

Expression of a Truncated, Kinase-Defective TGF- β Type II Receptor in Mouse Skeletal Tissue Promotes Terminal Chondrocyte Differentiation and Osteoarthritis

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Abstract. Members of the TGF- β superfamily are important regulators of skeletal development. TGF- β s signal through heteromeric type I and type II receptor serine/threonine kinases. When over-expressed, a cytoplasmically truncated type II receptor can compete with the endogenous receptors for complex formation, thereby acting as a dominant-negative mutant (DNIIR). To determine the role of TGF- β s in the development and maintenance of the skeleton, we have generated transgenic mice (MT-DNIIR-4 and -27) that express the DNIIR in skeletal tissue. DNIIR mRNA expression was localized to the periosteum/perichondrium, synovium, and articular cartilage. Lower levels of DNIIR mRNA were detected in growth plate cartilage. Transgenic mice frequently showed bifurcation of the xiphoid process and sternum. They also developed progressive skeletal degeneration, resulting by 4 to 8 mo of age in kyphoscoliosis and stiff and torqued joints. The histology of affected joints strongly resembled human osteoarthritis. The articular surface was replaced by bone or hypertrophic cartilage as judged by the expression of type X collagen, a marker of hypertrophic cartilage

normally absent from articular cartilage. The synovium was hyperplastic, and cartilaginous metaplasia was observed in the joint space.

We then tested the hypothesis that TGF- β is required for normal differentiation of cartilage in vivo. By 4 and 8 wk of age, the level of type X collagen was increased in growth plate cartilage of transgenic mice relative to wild-type controls. Less proteoglycan staining was detected in the growth plate and articular cartilage matrix of transgenic mice. Mice that express DNIIR in skeletal tissue also demonstrated increased Indian hedgehog (IHH) expression. IHH is a secreted protein that is expressed in chondrocytes that are committed to becoming hypertrophic. It is thought to be involved in a feedback loop that signals through the periosteum/perichondrium to inhibit cartilage differentiation. The data suggest that TGF- β may be critical for multifaceted maintenance of synovial joints. Loss of responsiveness to TGF- β promotes chondrocyte terminal differentiation and results in development of degenerative joint disease resembling osteoarthritis in humans.

ENDOCHONDRAL bone develops through a complex process whereby a cartilage model is replaced with bone (for reviews see 9, 20, 25). The cartilage template is formed from undifferentiated mesenchymal cells which condense and differentiate into chondrocytes. These cells then progress through a program of cell proliferation, maturation, and hypertrophy. Hypertrophic chondrocytes

represent the terminally differentiated phenotype and are replaced by bone cells in the final stages of endochondral bone development. While most of the cartilage model is eventually replaced with bone, cartilage is maintained on the articular surface (articular cartilage) and, in mouse, a small amount of the cartilage model persists in the growth plate. Longitudinal growth proceeds from the epiphyseal growth plate and must be intimately coordinated with appositional growth to maintain the shapes of individual bones. Thus, chondrocyte differentiation has to be strictly regulated so that the proper length and shape of the bone is maintained. The rate and extent of endochondral bone

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growth is regulated by Indian hedgehog (IHH)¹ and parathyroid hormone-related peptide (PTHrP), two secreted peptides (9, 35, for review see 74). IHH is expressed by cells that are committed to becoming hypertrophic and acts to increase PTHrP expression in the perichondrium and periarticular region. PTHrP inhibits further chondrocyte differentiation in cells that express the PTH receptor and are not yet committed to becoming hypertrophic. Control of skeletal development and maintenance is complex and likely involves several additional mediators.

Members of the TGF- β superfamily are secreted growth factors that regulate many aspects of development, including growth and differentiation (for reviews see 39, 44, 45). Mice and humans with mutations in certain members of the family (*Bmp5* and *Gdf5*) display a wide range of skeletal defects including reduced size of specific bones, brachypodism, and chondrodysplasia (30, 60, 65). TGF- β s are expressed in developing and adult skeletal tissue. TGF- β 1-3 mRNAs are expressed in condensing mesenchyme during the early stages of chondrocyte differentiation in the mouse and human (22, 41, 47, 52). TGF- β mRNAs were not detected in terminally differentiated chondrocytes, but TGF- β immunoreactivity was observed in the matrix surrounding these cells (24, 48). TGF- β is thought to play an important role in chondrogenic differentiation. Specifically, TGF- β promotes chondrogenesis in cultures of undifferentiated multipotent mesenchymal cells (14, 32, 36) but inhibits hypertrophic differentiation of chondrocyte cultures (4, 5, 29, 67) and in cultured mouse long bone rudiments (16).

Persistence of cartilage on the articular surface is necessary for proper joint function. Osteoarthritis is a noninflammatory disorder of synovial joints, often referred to as osteoarthrosis. Degeneration of the articular cartilage results in osteoarthritis, which is characterized by decreased proteoglycan and increased type X collagen in the cartilage matrix (for review see 23). That TGF- β might play a role in the function and maintenance of articular cartilage is suggested by its effects on chondrocyte differentiation and its expression in articular cartilage *in vivo* (18) and in organ culture (43). However, the effects of TGF- β on articular cartilage are not clear. TGF- β has been shown to both inhibit or stimulate proteoglycan synthesis and growth in articular chondrocytes in culture (for review see 66). Since cells in the articular cartilage normally do not differentiate past the stage of resting chondrocytes, and TGF- β inhibits terminal differentiation, it is possible that TGF- β plays a role in the maintenance of functional articular cartilage. On the other hand, repeated intra-articular injections of TGF- β into mouse knee joints result in the formation of osteophytes on the articular surface, suggesting a role for this peptide in the pathogenesis of osteoarthritis (69, 70).

Members of the TGF- β superfamily signal through a family of serine/threonine kinase receptors (for reviews see 15, 40, 64). Recently, a variety of type I and type II receptors for members of the TGF- β superfamily have been identified and characterized. TGF- β and related factors signal through a heteromeric cell surface receptor complex, which consists of two type II and two type I recep-

tors. Overexpression of a cytoplasmically truncated type II TGF- β receptor inhibits endogenous receptor function in a dominant way, most likely by interfering with endogenous receptor complex formation and function (11). Truncated type II receptors have been used to block TGF- β signaling in cells in culture (7, 11, 12, 53, 55) and in transgenic mice (6, 75). *In vivo*, overexpression of a truncated type II receptor is therefore thought to inactivate receptor function in a tissue-specific manner, depending on the promoter used, and thus to inhibit the response to all three TGF- β species while avoiding early embryonic lethality.

To study the role of TGF- β signaling *in vivo*, we have generated transgenic mice that express a cytoplasmically truncated, functionally inactive TGF- β type II receptor under the control of a metallothionein-like promoter, MT-DNIIR. Two mouse lines (MT-DNIIR-4, -27) demonstrated high basal levels of DNIIR expression in skeletal tissue. Heterozygous mice from these lines maintained on normal food and tap water developed joint abnormalities resembling osteoarthritis. We used this model system to test the hypothesis that TGF- β signaling is required for normal differentiation and maintenance of chondrocytes *in vivo*. Our results suggest that loss of responsiveness to TGF- β overrides the IHH-feed back loop and promotes terminal differentiation of chondrocytes resulting in osteoarthritis.

Materials and Methods

Generation and Identification of Transgenic Mice

The MT-DNIIR expression plasmid was used to generate transgenic mice (see Fig. 1). The EcoRI/XbaI fragment of p102 containing the truncated human TGF- β type II receptor (11) was inserted into the BamHI site of MT- β (78) by blunt end ligation. The HindIII/BglI fragment containing the transgene under the metallothionein promoter was microinjected into the pronuclei of single cell embryos from crosses of C57BL/6 and DBA mice (26). Mice were maintained on Purina mouse chow and tap water. Transgenic mice were identified by Southern blot (59) and PCR analyses of genomic DNA isolated from mouse tails by proteinase K digestion and phenol/chloroform extraction. For Southern blots, genomic DNA was digested with PstI/EcoRI and the BamHI/EcoRI rabbit β -globin fragment from MT- β was used as the probe. PCR was performed using primers to the FLAG epitope sequence: ATC GTC ATC GTC TTT GTA GTC and human TGF- β type II receptor: TCC CAC CGC ACG TTC AGA AG. Genomic DNA was amplified for 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 45 s, and elongation for 2 min at 72°C in reaction buffer containing 2 mM MgCl₂, 1 \times PCR buffer (Perkin Elmer, Blanchburg, NJ), 0.2 mM dNTPs (Pharmacia, Uppsala, Sweden), and 0.2 μ M of each primer.

Whole Mount Skeletal Preparation

Whole mount skeletal preparations of adult mice were prepared according to Selby (57). Briefly, mice that had been skinned and eviscerated were soaked in a series of KOH solutions over several days. Skeletal tissue was stained with 0.004% alizarin red in 1.9% KOH. The specimens were cleared in glycerin, benzyl alcohol, and ethanol and then stored in glycerin. Skeletons from 17.5-d post-coital fetal mice were double stained for cartilage and bone with alcian blue and alizarin red (33, 38). All carcasses were skinned and fixed in 95% ethanol for 72 h. Carcasses were then placed in 95% ethanol-alcian blue solution (20 h) for cartilage staining, followed by a 95% ethanol wash (8 h) and maceration in 0.35% KOH overnight. Bone was stained with alizarin red S in 0.2% KOH (4 to 6 h) followed by clearing in 95% ethanol:glycerin (1:1).

RNA Analysis

Mouse hindlimbs were removed and skin and muscle were trimmed away. The remaining skeletal tissue was frozen in liquid nitrogen and crushed

1. *Abbreviations used in this paper:* IHH, Indian hedgehog; PTHrP, parathyroid hormone-related peptide.

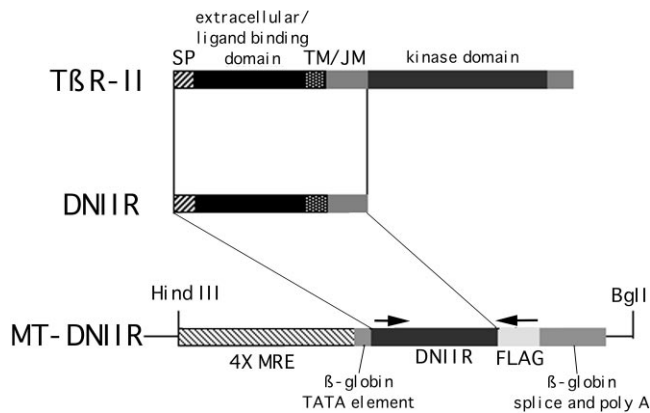


Figure 1. Map of the MT-DNIIR expression plasmid. The EcoRI/XbaI fragment of the human TGF- β type II receptor from the plasmid p102 containing a FLAG epitope tag and the signal sequence (SP), ligand binding, transmembrane (TM), and juxtamembrane (JM) domains of the receptor was inserted into the BamHI site of the MT- β metallothionein expression vector by blunt end ligation. The MT- β vector contains four metal responsive elements and the β globin TATA element, splice sites, and polyadenylation signal (69). The HindIII/BglI fragment was injected into single cell embryos. Arrows mark the location of primer sequences used for PCR and RT-PCR analysis.

with a mortar and pestal into a fine powder. RNA was extracted from the bone powder by the procedure of Chomerymski and Sacchi (13). RNA was treated with RQ1 RNase-free DNase (Promega Biotech, Madison, WI) for 30 min at 37°C to remove contaminating genomic DNA. RNA was then extracted by phenol:chloroform extraction and ethanol precipitation. RNA concentration was determined spectrophotometrically. For RT PCR analysis, cDNA was synthesized from 1 μ g of total RNA pooled from the hind limbs of two to four mice using oligo dT primers as described in the GeneAmp RNA PCR kit (Perkin Elmer, Norwalk, CT). PCR amplification was performed using 5 μ l of the cDNA mix. The same conditions and primers described above for the amplification of DNIIR from genomic DNA were used, except primers to glyceraldehyde-3-phosphate dehydrogenase were added as an internal loading control. (3' gapdh: CAT GTA GGC CAT GAG GTC CAC CAC; 5' gapdh: TGA AGG GTG TGA ACG GAT TTG GC; Clontech, Palo Alto, CA). Samples from reactions performed in the absence of reverse transcriptase were also amplified to demonstrate that there was no contaminating genomic DNA in the RNA samples.

In Situ Hybridization

In situ hybridization was performed as described (47) on sections from mouse knee joints decalcified in EDTA. Briefly, hindlimbs were removed, and skin and muscles were trimmed away. The long bone was trimmed close to the joint. The joint was fixed overnight at 4°C in fresh 4% paraformaldehyde. The specimens were rinsed in DEPC-treated water and soaked in 0.1 M Tris, pH 7.5, 10% EDTA tetrasodium salt, 7.5% polyvinyl pyrrolidone, and 1 μ l/ml DEPC for 2 to 7 d at 4°C. The joints were dehydrated through ethanol and xylene and then embedded in paraffin. If the tissue remained too hard to section, the block was soaked in the EDTA decalcification solution for an additional day. Sections were hybridized to ³⁵S-labeled sense and antisense riboprobes. The MT-DNIIR plasmid was linearized with EcoRI, and the antisense probe was made with T7 polymerase. The sense probe was made from XbaI-linearized plasmid and T3 polymerase. The IHH plasmid was the kind gift of Andy McMahon (Harvard University, Cambridge, MA). The antisense probe was made from XbaI-linearized plasmid using the T7 polymerase. The PTH receptor probe was the kind gift of Henry Kronenberg (Harvard Medical School, Boston, MA). The antisense probe was made from the EcoRI-linearized plasmid using T3 polymerase. Slides were exposed to photographic emulsion at 4°C for 2 wk and then developed with D19 developer, fixed in 1% acetic acid, and cleared in 30% sodium thiosulfate. Sections were counterstained with 0.2% toluidine blue. Kodak Ektachrome

film was used to take photographs under phase contrast, bright field, and dark field illumination using a microscope (Zeiss, Thornwood, NY).

Histology and Immunohistochemistry

Mouse hindlimbs were fixed overnight at 4°C in 4% paraformaldehyde and then decalcified overnight at 4°C in Surgipath decalcifying solution. Specimens were dehydrated and embedded in paraffin. For routine histological analysis, sections were stained with hematoxylin and eosin using standard procedures. To visualize proteoglycans, sections were stained with safranin O. Briefly, deparaffinized sections were rehydrated and stained with hematoxylin for 3 min. The slides were washed in tap water, and the hematoxylin was differentiated in acid alcohol and blued in lithium carbonate. Sections were then stained with a 1:5,000 dilution of aqueous fast green for 3 min, washed briefly in 1% acetic acid, and stained in 0.1% safranin O for 3 min. The sections were quickly dehydrated, cleared, and mounted.

Immunohistochemical staining of type X collagen was performed using polyclonal antibodies to mouse type X collagen that were a generous gift from Tim Pfordte and Bjorn Olsen (Harvard Medical School, Boston, MA). Sections were dewaxed, rehydrated, and digested with 1 mg/ml hyaluronidase in PBS at 37°C for 45 min. Immunohistochemistry was performed using the Vectastain Elite immunoperoxidase staining kit (Vector Laboratories, Hercules, CA) according to the manufacturer's instructions. The color reaction was performed using the DAB substrate kit from Vector Laboratories. Sections were counterstained with hematoxylin.

Results

Construction of MT-DNIIR Transgenic Mice

An expression plasmid (MT-DNIIR; Fig. 1) containing the coding sequence for the truncated human TGF- β type II receptor (11) and metal responsive regulatory elements from the human metallothionein II promoter (78) was constructed. The truncated receptor contained the extracellular, transmembrane, and juxtamembrane domains of the TGF- β type II receptor. Most of the cytoplasmic domain including the kinase domain was deleted. This mutant receptor is able to bind ligand and interact with type I receptors and acts as a dominant-negative mutation in cells in culture and in transgenic mice (11, 75). The promoter contained four metal responsive elements and a β -globin TATA element, splice sites, and polyadenylation signal. This promoter has been shown to regulate gene expression with heavy metals in cells in culture (78). The HindIII/BglI fragment of the MT-DNIIR expression plasmid was injected into single cell embryos, and five transgenic mouse lines (MT-DNIIR-4, -15, -27, -28, -30) were established.

Skeletal Defects in MT-DNIIR Transgenic Mice

Signs of skeletal abnormalities were observed in mice from two of the MT-DNIIR transgenic lines, MT-DNIIR-4 and MT-DNIIR-27, maintained on normal food and tap water. Defects were observed in heterozygous mice. Skeletal defects were apparent by 3 mo of age and became progressively worse as the mice aged. MT-DNIIR-4 mice demonstrated kyphoscoliosis and stiffness in the hindlimb joints. In some cases, hindlimbs were torqued laterally at varying angles. The xiphoid process of the sternum protruded out from the chest and was visible under the skin. MT-DNIIR-27 mice demonstrated stiffness in the knees. No differences in bone density were detected on X-ray films (data not shown). To further characterize the nature of the skeletal defects, alizarin red whole mount skeletal preparations of adult

Table I. Summary of Skeletal Defects Identified by Alizarin Red Whole Mount Skeletal Preparations

Line	Xiphoid/sternum*	Knee [‡]	Other joints [§]	Vertebrae	Tumoral calcinosis
WT	0/11	0/11	0/11	0/11	0/11
MTR27	0/9	2/9	1/9	0/9	0/9
MTR4	11/19	13/19	7/19	11/19	4/19

*Bifurcated sternum or xiphoid process. Fig. 2, A and B.

[‡]Calcified tissue in knee joint. Fig. 2, C and D.

[§]Calcified tissue in shoulder or pelvis joint. Fig. 2, G and H.

^{||}Misshapen or fused cervical or thoracic vertebrae. Fig. 2, I and J.

mice (4 to 8 mo of age) were performed (Table I; Fig. 2). A summary of skeletal defects observed in whole mount skeletal preparation of adult wild-type and heterozygous transgenic mice is shown in Table I. Representative preparations are shown from adult wild-type, MT-DNIIR-4, and

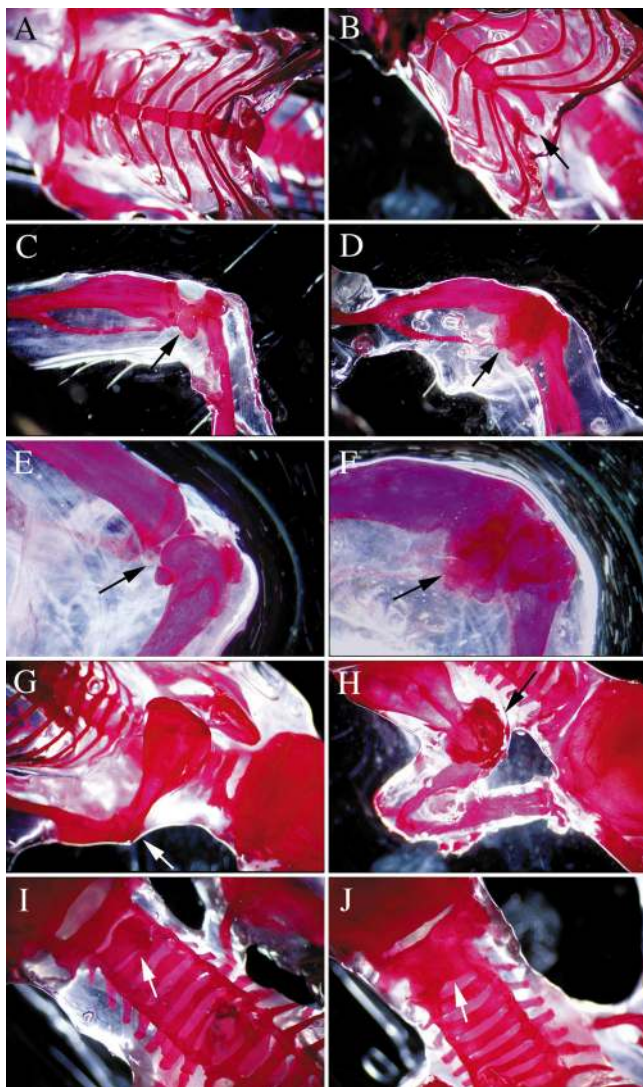


Figure 2. Skeletal defects in MT-DNIIR transgenic mice. Photographs of alizarin red whole mount skeletal preparations from adult wild-type (A, C, E, G, and I) MTR-DNIIR-4 (B, D, H, and J), and MT-DNIIR-27 (F) mice. Arrows point to xiphoid process (A and B), knee (C–F), shoulder (G and H) joints, and cervical vertebrae (I and J).

MT-DNIIR-27 mice (Fig. 2). The xiphoid process of adult MT-DNIIR-4 transgenic animals was bifurcated. Knee and shoulder joints of MT-DNIIR-4 and -27 mice were disorganized and contained excess calcified tissue. MT-DNIIR-4 vertebrae were often misshapen and appeared fused. Sternal defects and tumoral calcinosis were only found in the MT-DNIIR-4 line; therefore, these observations must be considered tentative. However, the presence of joint defects in two transgenic lines suggests the reasonable hypothesis that all the chondrocyte abnormalities in MT-DNIIR-4 mice are not simply insertional effects. Skeletal defects were not detected in wild-type mice.

Expression and Localization of DNIIR mRNA

Since transgenic mice developed skeletal defects in the absence of exogenously added zinc, we sought to determine if expression of the transgene in the MT-DNIIR-4 and -27 lines correlated with the skeletal phenotype and if the hybrid promoter allowed leaky expression in the absence of zinc. We thus prepared adult skeletal mRNA from hind limbs of mice maintained on normal food and tap water and examined using RT-PCR analysis whether DNIIR mRNA was expressed in transgenic (MTR4, -15, -27, -28, -30) and wild-type mice (Fig. 3). Transgene-specific (DNIIR) primers were used to amplify mutant but not endogenous type II receptor cDNA sequences. Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize for the amount of cDNA used in each reaction. As shown in Fig. 3, DNIIR mRNA was expressed in skeletal tissue from MT-DNIIR-4 and -27 mice in the absence of exogenously added zinc. DNIIR mRNA was not detected in MT-DNIIR-27 mice that did not exhibit skeletal defects (data not shown). There was very little basal expression of the DNIIR transgene in skeletal tissue from MT-DNIIR-15, -28, and -30 mice. We have also characterized DNIIR mRNA expression in other tissues in each of the MT-DNIIR mouse lines (data not shown). Our analyses showed that DNIIR mRNA was expressed in different sets of tissues depending on the individual MT-DNIIR mouse line. This heterogeneity in expression pattern is most likely due to the presence of only minimal gene regulatory elements in this version of the metallothionin promoter, which may make its transcriptional activity sensitive to DNA surrounding the transgene integration site. However, the skeletal phenotype is most likely not due to disruption of an unknown gene for several reasons. First, the skeletal phenotype is observed in heterozygous mice. Second, two separate lines of mice demonstrated a similar skeletal defect and, third, the sites of constitutive expression of the DNIIR mRNA correlated with skeletal abnormalities. All of our experiments outlined below were performed with heterozygous MT-DNIIR-4 mice maintained on normal food and tap water.

The expression of DNIIR mRNA in adult skeletal tissue was localized using in situ hybridization (Fig. 4). Sections of knee joints from wild-type and MT-DNIIR mice were hybridized to an antisense ³⁵S-labeled riboprobe corresponding to the extracellular domain of the human TGF- β type II receptor. DNIIR mRNA expression was localized to the articular cartilage, synovium, periosteum, and perichondrium of MT-DNIIR-4 mice at 8 wk of age (Fig. 4,

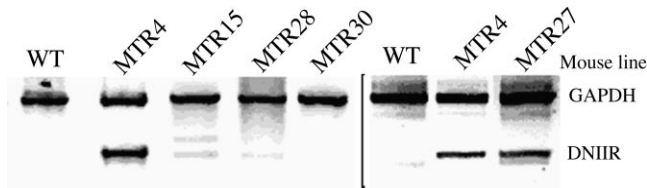


Figure 3. RT-PCR analysis of DNIIR mRNA expression in MT-DNIIR transgenic mouse lines. RNA isolated and pooled from the hind limbs of two to four wild-type (WT) or transgenic (*MT-DNIIR-4*, *-15*, *-27*, *-28*, *-30*) mice maintained on normal food and tap water was analyzed by RT-PCR. To specifically amplify the truncated DNIIR cDNA, primers targeted to FLAG epitope sequences were used (Fig. 1). Amplifications of GAPDH was used as an internal control. Two separate assays are shown separated by the black bar.

A–D). A low level of DNIIR mRNA was detected in the lower hypertrophic zone growth plate of transgenic mice (Fig. 4 *D*). At 6 mo of age, DNIIR mRNA was localized to hyperplastic synovium filling the joint space, especially near areas surrounding cartilage metaplasia (Fig. 4 *E*). DNIIR was also localized to the periosteum (data not shown). No hybridization was detected in sections from wild-type joints (Fig. 4 *F*). Hybridization was also not detected to an ^{35}S -labeled sense probe in sections from wild-type or transgenic joints (data not shown).

Developmental Defects in *MT-DNIIR* Mice

To determine if skeletal defects were due to alterations in embryonic skeletal development, alizarin red S/alcian blue whole mount skeletal preparations from 17.5-d post-coital wild-type and *MT-DNIIR* mice were performed. Fusion of the sternum was incomplete in 64% (23/36) of *MT-DNIIR* mice (Fig. 5, *C* and *D*). The degree of sternal bifurcation varied. The most severe cases extended caudally from the fifth rib, and the least severe cases involved only the xiphoid process. Defects in the size or shape of the long bones or vertebrae were not detected in 17.5-d post-coital mice. Since sternal development and fusion occur between 12 and 15 d post-coitum, expression and localization of DNIIR mRNA were examined by in situ hybridization in 12.5-d post-coital *MT-DNIIR* embryos (Fig. 5). Sections of wild-type and transgenic embryos were hybridized to an ^{35}S -labeled antisense DNIIR riboprobe. Expression was localized to the mesenchyme of the thoracic body wall in transgenic embryos (Fig. 5 *B*). There was no difference in DNIIR mRNA expression in *MT-DNIIR* embryos from mothers maintained on 25 mM ZnSO_4 or tap water. No hybridization was detected in sections from wild-type embryos (Fig. 5 *A*) or in sections hybridized to an ^{35}S -labeled sense riboprobe (data not shown). These data suggest expression of the DNIIR mRNA in the mesenchyme of the thoracic body wall during sternal development results in incomplete fusion of the sternum (Fig. 5 *D*). Since no

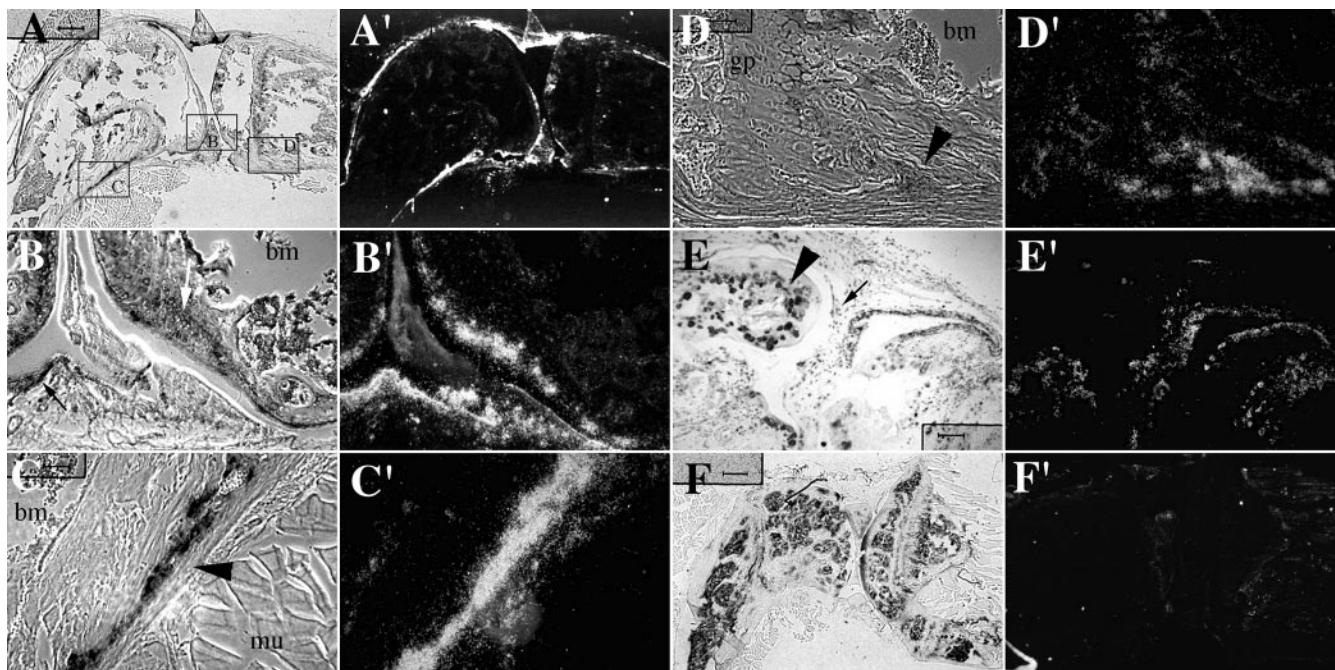


Figure 4. Localization of DNIIR mRNA in skeletal tissue. Sections of knee joints from 8-wk *MT-DNIIR* transgenic mice were hybridized to an ^{35}S -labeled antisense DNIIR riboprobe (*A–D*). Boxes shown in *A* delineate the approximate locations on the joints shown in (*B–D*). DNIIR expression was detected in the articular cartilage (*B*, white arrow), synovium (*B*, black arrow), and periosteum/perichondrium (*C* and *D*, black arrowhead) of transgenic mice. Representative images from analysis of two different mice are shown. In 6-mo-old transgenic mice, DNIIR expression was detected in hyperplastic synovium (*E*, small, black arrow) surrounding areas of cartilage metaplasia (*E*, large, black arrowhead). A representative image from two separate mice is shown. No hybridization was detected in wild-type tissue (*F*) or in transgenic mice with an ^{35}S -labeled sense riboprobe (data not shown). Toluidine blue-stained bright field (*A–F*) and dark field (*A'–F'*) images are shown. Bars: (*A* and *F*) 400 μm ; (*B–E*) 100 μm .

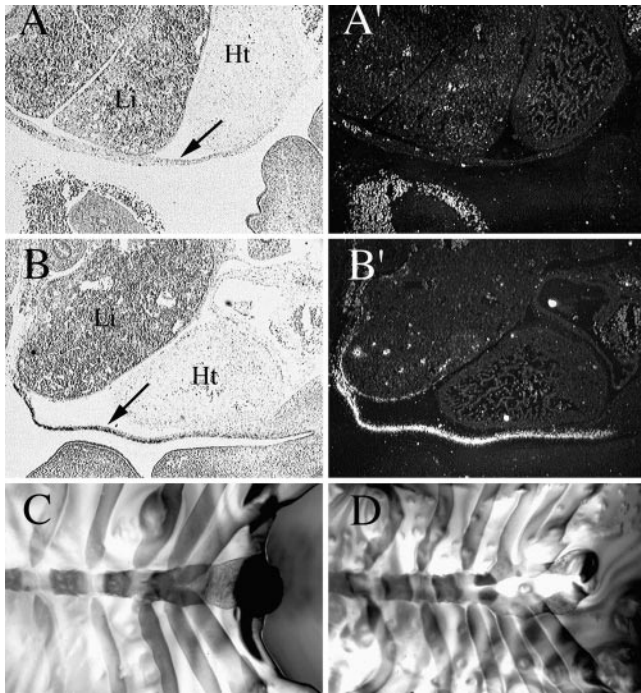


Figure 5. Expression of DNIIR mRNA in embryos. Sections of 12.5-d post-coital wild-type (A) and MTR-DNIIR transgenic (B) embryos were hybridized to an ^{35}S -labeled DNIIR riboprobe. DNIIR mRNA expression was detected in the mesenchyme of the thoracic body wall (A and B, arrow) in MT-DNIIR transgenic but not wild-type embryos at the time the sternum begins to develop. Toluidine blue-stained bright field (A and B) and dark field (A' and B') images are shown. Li, liver, Ht, heart. Bifurcated sternum in 17.5-d post-coital MT-DNIIR-4 mice. Alizarin red/alcian blue-stained skeletal preparations of wild-type (C) and MT-DNIIR transgenic (D) embryos at 17.5 d post coitus. Bar, 200 μm .

other skeletal defects were detected in 17.5-d post-coital mice, and DNIIR mRNA in the embryo was limited to the thoracic body wall, the other defects observed in adult transgenic mice (Table I, Fig. 2) are probably not a secondary result of early developmental defects in patterning of the cartilage model.

Histology of Transgenic Skeletal Tissue

To determine the effects of DNIIR mRNA expression on joint histology, sections from wild-type and MT-DNIIR mouse knee joints at 4 wk (Fig. 6) and 6 mo (Fig. 7) of age were stained with hematoxylin and eosin. Some degeneration of the articular surface was observed at 4 wk. Patches of the articular surface were denuded of cartilage (Fig. 6 B), and hypertrophic cells were observed in the articular cartilage (Fig. 6 D). Furthermore, disorganized zones of cartilage were often seen in the epiphyseal head of long bones from mice at all ages (Fig. 6 B, black arrow). Alterations in the organization and histology of the growth plate were apparent at 4 (Fig. 6, E and F), and 8 wk (Fig. 8, C and D). In wild-type mice, cells in the growth plate exhibited normal columnar organization, and resting, proliferative, and hypertrophic zones were clearly demarcated (Fig. 6 E). In 4-wk-old transgenic mice, the hypertrophic zone was thicker and the cells in this zone were not organized in columnar arrays (Fig. 6 F). Resting and proliferat-

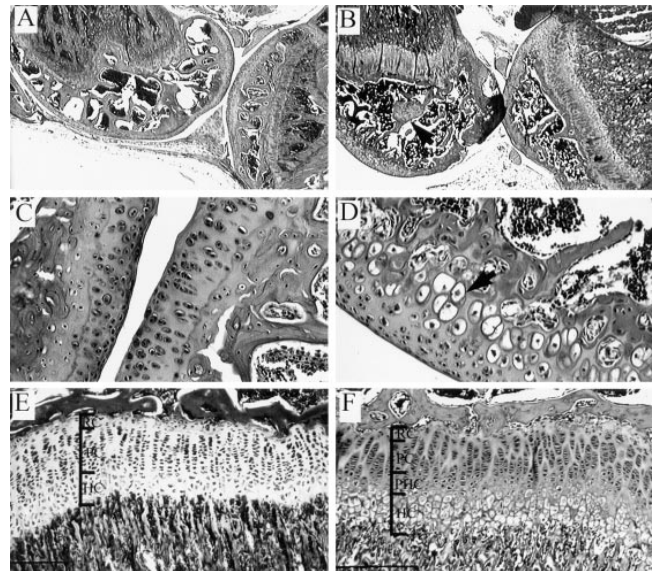


Figure 6. Knee joint histology in young MT-DNIIR mice. Images of hematoxylin- and eosin-stained sections from wild-type (A, C, and E) and MT-DNIIR (B, D, and F) knee joints at 4 wk of age. Disorganized cartilage islands were often observed in the transgenic epiphysis (B, black arrowhead). Hypertrophic cells were located in the deep zones of the articular cartilage in transgenic (D) but not wild-type (C) mice. Resting (RC), proliferating (PC), and hypertrophic (HC) cells were easily detectable in the wild-type growth plate (E). In transgenic mice, the histology of the growth plate was altered. The hypertrophic zone was thicker, and two distinct cell populations were observed, hypertrophic cells (HC) and smaller, round prehypertrophic cells (PHC). Bars: (A and B) 400 μm ; (C and D) 50 μm ; (E and F) 77 μm .

ing zones were visible, but the cells in the proliferating zone were sometimes grouped into clusters. A population of small, round cells not readily detectable in wild-type mice (Fig. 6 E) was located between the proliferating and hypertrophic zones (Fig. 6 F, PHC). In 8-wk-old transgenic mice, very few proliferating cells were seen. Most of the cells in the growth plate appeared hypertrophic and abnormally round (see Fig. 8 D).

The histology of joints from older mice was very similar to that observed in osteoarthritis (23; Fig. 7). In joints with mild degeneration, articular cartilage appeared fibrillated and disorganized (Fig. 7, B and D), and deeper chondrocytes were grouped into clusters (Fig. 7 D). As degeneration progressed, articular cartilage was replaced with hypertrophic cartilage and bone. Early osteophytes, which represent areas of new endochondral ossification, were present as outgrowths of chondroid tissue in the articular margins (Fig. 7 F). Apparently detached fragments of bone covered with cartilage were detected in the joint space (Fig. 7, B and E). The synovium was hyperplastic and thrown into folds (Fig. 7 E). Cartilaginous metaplasia was observed in synovium filling the joint space (Fig. 4 E). The growth plate was often disorganized or undetectable (Fig. 7 E) in bones from the older transgenic mice, while age-matched wild-type mice maintained a small but organized growth plate. In the most severe cases (data not shown), the femur was pressed into the tibia and the entire surface of the tibia was destroyed. The data suggest that altered responsiveness to TGF- β results in cartilage disor-

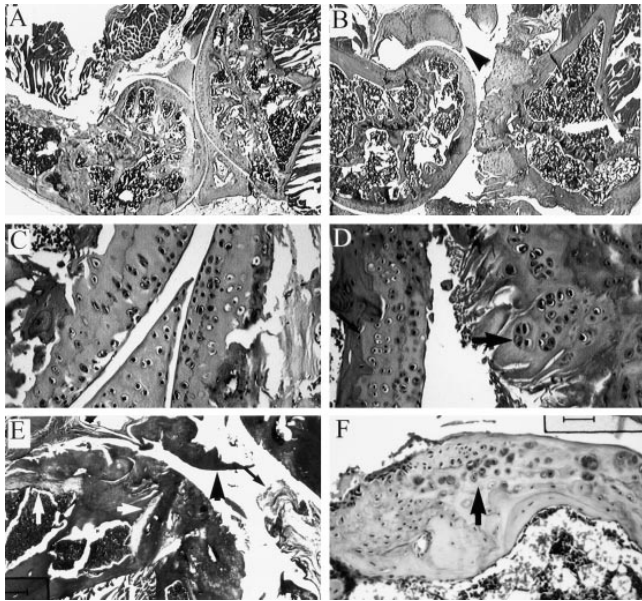


Figure 7. Knee joint histology in older MT-DNIIR mice. Joints from 6-mo-old transgenic mice with joint damage (*B* and *D–F*) and from wild-type mice (*A* and *C*) are shown. Cartilage was observed in the joint space (*B* and *E*, arrowheads) and the synovium was hyperplastic (*E*, arrow) in transgenic mice. The articular surface of wild-type mice was smooth and organized (*C*). In transgenic mice, the articular surface was fibrillated and chondrocytes were grouped into clusters (*D*, arrow). Early osteophytes were also present on the articular surface (*F*). The growth plate was often undetectable or highly disorganized (*E*, white arrows) relative to the wild-type growth plate (*A*). Bars: (*A* and *B*) 400 μm ; (*E*) 270 μm ; (*C*, *D*, and *F*) 50 μm .

ganization leading to a progressive degeneration resembling osteoarthritis.

DNIIR Expression Results in Altered Proteoglycan and Type X Collagen Expression

Our analyses suggest that MT-DNIIR transgenic mice demonstrate osteoarthritis, but we did not detect any changes in the shape of long bones in newborn mice. Based on this phenotype, we propose that altered responsiveness to TGF- β disrupts normal chondrocyte differentiation that later results in degeneration of the joint and the osteoarthritis phenotype. To test this hypothesis we histologically characterized the expression of proteoglycan, which is expressed by chondrocytes, and type X collagen in joints of 8-wk-old wild-type and MT-DNIIR mice (Fig. 8, *A–G*). Safranin O stains proteoglycans in cartilage matrix. Articular cartilage in wild-type mice showed intense staining of proteoglycan with safranin O (Fig. 8 *A*). In contrast, staining was reduced and patchy in the articular cartilage of MT-DNIIR mice (Fig. 8 *B*). In wild-type mice, safranin O stain extended throughout the growth plate into the bone trabeculae (Fig. 8 *C*). However, the staining intensity was decreased in MT-DNIIR mice from the resting and proliferative zones to the hypertrophic zone, and staining was not detected in bone trabeculae of MT-DNIIR mice (Fig. 8 *D*). Type X collagen is a marker of chondrocyte differentiation and is localized primarily to

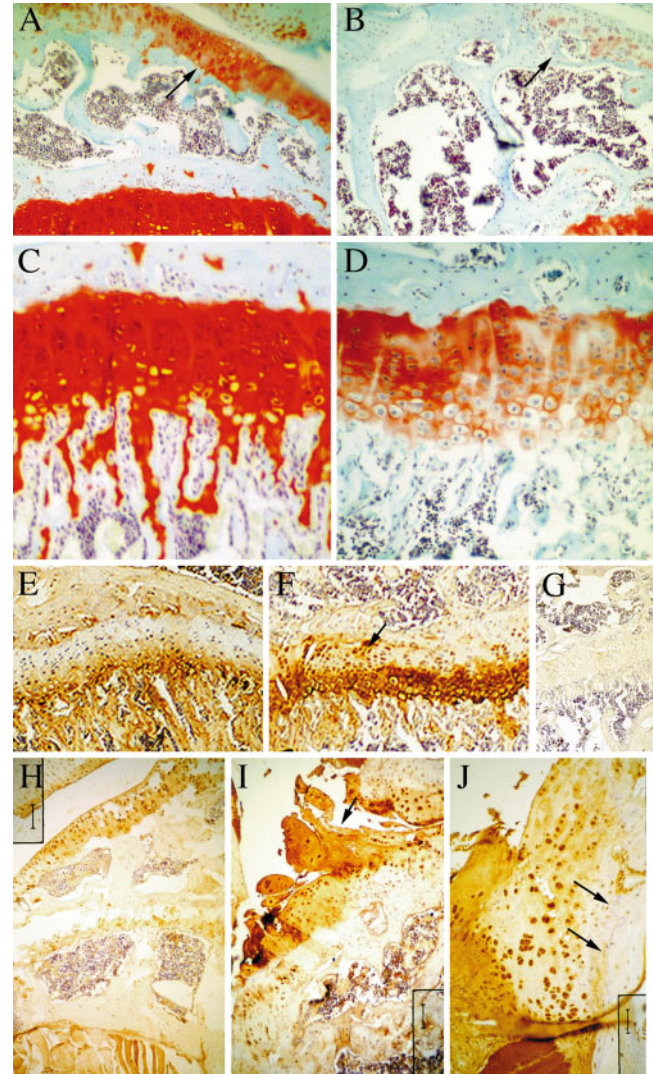


Figure 8. Localization of proteoglycans and type X collagen in the knee joint. Sections from 8-wk-old wild-type (*A* and *C*) and MT-DNIIR (*B* and *D*) knee joints stained with safranin O (*A–D*). Images at 150 \times (*A* and *B*) show staining in the articular surface (arrows). Images *C* and *D* focus on staining in the growth plate. There was intense proteoglycan staining in the articular cartilage of wild-type mice (*A*) while staining was less intense and patchy on the articular surface of transgenic mice (*B*). Staining was also less intense in the transgenic growth plate (*D*) relative to the wild-type growth plate (*C*). Sections from 8 wk (*E–G*) and 6-mo-old (*H–J*) wild-type (*E* and *H*) and MT-DNIIR (*F*, *G*, *I*, and *J*) were used for immunohistochemical staining of type X collagen (*E–J*). There was increased type X collagen staining in the transgenic growth plate at 8 wk (*F*) relative to wild-type controls (*E*). Intracellular staining was detected in transgenic chondrocytes in the upper zones of the growth plate (*F*, arrow). Type X collagen immunoreactivity was not readily detectable in articular cartilage from wild-type mice at 6 mo of age (*H*) but was detected in fibrillated cartilage (*I*, arrow) from older (6 mo) transgenic mice. Chondrocytes in osteophytes also stained for type X collagen (*J*). Arrows represent the original joint lining (*J*). No staining was detected in the absence of primary antibody (*G*). Bars: (*A* and *B*) 66 μm ; (*C* and *D*) 25 μm ; (*E* and *F*) 66 μm ; (*G*) 200 μm ; (*H* and *I*) 100 μm ; (*J*) 50 μm .

the matrix of hypertrophic, terminally differentiated chondrocytes (54). Intracellular type X collagen is characteristic of nonproliferating, prehypertrophic chondrocytes. In wild-type mice, type X collagen was localized to the matrix of the hypertrophic zone of the growth plate, which was one or two cells thick (Fig. 8 E). Type X collagen was not detected intracellularly in wild-type mice. In MT-DNIIR mice, type X collagen staining was more intense and was localized to a broad area of the growth plate matrix that was four to six cells thick (Fig. 8 F). Type X collagen staining was also visible within cells throughout the growth plate (Fig. 8 F). In humans, type X collagen is expressed in osteoarthritic cartilage. It is localized to sites of newly formed osteophytic and repair cartilage, and marks areas of endochondral bone formation (72). We therefore used immunohistochemistry to determine the localization of type X collagen in older (6 mo) transgenic mice with the osteoarthritis phenotype (Fig. 8, I and J). Little type X collagen was detected in joints from wild-type mice (Fig. 8 H). By contrast, in the joints of older MT-DNIIR mice, type X collagen was localized to fibrillated articular cartilage (Fig. 8 I), osteophytes (Fig. 8 J), and cartilage growing in the joint space (data not shown). These data suggest that expression of the DNIIR resulted in defects in chondrocyte differentiation so that there was less proteoglycan but more type X collagen localized to the cartilage matrix. This indicates that loss of responsiveness to TGF- β promotes terminal differentiation of chondrocytes.

Effects of Altered Responsiveness to TGF- β on IHH Expression

IHH is a secreted protein expressed in chondrocytes committed to become hypertrophic and is thought to regulate cartilage differentiation (73, 74). When misexpressed in chick, IHH has been reported to induce PTHrP in perichondrial cells, which inhibits further differentiation of chondrocytes that express the PTH receptor; therefore, the negative-feedback effect of IHH on chondrocyte differentiation is indirect and is mediated by the perichondrium. Since the DNIIR is expressed in perichondrial cells (Fig. 4, B and C) and to gain insight into the role of TGF- β in chondrocyte differentiation, we examined IHH and PTH receptor expression in MT-DNIIR mice. Sections from 8-wk-old wild-type and transgenic knee joints were hybridized to ³⁵S-labeled riboprobes. MT-DNIIR mice demonstrated higher levels of IHH expression in the growth plate relative to wild-type controls (Fig. 9, A and B). Expression was higher in each cell and IHH localized to a broader band of cells in the MT-DNIIR growth plate. PTH receptor was localized to prehypertrophic cells in wild-type and MT-DNIIR mice (Fig. 9, C and D). There was little difference in PTH receptor expression in the growth plate; however, there appeared to be a higher level of PTH receptor expression in osteoblasts in transgenic mice (Fig. 9 C). The altered expression of IHH suggests loss of responsiveness to TGF- β s, overrides the IHH feedback loop, and promotes commitment to terminal differentiation.

Tumoral Calcinosis in MT-DNIIR-4 Mice

In humans, tumoral calcinosis is a heritable disease charac-

terized by benign, calcified tumor-like periarticular masses (19). Tumoral calcinosis-like lesions were found in MT-DNIIR-4 mice. The firm, rubbery masses were found most often near the cervical vertebrae (Fig. 10, A and B), although masses were also seen all along the vertebral column, in the axilla and pelvis, and in the paws of transgenic mice. Similar lesions were never seen in wild-type mice. Alizarin red staining suggested that the lesions were calcified (Table I; Fig. 10 B). The encapsulated lesions consisted of a dense fibrous network with one or more spaces filled with white, calcareous material. Histologically, these spaces appeared necrotic (Fig. 10 C) and were surrounded by poorly differentiated cells, calcified material (Fig. 10 D), and osteoclast-like multinucleated giant cells (Fig. 10 E). The tumoral calcinosis-like masses expressed DNIIR mRNA as determined by RT-PCR analysis (Fig. 10 F). RNA from Mv1Lu cells stably transfected with a DNIIR expression plasmid, wild-type, and MT-DNIIR-4 skeletal tissue was used as controls. The data suggest that alterations in responsiveness to TGF- β may contribute to the formation of tumoral calcinosis.

Discussion

We have generated transgenic mice that express a truncated, kinase-defective TGF- β type II receptor, which acts as a dominant-negative inhibitor of TGF- β receptor signaling, in articular cartilage, periosteum/perichondrium, synovium, and in the lower zones of the growth plate. Our transgenic mice developed a progressive osteoarthritis-like disease. Besides defects in closure of the sternum, no other developmental defects were detected, suggesting that the osteoarthritis was due to a defect in maintenance of the skeletal system. Besides an anomalous, disorganized, and hypertrophic cartilage pattern, the young mice had decreased proteoglycan and increased type X collagen expression in the cartilage matrix. Our transgenic mice also demonstrated increased IHH expression, suggesting that loss of responsiveness to TGF- β results in a defect in the coordination of chondrocyte differentiation such that terminal differentiation is promoted.

Recently the dominant-negative strategy has been used to characterize the role of TGF- β s in homeostasis of skin and pancreas (6, 75). In addition, dominant-negative FGF receptor mutations have been used in transgenic mice to characterize the role of these factors in skin and lung development (49, 77). The advantages of using the dominant-negative strategy include: (a) the function of the receptor can be inhibited in specific tissues at specific times depending on the DNA regulatory elements employed. This strategy reduces the problem of embryonic lethality that is associated with targeted deletion of TGF- β ligands (28, 31, 50, 58) or the TGF- β type II receptor (46) and allows for the characterization of TGF- β function in adult mice. (b) Since signaling by all three TGF- β isoforms is mediated by the TGF- β type II receptor, signaling by all TGF- β isoforms is inhibited, avoiding problems with functional redundancy observed with targeted deletion of the TGF- β ligands (28, 31, 50, 58). There are also several disadvantages to the dominant-negative strategy. First, the dominant-negative effect requires high levels of expression of the mutant protein. Fortunately, the endogenous

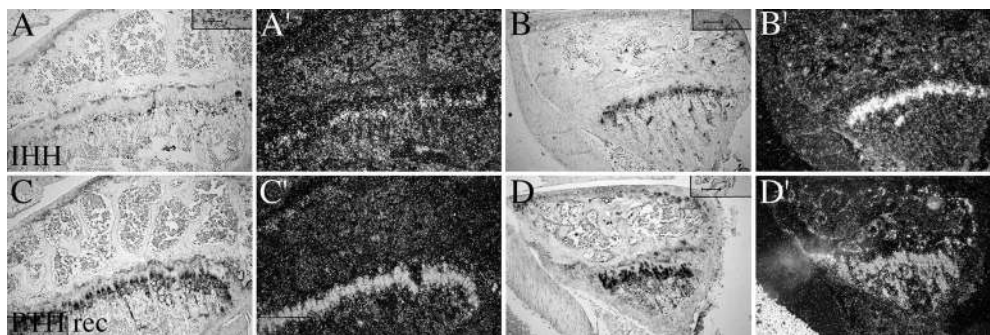


Figure 9. IHH and PTH receptor expression. Sections of knee joints from 8 wk wild-type (A and C) and MT-DNIIR transgenic (B and D) mice were hybridized to ³⁵S-labeled antisense IHH (A and B) and PTH receptor (C and D) riboprobes. Bars, 100 μm.

TGF-β type II receptor is normally expressed at very low levels. Second, the possibility exists that signaling by other members of the TGF-β superfamily could be inhibited. A dominant-negative activin receptor was shown to block signaling by Vg-1, another TGF-β family member, in *Xenopus* embryos (56). However, the skeletal phenotype of the MT-DNIIR-4 mice does not overlap with the skeletal phenotype of mice with mutations in BMP-5, BMP-7, or

GDF-5 (27, 30, 60), suggesting that the TGF-β DNIIR does not block signaling through these proteins.

Osteoarthritis

Osteoarthritis is a degenerative joint disease characterized by destruction of the articular cartilage (for review see 23). Pathological features of osteoarthritis include fibrillation of the articular cartilage, clustering and proliferation of articular chondrocytes, endochondral ossification of the articular surface (osteophytes), and cartilaginous metaplasia in the synovium and joint space. Remarkably, joints of MT-DNIIR mice display all of these pathological features. Reduction in the proteoglycan content of articular cartilage is one of the first changes observed in osteoarthritis, a feature also observed in our transgenic mice. It has been shown that TGF-β1 stimulates proteoglycan synthesis in normal and osteoarthrotic articular cartilage explants, suggesting that TGF-β plays a role in maintenance and repair of articular cartilage (34). TGF-β1 suppresses arthritis in some experimental animal models (8), but others have proposed that TGF-β is pathogenic for osteoarthritis (17, 69, 70). Intra-articular injection of TGF-β1 into murine knee joints stimulated proteoglycan synthesis but also resulted in disorganization of articular cartilage and formation of osteophytes (69, 70). Injection of TGF-β2 into rabbit joints resulted in decreased proteoglycan levels in the cartilage (17). Unfortunately, this model is complicated by the fact that TGF-β also induces inflammation in the joint (1, 17, 21, 69), and inflammatory cytokines are known to stimulate destruction of articular cartilage. In addition, constant over-stimulation with TGF-β could result in negative feedback regulation of the TGF-β response. Shifts in receptor expression and loss of responsiveness to various growth factors have been detected in cartilage from inflamed knee joints (71). Thus the relevance of these findings to the pathogenesis of osteoarthritis is unclear. Our data support the hypothesis that TGF-β plays a role in maintaining articular cartilage. Loss of responsiveness to TGF-β is likely the basis of the reduced proteoglycan expression and osteoarthritis observed in the MT-DNIIR mice.

Most cells in normal articular cartilage are mature chondrocytes arrested at a stage before terminal hypertrophic differentiation. Type X collagen is a marker for hypertrophic cartilage and has been detected in fibrillated cartilage from human osteoarthritis patients (72). This finding suggests that focal premature chondrocyte differentiation is present in osteoarthritic cartilage. TGF-β has been shown to inhibit terminal differentiation of chondrocytes

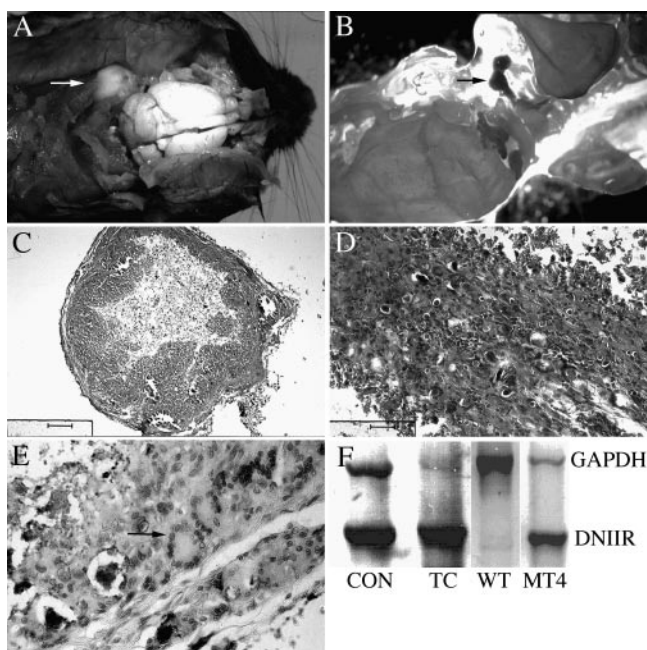


Figure 10. Tumoral calcinosis-like lesions in MT-DNIIR transgenic mice. Photograph of a large tumoral calcinosis-like lesion (arrow) from the cervical vertebrae of an MT-DNIIR transgenic mouse (A). Whole mount skeletal preparation showing alizarin red-stained tumoral calcinosis-like lesion (arrow) from the cervical vertebrae of an MT-DNIIR transgenic mouse (B). Images of hematoxylin- and eosin-stained sections from tumoral calcinosis lesion (C–E). The tumors were encapsulated and necrotic in the center (C). The tumors consisted primarily of poorly differentiated mesenchyme (B) and large multinucleated giant cells (E). Expression of DNIIR mRNA in tumoral calcinosis lesions (F). RNA from Mv1Lu cells that expressed the truncated receptor (CON), a tumoral calcinosis lesion (TC), and the hind limb of a wild-type (WT) or MT-DNIIR-4 (MT4) transgenic mice was used in RT-PCR analysis. Amplification of GAPDH cDNA was used as an internal control. The tumoral calcinosis lesion expressed high levels of the DNIIR mRNA. Bars: (C) 200 μm; (D) 100 μm; (E) 50 μm.

in culture (4, 5, 29, 67). Accordingly, the articular surface of joints in 4- and 8-wk-old MT-DNIIR mice had areas of articular cartilage that appeared hypertrophic and disorganized. Type X collagen was localized to fibrillated articular cartilage, osteophytes, and cartilage in the joint space of older MT-DNIIR mice. These observations suggest that loss of responsiveness to TGF- β in the articular cartilage results in inappropriate terminal differentiation of the chondrocytes. These changes may be mechanistically involved in degeneration of the joint, since this may alter the biomechanical properties of the articular cartilage.

The truncated TGF- β type II receptor was also expressed in the synovium of the transgenic mice. As a result, the synovium of the MT-DNIIR mice appeared hyperplastic, and cartilage metaplasia was observed in the joint space. The physiological effects of the transgene are most likely direct and not due to inflammation, since no inflammation was detected in the synovium or the joint space. Synovium secretes proteases that are known to degrade the articular matrix (25), and the expression of various proteases can be downregulated by TGF- β . In addition to the consequences of reduced responsiveness to TGF- β in the articular cartilage, excess synovium could contribute to the formation of osteoarthritis in MT-DNIIR mice by increasing the amount of protease present in the joint space.

Chondrocyte Differentiation

Changes in the shape of the epiphysis due to deregulated chondrocyte differentiation would also contribute to the formation of osteoarthritis. Indeed, loss of responsiveness to TGF- β resulted in increased hypertrophic differentiation as measured by an increase in type X collagen immunoreactivity and an increase in IHH expression in the MT-DNIIR growth plate. These data are consistent with observations showing that TGF- β prevents hypertrophic differentiation in chondrocytes grown in suspension and pellet cultures as well as in long bone rudiment organ cultures (4, 5, 16, 29, 67). IHH is normally expressed in cells committed to becoming hypertrophic located in the region of transition between the proliferating and hypertrophic zones (73, 74). Mis-expression of IHH in chick cartilage results in inhibition of chondrocyte hypertrophy and an increase in *patched*, *gli*, and *PTHrP* expression in perichondrium and periarticular cartilage (73). Mice that overexpress PTHrP in cartilage exhibit delayed chondrocyte differentiation (3, 76). PTHrP inhibits differentiation in cells that express the PTH receptor in the transition zone between proliferating and IHH-expressing chondrocytes (3, 35, 73, 76). Thus the increased expression of IHH concomitant with the increased hypertrophic differentiation in MT-DNIIR mice suggests that loss of responsiveness to TGF- β overrides this inhibitory feedback loop mediated by IHH and PTHrP. In this context, TGF- β could act directly on components of the IHH feed back loop, including expression of *patched*, *gli*, and *PTHrP*, that are located in the periosteum/perichondrium. TGF- β has been shown to induce PTHrP expression in articular chondrocyte cultures (68). Alternatively, loss of TGF- β responsiveness could promote differentiation through an independent pathway, and IHH expression would be a consequence of increased chondrocyte differentiation. In the latter case, the stimula-

tory actions of the DNIIR would be out of balance with the normal inhibitory actions of IHH, resulting in a net increase of hypertrophic cartilage.

Mice in which either the *PTHrP* or *PTH receptor* genes have been inactivated demonstrate premature cartilage hypertrophy resulting in a decrease in the amount of resting and proliferating cartilage and an overall increase in the amount of endochondral bone formation (2, 35). Our MT-DNIIR mice showed an increase in the amount of hypertrophic cartilage. Since PTHrP stimulates expression of *bcl-2* and delays terminal differentiation and apoptosis in chondrocytes (3), and TGF- β is known to induce apoptosis, it is likely that inhibition of TGF- β receptor signaling may delay the normal program of cell death associated with hypertrophic cartilage differentiation, and in this way increase the amount of hypertrophic cartilage.

Development of the Sternum

Besides an osteoarthritic phenotype in the joints, MT-DNIIR-4 mice also exhibit defects in sternal development. The sternum normally develops from lateral mesoderm that forms a pair of condensations between the clavicle at the level of the first pair of ribs at 12 d post coitus (10). Mesodermal condensations elongate and move ventromedially over the next 3 d. As the condensations elongate caudally, cells differentiate into procartilage. The procartilage does not differentiate into cartilage until the two sternal rudiments have fused. Fusion and differentiation start at the cranial end and move caudally, and this may account for the range of sternal malformations observed in MT-DNIIR mice. Bifurcation of the sternum was most common at the caudal end of the sternum. Defects in fusion of the xiphoid process were most common, but bifurcation was detected up to the level of the fifth rib. While rare, cases of congenital cleft sternum have been described in humans (51, 63), but the molecular basis of this defect is not known. Mice with mutations in the *Bmp5* gene demonstrate bifurcation of the xiphoid process, and BMP-7 null mice have holes in the xiphoid cartilage (27, 30). BMP-5 is thought to regulate mesoderm condensation at the future sites of cartilage rudiments, and defects in this process result in altered shape and size of specific bones (30, 60). Embryos homozygous for null mutations in both *Bmp5* and *Gdf5* result in disruption of sternebrae within the sternum and abnormal formation of fibrocartilage joints between the sternebrae and ribs (60). MT-DNIIR newborn mice do not have detectable defects in the size or shape of specific bones or defects in the formation of the fibrocartilagenous joints of the sternebrae. How defective TGF- β receptor signaling results in bifurcation of the xiphoid process is as yet unclear. However, TGF- β has been identified as a factor in caudal sternum cells that inhibits terminal differentiation (5). Therefore, loss of responsiveness to TGF- β may stimulate terminal chondrocyte differentiation, which in turn may interfere with fusion of the sternal rudiments in MT-DNIIR mice.

Tumoral Calcinosis

In humans, tumoral calcinosis is a benign, soft tissue tumor of uncertain origin. It is a heritable disease characterized by periarticular pseudotumors (19) primarily located near

the hip, shoulder, or elbow. Tumoral calcinosis involving the vertebrae is rare in humans, but a few cases have been reported (42). These benign masses are surrounded by a well defined capsule surrounding a chalky fluid and consist of fibrous tissue, inflammatory elements, and multi-nucleated, osteoclast-like giant cells. MT-DNIIR-4 mice developed pseudotumors of the paravertebral region, axilla, pelvis, and paws that histologically resemble tumoral calcinosis. The tumors were primarily located around the cervical vertebrae but were also seen near thoracic vertebrae, the pelvis, the axilla, and the paws. These lesions expressed high levels of the truncated receptor. The dominant-negative inhibition of endogenous receptor signaling may be at the basis of tumor development since inactivation of the type II receptor has been detected in several types of tumors (37). Furthermore, restoration of the type II receptor by stable transfection suppressed the tumorigenicity of receptor negative cells (37, 62), suggesting that the type II receptor may function as a tumor suppressor. It will be interesting to determine if mutations in members of the TGF- β superfamily or their receptors exist in human tumoral calcinosis lesions.

In summary, we have generated transgenic mice that express a dominant loss of function mutation in the type II receptor to address the role of endogenous TGF- β s in skeletal development and maintenance. Our data suggest that endogenous TGF- β s maintain cartilage homeostasis by preventing inappropriate chondrocyte differentiation. Previous experiments using in vitro models of chondrocyte differentiation have been difficult to interpret, with often contradictory conclusions possibly due to variation in the precise culture conditions used. Since interactions between cells, extracellular matrix, and growth factors are complex, we believe experimentation in vivo may be more informative, since these interactions are preserved. Our transgenic mouse model can be used to further understand the role of TGF- β s in chondrocyte differentiation and in the pathogenesis of osteoarthritis.

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