Transforming Growth Factor- α and Epidermal Growth Factor Expression in Human Fetal Gastrointestinal Tract

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ABSTRACT. Epidermal growth factor (EGF) and transforming growth factor- α (TGF- α) are mitogenic to the intestinal epithelium. To further clarify their role in the developing human fetal gut, their expression was studied in fetuses at 15 to 20 wk of gestation. TGF- α mRNA was present throughout the gastrointestinal tract, most abundantly in the duodenum. EGF mRNA could be detected only with ribonuclease protection assay and reverse transcription-polymerase chain reaction analysis. The effect of EGF and TGF- α on TGF- α mRNA expression was studied by culturing explants of fetal jejunum, ileum, and colon for 7 d in Leibowitz L-15 medium supplemented with 100 μ g/L of either EGF or TGF- α . EGF receptor-like immunoreactivity was detected in both the villi and the crypts. In the jejunum, exogenous EGF up-regulated TGF- α mRNA 3-fold. However, exogenous TGF- α reduced its own mRNA by 40%. No mature 6-kD TGF- α was detected in the culture medium by Western blotting, but precursor forms of approximately 30 and 68 kD were present. The ileum and colon did not respond to either growth factor. Besides the gut, TGF- α was expressed in the gallbladder, salivary gland, adrenals, brain, kidney, liver, and placenta. The data imply an important role for TGF- α and EGF in the developing intestine. (*Pediatr Res* 33: 481–486, 1993)

Abbreviations

EGF, epidermal growth factor
EGF-R, epidermal growth factor receptor
GI, gastrointestinal
TGF- α , transforming growth factor- α
PCR, polymerase chain reaction
AP, alkaline phosphatase
RNase, ribonuclease

EGF and TGF- α are mitogenic peptides that also inhibit gastric acid production (1, 2). In dogs, TGF- α is synthesized by parietal cells of the stomach (3). Many GI tumors (4) and cell lines as well as the normal human adult colon (5) synthesize TGF- α mRNA. Immunoreactive TGF- α has been detected in mucosal cells of human fetal (6) and adult (7) gut and in colorectal neoplasia (8). Rat intestinal epithelial cell line IEC-6 also expresses TGF- α transcripts and an EGF-R binding substance (9).

EGF is present in salivary, gastric, duodenal, pancreatic, and amniotic fluids (10). Immunohistochemically, EGF has been

found in human fetal (6, 11) and adult (12) intestine. In fetal rhesus monkey (13) and suckling rabbits (14), EGF stimulates proliferation of the intestinal epithelium. However, in fetal mice (15) and newborn rats (16), it has no effect on DNA synthesis. Therefore, the effects of EGF on small intestinal cell proliferation and maturation seem to vary with the developmental stage (17). In cultured human fetal jejunal explants, EGF increases lactase production and decreases DNA synthesis (17). DNA synthesis is also diminished by EGF in human fetal colon explants (18).

EGF-R binds both EGF and TGF- α . Binding studies with 125 I-EGF show that in the human fetus EGF-R is present in the epithelial cells of both the small intestine and colon from the 12th wk of gestation (19). Immunoreactive EGF-R is detectable in the villous epithelial cells from the 16th wk of gestation (20).

EGF up-regulates TGF- α mRNA in human colon carcinoma (21) and in rat intestinal epithelial cell lines (22) during 2 to 48 h of exposure. However, for a study of intestinal development, a longer stimulation time is needed to mimic the *in vivo* situation *in utero* with continuous passage of amniotic fluid through the fetal gut. The present study was undertaken to clarify the expression and role of EGF and TGF- α in the developing human fetal GI tract. It also explores whether these peptides regulate TGF- α mRNA production in long-term organ explant cultures.

MATERIALS AND METHODS

Fetal tissues. Tissues were obtained from prostaglandin-induced legal abortions at 15 to 20 wk of gestation. The study was approved by the Ethical Committee of the Hospital. Delay from delivery room to liquid nitrogen or cell culture was 1 h. The gestational age was estimated from the fetal foot length (23). The intestines were dissected from the surrounding mesentery and stored in liquid nitrogen at -70°C for RNA extraction or further processed for organ explant cultures. Other tissues (submandibular salivary gland, pancreas, gallbladder, liver, adrenals, kidney, lungs, thyroid gland, brain, cerebellum, spinal cord, skin, testes, femur, and decidua from a 2nd-trimester placenta) were stored similarly.

Organ explant cultures. The intestine was divided into jejunum, ileum, and colon. These fragments were split longitudinally, cut into 3×7 mm pieces, and placed in Petri dishes on lens papers (24). Explants were cultured overnight in (medium Dulbecco's minimum essential medium-10% FCS-penicillin-streptomycin) and starved for 4 h in Leibowitz L-15 medium (Gibco, Paisley, UK) before stimulations with recombinant human EGF 100 μ g/L (Bachem, Bubendorf, Switzerland) and recombinant human TGF- α 100 μ g/L (Bachem) in Leibowitz L-15 medium. The media were renewed after d 1 of culture and then every 2 d. The explants were grown for 7 d and then processed for RNA extraction or immunohistochemistry. The morphology was preserved sufficiently after 7 d of culture and intense staining with hematoxylin was localized to the crypts.

RNA extraction and Northern analysis. Total cellular RNA

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Fig. 1. Southern blots from TGF- α PCR. Reverse transcribed RNA from human fetal (15 to 20 wk of gestation) intact (30 cycles) and cultured (60 cycles) gut (A) and other fetal tissues (30 cycles; B) were amplified using TGF- α -specific primers. Autoradiography after Southern transfer and hybridization with a 32 P-labeled TGF- α cDNA probe showed a single band of 262 bp. On the left is shown a 271-bp band from the size marker Φ XRF174/*Hae*III. The cultured intestine was grown in medium without (Co, control) or with supplementation of EGF or TGF- α (100 μ g/L both).

Fig. 2. RNase protection assay from intact and cultured human fetal gut. Fifty μ g of RNA from fractionated intestine (17 wk of gestation) and 30 μ g RNA from cultured intestine were hybridized with a 32 P-labeled TGF- α cRNA. After RNase treatment, 262-bp-protected fragments were obtained. A cold TGF- α cRNA sense strand was used as a positive hybridization control.

was isolated by guanidine isothiocyanate extraction and cesium chloride centrifugation (25), measured spectrophotometrically at 260 nm, and stored at -20°C until use. From a few tissues (fetal adrenal, brain, and placenta), polyadenylated RNA also was isolated using oligo(dT) columns (Pharmacia, Uppsala, Sweden).

RNA derived from A431 cells was used as a positive hybridization control for the TGF- α probe (4).

Ten to 30 μ g of total RNA (occasionally 2 to 8 μ g polyadenylated RNA) were denatured in glyoxal and DMSO and loaded into a 1.5% agarose gel (26). After electrophoresis, the RNA was transferred to a Hybond-N filter (Amersham, Buckinghamshire, UK) by capillary blotting. The filters were hybridized with EGF or TGF- α cRNA and 28S or γ -actin cDNA probes as described (27). The filters were washed in $0.1 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.15 M NaCl and 0.015 M sodium citrate) with 0.1% SDS (three times for 20 min at 65°C for cRNA and at 50°C for cDNA probes) and subjected to autoradiography using Trimax T16 (3M, Ferrania, Italy) intensifying screens and Hyperfilm MP films (Amersham) for 1 to 4 wk at -70°C . Northern blots were usually done from three to six fetuses.

Dot blot. Total RNA (2.5 to 5 μ g) was denatured in formaldehyde at 60°C for 15 min and blotted as described (26). The filters were hybridized as mentioned above. The signals obtained were scanned densitometrically. TGF- α readings were correlated with 28S readings. Results are expressed as mean $\pm$ SEM of four to five explant cultures (from four to five different fetuses).

Probes. A 925-bp *Eco*RI fragment of human TGF- α (28) and a 555-bp *Eco*RI fragment of human EGF (29) cDNA were subcloned into pGEM7(z) ribovectors (Promega Biotechnology,

Fig. 3. Northern blot analysis of TGF- α expression in human fetus. Total RNA (30 μ g) from the human fetal GI tract and other tissues (2–5 μ g polyadenylated RNA from brain, placenta, adrenals, and A431 cells) were subjected to electrophoresis and subsequently blotted onto a nylon filter and hybridized with a 32 P-labeled TGF- α cRNA probe. The correct transcript size is shown on the right. Integrity of RNA samples was controlled with a 28S or a γ -actin (polyadenylated RNA samples) cDNA probe. RNA derived from A431 cells was used as a positive hybridization control for the TGF- α probe.

Fig. 4. Southern blots from EGF PCR. Reverse transcribed RNA from human fetal tissues (15 to 20 wk of gestation) was amplified using EGF-specific primers (30 cycles). Autoradiography after Southern transfer and hybridization with a 32 P-labeled EGF oligoprobe produced a 368-bp band. On the left are shown size markers from Φ X174RF/*Hae*III.

Fig. 5. RNase protection assay from intact and cultured human fetal gut. Fifty μ g of RNA from human fractionated intestine (17 wk of gestation) was hybridized with a 32 P-labeled EGF cRNA. After RNase treatment, 555-bp-protected fragments were obtained. A cold EGF cRNA sense strand was used as a positive hybridization control.

Madison, WI) and labeled with [32 P]-UTP (1000 Ci/mmol, Amersham) by *in vitro* transcription using SP6 or T7 RNA polymerases (30). The correct size of the labeled probes was checked in a sequencing gel. TGF- α PCR fragments from human fetal decidua (1st trimester) and fetal duodenum (2nd trimester) were also subcloned into pGEM7(z) ribovectors as described (27). [32 P]-dCTP random prime-labeled (Oligolabeling Kit, Pharmacia, Uppsala, Sweden) 28S or γ -actin cDNA (31) were used to verify the integrity of the RNA and to equalize the amounts of loaded RNA.

For PCR Southern blots, a 32 P-labeled 925-bp TGF cDNA probe, a 27-mer internal EGF oligoprobe (nucleotides 3440 to 3466 of human EGF precursor) (32), and a β -actin oligoprobe (nucleotides 312 to 338 of human β -actin cDNA) (33) were used for hybridizations. Oligoprobes were 3'-end-labeled with [32 P]-dCTP using terminal transferase (DuPont/New England Nuclear labeling system, NEP-100, Boston, MA).

Reverse transcription and PCR. One μ g of total RNA from each tissue was used for cDNA synthesis as described previously (34). For a negative control, water was used instead of RNA. PCR was performed according to the instructions provided with the GeneAmp DNA Amplification Reagent Kit (Perkin-Elmer-Cetus, Norwalk, CT). One μ L of reverse transcription mixture was combined with 1) 5 μ L of 10 $\times$ PCR-reaction buffer; 2) 37 μ L of water; 3) 4 μ L of dNTP mixture (0.2 μ M final concentration for each deoxynucleotide); 4) 50 pmol of antisense and sense strand primers each; and 5) 2.5 U of AmpliTaq (Perkin-Elmer-Cetus) enzyme. The reactions were heated to 95°C for 2 min and then immediately cycled (30 cycles: 94°C for 30 s for denaturation, 58°C for 30 s for annealing, and 72°C for 1 min 30 s for elongation) in a Hybaid thermal reactor (Teddington, UK).

The following oligonucleotide primers were synthesized: a 20-mer TGF- α sense primer (5'-CGCCCTGTTCTGCTCTGGGTA-3') corresponds to nucleotides 58 to 77, and a 20-mer antisense primer (5'-CTGGCTGGCAGCCACCGG-3') corresponds to nucleotides 300 to 319 (35). The expected size of the TGF- α PCR product is 262 bp. EGF 20-mer sense (5'-GACGCTGTCTGAACCAGGA-3') and 20-mer antisense (5'-CGATAGCAGCTTCTGAGTCC-3') primers create a 368-bp

fragment corresponding to nucleotides 3261 to 3628 of the EGF precursor (32). β -Actin sense (5'-CCCAGGCACCGGGCGT-GAT-3'; nucleotides 153 to 172) and antisense (5'-TCAAACAT-GATCTGGGTCAT-3'; nucleotides 396 to 415) primers create a 263-bp fragment (33). The β -actin PCR was done to verify the integrity of the reverse transcribed mRNA.

Ten- μ L aliquots of the PCR reaction products were size-fractionated in 2% agarose gel (Pharmacia, Sweden). *Hae*III-digested pBR322/ Φ XRF fragments were used as size markers. Southern transfer was done by capillary blotting the DNA to Hybond-N nylon membranes (Amersham). The membranes were hybridized with 32 P-dCTP-labeled TGF- α cDNA, EGF, or β -actin oligoprobes.

RNase protection assay. Protection assay was performed as described (27). Briefly, 30 to 80 μ g of fetal RNA samples were hybridized with 200 000 cpm of 32 P-labeled EGF and TGF- α riboprobes. Transfer RNA was used as a negative control and cold sense strands as positive controls. After treatments with RNaseA (40 mg/L; Sigma Chemical Co., MO) and RNaseT1 (375 000 U/L; Boehringer, Mannheim, Germany) and digestion with proteinase K (100 mg/L) and 0.5% SDS, the protected RNA-RNA duplexes were extracted with phenol and chloroform, precipitated with ethanol, and analyzed on denaturing 6% PAGE-urea gel. The EGF riboprobe contained 555-bp bases of EGF mRNA and 30 bases of the pGEM7(z) sequence. The TGF- α riboprobe contained 270 bases of TGF- α mRNA and 40 bases of the vector sequence, respectively.

Immunohistochemistry. Paraformaldehyde-fixed cultured jejunum, ileum, and colon explants were embedded in paraffin. Immunohistochemistry was performed using a Super Sensitive Multilink kit and a monoclonal EGF-R antibody (BioGenex, San Ramon, CA) (27). This antibody reacts with the 170-kD EGF-R transmembrane glycoprotein (obtained from A431 carcinoma cells). Briefly, the sections were incubated for 1 h with the antibody, washed twice in PBS-0.25% Triton X-100 (PBS-TX), and incubated with biotinylated anti-immunoglobulins for 20 min. The sections were then washed again in PBS-TX, and treated with AP-conjugated streptavidin for 20 min and with substrate solution for 25 min. Endogenous AP was blocked by including 10 mM levamisole (Sigma) and 25 mM phenylalanine in the substrate solution. Fast Red was used as a chromogen. EGF-R antibody was replaced by normal mouse serum for specificity controls. To exclude false-positive staining due to endogenous AP, the immunostaining was also performed with peroxidase-conjugated streptavidin (Dako, Copenhagen, Denmark) instead of AP. The results were the same with both enzyme conjugates. Before microscopic examination, the slides were counterstained with Mayer's hematoxylin.

Western immunoblot analysis. Two-mL samples from d-5 and d-7 intestine cultures were pooled, concentrated 40-fold with Centricon 3 (Grace & Co, Beverly, MA), and mixed with 2 $\times$ Laemmli buffer with β -mercaptoethanol. After 15% SDS-gel electrophoresis (36), the samples were electroblotted (250 mA for 1 h) to Hybond C-nitrocellulose paper (Amersham) using Bio-Rad Trans-Blot apparatus (37). Nonspecific binding was blocked by incubating the membrane overnight in 10% BSA-0.1% Tween 20 (Bio-Rad, Richmond, CA) in PBS. The membrane was incubated with the goat anti-human TGF- α (1:15000 dilution, Biotope), washed thoroughly in 0.1% Tween-PBS, and incubated with peroxidase-conjugated donkey anti-sheep IgG (Jackson, West Grove, PA). The immunoreaction was detected using the ECL system according to the manufacturer's instructions (Amersham). Recombinant TGF- α (Amgen) was used as a positive control. For specificity controls, staining was performed with preabsorbed antiserum (14 μ M TGF- α). Immunoreactions were either abolished or reduced.

RESULTS

Expression of TGF- α mRNA in intestine. Samples of total RNA from human fetal (15 to 20 wk of gestation) duodenum,

Fig. 6. Immunohistochemical localization of EGF-R in the cultured human fetal intestine. Hematoxylin staining of the cultured jejunal explants shows fairly well-preserved morphology (A). In the intact duodenum, EGF-R immunoreactivity is strong throughout the villous-crypt axis (red, B). In the cultured jejunal explants, the staining pattern is similarly seen in the epithelial (e) and goblet cells (g) as well as in the crypts (cr, C). Normal mouse serum does not stain these cells (arrows) (D). Bar = 100 μ m.

Fig. 7. Regulation of TGF- α mRNA in organ explants of human fetal intestine. Cultured jejunal, ileum, or colon was stimulated with EGF or TGF- α (100 μ g/L) for 7 d. RNA was isolated and analyzed for TGF- α mRNA expression using a dot-blot system. Hybridization signals were scanned and correlated to 28S mRNA expression. Results are expressed as mean $\pm$ SEM (four to five different fetuses).

Fig. 8. Western blot analysis of culture medium from jejunal explants. Concentrated media from control (C) and EGF- (E) and TGF- α - (T) stimulated cultures were run in 15% PAGE and blotted onto a nitrocellulose filter. Approximately 30-kD (bottom arrow) and 60- to 68-kD (top arrow) bands were detected with anti-TGF- α antiserum and the ECL detection system. Diffuse 6-kD band in TGF- α -stimulated culture represents the exogenous TGF- α (*). As a positive control, 5 μ g of recombinant TGF- α were used. A molecular size marker is shown on the left.

jejunum, ileum, and colon were assessed for TGF- α gene expression. The TGF- α -specific signal could be reverse transcribed and amplified from different parts of the intact GI tract and from cultured organ explants of the jejunum and the ileum (Fig. 1A).

Other positive tissues were submandibular salivary gland, pancreas, gallbladder, liver, adrenals, kidney, lungs, thyroid gland, brain (cerebellum, weakly), spinal cord, skin, testes, femur (weakly), and decidua from a 2nd-trimester placenta (Fig. 1B). An RNase protection assay was performed using a TGF- α riboprobe obtained from a decidual TGF- α PCR fragment. Protected hybrids were seen in both the fractionated and the cultured intestine (Fig. 2). A single band of 4.8 kb corresponding to TGF- α mRNA was also detected by Northern blotting; it was strongest in the duodenum (Fig. 3). TGF- α mRNA was only faintly visible in cultured jejunal organ explants (data not shown). TGF- α expression was also detected with Northern blotting from submandibular salivary gland, gallbladder, pancreas, kidney, adrenal, brain, liver, and placenta (10 wk of gestation) (Fig. 3). The integrity of the RNA samples was controlled by hybridization with a 28S or a γ -actin cDNA probe (not shown). RNA for PCR, RNase protection, and Northern blot analysis derived from different fetuses.

Expression of EGF mRNA in intestine. An EGF-specific 367-bp PCR fragment was amplifiable from duodenum, jejunum, ileum, and colon. Specificity was verified by hybridizing the generated Southern blots with an internal oligoprobe (Fig. 4). Other tissues positive in EGF PCR were skin, kidney, liver, submandibular salivary gland, pancreas, thyroid gland (not shown), and, faintly, femur (comprised also bone marrow; Fig. 4). The sensitivity of Northern blot analysis was insufficient to detect any EGF mRNA from the intestine. Using RNase protection assay, however, EGF transcripts could be located in duodenum, small intestine, and colon, respectively, corresponding to the PCR results (Fig. 5).

Regulation of TGF- α mRNA by EGF and TGF- α in intestine organ cultures. To further elucidate the function of EGF and TGF- α in fetal gut, organ cultures were studied. Explants from the jejunum, ileum, and colon (five different fetuses of 15 to 19 wk of gestation) were grown in Leibowitz L-15 medium supplemented with EGF (100 μ g/L) or TGF- α (100 μ g/L) for 1 wk. The presence of immunoreactive EGF-R in the intestine was demonstrated by an MAbs against the extracellular domain of the receptor. In the intact small and large intestine, EGF-R-like immunoreactivity was detected in the crypts, villi (being strongest in the apical region and in the brush border, although also in the basolateral region of the epithelial cells), capillary vessel walls, and in lamina propria. In the cultured jejunum, EGF-R-like immunoreactivity was localized to the goblet cells and epithelial cells of the villi and to the crypts (Fig. 6). In the cultured ileum and colon, the villous epithelium was only weakly positive. Endogenous AP was blocked during the staining procedure by levamisole and phenylalanine (38). For specificity controls, the antibody was replaced with normal mouse serum, and no staining was then seen. Furthermore, identical staining patterns were obtained when a peroxidase-conjugated rabbit anti-mouse antiserum was used instead of an AP-conjugated antiserum (not shown). This precluded the possibility that the intense staining would represent endogenous AP.

Because the amount of RNA obtained from the cultures was too low for Northern blot analysis, the regulation of TGF- α mRNA was studied in a dot-blot system. In the jejunum, EGF up-regulated TGF- α mRNA by approximately 3-fold, but TGF- α down-regulated its own mRNA by 40%. The ileum and colon did not respond to either growth factor (Fig. 7). To study whether the TGF- α mRNA is translated to protein, Western blot analysis was performed from the concentrated culture media. No mature TGF- α peptide was detectable (apart from the exogenous TGF- α), but precursor forms of approximately 30 kD and 60 to 68 kD were found (Fig. 8). When an antiserum preabsorbed with excess TGF- α was used, these immunoreactive bands were abolished. The amount of protein in the control and EGF/TGF- α -stimulated organ explants could not be equalized owing to limited material; therefore, no difference was seen between the control and stimulated cultures.

DISCUSSION

The present article reports the expression of EGF and TGF- α mRNA in the human fetal 2nd-trimester GI tract. TGF- α expression was strongest in the duodenum, gallbladder, pancreas, and submandibular salivary gland. It was expressed more abundantly in the intestine than EGF, because EGF mRNA was detectable only with the sensitive PCR and RNase protection assays, but TGF- α mRNA was also demonstrated by Northern blot analysis. TGF- α is synthesized during early murine development (10), but from human fetuses there is little evidence of this (27). EGF mRNA has not been previously detected during fetal development, probably because of insensitivity of methods. Nevertheless, the present data reveal a low-level expression of EGF in the 2nd-trimester human fetal intestine. Thus, TGF- α appears to be the major EGF-like peptide in 15- to 20-wk-old human fetuses.

Human fetal jejunum and colon grow in organ culture (39,40). At the 18th wk of gestation, proliferating epithelial cells in the cultured and intact intestine are confined to the crypts. In the present study, the morphology of the explant cultures was preserved after 7 d in culture, and staining with hematoxylin was localized to the crypts (Fig. 7A). Immunoreactive EGF-R was also strongly detected in the villi, crypts, and capillary vessel wall in both the intact and the cultured intestine (Fig. 7B-C). Furthermore, TGF- α mRNA was inducible with exogenous EGF. This agrees with previous results with adult human and animal cell cultures; namely, TGF- α mRNA was up-regulated by EGF and/or TGF- α (5 to 100 μ g/L) in most short-term (2- to 48-h) stimulation experiments with rat intestinal epithelial cells (22), regenerating liver (41), carcinoma cells from human pancreas (42), and cultured human keratinocytes (43). These effects were transient, usually declining within 24 h. The mechanism behind the TGF- α mRNA rise may be increased transcription (44). In well-differentiated quiescent colon carcinoma cells, EGF also up-regulated TGF- α mRNA, but was without effect on TGF- α synthesis in poorly differentiated cells (21, 45). In the present study, exogenous TGF- α reduced its own mRNA expression by 40%. This differs from the above-mentioned reports on adult and malignant cells, probably owing to the longer observation time used in the present experiments. Why EGF and TGF- α have different effects on TGF- α mRNA remains unclear. Recently, TGF- α binding was found to be increased in a pancreatic cancer cell line at alkaline pH. At neutral pH, TGF- α binding was markedly less than EGF binding (46). Therefore, EGF and TGF- α may regulate signal transduction pathways differently in different cell types through dissimilar interactions with the receptor. In addition, the intestine may respond differently to different doses of EGF and TGF- α . Unfortunately, the limited material available did not allow detailed dose-response studies.

Surprisingly, in the ileum and colon, TGF- α mRNA synthesis did not change after stimulations with EGF and TGF- α . According to our Northern blot results, there are no substantial differences in TGF- α expression between the intact jejunum, ileum, and colon. However, we found previously that the ileum and colon contain less TGF- α -like immunoreactivity than the duodenum and jejunum (6). Furthermore, it has been shown that, in fetal rhesus monkey, the growth-promoting activity of EGF is largely restricted to the upper intestine (13). Therefore, the 2nd-trimester ileum and colon may require higher concentrations of EGF/TGF- α than the jejunum to obtain similar effects. This could be due to a difference in receptor density. In autoradiographic studies, the human fetal colon exhibited more EGF-binding capacity than the small intestine (19). However, my unpublished results show that the EGF-R immunoreactivity is rather equally distributed between jejunum, ileum, and colon (two to three fetuses). In the present study, a strong EGF-R immunoreaction is located both in the crypts and in the brush border. The mapping of EGF-R to the apical surface is contrary to the 125 I-EGF binding study of Ménard and Pothier (47). Methodologic differences could explain the partially different

results. Furthermore, the absence of labeled EGF in the villi observed by Ménard and Pothier could also result from the already occupied EGF receptors by endogenous TGF- α or EGF.

EGF and TGF- α are both mitogens. In the human fetal jejunum and colon, EGF stimulates proliferation and differentiation (17, 18). EGF also promotes proliferation of the small intestinal mucosa in neonatal microvillous atrophy (48). The present demonstration of expression of both TGF- α and EGF in the human fetal GI tract further implies an important role for these peptides in the developing gut. Introduction of EGF-like peptides into the treatment of very premature newborns may in the future help to prevent GI problems caused by the immature intestines of these babies.

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REFERENCES

- Dembinski A, Drozdowicz D, Gregory H, Konturek SJ, Warzecha Z 1986 Inhibition of acid formation by epidermal growth factor in isolated rabbit gastric glands. *J Physiol (Lond)* 378:347-357
- Rhodes JA, Tam JP, Finke U, Saunders M, Bernanke J, Silen W, Murphy RA 1986 Transforming growth factor α inhibits secretion of gastric acid. *Proc Natl Acad Sci USA* 83:3844-3846
- Beauchamp RD, Barnard JA, McCutchen CM, Cherner JA, Coffey RJ 1989 Localization of transforming growth factor α and its receptor in gastric mucosal cells. Implications for a regulatory role in acid secretion and mucosal renewal. *J Clin Invest* 84:1017-1023
- Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger WH 1987 Synthesis of messenger RNAs for transforming growth factors α and β and the epidermal growth factor receptor by human tumors. *Cancer Res* 47:707-712
- Malden LT, Novak U, Burgess AW 1989 Expression of transforming growth factor alpha messenger RNA in the normal and neoplastic gastrointestinal tract. *Int J Cancer* 43:380-384
- Miettinen PJ, Perheentupa J, Otonkoski T, Lähdenmäki A, Panula P 1989 EGF- and TGF α -like peptides in human fetal gut. *Pediatr Res* 26:25-30
- Cartledge SA, Elder JB 1989 Transforming growth factor α and epidermal growth factor levels in normal human gastrointestinal mucosa. *Br J Cancer* 60:657-660
- Tanaka S, Imanishi K, Yoshihara M, Haruma K, Sumii K, Kajiyama G, Akamatsu S 1991 Immunoreactive transforming growth factor alpha is commonly present in colorectal neoplasia. *Am J Pathol* 139:123-129
- Koyama S-Y, Podolsky DK 1989 Differential expression of transforming growth factors α and β in rat intestinal epithelial cells. *J Clin Invest* 83:1768-1773
- Carpenter G, Wahl MI 1991 The epidermal growth factor family. In: Sporn MB, Roberts AB (eds) *Peptide Growth Factors and Their Receptors I*. Springer-Verlag, New York, pp 69-171
- Poulsen S, Nexø E, Skov Olsen P, Hess J, Kirkegaard P 1986 Immunohistochemical localization of epidermal growth factor in rat and man. *Histochemistry* 85:389-394
- Elder JB, Williams G, Lacey E, Gregory H 1978 Cellular localization of human urogastrone/epidermal growth factor. *Nature* 271:466-467
- Read LC, Tarantal A, George-Nascimento C 1989 Effects of recombinant human epidermal growth factor on the intestinal growth of fetal rhesus monkey. *Acta Paediatr Scand [Suppl]* 351:97-103
- O'Loughlin EV, Chung M, Hollenberger M, Hayden J, Zahavi I, Gall DG 1985 Effect of epidermal growth factor on ontogeny of the gastrointestinal tract. *Am J Physiol* 249:G674-G678
- Beaulieu JF, Ménard D, Calvert R 1985 Influence of epidermal growth factor on the maturation of the fetal mouse duodenum in organ culture. *J Pediatr Gastroenterol Nutr* 4:476-481
- Foltz-Jourdainne C, Raul F 1990 Effect of epidermal growth factor on the expression of digestive hydrolases in the jejunum and colon of newborn rats. *Endocrinology* 127:1763-1769
- Ménard D, Arsenault P, Pothier P 1988 Biologic effects of epidermal growth factor in human fetal jejunum. *Gastroenterology* 94:656-663
- Ménard D, Corriveau L, Arsenault P 1990 Differential effects of epidermal growth factor and hydrocortisone in human fetal colon. *J Pediatr Gastroenterol Nutr* 10:13-20
- Pothier P, Ménard D 1988 Presence and characteristics of epidermal growth factor receptors in human fetal small intestine and colon. *FEBS Lett* 228:113-117
- Oliver AM 1988 Epidermal growth factor receptor expression in human foetal tissues is age-dependent. *Br J Cancer* 58:461-463
- Mulder KM, Zhong Q, Choi HG, Humphrey LE, Brattain MG 1990 Inhibitory effects of transforming growth factor β on mitogenic response, transforming growth factor α and c-myc in quiescent, well-differentiated colon carcinoma cells. *Cancer Res* 50:7581-7586
- Suemori S, Ciacci C, Podolsky DK 1991 Regulation of transforming growth factor expression in rat intestinal epithelial cell lines. *J Clin Invest* 87:2216-2221
- Munsick RA 1984 Human fetal extremity lengths in the interval from 9 to 21 menstrual weeks of pregnancy. *Am J Obstet Gynecol* 149:883-887
- Ménard D, Arsenault P 1985 Explant culture of human fetal small intestine. *Gastroenterology* 88:691-700
- Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294-5299
- Voutilainen R, Tapanainen J, Chung B-C, Matteson KJ, Miller WL 1986 Hormonal regulation of P450scc (20, 22-desmolase) and P450c17 (17 α -hydroxylase/17, 20-lyase) in cultured human granulosa cells. *J Clin Endocrinol Metab* 63:202-207
- Miettinen PJ, Heikinheimo K 1992 Transforming growth factor alpha and insulin gene expression in human fetal pancreas. *Development* 113:833-840
- Murray JC, DeHaven CR, Bell GI 1986 RFLPs for epidermal growth factor (EGF), a single copy sequence at 4q25-4q27. *Nucleic Acids Res* 14:5117
- Murray JC, Buetow KH, Bell GI 1986 RFLPs for transforming growth factor alpha (TGFA) gene at 2p13. *Nucleic Acids Res* 14:7126
- Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR 1984 Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res* 12:7035-7056
- Arnheim N 1979 Characterization of mouse ribosomal gene fragments purified by molecular cloning. *Gene* 7:83-96
- Bell GI, Fong NM, Stempien MM, Wormsted MA, Caput D, Ku L, Urdea MS, Rall LB, Sanchez-Pescador R 1986 Human epidermal growth factor precursor: cDNA sequence, expression *in vitro* and gene organization. *Nucleic Acids Res* 14:8427-8446
- Ponte P, Ng S-Y, Engel J, Gunning P, Kedes L 1984 Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. *Nucleic Acids Res* 12:1687-1696
- Voutilainen R, Ilvesmäki V, Miettinen PJ 1991 Low expression of 3 β -hydroxy-5-ene steroid dehydrogenase gene in human fetal adrenals *in vivo*; adrenocorticotropin and protein kinase C-dependent regulation in adrenocortical cultures. *J Clin Endocrinol Metab* 72:761-767
- Derynck R, Roberts AB, Winkler ME, Chen EY, Goeddel DV 1984 Human transforming growth factor- α : precursor structure and expression in *E. coli*. *Cell* 38:287-297
- Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685
- Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76:4350-4354
- Ménard D, Pothier P 1987 Differential distribution of digestive enzymes in isolated epithelial cells from developing human fetal small intestine and colon. *J Pediatr Gastroenterol Nutr* 6:509-516
- Arsenault P, Ménard D 1987 Cell proliferation in developing human jejunum. *Biol Neonate* 51:297-304
- Arsenault P, Ménard D 1989 Cell proliferation during morphogenesis of the human colon. *Biol Neonate* 55:137-142
- Mead JE, Fausto N 1989 Transforming growth factor α may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc Natl Acad Sci USA* 86:1558-1562
- Glinnmann-Gibson BJ, Korc M 1991 Regulation of transforming growth factor- α mRNA expression in T₃T₄ human pancreatic carcinoma cells. *Pancreas* 6:142-149
- Coffey RJ, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL, Pittelkow MR 1987 Production and autoinduction of transforming growth factor- α in human keratinocytes. *Nature* 328:817-820
- Raja RH, Paterson AJ, Shin TH, Kudlow JE 1991 Transcriptional regulation of the human transforming growth factor- α gene. *Mol Endocrinol* 5:514-520
- Mulder KM 1991 Differential regulation of c-myc and transforming growth factor- α messenger RNA expression in poorly differentiated and well-differentiated colon carcinoma cells during establishment of a quiescent state. *Cancer Res* 51:2256-2262
- Korc M, Chandrasekar B, Shah GN 1991 Differential binding and biological activities of epidermal growth factor and transforming growth factor α in a human pancreatic cancer cell line. *Cancer Res* 51:6243-6249
- Ménard D, Pothier P 1991 Radiographic localization of epidermal growth factor receptors in human fetal gut. *Gastroenterology* 101:640-649
- Walker-Smith JA, Phillips AD, Walford N, Gregory H, Fitzgerald JD, McCullagh K, Wright NA 1985 Intravenous epidermal growth factor/urogastrone increases small-intestinal cell proliferation in congenital microvillous atrophy. *Lancet* 2:1239-1240