Transforming growth factor-alpha (TGF-α) and insulin gene expression in human fetal pancreas

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Summary

Transforming growth factor-alpha (TGF-α) mRNA is expressed in several pancreatic cancer cell lines, but its expression during normal fetal pancreas development has not been studied. We investigated the expression of TGF-α, its receptor (EGF-R) and insulin mRNA and their corresponding peptides in human fetal pancreata (15-20 gestation weeks). Polymerase chain reaction (PCR) and RNAase protection analysis revealed that TGF-α and insulin mRNAs were detectable in pancreas during the developmental span studied. In northern blot analysis a single band of 4.8 kilobases (kb) corresponding to the TGF-α transcript and a 0.6 kb for the insulin mRNA were detected in the pancreas. Using in situ hybridization, TGF-α mRNA expression was seen in a low copy number in both the exo- and endocrine pancreas. By immunohistochemistry TGF-α-immunoreactive cells were detected in the ducts, acini and islets showing that the mRNA was translated into protein. By contrast, insulin transcripts were detected in a high copy number, restricted to the islets of Langerhans. However, monoclonal insulin antibody detected less insulin containing cells than could be expected from the mRNA pattern suggesting that fetal β-cells rapidly secrete insulin instead of storing it in the secretory granules. Alternatively, the translation of insulin mRNA could be inefficient. By double labeling the pancreas sections with polyclonal TGF-α antiserum and monoclonal insulin antibody the TGF-α- and insulin-like immunoreactivity was localized to β-cells. Furthermore, mRNA for the TGF-α receptor, EGF-R, together with EGF-R-immunoreactive cells were also present in pancreas. The results suggest that TGF-α may participate auto- and/or paracrinically in the development of human fetal pancreas.

Key words: β-cell, development, EGF-R, fetal, glucagon, insulin, pancreas, somatostatin, TGF-α.

Introduction

Transforming growth factor alpha (TGF-α) is a mitogenic 50 amino acid polypeptide (Derynck et al., 1984) which is structurally related to epidermal growth factor (EGF) and binds to the same receptor (EGF-R). TGF-α was long thought to be a fetal form of EGF, but it is now known to be present also in normal adult tissues (Beauchamp et al., 1989; Madtes et al., 1988; Markowitz et al., 1990; Skinner et al., 1989; Wong et al., 1990) as well as in malignant tumors (Derynck et al., 1987; Mydlo et al., 1989). During fetal development TGF-α mRNA can be found in rat decidua (Han et al., 1987) and in several fetal mouse tissues (Wilcox and Derynck, 1988) and in preimplantation mouse embryos (Rappolee et al., 1988). TGF-α-like-immunoreactivity can be detected in human fetal intestine (Miettinen et al., 1989). Many pancreatic cancer cell lines express TGF-α (Smith et al., 1987) and overexpress EGF-R mRNA (Korc et al., 1986). In addition, EGF-R-like immunoreactivity can be found from ducts and acini in normal

adult pancreas (Damjanov et al., 1986). In transgenic mice overexpression of TGF-α leads to epithelial hyperplasia of liver, pancreas, intestine, mammary and coagulation glands (Jhappan et al., 1990; Matsui et al., 1990; Sandgren et al., 1990) and postnatally it also promotes proliferation of pancreatic acinar cells and fibroblasts (Sandgren et al., 1990).

Insulin is a 51 amino acid peptide, which regulates glucose homeostasis. However, during fetal development it probably acts more as a growth factor (DePablo et al., 1985; DePablo and Roth, 1990; Hill and Milner, 1985; Philipps et al., 1991) than as a regulator of the fetal glucose metabolism. Insulin also stimulates growth of the AR42J pancreatic acinar carcinoma cells and increases their amylase synthesis (Mössner et al., 1987). Hill and Milner suggest that insulin stimulates fetal growth by promoting uptake and utilization of a nutrient at a cellular level, by modulating the release of insulin-like growth factors (IGFs) or other growth factors from fetal tissues, or by exerting a direct anabolic effect via either insulin or IGF receptors (Hill

and Milner, 1985). In man, the only site for insulin synthesis is β-cells in pancreatic islets of Langerhans; in chicken embryos the insulin gene is expressed also extrapancreatically (liver) and prepancreatically even before the differentiation of β-cells occurs, further supporting a developmental role for insulin (DePablo et al., 1982; Serrano et al., 1989).

The pancreas derives from endoderm. Single glandular structures can be seen at 7 weeks gestation and discrete lobules and acini from 14-16 weeks onwards. Endoderm-mesoderm interaction is needed for pancreas to differentiate (Go et al., 1986) but otherwise the development of human endo- and exocrine pancreas is poorly known. To further clarify pancreatic differentiation, we studied the expression of TGF-α, its receptor and insulin in human fetal pancreas.

Materials and methods

Tissues

Fetal tissues were obtained from legal abortions induced with prostaglandins. The study was approved by the Ethical committee of Helsinki Maternity Hospital. The delay from delivery room to laboratory was approximately 1-2 hours. The gestation age was estimated from the fetal foot length (Munsick, 1984). The pancreata were dissected from the surrounding fat tissue, snap frozen in liquid nitrogen and stored at $-70°C$ for further use.

RNA extraction and northern analysis

Total cellular RNA was isolated by guanidium isothiocyanate extraction and cesium chloride centrifugation (Chirgwin et al., 1979), measured spectrophotometrically at 260 nm, and stored at $-20°C$ until use. 10-30 μg of total RNA was denatured in glyoxal and dimethylsulfoxide and loaded onto a 1.5% agarose gel. After electrophoresis the RNA was transferred onto Hybond-N filter (Amersham, UK) by capillary blotting (Thomas, 1980). Filters were hybridized in 0.1% SDS, 5× SSPE (1× SSPE is 0.15 M NaCl, 10 mM sodium phosphate, and 1 mM EDTA, pH 7.4), 50% formamide, 5× Denhardt's solution (0.1% Ficoll 400, 0.1% BSA, 0.1% polyvinylpyrrolidone 360), 0.1 mg/ml denatured herring sperm DNA and 0.1 mg/ml torula RNA with TGF-α-, EGF-R- and insulin-cRNA (65°C 16 hours) and γ-actin-cDNA probes (42°C 16 hours). Filters were washed in 0.1× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate) with 0.1% SDS (three times 20 minutes in 65°C for cRNA and 50°C for cDNA probes) and subjected to autoradiography using Trimax T16 intensifying screens (3M, Ferrania, Italy) and Hyperfilm MP films (Amersham, UK) for 1-4 weeks at $-70°C$.

Probes

925bp EcoRI fragment of human TGF-α cDNA (phTGF1-10-925, obtained from G.I. Bell) and 1.84 kbp EcoRI fragment of human epidermal growth factor receptor (phHER-A64-1; Ullrich et al., 1984) were subcloned into pGEM7(+z) ribovectors (Promega Biotec, Madison, WI) and labeled with [32P]UTP (1000 Ci/mmol, Amersham, Buckinghamshire, UK) or 35S-UTP by in vitro transcription using SP6 or T7 RNA polymerases (Melton et al., 1984). The correct size of the labeled probes were controlled in a sequencing gel. Also 270 bp TGF-α PCR fragments from human fetal decidua (first trimester) and fetal duodenum (second trimester) were

subcloned into EcoRV/SphI digested pGEM7(+z) ribovectors and used as cRNA probes. All TGF-α riboprobes gave similar results in northern blot analysis. [32P]dCTP random priming labeled (Oligolabelling Kit, Pharmacia, Uppsala, Sweden) γ-actin (Gunning et al., 1983) was used to verify the integrity of RNA and to equalize the loaded RNA amounts. Insulin cRNA probe was made by subcloning a PCR fragment containing nucleotides 134-395 of human preproinsulin mRNA (Bell et al., 1979) into EcoRI/BamHI digested pGEM9(+z) ribovector. It was labeled similarily to TGF-α.

Reverse transcription (RT)

One μg of total RNA from each tissue was used for cDNA synthesis (Saiki, et al., 1985). The RNA and 0.2 μg of oligo(dT)15 primer (Boehringer Mannheim, Mannheim, Germany) were incubated with 100 units Moloney murine leukemia virus reverse transcriptase (MMLV-RT) (BRL, Gaithersburg, MD), 2 μl 5× RT reaction buffer (BRL), the four dNTPs (Cetus, final concentration 0.5 mM for each), and 5 units RNAase inhibitor (Amersham, UK) in 10 μl reaction volume at $+37°C$ for 60 minutes. After incubation the solution was heated to 93°C for 5 minutes and flash cooled to 4°C. Fifty units of fresh MMLV-RT were added and the RT repeated for a second 60 minutes period. The samples were then stored on ice or frozen until used in the polymerase chain reaction.

Polymerase chain reaction

Oligonucleotide primers specific for human TGF-α were synthesized in the Cancer Biology laboratory, University of Helsinki. A 20-mer TGF-α sense primer (5'-CGCCCTGTTCGCTCTGGGTA-3') corresponds to nucleotides 58-77 (Derynck et al., 1984) and 21-mer antisense primer (5'-CTGGCTGGCAGCCACCACGG-3') corresponds to nucleotides 300-319. The expected size of the TGF-α PCR product was 270 bp. Primers for human insulin were also synthesized: sense primer (5'-TGTGAACCAACACCTGTG-3') corresponds to nucleotides 134-151 and antisense primer (5'-CGTCTAGTTGCAGTAGT-3') to nucleotides 379-395 in human preproinsulin (Bell et al., 1979). The expected size of the insulin PCR product was 260 bp. β-actin primers were as follows: sense primer 5'-CCCAGGCACCAGGGCGTGAT-3' (nucleotides 153-172) and antisense primer 5'-TCAAA-CATGATCTGGGTCAT-3' (nucleotides 396-415) creating a 263 bp fragment (Ponte et al., 1984).

PCR was performed according to the instructions provided with the GeneAmp DNA Amplification Reagent Kit (Perkin Elmer Cetus, Norwalk, CT). One μl of RT mixture was combined with: (1) 5 μl 10× PCR-reaction buffer; (2) 37 μl water; (3) 4 μl dNTP mixture (0.2 μM final concentration for each deoxynucleotide); (4) 50 pmol antisense and sense strand primers each; and (5) 2.5 units AmpliTaq enzyme. After addition of 50 μl of mineral oil (Perkin Elmer), the reactions were heated to 95°C for 5 minutes and then immediately cycled (30 cycles: 94°C for 30 seconds for denaturation, 58°C for 30 seconds for annealing and 72°C for 1.5 minutes for elongation) using Hybaid thermal reactor (Teddington, UK). Five μl aliquots of the PCR reaction products were size fractionated in 2% agarose gel (Pharmacia, Uppsala, Sweden) and stained in ethidium bromide. Standard size markers were pBR322/ΦXRFHaeIII fragments. Southern transfer was done by capillary blotting the DNA to Hybond-N nylon membranes (Amersham, UK). The membranes were hybridized with [32P]dCTP labeled TGF-α, insulin and β-actin cDNA probes. The rest of the TGF-α PCR products (45 μl) were purified with Geneclean kit (Bio 101, distributed by Stratech Scientific Ltd, London, UK) after electrophoresis in

2% low melting point agarose (BRL). Part of the purified PCR fragments were further used for asymmetric PCR (Allard et al., 1991). Single stranded TGF-α PCR products were purified with Centricon 30 (Amicon, Danvers, CA, USA) and sequenced using Sequenase 2000 kit (USB, Cleaveland, Ohio, USA).

In situ hybridization

Frozen sections of 5 μm were cut on aminoalkylsilane pretreated microscope slides (Rentrop et al., 1986). The sections were immediately fixed in freshly prepared 4% paraformaldehyde (PFA) with 5 mM MgCl₂ in 0.1 M phosphate buffered saline, pH 7.4 (PBS) for 15 minutes at room temperature (RT), washed in 70% and 100% ethanol, respectively for 5 minutes each, air-dried for 10 minutes at RT, frozen and stored at −70°C in boxes containing desiccant. In situ hybridization was performed according to Cox et al. (1984) with minor modifications. Briefly, frozen specimens were rehydrated in Tris-buffered saline, pH 7.4 (TBS) at RT and treated with proteinase K 0.5 μg/ml (Boehringer Mannheim) for 5 minutes at +37°C. Then the slides were washed with glycine (0.1 M in PBS) for 5 minutes at RT, postfixed in 4% PFA-5 mM MgCl₂, rinsed in 50% FA-2× SSC (deionized formamide-2× standard saline citrate: 0.3 M NaCl-0.03 M sodium citrate). The sections were then acetylated in freshly prepared 0.25% acetic anhydride in 0.1 M triethanolamine (TEA, pH 8.0) for 10 minutes at RT and rinsed again in 50% FA-2×SSC. Prehybridization was done at 50°C for 30-60 minutes in hybridization buffer (50% FA, 10% wt/vol dextran sulphate, 1× Denhardts' solution [0.02% Ficoll 400, 0.02% BSA, 0.02% polyvinylpyrrolidone 360], 300 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl, 10 mM dithiotreitol [DTT], and 200 μg/ml E. coli tRNA). Prehybridization mixture was removed and approximately 1-2 × 10⁶ cts/minute of the ³⁵S-labeled antisense or sense cRNA probe in 30 μl new hybridization buffer was applied on the sections. Sections were hybridized for 16-18 hours at 50°C. The posthybridization washes were as follows: (1) twice in 50% FA - 2× SSC - 10 mM DTT at 50°C for 30 minutes; (2) 3 times in NTE (0.15 M NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA) at 37°C for 10 minutes; (3) RNAase A (100 μg/ml; Sigma) and RNAaseT1 (2 μg/ml; Boehringer Mannheim) in NTE at 37°C for 30 minutes; (4) NTE at 37°C for 10 minutes; (5) twice 50% FA - 1× SSC - 10 mM DTT at 37°C for 30 minutes. After washes the slides were dehydrated in 70% and 95% ethanol with 0.3 M ammonium acetate, air dried and dipped in NTB-2 film emulsion (Kodak). Sections were exposed in light safe boxes for 10 days to 6 weeks at 4°C and developed in D-19 developer (Kodak). Before dark- and light-field microscopy analysis the sections were counterstained in Harris' hematoxylin.

RNAase protection assay

Protection assay was performed as described (Zinn et al., 1983). Briefly, 30-80 μg of fetal RNA samples were hybridized with 200000 cts/minute of ³²P-labeled TGF-α riboprobe in 30 μl hybridization buffer [40 mM 1,4-piperazinediethanesulphonic acid (pH 6.4), 1 mM EDTA, 400 mM NaCl, and 80% (v/v) deionized formamide] at 42°C overnight. tRNA was used as a negative control. Samples were then treated with RNAaseA (40 μg/ml; Sigma, MO.) and RNAaseT1 (375 units/ml; Boehringer Mannheim) for 1 hour at 30°C and digested with proteinase K (100 μg/ml) and 0.5% SDS for 15 minutes at 37°C. Protected RNA-RNA duplexes were extracted with phenol and chloroform and precipitated with ethanol before analysis on denaturing 6% PAGE-urea gel.

Immunohistochemistry

The paraformaldehyde fixed pancreata were embedded in paraffin. The immunohistochemistry was done as described (Miettinen et al., 1989). Briefly, the sections were first incubated with sheep anti-TGF-α (Chemicon, Temecula, CA, USA) and mouse anti-human insulin (Amersham), and then with the second antibodies (TRITC-conjugated donkey anti-sheep and FITC-conjugated donkey anti-mouse IgG 1:400, Jackson, West Grove, PA, USA). The immunoreactivity was analyzed by fluorescence microscopy. Normal sheep and mouse sera were used as negative controls. Specificity of the reactions was controlled by preabsorbing the antisera with 10 μM TGF-α (Promega), 1 μM insulin (Novo, Denmark) or both. Sections were also stained with anti-human glucagon and anti-human somatostatin (both from Dako, Copenhagen, Denmark). Epidermal growth factor receptor immunoreactions were done using a monoclonal anti-EGF-R antibody and alkaline phosphatase supplied in Stravigen Super Sensitive kit according to the manufactures instructions (Bio Genex Laboratories, San Ramon, CA, USA).

Results

The presence of TGF-α mRNA in pancreas was first studied by PCR. Human fetal pancreas RNA from four different fetuses (gestation ages 15-19 weeks) was reverse transcribed and subjected to PCR using specific human TGF-α primers. After 30 cycles of amplification a single band of expected 270 bp size was seen in ethidium bromide stained polyacrylamide gel (Fig. 1A). After Southern transfer and hybridization with full-
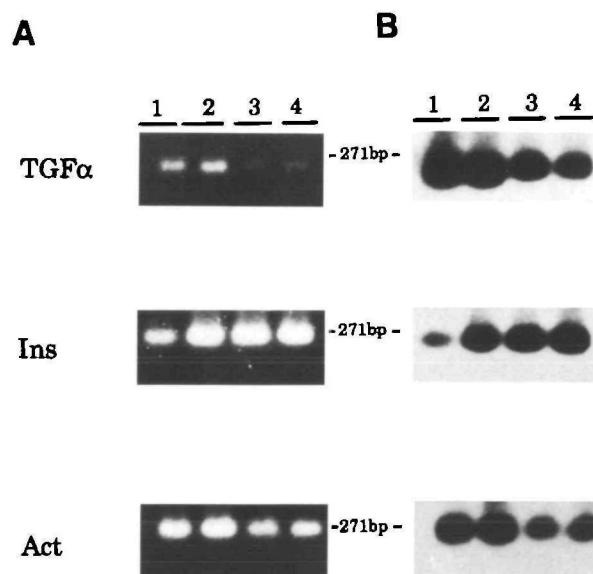

Fig. 1. PCR of human fetal pancreas using TGF-α, insulin and β-actin primers. Total RNA from second trimester human fetal pancreata (Lane 1, 17 weeks gestation; Lane 2, 16 weeks; Lane 3, 19 weeks; Lane 4, 17 weeks) were reverse transcribed and subjected to PCR.
(A) Ethidium bromide-stained gel of PCR products.
(B) Autoradiography after Southern transfer and hybridization with ³²P-labeled TGF-α, insulin and β-actin cDNA probes. 271 bp band from the size marker ΦXRF174/HaeIII is shown in the middle.
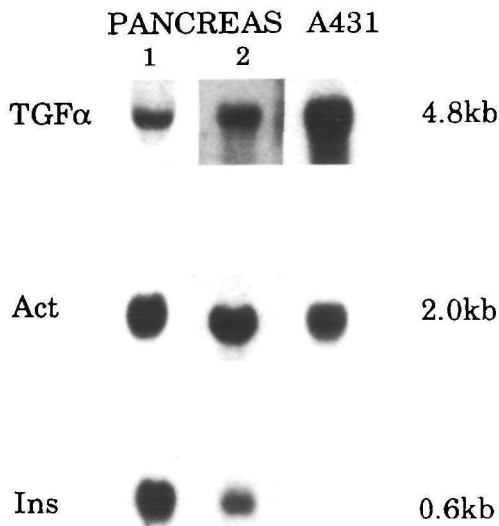
Fig. 2. Northern blot analysis of TGF-α expression in human fetal pancreas. 30 μg RNA from human fetal pancreas (Lane 1, 15 weeks gestation; Lane 2, 20 weeks) and A431 cells were subjected to electrophoresis and subsequently blotted onto nylon filter and hybridized with ^{32}P-labeled TGF-α cRNA, insulin and γ-actin cDNA probes. The obtained transcript sizes are shown on the right.

length TGF-α cDNA probe, the same 270 bp band could be visualized (Fig. 1B). For specificity control, the amplified TGF-α PCR-fragment was purified using ultrafiltration and further amplified using only the sense primer. The resulting ssDNA was confirmed to be TGF-α by sequencing it (data not shown). Degradation of pancreas RNA was excluded by β-actin PCR and insulin expression was verified by insulin PCR (Fig. 1).

A 4.8 kb band corresponding to TGF-α mRNA was detected in northern blot analysis from second trimester human fetal pancreata (gestation ages 15 and 20 weeks). Positive results were obtained using both a TGF-α cDNA subcloned into pGEM-ribovector and a TGF-α PCR fragment (nucleotides 58-319) from fetal duodenum also subcloned into pGEM-ribovector (see Materials and methods) as probes. A431 vulvar carcinoma cells were used as a positive hybridization control (Fig. 2). Insulin and actin expressions are shown for

comparison. The expression of TGF-α mRNA was more abundant in pancreas as compared to a weak signal obtained from brain, liver, kidney and lungs (data not shown).

In RNAase protection assay a protected TGF-α RNA-RNA hybrid of expected size was detected from fetal pancreas. When RNAase was included in the reaction, no signal was seen (Fig. 3).

In situ hybridization using the antisense insulin cRNA probe revealed that insulin mRNA is restricted to the Langerhans islets (Fig. 4A,B). Sense strand insulin cRNA gave no hybridization (Fig. 4C). The amount of immunoreactive insulin-containing cells is smaller (Fig. 4D) than could be deduced from the mRNA level (Fig. 4A). TGF-α mRNA was detected in a low copy number diffusely distributed throughout the pancreas (Fig. 4E,F) corresponding to the immunohistochemical pattern obtained with the polyclonal TGF-α antiserum (Fig. 4H). Sense probe gave a weak background hybridization (Fig. 4G).

TGF-α immunoreactive cells were seen in both the exocrine and endocrine pancreas. The acinar cells and ducts were positive in the majority of the studied pancreata (Figs 4H, 5A,B). Mesenchyme was negative. The gestation ages of the fetuses were between 16 and 20 weeks; during this time interval no age related differences in staining pattern could be detected. All Langerhans islets contained TGF-α-like immunoreactive cells. By double labeling the pancreas sections with monoclonal insulin antibody and polyclonal TGF-α antiserum, the TGF-α- and insulin-like immunoreactivity could be localized to the same cells - namely β-cells (Fig. 4D,H). Insulin-like immunoreactivity was mainly seen in the centre of the mantled islets, few positive cells where seen outside the islets (Figs 4D,5E). Glucagon-like immunoreactivity was mainly detected in the periphery of the islets (Fig. 5C) and did not colocalize with insulin or TGF-α positive cells (Fig. 5E,F). Only few somatostatin positive cells were seen in the periphery of the islets (Fig. 5D). The specificity was controlled by preabsorbing the antibodies with excess of recombinant TGF-α, glucagon and insulin prior staining the sections; no immunoreactive staining could be seen after preabsorbtion.

The expression of EGF-R mRNA in pancreas was also studied. 10.5 kb, 5.8 kb and 2.8 kb bands specific
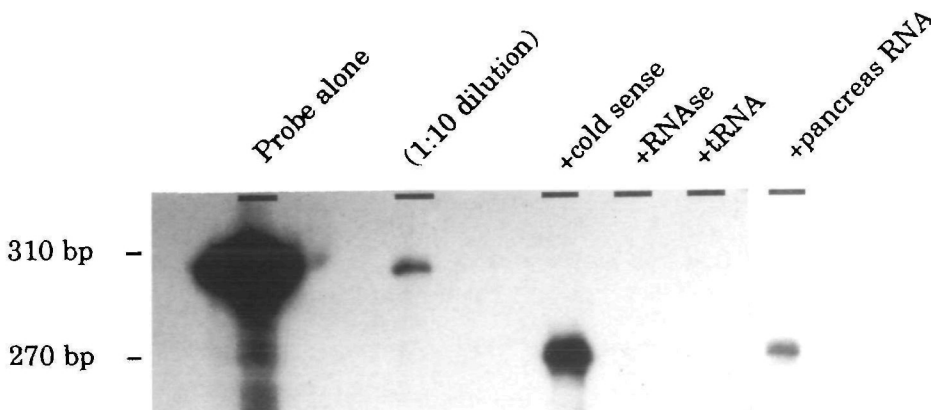

Fig. 3. RNAase protection assay from human fetal pancreas. 50 μg RNA from 17 weeks gestation human pancreas was hybridized with a 310 bp long fragment of ^{32}P-labeled TGF-α cRNA. After RNAase treatment a 270 bp protected fragment was obtained. A 40 bp difference resulted from ribovector sequence labeling. Hybridization with tRNA was used as the negative control and cold TGF-α cRNA sense strand hydridization as the positive control.
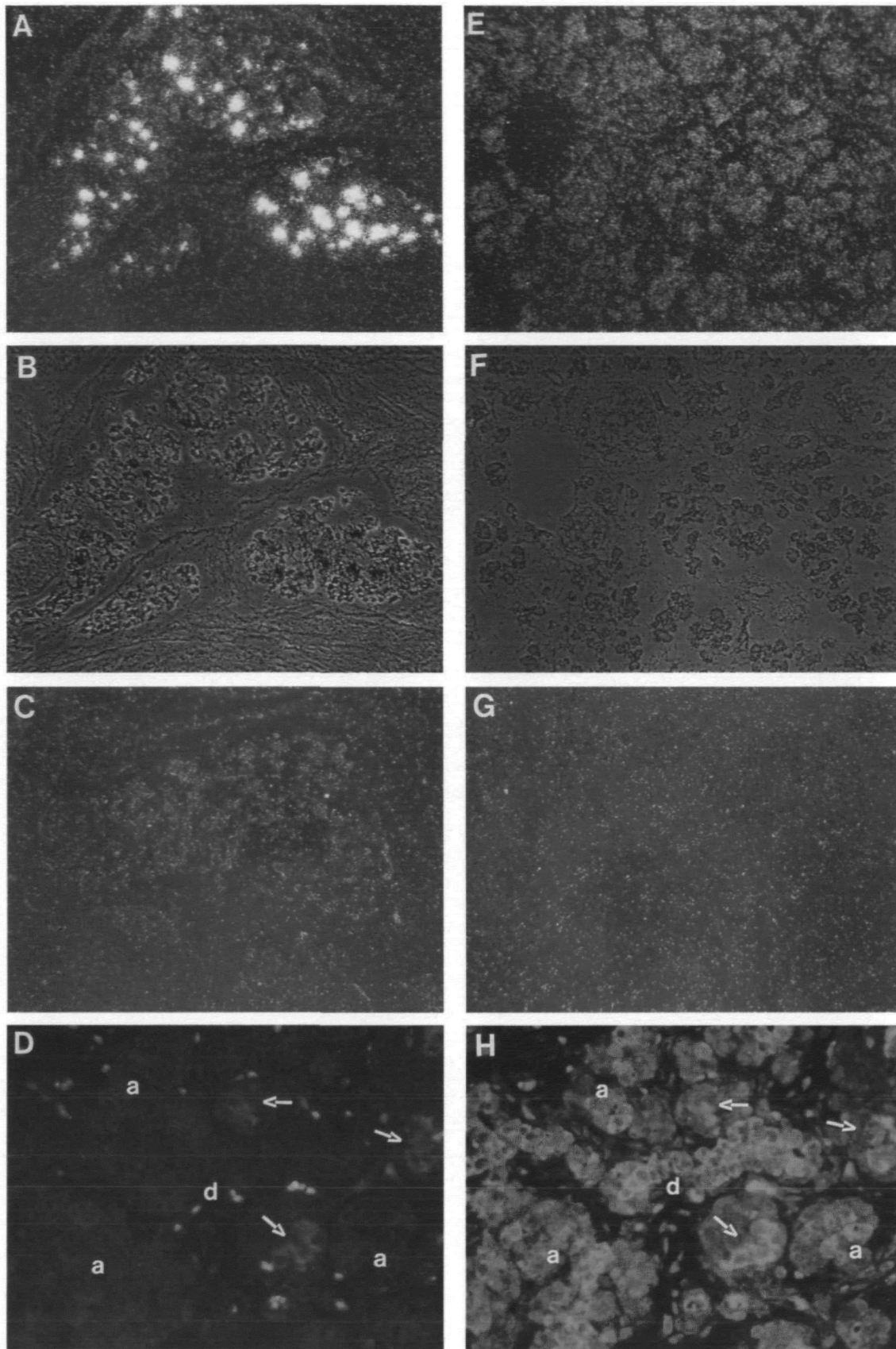
Fig. 4. In situ hybridization and immunohistochemical localization of TGF-α and insulin mRNAs and peptides in human fetal pancreas (20 gestation weeks). Insulin mRNA expression is limited to Langerhans islets [antisense probe, 10 day exposure; dark-field (A), phase contrast (B)]. No hybridization above background is seen with the corresponding insulin sense strand probe (C). TGF-α mRNA is expressed in low copy number in both the exo- and endocrine pancreas [antisense probe, 6 weeks exposure; dark-field (E), phase contrast (F)]. A weak background hydridization was obtained with the TGF-α sense strand probe (G). Double labeling the pancreas with rhodamine-conjugated insulin antibody (D) and fluorescein-conjugated TGF-α antiserum (H) shows colocalization of insulin and TGF-α to β-cells (arrows). TGF-α-immunoreactive cells are also seen in the acini (a) and ducts (d).
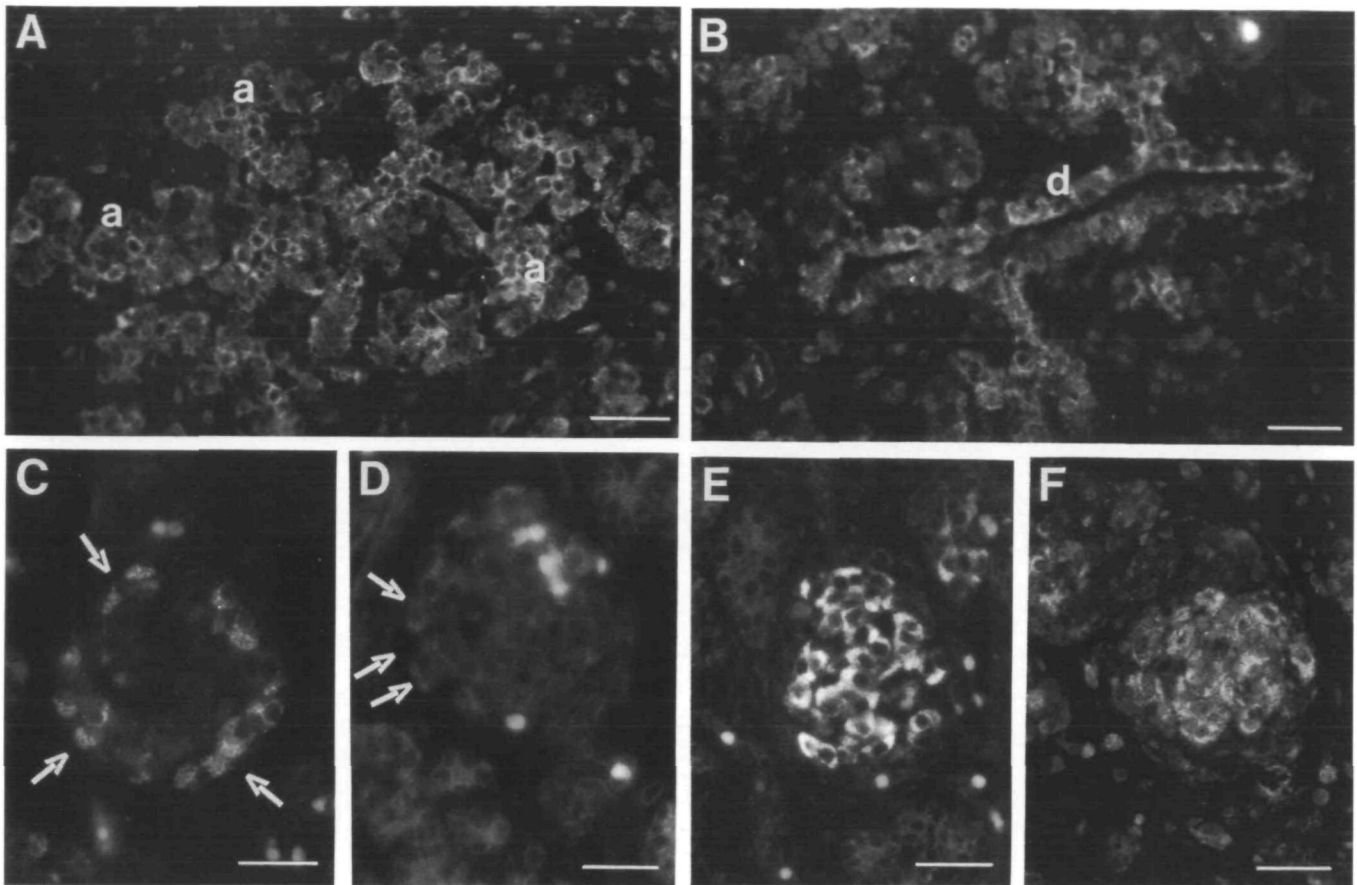
Fig. 5. Immunoreactive TGF-α, insulin, glucagon and somatostatin cells in human fetal pancreas (16 weeks gestation). TGF-α-immunoreactive cells are detected in the acini (A; a=acini) and ducts (B; d=ducts). Glucagon (C) and somatostatin immunoreactive cells (D) are located in the periphery of the islet as compared to the central distribution of insulin (E) and TGF-α containing cells (F). Bar=80 μm.

for EGF-R were detectable (Fig. 6A). Both exo- and endocrine pancreas synthesized the mRNA as shown by in situ hybridization (Fig. 6B,C). The strongest mono-clonal EGF-R antibody staining was in the cytoplasm of islet cells. Weaker immunoreactivity was seen in the acini and ducts as well as in the stroma (Fig. 6D).

Discussion

We report for the first time the expression of TGF-α and its receptor mRNA in human fetal pancreas. We also show that TGF-α and EGF-R immunoreactive cells are seen suggesting that the mRNAs are translated into protein. Furthermore, since TGF-α mRNA expression level is higher in pancreas than in various other tissues studied (data not shown) we suggest that TGF-α may be important in the development of the pancreas.

At the end of the first trimester (10-12 gestation weeks), the pancreatic acini appear and endocrine pancreas begins to develop by epithelial-mesenchymal interactions. Glucagon, insulin and finally somatostatin containing cells are detected by the 8-11th gestation week (Go et al., 1986). Islets originate from ductal precursor cells (Pictet et al., 1972) so that each islet

results from several independent cells (Deltour et al., 1991). During the second trimester, exocrine cells are distinguished from the ductal epithelium and by the 22nd gestation week lobules of exocrine acini and islets are separated by abundant mesenchyme. Blood vessels are formed between acini and islets. Lipase, amylase and tryptic activity can be detected from the 22nd gestation week. So, differentiation and cellular prolifer-ation take place concurrently with strong expression of TGF-α in both the exocrine and endocrine pancreas. Also the islet diameter increases by up to four times its original size during weeks 16-20, thereafter becoming smaller again (Hahn von Dorsche et al., 1988). The expression of the TGF-α receptor, EGF-R, in pancreas further suggests that TGF-α may function as an auto- and paracrine growth factor regulating these epithelial-mesenchymal interactions or it may function mitogeni-cally on the acini, ducts and islets.

As expected, insulin mRNA was detected only in the fetal pancreas during 15-20 gestation weeks. Despite earlier reports of insulin gene expression during organogenesis of chicken and rat liver and brain (DePablo et al., 1982; Muglia and Locker, 1984; Serrano et al., 1989), we were unable to detect any insulin mRNA in the corresponding tissues even by
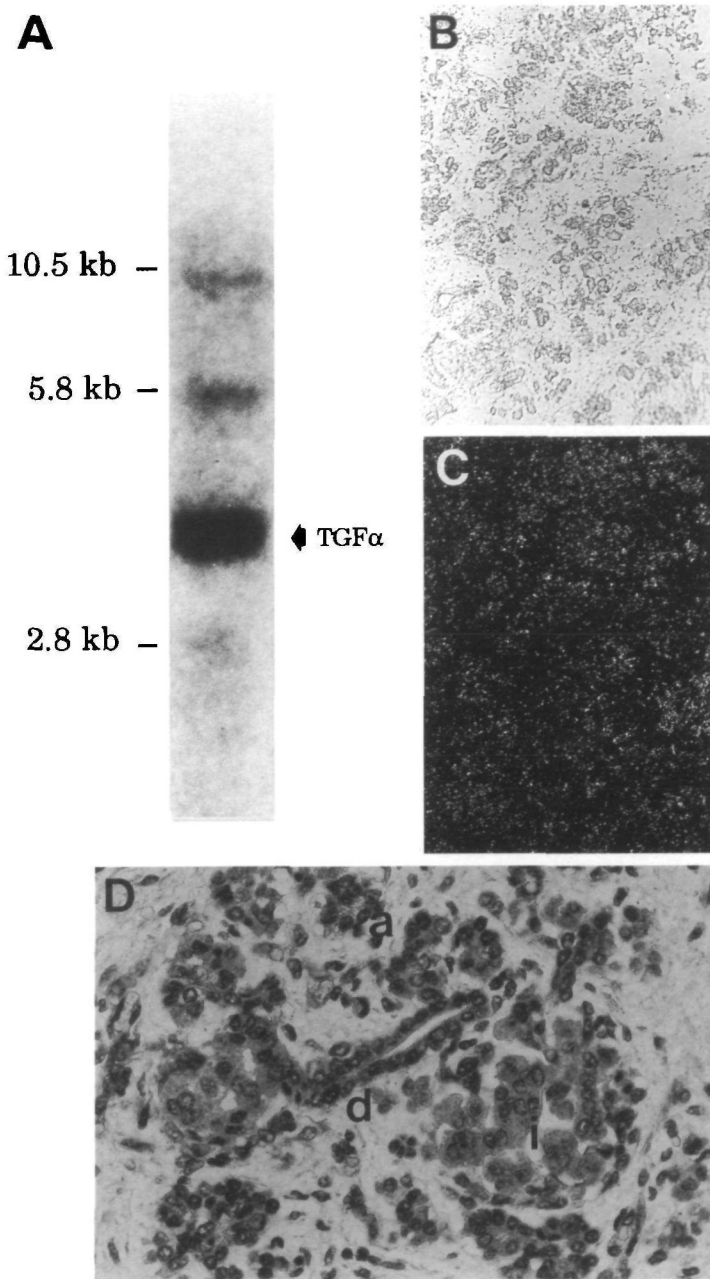
A

10.5 kb —

5.8 kb —

◀ TGFα

2.8 kb —

B

C

D

Fig. 6. Expression of EGF-R mRNA and EGF-R immunoreactive cells in human fetal pancreas (16–20 weeks gestation). 15 μg RNA from human fetal islets was subjected to electrophoresis and subsequently blotted onto nylon filter and hybridized with ^{32}P-labeled EGF-R cRNA probe. The obtained transcript sizes are shown on the left (A). TGF-α mRNA was probed simultaniously (arrow on the right). In situ hybridization of human fetal pancreas shows the diffuse expression of EGF-R mRNA throughout the pancreas [antisense probe; 3 weeks exposure; phase contrast (B) and dark-field (C)] corresponding to the pattern obtained with the monoclonal EGF-R antibody (red to pink staining; D). Acini=a; ducts=d; islets=i.

using PCR analysis. In rat embryos the insulin amount in pancreas increases parallel to the rise in insulin mRNA (Kakita et al., 1983). In our present study,

however, the amount of immunoreactive insulin-containing cells in pancreas is smaller than could be deduced from the mRNA level in β-cells. This suggests that either the translation of insulin mRNA is inefficient or fetal β-cells do not store insulin for a long period. The latter hypothesis is supported by studies showing, that the granularity of β-cells decreases and fetal blood insulin levels rise during 15-16 gestation weeks (Pronina and Sapronova, 1976). At this stage of development insulin might acquire altered functions. It might be increasingly distributed via blood circulation to function endocrinically as a growth factor since insulin and IGF I receptors are first detectable from the 15th gestation week (Sara et al., 1983). The glucoregulatory role for insulin is unlikely during the second trimester because β-cells are not sensitive to glucose until the 28th gestation week (Milner and Hill, 1987). We cannot exclude the possibility that the low amount of insulin immunoreactivity is an artefact resulting from the fetus's response to stress and prostaglandins used in the abortion - although it is questionable whether prostaglandins would reach significant concentrations in the fetus.

We have previously shown that fetal duodenum contains more immunoreactive TGF-α than other parts of the intestine (Miettinen et al., 1989). In the present study, TGF-α-like immunoreactivity was found also in the pancreatic ducts. The ductally produced TGF-α might flow into duodenum and function there paracrinically, increasing the TGF-α production of the Paneth cells and thereby increase their growth rate. A paracrine/autocrine stimulatory loop between pancreas and intestine is possible since in T3T4 pancreas carcinoma cells TGF-α upregulates its own mRNA production during a short term stimulation (Glinsmann-Gibson and Korc, 1991).

The localization of TGF-α into β-cells is interesting. These cells are known to secrete a variety of proteins besides insulin (Nishi et al., 1990). Insulin colocalization with TGF-α and the existence of insulin-degrading enzyme (IDE) that degrades insulin as well as TGF-α (Gehm and Rosner, 1991), suggests interaction between these two peptides. According to Sjöholm and co-workers (Sjöholm et al., 1990) TGF-α did not effect the insulin content of cultured fetal rat β-cells or their ability to secrete insulin. Neither did our preliminary results on human fetal islet-like cell clusters show any alteration in insulin mRNA expression after TGF-α stimulation. Nevertheless, insulin could inhibit the degradation of TGF-α by being a competitive substrate for IDE and thereby increase the local bioavailability of TGF-α.

To conclude, human fetal pancreas expresses TGF-α and EGF-R in both the exo- and endocrine parts. This argues in favor of TGF-α being one of the factors involved in pancreatic differentiation and growth.

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