Transforming Growth Factor-β Up-regulates the β₃ Integrin Subunit Expression via Sp1 and Smad Signaling*

Integrin-mediated cell-matrix interactions play important roles in regulating cell function. Since transforming growth factor-β (TGF-β) modulates many osteoblast activities, we hypothesized that the growth factor acts in part by modulating integrin expression. TGF-β increased cell adhesion to vitronectin and up-regulated the surface level of α₅β₃ via increasing β₃ protein synthesis by a transcriptional mechanism. Promoter activity analysis demonstrated that a TGF-β-responsive element resides between nucleotides −63 and −44. Electrophoretic mobility shift assay and immunoprecipitation/Western studies indicated that the nuclear complex formed using the −66/−42 oligonucleotide contained both Sp1/Sp3 and Smad proteins. Since nuclear Sp1/Sp3 levels were not altered, whereas Smad levels were increased by TGF-β, we investigated the roles of Smad proteins in the up-regulation of β₃ gene activation. Co-transfection of cells with β₃ promoter reporter construct and expression vectors for Smad3, Smad4, and Sp1 increased the stimulatory effect of TGF-β. Furthermore, expression of dominant negative Smad3 or Smad4 in cells decreased or abolished the stimulation of β₃ promoter activity by TGF-β. Smad4 mutant also inhibited the up-regulation of surface β₃ level by TGF-β. Thus, TGF-β increases expression of the integrin β₃ gene by mechanisms involving Sp1/Sp3 and Smad transcription factors.

TGF-β³ belongs to the superfamily of transforming growth factors, which modulate a wide variety of cellular activities (1–3). Originally identified as a factor that stimulates the growth of rat kidney fibroblasts, TGF-β was later reported to induce bone formation, accelerate fracture healing, and play an important role in cranial suture formation (3–11). TGF-β is secreted by both osteoclasts and osteoblasts, stored in bone matrix in a latent form, and activated by osteoclasts during bone resorption (3, 12, 13). The activated TGF-β can function as a chemoattractant to induce the migration of osteoblasts to bone surface (14–17), modulates proliferation of osteoprogenitor cells (18), and accelerates osteoblast differentiation (3, 19).

Interactions between integrins and extracellular matrix play fundamental roles in cell migration, proliferation, and differentiation (20–22). Integrins are a family of type I transmembrane glycoproteins consisting of non-covalently associated α and β chains. Osteoblasts express a repertoire of integrins including α₁β₁, α₂β₁, αβ₁, αβ₁, αβ₂, αβ₃, and αβ₅ (23–27). The roles of β₁-containing integrins on osteoblast function have been well documented (28–32). Whereas collagen and fibronectin are the ligands for several β₁ integrins, other bone matrix proteins, such as osteopontin, bone sialoprotein, and vitronectin can interact with αβ₂ (33–36). When osteoblasts are cultured on osteopontin or bone sialoprotein, their proliferation and differentiation profiles are altered (37, 38). Thus αβ₅ appears to play important roles on osteoblast activities.

Expression of integrins can be regulated by cytokines and growth factors (39). TGF-β increases the expression of α₁β₁, α₁β₂, and several β chains containing integrins in a variety of cells (40–42). Little is known about the underlying signal transduction mechanisms. It has been well established that Smad proteins mediate the signal transduction for TGF-β (43–46). Upon TGF-β binding to its receptors, Smad2 and Smad3 are activated via phosphorylation at the C-terminal end. These pathway-restrictive Smads then form complexes with Smad4, and these complexes migrate into the nucleus, where they exert transcriptional activities either directly or indirectly. Since TGF-β can induce osteoblast migration and differentiation and αβ₅, found on osteoblasts, has been reported to mediate cell migration, adhesion, and function in other cell systems (33, 47, 48), we hypothesized that TGF-β modulates osteoblast activities in part via regulating expression of αβ₅, and this regulation is dependent on Smad signals. We report here that TGF-β augments surface expression of αβ₅ by enhancing transcription of the β₅ subunit gene in murine osteoblastic cell line MC3T3-E1, via a mechanism that requires both Sp1/Sp3 and Smad proteins. The higher surface levels of the integrin result in increased adhesion of growth factor-treated osteoblasts to vitronectin.

EXPERIMENTAL PROCEDURES

Materials—TGF-β2 was generously provided by Dr. Nico C. Cerletti (Novartis Pharma AG, Basel, Switzerland). [³²P]dATP, [³²P]ATP, Megaprime DNA labeling systems, Poly(dI-dC), and ECL kit were from Amersham Pharmacia Biotech. ¹²⁵I-NaI and Tran³²S-label were from ICN (Costa Mesa, CA). AEBSF (4-(2-aminoethyl)benzenesulfonyl fluoride) and mithramycin were from Calbiochem. All chemicals for SDS-PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; kb, kilobase pair.

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‡The abbreviations used are: TGF-β, transforming growth factor-β; AEBSF, 4-(2-aminoethyl)benzenesulfonyl fluoride; BSA, bovine serum albumin; DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIFBS, heat-inactivated fetal bovine serum; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; kb, kilobase pair.

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polyacrylamide gel electrophoresis (PAGE) and protein assays were from Bio-Rad. Ultra RiboSep mRNA isolation kit was from Collaborative Biomedical Products (Bedford, MA). The Immobilon-P membrane for protein transfer was a product of Millipore (Bedford, MA). Recombinant protein A-Sepharose 4B was from Zymed Laboratories Inc. (South San Francisco, CA). Polyacrylamide gel was from NOVO (San Diego, CA). Normal goat IgG (sc-2028), normal rabbit IgG (sc-2027), and antibodies against Egr-1 (sc-110X), Sp1 (sc-59G, sc-59X), Sp3 (sc-644X), and Smad (sc-17c) (sc-6030), which recognizes Smad1, -2, -3, -5, and -8, and Smad4 (B-8) (sc-7966), were from Santa Cruz Biotechnology (Santa Cruz, CA). A polyclonal antibody against the β3 integrin cytoplasmic tail was kindly provided by Dr. Lou P. Reichardt (University of California, San Francisco, CA). DEAE-dextran, consen- sus oligonucleotides for Sp1 and AP-1, recombinant Sp1 protein, β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer kit, and reagents for labeling oligonucleotides were from Promega (Madison, WI). Site-directed Mutagenesis kit was from Stratagene (La Jolla, CA). LipofectAMINE Plus reagent was from Life Technologies, Inc. Human vitronectin and all other reagents were from Sigma.

Cell Culture—The murine osteoblastic cell line MC3T3-E1 was cul- tured in α-minimum Eagle's medium with 10% heat-inactivated fetal bovine serum (HIFBS) until confluence. After changing to medium containing 0.2% HIFBS overnight, cells were treated with either vehicle or TGF-β, which was dissolved in 4 mM HCl containing 1 mg/mL bovine serum albumin (BSA). Only cells less than passage 22 from our stocks were used. Cell Adhesion—Adhesion of cells to vitronectin was performed as described previously (34). MC3T3-E1 cells were treated with TGF-β (1 ng/mL) or vehicle for 24 h. Single cell suspensions obtained after collage- nanase and trypsin/EDTA digestion were washed three times with serum-free α-minimum Eagle's medium supplemented with 0.1% BSA and allowed to recover for 30 min on a rotating platform at 37 °C. 1 × 106 cells were seeded to each well of 48-well Costar plates, which were pre-coated with vitronectin (5 μg/mL, 0.25 mL/well) or BSA. After incu- bation at 37 °C for 1 h, wells were washed three times with PBS, and the number of adherent cells measured by absorbance at 630 nm after staining the cells with 0.5% toluidine blue in 4% paraformaldehyde and dissolving the blue stain in 1% SDS.

Surface and Metabolic Labeling and Immunoprecipitation—Cells in p-150 culture dishes were treated with TGF-β (1 ng/mL) or vehicle for 24 h, washed with PBS, and surface-labeled by treatment with lactoper- oxidase (20 μg) and glucose oxidase (0.05 units) in 1 mL of 5 mM phosphate buffered saline containing 125I-NaI (250 μCi/mL) as described previously (34). Cell layers were extracted with 1 mL of cell lysis buffer (10 mM Tris-HCl, pH 8.5, 0.15 M NaCl, 1 mM CaCl2, 0.02% NaN3, 2% poly(dI-dC)). DEAE-dextran, consensus oligonucleotides for Sp1 and AP-1, recombinant Sp1 protein, β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer kit, and reagents for labeling oligonucleotides were from Promega (Madison, WI). Site-directed Mutagenesis kit was from Stratagene (La Jolla, CA). LipofectAMINE Plus reagent was from Life Technologies, Inc. Human vitronectin and all other reagents were from Sigma.

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay—Nuclear extracts from MC3T3-E1 cells, previously treated with 1 ng/mL TGF-β or vehicle, were prepared as described (35). 2–4 × 106 cells were pelleted by a 10 min 100 g spin at 4 °C, washed once with ice-cold 5 mM Tris-HCl, pH 7.4, 0.15 mM NaCl, 1 mM EDTA, and 20% glycerol, and fixed under UV light. mRNA on the membrane was hybridized with Megaprobe labeled [α-32P]PdNA for mouse β3 integrin and [α-32P]PdNA for glyceraldehyde-3-phosphate dehydro- genase (GAPDH) for loading variation. Bands of mRNA were visualized by autoradiography and quantitated by image analysis using ISS SepraScan.

β3, Promoter Luciferase Reporter Constructs—Generation of progress- ive 5′- to 3′-deletion constructs of murine β3 promoters has been described previously (49). Briefly, a 1-kb fragment was isolated from the AccI-digested products of the β3 genomic 9-kb fragment. Deletion con- structs of this 1-kb fragment were obtained by using the Exonuclease III/Mung Bean Nuclease kit from Stratagene. All the fragments were subcloned into pGL3-basic vector containing the luciferase reporter gene. The promoter constructs used in this study spanned from −875, 0 to −63 (m1), −63 to −42 (m2), −42 to −30 (m3), −30 to −21 (m4), −21 to −10 (m5), and −10 to +250 (m6) in the 5′-3′ direction. To test the effects of various expression vectors on promoter activities, cells were transfected with β3 promoter (0.2 μg/well) together with indicated expression vector (0.2 μg/well for each vector) or empty vector (0.2 μg/well) using LipofectAMINE Plus reagent according to the protocols provided by the manufacturer. After overnight incubation, cells were treated and analyzed as de- scribed above.

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay—Nuclear extracts from MC3T3-E1 cells, previously treated with 1 ng/mL TGF-β or vehicle, were prepared as described (35). 2–4 × 106 cells were pelleted by a 10 min 100 g spin at 4 °C, washed once with ice-cold 5 mM Tris-HCl, pH 7.4, 0.15 mM NaCl, 1 mM EDTA, and 20% glycerol, and fixed under UV light. mRNA on the membrane was hybridized with Megaprobe labeled [α-32P]PdNA for mouse β3 integrin and [α-32P]PdNA for glyceraldehyde-3-phosphate dehydro- genase (GAPDH) for loading variation. Bands of mRNA were visualized by autoradiography and quantitated by image analysis using ISS SepraScan.

β3 Promoter Luciferase Reporter Constructs—Generation of progress- ive 5′- to 3′-deletion constructs of murine β3 promoters has been described previously (49). Briefly, a 1-kb fragment was isolated from the
FIG. 1. TGF-β enhances adhesion of osteoblasts to vitronectin. MC3T3-E1 cells were treated with either vehicle (Control) or TGF-β for 24 h. A single cell suspension, obtained after collagenase and trypsin/EDTA digestion, was pipetted into wells pre-coated with vitronectin. After 1 h of adhesion, non-adherent cells were removed and the number of adherent cells measured after fixing and staining.

then blotted to an Immobilon-P membrane. Western analysis was performed by incubating the membrane with rabbit anti-Sp1 or Sp3 antibody (1:2000) or anti-pan-Smad antibodies (an equal mixture of anti-Smad antibody (C-17) and anti-Smad4 antibody (B-8)) (1:150), followed by horseradish peroxidase-conjugated secondary antibodies (1:2000), according to the rapid detection protocol provided by Millipore. Sp1, Sp3, and Smad proteins were visualized by enhanced chemiluminescence using an ECL kit. To detect Smad proteins in complexes with Sp1 and Sp3, RIPA buffer-diluted nuclear extracts (100 μg of protein) were first immunoprecipitated with goat anti-Sp1 antibody (5 μl) and rabbit anti-Sp3 antibody (1 μl), respectively, and extracted with protein A-Sepharose. The extracts were subjected to SDS-PAGE, transferred to membranes, and probed with anti-Smad4 antibody (1:500).

Generation of MC3T3-E1 Mutants Expressing Dominant Negative Smad3 and Smad4 Proteins—MC3T3-E1 cells were transfected with pcDNA3 plasmid carrying FLAG-tagged dominant negative Smad3 or Smad4 cDNA (51) using LipofectAMINE Plus reagent. Stably transfected cell lines were generated by incubation in medium containing G418 (1 mg/ml) and used within five passages. Expression of these dominant negative Smad proteins was verified by Western blot analysis using anti-FLAG antibody.

Statistical Analysis—Statistical analysis was performed using Student’s t test. Each experiment was performed at least twice. The data were presented as mean ± S.E.

RESULTS

TGF-β Increased Adhesion of MC3T3-E1 Cells to Vitronectin—Treatment of MC3T3-E1 cells with TGF-β for 24 h increased cell adhesion to vitronectin to 2.9-fold of the control level (Fig. 1). Since adhesion of osteoblasts (34) and other cell types (47, 48, 52) to vitronectin is dependent on α5β1, these data suggested that TGF-β up-regulated the expression or the activities of α5β1 integrin on the surface of osteoblasts.

TGF-β Increased α5β1 Expression on Murine Osteoblastic Cells—Surface labeling of MC3T3-E1 cells, followed by immunoprecipitation with a polyclonal antibody against the cytoplasmic tail of β1 and SDS-PAGE indicated that TGF-β treatment increased surface levels of β1 (and hence α5β1) to 2.7-fold of the control level (Fig. 2A). To determine whether this increase in surface expression was derived via increased synthesis, cells were metabolically labeled with Tran35S-label during treatment with TGF-β or vehicle, and immunoprecipitation was repeated as described above. As shown in Fig. 2B, TGF-β up-regulated the synthesis of β1 protein (6.6-fold of control level). Consistent with the increased β1 protein synthesis, normalized Northern blot analysis demonstrated that TGF-β increased β1 steady-state mRNA level to 2.3-fold of the control level (Fig. 2C). Thus, TGF-β stimulated expression of α5β1 by increasing β1 mRNA level and protein synthesis.

TGF-β Up-regulated β1 mRNA by a Transcriptional Mechanism—To examine whether up-regulation of β1 mRNA by TGF-β resulted from increased gene transcription, MC3T3-E1 cells were transfected with progressive 5’ end-deleted β1 promoter constructs carrying luciferase reporter gene. As demonstrated in Fig. 3, TGF-β stimulated the activities of all the promoter constructs that included the first 63 nucleotides upstream of the transcription start site. Further deletion of the promoter construct at the 5’ end to -43 or -28 eliminated TGF-β-mediated up-regulation (Fig. 3). Thus, TGF-β stimulated β1 expression via transcriptional mechanism(s), and a TGF-β-responsive element resided between nucleotides -63 and -44 (−63/−44) of the murine β1 integrin gene.

TGF-β Increased Binding of Nuclear Factors to the Oligonucleotide −66/−42—To confirm that a TGF-β-responsive element was indeed present between nucleotides -63 and -44 in the β1 proximal promoter, EMSA was performed after incubating the nuclear extracts, derived from either control or TGF-β-treated cells, with double-stranded radiolabeled oligonucleotide −66/−42. As shown in Fig. 4, three bands were observed in all samples tested. Two bands migrated as a closely spaced doublet, and the third was a faint, more rapidly migrating band. Treatment with TGF-β for 6 or 24 h increased the band intensities as compared with the corresponding control levels (Fig. 4). The stimulation by TGF-β tapered off after 48 h (Fig. 4). These data indicated that TGF-β stimulated the binding of nuclear factor(s) to the region −66/−42 of the murine β1 integrin gene.

The TGF-β-responsive Element Was an Sp1/Sp3 Site Residing between Nucleotides −53 and −48—Sequence analysis of the β1 proximal promoter (49) revealed the presence of three potential Sp1/Sp3-responsive elements, between nucleotides −53/−48, −24/−19, and +28/+33 (Fig. 5). The promoter analysis described above (Fig. 3) indicated that the site at −53/−48 was the likely TGF-β-responsive element, whereas the remaining two Sp1/Sp3-like sites were inactive. To confirm this hypothesis, we used site-specific mutagenesis to generate promoter luciferase constructs carrying mutation at each of the three Sp1/Sp3-like sites. As expected, TGF-β stimulated the wild type promoter activity (Fig. 6A). When the nucleotides GG at −52 and −51 positions of the −53/−48 were mutated to TT
FIG. 3. TGF-β stimulates β5 promoter activity, and a TGF-β-responsive element resides between nucleotides −63 and −44. MC3T3-E1 cells were transfected with the indicated β5 promoter luciferase reporter constructs, all of which terminated at +110. After overnight recovery, cells were treated with either vehicle (Control) or TGF-β for 24 h, and luciferase activity was measured and normalized with activity of co-transfected β-galactosidase. *, p < 0.001 when compared with the corresponding control value.

FIG. 4. TGF-β increases the binding of nuclear factors to −66/−42 oligonucleotide. Cells were treated with either vehicle (C) or TGF-β (T) for the indicated period. Nuclear extracts were isolated and incubated with radioactive end-labeled double-stranded −66/−42 oligonucleotide. Mixtures were separated on a 4–20% polyacrylamide gel and visualized after autoradiography. NC, no nuclear extract added.

Fig. 5. The proximal promoter sequence of β5 integrin subunit showing the presence of three putative Sp1/Sp3 sites (underlined).

(m1, Fig. 6B), TGF-β stimulation was abolished (Fig. 6A). In contrast, mutation of the other two sites (m2 and m3, Fig. 6B) did not affect TGF-β stimulation, although the reporter activity of m2 was very low, only 5% of wild type activity (Fig. 6A). Thus, the region −53/−48 is the sole TGF-β-responsive element in the β5 integrin promoter.

To confirm that the −53/−48 site binds proteins of the Sp1/Sp3 family, we carried out a series of EMSA-based studies. As expected, TGF-β increased the binding of all three bands (A–C) to radioactive oligonucleotide −66/−42 in EMSA (lanes 1 and 2, Fig. 7). Preincubation of nuclear extracts with 100-fold unlabeled −66/−42 oligonucleotide inhibited binding of nuclear extracts to labeled probe (lanes 3 and 4). Consistent with the region being an Sp1/sp3 site, addition of 100-fold excess consensus Sp1 oligonucleotide abolished all three bands in both control and TGF-β-treated cells (lanes 5 and 6), whereas preincubation of the nuclear extract with 100-fold consensus AP-1 oligonucleotide failed to prevent complex formation (lanes 7 and 8). These combined data indicated that A–C bands in EMSA were specific complexes formed between oligonucleotide −66/−42 and the members of Sp1 family present in the nuclear extracts.

Since both Sp1 and Sp3 bind to the same DNA consensus sequence, we employed supershift EMSA to identify the proteins bound to −66/−42. Nuclear extracts were preincubated with either anti-Sp1 antibody or anti-Sp3 antibody before ad-
We detected supershifted bands using anti-pan-Smad antibodies, and Sp3 antisem proteins were bound to the responsive element. Since no activated Smad proteins were available for interaction with Sp1/Sp3, the Smad proteins in nuclear extracts were immunoprecipitated with anti-Smad antibodies in both control and TGF-β-treated samples. Thus, the reduction of the band intensities by pan-Smad antibodies in both control and TGF-β-treated samples appeared to be specific. Moreover, Egr-1 did not appear to be involved in TGF-β-mediated up-regulation of β5 integrin.

To confirm further the interactions between Sp1/Sp3 and Smad proteins, nuclear extracts were immunoprecipitated with anti-Sp1 or anti-Sp3 antibody followed by Western blot analysis for Smad4 protein. As demonstrated in Fig. 10, Smad4 binds to Sp1 and Sp3 in the nuclear extracts of both control and TGF-β-treated cells, whereas nonspecific anti-rabbit and anti-goat IgG did not yield any Smad4 bands. Thus, Smads by translocating to the nucleus, where they associate with Sp1 and Sp3, are likely involved in regulating the TGF-β-dependent increase in transcription of the murine β5 integrin gene. To test this possibility, we co-transfected expression vectors for Sp1 (kindly provided by Dr. Sunil Srivastava, University of Cincinnati, Cincinnati, OH), Smad3, and Smad4 (51) with β5 promoter reporter construct and determined the outcome by luciferase assay. As expected, TGF-β-stimulated β5 promoter activity in cells co-transfected with the empty vector pcDNA3 (basal group) (Fig. 11). Sp1 expression vector alone had no effect on the promoter activity in the Control cells but significantly, although only slightly, increased up-regulation by TGF-β as compared with the basal group (26.12 ± 1.41 versus 21.01 ± 0.42, p < 0.05). Likewise, co-transfection with expression vectors of Smad3 and Smad4 had no effect on the β5 promoter activity in the Control cells but increased slightly the up-regulation by TGF-β as compared with the basal group (30.38 ± 2.12 versus 21.01 ± 0.42, p < 0.05). When cells were co-transfected with all three expression vectors (Sp1 + Smad3 + Smad4), Control and TGF-β-stimulated β5 promoter activities were increased to 1.9- and 3.3-fold of their respective basal activity. These data confirmed that all three transcription factors (Sp1, Smad3, and Smad4) were involved in the up-regulation of β5 by TGF-β.

To verify further the roles played by Smad3 and Smad4 in TGF-β up-regulation of β5, MC3T3-E1 cell lines stably expressing dominant negative FLAG-tagged Smad3 (Smad3 m) or Smad4 (Smad4 m) were generated. The inset in Fig. 12 demonstrated expression of the dominant negative forms of Smad3 and Smad4, as evidenced by the positive Western signals using an antibody against the FLAG epitope. Whereas cells carrying...
control vector (pcDNA3) maintained the up-regulation of $\beta_5$ gene expression by TGF-$\beta$, those expressing mutant Smad3 and Smad4 exhibited reduced and abolished stimulation of promoter activity, respectively (Fig. 12). Surface labeling showed that the induction of $\beta_5$ by TGF-$\beta$ was greatly diminished in the Smad4 cells, whereas the up-regulation of surface $\beta_5$ level by TGF-$\beta$ was maintained in MC3T3 cells carrying pcDNA3 empty vector (Fig. 13). Thus, Smad proteins play an essential role in TGF-$\beta$ up-regulation of the $\beta_5$ gene.

**DISCUSSION**

We have demonstrated that TGF-$\beta$ up-regulates adhesion of murine osteoblasts to vitronectin and enhances surface expression of the integrin $\alpha_\beta_{53}$ by a transcriptional mechanism targeting the rate-limiting $\beta_5$ integrin subunit. We identify a TGF-$\beta$-responsive element to a specific Sp1/Sp3 site between nucleotides $-63$ to $+110$ and $\beta$-galactosidase. After overnight recovery, cells were treated with either vehicle (Control) or TGF-$\beta$ for 24 h, and luciferase activities were measured and normalized with $\beta$-galactosidase activities. The inset demonstrates the expression of Smad3 and Smad4 as measured by the anti-FLAG antibody in Western blot analysis. *, $p < 0.01$ as compared with the corresponding control value.
stimulation by TGF-β. The αvβ5-responsive element at −24/−19 is dispensable for basal expression, since mutation at this site results in a very low (5% of wild type) promoter activity (Fig. 6). In contrast, the Sp1/Sp3 site at +28/+31 is not involved in regulating β5 expression. The differential responsiveness of the three Sp1/Sp3 sites in the β5 promoter toward TGF-β stimulation may derive from the fact that variations in sequence of the regions flanking the three Sp1/Sp3 binding domains may lead to altered DNA conformation and hence function. Similar findings have been reported for several other promoters, including those of p21, p15INK4B, and α1(I) procollagen (58–60).

Although we have reported previously that αvβ5 mediates attachment of osteoblasts to vitronectin (34), whether the integrin plays other roles in osteoblast function is unknown. αvβ5 may promote osteoblast migration, since interaction between αvβ5 and vitronectin induces locomotion in several cell systems (48, 62). In bone formation, osteoprogenitor cells in the bone marrow must migrate to remodeling sites on the bone surface. The stimulation of αvβ5 by TGF-β is consistent with the chemotactic and osteogenic activities of TGF-β (3, 14–17, 19). αvβ5 can also function as an endocytic receptor for vitronectin and is involved in phagocytosis of apoptotic cells and rod outer segment (63–66). Thus, αvβ5 may also mediate the reported ability of osteoblasts to clear the frayed organic matrix via endocytosis and/or phagocytosis in the resorption lacuna left behind by osteoclasts during the remodeling cycle (67–69). Recently, the TGF-β1, -2, and -3 gene knockout mice have been generated (70–72). Of note is that the TGF-β2 knockout mice show significant defects in both endochondral and membranous ossification in vivo (71). Since the formation of these tissues requires migration, proliferation, and differentiation of osteoblast precursor cells and integrins-mediated cell-matrix interactions govern these processes, there exists a possibility that the expression of β5 and/or other integrins are compromised in TGF-β2 null osteoblast precursor cells. Future experiments analyzing the expression patterns of integrins using TGF-β2-deficient osteoblasts may shed some light on this matter.

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