Insulin-like growth factor-binding protein-5 (IGFBP-5) Stimulates Growth and IGF-I Secretion in Human Intestinal Smooth Muscle by Ras-dependent Activation of p38 MAP Kinase and Erk1/2 Pathways*

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Insulin-like growth factor-binding protein-5 (IGFBP-5) and insulin-like growth factor-I (IGF-I) are produced by human intestinal smooth muscle cells. Endogenous IGF-I stimulates growth and increases IGFBP-5 secretion. IGFBP-5 augments the effects of IGF-I by facilitating interaction of IGF-I with the IGF-I receptor tyrosine kinase. Andress (Andress, D. L. (1998) Am. J. Physiol. 274, E744–E750) and Berfield et al. (Berfield, A. K., Andress, D. L., and Abrass, C. K. (2000) Kidney Int. 57, 1991–2003) have shown that in osteoblasts and kidney mesangial cells, IGFBP-5 stimulates proliferation and filopodia formation independently of IGF-I, presumably by activating a distinct IGFBP-5 receptor serine kinase. The present study determined whether IGFBP-5 exerts direct effects on growth in human intestinal smooth muscle cells and identified the intracellular signaling pathways involved. IGFBP-5 caused a concentration-dependent increase in [3H]thymidine incorporation and an increase in IGF-I secretion that occurred independently of IGF-I and the IGF-I receptor tyrosine kinase. IGFBP-5-induced phosphorylation of p38 MAP kinase, which was abolished by SB203580, or expression of a dominant negative Ras mutant, Ras(S17N), and phosphorylation of Erk1/2, which was abolished by a Raf1 kinase inhibitor, U1026, or expression of Ras(S17N). IGFBP-5-stimulated [3H]thymidine incorporation and IGF-I secretion were partly inhibited by SB203580 or U1026 and abolished by the combination of the two inhibitors or by expression of Ras(S17N). These data show that IGFBP-5 stimulates growth and IGF-I secretion in human intestinal smooth muscle cells by activation of p38 MAP kinase-dependent and Erk1/2-dependent pathways that are independent of IGF-I. A positive feedback mechanism therefore links IGFBP-5 and IGF-I secretion that reinforces their individual effects on growth.

Insulin-like growth factor-I (IGF-I)1 mediates three distinct regulatory effects on cell growth by activation of the IGF-I receptor: IGF-I stimulates proliferation of cells and is required for sustained growth of many cells (1); transformation and maintenance of the transformed state also require IGF-I receptor activation in some cells (2); and IGF-I protects cells from apoptosis (3). The central role of IGF-I in the regulation of smooth muscle cell growth in both the normal and pathologic states is manifested by the hyperplasia of intestinal and vascular smooth muscle in transgenic animals overexpressing a human IGF-I cDNA (4, 5). The effects of IGF-I are modulated by IGF-binding proteins. Six IGF-binding proteins (IGFBP-1–6) have been identified that can either augment the effects of IGF-I by facilitating the interaction of IGF-I with its cognate receptor or inhibit the effects of IGF-I by diminishing the interaction of IGF-I with its receptor (6). The presence and effect of each IGF binding protein, however, is both tissue- and species-specific.

IGFBP-1, IGFBP-3, and IGFBP-5 indirectly influence cell growth by modulating the interaction of IGF-I with the IGF-I receptor and also directly influence cell growth by interacting with distinct cell surface receptors. IGFBP-1 interacts with the α5β1 integrin receptor expressed by placental cells and Chinese hamster ovary cells (7). IGFBP-3 interacts with the Type V TGF-β receptor expressed in T47D breast cancer cells and mink lung epithelial cells (8). Recently, an IGFBP-5-specific receptor has been characterized in mouse osteoblasts and rat kidney mesangial cells (9). IGFBP-5 binds with high affinity to this ~420-kDa membrane-bound receptor protein and elicits autophosphorylation of serine residues (10). One intracellular signaling pathway coupled to this receptor is the small G-protein, Cdc42, through which IGFBP-5-dependent mesangial cell fipolidation formation is mediated (11).

Human intestinal smooth muscle cells produce IGF-I, and three IGF-binding proteins, IGFBP-3, IGFBP-4 and IGFBP-5, each of which plays an autocrine role in the regulation of growth in human intestinal muscle cells (12, 13). Binding of IGF-I to the IGF-I receptor tyrosine kinase activates distinct PI3-kinase-dependent and Erk1/2-dependent pathways that stimulate both proliferation and IGFBP-5 production (13, 14). IGF-I-dependent stimulation of growth in these cells is inhibited by the indirect actions of IGFBP-3 and IGFBP-4 and is augmented by the indirect actions of IGFBP-5 (12, 13). IGF-I and IGFBP-5 expression is increased within the intestinal muscle layer in regions of active inflammation and stricture in Crohn's disease and in models of experimental enterocolitis (15, 16). It is not known whether an IGFBP-5-specific receptor is expressed by human intestinal muscle cells or what role this receptor plays in the regulation of growth.

This study shows that an IGFBP-5 receptor is present in human intestinal smooth muscle cells. Binding of IGFBP-5 to its cognate receptor activates both the p38 MAP kinase and

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Erk1/2 signaling cascades. Activation of these pathways by IGFBP-5 mediates jointly stimulation of growth and secretion of IGF-I. Thus, dual stimulatory pathways link IGF-I and IGFBP-5 secretion, reinforcing their individual abilities to stimulate growth of human intestinal muscle cells.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human IGFBP-5, the dominant negative Ras(S17N) mutant in pUSEpamp(+) vector, and antibodies to the signaling intermediates, p38 MAP kinase, MKK3/6, Ras, Raf1, MKK1/2, and Erk1/2, were obtained from Upstate Biotechnology (Lake Placid, NY). The membrane and soybean tryptophan kinase inhibitor were obtained from Worthington Biochemical Inc (Freehold, NJ); Dulbecco's modified Eagle's medium (DMEM) was obtained from Mediatech Inc. (Herndon, VA); fetal bovine serum was obtained from Summit Biotechnologies, Inc. (Fort Collins, CO); [3H]thymidine (specific activity, 6 Ci/mmol) was obtained from Amersham Biosciences; [125I]IGF-I radioimmunoassay kit was obtained from Peninsula Laboratories (San Carlos, CA); Western blotting materials and protein assay kit were obtained from BioRad Laboratories; plastic cultureware was obtained from Corning (Corning, NY); antibodies to the phosphorylated isoforms of Raf1 (Ser259), MKK1/2 (Ser180/Ser211), Erk1/2 (Thr202/Tyr204), MKK3/6 (Ser189/Ser207), and p38 MAP kinase (Thr180/Tyr182) were obtained from Cell Signaling Technology (Danvers, MA); IGF-I was obtained from the National Hormone and Peptide Program (Nashville, TN); U1026, SB203580 and the Raf1 kinase inhibitor, 5-iodo-3-(3,5-dibromo-4-hydroxyphenyl)methylenel-2-indolone were obtained from Calbiochem. All other chemicals were obtained from Sigma.

Isolation and Culture of Muscle Cells from Human Jejunum—Muscle cells were isolated from the circular muscle layer of human jejunum as described previously (12, 13, 17). Segments of normal jejunum were obtained from patients undergoing surgery according to a protocol approved by the Institutional Committee on the Conduct of Human Research. Briefly, muscle cells were isolated by enzymatic digestion overnight at 37 °C in Dulbecco's modification of Eagle's medium containing 10% fetal bovine serum (DMEM-10), penicillin 200 units/ml, streptomycin 200 μg/ml, gentamycin 100 μg/ml, and amphotericin B 2 μg/ml, with added 0.33% collagenase (CLS type II), and 0.1% soybean trypsin inhibitor. The cells were plated at a concentration of 5 × 10⁶ cells/ml in DMEM-10 with antibiotics and incubated in a 10% CO₂ environment at 37 °C. Medium was replaced every 3 days. Studies were performed in first passage after 14 days, at which time the cells are post-confluent and the production of endogenous IGF-I and IGFBP-5 are low (18).

[3H]Thymidine Incorporation Assay—Proliferation of smooth muscle cells was measured by [3H]thymidine incorporation as described previously (14, 18, 19). Briefly, the cells were washed free of serum and incubated for 24 h in serum-free DMEM. The quiescent muscle cells were incubated for an additional 24 h with a maximally effective concentration of IGFBP-5 (50 nM) for 2 min in the presence of the IGF-I receptor antagonist did not represent the number of experiments on cells from a single primary culture. Statistical significance was tested by Student's t test for either paired or unpaired data as appropriate.

RESULTS

IGFBP-5 Stimulates Growth and IGF-I Secretion Independently of IGF-I—Our previous work has shown that IGFBP-5 augments, in a concentration-dependent fashion, the stimulatory effects of IGF-I on the growth of human intestinal smooth muscle cells in culture (15). In the present study, we hypothesized that, in addition to its IGF-I-dependent effects, IGFBP-5 might exert direct effects on the growth of human intestinal smooth muscle cells. Cells were examined during the post-confluent phase of culture, when the endogenous levels of both IGFBP-5 and IGF-I are lowest, and in the presence of an IGF-I receptor antagonist, IGF-1 analog (1 μM) (17, 23). IGF-I analog, an IGF-I receptor antagonist that blocks the ability of IGF-I to activate the phosphotyrosine kinase used the IGF-I receptor tyrosine kinase, was used to eliminate the effects of IGFBP-5 mediated by facilitation of IGF-I binding to its cognate receptor. We have previously shown that in the presence of this antagonist, the ability of IGF-I to cause phosphorylation of its receptor is abolished. Incubation of quiescent muscle cells with IGFBP-5 (50 nM) for 2 min in the presence of the IGF-I antagonist did not decrease protein synthesis in quiescent cells.
elicit IGF-I receptor phosphorylation (1.03 ± 0.10% of basal). The results of these initial studies implied that effects attributed to IGFBP-5 represented its IGF-I-independent effects, i.e. when the IGF-I-dependent effects of IGFBP-5 were abolished in the presence of the IGF-I receptor antagonist.

In normal human intestinal smooth muscle cells, incubation of quiescent muscle cells with IGFBP-5 for 24 h directly caused concentration-dependent (0.5–50 nM IGFBP-5) increase in [3H]thymidine incorporation (50 nM, 145 ± 9% above basal; basal, 84 ± 3 cpm/mg protein) (Fig. 1). Incubation of human intestinal muscle cells for 24 h with 50 nM IGFBP-5, increased the secretion of IGF-I by 90 ± 12% above basal levels (basal, 3.1 ± 0.2; IGFBP-5, 5.7 ± 0.6 pmol/mg protein/24 h, p < 0.05). The ability of IGFBP-5 to stimulate thymidine incorporation and IGF-I secretion in the presence of the IGF-I receptor antagonist implied that these effects were distinct from those mediated via the IGF-I receptor by augmentation of IGF-I agonist.

IGFBP-5 Activates the p38 MAP Kinase Signaling Pathway—Activation of p38 MAP kinase was measured using a phospho-specific antibody recognizing the Thr180/Tyr182 phosphorylated (activated) p38 MAP kinase isoform. IGFBP-5 caused rapid, time-dependent phosphorylation of p38 MAP kinase, which attained a maximum within 5 min and declined to lower levels by 60 min (Fig. 2A). The increase in p38 MAP kinase phosphorylation, measured at the 5 min maximum, was concentration-dependent (0.5–50 nM IGFBP-5) (Fig 2B).

IGFBP-5-induced phosphorylation of p38 MAP kinase was abolished by the selective p38 MAP kinase inhibitor, SB203580 (1 μM), but was not affected by either the Raf1 kinase inhibitor, 5-iodo-3-[3,5-dibromo-4-hydroxyphenyl]methylene]-2-indolone (10 nM), or the MKK1/2 inhibitor, U1026 (10 μM) (Fig. 2C) (24).

Activation of the homologs MKK3/6 by IGFBP-5 followed a similar time-course to that of p38 MAP kinase. IGFBP-5 elicited prompt, time-dependent phosphorylation of MKK3/6 (Ser189/Ser207) that was maximal within 5 min and declined to lower levels within 60 min (Fig. 3A). When measured at the 5 min maximum, phosphorylation was also concentration-dependent (0.5–50 nM IGFBP-5) (Fig 3B). The increase in MKK3/6 phosphorylation induced by IGFBP-5 was not affected by the Raf1 inhibitor, the MKK1/2 inhibitor, or the p38 MAP kinase inhibitor (Fig 3C).

IGFBP-5 Activates the Erk1/2 Signaling Pathway—Incubation of quiescent muscle cells with IGFBP-5 in the presence of the IGF-I receptor antagonist elicited a prompt, time-dependent phosphorylation of both Erk1/2 isoforms on Thr202/Tyr204. Phosphorylation was maximal within 5 min and declined to lower levels within 60 min (Fig. 4A). When measured at the 5 min peak, IGFBP-5-induced phosphorylation was concentration-dependent (0.5–50 nM IGFBP-5) (Fig. 4B). Erk1/2 phosphorylation was abolished in the presence of either the Raf1 kinase inhibitor (10 nM) or the MKK1/2 inhibitor, U1026 (10 μM), but was not affected by the p38 MAP kinase inhibitor, SB203580 (1 μM) (Fig. 4C).

IGFBP-5 elicited a similar time-dependent increase in (Ser217/Ser221)MKK1/2 phosphorylation that was prompt, attained a maximum within 5 min, and declined to lower levels within 60 min (Fig. 5A). Phosphorylation of MKK1/2 by IGFBP-5 was also concentration-dependent (Fig 5B).
crease in MKK1/2 phosphorylation induced by IGFBP-5 was abolished in the presence of the Raf1 kinase inhibitor (1 nM) and the MKK1/2 inhibitor, U1026 (10 μM), but was not affected by the p38 MAP kinase inhibitor, SB203580 (1 μM) (Fig. 5C).

The effect of IGFBP-5 on (Ser^{259})Raf1 phosphorylation was also examined. The addition of 50 nM IGFBP-5 elicited time-dependent phosphorylation of Raf1 that was maximal within 5 min and declined to lower levels after 60 min (Fig. 6A). When measured at the peak (5 min), IGFBP-5-induced Raf1 phosphorylation was concentration-dependent (0.5–50 nM IGFBP-5) (Fig. 6B). The phosphorylation of Raf1 induced by IGFBP-5 was abolished in the presence of the Raf1 kinase inhibitor (10 nM) but was not affected by the MKK1/2 inhibitor, U1026 (10 μM), or the p38 MAP kinase inhibitor, SB203580 (1 μM) (Fig. 6C).

IGFBP-5 Activates Ras—Two methods were used to identify the role of Ras in the signaling pathways activated by IGFBP-5. The first method measured IGFBP-5-induced Ras activation as Ras-GTP using an immunoprecipitation-based assay as described under “Experimental Procedures” (20). The second method identified the requirement for Ras activation in the signaling pathways leading to p38 MAP kinase and Erk1/2 activation by expression of a dominant negative Ras(S17N) mutant in human intestinal muscle cells (22).

Incubation of quiescent human intestinal smooth muscle cells with IGFBP-5 caused time-dependent activation of Ras that was rapid, occurring within 30 s, sustained for up to 2 min, and then declined rapidly to lower levels by 5 min (Fig. 7A).

Activation of Ras by IGFBP-5 (0.5–50 nM), measured at the 2
min maximum, was also concentration-dependent (Fig. 7B).

The requirement for Ras activation in IGFBP-5-induced p38 MAP kinase and Erk1/2 activation was examined in cells transiently transfected with a dominant negative Ras(S17N) mutant. In these cells, the ability of 50 nM IGFBP-5 to stimulate p38 MAP kinase phosphorylation was abolished (110 ± 8% inhibition versus vector transfected control, \( p < 0.05 \)) (Fig. 8A). In cells expressing the dominant negative Ras mutant, the ability of 50 nM IGFBP-5 to stimulate Erk1/2 phosphorylation was also abolished (98 ± 8% inhibition versus vector transfected control, \( p < 0.05 \)) (Fig. 8B). The dominant negative effect of the Ras(S17N) mutant on Ras activation was confirmed in separate studies. Incubation of quiescent muscle cells transiently transfected with empty vector for 2 min with 50 nM IGFBP-5 elicited an increase in activated, GTP-bound Ras (270 ± 40% above basal), whereas in cells expressing RAS(S17N), the ability of IGFBP-5 to activate Ras was abolished (9 ± 2% above basal, \( p < 0.05 \) versus empty vector).

**IGFBP-5 Stimulates Growth via Ras-dependent Activation of the p38 MAP Kinase and Erk1/2 Pathways**

The role of Ras and of the p38 MAP kinase and Erk1/2 signaling pathways in IGFBP-5-induced proliferation was investigated using two techniques. The involvement of p38 MAP kinase and Erk1/2 in IGFBP-5-stimulated growth was determined using the p38 MAP kinase inhibitor, SB203580 (1 \( \mu \)M), and the MKK1/2 inhibitor, U1026 (10 \( \mu \)M). The involvement of Ras in IGFBP-5-stimulated growth was determined after transient transfection of the dominant negative Ras(S17N) mutant (22).

Activation of the p38 MAP kinase pathway by IGFBP-5 was
coupled to an increase in [3H]thymidine incorporation. In the presence of the p38 MAP kinase inhibitor, SB203580 (1 μM), the ability of 50 nM IGFBP-5 to stimulate [3H]thymidine incorporation was inhibited 69 ± 5% (p < 0.05) (Fig. 9). At the 1 μM concentrations used in the present study, SB203580 selectively inhibits p38 MAP kinase activation without affecting other protein kinases (25, 26).

The Erk1/2 pathway was also activated by IGFBP-5 and led to an increase in [3H]thymidine incorporation. In the presence of the MKK1/2 inhibitor, U1026 (10 μM) (26), the increase in [3H]thymidine incorporation induced by 50 nM IGFBP-5 was also partly inhibited, 40 ± 6%, (p < 0.05) (Fig. 9). At the 10 μM concentrations used in the present study, U1026 has been previously shown to be highly selective for MKK1/2 inhibition without affecting other protein kinases. In cells transfected with the dominant negative Ras(S17N) mutant, the ability of 50 nM IGFBP-5 to stimulate [3H]thymidine incorporation was also abolished (vector, 158 ± 6% above basal; Ras(S17N), 10 ± 8% above basal).

In the presence of the combination of the p38 inhibitor, SB203580 (1 μM), and the MKK1/2 inhibitor, U1026 (10 μM),

**FIG. 7.** IGFBP-5 activates Ras. A, incubation of confluent human intestinal muscle cells with 50 nM IGFBP-5 elicits time-dependent association of Ras with GTP (Ras-GTP). Inset, representative affinity precipitation (Ras-GTP) and immunoblot analysis of Ras in IGFBP-5-stimulated muscle cells. B, IGFBP-5 elicits concentration-dependent Ras activation when measured at the 5 min peak. Inset, representative affinity precipitation (Ras-GTP) and immunoblot analysis of Ras in IGFBP-5-stimulated muscle cells. Ras-GTP was immunoprecipitated from cell lysates using the Raf-binding domain of Ras-GTP (Raf-RBD), and the resulting immunoprecipitates (IP) subjected to Western blot analysis (WB) for Ras. Results are expressed in relative densitometric units. A.U., arbitrary units. Values represent the means ± S.E. of 3–5 experiments. *, p < 0.05 versus basal levels.

**FIG. 8.** IGFBP-5-induced p38 MAP kinase and Erk1/2 activation is Ras-dependent. A, IGFBP-5-induced p38 MAP kinase phosphorylation is abolished after the transient transfection of a dominant negative Ras(N17) mutant. Inset, representative Western blot analysis of IGFBP-5-dependent p38 MAP kinase phosphorylation. B, IGFBP-5-induced Erk1/2 phosphorylation is abolished after the transient transfection of a dominant negative Ras(N17) mutant. Inset, representative Western blot analysis of IGFBP-5-dependent Erk1/2 phosphorylation. Results are expressed in relative densitometric units. A.U., arbitrary units. Values represent of mean ± S.E. of three separate experiments.

**FIG. 9.** IGFBP-5-induced [3H]thymidine incorporation is mediated jointly by p38 MAP kinase and Erk1/2 activation. Incubation of human intestinal muscle cells for 24 h with 50 nM IGFBP-5 elicits an increase in [3H]thymidine incorporation that is partly inhibited by the p38 MAP kinase inhibitor, SB203580 (1 μM), or the MKK1/2 inhibitor, U1026 (10 μM), and is nearly abolished by the combination of the two inhibitors. Results are expressed as percent increase above basal levels. Values represent means ± S.E. of four separate experiments. *, p < 0.05 versus basal.

the ability of 50 nM IGFBP-5 to stimulate [3H]thymidine incorporation in human intestinal muscle cells was nearly abolished at 89 ± 2% inhibition (p < 0.01) (Fig. 9). The results suggest that activation of these two Ras-dependent pathways, p38 MAP kinase and Erk1/2, in human intestinal smooth muscle cells...
were involved (14). 4) Incubation of muscle cells with IGFBP-5 causes a p38 MAP kinase- and Erk1/2-dependent increase in IGF-I secretion. 5) In the presence of the IGF-I receptor antagonist, IGFBP-5 does not elicit IGF-I receptor phosphorylation. IGFBP-5 has been shown to stimulate [3H]thymidine incorporation in osteoblast and bone cells independently of IGF-I and the IGF-I receptor (27). The mechanisms mediating the direct, proliferative effects of IGFBP-5 were not delineated. On the basis of the current study and previous work, two potential mechanisms have been identified. The first involves a membrane-bound, IGFBP-5-specific receptor first characterized by Andress in osteoblast cells (9–11), and the second involves nuclear transport of IGFBP-5 and direct nuclear effects mediated in the fashion of a ligand-dependent transcription factor. The latter mechanisms was identified in T47D breast cancer cells by Scheldich and colleagues (28, 29). The carboxy tail of IGFBP-5 (and IGFBP-3, which also possesses IGF-I-independent effects (30, 31)) share a common nuclear localization sequence, IGFBP-3-(215–232) and IGFBP-5-(201–218). This sequence has been shown to be required for the importin-β-dependent nuclear translocation of both IGFBP-5 and IGFBP-3 (8, 29). Although this portion of IGFBP-5 also has been shown to participate in binding to the IGFBP-5 receptor, deletional studies have demonstrated that this sequence of the IGFBP-5 peptide is not required for activation of its cell surface receptor (27). Nuclear transport of IGFBP-5 was not specifically addressed in the current study; however, the ability of the p38 and M KK1/2 inhibitors in combination to abolish both proliferation and IGF-I secretion in response to IGFBP-5 suggests that if nuclear transport of IGFBP-5 does occur in these cells, it is not required for IGFBP-5-stimulated proliferation or IGF-I secretion. The intracellular signaling cascades coupled to activation of the IGFBP-5 receptor are largely unknown but appear to begin following the autophosphorylation of serine residues of the receptor (10). In rat glomerular mesangial cells, activation of the IGFBP-5 receptor results in Cdc42-GTPase-activating protein aggregation, reorganization of the actin cytoskeleton, and filopodia formation (11). Whether this signaling pathway is involved in the proliferative responses to IGFBP-5 is unknown. Growth factors utilize distinct small GTPases to mediate specific cytoskeletal reorganizations such as lamellipodium formation (mediated via Rac), stress fiber formation (mediated via Rho), or filopodia formation (mediated via Cdc42). The small G-proteins Rho and Cdc42 are expressed by intestinal smooth muscle cells and mediate neurotransmitter-induced contraction (32). In human intestinal smooth muscle and other muscle cell types, the proliferative effects of IGFBP-5 are mediated by the activation of distinct Raf-Erk1/2 and p38 MAP kinase pathways. In COS-7 and HeLa cells, the small GTPases Rac, Rho, and Cdc42 are requisite cofactors in Ras-dependent Raf activation and subsequent activation of Erk1/2 (33–35). These monomeric G-proteins have also been shown to play a role in the activation of MKK3/6 leading to p38 MAP kinase activation (36, 37). It remains to be determined whether IGFBP-5-induced growth mediated by the Raf-Erk1/2 or p38 MAP kinase pathways involves the participation of the GTPases Rho, Rac, and Cdc42. Based on our previous work and the results of this current study we propose the following model whereby a positive feedback mechanism links IGF-I and IGFBP-5 (Fig 11). IGF-I, acting via its cognate receptor and facilitated by the presence of IGFBP-5, activates the PI3-kinase and Erk1/2 pathways that jointly mediate increased proliferation and secretion of IGFBP-5 (14, 17). IGFBP-5, in turn, acting via its cognate receptor, activates the p38 MAP kinase and Erk1/2 pathways.
that jointly mediate increased proliferation and further secretion of IGF-I.

The concomitant presence of IGF-I and IGFBP-5 and the resultant interplay between their signaling pathways may be important factors in mediating their individual effects. We and others (13, 38) have shown that IGFBP-5 protein levels are highly sensitive to protein synthesis inhibitors. Inhibition of RNA stabilizing factor translation by inhibitors such as cycloheximide leads to rapid IGFBP-5 mRNA degradation and results in IGFBP-5 levels falling rapidly to undetectable levels. The 3’-untranslated region of IGFBP-5 mRNA contains several adenosine-uridine-rich elements (38), which confer instability to IGFBP-5 mRNA but can be stabilized by binding cytoplasmic proteins. Hou and colleagues (38) have suggested that IGF-I might stimulate the production of AU-binding proteins that stabilize IGFBP-5 mRNA. In NIH 3T3 cells, the regulation of mRNA abundance is a dynamic process in which stabilizing and destabilizing AU-binding proteins compete (39). In these cells, PI3-kinase and p38 MAP kinase function by activating pathway specific stabilizing AU-binding proteins that regulate interleukin-3 mRNA levels (39). We have previously shown that in human intestinal muscle cells, IGF-I increases IGFBP-5 levels via Erk1_2-dependent and PI3-kinase-dependent mechanisms (17). We hypothesize that the ability of IGF-I to increase IGFBP-5 mRNA levels in human intestinal muscle cells might reflect in part the ability of IGF-I-activated signaling intermediates, such as PI3-kinase, to act as stabilizing kinases for IGFBP-5 mRNA.

The results of this study show that IGFBP-5 stimulates the secretion of IGF-I in intestinal muscle cells by activation of distinct p38 MAP kinase-dependent and Erk1_2-dependent pathways. The IGF-I gene has two alternative first exons that are spliced to a common block of exons that contain the mature peptide-containing sequence. In most tissues, IGF-I gene expression is regulated through activation of exon 1 (40). Several potential regulators of IGF-I gene transcription are known to be downstream targets for p38 MAP kinase including members of the CCAAT/enhancer-binding protein (C/EBP) and ATF/CREB family; downstream targets of Erk1_2 also include members of the ATF/CREB family and the AP-1 family (40–43). The specific transcriptional regulators of the IGF-I gene in human intestinal smooth muscle remain to be determined.

The positive feedback loop linking IGFBP-5 and IGF-I secretion may have clinical importance in the setting of intestinal inflammation, e.g. Crohn’s disease. The presence of increased IGF-I and IGFBP-5 expression by intestinal smooth muscle cells in animal models of enterocolitis has been appreciated for a number of years (15), and this observation has recently been extended to include Crohn’s disease in humans (16). The expression of both IGF-I and IGFBP-5 is increased in parallel in regions of active inflammation and stenotic formation. In addition to muscle proliferation induced by IGF-5 and IGF-I, IGFBP-5 also stimulates collagen secretion (44). The mechanisms responsible for initiating this response have yet to be fully elucidated, but the resulting muscle hyperplasia and extracellular matrix production may be responsible, in part, for luminal narrowing and stenotic formation in the intestine.

In summary, the present paper shows that IGFBP-5 exerts direct, IGF-1-receptor-independent effects in human intestinal smooth muscle cells. By jointly activating p38 MAP kinase and Erk1_2, IGFBP-5 stimulates both muscle cell proliferation and IGF-I secretion. A positive feedback mechanism linking IGFBP-5 and IGF-I secretion in these cells reinforces their individual ability to cause intestinal smooth muscle cell growth.

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