, Dx + NA nor Dx + 3AB was ineffective. The addition of PARP inhibitors to the combination of IL-6 + Dx did not additionally increase the promoter activity (Fig. 3A). These promoter assays revealed that the induction of the *HGF* mRNA occurred at the transcriptional level. We previously reported that the combined addition of IL-6/Dx induced the *Reg I* gene activation in RINm5F cells [11]. To investigate whether the *HGF* promoter was activated by Reg I protein in RINm5F cell culture medium, we added

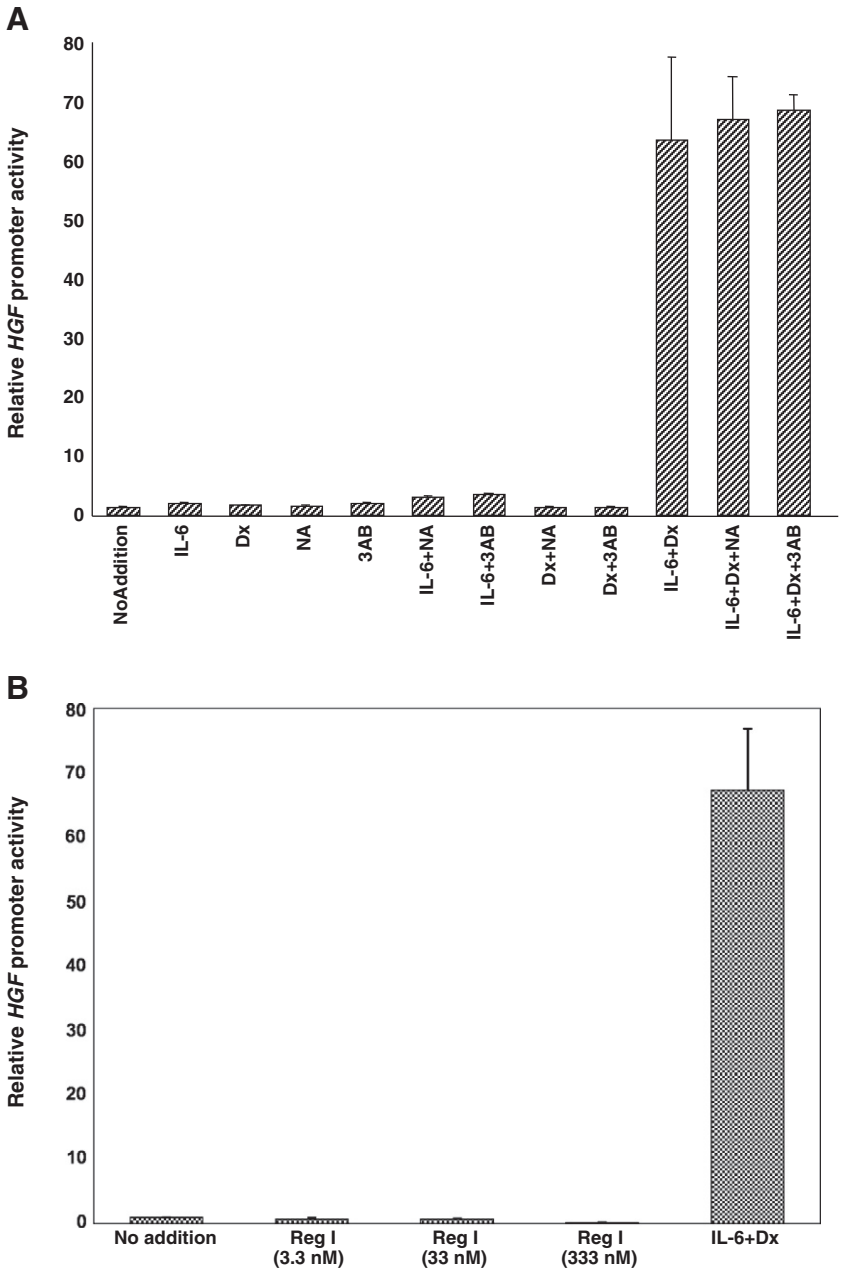

Fig. 3. The rat *HGF* promoter activity in RINm5F cells. (A) Induction of the *HGF* promoter by IL-6 + Dx. The promoter activity was normalized for variations in transfection efficiency using β-galactosidase activity as an internal standard and was expressed relative to the activity of pGL3-Basic. Values are mean ± S.E. of triplicate experiments. (B) Reg I protein did not induce the *HGF* promoter activation. The promoter activity was expressed relative to the activity of the reporter plasmid containing the full length of the *HGF* promoter (−1336 to +59) with no addition. Values are mean ± S.E. of triplicate experiments.

increasing concentrations of Reg I protein in the RINm5F culture medium and analyzed the *HGF* promoter activity. As shown in Fig. 3B, the addition of Reg I protein did not activate the *HGF* transcription. These results indicate that the *HGF* promoter activation by the combined addition of IL-6 + Dx is not mediated by Reg I protein in the medium and that the promoters for *HGF* and *Reg I* are independently activated by the combined addition of IL-6 + Dx in pancreatic β cells.

3.4. Localization of IL-6/Dx-responsive region in the HGF promoter

In order to identify the region necessary for the induction of the *HGF* gene by IL-6 + Dx, progressive deletions of the *HGF* promoter were performed. The deletion down to position −96 did not alter significantly the expression of the reporter gene induced by the combined addition of IL-6 + Dx, but an additional deletion to nucleotide −92 caused a remarkable decrease of promoter activity (Fig. 4A), indicating that the region from −96 to −92 is essential for the IL-6 + Dx-sensitive *HGF* promoter activities. The 5′-upstream region of the rat *HGF* gene (−148 to −1) was highly conserved in human (94.6%), mouse (98.0%), and chicken (79.2%) *HGF* genes. A computer-aided search for sequences similar to known *cis*-acting elements revealed that there were four possible binding sites for transcription factors: signal transducer activator of the transcription family (STATx), nuclear respiratory factor 2 (Nrf-2), GATA transcription factor (GATA), and heart shock factor 2 (HSF-2) (Fig. 4B).

3.5. STAT3 is a key factor for HGF transcription

To map out the *cis*-elements of *HGF* promoter that are responsible for the IL-6 + Dx-induced *HGF* transcription, site-directed mutagenesis

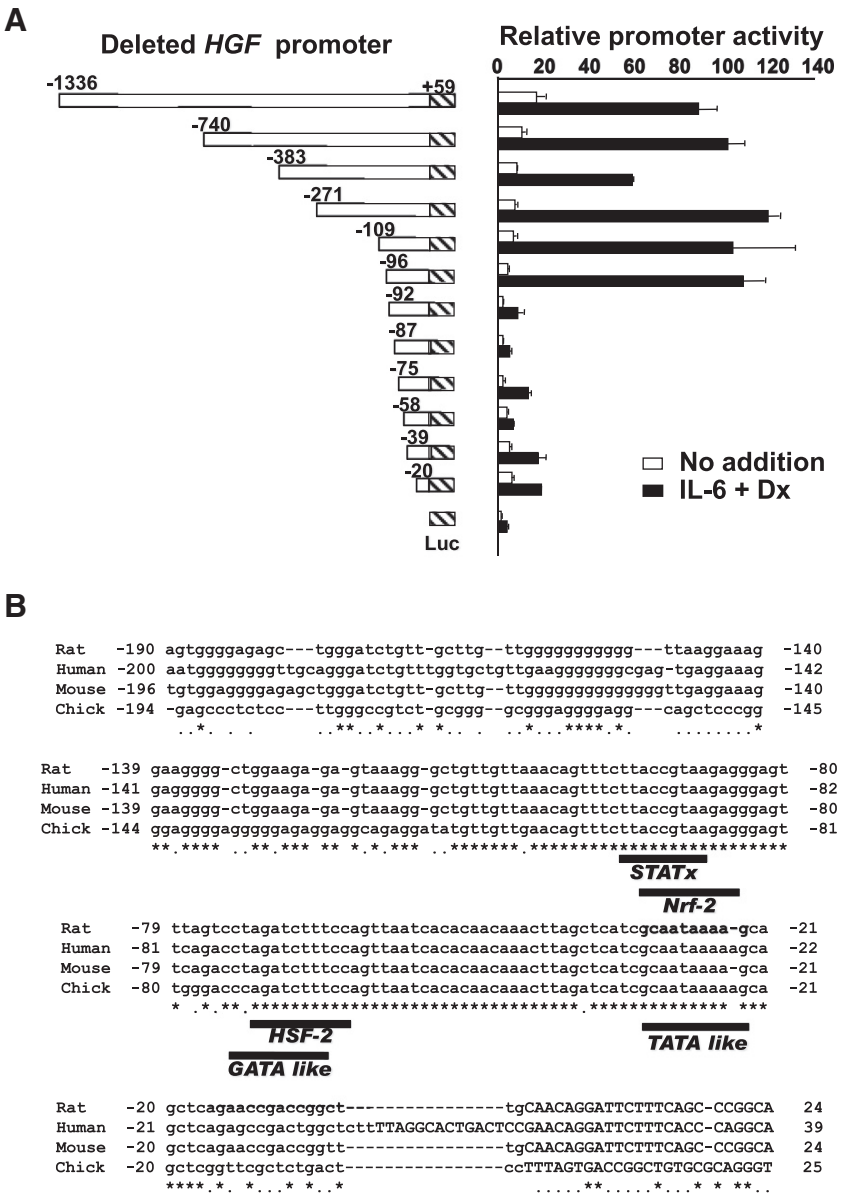

Fig. 4. Localization of IL-6/Dx-responsive region in the *HGF* promoter. (A) The promoter activity on deleted promoter of rat *HGF* gene. A series of luciferase constructs containing promoter fragments with various 5′-ends were transfected into RINm5F cells. The promoter activity was normalized and was expressed relative to the activity of pGL3-Basic. Values are mean ± S.E. of triplicate experiments. (B) Alignment of the 5′-flanking regions of rat (D14341), human (M75967), mouse (L25131), and chicken (AADN01083937) *HGF* genes. Residues that are conserved among all the genes are designated by asterisks. Residues that are conserved between 3 of 4 genes are designated by dots. Possible binding sites for transcription factors in the promoter region of −96 construct were underlined and labeled.

of the possible transcription factor binding sites was conducted within the luciferase construct of "−1336". STAT-M1, which altered STAT binding motif, STAT-M2, which altered binding motifs of STAT and Nrf-2, and GATA-M, which destroyed binding motifs of GATA and HSF-2 were constructed (Fig. 5A). As shown in Fig. 5B, STAT-M1 and STAT-M2 showed remarkable reductions in promoter activities and GATA-M showed almost the same promoter activity as that of −1136 construct. Comparison between STAT-M1 and STAT-M2 showed that STAT-M2 did not cause a further decrease of the *HGF* promoter activity, despite of additional mutation in Nrf-2 binding site, indicating that the STAT binding element (Figs. 4B and 5A) is the most important for the *HGF* transcriptional activation by the combined stimulation of IL-6 + Dx.

To investigate specific binding of STAT transcription factor (STAT3) to potential STAT binding element in vivo, we used ChIP assays to identify the binding state of STAT3 to the predicted STAT3 binding element in the *HGF* promoter in rat RINm5F and human 1.1B4 β cells after IL-6 + Dx treatment. As shown in Fig. 6A, the in vivo physical interaction of STAT3 to the rat *HGF* promoter in response to the IL-6 + Dx stimulation was demonstrated. Further increment of STAT3 binding by IL-6 + Dx + NA was not observed, which is completely consistent with the results of promoter assays. The induction of *HGF* mRNA by IL-6 + Dx was also demonstrated in human 1.1B4 β cells by real-time RT-PCR (Fig. 6B). Furthermore, in vivo physical interaction of STAT3 to the STAT binding element of the human *HGF* promoter was confirmed in human 1.1B4 pancreatic β cell line (Fig. 6C).

These data provided evidence that STAT3 bound to the *HGF* promoter and regulated transcription in pancreatic β cells in response to inflammatory stimulus such as IL-6 + Dx treatment, and this regulation was most likely mediated via the STAT binding site of *HGF* promoter.

4. Discussion

In the respect of the pathogenesis of type 1 diabetes, the extensive destruction and depletion of β cells, their treatment should be based

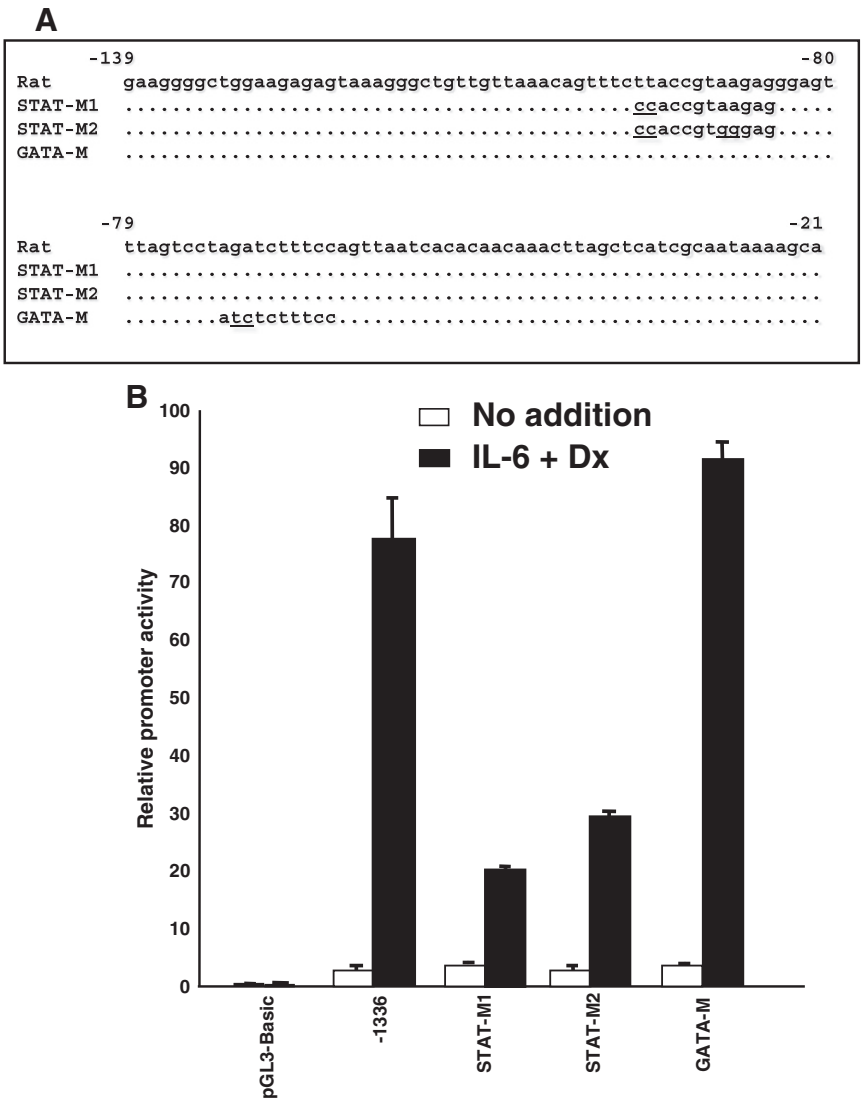

Fig. 5. Effects of site-directed mutations on the *HGF* promoter activity. (A) Three site-directed mutations, STAT-M1 (destroyed STAT), STAT-M2 (destroyed STAT and Nrf-2) and GATA-M (destroyed GATA and HSF-2), are indicated. (B) STAT-M1 markedly decreased the promoter activity induced by the combination of IL-6 + Dx. STAT-M2 did not cause a further decrease. GATA-M showed no significant change from the normal construct, −1336. Values are mean ± S.E. of triplicate experiments.

on the protection from further destruction and/or the proliferation or regeneration of β cells [2]. Reg I has been demonstrated to proliferate and/or regenerate β cells to ameliorate animal models of type 1 diabetes [8–10] and expected to apply to the treatment of human diabetes. In spite of its growth promoting activity on β cells, it was revealed that high concentrations of Reg I protein induced apoptosis of β cells [12]. For the application of Reg I protein to the treatment of human diabetes, it is therefore important to use only favorable characteristics of Reg I protein, controlling its bad side. In this study, we have demonstrated that HGF expression was induced in rat and human β cells by the combined stimulation of IL-6 + Dx and that HGF attenuated the high concentrations Reg I-induced apoptosis resulting in an increase of the β cell numbers in an autocrine and/or paracrine manner.

HGF is well known as a mesenchyme-derived multifunctional protein that plays a critical role in cell survival, proliferation, migration, and differentiation [28]. Earlier studies demonstrated that HGF receptor, a receptor tyrosine kinase encoded by c-*met* proto-oncogene, was expressed in various cells of epithelial origin including pancreatic β cells [29,30]. HGF is also shown to promote β cell differentiation, proliferation, and regeneration [29,30] and to increase the expression of *REG Iα* mRNA in human fetal pancreatic cells [31]. Transgenic mice overexpressing *HGF* in β cells resisted the diabetogenic effects of streptozotocin [14] and administrations of *HGF* expression vectors attenuated β cell destruction and hyperglycemia [32]. Intraperitoneal injection of HGF also exhibited a favorable effect for amelioration of hyperglycemia in diabetic mice receiving a marginal mass of islet grafts [33]. These observations led us to speculate that HGF might play a critical role in promoting β cell survival after injurious stimuli. When β cells receive injurious stimuli such as inflammation, *Reg I* gene transcription initiates via IL-6 + glucocorticoids [11] and islets could recover their cell mass by proliferation/regeneration of β cells by Reg I protein. Promoters for *HGF* gene were also activated by the combined addition of IL-6 + Dx in β cells. This means β cells produce both Reg I and HGF under inflammatory situations such as in 90% pancreatectomy and in autoimmune models of diabetes [5,34]. Thus, HGF could enhance the Reg I-induced proliferation/regeneration of β cells efficiently by inhibiting the high concentrations of Reg I-induced apoptosis. As HGF attenuates the Reg I-induced apoptosis, administration of HGF protein or introduction of *HGF* expression vector seems to have an immediate effect on efficient Reg I-induced β cell regeneration. However, exogenous HGF protein has a very short half-life in blood circulation, presumably owing to its rapid clearance by the liver in vivo [35]. Although recent progress of viral gene delivery systems [15] has enabled a prolonged expression of foreign genes, the foreign gene expression is

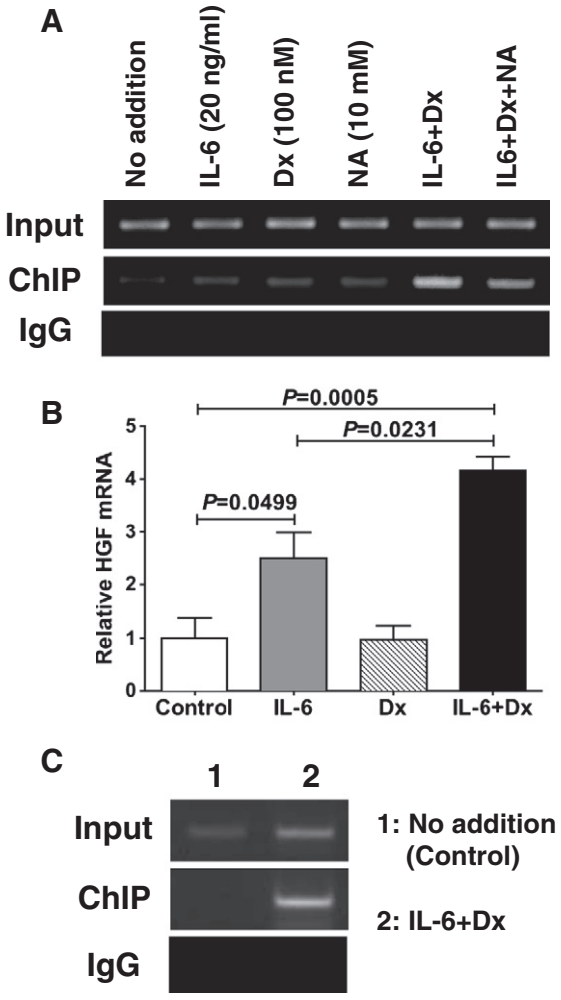

Fig. 6. STAT3 localization on the *HGF* promoters. (A) ChIP assay showing IL-6 + Dx increases in STAT3 binding to the *HGF* promoter. Nuclear proteins bound to genomic DNA in RINm5F cells were cross-linked and subjected to a ChIP assay using an antibody against STAT3, and oligonucleotide primers to PCR amplify the rat *HGF* promoter. Input row was the DNA fragment amplified from the extracts before immunoprecipitation. In the control immunoglobulin G (IgG) reaction, PCR was done in the eluates from beads collected after preclearing of these extracts with normal rabbit serum. (B) Real-time RT-PCR of human *HGF* mRNA. 1.1B4 cells were treated for 24 h with Dx, IL-6 or combinations thereof. All expression data were determined and shown as means ± S.E. (n = 4). (C) ChIP assay showing IL-6 + Dx increases in STAT3 binding to the *HGF* promoter. Nuclear proteins bound to genomic DNA in 1.1B4 cells were cross-linked and subjected to a ChIP assay using an antibody against STAT3, and oligonucleotide primers to PCR amplify the human *HGF* promoter. Input row was the DNA fragment amplified from the extracts before immunoprecipitation. In the control immunoglobulin G (IgG) reaction, PCR was done in the eluates from beads collected after preclearing of these extracts with normal rabbit serum.

still transient and repeated injections are usually difficult because of the strong antigenicity and cytotoxicity. Therefore, the activation of endogenous *HGF* gene should be ideal. Our findings that the combined stimulation of IL-6 + Dx induces endogenous genes for Reg I and HGF in β cells therefore may provide a new strategy for inducing the growth of the β cell mass to ameliorate diabetes mellitus.

Up-regulation/down-regulation of *HGF* mRNA and regulation of *HGF* promoter have been analyzed in a variety cells [36], however the induction *HGF* mRNA in pancreatic β cells has not been reported. In this study, we revealed the activation of the *HGF* gene in β cells by IL-6 + Dx possibly via involvement of STAT3. We have previously found that PARP inhibitors enhanced the *Reg I* gene expression induced by IL-6 + Dx [11]. However, the results of luciferase assays showed that PARP inhibitors did not activate *HGF* promoter. Furthermore, the *cis*-elements of *HGF* promoter were different from that of the *Reg I* promoter; Possible PARP binding sequence, which was found in rat *Reg I* promoter [11], is not found in *HGF* promoters (Fig. 4B). These results indicate that the transcriptional mechanisms of *HGF* and *Reg I* genes are distinct from each other, although their activation occurs simultaneously by IL-6 + Dx. The simultaneous expression of Reg I and HGF in β cells raises a hypothesis that Reg I, induced by IL-6 + Dx, but not IL-6 + Dx activates the *HGF* promoter or vice versa. The earlier study that HGF increased the *REG Iα* mRNA in human fetal pancreatic cells [29] seems to support this idea. However, in addition to the ineffectiveness of Reg I protein on the *HGF* promoter activation (Fig. 3B), rat *Reg I* promoter (−2303 to +25) was not activated by the stimulation of HGF (5 to 25 ng/ml) (data not shown), suggesting that these two genes are independently activated by IL-6 + Dx through different intracellular mechanisms.

In the present study, it was revealed that both Dx and IL-6 was required for *HGF* transcription in pancreatic β cells via STAT3 activation. Requirement of both IL-6 and Dx for STAT3 activation was also reported in rat serine protease inhibitor-3 gene transcription [37]. However, in most cases, IL-6 induces STAT(3) activation and glucocorticoids such as Dx suppresses STAT(3) activation [38]. Although why glucocorticoid is required for STAT3 activation in addition to IL-6 remains unknown, simultaneous transcriptional activation of *HGF* and *Reg I* by the combined addition of IL-6 and Dx in pancreatic β cells may be a novel β cell regeneration strategy/therapy.

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