Intestinal fibroblasts regulate intestinal epithelial cell proliferation via hepatocyte growth factor

MICHAEL GÖKE, MICHYUKI KANAI, AND DANIEL K. PODOLSKY
Gastrointestinal Unit and Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Göke, Michael, Michiyuki Kanai, and Daniel K. Podolsky. Intestinal fibroblasts regulate intestinal epithelial cell proliferation via hepatocyte growth factor. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G809–G818, 1998.—Although the presence of subepithelial intestinal fibroblasts has been well recognized, the effects of fibroblasts on intestinal epithelial cell (IEC) growth are incompletely understood. In vitro studies were undertaken to evaluate the effects of fibroblasts on the proliferation of model IEC lines. IECs (Caco-2, T84, and IEC-6) were grown alone or in the presence of irradiated fibroblasts or by Transwell coculture technique with fibroblasts and epithelial cells separated by a porous filter. Cell proliferation was assessed by [3H]thymidine incorporation into DNA and in coculture with fibroblasts and epithelial cells. Cocultures were carried out directly on the mucosal side of Transwell filters in primary cultures of rat colon fibroblasts and in cocultures with human intestinal epithelial cell models through paracrine action. Further evidence for the growth-promoting effects of fibroblasts in both fibroblasts and fibroblast-conditioned medium is provided by recombinant human HGF dose dependently promoted IEC proliferation. HGF mRNA and protein expression were restricted to fibroblasts. High levels of c-met expression were found in Caco-2 and T84 cells; in contrast, expression in fibroblasts was weak. In summary, fibroblasts stimulate IEC proliferation through a paracrine mechanism mediated predominantly by HGF.

THE EPITHELIUM OF THE INTESTINAL TRACT UNDERGOES RAPID AND CONTINUED TURNOVER THROUGHOUT LIFE (26, 37). Various peptide growth factors present in the intestinal mucosa are known to modulate epithelial cell proliferation. Transforming growth factor-α (TGF-α) is a strong mitogen and TGF-β1 a potent inhibitor of intestinal epithelial cell growth (18, 22, 37). Insulin-like growth factor I (IGF-I) and IGF-II, hepatocyte growth factor (HGF), and members of the fibroblast growth factor (FGF) family, including acidic FGF (aFGF) and keratinocyte growth factor (KGF), have been demonstrated to increase intestinal epithelial cell proliferation in vitro and in vivo (7, 8, 12, 14, 19, 34, 35, 37, 38, 48).

Despite increasing information about factors affecting intestinal epithelial cell proliferation, the understanding of mechanisms regulating intestinal epithelial cell growth is incomplete. In particular, the role of other cell populations commonly present in close proximity to the regenerative intestinal epithelial cell compartment is unknown. Because growth and differentiation of hematopoietic cells depend, for example, on stromal cell-derived stem cell factor (c-kit ligand; Refs. 10, 56), it is also possible that growth of intestinal epithelial cells is modulated by other cell populations underlying the epithelium.

The presence of a fibroblast sheet underlying the intestinal epithelium has been well recognized (5, 9, 36), but its interaction with the overlying epithelium has not been well characterized. Epithelial-mesenchymal interactions are thought to be critical for formation of the basement membrane in the intestinal mucosa (15, 43, 53, 55). In vitro studies indicate that intestinal fibroblasts modulate electrical resistance of intestinal epithelial cells, e.g., chloride secretory responses of T84 colonic epithelial cells to bradykinin, serotonin, hydrogen peroxide, and histamine are enhanced in the presence of fibroblasts (1). Other reports (16, 44, 47) have demonstrated that mesenchymal cells promote differentiation and organization of intestinal epithelial cells in vitro, an effect that appears to be mediated through TGF-β1. In addition, colony formation of human colon tumor cells in soft agarose was enhanced by mouse embryonic salivary gland mesenchyme in vitro (3). Another recent study (11) described the stimulatory effects of intestinal fibroblasts on morphogenesis, differentiation, and growth of rat fetal gut endoderm in a coculture system.

These data suggest that fibroblasts underlying the intestinal epithelium could play an important role in regulating rapid intestinal epithelial cell turnover. However, understanding of the effects of adult human intestinal fibroblasts on human intestinal epithelial cell proliferation remains incomplete. Most importantly, previous studies have provided limited insight into the mechanistic basis through which fibroblasts modulate intestinal epithelial populations. Thus interaction might be achieved through cell-to-cell contact-dependent interactions, indirectly through modulation of the extracellular matrix milieu of the intestinal epithelial cells by fibroblasts, or by production of soluble regulatory peptides. In this report, we demonstrate that fibroblasts predominantly regulate intestinal epithelial cell models through paracrine action. Furthermore, we find that HGF is the dominant factor produced by fibroblast populations that promotes intestinal epithelial cell proliferation through c-met (HGF receptor) present on the epithelial population.

METHODS

Growth factors and antibodies used. Rabbit neutralizing polyclonal anti-bovine aFGF and neutralizing polyclonal goat anti-human HGF antibodies were a generous gift from R&D...
L-glutamine, penicillin, and streptomycin, as described above, were purchased from R&D Systems. The monoclonal antibody was obtained from Dr. T. Jake Liang (National Institutes of Health, Bethesda, MD) (41). Recombinant human HGF was purchased from R&D Systems. The monoclonal anti-human HGF antibody was a generous gift from Dr. Jeffrey S. Rubin (National Institutes of Health, Bethesda, MD) (24).

Cells used. CCD-18 cells (normal human colon fibroblasts, American Type Culture Collection [ATCC] CRL 1459, passages 8-12; Ref. 23) and CCD-37 cells (normal human lung fibroblasts, ATCC CRL 1496, passages 5-8) were obtained from ATCC (Rockville, MD) and cultured in MEM containing Earle's salts, nonessential amino acids ( Gibco-BRL, Gaithersburg, MD), 4.5 g/l glucose, 4 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 10% heat-inactivated fetal bovine serum (FBS). Human skin explant-derived normal fibroblasts were kindly provided by Dr. Michael N. Moran (Dermatology Unit, Massachusetts General Hospital), using a previously described technique (25) and cultured (passages 2-6) in DMEM (Mediatech, Herndon, VA) containing L-glutamine, penicillin, and streptomycin, as described above, and 10% FBS. Caco-2 (human colon adenocarcinoma, ATCC HTB 37) cells at passages 22-38 were cultured in medium used for CCD-37 cells, except that 20% FBS was used. T84 cells (human colon carcinoma, ATCC CCL 248) at passages 55-65 were cultured in a 1:1 mixture of Ham's F-12 medium and DMEM (Cellgro) containing L-glutamine, penicillin, streptomycin, and 5% FBS. IEC-6 cells were cultured as described previously (13, 40) and used at passages 15-20. T84, Caco-2, and IEC-6 cells were cultured in 24-well plates or on Transwell filters (Corning-Costar, Cambridge, MA; diameter, 6.5 mm; pore size, 3.0 µm) in 24-well plates (Corning-Costar).

Intestinal epithelial cell-fibroblast direct coculture experiments. Caco-2 and T84 cells were cultured directly on top of irradiated (3,000 rad, Cs irradiator MKI-30; J. L. Shepherd Associates, Glendale, CA) confluent monolayers of normal colon (CCD-18), lung (CCD-37), or explant-derived skin fibroblasts, ATCC CRL 1496, for 24 h. Intestinal epithelial cells grown in Transwells was assessed. After 20 h, [3H]thymidine was added (1.8 µCi/well of 24-well plate or 0.8 µCi/apical Transwell compartment) for 4 h. Incorporation of radiolabeled thymidine was determined as described previously (54). Measurements were performed in triplicate. [3H]thymidine incorporation assay results of coculture Transwell experiments were validated by cell counting after trypsinization and trypan blue exclusion.

Assessment of HGF and c-met mRNA expression. A human HGF cDNA fragment was isolated by RT-PCR using total RNA from IEC-6, Caco-2, and T84 cells and colon, lung, and skin fibroblasts as a template for RT into cDNA. The RT mixture (1 µg RNA, 5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1 mM dNTPs, 1 µg RNase inhibitor, 2.5 U/µl murine leukemia virus reverse transcriptase, and 2.5 µM random hexamer primers in a total volume of 20 µl; reagents from Perkin-Elmer Cetus, Norwalk, CT) was incubated at 42°C for 15 min, heated at 99°C for 5 min, and then cooled at 5°C for 5 min. PCR amplification was performed after RT reaction for 40 cycles, using the following human HGF primers: 5', 5'-CAC-GTGT-TGG-GAT-TCT-CAG-TAT-3'; and 3', 5'-CCT-ATG-ATT-GTG-CGT-GTT-GGA-3' (50). These primers represent the sense sequence in the K3 (exon 8) domain of the α-chain (nucleotides 979-1000) and the antisense sequence in the 5' end (exon 13) of the β-chain (nucleotides 1497-1518) of human HGF. The PCR mix contained 2 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.2 µM random hexamer primers, 2.5 U/µl Taq DNA polymerase, and a total volume of 100 µl (reagents from Perkin-Elmer Cetus). The amplification profile was as follows: denaturation at 94°C for 1 min, primer annealing at 57°C for 1.5 min, and extension at 72°C for 1 min. The 538-bp PCR fragment and its restriction digest products (253-bp and 285-bp AFL3 fragments and 475-bp and 63-bp DRA2 fragments) were visualized on a 1.5% agarose gel. Ligation of the purified (PCR-Quia-Quick gel extraction, Quiagen) 538-bp cDNA fragment into pCR II 3.9-µl vector (Invitrogen, San Diego, CA) and transformation of E. coli (Streptomyces 8-6) by random-primed [32P]CTP-DNA labeling (Ready-To-Go kit, Pharmacia, Piscataway, NJ) of the above-described RT-PCR-generated purified 538-bp fragment and removal of unincorporated nucleotides by spin columns (Probe Quant, Meridian Diagnostics, Cincinnati, OH) was performed and DNA was extracted. The 538-bp fragment was sequenced using 35S-ATP and Sequenase 2.0 according to the manufacturer's suggested protocol (Promega Corporation, Madison, WI)

Total cellular RNA was isolated by a modified single-step extraction technique (4) using Trizol reagent (Gibco-BRL), electrophoresed in 1.0% formaldehyde agarose gels, and blotted onto nylon transfer membranes (MSI, Westboro, MA), as described previously (13). A human HGF cDNA probe was prepared by random-primed [32P]CTP-DNA labeling (Ready-To-Go kit, Pharmacia, Piscataway, NJ) of the above-described RT-PCR-generated purified 538-bp fragment and removal of unincorporated nucleotides by spin columns (Probe Quant, Meridian Diagnostics, Cincinnati, OH) was performed and DNA was extracted. The 538-bp fragment was sequenced using 35S-ATP and Sequenase 2.0 according to the manufacturer's suggested protocol (Promega Corporation, Madison, WI).
Tris·HCl, pH 7.4, 150 mM NaCl, and 1% Nonidet P-40 were extracted by scraping cells into 2 mM EDTA, 50 mM centrifugation at 10,000 µg/ml leupeptin, and 2 mM phenylmethylsulfonyl fluoride, ethylmaleimide, 2 µg/ml aprotinin, 4 µg/ml pepstatin, 10

Assessment of HGF and c-met protein expression. Proteins were extracted by scraping cells into 2 mM EDTA, 50 mM Tris·HCl, pH 7.4, 150 mM NaCl, and 1% Nonidet P-40 containing protease and phosphatase inhibitors (2 mM N-ethylmaleimide, 2 µg/ml apronin, 4 µg/ml pepstatin, 10 µg/ml leupeptin, and 2 mM phenylmethylsulfonyl fluoride, 100 mM sodium fluoride, 200 µM sodium orthovanadate, and 10 mM tetrasodium pyrophosphate). Extracts were cleared by centrifugation at 10,000 g for 15 min and stored at −80°C. Protein concentration in each sample was determined by colorimetric Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Electrophoresis of equal amounts of proteins in 8.0% polyacrylamide gels was performed under reducing conditions (5% vol/vol 2-mercaptoethanol) according to Laemmli (23). Proteins were electroeluted onto polyvinylidene difluoride-Immobilon P transfer membranes (Millipore, Bedford, MA) in transfer buffer (50 mM Tris, 0.38 M glycine, 10% vol/vol methanol) for 15 h at 35 mA and then stained (0.1% Ponceau red, 1% acetic acid). After washing, membranes were blocked in 1× Tris-buffered saline (TBS), 0.05% Tween 20, and 5% nonfat dry milk (Western blocking buffer) at 4°C overnight. Blots were incubated with primary antibody (monoclonal mouse anti-human c-met and rabbit polyclonal anti-human HGF antibody, respectively) diluted 1:1,000 in Western blocking buffer for 1 h at room temperature. After washing in 1× TBS and 0.05% Tween 20, hybridization with the secondary antibody (sheep anti-mouse immunoglobulin, horseradish peroxidase-linked antibody diluted 1:10,000 from Amersham Life Science) was performed for 45 min at room temperature. After washing in 1× TBS and 0.05% Tween 20, signals were detected by chemiluminescent reagents (DuPont NEN, Boston, MA) and autoradiography.

Statistical analysis. Experiments were performed at least in triplicate. For statistical analysis of proliferation assays, data are expressed as means ± SE. Statistical significance between different groups was evaluated by the two-tailed t-test for independent samples (30).

RESULTS
Effects of colon fibroblasts on intestinal epithelial cell proliferation in direct coculture. The normal human colon fibroblast cell line CCD-18 was used in coculture experiments to evaluate effects of intestinal fibroblasts on proliferation of intestinal epithelial cells. Human colon cancer-derived epithelial cells (Caco-2 and T84, respectively) were grown directly on top of confluent colon fibroblast monolayers. Fibroblasts were irradiated before direct coculture experiments to avoid confounding the determination of intestinal epithelial cell incorporation of [3H]thymidine. Previous measurements of cell proliferation in irradiated confluent fibroblast monolayers confirmed very low [3H]thymidine incorporation, indicating that irradiated fibroblast monolayers contribute <0.3% of the counts measured in direct cocultures of epithelial cells and fibroblasts (data not shown).

As demonstrated in Fig. 1, cocultures of intestinal epithelial cells with colon fibroblasts resulted in increased [3H]thymidine incorporation compared with intestinal epithelial monocultures. Incorporation of radiolabeled thymidine was significantly higher in direct cocultures of Caco-2 cells with colon fibroblasts compared with Caco-2 cells alone (53,840 ± 2,773 vs. 17,710 ± 525 cpm; P = 0.017; Fig. 1A). [3H]thymidine

![Graph A](http://example.com/grapha.png)

![Graph B](http://example.com/graphb.png)

![Graph C](http://example.com/graphc.png)

![Graph D](http://example.com/graphd.png)
incorporation was also higher in cocultures of T84 cells with colon fibroblasts compared with T84 cells alone (84,210 ± 3,072 vs. 32,880 ± 2,097 cpm; P = 0.001; Fig. 1C).

Nonintestinal fibroblast populations obtained from lung and explant-derived skin fibroblasts also stimulate Caco-2 and T84 cell proliferation in direct coculture, although to a lesser extent, as evaluated by [3H]thymidine incorporation. The relative increases in Caco-2 and T84 cell proliferation induced by colon, lung, and skin fibroblasts are depicted in Fig. 1, B and D.

Effects of colon fibroblasts on intestinal epithelial cell proliferation in Transwell coculture and effects of fibroblast-conditioned medium on intestinal epithelial cell proliferation. To determine whether the effects of fibroblasts on epithelial cell proliferation were dependent on cell-to-cell contact, studies were carried out using the Transwell system in which the two cell populations are grown in proximity but without direct contact. Significantly higher [3H]thymidine incorporation was also observed in intestinal epithelial cells when cocultured with colon fibroblasts in Transwell coculture. The fact that Caco-2 or T84 cells were first seeded in the upper surface of the Transwell membrane for 24 h ensures that the effects of fibroblasts on epithelial cell thymidine incorporation reflect increased proliferation and not increased attachment of intestinal epithelial cells. As shown in Fig. 2A, incorporation of the radiolabel in Caco-2 cells cocultured in the Transwell system with colon fibroblasts was higher compared with Caco-2 cells grown alone (21,540 ± 890 vs. 6,978 ± 1,168 cpm; P = 0.0002). Similar results were found for T84 cells (14,480 ± 658 vs. 5,958 ± 557 cpm; P = 0.0001) as demonstrated in Fig. 2B. The effects of Transwell coculture on intestinal epithelial proliferation were determined by thymidine incorporation, which was corroborated by direct cell counting of epithelial cells in the apical Transwell compartment after coculture with fibroblasts. The number of Caco-2 and T84 cells after coculture with intestinal fibroblasts was 2-fold and 1.6-fold higher, respectively, compared with Caco-2 and T84 cells grown in the absence of intestinal fibroblasts.

After observation of increased intestinal epithelial cell proliferation in the presence of fibroblasts in Transwell coculture, the effects of conditioned medium harvested from serum-starved colon fibroblast cultures were studied to confirm the inference that soluble factors mediate fibroblast regulation of intestinal epithelial cells. Caco-2 and T84 cells were grown on Transwells in the presence of conditioned medium collected from serum-starved colon fibroblasts. As shown in Fig. 2A, conditioned colon fibroblast medium significantly stimulated [3H]thymidine uptake by Caco-2 cells (20,670 ± 831 cpm; P = 0.0002) compared with Caco-2 cells grown in fresh serum-deprived (control) medium (Fig. 2A). Conditioned colon fibroblast medium also stimulated [3H]thymidine incorporation in T84 cells (13,540 ± 3,285 cpm; P = 0.029) compared with T84 cells incubated with fresh serum-deprived medium (Fig. 2B). Lung and skin explant-derived fibroblasts also significantly promoted Caco-2 and T84 cell proliferation in Transwell coculture as assessed by [3H]thymidine incorporation (data not shown).

Effects of neutralizing anti-HGF antibodies on fibroblast-stimulated intestinal epithelial cell proliferation. The results above indicate that fibroblasts regulate intestinal epithelial cells predominantly through production of soluble factor(s). It is known that fibroblasts produce several mitogenic growth factors, including aFGF and HGF. Therefore, the effects of neutralizing anti-aFGF and anti-HGF antibodies on proliferation of intestinal epithelial cells cultured in the presence of colon fibroblasts in the Transwell coculture technique...
were assessed. As shown in Fig. 3, the enhanced proliferation of Caco-2 (Fig. 3A) and T84 cells (Fig. 3B) found in the presence of colon fibroblasts in Transwell coculture was significantly inhibited by addition of neutralizing anti-human HGF antibodies. In contrast, neutralizing anti-aFGF antibodies and normal (control) IgG had no inhibitory effect. Neutralizing anti-HGF antibodies also diminished the increase in proliferation of Caco-2 cells cocultured with lung or skin fibroblasts in the Transwell system (data not shown).

The results of the epithelial-mesenchymal Transwell coculture studies in the presence of neutralizing antibodies described above indicate that HGF is a mediator of fibroblast regulation of intestinal epithelial cell proliferation. To confirm this, the effects of colon fibroblast-conditioned medium in the presence and absence of neutralizing anti-human HGF antibodies on proliferation of intestinal epithelial cells were evaluated. Parallel to the results of the Transwell coculture experiments, the stimulatory effects of conditioned colon fibroblast medium on [H]thymidine uptake by Caco-2 cells were also substantially inhibited by neutralizing anti-human HGF antibody, an effect that appeared to be dose dependent as shown in Fig. 4A. Similar inhibition of the proliferation promoting activity of colon fibroblast-conditioned medium in the presence of neutralizing anti-HGF antibodies was observed for T84 cells as demonstrated in Fig. 4B.

Effect of human recombinant HGF on intestinal epithelial cell proliferation. The observation that addition of neutralizing anti-HGF antibodies blocked increased proliferation of Caco-2 and T84 cells in Transwell cocultures with fibroblasts and decreased the
growth-enhancing effects of colon fibroblast-conditioned medium suggested that HGF might play a role in enhanced proliferation of intestinal epithelial cells cocultured with fibroblasts. Indeed, HGF dose dependently stimulated uptake of tritiated thymidine in Caco-2 cells (Fig. 5A) as well as in T84 cells (Fig. 5B) in a manner similar to that previously observed in IEC-6 cells (7). This effect could be antagonized by neutralizing anti-HGF antibodies. Of note, anti-HGF antibodies alone had no significant effect on Caco-2 or T84 cell proliferation.

Effects of colon fibroblasts on IEC-6 cell proliferation. After observing that fibroblasts and fibroblast-conditioned media stimulate proliferation of transformed human intestinal model cell lines, we studied the effects of colon fibroblasts on proliferation of nontransformed intestinal epithelial cells. Because no human nontransformed intestinal epithelial cell lines are available, the nontransformed rat epithelium-derived cell line IEC-6 was used. IEC-6 cells were cultured with CCD-18 cells in Transwell coculture technique. Colon fibroblasts significantly enhanced [3H]thymidine incorporation of IEC-6 cells (14,280 ± 2,117 vs. 6,050 ± 555 cpm; P = 0.023; Fig. 6) as previously observed for the human colon cancer-derived cell lines. This increase could be blocked by neutralizing anti-HGF antibodies but not control IgG. The parallel effects in nontransformed rat intestinal epithelial cells indicate that the effects observed in models using human colon cancer-derived cell lines may have general relevance.

Expression of c-met and HGF transcripts in fibroblasts and intestinal epithelial cells. After observing the effects of HGF and anti-HGF antibody on proliferation of intestinal epithelial cells in mono- and coculture, we evaluated the expression of HGF in fibroblasts and the presence of c-met HGF receptors on intestinal epithelial cells. c-met HGF receptor and HGF steady-state mRNA expression were assessed by Northern blot analysis using total RNA extracted from intestinal epithelial cells and fibroblasts. As demonstrated in Fig. 7, c-met transcripts were readily detectable in Caco-2 and T84 cells. A c-met mRNA signal was also evident in IEC-6 cell RNA after long exposure (not shown). Weak expression of c-met mRNA was observed in colon, lung, and skin fibroblasts. Conversely, high levels of HGF mRNA expression were found in colon and lung fibroblasts. In RNA extracted from skin fibroblasts, HGF mRNA was detected by Northern blotting after longer exposure as well as by RT-PCR (data not shown). In contrast, HGF mRNA was not found in any of the epithelial cells tested, either by Northern blotting or RT-PCR. (data not shown).
Expression of c-met and HGF proteins in fibroblasts
and intestinal epithelial cells.

HGF protein expression in colon, lung, and skin fibroblast lysates was confirmed by Western blotting using recombinant human HGF as a positive control. Paralleling mRNA expression, HGF protein was found in all three fibroblast populations tested (Fig. 8B). Differences in the molecular weight observed for HGF protein in the fibroblast lysates compared with recombinant HGF protein may be explained by previously described posttranslational modifications of HGF (17, 42, 52). The presence of c-met receptor protein in Caco-2 and T84 cell lysates was confirmed by Western blotting using an anti-human c-met antibody as shown in Fig. 8A. No c-met receptor protein was detected in rat-derived IEC-6 cell lysates using the anti-human c-met antibody.

DISCUSSION

This study demonstrates that human intestinal fibroblasts stimulate proliferation of Caco-2 and T84 cells, model cell lines resembling primary intestinal epithelial cells (6, 32). The observation that this stimulation was evident in direct coculture experiments with cell contact between fibroblasts and epithelial cells as well as in Transwell coculture experiments in which there was no immediate contact between cell types suggests that the growth stimulatory effects are mediated primarily through paracrine mechanisms. Insofar as the stimulating activity was entirely provided by conditioned medium, cell-to-cell interaction and indirect modulation through production of extracellular matrix components do not appear to play a significant role in mediating fibroblast regulation of intestinal epithelial proliferation.

In the context of conflicting data regarding the tissue specificity of differentiation-promoting effects of mesenchymal cells on intestinal epithelial cells (44, 47), it should be noted that proliferation of Caco-2 and T84 cells was also increased by human fibroblasts derived from lung or skin, although to a lesser extent. These findings indicate that the fibroblast-mediated paracrine stimulatory effect on intestinal epithelial cell growth is tissue nonspecific vis-à-vis the source of fibroblasts. The demonstration of increased proliferation of intestinal epithelial cells when cultured in the presence of conditioned medium collected from colon-, lung-, and skin-derived fibroblasts provides further support for the concept of fibroblast-mediated paracrine and tissue-nonspecific stimulation of intestinal epithelial cell proliferation. This is consistent with previous in vitro studies that demonstrated increased colony formation of human colon tumor cells in soft agarose in the presence of mouse embryonic salivary gland mesenchyme (3) or induction of rat fetal gut endoderm growth by intestinal mucosal fibroblast lines (11). Collectively, these observations are consistent with a role for subepithelial fibroblasts in regulating the continued high cell turnover rate of epithelium in the adult intestinal tract throughout life (26, 37).

The ability of neutralizing anti-HGF antibody to block epithelial cell proliferation in epithelial-mesenchymal coculture models indicates that HGF plays an important role in mediating the growth-promoting...
effects of fibroblasts from colon, lung, or skin on Caco-2 and T84 cells. Neutralizing anti-HGF antibodies also dose dependently decreased the stimulatory effect of conditioned medium harvested from serum-starved colon fibroblast cultures on Caco-2 and T84 cell proliferation. The role of HGF in stimulating intestinal epithelial cell proliferation is supported by the observed dose-dependent increase in Caco-2 and T84 cell proliferation induced by recombinant HGF. Of note, the effects of 50 ng/ml HGF were comparable to the effects of intestinal fibroblast-conditioned medium. The finding of HGF stimulation of Caco-2 and T84 cell proliferation is consistent with previous studies documenting the growth-promoting properties of HGF on other intestinal epithelial cells, including IEC-6 cells (7, 12) and nonintestinal epithelial cell populations, including gastric epithelial cells, hepatocytes, biliary epithelial cells, renal tubular epithelial cells, bronchial epithelial, and alveolar type II cells, epidermal melanocytes, and keratinocytes (12, 20, 21, 27–29, 31, 45, 50). The present studies of the rat IEC-6 cell line coculture experiments with human intestinal fibroblasts confirm the effects observed in the human tumor-derived cell lines in a nontransformed intestinal epithelial model cell. The similarity of results obtained in coculture of intestinal fibroblasts with IEC-6 cells lends further support to the inference that HGF may play an important role in stimulating proliferation of nontransformed intestinal epithelial cells by intestinal fibroblasts. Conversely, the present data indicate that aFGF is not a significant mediator of fibroblast regulation of intestinal epithelial cell proliferation. However, these studies do not exclude a small role for other fibroblast-derived factors, e.g., KGF, IGFs, or yet undefined factors, in contributing to fibroblast stimulation of intestinal epithelial cell growth.

The relevance of the effects of HGF in coculture experiments is further supported by the presence of HGF and c-met transcripts and proteins in fibroblast and intestinal epithelial cell populations. Consistent with expression studies in other epithelial cell populations, HGF mRNA and protein expression were present in intestinal, lung, and skin fibroblasts but not in intestinal epithelial cells. Similarly, HGF mRNA and protein expression have been observed in rabbit gastric fibroblasts but not gastric epithelial cells (50). In contrast, c-met receptor mRNA and protein were found in Caco-2 cells as well as in T84 cells, the latter confirming previous observations (33).

The pattern of HGF and c-met expression parallels observations that HGF is expressed by mesenchymal but not epithelial cells of the gastrointestinal tract (12, 39). During mouse development c-met transcripts have been found predominantly in epithelial cells, whereas the cognate ligand HGF was expressed in mesenchymal cells (46). c-met expression has been found in transformed as well as in nontransformed epithelial cells (12, 33, 46). In addition, the present data indicate that transformed intestinal epithelial cells such as T84 and Caco-2 cells respond to HGF in a fashion similar to that previously described for nontransformed intestinal epithelial cells (7, 12). The failure to detect c-met protein expression in rat-derived IEC-6 cells may reflect absence or limited cross-species reactivity by the antibody prepared against human c-met HGF receptor. Alternatively, c-met protein expression in IEC-6 cells may be very low, given the c-met mRNA expression as demonstrated in the Northern blot experiments. Despite the inability to detect c-met protein expression in unstimulated IEC-6 cells, the earlier observation of dose-dependent stimulation of IEC-6 cell proliferation might reflect HGF-induced expression of its own receptor. Indeed, c-met mRNA and protein expression in epithelial cells have been demonstrated to be inducible in a time- and dose-dependent manner by HGF, and c-met promoter activity has been shown to be enhanced by HGF stimulation (2).

Collectively, the present data are consistent with the concept that fibroblasts are able to promote intestinal epithelial cell proliferation in addition to their effects on secretory responses, differentiation, and morphogenesis. This effect appears to be predominantly mediated by the paracrine action of HGF. Thus the proximity of the intestinal fibroblast sheet to the intestinal epithelial stem cell compartment may contribute to continued high proliferative activity found in the intestinal crypt via HGF. To evaluate the potential implication of the described findings for human diseases, further studies should examine the functional role of HGF in diseases characterized by altered intestinal epithelial cell growth. In addition, fibroblast-derived HGF may mediate intestinal wound healing in inflammatory conditions, e.g., inflammatory bowel diseases, since HGF expression has been found to be upregulated by proinflammatory cytokines, such as IL-1α and -1β, TNF-α, and prostaglandins (49, 51), factors that are present in high local concentration at sites of inflammation.

We express our appreciation to Dr. T. J. Jake Liang (NIH) and Dr. A. Ewing-Reid (Massachusetts General Hospital) for generously providing the plasmid PEG c-met-XS and the anti-c-met monoclonal antibody, to Dr. J effrey S. Rubin (NIH) for providing anti-HGF antibody, to Dr. Douglas R. Taupin (Massachusetts General Hospital) for DNA sequencing, and to Dr. Michael N. Moran (Massachusetts General Hospital) for providing human skin explant fibroblasts. These studies were supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-41557 and DK-43351 (to D. K. Podolsky), a fellowship grant from the Else Kröner-Fresenius-Stiftung (to M. Göke), and a research stipend from Pharmacia (to M. Göke).

Address for reprint requests: D. K. Podolsky, Gastrointestinal Unit, GRJ-719, Massachusetts General Hosp., 55 Fruit St., Boston, MA 02114.

Received 23 July 1997; accepted in final form 9 January 1998.

REFERENCES


