A Short Isoform of Col9a1 Supports Alveolar Bone Repair

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Bone wound created in intramembranous alveolar bone heals without the formation of cartilage precursor tissue. However, the expression of cartilage collagen mRNAs has been suggested. In this report, we examined the expression and the potential role of type IX collagen in bone restoration and remodeling. The sequence specific polymerase chain reaction demonstrated the exclusive expression of short transcriptional isoform of $\alpha 1(\text{IX})$ collagen (Col9a1) in alveolar bone wound healing, while the long isoform of Col9a1 transcript was absent. Type IX collagen was immunolocalized in the preliminary matrix organized in granulation tissue before trabecular bone formation in tooth extraction socket. In Col9a1-null mutant mice, there were considerable variations in alveolar bone wound healing with the absence of or abnormally organized trabecular bone. Occasionally, unusual apposition of cortical-bone-like layers in bone marrow space was observed. The Col9a1-null mice indicated no growth retardation, and their facial and long bones maintained the normal size and shape. However, the primary spongiosa region of adult Col9a1 mutant mice showed an abnormal trabecular bone structure associated with abnormal immunostaining with the hypertrophic cartilage specific type X collagen antibody. These data suggest that type IX collagen short transcriptional variant is involved in the restoration and remodeling processes of trabecular bone. (Am J Pathol 1999, 155:1993–1999)

Trabecular bone is interconnecting thin osseous plates contributing to bearing compression force at the terminal end of adult axial bones and the inner architecture of vertebral bones.¹ In the long bone, embryonic development of trabecular bone requires a sequential collabora-

tion between cartilage and bone tissues. The unique geometry of trabecular bone is guided, at least initially, by the calcified cartilage matrix remaining after the removal of hypertrophic chondrocytes. The abnormal size and shape of bones in patients and animals with cartilage disorders such as achondrodysplasia suggest that cartilage extracellular matrix is crucial for proper bone growth and development.²

Wounds created in the bone marrow space by bone ablation in the long bone³ and by tooth extraction in the alveolar bone^{4,5} undergo a sequential healing process that restores trabecular bone structure without cartilage precursor tissue formation. Histological and ultrastructural studies report the formation of a preliminary matrix composed of thin collagen fibers that are synthesized during the initial stages of wound healing before trabecular bone restoration.^{6,7} The preliminary matrix is formed within the granulation tissue and connects the neighboring bone and soft tissues. Disturbance of the preliminary matrix results in the lack of trabecular bone restoration. However, the composition and functional role of this initial preliminary matrix have not yet been fully elucidated.

Studies of the rat tooth extraction model has suggested the temporal expression of cartilage collagen types II and IX at the steady-state transcriptional level during the early stages of wound healing.^{4,5} The $\alpha 1(\text{IX})$ collagen (Col9a1) gene has two independent promoter/transcriptional start sites that are used to synthesize long and short isoforms⁸ in a tissue-specific fashion, including osteoprogenitor cells.⁹ In this study, we focused on type IX collagen as a candidate component of the preliminary matrix appearing before trabecular bone restoration. We demonstrated that the short type IX collagen isoform was likely to be involved in the preliminary matrix during trabecular bone restoration, and that the null mutation of the Col9a1 alleles in mice significantly disturbed the restoration and remodeling of trabecular bone with minimal effects on the cortical bone.

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Materials and Methods

Cloning and Characterization of the Downstream Promoter/Transcriptional Start Site of the Rat Col9a1 Gene

A polymerase chain reaction (PCR) product spanning the entire intron sequence between exons 6 and 7 of Col9a1 was amplified from rat genomic DNA using two flanking primers derived from the coding sequences of exon 6 (5'-CUA CUA CUA CUA CGG AGA GAG ACG TGC-3') and exon 7 (5'-CAU CAU CAU CAU CTC ATC GGT GGT CTG-3'). A PCR product was cloned and sequenced.

The downstream transcriptional start site was characterized using a combination of the RNase protection assay and the primer extension assay. The isolated PCR fragment of genomic DNA (HR1) containing tentative exon 1* was used to generate anti-sense and sense riboprobes. An extension primer was designed to encode the reverse and complementary sequence of the 3' end of the alternative exon 1* (5'-TTG AGC AGC ACA TAA CG-3'). RNase protection and primer extension experiments were performed using poly(A)+ RNA isolated from rat calvarial osteoprogenitor cells, which contains only short isoform of Col9a1.^{4,9} The protected riboprobe and the primer extended products were analyzed on a 5% sequence gel. The downstream transcriptional start site in the HR1 sequence was identified as simultaneously indicated by RNase protection and primer extension assays.

Expression of Col9a1 Transcripts during Alveolar Bone Wound Healing

Forty-day-old Sprague-Dawley rats were anesthetized by an intramuscular injection of ketamine (1.5 mg/100 g body weight) and rompun (0.824 mg/100 g body weight). Maxillary molars were extracted with a dental explorer under a dissection microscope.¹⁰ The alveolar bones containing the healing extraction socket were harvested before tooth extraction and at the 4-day and 7-day post-extraction periods, and total RNA for each sample was prepared separately. A total RNA sample was also isolated from adult rat epiphyseal tissues containing hyaline cartilage and subarticular bone.

Long and short transcripts of the Col9a1 gene were identified by reverse transcriptase PCR using the combinations of a common 3' primer (5'-CCG GAA CTC CAG GAG GC-3') and one of the following specific 5' primers: 5'-GTA GAC TTC AGG ATT CCA-3' for the long Col9a1 transcript or 5'-TTG CAA CCA CTA CCC TG-3' for the short Col9a1 transcript (based on the rat alternative exon 1* sequence determined in the present study). The PCR products representing the long (583 bp) and the short (261 bp) Col9a1 transcripts were identified by Southern blotting using a radiolabeled oligonucleotide probe 5'-AGA GGT CCT CCG GGT GAG CAG GGG-3'. For a housekeeping control gene, GAPDH primers were used in a PCR experiment.

In Situ Hybridization

The healing maxillary alveolar bone tissues were harvested at day 4 postextraction and immediately fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), pH 7.4, for 2 days at 4°C. The specimens were treated for conventional paraffin embedding after decalcification. Some histological sections stained with hematoxylin and eosin (H&E) were examined for wound healing and bone formation. For *in situ* hybridization, the optimal method for the rat alveolar bone tissue has been previously established.⁴ An 800-bp cDNA encoding rat Col9a1, EKT101, was radiolabeled with ³⁵S dATP by nick translation and served as probe. After counterstaining with Harri's alum-hematoxylin, *in situ* hybridization sections were examined under a light microscope.

Immunohistology

Eight-micron sagittal sections of the 4-day postextraction specimen were cut and mounted on silane-prep slides. Deparaffinized sections were treated with 10 mg/ml hyaluronidase in 0.1 mol/L sodium acetate buffer at 37°C for 1 hour. This was followed by an incubation in 1% hydrogen peroxide-methanol at room temperature for 30 minutes to block endogenous peroxide. Sections were treated with Tris-buffered saline (TBS) and then blocked with 10% normal goat serum in TBS at room temperature for 30 minutes in a humid chamber. Monoclonal antibodies (mAbs) B3-1 and D1-9 were applied and incubated at 4°C for 2 hours in a humid chamber. mAbs B3-1 and D1-9 were generated against the bovine low molecular weight pepsin-digested triple helical fragment containing Col9a1, Col9a2, and Col9a3 (kindly provided by Dr. Ye, University of Tennessee at Memphis and VA Medical Center, Memphis, TN). B3-1 and D1-9 cross-react with type IX collagen of different species including chicken, bovine, rat, and human.¹¹ The slides were rinsed with TBS, and secondary antibody was applied. A standard avidin-biotin complex/immunoperoxidase protocol was used to visualize the immunolocalization of rat type IX collagen.

Genotyping of Mice with Inactivated $\alpha 1$ (IX) Alleles

Type IX collagen knockout mice have been generated and described.¹² The *neo* gene was inserted into exon 8 of the Col9a1 gene by homologous recombination, resulting in inactivation of the Col9a1 alleles. Homozygous knockout mice have lost the ability to synthesize both the short and long forms of the Col9a1 chain. The skeletal development and gross abnormality of mice were evaluated by lateral radiographs.

Genotype was established using 1 cm of tail tendon harvested from each offspring (at about 3 weeks of age) of heterozygous mutant parents. The tail tendon was transferred into a 700- μ l lysis mix containing 10 mg/ml proteinase K in 100 mmol/L Tris-HCl, pH 8.5, 5 mmol/L EDTA, 0.2% sodium dodecyl sulfate, and 200 mmol/L

Figure 1. A: Diagram of the $\alpha 1(\text{IX})$ collagen gene containing the upstream (UP-Pm) and downstream (DW-Pm) promoter/transcriptional start sites. The alternative use of these promoter/transcriptional start sites results in generation of long and short $\alpha 1(\text{IX})$ collagen transcripts differing in the amino-terminal NC4 domain encoded by exons 1–7. **B:** The nucleotide sequence of the rat $\alpha 1(\text{IX})$ collagen downstream promoter/transcriptional start site. Exon 6, the alternative exon 1*, and exon 7 are highlighted by overline with the translated amino acid sequence. The consensus DNA sequences, i.e., TATA box, CAAT boxes, and intron donor and recipient sites, are underlined.

NaCl at 55°C for 6 hours. Supernatant was collected after 30 minutes' centrifugation and transferred to 700 μl of ice-cold isopropanol. Precipitated DNA was removed and suspended in 10 mmol/L Tris, pH 7.5, 0.1 mmol/L EDTA. Thirty-microliter samples were digested with *Eco*RI overnight. Genomic fragments were separated by electrophoresis run at 30 V for 6 hours. Southern blot analysis was performed with a radiolabeled DNA probe located in Col9a1 between the *Hind*III and *Aba*I sites.

Histological and Immunohistological Examinations of Trabecular Bone Restoration and Remodeling

Littermates of 4- to 5-week-old wild-type mice ($n = 10$) and homozygous Col9a1-null mutant mice ($n = 10$) were used. Animals received an intramuscular injection of ketamine (1.5 mg/100 g body weight) and rompun (0.824 mg/100 g body weight) for anesthesia, and unilateral maxillary molars were extracted with a dental explorer. Animals were sacrificed at 7 and 14 days after extraction. Maxillae were harvested and prepared for histological evaluation. Coronal and sagittal sections were cut. In coronal sections, maxillary tissues were oriented to provide standardized sections perpendicular to the midpalatal suture mesial to

the remaining second molar. Sagittal sections were made along the midpalatal suture. Sections were stained with H&E and examined under a light microscope.

Mandibular condyles of 8-week-old homozygous Col9a1-null mutants and normal mice were selected for examination of trabecular bone remodeling. The histological sections were prepared as above, stained with Alcian blue, and counterstained with H&E. The mandibular condyles were further examined immunohistochemically with anti-type X collagen antibody.¹³ Harvested condyles were embedded in OCT media and frozen at -80°C . Ten-micron sections were prepared in a cryostat and subjected to an immunohistological protocol as described above.

Results

The isolated rat genomic DNA clone, HR1, contained an 824-bp insert, which included the 764-bp intron sequence between exons 6 and 7 of the Col9a1 gene (Figure 1). The alternative exon 1*, a 212-bp sequence, was identified within the intron sequence, which contained an open reading frame started by ATG (Met) followed by a leader peptide sequence. The 5' end cap site of exon 1* was defined by a combination of RNase pro-

Figure 2. Southern blot analysis of the PCR products depict the presence of short $\alpha 1(\text{IX})$ transcript ($\alpha 1(\text{IX})\text{S}$) (**A**) and the absence of long $\alpha 1(\text{IX})$ transcript ($\alpha 1(\text{IX})\text{L}$) (**B**). The RNA samples from the wound healing alveolar bone tissue were harvested immediately after tooth extraction (0) and at days 4 (4) and 7 (7) of healing periods. The RNA sample from adult rat tibia (T) was also used. When the root of a tooth (t) is removed from the alveolar bone (**C**), a sequential wound healing process is activated. During day 4 of the healing period, the granulation tissue gives rise to a preliminary matrix (*) as an extension from the residual alveolar bone (**D**, ab). The preliminary matrix is further organized to form trabecular bone in the extraction socket at day 7 after extraction (**E**, arrows).

Figure 3. **A:** A day 4 postextraction socket showed the new trabecular bone (tb) that was formed after the preliminary matrix. **B:** *In situ* hybridization showed that cells synthesizing $\alpha 1(\text{IX})$ collagen mRNA (arrows) formed a cluster near the alveolar bone and appeared to associate with relatively unorganized preliminary matrix (*) ($\times 80$, hematoxylin counterstaining with a blue filter during microphotography). **C:** Immunohistology showed that the preliminary matrix (*) contained the type IX collagen epitope. The distribution of the type IX collagen was indicative of a pattern of trabecular bone ($\times 100$, hematoxylin counterstaining).

tection assay and primer extension assay (data not shown). From the cap site, a TATA consensus sequence was found at -30 bp, and the CCAAT transcription factor/nuclear transcription factor-1 (CTF/NF-1) sites were found at -62 and -98 bp (Figure 1).

Tooth extraction in rats and mice created a maxillary alveolar bone wound, which healed by intramembranous bone formation without a cartilage template formation. The granular tissue in the wound site was organized at approximately day 4 after extraction, followed by active trabecular bone formation starting at approximately day 7 (Figure 2). In the RT-PCR analysis, short Col9a1 mRNA was identified in the healing alveolar bone. Its steady-state level appeared to be temporarily up-regulated at day 4. The primer set for long Col9a1 mRNA failed to amplify the corresponding PCR product in the healing alveolar bone. Both the long and short isoforms of Col9a1 mRNA were present in epiphyseal tissue containing hyaline cartilage and subchondylar bone (Figure 2).

The organized granulation tissue at day 4 after extraction contained the less cellular, amorphous extracellular matrix stained a bright pink color by H&E (Figure 3A). This preliminary matrix appeared to be connected to the alveolar bone surface. In some areas, immature trabecular bone was seen as an extension from the alveolar bone surface (Figure 3, B and C).

The spatial expression of type IX collagen was examined in the day 4 postextraction specimens by *in situ* hybridization. Col9a1 transcripts appeared to be synthesized by a cluster of cells that surrounded the amorphous preliminary matrix (Figure 3B).

Immunohistochemistry experiments with anti-type IX collagen antibody indicated that type IX collagen was a component of the preliminary matrix (Figure 3). Within this

amorphous region of the preliminary matrix, type IX collagen immunostaining resembled the trabecular bone structure and the immunostaining-negative areas were associated with small blood vessels. The Type IX collagen immunostaining was also found over the new trabecular bone and on the surface of newly formed trabecular bone matrix. However, in old bone and cortical bone, type IX collagen immunostaining was not observed (data not shown).

Col9a1-null mice have been generated by homologous recombination of a *neo* gene inserted into exon 8 (Figure 4A), resulting in the lack of expression of both the long and short Col9a1 mRNAs.¹² The Col9a1-null mice exhibit minimal developmental and pathological phenotypes, and hyaline cartilage was developed and maintained normally. Wild-type (+/+) and homozygous Col9a1-null (-/-) mice, as determined by genomic Southern blot analyses (Figure 4B), indicated similar skeletal features on radiographs (Figure 4C). The size and shape of bones including the mandible did not show significant difference between the mutants and controls.

Tooth extraction was performed in homozygous Col9a1-null mutants and control mice. At day 7, the control mice exhibited well organized trabecular bone restoration in the healing site, whereas the extraction socket of Col9a1-null mice showed considerable variability. It was often observed as a thick cortical-bone-like tissue formed directly on the bony wall that was lined by a layer of osteoblast-like cells (Figure 5). The restored trabecular bones frequently exhibited unusual shapes. Some sockets showed minimal bone formation activity and were filled with dense fibrous tissues with large cells. At day 14, the sockets of normal mice were healed with more matured trabecular bone, whereas the healing alveolar

Figure 4. **A:** The inactivation of the $\alpha 1(\text{IX})$ collagen gene alleles was achieved by inserting a *neo* gene in exon 8, which is located downstream of the alternative promoter. **B:** Wild-type mice (+/+) carried the 3.5-kb *EcoRI* restriction fragment of genomic DNA containing normal exon 8, whereas the *neo* gene homologous recombination resulted in the 3.0-kb *EcoRI* restriction fragment, which was seen in heterozygous (+/-) and homozygous (-/-) mutant mice. **C:** As compared to wild-type mice (+/+), homozygous *Col9a1*-null mice (-/-) exhibited no skeletal abnormalities in size and shape.

bone of *Col9a1*-null mice was composed of disorganized bone structure (Figure 5). Histological examination of mandibular condyles revealed that the primary spongiosa of *Col9a1*-null mutants retained Alcian blue-stained hypertrophic cartilage matrix that was impregnated within the bone trabeculae. Immunostaining of type X collagen demonstrated its distinct localization within the hypertrophic cartilage zone of normal mice, whereas *Col9a1*-null mutants exhibited positive immunostaining not only in the hypertrophic cartilage zone but also in the entire primary spongiosa, including the trabecular bone structure (Figure 5).

Discussion

Type IX collagen is a heterotrimer composed of three polypeptide chains, *Col9a1*, *Col9a2*, and *Col9a3*, that are derived from separate genes located on human chromosomes 6q12-q14,^{14,15} 1p32.3-p33,^{16,17} and 20q13.3,¹⁸ respectively. The *Col9a1*, *Col9a2*, and *Col9a3* chains exhibit the characteristic structure of the fibril-associated

Figure 5. **A:** In wild-type mice, trabecular bone (tb) pattern was established in a typical tooth extraction socket at day 7 (40 $\times$, H&E). **B:** At day 14, the extraction socket of wild-type mice was filled with trabecular bone (tb) (40 $\times$, H&E). **C:** Trabecular bone in the wild-type socket maintained the bone marrow space (100 $\times$, H&E). **D:** In homozygous *Col9a1*-null mutant mice, trabecular bone pattern was not well developed in the tooth extraction socket at day 7 (40 $\times$, H&E). **E:** Disorganized bone remodeling appeared in *Col9a1*-null mutant mice resulting in disturbed wound healing observed in an extraction socket at day 14 (40 $\times$, H&E). **F:** *Col9a1*-null mutant mice often exhibited a layer of cortical-type bone in the extraction socket, which was lined by osteoblast-like cells (arrowheads) (100 $\times$, H&E). **G:** Cross-section of the mandibular condyle from an adult wild type mouse, depicting the matured trabecular bone structure (20 $\times$, Alcian blue with H&E). **H:** Immunostaining with anti-type X collagen antibody was limited to hypertrophic cartilage zone (arrow) in the wild-type mandibular condyle. The primary spongiosa of trabecular bone was completely negative for the type X collagen immunostaining (20 $\times$). **I:** Mandibular condyle trabecular bone of a *Col9a1*-null mutant mouse was composed of unusual lamellar bones associated with unresorbed cartilage matrix stained with Alcian blue (20 $\times$, Alcian blue with H&E). **J:** The anti-type X collagen antibody stained not only the hypertrophic cartilage zone but also the entire area of primary spongiosa and trabecular bone (arrow, 20 $\times$).

collagens with interrupted triple helices and consist of short triple-helical or collagenous domains (COL1–3) that are flanked by non-triple-helical domains.¹⁹ As compared to the *Col9a2* and *Col9a3* chains, the *Col9a1* gene contains seven additional exons at its 5' end that encode for a large amino-terminal globular domain (NC4).^{20,21} In the chicken *Col9a1* gene, an alternative promoter/transcriptional start site has been identified within the intron between exons 6 and 7. The short *Col9a1* collagen transcript is driven from this alternative downstream promoter, and thus lacks the entire coding sequence for the NC4 domain.⁸ The newly characterized rat *Col9a1* gene in this study showed homologous gene structure at the downstream transcriptional start site. The Pustell DNA matrix analysis between the downstream promoter region of the chicken and rat *Col9a1* collagen gene revealed six highly conserved sequences ranging in size from 7 to 15 bp located at conserved distances from the cap site (data not shown).

Examination of the rat tooth extraction model undergoing bone wound healing has suggested expression of type II collagen and short type IX collagen transcripts without formation of cartilage tissue. The lack of the NC4 domain in the short type IX collagen molecule has been postulated to prevent, in part, the formation of cartilage tissue in the extraction socket.⁴ However, because type IX collagen is not a matrix component of mature bone of either endochondral or intramembranous origin,^{22–25} the role of short type IX collagen in bone wound healing has not been established. This study further characterized the expression pattern of the short Col9a1 collagen transcript during the early bone remodeling stages. A combination of *in situ* hybridization and immunohistological studies indicated the strong association between type IX collagen and the relatively unorganized amorphous fibrous matrix in the tooth extraction socket. The histological characteristics of type IX collagen-containing matrix were unlike either bone or cartilage; rather, they resembled the description of preliminary matrix that has been reported during bone marrow ablation wound healing as a prerequisite for intramembranous trabecular bone restoration.⁷ The nature of the preliminary matrix has not been well understood. This study demonstrated, for the first time, that type IX collagen was a protein component of the preliminary matrix. The monoclonal antibodies used in this study recognize the cognitive epitopes of the type IX collagen heterotrimer structure containing Col9a1, Col9a2, and Col9a3. Because type IX collagen is found covalently associated with type II collagen fibril in hyaline cartilage,^{26,27} type IX collagen in the preliminary matrix may also form a composite collagen fibril with type II collagen. The missing amino-terminal globular domain from the short type IX collagen molecule due to Col9a1 alternative transcription may determine the unique surface topography and chemistry of the preliminary matrix. It is tempting to speculate that the preliminary matrix may contribute to the osteoblast homing through the type II/IX collagen composite fibril.

Mice carrying abnormal Col9a1 chains exhibit early onset of osteoarthritic cartilage degradation.^{28,29} Immortalized chondrocytes lacking the Col9a1 chain synthesize thicker, or fused, type II collagen fibrils *in vitro*.³⁰ However, investigation of Col9a1-null mutant mice has shown only minimal pathological phenotypes in cartilage tissues.¹² The young adult Col9a1-null mutant mice (4–8 weeks old) did not show observable growth retardation or abnormalities in skeletal size and shape, including craniofacial bones. In the present study, Col9a1-null mutant mice were combined with the tooth extraction model to address the role of type IX collagen in bone wound healing. Unlike the consistent trabecular bone formation in the wild-type mice, the mutant tooth extraction socket showed various healing patterns. Trabecular bone restoration was often disturbed, and, in some cases, unusual new cortical-bone-like formation took place in the tooth extraction socket. Due to alternative mRNA processing, the primary structure of the short Col9a1 chain resembles that of the Col9a2 and Col9a3 chains.^{8,31} It is conceivable that the missing short Col9a1 chain can be effectively substituted with the Col9a2 or Col9a3 chain. Al-

though this potential compensation phenomenon must be addressed, our data support that the Col9a1-null mutation primarily affects the sound restoration of trabecular bone.

Mandibular condyles of 8-week-old homozygous Col9a1-null mutants and normal mice were selected for examination of trabecular bone remodeling. Mandibular condyle is initially organized as a mass of secondary cartilage, which lacks an active proliferation. Chondrocyte hypertrophy occurs at around the birth in rats and mice, and type X collagen is synthesized similarly to primary cartilage.³² The formation of trabecular bone rapidly replaces hypertrophic cartilage, which becomes only several cell layers by postnatal day 14. Type X collagen is an exclusive product of hypertrophic chondrocytes.³³ Therefore, the extended immunostaining of type X collagen found in both the hypertrophic cartilage zone and the primary spongiosa of Col9a1-null mice is a unique observation, and has not been reported previously.

Type X collagen has been reported to form hexagonal matrix aggregate *in vitro*.³⁴ Type X collagen shares the structural homology with type VIII collagen,³⁵ which forms the hexagonal matrix of cornea endothelial Descemet's membrane.³⁶ The postulated functions of type X collagen during endochondral ossification span from a structural component rigidly supporting the hypertrophic cartilage matrix column^{32,37} to an angiogenic factor.³⁸ Although the exact role of type X collagen is not yet fully elaborated, it is well established that type X collagen is localized predominantly at the matrix/cell interface within the hypertrophic cartilage zone.³⁹ Removal of hypertrophic chondrocytes exposes the type X collagen membrane to the invading osteogenic cells, and the immediate disappearance of type X collagen during formation of the primary spongiosa bone may therefore be explained by an active matrix remodeling.

Our tooth extraction study suggests the involvement of Col9a1 in the preliminary matrix, which may provide a temporary scaffold and homing site for osteoblasts responsible for trabecular bone formation. The Col9a1 mutation may alter this homing mechanism of the osteoblasts. The interference of osteoblast homing may then limit the recruitment of osteoclasts, resulting in the abnormal retention of hypertrophic cartilage matrix containing type X collagen. Further studies are needed to address these hypotheses.

Differentiated osteoblasts contribute differently to adult cortical and trabecular bones, which vary in matrix components^{40–42} and metabolic reactions such as postmenopausal osteoporosis and diabetes.^{43,44} To date, little is known about how adult trabecular and cortical bones undergo distinct remodeling processes. Our data suggest that there may be heterogeneous bone remodeling processes and that the transient expression of short type IX collagen by its osteoblasts is required for trabecular bone remodeling.

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