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Inhibition of TGF-β receptor signaling in osteoblasts leads to decreased bone remodeling and increased trabecular bone mass

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SUMMARY

Transforming growth factor-β (TGF-β) is abundant in bone matrix and has been shown to regulate the activity of osteoblasts and osteoclasts in vitro. To explore the role of endogenous TGF-β in osteoblast function in vivo, we have inhibited osteoblastic responsiveness to TGF-β in transgenic mice by expressing a cytoplasmically truncated type II TGF-β receptor from the osteocalcin promoter. These transgenic mice develop an age-dependent increase in trabecular bone mass, which progresses up to the age of 6 months, due to an imbalance between bone formation and resorption during bone remodeling. Since the rate of osteoblastic bone formation was not altered, their increased trabecular bone mass is likely due to decreased bone resorption by osteoclasts. Accordingly, direct evidence of reduced osteoclast activity was found in transgenic mouse skulls, which had less cavitation and fewer mature osteoclasts relative to skulls of wild-type mice. These bone remodeling defects resulted in altered biomechanical properties. The femurs of transgenic mice were tougher, and their vertebral bodies were stiffer and stronger than those of wild-type mice. Lastly, osteocyte density was decreased in transgenic mice, suggesting that TGF-β signaling in osteoblasts is required for normal osteoblast differentiation in vivo. Our results demonstrate that endogenous TGF-β acts directly on osteoblasts to regulate bone remodeling, structure and biomechanical properties.

Key words: TGF-β, Osteoblast, Bone, Transgenic mouse, Biochemical property

INTRODUCTION

Bone consists of a highly structured extracellular matrix containing osteoblasts, osteocytes and osteoclasts. Osteoblasts, which are of mesenchymal origin, synthesize and secrete bone matrix, which subsequently mineralizes. Once embedded in bone matrix, osteoblasts differentiate into osteocytes. In contrast, osteoclasts, which are of hematopoietic lineage, resorb mineralized bone. Bone structure and integrity are maintained through bone remodeling, a continuous process of bone resorption and deposition, which is coordinated through the relative activities of osteoblasts and osteoclasts (Parfitt, 1994, for review, see Erlebacher et al., 1995). How these activities are coupled is not fully understood, but defects in this process, due to ageing or metabolic bone disease, lead to progressive changes in bone mass and quality and are the basis for the increased fracture risk of patients with osteoporosis (for review, see Manolagas and Jilka, 1995).

Transforming growth factor-β (TGF-β) is the prototype for a family of proteins, several of which regulate bone and cartilage differentiation (for reviews, see Bonewald and Dallas, 1994; Centrella et al., 1994). Several lines of evidence have implicated TGF-β, which is deposited in bone matrix by osteoblasts and is released during osteoclastic resorption (Oreffo et al., 1989; Pfeilschifter and Mundy, 1987), as an important regulator of osteoblast and osteoclast activity during bone growth and remodeling (for reviews see Bonewald and Dallas, 1994; Centrella et al., 1994). Both osteoblasts and osteoclasts synthesize and respond to TGF-β (Seyedin et al., 1985, 1986; Robey et al., 1987; Pelton et al., 1991), yet the exact nature of the response appears to depend on experimental and physiological conditions. Thus, TGF-β inhibits or stimulates osteoblast differentiation and function, depending on the cell system and culture conditions (reviewed by Centrella et al., 1994; Iba et al., 1996). The dramatic changes in levels and relative ratios of type I and type II and TGF-β receptors, which occur as cells within the osteoblast lineage differentiate, may explain the correlation between the differentiation state and their response to TGF-β (Gazit et al., 1993; Centrella et al., 1995). The osteoclastic response to TGF-β is also complex and may similarly depend on the differentiation stage of the cells. Thus, TGF-β inhibits

early osteoclastic differentiation from bone marrow monocytes, yet stimulates bone resorption by differentiated osteoclasts (Tashjian et al., 1985; Chénu et al., 1988; Pfeilschifter et al., 1988; Dieudonné et al., 1991; Hattersley and Chambers, 1991). In vivo, TGF-β has been implicated as a regulator of endochondral ossification, not only during formation of the skeleton (Carrington et al., 1988), but also during fracture healing. Accordingly, TGF-β is expressed at all stages during fracture repair, and exogenous TGF-β has dramatic effects on gene expression and differentiation of cartilage and bone cells during bone repair (reviewed in Bostrom and Asnis, 1998 and Rosier et al., 1998). Furthermore, in vivo administration of TGF-β induces rapid closure of skull defects (Beck et al., 1991), callus formation in normal bone, and increased bone formation and strength during rat tibial fracture repair (Mackie and Trechsel, 1990; Marcelli et al., 1990; reviewed in Bostrom and Asnis, 1998 and Rosier et al., 1998). Taken together, these findings illustrate that TGF-β can regulate osteoblast and osteoclast function in vitro and in vivo, but the roles of endogenous TGF-β in the activities of these two cell types in vivo and in bone remodeling remain unclear.

TGF-β exerts its many activities by interacting with a heterotetrameric complex of two types of cell surface receptors, the type I and type II TGF-β receptors, which are both transmembrane serine/threonine kinases (for review, see Derynck and Feng, 1997). Ligand binding induces phosphorylation of type I cytoplasmic domains by type II receptors, a critical event that results in activation of the receptor complex and subsequent downstream signaling (Wrana et al., 1994). Since both receptor types are required for TGF-β signaling, overexpression of cytoplasmically truncated versions of type I or type II receptors results in dominant negative inhibition of TGF-β responsiveness (Brand et al., 1993; Brand and Schneider, 1995; Chen et al., 1993; Miettinen et al., 1994).

To better characterize the role of TGF-β in skeletal development and remodeling in vivo, transgenic mouse models with either altered TGF-β expression or TGF-β responsiveness have been generated. In one model, expression of a truncated type II TGF-β receptor and consequent inhibition of TGF-β responsiveness in cartilage and the synovium altered chondrocyte differentiation and caused a pattern of joint degeneration that resembled human osteoarthritis (Serra et al., 1997). In another mouse model generated in our laboratory, overexpression of TGF-β2 in osteoblasts caused increased osteoblast and osteoclast activity with an age-dependent, progressive loss of bone mass and spontaneous fractures, in a manner resembling high turnover osteoporosis (Erlebacher and Derynck, 1996). Thus, high levels of active TGF-β can stimulate bone remodeling in vivo. However, the role of endogenous TGF-β in bone formation, growth and remodeling is still not known. To address this question, we generated and characterized transgenic mice with osteoblast-specific expression of a cytoplasmically truncated version of the type II TGF-β receptor, which has been shown to act as a dominant negative inhibitor of TGF-β signaling (Brand et al., 1993; Chen et al., 1993; Filvaroff et al., 1994). The resultant transgenic mice, RIIDN mice, had an age-dependent and dramatic increase in trabecular bone mass. Interestingly, transgenic mice showed normal rates of osteoblastic bone deposition, suggesting that TGF-β signaling in osteoblasts indirectly regulates osteoclastic bone resorption. Thus, osteoblastic responsiveness to TGF-β is required for normal coupling of bone formation and resorption during bone remodeling. Finally, cortical bone of RIIDN mice showed increased toughness, and their trabecular bone was stiffer and stronger, indicating that endogenous TGF-β regulates the biomechanical properties of bone. Taken together, these results establish endogenous TGF-β as a key regulator of bone remodeling, trabecular architecture and skeletal biomechanics.

MATERIALS AND METHODS

Expression plasmid

The expression plasmid pOc-RIIDN, derived from pcDNAII (Invitrogen), contains a 1.8 kbp rat osteocalcin promoter fragment (Baker et al., 1992), a 0.65 kbp fragment containing the second intron of the rabbit β-globin gene (O'Hare et al., 1981), the cDNA sequence for the extracellular and transmembrane region of the human type II TGF-β receptor (RIIDN) (Lin et al., 1992; Chen et al., 1993), followed by a Myc epitope tag, and a 0.63 kbp 3′ fragment of the human growth hormone gene that contains the polyadenylation signal. This plasmid, when transfected into ROS 17/2.8 osteosarcoma cells, expressed mRNA for the cytoplasmically truncated type II receptor mRNA, as assessed by reverse transcriptase and polymerase chain reactions (RT-PCR) (data not shown).

Transgenic mice

Transgenic RIIDN mice were generated following standard techniques (Hogan et al., 1986) using (C57BL/6J ×DBA/2) F_1 (B6D2F1) mice (Jackson Laboratories). The 3.6 kbp DNA fragment containing the osteocalcin promoter and RIIDN cDNA sequence was excised from pOc-RIIDN with *Hin*dIII and purified using Geneclean. Southern blot analysis of tail DNA (Laird et al., 1991) with the β-globin intron fragment as hybridization probe was used to identify transgenic founder mice. Nine lines of transgenic mice were established, and mice were tested for genomic insertion of the transgene using non-radioactive dot-blot analyses (Boehringer Mannheim; Engler et al., 1993).

Mice with targeted inactivation of TGF-β1 have been described previously (Shull et al., 1992). A male TGF-$\beta1^{+/-}$ founder on a CF1 × 129/SvJ background was purchased from Jackson Laboratories (Bar Harbor, ME) and crossed to (C56BL6/J × 129/SvJ) F_1 females. Offspring males were aged to 6 months and genotyped as described (Diebold et al, 1995). Quantitation of bone matrix associated TGF-β1 quantities was performed using a TGF-β1-specific ELISA as previously described (Erlebacher and Derynck, 1996).

Analysis of transgene expression

RNA was extracted from femurs and humeri after removal of soft tissue, epiphyses and bone marrow. Frozen bones were crushed with mortar and pestle in liquid nitrogen, homogenized on ice in an Omni 200 tissue homogenizer (Omni International) for 30 seconds in guanidine hydrochloride and RNA was prepared by standard methods (Chomczynski, 1987).

To measure expression of the transgene mRNA, total RNA from individual mice from each line was analyzed by RT-PCR. 0.5-1 μg of total RNA were incubated with (or without as a control) MMLV reverse transcriptase (Gibco-BRL) in 1× PCR buffer, 2.5 mM $MgCl_2$, 1 mM dNTPs, 100 pmol random hexanucleotides (Pharmacia), 1 mM DTT, 0.5 μl RNasin (Promega) for 10 minutes at room temperature,

30 minutes at 37°C and 30 minutes at 42°C. To detect expression of the transgene by PCR, the 5′ primer (5′-CATCGTCCTGTGGA-CGCGTATC-3′) and 3′ primer (5′-CGGATATCAGCTTCTGCT-CACC-3′) were used to generate a product of approximately 550 bp. 1 μl of RT reaction was used for amplification by PCR cycling in a 50 μl reaction at a final concentration of 1× PCR buffer (Gibco-BRL), 2 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol of each primer and 0.25 μl *Taq*I polymerase (Gibco-BRL). Half of this reaction was analyzed in a 2% TBE agarose gel containing ethidium bromide.

For analysis of expression of RIIDN protein, freshly dissected calvariae of transgenic mice were minced and incubated in RIPA (0.15 M NaCl, 10 mM phosphate buffer, pH 7, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with protease inhibitors (20 μg/ml aprotinin, 20 μg/ml leupeptin, 1 mM PMSF, 20 μg/ml pepstatin) at 4°C for 1 hour. After addition of sample buffer (50 mM TrisCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10 % glycerol), samples were analyzed in 15% polyacrylamide gels, transferred to nitrocellulose and blots were probed with the 9E10 antibody against the Myc epitope tag (Evan et al., 1985) using chemiluminescence.

X-ray analysis

X-ray radiographs of animals were taken using a Hewlett-Packard faxitron model 43805N and Kodak X-OMAT TL film. Exposure was for 1 minute at 35 kVp.

Histology

Bones were fixed in 2% paraformaldehyde/PBS at 4°C for 24-48 hours, decalcified for 4 hours in 22.5% formic acid, 10% sodium citrate at room temperature and then embedded in paraffin. 5 μm sections were stained with hematoxylin and eosin using standard procedures (Sheehan and Hrapchak, 1980).

Analysis of calvariae

Histochemical staining for tartrate-resistant acid phosphatase (TRAP) activity was used to detect the presence of osteoclasts on calvarial surfaces using the leukocyte acid phosphatase kit (Sigma 387-A).

Histomorphometry

Male RIIDN and control B6D2 F2 mice were injected intraperitoneally with 10 mg/kg calcein (Sigma) 12 days prior to killing and with 25 mg/kg tetracycline 1 day prior to killing. Longitudinal, undecalcified 4.5 μm sections were prepared from femurs, which were fixed in 70% ethanol, stained en bloc in the Villanueva bone stain (osteochrome stain, Polysciences Inc., Niles, IL) and embedded in methylmethacrylate. Some sections were stained for mineralized bone by the Von Kossa method and counterstained with nuclear fast red (Sheehan and Hrapchak, 1980). Histomorphometric analyses were performed on unstained sections viewed under UV light at a 200× magnification. The trabecular bone volume/total marrow space volume, mineralizing surface/total trabecular bone surface (MS/BS) and eroded surface/total trabecular bone surface (ES/BS) were measured as described (Weibel, 1969; Parfitt et al., 1987), using a 200×200 μm grid reticule with 20 μm subdivisions to systematically scan over the entire bone marrow cavity. Thus, about 10 mm^2 total area was scanned per section covering the distal 7 mm of the femoral metaphysis. Average trabecular bone density values were calculated for each consecutive 200 μm-thick cross-sectional slice of the femur.

The mineral apposition rate was measured from photomicrographs of all visible double-labeled surfaces as described previously (Erlebacher and Derynck, 1996). A total of 5 RIIDN mice and 5 control B6D2 F2 mice were analyzed. Measurements are shown as means ±s.d., except for trabecular bone densities, which are shown as mean ±s.e.m.

Osteocyte density measurements

Osteocyte densities were determined as previously described (Erlebacher et al., 1998) from hematoxylin- and eosin-stained sections of decalcified bones. Osteocyte densities in epiphyseal trabecular bone were measured in two longitudinal sections spaced 200 μm apart, and osteocyte densities in cortical bone were determined in the femoral diaphysis at the level of the third trochanter using two cross-sections spaced 200 μm apart.

Biomechanical studies

Two types of experiments measured the biomechanical properties of bones. Three-point bending of the femoral diaphysis evaluated the properties of cortical bone, whereas axial compression of tail vertebrae allowed an assessment of the properties of trabecular bone. The data were used to calculate strength, stiffness and energy to failure for each specimen.

Specimen preparation

The femurs and tail vertebrae were harvested from freshly killed mice and surrounding soft tissues were removed. The samples were X-ray imaged, wrapped in saline-soaked gauze, sealed in plastic bags and stored at –20° C until time of testing.

Three-point bending

The femurs were thawed to room temperature and placed in a custom-made three-point bending fixture (lower support separation is 8.5 mm). Specimens were aligned onto the lower support such that the bending axis corresponded with the sagittal plane. The upper support was brought in contact with the femur, which was then loaded to failure at a displacement rate of 1 mm/minute using a servohydraulic materials testing system (MTS Bionix Model 858). The applied force was measured with a calibrated load cell (Sensotec Model 31), while the support travel was measured using the LVDT system. After testing, the anterior-posterior and medial-lateral dimensions of the femurs were measured at the periosteal and endosteal surfaces of the fracture site using digital callipers. These dimensions were used to calculate the area moment of inertia.

Compression

Before testing, the height and transverse dimensions of the vertebra were measured using digital callipers. Each tail vertebra was then placed in a cylindrical compression chamber, which maintained alignment of the long axis of the vertebra with the axis of compressive loading. As with the femurs, the vertebrae were loaded to failure with a load platten displacement rate of 1 mm/minute.

Mineral density

Tissue and ash densities were measured for unfractured diaphyseal cortical bone. 5 mm long sections of diaphyseal cortical bone were removed using a precision diamond saw (Exakt) and ultrasonically cleaned for 40 minutes using a detergent solution (Alkinox) to remove traces of bone debris and marrow. The specimens were then weighed in air using a precision balance. They were then submerged in water and vacuum degassed for 1 hour to remove traces of air. The specimens were weighed again while still submerged. The tissue volume of the specimen was then calculated using Archimedes' principle, $V= (W_a-W_s)/\rho$, in which V is the specimen tissue volume, W_a and W_s are the specimen weights in air and in water, respectively, and ρ is the density of water. After weighing, the specimens were heated at 600°C for 18 hours and the ash was weighed. The tissue density was defined as the in-air weight divided by the tissue volume. Using this method, we assessed ash densities of tail vertebrae immediately adjacent and distal to the ones chosen for mechanical testing.

Data analysis

For the specimens tested in three-point bending, the bending strength

was determined from maximum bending stress supported by the specimen. The energy dissipated prior to failure was calculated as the area under the bending moment versus displacement curve up to the point of failure. All statistical analyses were done using SYSTAT (version 5.0). Standard analysis of variance was used to compare specimen group means and to estimate the effect of the specimen variables (transgenic or wild-type) on the parameters of interest (strength, stiffness, energy to failure and ash density).

Ovariectomy

2-month-old females were anesthesized with ketamine hydrochloride and xylazine (by intraperitoneal injection) at 25 and 5 mg/kg, respectively. Bilateral removal of ovaries was by incision into the abdominal flank and cutting at the uterine horn (Bain et al., 1993). Control mice underwent the same procedure except for excision of the ovaries. Immediately after killing, the animals were weighed, bones were fixed, and uteri were weighed. As expected (Bain et al., 1993), uterine weights decreased significantly following ovariectomy, confirming complete absence of ovarian tissue.

RESULTS

Expression of a cytoplasmically truncated type II TGF-β receptor from the osteocalcin promoter in transgenic mice

The functional cell surface complex of TGF-β receptors consists of two type II and two type I receptors. Following ligand binding, the cytoplasmic domains of the type II receptors phosphorylate and thereby activate the type I receptors to initiate downstream signaling (for review, see Derynck and Feng, 1997). To inhibit TGF-β receptor signaling, we constructed an expression vector for a cytoplasmically truncated type II receptor with a C-terminal Myc epitope tag to allow antibody-based detection (Chen et al., 1993). Expression of this truncated receptor, RIIDN, is known to interfere with TGF-β-induced signaling in a dominant negative manner (Fig. 1A, Chen et al., 1993). To direct expression of RIIDN in transgenic mice specifically in osteoblasts, we inserted the transgene under the control of a 1.8 kbp rat osteocalcin promoter segment, thus creating plasmid pOc-RIIDN (Fig. 1B). This promoter segment is known to direct high level expression of a transgene specifically in osteoblasts and not in other cell types (Baker et al., 1992). In addition, we have previously used the identical expression plasmid (driving expression of TGF-β2 instead of RIIDN) to generate transgenic mice that overexpress active TGF-β2 in osteoblasts and verified the osteoblast-specific expression of the transgene in these mice (Erlebacher and Derynck, 1996). Expression of the truncated type II receptor from pOc-RIIDN was verified in transfected ROS17/2.8 osteosarcoma cells (data not shown).

We established nine independent lines of transgenic mice that carry the pOc-RIIDN transgene stably integrated into their germline genome. We used RT-PCR to assess expression of RIIDN mRNA (Fig. 1C) and found that eight of the nine lines expressed the transgene in bone and not in a variety of other tissues (data not shown). The expression level of the truncated receptor protein was assessed by western blotting of total protein extracts of calvariae using an antibody for the C-terminal myc epitope tag (Fig. 1D). Persistent high-background problems prevented unambiguous immunohistochemical analyses of RIIDN transgene expression using the myc tag antibody (data not shown). While all eight lines expressed the truncated receptor, the four

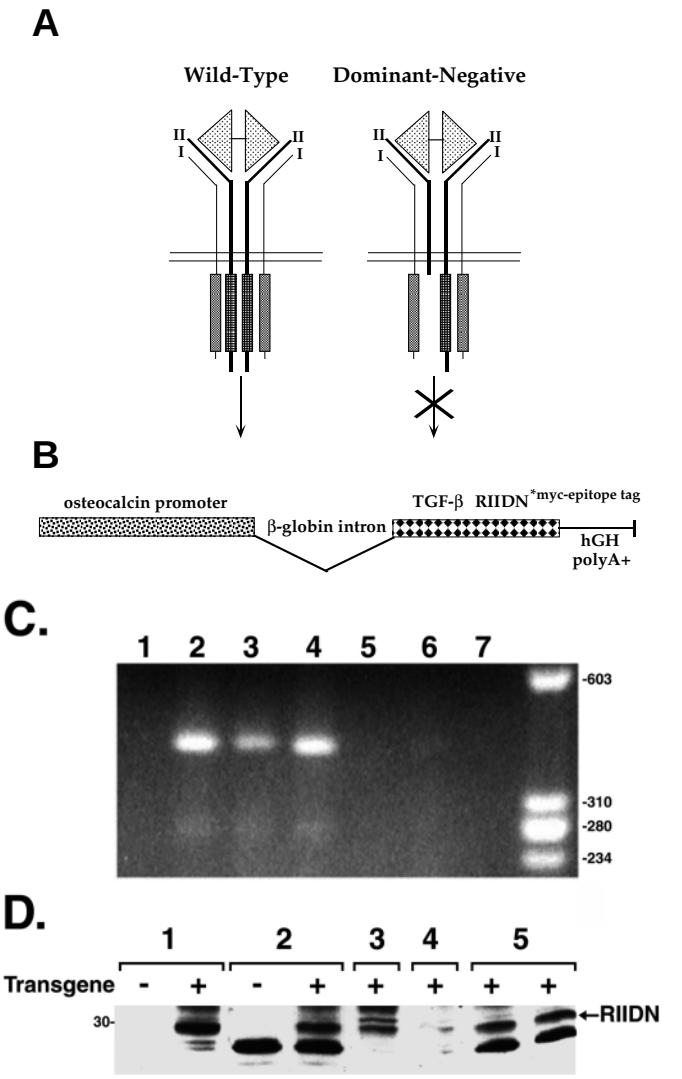


Fig. 1. (A) Diagram of wild-type and dominant-negative (RIIDN) forms of the type II TGF-β receptors. The cytoplasmically truncated type II receptor acts as a dominant negative inhibitor of signaling by the TGF-β receptor complex. (B) Diagram of expression plasmid pOc-RIIDN used to create RIIDN transgenic mice. Note the inclusion of a myc-epitope tag for detection of expression of the transgene. (C) RT-PCR analysis of expression of the RIIDN transgene mRNA in bone RNA. Lanes 2-4 show the PCR product from different mouse lines that are positive for the transgene, whereas lane 1 used the same RNA as in lane 4 in a reaction without reverse transcriptase. In lane 5, the RT-PCR reaction was performed on RNA from a mouse that was negative for the transgene. In lanes 6 and 7, the reaction was done using RNA from mice that were positive for the transgene, but apparently did not express the transgene mRNA. (D) Western blot analysis of protein extracts from the skulls of transgene-positive (+) and transgene-negative littermates (–) from five lines (1-5) using anti-myc tag antibody to recognize the RIIDN protein (arrow). The large number of non-specific bands may be related to the crude nature of the bone extract.

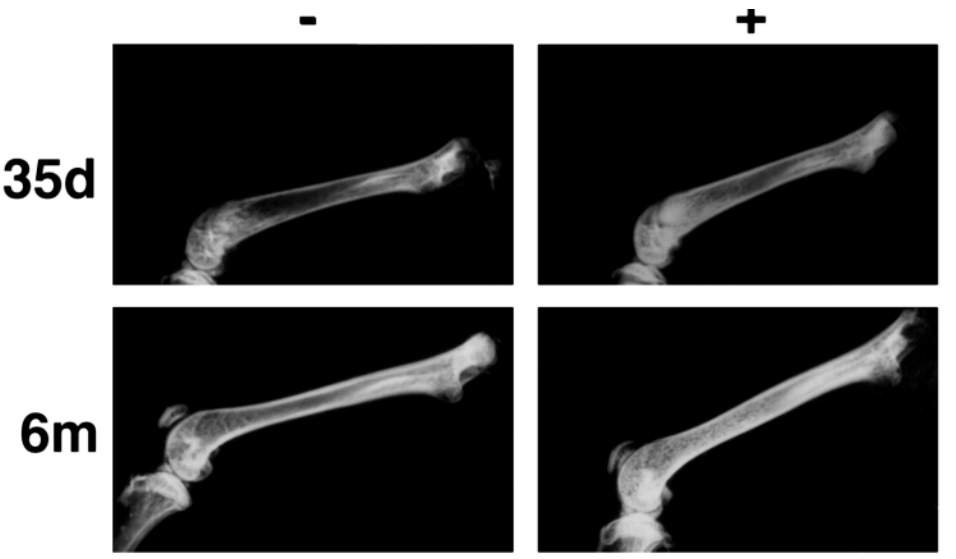


Fig. 2. X-ray analysis of femurs of 35-day-old (top) and 6-month-old (bottom) transgenic mice (+) and nontransgenic (–) littermates. Note the widening in the metaphyses in transgenic bone and the extension of trabecular bone further into the bone marrow cavity in the transgenic mice. This progressive phenotype is more apparent at 6 months than at 35 days.

mouse lines with the highest expression level of the truncated receptor were selected for further characterization of the skeletal phenotype. The functionality of the truncated receptor was apparent from its ability in genetic crosses to inhibit the increase in osteocyte density and correct the cortical bone defect and spontaneous fractures caused by TGF-β2 overexpression in D4 transgenic mice, without affecting the level of expression of the TGF-β2 transgene (Erlebacher et al., 1998; unpublished data). Thus, the truncated receptor (RIIDN) acts as a dominant negative inhibitor of TGF-β-induced signalling in vivo, even in the presence of 16-fold higher than normal levels of TGF-β2.

RIIDN transgenic mice show an increase in trabecular bone mass

X-ray analyses of long bones showed that transgenic mice had a progressive increase in trabecular bone mass as they aged, relative to wild-type mice. This effect was apparent in the metaphyses of femurs of 35-day-old mice, became more pronounced in mice up to 6 months of age (Fig. 2) and decreased (relative to 6-month-old transgenic mice) by 9 months of age (data not shown). Among the different transgenic mouse lines, the mice with the highest level of transgene expression had generally the highest increase in trabecular bone (data not shown). Histological analyses of femurs showed that RIIDN mice had more trabecular bone, which extended further into the bone marrow cavity (Fig. 3), consistent with their radiographic appearance. Quantitative histomorphometry of longitudinal sections of distal femoral metaphyses showed that 6-month-old RIIDN mice had a higher than normal percentage of trabecular bone volume

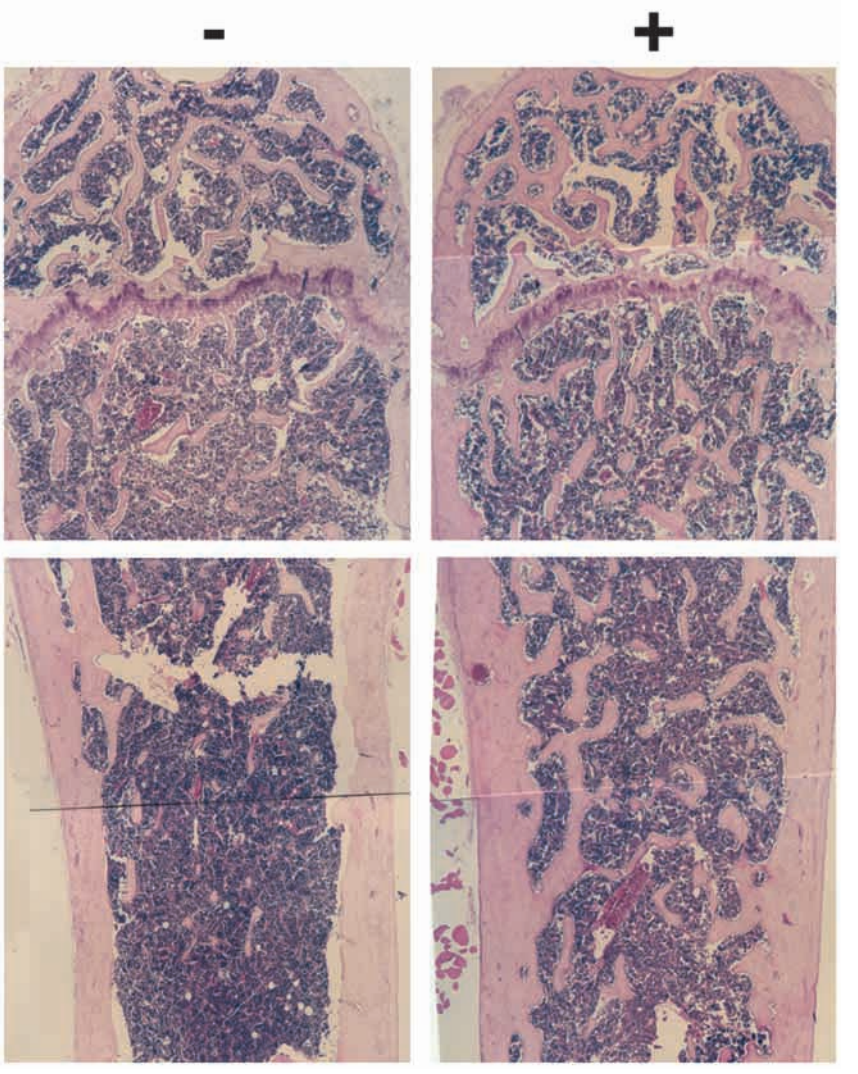


Fig. 3. Histological appearance of transgenic bone. Longitudinal sections through the femurs of 6-month-old transgenic (+) and wild-type (–) mice. RIIDN bone shows increased trabecular bone that extends further down the bone marrow cavity towards the diaphysis. The epiphyseal growth plate appears normal.

per total marrow volume at a given distance from the growth plate (Fig. 4). Furthermore, trabecular bone extended 2 mm further down the length of the distal femur in these mice than in wild-type femurs. The increases in these two parameters resulted in a 2.8-fold increase in total trabecular bone volume. Interestingly, the density of trabecular bone closest to the growth plate was similar to that of wild type, suggesting that the initial generation of trabecular bone was not affected in RIIDN mice, but rather that its subsequent turnover was altered.

We previously observed that increased TGF-β2 expression in osteoblasts results in increased osteocyte number (Erlebacher and Derynck, 1996) due to a direct stimulatory effect of osteoblastic TGF-β2 on the rate of osteoblast differentiation (Erlebacher et al., 1998). To determine the effect of endogenous TGF-β on osteoblast differentiation, we measured the osteocyte density in RIIDN mice and found that the osteocyte density in the femoral epiphyses of RIIDN mice was lower than in wild-type mice. Thus, 35-day-old RIIDN mice (n=5) had an osteocyte density of 60 000±10 600 cells/mm^3, which is significantly lower than the osteocyte density of 80 000±6800 cells/mm^3 in wild-type mice (n=6, P<0.005) (Erlebacher et al., 1998). This observation therefore suggests that endogenous TGF-β regulates osteocyte density. In contrast to the femoral epiphysis, the osteocyte density in transgenic cortical bone was not significantly different from that of wild type (data not shown), and cortical diaphyseal bone in RIIDN mice was histologically indistinguishable from wild type (Fig. 3). Nevertheless, the truncated receptor is clearly expressed and functional in cortical bone of RIIDN mice, since crossing RIIDN mice with mice overexpressing TGF-β2 rescues the increase in osteocyte density in cortical bone relative to that of mice overexpressing TGF-β2 alone (Erlebacher et al., 1998). We therefore conclude that osteoblastic responsiveness to endogenous TGF-β is a determinant of osteocyte density in epiphyses but not in diaphyses. Since overexpression of TGF-β2 in osteoblasts results in a higher than normal osteocyte density due to an increased rate of osteoblast differentiation (Erlebacher et al., 1998), the finding of decreased osteocyte density in RIIN mice suggests that endogenous TGF-β determines the rate of osteoblast differentiation in vivo.

Overexpression of TGF-β2 in osteoblasts in transgenic mice also leads to irregular bone mineralization and areas of unmineralized cortical bone (Erlebacher and Derynck, 1996). However, von Kossa staining of undecalcified sections of RIIDN bone did not reveal differences in mineralization (data not shown). In addition, the mineral densities of transgenic trabecular bone and cortical bone were very similar to wild type, i.e. 1.53 mg/mm^3 versus 1.50 mg/mm^3 for trabecular bone and 1.86 mg/mm^3 versus 1.90 mg/mm^3 for cortical bone. Interestingly, the mineral density of trabecular bone was always lower than that of cortical bone in both types of mice.

RIIDN mice show changes in skull bone phenotype

Besides the long bones, which develop through endochondral bone formation, the parietal bones in the skull, which form by intramembranous ossification, were also altered in RIIDN mice. Since osteoclasts resorb bone during calavariae growth and development, the pattern and extent of cavitation is indicative of osteoclastic activity. As shown in Fig. 5A, X-ray analyses of calvarial bones of transgenic mice indicated decreased cavitation relative to wild-type mice. A similar decrease in cavitation was evident in male mice heterozygous for an inactivated TGF-β1 gene (TGF-β1$^{+/-}$ mice) (Fig. 5B), which have a 50% decrease in the amount of bone-matrix associated TGF-β1 (A. E. et al., unpublished results). The decreased cavitation in RIIDN transgenic mice strongly suggests a decrease in calvarial bone resorption. Whole-mount histochemical staining of skull bones for tartrate-resistant acid phosphatase (TRAP), a marker of mature osteoclasts, indicated that calvariae of transgenic mice had fewer osteoclasts at their surface than those of wild-type mice (Fig. 6). Thus, osteoclast number and activity in the skull depend on osteoblastic responsiveness to TGF-β.

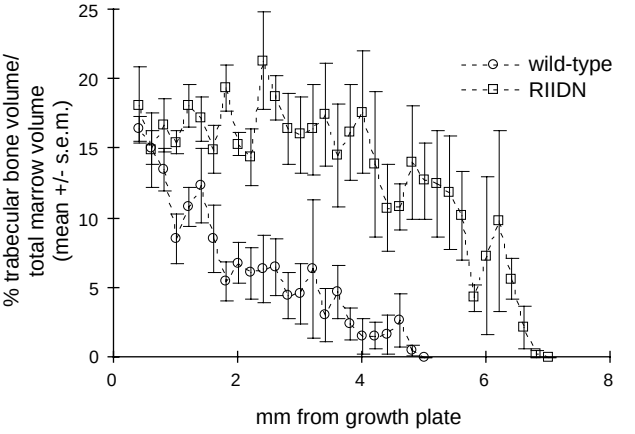


Fig. 4. Trabecular bone volume as a fraction of total bone volume. The volume is measured at increasing distance from the growth plate in the metaphysis of the femur of 6-month-old mice. Values represent the mean ± s.e.m. of n=5 wild-type mice (circles) and n=5 RIIDN mice (squares).

Finally, we evaluated the histology of the skulls at the posterior frontal suture, since TGF-β is known to be expressed by differentiating osteoblasts at the site of suture closure and appears to play a role in promoting suture closure (reviewed in Cohen 1997; Most et al., 1998). Mice expressing the truncated type II TGF-β receptor (RIIDN) showed a pattern of suture closure that was similar to that of their wild-type littermates (data not shown), suggesting that full responsiveness of mature osteoblasts to TGF-β is not required for suture closure.

Besides the changes in skeletal phenotype, transgenic mice appeared healthy and had normal size, body weight, fertility and life span. All organs were macroscopically normal at autopsy. These findings are consistent with the osteoblast-specific expression of the truncated receptor from the osteocalcin promoter (Baker et al., 1992; Erlebacher and Derynck, 1996).

Kinetic analyses of bone turnover

The phenotype of RIIDN mice suggested an imbalance between the rates of osteoblastic bone formation and

osteoclastic bone resorption. The rate of bone deposition can be measured histomorphometrically from bone sections of mice injected sequentially with two fluorochrome labels that incorporate into bone matrix at sites of mineralization (Parfitt, 1983). Comparison of RIIDN with wild-type bones indicated that the distance between the two fluorochrome labels, and, thus, the mineral apposition rate were similar at day 35 in the femoral epiphysis (1.7±0.1 μm/day for RIIDN [*n*=4] and 1.7±0.2 μm/day for wild type [*n*=5]) and at 6 months in the femoral metaphysis (2.76±1.02 μm/day (*n*=5) for wild-type mice and 2.25±0.95 μm/day for RIIDN mice (*n*=4)) (Erlebacher et al., 1998). In the femoral metaphysis at 6 months, the mineralizing surface per total trabecular bone surface (MS/BS), which reflects the percentage of bone surface undergoing formation, was also similar in RIIDN mice compared to wild type (32.8±7.7% versus 34.9±7.2%), and no variations in this parameter at different distances from the growth plate were found (data not shown). Multiplying these two parameters therefore reveals no significant differences in the rate of bone formation per unit bone surface in RIIDN compared to wild-type mice.

In contrast to the rate of bone formation, the rate of osteoclastic resorption can not be directly measured. However, the density of osteoclasts in rodents parallels closely the fraction of eroded bone surface (Beaudreuil et al., 1995). We therefore determined the percentage of eroded surface per total trabecular bone surface (ES/BS) in long bones by histomorphometry, and found no significant difference between RIIDN and wild-type mice (2.7±1.1% versus 5.6±3.7%, P=0.07). Similarly, no difference in osteoclast numbers between wild-type and RII DN mice were apparent, as assessed by histochemical TRAP staining of long bones (data not shown). This contrasts with the apparent decrease in osteoclast number on calvarial surfaces (Fig. 6). Another measure of bone resorption is the perpendicular rate of bone resorption per resorbing surface, which is the osteoclast analogue of the mineral apposition rate. Unfortunately, this rate cannot be measured by histomorphometry. However, the dramatic increase in trabecular bone volume in RIIDN mice, relative to wild-type mice, without significant changes in the rate of bone formation or fraction of eroded bone surface, suggest that the rate of bone resorption per osteoclast is decreased in RIIDN trabecular bone. Accordingly, we have previously found that transgenic mice that overexpress TGF-β2 have increased resorption in their long bones without a change in osteoclast number, suggesting that TGF-β can increase activity per osteoclast (Erlebacher et al., 1996, 1998).

Altered biomechanical properties of the transgenic bones

We next determined whether the skeletal alterations caused by osteoblastic expression of truncated type II TGF-β receptor were accompanied by changes in biomechanical properties. To test femoral cortical bone, we used a three-point bending test whereby dissected femurs were placed on a platform and deflected by a loaded bar from above (Fig. 7). The mechanical properties were then calculated based on the force required to break the bone. Although RIIDN femurs had normal load, stiffness and strength, they had a significantly higher energy to failure than did wild-type femurs. Thus, long bones of RIIDN mice were tougher, i.e. less brittle, than their wild-type counterparts and could absorb more energy before breaking. This change is most likely due to altered matrix composition or architecture of cortical bone in RIIDN mice, since the mineralization of transgenic bone was the same as wild type and no significant differences in their size were found (data not shown).

To test trabecular bone, isolated vertebral bodies, which serve as a model for trabecular bone mechanics due to their extensive and predominant trabecular bone architecture, were loaded to failure in a cylindrical compression chamber. In contrast to the femurs, the vertebral bodies of transgenic mice had a normal energy to failure, indicating normal toughness. However, vertebral bodies of RIIDN mice were able to bear more load and were significantly stiffer and stronger than those

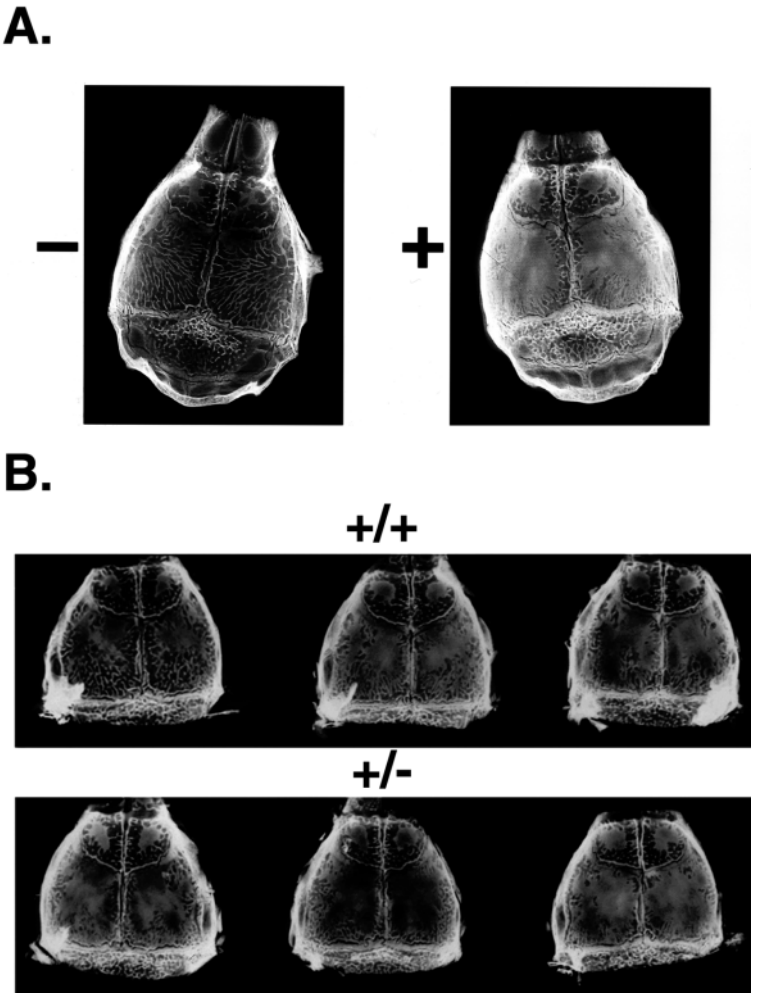


Fig. 5. X-ray analysis of skulls of RIIDN transgenic (A) and TGF-β1$^{+/-}$ (B) mice. (A) Radiographs of calvariae of transgenic (+) and non-transgenic (–) mice. Skulls of transgenic mice contain less cavitation in their parietal bones and are more radio-opaque than their wild-type counterparts.
(B) Radiographs of calvariae of wild-type (+/+) or heterozygous TGF-β1 (+/–) mice. The TGF-β1$^{+/-}$ skulls contain less cavitation in their parietal bones than their wild-type littermates. Some variability was observed.

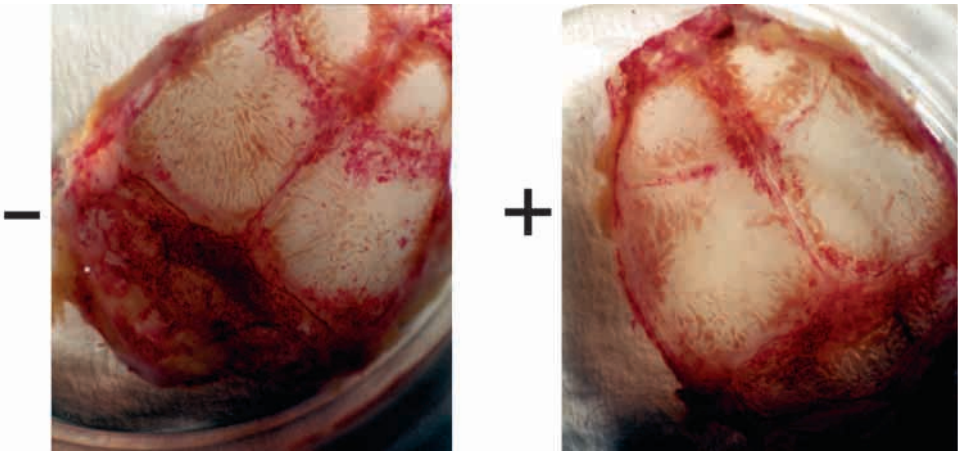
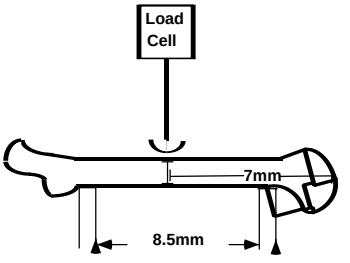


Fig. 6. Histochemical identification of osteoclasts in skulls. Whole-mount preparations of calvariae stained for tartrate-resistant acid phosphatase activity (red), an osteoclast-specific marker. Although the location of osteoclasts is normal in transgenic mice (+), they have more osteoclasts than their wild-type littermates (–).

of wild-type mice (Fig. 8). These changes could not be explained by alterations in size or shape of the vertebral bodies, since RIIDN vertebral bodies had normal length, width and height (data not shown).

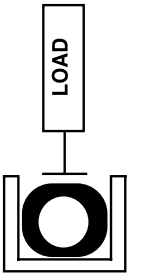
Effect of ovariectomy on bone mass

Estrogen deficiency, which causes a loss of bone mass due to a net increase in bone resorption over bone formation in experimental animals (Wronski et al., 1985, 1986), is thought to be the primary cause of postmenopausal osteoporosis in humans (Cauley et al., 1995; Marcus, 1996). While the exact mechanism underlying the effects of estrogen on bone structure remains unclear, TGF-β has been implicated in this pathway (Finkelman et al., 1992; Ikeda et al., 1993; Hughes et al., 1996). Since the phenotype of RIIDN mice suggested that decreased osteoblastic responsiveness to TGF-β leads to decreased resorption, and because TGF-β2 overexpression increases bone resorption (Erlebacher and Derynck, 1996), we tested whether bone loss caused by estrogen deficiency required normal TGF-β signaling in osteoblasts. We therefore analyzed the skeletal effects of ovariectomy in transgenic RIIDN mice and wild-type mice. All ovariectomized mice showed a decrease of about 80% in uterine weight (0.096 g to 0.018 g) indicating a loss of ovarian function, as previously reported (Bain et al., 1993), and transgenic and wild-type mice had similar weights before (24.45 g versus 24.81 g) and after (30.36 g versus 28.25 g) ovariectomy. X-ray analyses of femurs from ovariectomized wild-type mice showed the expected loss of trabecular bone, particularly in the metaphyses (Fig. 9A). Similarly, transgenic RIIDN mice also had trabecular bone loss after ovariectomy (Fig. 9B); however, even after ovariectomy, the amount of trabecular bone remained higher in RIIDN bones than in wild-type bones (Fig. 9B).



	Wild-Type	RIIDN	
Load (N)	19.21±0.70	20.56±0.55	
Energy (N-mm)	3.32±0.26	4.13±0.21	*P*=0.019
Stiffness (N/mm)	101.42±3.63	102.79±2.85	
Strength (N/mm^2)	174±5	168±4	

Fig. 7. Mechanical properties of cortical bone in the femurs. Diagram of the three-point bending mechanism and values obtained for the different parameters. Numbers are represented as averages ± standard deviation of bones from 24 wild-type and 39 RIIDN mice.



	Wild-Type	RIIDN	
Load (N)	74.05±2.02	83.41±1.74	*P*=0.0007
Energy (N-mm)	15.04±2.18	17.49±1.87	
Stiffness (N/mm)	322.88±11.06	356.27±9.49	*P*=0.024
Strength (N/mm^2)	11.85±0.26	13.25±0.23	*P*=0.00015

Fig. 8. Mechanical properties of trabecular bone in tail vertebrae. Diagram of compression mechanism and values obtained for the different parameters. No differences in height or width of vertebral bodies were found by manual measurement (data not shown). Numbers are represented as averages ± standard deviation of measurements of 45 wild-type and 61 RIIDN bones.

Since the RIIDN transgenic mice had fewer osteoclasts at the surface of their skull bones, we examined osteoclast number in parietal bones of ovariectomized and sham-operated mice. As assessed by whole-mount histochemical staining for osteoclasts, ovariectomy induced an increase in osteoclast number in transgenic RIIDN mice, which was not apparent in wild-type mice (data not shown). Thus, both the number and activity of osteoclasts, although decreased in transgenic RIIDN skulls, can be induced following ovariectomy. These findings provide evidence that TGF-β responsiveness is not required for ovariectomy-induced bone resorption, even though endogenous TGF-β, through its direct effects on osteoblasts, can affect osteoclast number in skulls and osteoclast activity in skulls and long bones.

DISCUSSION

TGF-β is deposited at high levels in skeletal tissue and has significant, yet seemingly contradictory, effects on bone cell function (for review, see Centrella et al., 1994). The complexity of these effects depends on the cell type and differentiation stage, and on the duration and concentration of TGF-β. To better understand the role of TGF-β in bone development and remodelling, we have generated transgenic mice with altered levels of TGF-β ligand or responsiveness. Transgenic mice, which overexpress TGF-β2 specifically in osteoblasts, have an age-dependent loss of bone mass and spontaneous fractures, in a manner similar to high-turnover osteoporosis (Erlebacher and Derynck, 1996). This phenotype demonstrates that continuous, high levels of active TGF-β lead to increased osteoblast and osteoclast activity and to decreased bone integrity, but do not directly address the role of endogenous TGF-β in bone development and turnover. To this end, we generated transgenic mice with impaired osteoblastic responsiveness to TGF-β by overexpressing a dominant-negative form of the type II TGF-β receptor (RIIDN). Coexpression of this dominant negative receptor in mice overexpressing TGF-β2 rescued the increase in osteocyte density, the cortical bone defect and spontaneous fractures caused by overexpression of TGF-β2, without changing the level of TGF-β2 transgene expression (Erlebacher et al., 1998, unpublished data). Thus, the truncated type II TGF-β receptor (RIIDN) functions as a dominant negative inhibitor of TGF-β signaling in vivo, even in mice with 16-fold overexpression of TGF-β2 in their bones. In the current study, we have characterized four independent lines of RIIDN mice by measuring bone cell activity and biomechanical properties of long bones and vertebral bodies.

TGF-β regulates trabecular bone mass and osteoblast differentiation

The primary phenotype of RIIDN mice was an increase in trabecular bone volume that was apparent by postnatal day 35 and became more pronounced in mice up to 6 months of age. The age-dependent nature of this phenotype suggested a change in the balance between bone formation and resorption and thus a remodeling defect. The decrease in trabecular bone at 9 months of age, relative to that at 6 months, may be due to the normal age-related decreases in osteoblast number and remodeling rate, or a decrease in expression of the RIIDN transgene, which would be consistent with the decrease of endogenous osteocalcin expression at later age (Frenkel et al., 1997).

Although TGF-β can act on cells in both cortical and trabecular bone, the trabecular phenotype of RIIDN bones may result from relative differences in osteoblast density and activity and a consequent higher bone remodeling in trabecular bone, when compared to cortical bone. In addition,

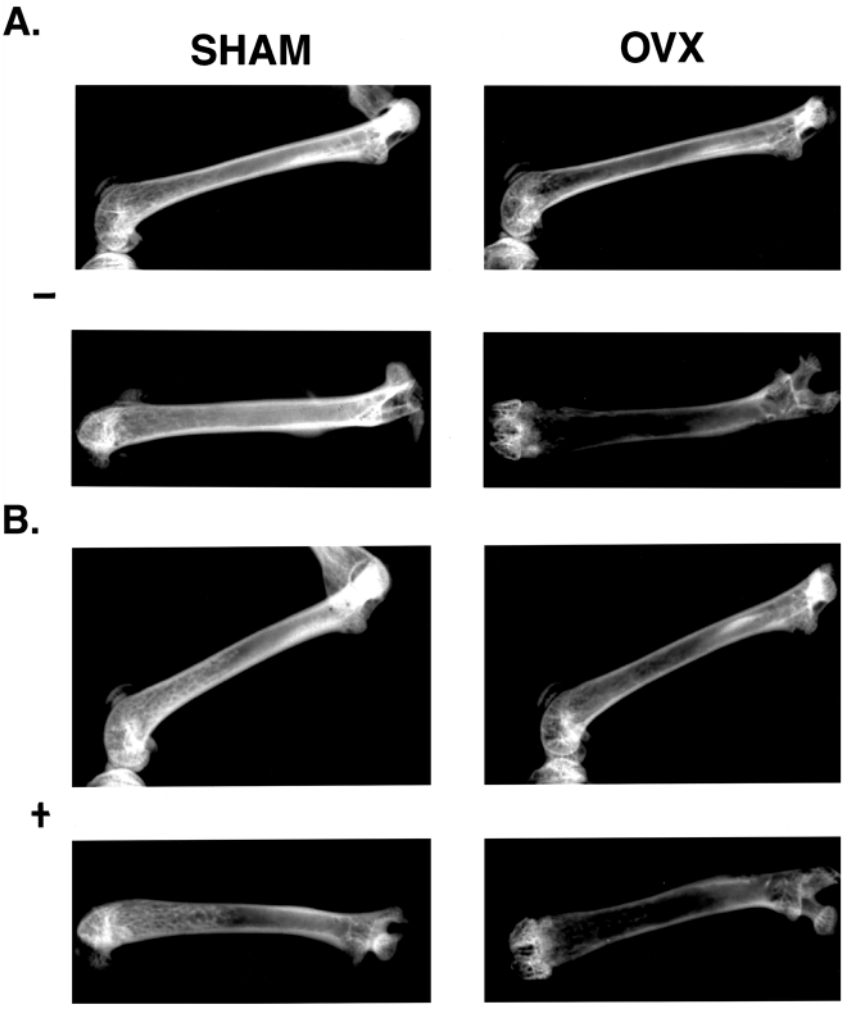


Fig. 9. X-ray analyses of femurs 4 weeks after sham operations (SHAM) or ovariectomies (OVX) of 2-month-old wild-type (–, A) or RIIDN transgenic (+, B) mice. Two views under a different angle are shown per bone. Note the loss of trabeculation in the metaphyses following ovariectomy in both wild-type and RIIDN mice. However, RIIDN mice have higher levels of trabeculation than wild-type controls both before and after ovariectomy.

Table 1. Schematic summary of bone phenotypes of transgenic mice expressing the TGF-β type II dominant negative receptor (RIIDN), or TGF-β2 (TGF-β2), or both (β2 × DN) as compared to wild-type mice (WT)

	Cortex	Trabec.	O.D.	MAR	O'Clast
RIIDN	↑	↑	↓	–	↓
WT	–	–	–	–	–
β2×DN	–	↓	–	↑	
↑TGF-β2	↓	↓	↑	↑	↑

Cortex, integrity of cortical bone; Trabec., amount of trabecular bone; O.D., osteocyte density; MAR, mineral apposition rate. O'Clast #, osteoclast activity on the calvarial surfaces. The increase in MAR in TGF-β2 mice is a homeostatic reaction to increased resorption and is thus not rescued by inhibition of TGF-β-induced signaling in osteoblasts in RIIDN mice (Erlebacher et al., 1998).

osteoclastic resorption is also different in cortical versus trabecular bone. Thus, the periosteum of cortical bone is devoid of osteoclastic activity, whereas all surfaces of trabecular bone undergo continuous bone resorption. Regional differences in endogenous TGF-β expression and/or a stronger expression of the transgene from the osteocalcin promoter in trabecular osteoblasts may also contribute to the predominantly trabecular phenotype in RIIDN mice. Consistent with our findings, altering the expression levels of TGF-β causes changes in trabecular bone volume. Thus, mice with a 50% decrease in TGF-β1 levels due to inactivation of one TGF-β1 allele, show increased trabecular bone volume, (A. E. and R. D., unpublished data), and mice that overexpress TGF-β2 show progressive loss of trabecular bone volume (Erlebacher and Derynck, 1996; Table 1). Similarly to the RIIDN mice, TGF-β1 knockout mice have changes in their proximal tibial metaphyses, but not in their midshaft diaphyses (Geiser et al., 1998). These similar spatial differences in RIIDN mice are therefore unlikely due to use of the osteocalcin promoter. In addition, expression from this promoter is higher in the diaphysis (Baker et al, 1992) or epiphysis (Erlebacher and Derynck 1996) than in the metaphysis, which is similar to the pattern of endogenous osteocalcin expression (Bronckers et al., 1985, Vermeulen et al., 1989). Besides the changes in trabecular bone, homozygous TGF-β1 knockout mice also have a bone modeling defect that results in altered bone shape and size, suggesting that TGF-β1 has additional effects on other cells (i.e. chondrocytes and osteoblastic precursors) involved in bone growth and development (Geiser et al., 1998). Our data indicate that the direct activity of endogenous TGF-β on osteoblasts regulates bone remodeling and trabecular architecture, without affecting bone growth, size or morphogenesis.

Histological and kinetic analyses of the bones of RIIDN mice emphasized the importance of TGF-β receptor signaling in osteoblast function. Osteoblast differentiation, as indicated by osteocyte density in trabecular bone, was decreased in RIIDN mice. Similarly, TGF-β2 overexpression increases the rate of osteoblastic differentiation in trabecular and cortical bone, which can be prevented by coexpression of the dominant negative receptor (RIIDN) (Erlebacher et al., 1998). Although increased TGF-β2 levels increase bone deposition and affect matrix mineralization (Erlebacher and Derynck, 1996, Erlebacher et al., 1998), these processes appeared normal in RIIDN mice and thus may not require osteoblastic responsiveness to endogenous TGF-β. Similarly, bone mineral density in TGF-β1 knockout mice is normal (Geiser et al., 1998). Thus, despite the seemingly contradictory effects of TGF-β on osteoblast differentiation in vitro (for review, see Centrella et al., 1994), our results indicate that endogenous TGF-β is a direct, positive regulator of osteoblast differentiation during bone development and turnover in vivo.

Osteoblastic responsiveness to TGF-β regulates bone resorption

In addition to their osteoblastic phenotype, RIIDN mice had decreased osteoclastic resorption. This was most apparent in the skull, where osteoclast number and osteoclast activity, as indicated by the degree of cavitation, were decreased. Similarly, TGF-$\beta1^{+/-}$ mice, which have decreased skeletal TGF-β1 levels (A. E. and R. D., unpublished observation), had decreased cavitation in their skulls. Conversely, mice overexpressing TGF-β2 have increased numbers of cranial osteoclasts (data not shown). Long bones of RIIDN mice also showed evidence of decreased resorption, as inferred from the increase in trabecular bone volume in the absence of changes in rate of bone formation. Accordingly, overexpression of TGF-β2 has the opposite effect, i.e. increased osteoclast activity and decreased trabecular bone mass (Erlebacher and Derynck, 1996). These changes in osteoclast activity in long bones of RIIDN and TGF-β2-overexpressing mice occur in the absence of alterations in osteoclast number in long bones, suggesting that the mechanism, by which TGF-β regulates osteoclast number on cranial bone surfaces, is distinct from how it affects osteoclast activity on trabecular bone surfaces. Our finding that osteoclast activity is regulated by osteoblast function may seem to contradict a recent study, which showed that osteoclasts do not require the concomitant presence and activity of osteoblasts (Corral et al., 1998). However, many of the cytokines and growth factors, such as TGF-β, produced by osteoblasts are deposited in bone matrix and can be released during subsequent osteoclastic resorption. In this way, osteoblasts (as well as osteoblastic precursors) could regulate osteoclast activity in a delayed manner.

Our finding that inhibition of TGF-β-induced signaling in osteoblasts of RIIDN mice leads to decreased osteoclast function suggests a key role for endogenous TGF-β in coordinating the activity of osteoblasts and osteoclasts. Thus, TGF-β stimulation of osteoblasts may lead to a secondary signal, which induces osteoclast differentiation and/or activity, similar to the indirect mechanism of action of parathyroid hormone (PTH) on bone resorption (Centrella et al., 1988; Oursler et al., 1991; Pfeilschifter et al., 1995). In fact, TGF-β induces osteoblastic secretion of M-CSF, a regulator of osteoclast maturation and activity (Takaishi et al., 1994). The expression of the osteoclastic inhibitor osteoprotegerin (OPG) by osteoblastic/stromal cells is also transiently increased by high levels of TGF-β. Although it is difficult to reconcile this with some reports on the effects of TGF-β on bone resorption in vitro (Tashjian et al., 1985; Chénu et al., 1988; Pfeilschifter et al., 1988; Shinar and Rodan 1990; Dieudonné et al., 1991; Hattersley and Chambers, 1991), a regulatory interaction

between TGF-β and the OPG/OPGL system (reviewed in Filvaroff and Derynck, 1998) is suggested by the similarities in phenotypes between OPG-deficient mice (Bucay et al., 1998) and TGF-β2-overexpressing mice (Erlebacher and Derynck, 1996). Both types of mice show increased osteoblast differentiation and activity, cortical bone porosity, progressive loss of trabecular bone and spontaneous fractures, albeit to a different extent. However, trabecular osteoclast density is increased in OPG-deficient mice (Bucay et al., 1998), but not in TGF-β2-overexpressing mice (Erlebacher and Derynck, 1996). The similarities in phenotype may be due to increased release and activation of latent bone matrix-bound TGF-β during osteoclastic resorption (Pfeilschifter and Mundy, 1987; Oreffo et al., 1989) in OPG-deficient mice.

Despite their increase in trabecular bone volume, RIIDN mice were still susceptible to ovariectomy-induced bone loss. These results suggest that estrogen deficiency does not require full TGF-β signaling in mature osteoblasts to have deleterious effect on the skeleton, although alterations in TGF-β production or responsiveness, or changes in transgene expression after ovariectomy can not be ruled out. The continued sensitivity of RIIDN mice to ovariectomy, however, is most likely due to the fact that estrogen deficiency in rodents causes an increase in osteoclast number (for review, see Kalu et al., 1993), which could then overcome the decrease in osteoclast activity in RIIDN mice. Our findings that TGF-β can regulate the rate of bone resorption in trabecular bone without changing osteoclast number indicate that bone resorption can be regulated through multiple mechanisms, and suggest that pathological bone resorption might have multiple independent components.

The osteoblastic response to TGF-β regulates biomechanical properties of bone

Our studies also reveal an important role for endogenous TGF-β in determining biomechanical properties of bone. Although bone strength was normal in long bones of RIIDN mice, the energy required to break their bones was increased. This latter parameter, which reflects the ability of a bone to absorb energy and is therefore a measure of its toughness, is considered a relevant clinical measure of human bone quality (Pereira et al., 1995). Similar studies in femurs of 24-month-old transgenic mice, expressing a mutated form of type I procollagen, showed no difference in strength, but a decreased energy to failure relative to wild-type controls (Pereira et al., 1993, 1995). Since these mice had a decreased collagen content and increased mineral/collagen ratio in their bones, these results demonstrate that changes in collagen content can affect bone fragility without altering bone strength (Pereira et al., 1993, 1995). Since collagen I synthesis by osteoblasts is regulated by TGF-β in vitro (Rosen et al., 1988; Breen et al., 1994) and mineralization appears to be normal in RIIDN mice, the increased energy in our RIIDN mice may in part be due to changes in matrix composition or architecture of transgenic cortical bone.

Unlike femoral cortical bone, vertebral bodies of RIIDN mice had normal energy, but increased load, strength and stiffness. Since trabecular bone of vertebral bodies of mice accounts for most of the biomechanical properties in compression experiments, the enhanced strength and stiffness are most likely due to the increased trabecular bone volume. While we can not rule out the possibility that the trabecular bone in RIIDN mice has an altered matrix composition or structure, no differences in histology or in energy to failure were detected, suggesting normal matrix integrity.

The changes in biomechanical properties of the RIIDN bones highlight the distinct effects of TGF-β in cortical and trabecular bone. In cortical bone, only the energy to failure was increased whereas, in trabecular bone, energy to failure was normal, yet stiffness and strength were increased. The basis for these differences in biomechanical changes between the two types of bones, although not clear, is not necessarily a mere consequence of their different architecture, but may be due to differences in the osteoblastic responses to TGF-β in these two types of bones and in regional differences in osteoclastic resorption, as discussed before.

Conclusions

Our current findings demonstrate that endogenous TGF-β is an important regulator of bone remodeling, through its effects on osteoblast differentiation and osteoclast function. The phenotype of the RIIDN mice, in which osteoblastic responsiveness to TGF-β has been inhibited, is in many ways the inverse of that of mice with increased expression of TGF-β2 ligand (Table 1). Thus, alterations in osteoblastic TGF-β expression or responsiveness shift the balance between bone deposition and resorption and lead to changes in trabecular bone volume. Furthermore, expression of the dominant negative type II receptor inhibits the increase in osteoblast differentiation, the defect in cortical bone and the occurrence of spontaneous fractures caused by overexpression of TGF-β2 (Erlebacher et al., unpublished data). Our results show that endogenous TGF-β regulates osteoblastic differentiation, osteoclastic resorption and biomechanical properties of bones through its effects on osteoblasts. Given the central role of TGF-β in maintaining normal bone homeostasis, and the recent reports linking TGF-β to human bone mass (Yamada et al., 1998) and turnover (Pfeilschifter et al., 1998), it is tempting to speculate that changes in TGF-β activity or responsiveness play a role in pathological bone conditions in humans.

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