Expression of Heparin-binding Epidermal Growth Factor-like Growth Factor during Pancreas Development

A POTENTIAL ROLE OF PDX-1 IN TRANSCRIPTIONAL ACTIVATION*

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The development of the pancreas appears to be regulated by various growth factors. We report here the expression of heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) in the developing pancreas. Immunostaining of fetal and neonatal rat pancreas, in which endocrine cells are visible as cell clusters often associated with primitive ducts or ductular cells, revealed that most of the cluster-forming cells and primitive ducts or ductular cells express HB-EGF protein. In contrast, the exocrine pancreas lacked HB-EGF expression. Based on findings that the expression pattern was similar to that of the homeodomain-containing transcription factor PDX-1 (IDX-1/STF-1/IPF1) and that the regulatory region of the HB-EGF gene contained sequences similar to the PDX-1-binding A element, we examined whether PDX-1 could be a potential activator of HB-EGF gene expression. The results of reporter gene analyses suggested that the HB-EGF gene promoter is PDX-1-responsive and that the activity of the promoter in pancreatic beta cell-derived βTC1 cells depends on the PDX-1 binding site-like sequences. Gel-mobility shift analyses using an anti-PDX-1 antibody indicated that PDX-1 is a specific and dominant binding factor for an A element-like sequence in the HB-EGF gene. These observations suggest the possible involvement of HB-EGF in pancreas development. While PDX-1 is essential for pancreas development, HB-EGF may function as a mediator of PDX-1 and thus be involved in the development of the endocrine pancreas.

The pancreas is an organ composed of two distinct cell populations: exocrine cells, which secrete digestive enzymes, and endocrine cells, which secrete hormones. The pancreas arises from the endoderm as a dorsal bud and a ventral bud which fuse together to form a single organ (1). Various growth factors expressed in the developing pancreas and its surrounding mesenchyme-derived cells are considered to be involved in the development of the endocrine and exocrine cells. Among those growth factors which have been shown to be expressed in pancreatic islets, the epidermal growth factor (EGF) family of growth factors such as EGF, transforming growth factor-α (TGF-α), and betacellulin can bind to the EGF receptor produced by pancreatic islet cells and have been shown to exert various effects on islet cell differentiation and proliferation (2, 3).

Heparin-binding EGF-like growth factor (HB-EGF) is a member of the EGF family which was purified initially from the conditioned medium of macrophage-like U937 cells (4, 5). It is synthesized as a membrane-anchored precursor (HB-EGF proform) that can be processed to release the soluble form. In terms of function, HB-EGF induces autophosphorylation of the EGF receptor and is known to be a potent mitogen for several cells (6–10). Clinically, HB-EGF may be involved in several diseases such as atherosclerosis (11) or carcinogenesis (12, 13). Like other EGF family growth factors (2, 3), HB-EGF seems to play a role in tissue development; during myogenesis, for example, HB-EGF gene expression is activated by MyoD (14). To clarify the molecular mechanism for pancreas development, we examined the expression of HB-EGF in fetal, neonatal, and adult pancreata. We found that HB-EGF is abundantly expressed in endocrine pancreas cells and primitive duct cells from which the endocrine cells are derived in fetal rats. We also found that the expression pattern in the developing pancreas was similar to that of the homeodomain-containing transcription factor PDX-1 (IDX-1/STF-1/IPF1). While the regulatory sequences of the HB-EGF gene contained sequences similar to the PDX-1 binding motif, we have shown that PDX-1 can bind to the 5'-flanking region of the HB-EGF gene and activates its promoter. Thus our present observations suggest that HB-EGF, which is expressed in the developing pancreas and may be regulated in part by PDX-1, is involved in pancreas development.

MATERIALS AND METHODS

Animals and Tissue Processing—Pregnant female rats were anesthetized by injection of pentobarbital (0.5 mg/kg of body weight) and the uterus resected and placed in 10% formalin buffered to pH 7.4 with 0.1 M sodium phosphate. The embryos (days 14 and 20) were removed and fixed for 5 h at 4 °C in the same solution. Next, pancreas were removed and fixed for 5 h at 4 °C, and the tissues were routinely processed to prepare paraffin sections.

Immunostaining of PDX-1, HB-EGF, Insulin, and Cytokeratin—The anti-PDX-1 antiserum was recently established by us (15) after immunizing a rabbit with the synthetic peptide SPQPSSIAPLRPQE repre-

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senting amino acid residues 269–282 of the PDX-1 peptide (16). An antibody recognizing the cytoplasmic domain of the HB-EGF proform was also produced by immunizing a rabbit with another synthetic peptide (precursor COOH-terminal residues 185–208) as previously reported (11). All procedures were carried out at room temperature unless otherwise specified. The sections were rinsed with 0.1 M Tris HCl buffer or 0.01 M phosphate-buffered saline three times before incubation at each step.

Immunohistochemical detection of HB-EGF protein was done using the streptavidin-biotin (LSAB) method (Dako LSAB Kit). The sections, which had been preincubated with 3% H₂O₂ solution for 10 min to block endogenous peroxidase, were incubated for 20 min with blocking agent, for 20 min with rabbit polyclonal anti-HB-EGF antibody immunodiluted 1:200 in phosphate-buffered saline containing 1% bovine serum albumin, and for 10 min with biotinylated swine anti-rabbit immunoglobulins in phosphate-buffered saline. They were then incubated with streptavidin conjugated to horseradish peroxidase in 0.05 M Tris-HCl buffer, and a positive reaction was visualized with 3-amino-9-ethylcarbazol. For the negative control, the primary antibody was preabsorbed with an excess amount of the peptide antigens. Before mounting, the sections were counterstained with Mayer’s hematoxylin.

For detection of PDX-1, the avidin-biotin complex method (Vectastain ABC Kit, Vector Laboratories Inc., Burlingame, CA) was used. Sections were incubated with rabbit anti-rat PDX-1 antibody diluted 1:2000 in phosphate-buffered saline containing 1% bovine serum albumin for 30 min and then incubated with secondary antibodies using biotinylated goat anti-rabbit IgG diluted 1:2000. These sections were then incubated with avidin-biotin complex reagent for 30 min. A positive reaction was visualized by incubating with peroxidase substrate solution containing 3,3’-diaminobenzidine (Zymed Laboratories Inc., San Francisco, CA) for 3–5 min.

For double immunostaining for HB-EGF and insulin and for PDX-1 and duct cell-specific cytokeratin, the indirect immunofluorescence method was used. The sections, which had been immunostained for HB-EGF or PDX-1 as described above, were further incubated for 30 min with guinea pig anti-porcine insulin antibody or rabbit anti-bovine epidermal cytokeratin antibody (Nichirei Co., Ltd., Tokyo, Japan), respectively. The latter antibody was shown to react selectively to duct cell cytokeratin previously (17). They were then incubated with fluorescein-conjugated goat anti-guinea pig IgG or goat anti-rabbit IgG F(ab’)2 (Dako), respectively.

Molecular Cloning of the Human HB-EGF Gene Promoter Region—Recombinant λ plaques were propagated in Escherichia coli at 37 °C, and approximately 8 × 10⁵ bacteriophage plaques from the human genomic library were screened by standard methods with specific oligonucleotides, which we described previously (18), using a [32P]-labeled 900-bp EcoRI-SmaI human HB-EGF cDNA fragment (λ-[32P]pdCTP, Amersham Corp.). Hybridization was performed at 37 °C in a buffer containing 50% formamide and 10% dextran sulfate, and the filters were washed twice for 10 min at room temperature and three times for 10 min at 48 °C in a solution containing 30 mM sodium chloride, 3 mM sodium citrate, 0.1% sodium dodecyl sulfate. Hybridizing plazides were detected by autoradiography. DNA from plaque-positive isolates was mapped after digestion with selected restriction enzymes and Southern hybridization with a [32P]-labeled HB-EGF cDNA fragment.

Preparation of Plasmids Used for Reporter Gene Analyses—HB-EGF gene promoter-luciferase reporter constructs were prepared by inserting various 5′-flanking sequences of the human HB-EGF gene into a promoterless vector, pA3Luc, which was a generous gift from Dr. W. M. Wood (University of Colorado Health Science Center, Denver, CO) and Dr. D. R. Helenius (University of California San Diego, San Diego, CA). To generate pHB-EGF/Luc880, pHB-EGF/Luc590, and pHB-EGF/Luc470 plasmids, the 880-, 590-, and 470-bp fragments of the human HB-EGF gene promoter region, respectively, were isolated from the λ phage DNA using appropriate restriction enzymes (see Fig. 4). After both ends of those DNA fragments were made blunt-ended using T4 DNA polymerase, they were individually ligated 5′ to the luciferase reporter gene at the Smal site in the pA3Luc plasmid. pA3Luc was the insertless pA3Luc plasmid used as a negative control. Site-directed mutagenesis at A element-like sequences located in the 5′-flanking region of the HB-EGF gene was performed by using a Transformation Site-directed mutagenesis kit (CLONTECH Laboratories Inc., Palo Alto, CA). PDX-1 expression plasmid containing nucleotides 221–1151 of the mouse PDX-1 cDNA (16) was prepared as described previously (15).

Cell Culture and DNA-mediated Gene Transfection—Mouse pancreatic beta cell-derived βTC1 cells and MIN6 cells and human hepatoblastoma cell line HepG2 cells were grown in RPMI 1640, Dulbecco’s modified Eagle’s medium, and EMEM medium (Nacalai Tesque, Japan), respectively, supplemented with 10% fetal calf serum (ICN Biomedical, Inc.), 100 units/ml penicillin, and 0.1 mg/ml streptomycin sulfate in a humidified atmosphere of 5% CO₂ at 37 °C. Transfection was performed by the calcium phosphate precipitation method followed 4 h later by a glycerol shock (19). HepG2 cells and βTC1 cells were replated at a density of 5 × 10⁵/10 cm diameter tissue culture dish 24 h before transfection. Then the cells were co-transfected with 4 µg of an HB-EGF promoter-reporter plasmid and 4 µg of an internal control, pMSVβGal plasmid, which contained the β. coli β-galactosidase gene driven by the murine sarcoma virus promoter (20). When required for HepG2 cells, 4 µg of the PDX-1 expression plasmid or the empty vector was also co-transfected.

Luciferase and β-Galactosidase Assays—Forty-eight hours after the transfection, the cells were harvested for luciferase and β-galactosidase assays. Preparation of cellular extracts and luciferase assays were performed using a PicoGene Kit (Toyo Ink Inc., Tokyo) as we reported previously (19). Light emission was measured by integration over 20 s of reaction using Lumat LB9501 (Belthold, Postfach, Germany). β-Galactosidase assays were also performed as described previously (19). The luciferase results were normalized with respect to transfection efficiency assessed from the results of the β-galactosidase assays.

Gel-mobility Shift Assay—Nuclear extracts of βTC1 cells and MIN6 cells were prepared following a reported procedure (21). Two micrograms of nuclear extract was incubated with 2 µg of poly(dI-dC) at 4 °C in 20 µl of reaction buffer (10 mM HEPES pH 7.8, 0.1 mM EDTA, 75 mM KCl, 2.5 mM MgCl₂, 1 mM dithiothreitol, and 3% Ficoll). The binding reaction was initiated by adding 5′ end [32P]-labeled double-stranded oligonucleotide probes and, when required, nonradioactive competitor oligonucleotides, followed by incubation at room temperature for 30 min. In some of the binding assays, anti-PDX-1 antisense or preimmune serum was added to the binding reactions 1 h before addition of the DNA probes. After the binding reactions, the samples were analyzed by separation on 5% polyacrylamide gel (150 µV, 1 h) in 1 × TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA) followed by autoradiography.

RESULTS

Immunohistochemical Detection of HB-EGF and PDX-1 in Developing Pancreas—To examine the possible implication of HB-EGF in pancreas development, immunohistochemical analyses of fetal rat pancreata were performed. As shown in Fig. 1, developing endocrine cells did not form islet-like cell clus-
PDX-1 and HB-EGF in Pancreas Development

Although the physiological significance is not known, clear subcellular localization of PDX-1 in the nucleus was observed in neonatal and adult rat pancreas but not in fetal rat pancreas (Fig. 1, A and B; Fig. 2A; Fig. 3B).

The co-localization of HB-EGF and PDX-1 in the pancreas, along with the existence of regions similar to the PDX-1 binding site (A element) in the 5′-flanking sequences of the human HB-EGF gene, suggested that PDX-1 may directly affect HB-EGF gene transcription in the pancreatic endocrine cells of the developing pancreas.

Evaluation of Promoter Activities of HB-EGF Gene in βTC1 Cells—To clarify the molecular basis of HB-EGF gene regulation in pancreatic islet cells, we searched for regions within the 5′-flanking region of the HB-EGF gene which are important for its promoter activity in pancreatic beta cells. By screening a λ phage genome library, we isolated and mapped an approximately 12-kilobase genome DNA fragment, containing a 6-kilobase fragment of the 5′-flanking DNA of the human HB-EGF gene (data not shown). Using this λ phage clone, reporter gene plasmids containing various lengths of the HB-EGF promoter linked to a luciferase reporter gene were constructed (Fig. 4).

Within the 880-bp 5′-flanking DNA contained in the pHBEFG/Luc880 plasmid, there were four A element-like regions to which PDX-1 may bind (arbitrarily named TAAT1, TAAT2, TAAT3, and TAAT4; Fig. 4). Among them, TAAT2 displayed a perfect match to the previously described CT box (A element) consensus (C/T(TAATG), while TAAT4 was a stretch of AT-rich sequences containing repeated TAAT sequences, which are known as an important core motif of the cis-acting element. The pHBEFG/Luc590 plasmid, containing 590-bp 5′-flanking DNA, had only two A element-like regions, including TAAT4 with repeated TAAT sequences (Fig. 4). The pHBEFG/Luc470 plasmid contained only 470-bp 5′-flanking DNA and lacked the A element-like sequences. The basal promoter activities of those 5′-flanking DNA of the human HB-EGF gene were evaluated in the beta cell-derived βTC1 cells and hepatoblastoma-derived HepG2 cells. Although both cells express the HB-EGF gene (data not shown), the former cells express PDX-1 but the latter cells do not. The results (Fig. 5B) indicated that, in HepG2 cells, all the fusion plasmids containing 470 bp or longer segments of 5′-flanking DNA induced comparable luciferase activity, which was well above the background (pA3/Luc0).

However, in βTC1 cells (Fig. 5A), a stepwise reduction of the promoter activity was observed; the pHBEFG/Luc470 plasmid containing no A element-like motifs displayed a promoter activity barely detectable over the background (pA3/Luc0). These observations indicate that certain cis motifs located between −880 and −590 and also between −590 and −470 function in a cell type-dependent manner; i.e. they are important for gen-

![Fig. 2. Localization of HB-EGF, PDX-1, insulin, and duct cell-specific cytokeratin in neonatal rat pancreas. A set of mirror-image sections from the pancreas of a neonatal rat (3 weeks old) were double-immunostained for HB-EGF (A) and insulin (C) and for PDX-1 (B) and duct cell-specific cytokeratin (D). HB-EGF and PDX-1 immunoreactivity was detected in cells forming an islet-like structure (A and B), which consisted of insulin-positive cells (C) and surrounding duct cells (D). Original magnification, ×210. Bar, 25 μm.](image1)

![Fig. 3. Immunohistochemical localization of HB-EGF and PDX-1 in adult rat pancreas. Pancreata from adult rats (8 weeks old) were immunostained for HB-EGF (A) and PDX-1 (B). A and B are a set of mirror-image sections. In adult pancreata, the endocrine cells could be recognized clearly as an isolated islet, and they were positive for both HB-EGF (A) and PDX-1 (B). Original magnification, ×90. Bar, 25 μm.](image2)
erating promoter activity in bTC1 cells but not in HepG2 cells.

Effect of PDX-1 Overexpression on HB-EGF Gene Promoter Activity—To discuss whether PDX-1 would be a possible factor that contributes to the cell type-dependent promoter activity of the HB-EGF gene, we investigated whether the HB-EGF gene is a potential target of PDX-1 transactivation. Exogenous expression of PDX-1 in HepG2 cells was induced by transient transfection of a PDX-1-expressing plasmid, and the effect on the HB-EGF promoter activity was evaluated. As shown in Fig. 6, PDX-1 expression in HepG2 cells caused a 20-fold increase in the promoter activity of the 880-bp promoter (pHB-EGF/Luc880). A slightly weaker induction (12-fold) was observed with the 590-bp promoter (pHB-EGF/Luc590). However, for the 470-bp 5'-flanking DNA which contained no A element-like sequences (pHB-EGF/Luc470), the PDX-1-responsive induction of the promoter activity was trivial, if any. These results indicate that the human HB-EGF promoter is PDX-1-responsive and suggest that the A element-like sequences located in the promoter may be important for the responsiveness.

Evaluation of Roles of A Element-like Sequences in the HB-EGF Gene Promoter—To examine whether the A element-like sequences function as cis-active elements, we disrupted those sequences and evaluated the effects on the basal promoter activity and PDX-1-induced promoter activation. Using the pHB-EGF/Luc 880 plasmid as a template, five mutant reporter plasmids, in which one or two of the four A element-like sequences were disrupted, were prepared (Mut1–5; Fig. 7). As shown in Fig. 8, Mut2 and Mut4 mutants, in which TAAT2 or TAAT4 is disrupted, respectively, revealed reduced basal promoter activity as evaluated in the beta cell-derived bTC1 cells, whereas Mut1 or Mut3 mutants showed no effects. The Mut5 mutant, in which both TAAT2 and TAAT4 are disrupted, revealed further decrease in the promoter activity. These results indicate that the human HB-EGF promoter is PDX-1-responsive and suggest that the A element-like sequences located in the promoter may be important for the responsiveness.

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![FIG. 4. Structures of HB-EGF-luciferase fusion plasmids. The pHB-EGF/Luc880, pHB-EGF/Luc590, and pHB-EGF/Luc470 plasmids contained the 880-, 590-, and 470-bp fragment, respectively, of the human HB-EGF gene promoter plus the first 68 bp of the exon 1 untranslated region, linked to a luciferase reporter. Four A element-like regions located in the HB-EGF gene 5'-flanking region are shown in solid boxes and named arbitrarily TAAT1, TAAT2, TAAT3, and TAAT4. Among them, TAAT2 matches the CT box (A element) consensus and TAAT4 contains repeats of TAAT motifs. The plasmid pA3/Luc0 is a negative control plasmid containing no HB-EGF sequences.](image)

![FIG. 5. Basal promoter activities of HB-EGF gene in bTC1 cells and HepG2 cells. Forty-eight hours after the transfection of an HB-EGF promoter-luciferase reporter plasmid into bTC1 cells (A) and HepG2 cells (B), luciferase and β-galactosidase assays were performed. The luciferase results were normalized with respect to transfection efficiency using the results of β-galactosidase assays and are expressed as relative light units with that of pHB-EGF/Luc880 arbitrarily set at 1. All data are presented as means ± S.D. of three independent experiments.](image)

![FIG. 6. Effect of exogenous PDX-1 expression on HB-EGF gene promoter. PDX-1 was expressed in HepG2 cells by transient transfection of PDX-1 expression plasmid, and the effect of PDX-1 on the HB-EGF gene promoter activity was examined. A reporter plasmid, pHB-EGF/Luc880, pHB-EGF/Luc590, or pHB-EGF/Luc470, and the pMSVβgal plasmid were co-transfected with either the PDX-1-expressing plasmid or the control plasmid (empty vector). Forty-eight hours after the transfection, luciferase and β-galactosidase assays were performed. The luciferase results were normalized with respect to transfection efficiency using the results of β-galactosidase assays. The results are expressed as n-fold induction over the basal promoter activity of each reporter plasmid obtained in HepG2 cells transfected with the control plasmid (empty plasmid). All data are presented as means ± S.D. of three independent experiments.](image)
promoter activity of the 880-bp promoter (pHB-EGF/Luc880, Fig. 6). The magnitude of the induction was lower in Mut2 and Mut4 mutants but not in the Mut1 or Mut3 mutant (Fig. 9). When the reporter plasmid had both TAAT2 and TAAT4 mutations (Mut5), the promoter activity was not substantially induced by PDX-1 overexpression (Fig. 9). These results together indicate that there are two A element-like sites which play a major role in generating promoter activity in βTC1 cells and in the PDX-1 responsiveness of the promoter: TAAT2, which matches the CT box (A element) consensus (C/TTAATG), and TAAT4, the repeated TAAT sequences.

Identification of A Element-like Region-Binding Factor in Beta Cell-derived Cells—Because two of the A element-like regions (TAAT2 and TAAT4) in the HB-EGF gene promoter were important for the PDX-1 responsiveness (Fig. 9), as well as for the basal promoter activity in βTC1 cells (Fig. 8), it seemed likely that PDX-1, which is expressed throughout beta cell development (22) (Figs. 1–3), binds to those regions and thus plays a primary role in generating promoter activity in the cells. This led us to perform gel-mobility shift analyses for those two A element-like regions using an anti-PDX-1 antibody. Nuclear extracts were isolated from two pancreatic beta cell-derived cell lines, βTC1 cells and MIN6 cells, and allowed to bind to two double-stranded oligodeoxynucleotide probes representing each of the two functional A element-like regions (Figs. 7–9) in the promoter. The results of the gel-mobility shift analyses (Fig. 10, A and C) revealed that there is one major nuclear factor in βTC1 cells and MIN6 cells which binds specifically to either of the A element-like regions (TAAT2 or TAAT4). Because the gel-shift complexes were supershifted by addition of an anti-PDX-1 antibody (Fig. 10, B and D), PDX-1 seems to be the dominant DNA-binding factor for the A element-like regions (TAAT2 and TAAT4) in the HB-EGF gene promoter in βTC1 cells and MIN6 cells. Since both TAAT2 and TAAT4 were shown to be important for the promoter activity, PDX-1 may play an essential role in the transcriptional activation of the HB-EGF gene in the endocrine pancreas.

DISCUSSION

In the present study, we identified the expression of HB-EGF in the developing pancreas. To date, various growth factors have been shown to be expressed in the pancreas and the surrounding mesenchyme-derived cells during development and also in adults. They include hepatocyte growth factor, expressed in mesenchyme-derived tissue, and EGF, TGF-α, betacellulin, nerve growth factor (NGF), and transforming growth factor-β1 expressed in the pancreas itself (2, 3, 24, 25). The growth factors expressed in the developing pancreas may function independently or in cooperation with other growth factors. Among them, most of the EGF family growth factors share a common receptor of the ErbB receptor family, EGF receptor (ErbB1), which has been known to be expressed also in the endocrine and exocrine pancreas and is thought to exert...

FIG. 7. Schematic representation of site-directed mutagenesis of A element-like sequences. Mutations were introduced to the A element-like sequences in the HB-EGF gene promoter within the pHB-EGF/Luc880 plasmid (Wild-type) and five mutant reporter plasmids (Mut 1–Mut 5) were obtained. In Mut1, Mut2, Mut3, and Mut4 plasmids, TAAT1, TAAT2, TAAT3, and TAAT4 was disrupted, respectively. In Mut5, both TAAT2 and TAAT4 were disrupted. Substituted nucleotides are underlined.

FIG. 8. Effects of mutations in A element-like regions on promoter activity in βTC1 cells. Effects of disruption of A element-like regions on the basal promoter activity in βTC1 cells were evaluated. A wild-type or mutant reporter plasmid was co-transfected with the pMSVβ-gal plasmid, and 48 h after the transfection, luciferase and β-galactosidase assays were performed. The data are expressed as relative light units with that of the wild-type plasmid arbitrarily set at 1. The luciferase results were normalized with respect to transfection efficiency using the results of β-galactosidase assays. All data are presented as means ± S.D. of three independent experiments.

FIG. 9. Effects of mutations in A element-like regions on PDX-1-responsive promoter activation. Effects of disruption of A element-like regions on the PDX-1 responsiveness of the HB-EGF gene promoter were evaluated. A wild-type or mutant reporter plasmid was co-transfected into HepG2 cells with PDX-1 expression plasmid or the control plasmid (empty vector). To allow normalization of the luciferase results, the pMSVβ-gal plasmid was also co-transfected. Forty-eight hours after the transfection, cellular extracts were obtained, and luciferase and β-galactosidase assays were performed. The results are expressed as n-fold induction over the basal promoter activity of each reporter plasmid obtained in HepG2 cells transfected with the control plasmid (empty plasmid). The luciferase results were normalized with respect to transfection efficiency using the results of β-galactosidase assays, and the data are presented as means ± S.D. of three independent experiments.
autocrine or paracrine effects. EGF seems to promote ductal development in the fetus (3) and increases the cell proliferation rate of fetal pancreatic cells (24). Also, another member of the EGF family, betacellulin, which was originally isolated from the conditioned medium of beta cell-derived hTERT cells (A and B) and MIN6 cells (C and D). For A and C, two kinds of double-stranded oligonucleotides were used as binding probes: a (sequence of sense strand: 5′-GTCAGCCATTAATGTC-CCCTG-3′) representing TAAAT (Fig. 7), and probe b (sequence of sense strand: 5′-GATAGATGAATTAATTATTTGATACTTGC-3′), representing TATAAT (Fig. 7). The ~P-labeled probe was incubated with the nuclear extracts and, when required, 50-fold excess of unlabeled wild-type or mutated-type competitor was added to the binding reactions. The sequences of the sense strand of the mutated-type competitors were 5′-GTCAGCCATTAATGTC-CCCTG-3′ for probe a and 5′-GATAGATGAATTAATTATTTGATACTTGC-3′ for probe b. For B and D, gel-mobility shift analyses were performed as described for A and C except that an anti-PDX-1 antiserum or preimmune serum was added to the binding reactions before addition of the binding probe. Products were electrophoresed at 150 V for 1 h on 5% polyacrylamide gels in 1 × TBE buffer, and dried gels were analyzed by autoradiography.

The expression pattern of HB-EGF during pancreas development is clearly in contrast to that of TGF-α, which can be detected in both endocrine and exocrine tissues (2), and also with NGF localized in nonendocrine cells surrounding the islets (25). Although the EGF receptor, a common receptor for HB-EGF and TGF-α, is expressed in both endocrine and exocrine tissues, the expression pattern of HB-EGF in the developing pancreas suggested that HB-EGF is likely to be exclusively involved in the development of the endocrine pancreas. It is difficult to clarify the in vivo physiological role of HB-EGF; however, evaluation of its effects using different tissue components of the developing pancreas, as previously done for EGF or other growth factors (3), may provide useful information. Also, mice homozygous for a targeted mutation in the HB-EGF gene, when they become available, would be useful for clarifying the physiological significance of HB-EGF expression during pancreas development.

Our present study has also shown that the expression of HB-EGF can be potentially regulated by the transcription factor PDX-1. It was originally isolated as IPF1 in mouse (16) and as STF-1/DIX-1 in rat (29, 30) and has been shown to bind to the A elements of insulin gene and to activate its transcription (1, 16, 22, 31, 32). PDX-1 is selectively expressed in pancreatic islets and in the duodenum. At an early stage of embryonic development, PDX-1 is initially expressed in the gut region where the foregut endoderm becomes committed to common pancreatic precursor cells. During the development of the pancreas, PDX-1 expression is maintained in multipotential precursors that coexpress several hormones, and later it becomes restricted to beta cells. Mice homozygous for a targeted mutation in the PDX-1 gene have been shown to lack a pancreas (1, 33, 34). These observations support the crucial role of PDX-1 in pancreas development.

Although data are accumulating for the expression pattern of PDX-1 during development, it is largely unknown how PDX-1 drives pancreas development. Because PDX-1 is a DNA-binding transcription factor, its action should be exerted by trans-activating genes through its binding to their regulatory sequences. To date, only four beta cell-specific genes, insulin, glucokinase, IAPP, and Glut2 genes, all of which have the A element-like motifs in their regulatory region, are known as putative targets of the PDX-1 regulation (15, 28, 35–37). However, the simple induction of these four genes is unlikely to be enough to explain all of the in vivo effects of PDX-1. Recent data obtained with PDX-1-deficient mice showed that PDX-1 is essential for induction of the morphogenesis of pancreatic epithelium as well as the progression of differentiation of the endocrine cells (34). These observations cannot be rationally explained by the already known function of PDX-1 as a transcription factor of those four genes. Therefore, we are tempted to consider that PDX-1 may induce gene expression of one or more growth factors and that the growth factor(s) may, in turn, exert physiological effects on pancreas development. Thus, HB-EGF, which was shown to co-localize with PDX-1 during development and also revealed potential PDX-1 responsiveness in its promoter activity, may be a good candidate for such growth factors which would mediate PDX-1 effects.

Since AT-rich regions are known to be binding sites for a wide range of homeoproteins, we assume it unlikely that PDX-1 is the only transcription factor involved in HB-EGF gene transcription in the pancreas. To date, various homeodomain-containing transcription factors as well as basic helix-loop-helix proteins, which are either beta cell-specific or ubiquitous, are known to be expressed in pancreatic islets. To fully understand the regulation of HB-EGF gene expression during pancreas development, the possible involvement of those factors in HB-EGF gene activation needs to be studied.

In conclusion, HB-EGF is expressed in the developing pancreas and may be involved in the differentiation or growth of endocrine cells. Also, in our present study, PDX-1 was suggested as being a possible regulator of the HB-EGF gene expression in the pancreas. PDX-1-responsive induction of the...
growth factor gene expression, if it occurs in vivo, may at least partially explain the essential and extensive roles of PDX-1 in the morphogenesis of the pancreas, as suggested by phenotypes of PDX-1-deficient mice.

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