Regulation of IGFBP-4 levels in human intestinal muscle by an IGF-I-activated, confluence-dependent protease

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Received 8 February 2000; accepted in final form 20 May 2000

Kuemmerle, John F., and Baqiong Teng. Regulation of IGFBP-4 levels in human intestinal muscle by an IGF-I-activated, confluence-dependent protease. Am J Physiol Gastrointest Liver Physiol 279: G975–G982, 2000.—Human intestinal smooth muscle cells in culture produce insulin-like growth factor-I (IGF-I), IGF binding protein-3 (IGFBP-3), IGFBP-4, and IGFBP-5, which can modulate the effects of IGF-I on growth. This study examined the role of IGFBP-4 on IGF-I-induced growth and the mechanisms regulating IGFBP-4 levels. IGFBP-4 inhibited IGF-I-induced [3H]thymidine incorporation. IGFBP-4 mRNA levels were not altered by IGF-I. IGF-I caused a concentration-dependent activation of an endogenous IGFBP-4 protease, resulting in time-dependent degradation of intact IGFBP-4 into inactive fragments. Protease activity was measured in a cell-free assay using smooth muscle cell conditioned medium containing the IGFBP-4 protease. The protease was inhibited by EDTA and benzamidine. Protease activity was highest in proliferating cells and lowest in postconfluent cells. The role of endogenous IGF-I in regulating IGFBP-4 degradation was confirmed by the presence of a protease in medium conditioned by vascular smooth muscle cells that degrades intact IGFBP-4 into inactive 14- and 18-kDa fragments. The binding of IGF-I to IGFBP-4 initiates this process of regulated proteolysis (6, 12).

Within the gastrointestinal tract, human intestinal smooth muscle and rat colonic smooth muscle produce IGF-I (14, 15, 30–32). The importance of IGF-I in the regulation of growth in vivo is exemplified by the increased thickness of the muscularis propria in rats infused with IGF-I (28) and in transgenic mice overexpressing a human IGF-I cDNA (21). In vitro, endogenous IGF-I stimulates the growth of human intestinal muscle (14, 15). The production of IGF-I and its ability to stimulate growth depend on the phase of culture: the production of IGF-I and its ability to stimulate growth are highest in proliferating cells and decline progressively with time in culture (14). Human intestinal muscles express IGFBP-3, which inhibits IGF-I-induced growth, and IGFBP-5, which augments IGF-I-induced growth (1). The production of IGFBPs is also determined by the phase of growth in culture. IGFBP-5 production is highest in proliferating cells and declines progressively during growth in culture, whereas IGFBP-3 production is lowest in proliferating cells and increases progressively during growth in culture (1). IGFBP-4 is also expressed by human intestinal muscle, and its production remains constant during culture (1). Although endogenous IGF-I can elicit a small increase in IGFBP-4 production, the mechanisms regulating IGFBP-4 levels in these cells during growth in culture has not been determined. The effects of IGFBP-4 on IGF-I-induced growth of intestinal smooth muscle are also not known.

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In the present study, the regulatory interplay between IGFBP-4 and endogenous IGF-I was examined in cultures of human intestinal smooth muscle cells. An IGFBP-4 protease secreted during growth in culture was identified and characterized. The results show that IGFBP-4 inhibits IGF-I-induced growth of human intestinal muscle cells. Although IGF-I does not alter IGFBP-4 mRNA expression, levels of secreted IGFBP-4 are determined by the confluence-dependent production of a cation-dependent serine protease that is activated by endogenous IGFBP-4. We hypothesize that this is the predominant regulatory mechanism determining IGFBP-4 levels in human intestinal smooth muscle cells.

METHODS

Preparation and culture of human intestinal muscle cells. Smooth muscle cells of normal human jejunum were isolated and cultured, as described previously (1, 14–16), from surgical specimens obtained by a protocol approved by the Institutional Committee on the Conduct of Human Research. Tissue slices from the circular muscle layer were digested in a medium consisting of (in mM) 120 NaCl, 4 KCl, 2.6 KH2PO4, 2 CaCl2, 0.6 MgCl2, 25 HEPES, and 14 glucose with 2.1% Eagle’s essential amino acid mixture, 0.2% collagenase (CLS type II), and 0.1% soybean trypsin inhibitor. Partially digested muscle cells were dispersed in enzyme-free medium. Muscle cells were harvested by filtration (500-μm Nitex). The cells were washed twice by centrifugation for 5 min at 150 g and resuspension in Hanks’ medium containing 200 U/ml penicillin, 200 μg/ml streptomycin, 100 μg/ml gentamicin, and 2 μg/ml amphotericin B. Muscle cells were resuspended in DMEM containing 10% fetal bovine serum (DMEM-10) and antibiotics and plated at a density of 5 × 10^4 cells/ml. The medium was replaced every 3 days. Once confluence was attained, primary cultures were passaged and seeded at a density of 2.5 × 10^5 cells/ml. All subsequent studies were performed on first passage cultured cells on day 3 of culture, when cells were rapidly proliferating, on day 7 of culture, when the cells attained confluence, or on day 14 of culture, when cells were postconfluent and attained a “hill-and-valley” architecture (14, 29). We have previously shown that these cells express a phenotype characteristic of intestinal smooth muscle as determined by immunostaining for smooth muscle markers and expression of γ-enteric actin (14, 29). Epithelial cells, endothelial cells, neurons, and interstitial cells of Cajal are not detected in these cultures (29).

Northern blot analysis of IGFBP-4 mRNA expression. Northern blot analysis was performed as described previously (29). Briefly, total RNA was prepared from muscle cells lysed in Ultraspec RNA isolation reagent. Eighteen micrograms of total RNA were fractionated by electrophoresis on a 1% agarose gel and transferred to a nylon membrane. A partial cDNA (505 bp) encoding human IGFBP-4 (27), excised from the pHBP4-503 plasmid (obtained from Dr Shunichi Shimisaki) using appropriate restriction enzymes, was labeled with ³²P using random hexamers and used as a probe. Hybridization was carried out overnight at 42°C under standard conditions: 50% formamide, 6 × SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), 0.5% SDS, 20 mM Na₂HPO₄ (pH 7.0), 5 × Denhardt’s reagent, and 100 μg/ml herring sperm DNA and then washed with 2× SSC and 0.1% SDS for 30 min at room temperature, followed by washing in 0.2× SSC and 0.1% SDS at 55°C. Autoradiography was performed at −80°C. The resulting bands were quantitated with densitometry. Blots were rehybridized with a cDNA probe (~1000 bp) of human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to control for the amount of RNA loaded per lane.

Preparation of conditioned medium. Conditioned medium was prepared as described previously from confluent muscle cells for measurement of soluble IGFBP-4 (1, 15). The cells were washed and incubated in serum-free DMEM for 48 h. The conditioned medium was removed, concentrated 10-fold in Centricon-10 tubes at 4°C, and used for measurements of IGFBP-4 secretion and IGFBP-4 protease activity.

Measurement of IGFBP-4 by Western blot analysis. IGFBP-4 production was measured by Western blot analysis as described previously (1, 15). Concentrated conditioned medium was added to loading buffer (62.5 mM Tris, pH 6.8, with 2% SDS, 25% glycerol, 0.01% bromphenol blue, and 5% β-mercaptoethanol) to provide samples derived from equal amounts of total cellular protein (200 μg/30 μl). Proteins separated by SDS-PAGE on 15% polyacrylamide gels were electroblotted to nitrocellulose membranes in 25 mM Tris (pH 8.3) and 192 mM glycine, with 20% methanol and 0.1% SDS added. Membranes were incubated overnight at 4°C with a 1:1,000 dilution of an antibody to IGFBP-4 displaying <1% cross-reactivity with other IGFBPs. Membranes were then incubated in a 1:1,000 dilution of goat anti-rabbit IgG-horseradish peroxidase conjugate. Bands were visualized with chemiluminescence and quantitated by densitometry.

Measurement of IGFBP-4 protease activity. The activity of an IGFBP-4 protease secreted by muscle cells was measured in medium conditioned by human intestinal muscle cells during three phases of growth in culture: in proliferating cells (on day 3 of culture), confluent cells (on day 7 of culture), or postconfluent cells (on day 14 in culture). IGFBP-4 protease activity was measured as the degradation of authentic recombinant human IGFBP-4 by modification of the methods of Duan and Clemmons (6) and Kamyar et al. (12). Measurements were made in a cell-free system consisting of 35 μl of concentrated medium conditioned by human intestinal muscle cells (containing the IGFBP-4 protease), 50 ng/ml authentic recombinant human IGFBP-4, and 10 nM IGF-I to activate the protease (which is within the range of endogenous IGF-I production; Ref. 14), to which various test agents were added in a reaction volume of 50 μl. The mixture was incubated at 37°C for increasing periods of time from 0 to 48 h. The reaction was terminated by the addition of sample buffer (62.5 mM Tris, pH 6.8, with 2% SDS, 25% glycerol, 0.01% bromphenol blue, and 5% β-mercaptoethanol). Proteins were separated by SDS-PAGE on 15% polyacrylamide gels and electroblotted to nitrocellulose membranes in 25 mM Tris (pH 8.3) and 192 mM glycine, with 20% methanol and 0.1% SDS added. Membranes were incubated overnight at 4°C with a 1:1,000 dilution of an antibody to IGFBP-4. Protein bands corresponding to intact (24 kDa) and degraded (18 kDa) IGFBP-4 were visualized by enhanced chemiluminescence and quantitated by densitometry.

Measurement of IGFBP-4 protease activity. The activity of an IGFBP-4 protease secreted by muscle cells was measured in medium conditioned by human intestinal muscle cells during three phases of growth in culture: in proliferating cells (on day 3 of culture), confluent cells (on day 7 of culture), or postconfluent cells (on day 14 in culture). IGFBP-4 protease activity was measured as the degradation of authentic recombinant human IGFBP-4 by modification of the methods of Duan and Clemmons (6) and Kamyar et al. (12). Measurements were made in a cell-free system consisting of 35 μl of concentrated medium conditioned by human intestinal muscle cells (containing the IGFBP-4 protease), 50 ng/ml authentic recombinant human IGFBP-4, and 10 nM IGF-I to activate the protease (which is within the range of endogenous IGF-I production; Ref. 14), to which various test agents were added in a reaction volume of 50 μl. The mixture was incubated at 37°C for increasing periods of time from 0 to 48 h. The reaction was terminated by the addition of sample buffer (62.5 mM Tris, pH 6.8, with 2% SDS, 25% glycerol, 0.01% bromphenol blue, and 5% β-mercaptoethanol). Proteins were separated by SDS-PAGE on 15% polyacrylamide gels and electroblotted to nitrocellulose membranes in 25 mM Tris (pH 8.3) and 192 mM glycine, with 20% methanol and 0.1% SDS added. Membranes were incubated overnight at 4°C with a 1:1,000 dilution of an antibody to IGFBP-4 displaying <1% cross-reactivity with other IGFBPs. Membranes were then incubated in a 1:1,000 dilution of goat anti-rabbit IgG-horseradish peroxidase conjugate. Bands were visualized with chemiluminescence and quantitated by densitometry.

[³H]thymidine incorporation assay. The effect of IGFBP-4 on IGF-I-induced growth of cultured muscle cells was examined by measurement of [³H]thymidine incorporation as described previously (1, 13–15). Confluent cells were incubated for 24 h in serum-free DMEM. Cells were incubated an additional 24 h with a submaximal concentration (1 nM) of IGF-I in the presence of increasing concentrations (0.5–50 nM) of authentic IGFBP-4. IGF-I and IGFBP-4 were added simultaneously. [³H]thymidine (1 μCi/ml) was added for the final 4 h, and its incorporation was measured in the perchloric acid-extractable pools using 2 N perchloric acid (30 min at
60°C). After solubilization, protein in the cell residue was measured using the Bio-Rad protein reagent (Hercules, CA). [3H]thymidine incorporation was expressed as counts per minute (cpm) per microgram of protein.

Statistical analysis. Values represent means ± SE of n experiments where n represents the number of experiments on cells derived from separate primary cultures. Statistical significance was tested by Student’s t-test for either paired or unpaired data. Bands corresponding to the regions of interest on Western and Northern blots were measured with computerized densitometry and analyzed using Scion Image (National Institutes of Health, Bethesda, MD), and the results were reported in arbitrary units above background.

Materials. Recombinant human IGF-I, IGF-II, and IGFBP-4 were obtained from Austral Biologicals (San Ramon, CA), and collagenase and soybean trypsin inhibitor were from Worthington Biochemical (Freehold, NJ). [3H]thymidine (6 Ci/mmol) and [α-32P]dCTP (3,000 Ci/mmoll were obtained from NEN (Boston, MA), and DMEM and Hanks’ medium were obtained from Mediatech (Herndon, VA). Fetal bovine serum was obtained from Summit Biotechnologies (Fort Collins, CO), and protein assay reagent and Western blotting materials were from Bio-Rad. Arg3-substituted IGF-I (R3-IGF-I) and rabbit polyclonal antibody to IGFBP-4 were obtained from Upstate Biotechnology (Lake Placid, NY). IGF-I Analog was obtained from Bachem (Torrance, CA), and plastic cultureware was obtained from Corning (Corning, NY). Centricon-10 ultrafiltration tubes were obtained from Millipore (Bedford, MA), and Ultraspec RNA isolation reagent was from Biotecx Laboratories (Houston, TX). All other chemicals were obtained from Sigma Chemical (St. Louis, MO).

RESULTS

Regulation of IGFBP-4 expression. We (1, 15) have previously shown that IGFBP-4 is expressed by human intestinal muscle cells and that endogenous IGF-I causes an increase in production of IGFBP-4. In the present study, the regulation of IGFBP-4 expression by IGF-I was examined by Northern blot analysis. The effect of IGF-I mediated by activation of the IGF-I receptor in the absence of interference from IGFBPs was examined in confluent cultures of human intestinal muscle cells treated with R3-IGF-I, which does not interact with IGFBPs (10). Incubation of muscle cells for 48 h with increasing concentrations of R3-IGF-I did not alter the levels of IGFBP-4 mRNA when normalized to GAPDH levels (1 nM R3-IGF-I, 92 ± 15% of basal; 10 nM R3-IGF-I, 91 ± 14% of basal) (Fig. 1). The role of endogenous IGF-I was also examined by incubation of muscle cells with the IGF-I receptor antagonist IGF analog. IGF-I analog is a competitive peptide antagonist of IGF-I with the following structure (15, 23): H-Cys-Tyr-Ala-Ala-Pro-Leu-Cys-Pro-Ala-Lys-Ser-Cys-OH. Incubation of muscle cells for 48 h with increasing concentrations of the IGF-I receptor antagonist did not affect IGFBP-4 expression. Eighteen micrograms of total RNA prepared from treated cells were fractionated by electrophoresis and hybridized to a partial cDNA encoding human IGFBP-4 and rehybridized with a cDNA probe encoding human GAPDH to control for the amount of RNA loaded/lane as described in METHODS. Autoradiographs were quantitated with densitometry. Results are expressed in arbitrary densitometric units normalized to GAPDH expression. Values are means ± SE of 3 experiments.

Modulation of IGF-I-induced proliferation by IGFBP-4. The effect of IGF-I on IGF binding protein-4 (IGFBP-4) expression in human intestinal muscle cells. A: representative Northern blot of IGFBP-4 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. B: densitometric analysis of IGFBP-4 mRNA expression. Incubation of confluent cultures of human intestinal muscle cells for 48 h with increasing concentrations of Arg3-substituted IGF-I (R3-IGF-I) or IGF-I antagonist did not affect IGFBP-4 expression. Eighteen micrograms of total RNA prepared from treated cells were fractionated by electrophoresis and hybridized to a partial cDNA encoding human IGFBP-4 and rehybridized with a cDNA probe encoding human GAPDH to control for the amount of RNA loaded/lane as described in METHODS. Autoradiographs were quantitated with densitometry. Results are expressed in arbitrary densitometric units normalized to GAPDH expression. Values are means ± SE of 3 experiments.
and assayed in a cell-free system. In control experiments, incubation of 50 ng authentic IGFBP-4 with 10 nM IGF-I for up to 48 h did not cause significant degradation of IGFBP-4 (96 ± 4% degradation of IGFBP-4). In contrast, incubation of 50 ng of IGFBP-4 with 10 nM IGF-I for increasing periods of time (0–48 h) in the presence of medium conditioned by confluent (day 7 in culture) intestinal smooth muscle cells caused time-dependent degradation of IGFBP-4 (Fig. 3). Degradation of IGFBP-4 was maximal by 24 h (80 ± 4% of added IGFBP-4). Subsequent experiments were performed at the 24 h peak of degradation.

Incubation of 50 ng of IGFBP-4 and medium conditioned by confluent cells in the presence of increasing concentrations of IGF-I (0.1–100 nM) caused concentration-dependent degradation of IGFBP-4 (Fig. 4). Activation of the IGFBP-4 protease by IGF-II has been observed in vascular muscle cells (6, 12, 22), bone cells (7, 19), neurons (2), and fibroblasts (3). Because low levels of IGF-II (10–20 pM) are also secreted by cultured human intestinal muscle cells (15), the ability of IGF-II to initiate IGFBP-4 degradation was examined. Incubation of 50 ng of IGFBP-4 and medium conditioned by confluent cells in the presence of increasing concentrations of IGF-II (0.1–100 nM) caused concentration-dependent proteolysis of IGFBP-4 (Fig. 4). Significant degradation of IGFBP-4 initiated by IGF-II-dependent activation of the IGFBP-4 protease occurred in the presence of 10 nM and higher concentrations of IGF-II; these concentrations are ~500- to 1,000-fold higher than those attained by endogenous IGF-II in these cells (15).

The characteristics of the IGF-I-activated IGFBP-4 protease secreted by cultured intestinal muscle cells were examined in the cell-free system using medium conditioned by confluent cells and the degradation of IGFBP-4 measured in the presence and absence of the protease inhibitors (Fig. 5) EDTA (10 mM), benzamidine (50 mM), and phenylmethylsulfonyl fluoride (PMSF; 1 mM). In the presence of the cation-dependent protease inhibitor EDTA, the degradation of IGFBP-4 was significantly inhibited (80 ± 9% inhibition of degradation, $P < 0.01$). In the presence of the serine protease inhibitor benzamidine, degradation of IGFBP-4 was also inhibited (67 ± 7% inhibition of degradation, $P < 0.01$). In the presence of PMSF, the degradation of IGFBP-4 was not affected (4 ± 3% inhibition of degradation, $P > 0.05$; Fig. 5). The results implied that the IGFBP-4 protease secreted was a cation-dependent serine protease.

Confluence-dependent secretion of IGFBP-4 protease. The confluence dependence of IGFBP-4 protease secretion by human intestinal muscle cells was examined in proliferating (day 3 in culture), confluent (day 7 in culture), and postconfluent (day 14 in culture) cultures of muscle cells. Medium conditioned by cells growing during each phase of growth was incubated with 50 ng of authentic IGFBP-4 and 10 nM IGF-I for 24 h. IGFBP-4 protease activity declined progressively with time of culture. IGFBP-4 protease activity was highest in proliferating cells with 94 ± 1% of IGFBP-4 degraded after 24 h ($P < 0.01$), lower in confluent cells

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Fig. 2. IGFBP-4 inhibits IGF-I-induced proliferation of human intestinal muscle cells. Incubation of confluent cultures of human intestinal muscle cells for 24 h with a submaximal concentration of IGF-I (1 nM) increased [%H]thymidine incorporation 87 ± 11% above basal levels (basal: 104 ± 9 cpm/µg protein). Authentic human IGFBP-4 inhibited IGF-I-induced proliferation in a concentration-dependent fashion. Results are expressed as a % of 1 nM IGF-I-induced [%H]thymidine incorporation. Values are means ± SE of 3 separate experiments performed in triplicate. *$P < 0.05$, **$P < 0.01$ vs. 1 nM IGF-I alone.

Fig. 3. Time course of IGF-I-dependent IGFBP-4 proteolysis. A: representative Western blot of the time course of IGF-I-dependent proteolysis of intact (24-kDa) IGFBP-4. B: densitometric analysis of the time course of IGF-I-dependent IGFBP-4 proteolysis. Incubation of 50 ng of human IGFBP-4 with 10 nM IGF-I for increasing periods of time (0–48 h) in the presence of medium conditioned by human intestinal muscle cells caused time-dependent degradation of intact IGFBP-4 (24 kDa) into inactive IGFBP-4 fragments (18 kDa). Results are expressed as a % of control (0 h) value. Values are means ± SE of 3 separate experiments. *$P < 0.05$, **$P < 0.01$ vs. control values.
(85 ± 6% degradation after 24 h, $P < 0.01$), and lowest in postconfluent cells (20 ± 14% degradation after 24 h, $P < 0.05$; Fig. 6).

Specificity of IGF-I-induced activation of the IGFBP-4 protease. The specificity of IGF-I-induced activation of the IGFBP-4 protease was examined in two complimentary ways. In the first experiment, the ability of R3-IGF-I to activate the IGFBP-4 protease was examined in the cell-free system. The protease present in medium conditioned by confluent cells was only minimally activated by R3-IGF-I and little IGFBP-4 degradation occurred compared with that activated by IGF-I (maximal degradation: 13 ± 12% with 10 nM R3-IGF-I vs. 80 ± 4% with 10 nM IGF-I; Fig. 7).

In the second experiment, the ability of the IGF-I receptor antagonist to block IGF-I-induced activation of the protease was examined in the cell-free system. Authentic IGFBP-4 was incubated for 24 h with medium conditioned by confluent cells and 10 nM IGF-I in the presence and absence of the IGF-I receptor antag-
Endogenous IGF-I regulates IGFBP-4 levels in intact cell cultures. We (15) have previously shown that endogenous IGF-I causes a modest increase in IGFBP-4 production. Results obtained with R3-IGF-I and the inhibitory IGF-I receptor antibody 1H7 showed that incubation of confluent cultures with R3-IGF-I caused a concentration-dependent increase in IGFBP-4 production (43 ± 14% increase over basal) and incubation with 1H7 caused concentration-dependent inhibition of IGFBP-4 production (50 ± 4% inhibition of basal) from intact cells, implying that endogenous IGF-I causes a small increase in IGFBP-4 protein production (15).

In the present study, incubation of intact confluent cultures with the IGF-I receptor antagonist IGF analog elicited a concentration-dependent increase in IGFBP-4 levels (maximal increase: 69 ± 15% above basal with 1 nM antagonist; Fig. 8). This apparent paradox, that the inhibitory antibody 1H7 inhibits and the IGF-I receptor antagonist increases IGFBP-4 levels in intact cultures is explained by the ability of the antagonist to inhibit not only the binding of IGF-I to its receptor but also to inhibit the binding of IGF-I to IGFBP-4 (as shown above), thereby inhibiting IGF-I-activated IGFBP-4 proteolysis. The results imply that the predominant regulatory effect of endogenous IGF-I in intact cells is to determine IGFBP-4 levels by activation of IGFBP-4 proteolysis.

DISCUSSION

This study shows that IGFBP-4 inhibits IGF-I-induced growth of human intestinal smooth muscle cells. Levels of IGF-I-induced growth inhibitory IGFBP-4 are in turn regulated by IGF-I through two mechanisms. Endogenous IGF-I increases IGFBP-4 protein production by posttranslational mechanisms and without a concomitant change in gene expression. The lack of effect of IGF-I on IGFBP-4 gene expression is a consistent observation in the cell types previously examined (6, 12, 13). Levels of secreted IGFBP-4 are predominantly regulated by an endogenous IGF-I-activated, cation-dependent, serine protease in a confluence-dependent fashion as cells grow in culture. This system has not previously been examined in the human intestine, and this is the first demonstration of the operation of the confluence-dependent production of an IGFBP-4 protease in either human cells or in nontransformed cell lines. The present study cannot, however, differentiate between changes in the secretion of the IGFBP-4 protease and changes in protease activity.

The evidence supporting the presence of an endogenous IGF-I-activated IGFBP-4 protease that regulates the level of secreted IGFBP-4 in human intestinal muscle cells in a confluence-dependent fashion can be summarized as follows: 1) conditioned medium causes time-dependent degradation of IGFBP-4 into inactive fragments in the presence of IGF-I but not R3-IGF-I, 2) the IGF-I antagonist increases IGFBP-4 levels in intact cells, 3) the IGF-I antagonist inhibits IGF-I-activated IGFBP-4 degradation, 4) IGFBP-4 proteolytic activity is inhibited in the presence of EDTA, a cation-dependent protease inhibitor, and benzamidine, a serine protease inhibitor, and 5) the activity of the IGFBP-4 protease is confluence dependent with highest activity in rapidly growing cells (day 3 in culture) and lowest activity in postconfluent cells (day 14 in culture).

In all cell types that have been examined, including human intestinal smooth muscle, intact IGFBP-4 inhibits IGF-I-induced growth by binding IGF-I and inhibiting its interaction with the IGF-I receptor tyrosine kinase (11, 13). Although IGFBP-4 is expressed by all examined cell types (27), the presence of an IGFBP-4-specific protease has been identified only in some cells, including smooth muscle cells (6, 12, 22), transformed fibroblasts (3), bone-derived cells (7, 19), neuroblastoma (2), and prostate cells (17). Notably, vascular smooth muscle cells cultured from the rat and porcine aorta produce a similar cation-dependent serine protease that is secreted in a confluence-dependent fashion and regulates the levels of intact IGFBP-4 (6, 12, 22). In the presence of the IGFBP-4 protease, binding of IGF-I to IGFBP-4 initiates IGFBP-4 degradation.
IGFBP-4 protease activity results in low levels of intact IGFBP-4 and release from the inhibitory effects of IGFBP-4 on IGF-I-induced growth (Fig. 9). In contrast, in postconfluent cells (on day 14 in culture), the combination of low levels of IGF-I production and low IGFBP-4 protease activity results in higher levels of intact IGFBP-4 and inhibition of IGF-I-induced growth by intact IGFBP-4 (Fig. 9).

The confluence-dependent interplay between IGF-I and IGFBPs in human intestinal smooth muscle is not limited to IGFBP-4. Production of IGFBP-3, which inhibits IGF-I-induced growth, is confluence dependent in these cells, with the lowest levels produced by proliferating cells and the highest levels produced by postconfluent cells (1). IGFBP-5, which augments IGF-I-induced growth, exhibits a pattern opposite to that of IGFBP-3 but one similar to that of IGF-I, with the highest levels produced by proliferating cells and the lowest levels produced by postconfluent cells (1, 14). These interdependent processes, IGF-I, IGFBP-3, and IGFBP-5 production (1, 14) and IGFBP-4 proteolysis, act in concert to regulate human intestinal muscle cell growth in culture from proliferation to postconfluence.

In summary, human intestinal muscle cells produce an IGFBP-4 protease in a confluence-dependent fashion that is activated by endogenous IGF-I. This is the predominant regulatory mechanism determining IGFBP-4 levels in these cells. The autocrine pathway linking endogenous IGF-I, IGFBP-4, and the IGFBP-4 protease plays a role in regulating growth of human intestinal smooth muscle cells.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-49691.

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