Transforming Growth Factor-β Inhibition of Insulin-like Growth Factor-binding Protein-5 Synthesis in Skeletal Muscle Cells Involves a c-Jun N-terminal Kinase-dependent Pathway*

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The IGFBP family includes six structurally related soluble proteins that bind IGFs with a very high affinity, equal to or greater than that of IGFs for binding to the IGF-1R. IGFBP-5, the most highly conserved of all known IGFBPs, is produced in large amounts in muscle tissue and is up-regulated during differentiation in vitro (8–7). IGFBP-5-mRNA has been detected as early as embryonic day 10.5 in the rat embryo, especially in muscle progenitors, and is present in muscle cells in all regions of the embryo throughout fetal life (8). IGFBP-5 synthesis is induced by IGF following IGF-1R activation (7, 9) but seems to be repressed by several growth factors including TGF-β (transforming growth factor-β), fibroblast growth factor-2, and epidermal growth factor (10). Purified IGFBP-5 prevents the proliferation of interstitial cell precursors in vivo (11) and negatively regulates IGF-dependent proliferation and differentiation, in vitro (12). Moreover, the overproduction of IGFBP-5 in muscle cells by transfection inhibits differentiation whereas inhibition of IGFBP-5 production results in more extensive differentiation (4). Thus, the factors that regulate IGFBP-5 synthesis by muscle cells determine the bioavailability of IGFs and the IGFBP-5 synthesis induced during myogenic differentiation may regulate IGF activity by negative feedback. However, the mechanisms by which IGFBP-5 synthesis is regulated during myogenesis are still incompletely understood.

TGF-β plays an important role during myogenesis but has contrasting activities. In vivo, TGF-β expression is associated with myogenesis during the early stages of myoblast differentiation and during muscular regeneration (13). Consistent with this idea, myoblasts have been reported to require functional TGF-β receptors for fusion in vivo (14), raising the possibility that TGF-β may act as a competence factor in myogenic differentiation. However, the situation is probably more complicated as the effects of TGF-β on muscle differentiation depend on culture conditions and the combination of TGF-β with other growth factors. TGF-β has been shown to inhibit the differentiation of myoblasts, but only if the cells are cultured at high density (15). In contrast, TGF-β not only promoted differentiation when combined with other growth factors, such as fibroblast growth factor-2, but also blocked the effects of stimuli that negatively regulate myogenic differentiation (16, 17). However, it has often been reported that, in vitro, TGF-β is negatively regulated by IGFs, at the RNA and protein levels, during later stages of differentiation (18) and that both the TGF-β secreted by muscle cells and exogenous TGF-β inhibit myoblast fusion (19–23).

Skeletal muscle differentiation is a complex cellular process modulated by growth factors, which may act in an autocrine or paracrine manner to regulate proliferation and terminal differentiation. IGFs (insulin-like growth factors) stimulate myoblast replication but also induce terminal differentiation (1). IGFs (insulin-like growth factors) play critical roles in the control of myogenesis. Insulin-like growth factor binding protein-5 (IGFBP-5), by regulating the bioavailability of IGFs, is involved in controlling IGF-dependent differentiation. We investigated the effects of TGF-β on the IGFBP-5 production induced by IGFs in mouse myoblasts. TGF-β leads to a decrease in IGFBP-5 synthesis at both transcript and protein levels, and blocked muscle differentiation. The Smad proteins and the c-Jun N-terminal kinase (JNK) have been shown to be involved in TGF-β signaling pathways. We provide evidence that the JNK pathway, rather than Smad proteins, is involved in the response of muscle cells to TGF-β. This factor failed to stimulate the GAL4-Smad 2/3 transcriptional activities of the constructs used to transfect myoblasts. Moreover, stable expression of the antagonistic Smad7 did not abolish the inhibitory effect of TGF-β on IGFBP-5 production whereas expression of a dominant-negative version of MKK4, an upstream activator of JNK, did. We also showed, using a specific inhibitor, that the p38 mitogen-activated protein kinase (p38 MAPK) was not involved in the inhibition of IGFBP-5 production. Thus, TGF-β-mediated IGFBP-5 inhibition is independent of Smads and requires activation of the JNK signaling pathway.

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The abbreviations used are: IGF-1R type 1 insulin-like growth factor receptor; IGFBP, insulin-like growth factor-binding protein; Ins, insulin; GST, glutathione S-transferase, JNK, c-Jun NH2-terminal protein kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/extracellular signal-regulating kinase; MKK, MAPK kinase; MKK4DN, MKK4 dominant negative; SAPK, stress-activated protein kinase; TGF-β transforming-growth factor; FCS, fetal calf serum; PAGE, polyacrylamide gel electrophoresis.

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TGF-β signals by simultaneously contacting two transmembrane serine/threonine kinases known as the type I and type II receptors. The type II receptor can bind TGF-β directly, but cannot mediate responses in the absence of the type I receptor (24, 25). The intracellular pathways mediating the transmission of TGF-β signals from the membrane to the nucleus have recently begun to be elucidated. Smad2 and Smad3 proteins have been shown to mediate TGF-β signal transduction in a variety of species. On phosphorylation by the activated type I receptor at COOH-terminal serine residues, the Smad2 or Smad3 protein associates with the shared partner Smad4, and the resulting complex is translocated to the nucleus where they participate in transcriptional activation of target genes. Unlike Smad2, Smad3, and Smad4, the antagonistic Smad proteins which include Smad6 and Smad7, appear to regulate these pathways negatively by blocking ligand-dependent signaling (26–28). Recently, the role of Smad7 was revealed more complex as it is involved in cell death regulation (29–31).

The JNK (c-Jun N-terminal kinase) signaling pathway is also one of the downstream effectors required for TGF-β-mediated responses. TGF-β can activate the JNK pathway in a variety of cell lines (32–35). The activation of JNK by TGF-β signaling seems to be largely mediated by sequential activation of the mitogen-activated protein kinase kinase kinase, a MAP kinase kinase kinase, as it is involved in cell death regulation (29–31).

In this study, we analyzed the effects of TGF-β on IGFBP-5 production and on the differentiation induced by high concentrations of insulin which mimic the action of IGFs. We found that TGF-β inhibited IGFBP-5 induction and muscle differentiation. We present evidence that the inhibition of IGFBP-5 synthesis is independent of Smad proteins and requires the functional regulatory kinase, MKK4, an upstream activator of the c-Jun N-terminal kinase.

MATERIALS AND METHODS

Plasmids—Specific complementary DNA probe for mouse IGFBP-5 was generously provided by a gift from S. Drop (Erasmus University, Rotterdam, The Netherlands). The expression vectors encoding the dominant-interfering mutant form of MKK4 (pDNA3-FLAG-MKK4-Ala) (pcDNA3-FLAG-MK4-Ala) (pcDNA3-FLAG-MK4-Ala), GST-JNK (pGEX-4T1-c-Jun 1–79), and β-galactosidase (pcMV-5-LaCZ) have been described elsewhere (32, 33, 38). The p3TP-Lux reporter construct (a gift from Dr. Joan Massagué) contains three consecutive AP1 sites, and sequences from the plasminogen activator inhibitor promoter driving the expression of a luciferase reporter gene. To obtain plasmide encoding GAL4-Smad2 and GAL4-Smad3 fusion proteins, cDNAAs from pcMV5-Flag-Smad2 and pcMV5-Flag-Smad3 (generously provided by Dr. Wranan) were inserted into the pSG424 vector in-frame with an amino-terminal GAL4 (1–147) tag, using convenient sites in the vector polylinker. The expression vector for G15E1b-lux, which contained the luciferase reporter gene downstream from the DNA-binding elements of GAL4 and the E1B TATA box, has been described elsewhere (39).

Cell Culture—The C2 myoblast cell line stably transfected with a vector generating an antisense RNA complementary to the first 106 nucleotides of the mature IGF-II protein, called C2 anti-IGF-II (39), was cultured in proliferation medium, which consisted of Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.), supplemented with 20% (v/v) FCS (Institut Jacques Boy, Reims, France), 200 units/ml penicillin, 200 μg/ml streptomycin, and 200 μg/ml hygromycin B (Sigma) added for selection. For experiments, cells were cultured for 3 days in proliferation medium then incubated in low-serum medium containing 1% (v/v) FCS, for 1 day. These quiescent cells were transfected to serum-free-medium supplemented with 10–5 M bovine insulin (Sigma) to induce differentiation, in the presence or absence of human TGF-β (Sigma) at the concentrations indicated. The parental untransfected C2 myoblasts used in some experiments were induced to differentiate in a serum-free medium with 10–6 M insulin or in low-serum medium containing 1% FCS.

To generate C2 anti-IGF-II cells stably expressing the FLAG-Smad7 construct and the dominant-interfering mutant MKK4-Ala gene, cells were transfected with pcDNA3-FLAG-Smad7 or pcDNA3-FLAG-MK4-Ala using Lipofectamine™ (Life Technologies, Inc.). In regular medium (20% FCS), selection was initiated by adding 200 μg/ml hygromycin B (Sigma) and 400 μg/ml genetiscin (Life Technologies, Inc.). Single colonies obtained on day 15 were cloned. Control cells were transfected with the appropriate vector alone (control), selected in parallel with the other cells, and expanded as pools of stably transfected cells. The human hepatoma cell line HepG2, used as a positive control of infection with the GAL4-Smad2 and -3 constructs, was maintained in RPMI supplemented with 10% fetal calf serum.

Troponin T Immunodetection—Cells cultured in plastic tissue culture plates were fixed by incubation for 3 min with a 1:1 mixture of methanol and acetone and stored at −20 °C. Cells were incubated with antibodies as previously described (39): (i) 40 min with a monoclonal antibody against troponin T (Sigma) used at 1:200 dilution; (ii) 30 min with a streptavidin-conjugated peroxidase. The product of the peroxidase reaction was detected with a kit from Sigma. Cells were thoroughly washed with phosphate-buffered saline after each step.

JNK Kinase Assay—Cells were lysed in lysis buffer (25 mM HEPES, pH 7.5, 0.3 M NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM dithioretilol, 1% Triton-X-100, 0.5% sodium deoxycholate, 20 mM β-glycerophosphate, 0.1% phenylmethylsulfonyl fluoride, 100 μM leupeptin, 10 mM benzamidine, 100 μM aminotriazole, and 0.2% Triton X-100) and homogenized in a microhomogenizer. The cell homogenates were centrifuged at 10,000 × g for 30 min at 4 °C. The resulting supernatant was subjected to gel filtration on a column of Sepharose 6B (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Then, the samples were loaded onto a 12% polyacrylamide gel. For Flag-tagged MKK4DN, Flag-Smad7, endogenous JNK, troponin T, and troponin detections, cells were lysed with the same lysis buffer used for JNK kinase assays, and 40 μg of cellular protein was analyzed by SDS-PAGE in a 12.5% polyacrylamide gel.

Secreted IGFBP-5 was precipitated from 0.5-ml samples with 40% trichloroacetic acid and analyzed by SDS-PAGE in a 12.5% polyacrylamide gel. For Flag-tagged MKK4DN, Flag-Smad7, endogenous JNK, and troponin detections, cells were lysed with the same lysis buffer used for JNK kinase assays, and 40 μg of cellular protein was analyzed by SDS-PAGE in a 10% polyacrylamide gel. Protein concentration was determined using the Bradford assay (Bio-Rad), with bovine serum albumin as the standard.

The proteins were transferred to nitrocellulose membranes (Sartorius, Göttingen, Germany) and incubated with specific antibodies: the rabbit polyclonal anti-human IGFBP-5 antiserum (Upstate Biotechnology, Lake Placid, NY), the rabbit polyclonal anti-JNK antibody (Santa Cruz); the monoclonal antibody directed against the Flag epitope (anti-Flag M2, from Sigma), and the monoclonal anti-troponin T (Amersham Pharmacia Biotech). Immuno complexes were detected by enhanced chemiluminescence (ECL kit, Amersham Pharmacia Biotech). Northern blot Analysis—Total RNA was prepared from cultured cells using TRIZOL (Gibco-BRL). Total RNA (10 μg) was then subjected to gel electrophoresis and blotted as previously described (40).

Mouse IGFBP-5 sequences have been published (41), and we used a specific complementary DNA probe generously provided by S. Drop (Erasmus University, Rotterdam, The Netherlands), random primed with [α-32P]dCTP using the Rediprime labeling kit (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.
Myoblasts/H9252—hormonal stimulation.

TGF-β Abolishes IGF-dependent Muscle Differentiation and IGFBP-5 Induction—We first investigated the effect of TGF-β on the muscle differentiation triggered by insulin using a C2 cell line variant (C2 anti-IGF-II cell line) obtained by stably transfecting C2 myoblasts with a vector generating an antisense IGF-II mRNA (39). This well characterized cell line, which did not undergo muscle differentiation without the addition of exogenous IGF or insulin at high concentration (10−6 M), was used to investigate directly the relationships between IGF and TGF-β in the regulation of muscle differentiation. Consistent with previously published results, the insulin-induced differentiation of C2 anti-IGF-II was markedly inhibited by TGF-β (Fig. 1). Thus, in addition to inhibiting differentiation, TGF-β also inhibited IGFBP-5 induction in C2 anti-IGF-II cells (Fig. 1).

IGFBP-5 is produced and secreted by muscle cells early in differentiation (5–7). We therefore investigated whether TGF-β could also inhibit IGFBP-5 production. Exposure of the C2 anti-IGF-II myoblasts to TGF-β greatly decreased the amount of IGFBP-5 mRNA detected and caused a similar decrease in the amount of IGFBP-5 secreted (Fig. 2A). The inhibitory effect of TGF-β on the amount of IGFBP-5 mRNA and secreted protein was also observed using the parental C2 myoblast cell line (Fig. 2B). Thus, in addition to inhibiting differentiation, TGF-β also inhibited IGFBP-5 induction in response to IGF signaling.

TGF-β Did Not Activate Smad Protein in C2 Anti-IGF-II Myoblasts—It has been suggested that the activation of TGF-β signaling via the type I receptor may involve Smad2, Smad3, and Smad4 (44–46). Smad4 is the common mediator required by all pathways, whereas Smad2 and Smad3 are specific substrates of TGF-β receptors. So, to investigate the possible role of Smad proteins in mediating the effects of TGF-β on IGFBP-5 production, we studied the effect of TGF-β on the transcriptional activity of Smad2 and Smad3 in C2 anti-IGF-II cells. We first assessed the effects of TGF-β on the 3TP-Lux reporter construct, which contains the sequences from the plasminogen activator inhibitor-1 promoter that bind Smad3 and Smad4, along with three consecutive AP1 sites (47). The addition of TGF-β to C2 anti-IGF-II cells strongly induced transcription from the 3TP-Lux promoter (Fig. 3). To investigate directly whether Smad proteins were involved in the regulation of gene expression by TGF-β in these cells, we assessed the effect of TGF-β on the activity of the GAL4-Smad2 and GAL4-Smad3 constructs. The Smad2 and Smad3 proteins, if fused to the DNA-binding domain of the yeast transcription factor GAL4 (1–147), activate transcription of a luciferase reporter gene under the control of GAL4-binding sites linked to an E1b TATA box. Surprisingly, we observed that the addition of TGF-β had no significant effect on GAL4-Smad2 and GAL4-Smad3 transcriptional activities in C2 anti-IGF-II cells (Fig. 3). The lack of TGF-β activation of the GAL4-Smad2 and GAL4-Smad3 constructs was not due to a defect in expression as we demonstrated that TGF-β increased, under these experimental conditions, the expression of GAL4-Smad2 and GAL4-Smad3 in HepG2 cells (Fig. 3). These results suggest that, in C2 anti-IGF-II cells, TGF-β receptor activation may exert its inhibitory functions on IGF-induced functions by triggering a signaling pathway different from that involving Smad proteins. In support of this hypothesis, we generated stable independent clones of C2 anti-IGF-II cells stably expressing Smad7, the antagonistic Smad (Fig. 4A). We used these clones to assess the role of Smad proteins in the regulation of Smad proteins in the regulation by TGF-β of IGFBP-5 expression and myogenic differentiation. As expected, the overexpression of Smad7 did not interfere with the inhibitory effect of TGF-β on IGFBP-5 secretion (Fig. 4B) and troponin expression (Fig. 4C) induced by 10−6 M insulin (or 10−7 M IGF). In these cell lines Smad7 is functional since its overexpression sensitizes cells to apoptosis (Fig. 4D) and inhibits NF-κB activity (data not shown), two well established functions of Smad7 (30, 31).

TGF-β Activates the JNK Signaling Pathway—The JNK signaling pathway is also regulated by TGF-β. This pathway activates transcription of several genes via interactions of c-Jun with AP-1 sites, thereby enabling TGF-β to transmit signals from the cell surface to the nucleus. We first investigated the possible involvement of JNK in TGF-β-mediated responses in C2 anti-IGF-II cells by determining the activity of endogenous JNK from cells exposed to 2 ng/ml TGF-β for a variety of time periods. Endogenous JNK was isolated by immunoprecipitation using a specific antibody and its activity was determined in an in vitro kinase assay using GST-Jun as a specific substrate of JNK. Consistent with our previous reports in other
cell lines, TGF-β increased JNK activity in a time-dependent manner (Fig. 5). Activation of JNK by TGF-β was first detected after 1 h, peaked at 6 h (reaching a level 3 to 4 times higher than the initial level), and remained high for a prolonged period of time.

The delayed and persistent activation of JNK by TGF-β may be due to the accumulation of JNK protein in the cells. However, immunoblotting analysis of total cell lysates with anti-JNK antibody demonstrated that similar amounts of JNK proteins were present. Thus, the increase in JNK activity probably results from the phosphorylation and activation of JNK by an upstream kinase such as mitogen-activated protein kinase (MAPK) kinase 4 (MKK4, also called Sek1 or JNKK). MKK4 is a dual specificity protein kinase that phosphorylates JNK, resulting in its activation. If MKK4 acts downstream from the TGF-β receptor in the biochemical pathway leading to JNK activation in C2 anti-IGF-II cells, then expression of a dominant-negative mutant version of the MKK4 gene might abolish TGF-β-induced JNK activation. We investigated this possibility by generating independent clones of C2 anti-IGF-II cells stably expressing MKK4-Ala. MKK4-Ala (MKK4DN) encodes a dominant-negative mutant with defective JNK activation by TGF-β and other extracellular stimuli (45). Analysis of whole cell lysates from transfected C2 anti-IGF-II cells revealed that three representative clones of 12 isolated, produced large amounts of a Flag-MKK4DN fusion protein (Fig. 6). As expected, the expression of MKK4DN reduced the TGF-β-induced activation of JNK (Fig. 6), indicating that TGF-β signaling via MKK4 was specifically blocked in the transfected cells. Anti-JNK immunoblotting of cell lysates confirmed that the observed decrease in JNK activity was not due to low levels of JNK in the MKK4DN-transfected cells (Fig. 7).

To confirm the possible involvement of the JNK pathway in the TGF-β-dependent activation of gene expression, we determined the extent to which MKK4DN decreased the TGF-β-induced activation of the 3TP-Lux construct. The level of transcriptional activation of the 3TP-Lux promoter by TGF-β was significantly lower in cells expressing MKK4DN than in control myoblasts (Fig. 8). Taken together, these data provide strong evidence that the JNK pathway plays a critical role in the TGF-β-dependent activation of JNK in C2 anti-IGF-II myoblasts.
MKK4DN. 

FIG. 6. Production of Flag-MKK4DN in C2 anti-IGF-II cells. C2 anti-IGF-II cells were stably transfected with pcDNA3-Flag-MKK4-Ala, as described under “Materials and Methods.” Three clones of transfected cells (n°1, n°4, and n°12) were compared with myoblasts transfected with insert-free vector (control myoblasts, C). To demonstrate the synthesis of Flag-MKK4DN, 50 μg of total cellular protein was resolved by 10% SDS-PAGE and subjected to Western immunoblot analysis using a monoclonal antibody directed against the Flag epitope (anti-Flag M2). The arrow on the left indicates the position of the Flag-tagged MKK4DN.

FIG. 7. Dominant-negative mutant of MKK4 abolishes TGF-β-induced JNK activation. Myoblasts transfected with the insert-free vector and MKK4DN myoblasts (clone n°4) were exposed to TGF-β (2 ng/ml) for 6 h. Top panel, JNK was immunoprecipitated from cell lysates with anti-JNK antibody and the immunoprecipitates were used for kinase assays with GST-Jun (1-79) as the substrate. The phosphorylated proteins were resolved by SDS-polyacrylamide gel electrophoresis and detected by autoradiography. Bottom panel, whole cell lysate proteins were subjected to Western blot analysis with an anti-JNK antibody.

FIG. 8. MKK4DN production interferes with the TGF-β-induced transcriptional activation of the 3TP-Lux promoter. Stable clones expressing the MKK4DN construct (n°4 and n°12) and control cells were transiently transfected with 1 μg of p3TP-Lux and 0.1 μg of pCMVβ-Gal, as described in Fig. 3, left panel. Cells were treated with (black bars) or without (white bars) TGF-β for 16 h prior to lysis and then assayed for luciferase activity.

with MKK4DN to induce IGFBP-5 production upon insulin stimulation in the presence or absence of TGF-β. In the absence of TGF-β, cells harboring the dominant negative version of MKK4 responded to insulin stimulation by producing IGFBP-5 in amounts similar to those in control cells (Fig. 9), indicating that the JNK pathway is not required for the up-regulation of IGFBP-5 by insulin. In contrast, the inhibitory effect of TGF-β on insulin-induced IGFBP-5 expression was abolished in MKK4DN-expressing cells (Fig. 9), indicating that MKK4 plays a crucial role in the negative regulation of IGFBP-5 expression by TGF-β, probably through its ability to activate JNK.

Previous studies have shown that MKK4 phosphorylates and activates both JNK and p38 MAP kinases (48, 49). Moreover, p38 kinases are activated in C2 myoblasts in response to TGF-β (50). We evaluated the possibility that TGF-β inhibited IGFBP-5 production via the activation of p38 kinases. Treatment of C2 anti-IGF-II cells with a specific inhibitor of p38 kinases (SB 203580) did not abolish the inhibitory effect of TGF-β on IGFBP-5 production (Fig. 10). This inhibitor which also did not alter IGF-dependent IGFBP-5 induction, abolished the IGF-dependent differentiation of C2 anti-IGF-II (data not shown), as previously described (51). Thus, the inhibition of IGF-induced IGFBP-5 secretion by TGF-β appears to be independent of p38 kinase but likely dependent on JNK.

FIG. 9. IGFBP-5 induction in MKK4DN myoblasts is totally insensitive to TGF-β treatment. The MKK4DN (MKK4DN n°4, 12) clonal C2 anti-IGF-II cells and clones transfected with insert-free vector alone (control) were cultured as described under “Materials and Methods.” Cells in serum-free medium were untreated (0), treated with 10−6 M insulin (Ins) without or with 2 ng/ml TGF-β for 1 h before the addition of insulin (TGf + Ins). 24 h later, the conditioned media were tested for the presence of IGFBP-5 by western immunoblotting.

DISCUSSION

IGFBP-5 is the major IGFB produced by differentiated muscle cells in vitro and in vivo (3, 6, 9, 10). As TGF-β is known to inhibit differentiation in vitro, we analyzed its effects on IGFBP-5 production and investigated the signaling pathways involved.

Consistent with previous studies, we found that treatment of myoblasts with human TGF-β counteracted the stimulatory effect of IGFs on myoblast differentiation (21–23). We also found that TGF-β abolished the IGF-dependent synthesis of IGFBP-5 in C2 anti-IGF-II cells. To gain insight into the mechanism underlying the inhibitory effect of TGF-β on IGFBP-5 expression, we focused our analysis on the signaling pathways triggered by TGF-β, by investigating the involvement of Smad proteins and JNK in this process.

Our data provide evidence that human TGF-β cannot activate Smad proteins in C2 anti-IGF-II myoblasts. We assessed the activation by TGF-β of Smad proteins by measuring the
expression of Smad7 did not abolish the inhibitory effect of TGF-
translocation to the nucleus of Smad2 and -3, did not modify
high thereafter. Activation of the JNK cascade by TGF-
mediated inhibition of IGF-dependent IGFBP-5 secretion and differentiation. Our observations confirm our previous data (7) showing that IGFBP-5 induction by IGFs or insulin could be dissociated from differentiation. They also suggest that MKK4 protein kinase is involved in the pathway triggered by IGF-IR activation to promote differentiation whereas Smad proteins are not. Investigations are under way to identify the target of MKK4 which could be the p38 protein kinase. We are also investigating the possible role of IGFBP-5-independent inhibition of differentiation via this target, or otherwise.

Smad proteins are critical mediators of many of the effects of TGF-β in a variety of cell systems. However, by studying the mechanisms of IGFBP-5 regulation by TGF-β, we found that in C2 myoblasts, TGF-β acts on cell differentiation and IGFBP-5 secretion via a mechanism independent of Smad activation. The inhibitory effect of TGF-β on IGFBP-5 secretion, is mediated by the activation of MKK4 and probably JNK, an essential component of TGF-β signal transduction. The results presented here provide evidence of a further mechanism for the modulation of IGFBP-5 synthesis in muscle cells.

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