Skeletal Abnormalities in Mice Lacking Extracellular Matrix Proteins, Thrombospondin-1, Thrombospondin-3, Thrombospondin-5, and Type IX Collagen

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Thrombospondin-5 (TSP5) is a large extracellular matrix glycoprotein found in musculoskeletal tissues. TSP5 mutations cause two skeletal dysplasias, pseudoachondroplasia and multiple epiphyseal dysplasia; both show a characteristic growth plate phenotype with retention of TSP5, type IX collagen (Col9), and matrillin-3 in the rough endoplasmic reticulum. Whereas most studies focus on defining the disease process, few functional studies have been performed. TSP5 knockout mice have no obvious skeletal abnormalities, suggesting that TSP5 is not essential in the growth plate and/or that other TSPs may compensate. In contrast, Col9 knockout mice have diminished matrillin-3 levels in the extracellular matrix and early-onset osteoarthritis. To define the roles of TSP1, TSP3, TSP5, and Col9 in the growth plate, all knockout and combinatorial strains were analyzed using histomorphometric techniques. While significant alterations in growth plate organization were found in certain single knockout mouse strains, skeletal growth was only mildly disturbed. In contrast, dramatic changes in growth plate organization in TSP3/5/Col9 knockout mice resulted in a 20% reduction in limb length, corresponding to similar short stature in humans. These studies show that type IX collagen may regulate growth plate width; TSP3, TSP5, and Col9 appear to contribute to growth plate organization; and TSP1 may help define the timing of growth plate closure when other extracellular proteins are absent. (Am J Pathol 2008, 172:1664–1674; DOI: 10.2353/ajpath.2008.071094)

Thrombospondin-5 (TSP5), also known as cartilage oligomeric matrix protein, is the fifth member of the TSP gene family that includes trimeric (TSP1 and TSP2) and pentameric (TSP3, TSP4, and TSP5) protein members.1 First identified in cartilage and considered to be cartilage specific, TSP5 was subsequently found in other musculoskeletal tissues and is used as a marker for osteoarthritis and joint injury.2–9 TSP5 has been intensely studied, because mutations in this gene were shown to cause pseudoachondroplasia and multiple epiphyseal dysplasia (MED/EDM1).10–17 Mutations result in a dominant-negative effect on TSP5 causing massive retention of mutant TSP5 and other extracellular matrix (ECM) proteins, including types II and IX collagen and matrillin-3 (MATN3), in large rough endoplasmic reticulum cisternae.15,18–20 Recent studies show that these ECM proteins associate with TSP5 to form a structured intracellular matrix network.20 Interestingly, mutations in MATN3 (MED/EDM5) and all three type IX collagen (Col9) genes also cause MED phenotypes (MED/EDM2, -3, and -6) that are typically milder than the MED condition caused by TSP5 mutations.21

Different approaches have attempted to define the role of TSP5. Recent work suggests that TSP5 stimulates chondrocyte proliferation through the binding of granulin-epithelin precursor, an autocrine growth factor.22 Binding experiments demonstrate that TSP5 interacts with types I, II and IX collagens, MATN3, aggrecan, granulin-epithelin
The presence of an ordered intracellular matrix network in pseudoachondroplasia chondrocytes and colocalization of these proteins in the normal ECM suggest that TSP5 has a significant role in the ECM. These observations support a model in which TSP5, type IX collagen, and MATN3 interact and function to establish and maintain the homeostasis of the ECM. Furthermore, TSP1 and TSP3 are present in the growth plate and may play similar and/or redundant roles with TSP5 in ECM.

TSP1, TSP3, TSP5, and type IX collagen knockout mice were previously evaluated in other studies. Interestingly, loss of type IX collagen, TSP1, TSP3, TSP5, or MATN3 reportedly causes minor or no overt phenotypic changes, and life spans were normal. Type IX collagen knockout mice have early-onset osteoarthritis, suggesting that the loss of this protein affects matrix integrity and permits articular cartilage erosion. TSP1- and TSP2-null mice have wound-healing abnormalities and minor craniofacial dysmorphism, whereas TSP3 knockout mice show accelerated endochondral ossification and increased trabecular bone in the femoral head. The MATN3 knockout mouse has higher bone mineral density and increased prevalence of arthritis. In contrast, TSP5 knockout mice show no reported phenotypic abnormalities.

The TSP genes have been implicated in different disease processes, not limited to the growth plate. TSP3 is highly expressed in osteosarcomas and associated with metastasis. Single nucleotide polymorphic variants in TSP2 and TSP4 are associated with early-onset coronary disease, with the TSP4 A387P variant specifically associated with atherosclerosis. Only TSP5 mutations have been found to cause defined disease pathology, whereas variation in the other TSP genes contributes to the development of complex human disorders.

In this study, growth plates from single and combinatorial knockout mouse strains were systematically evaluated to elucidate the roles of type IX collagen and TSP1, TSP3, and TSP5 in the growth plate. We show that the loss of individual proteins affects growth plate size and organization, whereas compound loss exacerbates growth plate disorganization and causes short limb length.

### Materials and Methods

#### Knockout Mice

Four previously described knockout mouse lines, TSP1, TSP3, TSP5, and Col IX, were evaluated. Controls and compound knockout strains, TSP5/Col9, TSP3/5, TSP1/3/5, TSP3/5/Col9, TSP1/5/Col9, and TSP1/3/5/Col9, were generated and are shown in Table 1. Because the genetic background was not the same for all strains, controls were bred for each strain. Genotyping primers specific for each knockout strain are shown in Table 2 as is the original reference describing the knockout strain.

#### Histology and Immunohistochemistry

Hind limbs from control and knockout mice were obtained from 1- and 2-month-old mice, and the growth plate from upper tibias were analyzed in all studies. The limbs were fixed in Carson Millonig, pH 7.4 (10% formalin), or ethanol, and decalcified for 1 week in Immunocal formic acid decalifier solution (American Master Tech Scientific, Inc., Lodi, CA). After embedding in paraffin, 5-μm sections were stained with H&E, following standard protocols.

For immunohistochemistry, the sections were digested with 1 mg/ml pepsin A (Sigma-Aldrich, St. Louis, MO) in 0.1 N HCl for 30 minutes at room temperature for all antibodies except type X collagen. Sections for type X collagen staining were digested in 1 mg/ml hyaluronic acid decarboxylase (Sigma-Aldrich). After embedding in paraffin, 5-μm sections were stained with H&E, following standard protocols.

### Table 1. Mouse Knockout Strains Used and Control Background Strains

<table>
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<th>Strain</th>
<th>Background</th>
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<tr>
<td>TSP1/Col9</td>
<td>C57BL/6</td>
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<tr>
<td>TSP3/5/Col9</td>
<td>(129SvJ × C57BL/6) × C57BL/6</td>
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### Table 2. Genotype Primer Sequences

<table>
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<td>Hankenson et al32</td>
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<td>Col9</td>
<td>5'-GGATACGAGAGATGTGCAGTAC-3'</td>
<td>Nakata et al43</td>
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Growth Plate Abnormalities in ECM Knockout Mice 1665
AJP June 2008, Vol. 172, No. 6
To control for any possible strain differences, all growth plates from each strain were compared with their respective control background strain listed in Table 1. Upper tibial growth plates were obtained from at least three different mice of each strain. Growth plate organization was blindly assessed using the following methods. Sections were then incubated overnight at 4°C with the primary antibody. An affinity-purified goat polyclonal MATN3 antibody was used at 1:500 (R&D Systems, Minneapolis, MN) and goat polyclonal type II collagen antibody at 1:200. Affinity-purified donkey anti-goat biotin-conjugated secondary antibody (Chemicon International, Temecula, CA) was used at a dilution of 1:500 (for MATN3) or 1:300 (for type II collagen) for 30 minutes, followed by horseradish peroxidase-conjugated streptavidin (Chemicon International) for 30 minutes. The results were detected using 3,3′-diaminobenzidine (Sigma-Aldrich). A polyclonal rabbit primary TSP5 antibody was used at a 1:200 dilution (Kamiya Biomedical Co., Thousand Oaks, CA), and rabbit polyclonal type X collagen antibody was used at 1:200 (gift from Dr. Lunstrum, Shriners Hospital for Children, Portland, OR). Affinity-purified donkey anti-rabbit biotin-conjugated secondary antibody (Chemicon International) was used at a dilution of 1:400 (for both TSP5 and type X collagen) for 30 minutes, followed by horseradish peroxidase-conjugated streptavidin (Chemicon International) for 30 minutes. The results were also detected using 3,3′-diaminobenzidine (Sigma-Aldrich). For type IX collagen staining, a mouse monoclonal at 1:150 (Hybridoma bank at the University of Iowa) was incubated with sections overnight and detected using the Histomouse kit (Zymed Laboratories, South San Francisco, CA). All sections were examined, and images were captured using an Olympus BX51 light microscope (Olympus, Melville, NY) attached to a Spot RT camera Ver 4.6.4.2.

Growth Plate Analysis

To control for any possible strain differences, all growth plates from each strain were compared with their respective control background strain listed in Table 1. Upper tibial growth plates were obtained from at least three different mice of each strain. Growth plate organization was blindly assessed using the following methods. Sections were examined at ×100 magnification, and cells were designated as being arranged in columns or clusters using Image Pro (Media Cybernetics, Silver Spring, MD). Lone cells or cells arranged in small groups were classified as clustered to differentiate them from cells organized in columns (see Figure 2A). The growth plate was digitally outlined at ×40 magnification, and the area was measured using Image Pro. Measurements of the area were calibrated using a microscopic grid. Chondrocyte density in the growth plate was calculated by dividing the total number of chondrocytes by the area of the growth plate per 100 μm². The chondrocyte density and counts were averaged from at least three sections from different limbs from each strain and their respective controls. Similarly, five measurements spaced along the width of the growth plate were taken from three sections of different limbs using Image Pro software, and the measurements were then averaged (see Figure 3A).

Limb Length Measurements

Soft tissue was removed from hind limb, and the resulting skeletal tissue was fixed in 70% ethanol and then subjected to radiographical examination. All limbs were attached to a grid to ensure consistency and accuracy of measurements, and at least 10 hind limbs were measured for each strain. Measurements were made from end-to-end of both long bones in the hind limb (see example in Figure 4A, below) using Image Pro software. The measurements were converted to millimeters by measuring an object of known length and calculating the pixel-to-millimeter ratio.

Exercise Protocol

Two to four male mice from TSP5, Col9, and TSP5/Col9 knockout strains and the controls were subjected to an exercise protocol in which they were allowed to run from age 6 weeks to 5 months. The rodent wheel was held in place by a heavy metal plate, and the distance run by each mouse was recorded using a VDO C10+ magnetic bicycle monitor (Cytec, Rohrbach, Germany). Daily distances were recorded both manually and digitally from Monday through Friday, and distance traveled on Saturday and Sunday was recorded with the digital monitor only. All housing conditions and experiments were conducted in compliance with University of Texas Medical School at Houston animal care standards (protocol no. 02-022 and 05-020).

Results

Growth Plate Abnormalities

These experiments were performed to assess the roles of TSP1, TSP3, and TSP5, and type IX collagen in the growth plate. We first asked whether the loss of one or more of these extracellular proteins affected development and longevity. All offspring were liveborn, premature death was not observed, and there was no deviation from Mendelian expectations.

We next investigated what effect the loss of individual TSP proteins and type IX collagen had on growth plate organization and size. We evaluated the growth plates at 1 and 2 months of age because these time spans represent periods of rapid growth, after which the mouse growth plate is essentially quiescent and limb growth ceases. Chondrocyte growth plate disorganization was observed in all of the individual knockout strains at both ages with the least effect seen in the TSP1 strain (Figure 1A). In general, the columnar patterning was disrupted, and there was more clustering of chondrocytes. The TSP3/5-null growth plate was similar to those of the TSP3- and TSP5-null mice at 1 month (Figure 1A, panels E, L, G, N, and 1B, panels B and F). More disorganization...
was observed in the TSP5/Col9 knockout growth plate (Figure 1B, panels D and H) compared with either null strain alone (Figure 1A, panels B, I, G, and N). The most dramatic disorganization was observed in the TSP3/5/Col9 knockout growth plate (Figure 1C, panels D and H). This disorganization is similar to that observed when β1-integrin is inactivated in chondrocytes, resulting in chondrocyte rotation. The additional loss of TSP1 with
any of the other combinations did not appear to contribute to chondrocyte disorganization (Figure 1).

The organization of the chondrocytes in the growth plate was quantified by measuring the number of chondrocytes in columns or clusters. At 1 and 2 months, the controls had 65 to 85% of the chondrocytes in columns, compared with the knockout strains, which had 10 to 42% in columns (Figure 2). The TSP3/5/Col9 knockout had very few chondrocytes in columns (Figures 1C, panels D and I, and 2). These results suggest that TSP3, TSP5, and

**Figure 2.** Comparison of growth plate chondrocyte organization in knockout mice and controls. **A:** The method used in the classification of chondrocytes is shown and described in Materials and Methods. **B:** Comparison of percent chondrocytes organized into columns in all knockout strains and respective controls at 1 month of age. **C:** Comparison of percent chondrocytes organized into columns in all knockout strains and respective controls at 2 months of age. Black and gray bars are measurements for controls and strains, respectively. All knockout strains showed disrupted chondrocyte columns, with the TSP1 knockout the least affected and the TSP3/5/Col9 and TSP1/3/5/Col9 knockout strains the most affected. *P < 0.05. Upper tibial growth plates were used in all studies.

**Figure 3.** Loss of type IX collagen affects the width of growth plate. **A:** Measurements of growth plates was performed as shown on the images and described in Materials and Methods. PZ, proliferative zone; GP, growth plate. **B:** Comparison of growth plate width at 1 month. Black and gray bars are measurements for controls and strains, respectively, and the asterisk indicates a significant difference from the control (*P < 0.05). The overall growth plate of the Col9 and combinatorial knockout strains lacking Col9 tend to be wider compared with the controls. **C:** By 2 months, there is no difference in the width of the Col9 growth plate compared with the control; however, the TSP1, TSP5, and TSP3/5 growth plates are all more narrow than the control, whereas the growth plates of the other combinatorial mouse strains are wider than the control. *P < 0.05. Upper tibial growth plates were used in all studies.
type IX collagen play a role in the chondrocyte organization in the growth plate.

The widths of all of the growth plates are shown in Figure 3. Significantly wider growth plates were observed at 1 month in mouse strains lacking type IX collagen (Col9, TSP5/Col9, TSP1/5/Col9, and TSP1/3/5/Col9) (Figure 3B). However, by 2 months, only the growth plates from combinatorial knockout strains lacking type IX collagen (TSP5/Col9, TSP1/5/Col9, TSP3/5/Col9, and TSP1/3/5/Col9) remain wider (Figure 3C). These results suggest that type IX collagen plays a role in determining growth plate width. Interestingly, the growth plate of the compound quadruple knockout, TSP1/3/5/Col9, is twice the width of the control at 2 months (Figure 3C). Here, the additional loss of TSP1 has a significant impact on the width of the growth plate, suggesting that TSP1 loss, together with loss of other ECM proteins, may interfere with growth plate closure (Figure 1C). In contrast, the TSP3/5 growth plate is thinner than the control at both ages, whereas this observation was made in TSP1 and TSP5 knockout growth plates only at 2 months (Figure 3, B and C).

To assess the limb lengths in the single and compound knockout mice, limbs were collected at 1 month, and the long bones of the hind limbs were measured by radiographical examination, as shown in Figure 4A. Loss of type IX collagen resulted in a 5% reduction in limb length. An 8% reduction was observed with loss of both TSP3/5. The combinatorial knockout strains showed the most dramatic reductions ranging from 11 to 21%. Loss of TSP1 alone or in combination did not contribute to limb length reduction (Figure 4B).

We next asked whether the quantity and distribution of TSP5, types II and IX collagens, and MATN3 protein in growth plate were altered. All sections were immunostained simultaneously. MATN3 was diminished in type IX knockout growth plates and in all combinatorial Col9 knockout strains (Figure 5). MATN3 incorporation into ECM was not affected by loss of TSP1, TSP5, and TSP3/5 (Figure 5). TSP5 staining was mildly decreased in the Col9 knockout growth plate, which showed limited patchy staining in the hypertrophic zone (data not shown). These observations are consistent with previous findings. No intracellular protein retention was observed. Immunolocalization of types II and IX collagens were similar in null and control mice strains.

**Effect of Exercise on Articular Cartilage**

Previous studies showed that 9-month-old type IX collagen knockout mice develop arthritis more often than controls. To determine whether TSP5 knockout mice were more prone to arthritis, Col9, TSP5, and TSP5/Col9 knockout mice were subjected to exercise (Figure 6; Table 3). The mice ran an average of 960 ± 405 miles (range, 277 to 1488 miles) from 6 weeks to 5 months of age. There was no correlation between genotype and the distance the mice ran. Compared with the control (Figure 6A), the Col9 knockout nonrunner mice had flat articular cartilage (Figure 6D; Table 3). However, exercise further accentuated the flattening of the articular surface (Figure 6G). The TSP5 runner had very mild flattening (Figure 6H) compared with nonrunner and control (Figure 6, E and B) articular cartilage (Table 3). TSP5/Col9 knockout articular cartilage had cartilage flattening similar to Col9 single knockout mice, but in the absence of both TSP5 and Col9, fibrillation of the articular cartilage surface was also observed (Figure 6, F and I). Cartilage fibrillation is a hallmark of cartilage degeneration. These findings suggest that a combination of TSP5 and Col9 deficiency results in development of exercise-associated cartilage degeneration.

**Discussion**

Previous observations suggest that individual loss of TSP1, TSP3, TSP5, and type IX collagen causes modest to no alteration in murine skeletal development. In this work, we focused on the combinatorial role(s) that TSP1, TSP3, TSP5, and type IX collagen plays in the growth plate and on whether their functions are redundant. Toward this goal, we compared several parameters in single and combinatorial knockout mice: growth plate...
organization, chondrocyte density, growth plate width, and relative presence and distribution of TSP5, types II and IX collagen, and MATN3 in the ECM.

These studies led to several novel observations. First, a deficiency in any one of these individual proteins disrupts the columnar arrangement of chondrocytes in the growth plate, although the lack of TSP-1 only mildly perturbed chondrocyte organization. Increased growth plate width was associated with loss of type IX collagen at only 1 month of age and was found in combination strains lacking type IX collagen. In contrast, the growth plate is significantly narrower at 1 and 2 months in the TSP3/5 knockout strain, and their hind limbs were 8% shorter than the controls. A 20% reduction in limb length and almost complete loss of chondrocyte columnar patterning was observed in TSP3/5/Col9 knockout mice. Taken together, these results show that the loss of individual ECM proteins and combinatorial loss significantly impact growth plate organization and long bone growth.

TSP1 single- and TSP1/2 double-null mice recruit fewer macrophages and produce less monocyte chemoattractant protein in wounds, whereas both TSP2 and TSP3 knockout mice have thicker cortical bone. Additionally, TSP3 knockout mice show earlier ossification and more trabecular bone in the femoral head. Previous work with these null mice focused on assessing gross skeletal parameters, and identified subtle skeletal abnormalities. Here, a detailed study of growth plates and long bones has revealed both subtle alterations in single-null strains and more dramatic alterations in compound-null mice. The TSP1-null growth plate shows the least disorganization followed by that in TSP3-null growth plates (Figure 2). In contrast to a previous report that TSP5 knockout mice had no growth plate abnormalities, using quantitative morphometry, we demonstrate significant growth plate disorganization (Figure 3). However, none of the single TSP knockout mice showed any reduction in limb length, despite an abnormal growth plate organization. This finding suggests that the individual loss of any of these three TSP proteins is not sufficient to disrupt long bone development or growth.

In contrast to the TSP-null mouse strains, previous studies of type IX collagen knockout mice showed early-onset osteoarthritis, less MATN3 incorporation into

Figure 5. Distribution of MATN3 in knockout growth plates. Growth plates were simultaneously immunostained with an antibody to MATN3, as described in Materials and Methods. Control sections are to the left for all strains (A, D, F, H, J, and L). MATN3 was markedly diminished in the Col9 knockout growth plate and in all compound knockout growth plate strains that do not synthesize type IX collagen (B, K, N, O, and P). Interestingly, the Col9 knockout growth plate shows light immunostaining throughout the proliferative zone and darker staining in the hypertrophic zone (B). Loss of TSP1, TSP3, and TSP5 does not affect the quantity or distribution of MATN3 (C, E, and G). Loss of TSP5/5 (I) and TSP1/3/5 (M) are also shown. Scale bar = 100 μm. Upper tibial growth plates were used in all studies.
Figure 6. Articular cartilage from hind limbs from knockout mice subjected to exercise regimen. Col9, TSP5, TSP5/Col9 knockout, and control mice were subjected to a running exercise regimen, as described in Materials and Methods. The H&E-stained articular cartilage sections are shown. All of the wild-type running mice (A, B, and C) had normal articular cartilage. The nonrunner TSP5 knockout mice (E) had relatively normal articular cartilage, whereas the articular cartilage of the runners (H) was mildly flattened. At the end of the exercise regimen, the Col9 knockout nonrunner (D) had mild flattening, whereas the runner (G) had severe flattening (asterisk) of the articular cartilage. Interestingly, the TSP5/Col9 knockout nonrunner (F) had flattened articular cartilage (asterisk), and exercise caused additional flattening (asterisk) and fraying (arrow) of the cartilage in the runner (I). A closer view of the articular cartilage cap is shown below each panel (a–i). Scale bar = 100 μmol/L.
the matrix, degraded type II collagen fibrils, and slower fracture repair. We observed similar findings and, in addition, found wider growth plates and significant limb shortening at 1 month of age. Lower chondrocyte density was found in these mice, although the growth plate was wider, suggesting that although there is a similar number of chondrocytes, they are distributed over a larger area. This may indicate that there is more interterritorial matrix or less dense matrix because of absent matrix proteins in these growth plates. Furthermore, the ratio of the proliferative zone to the total width of the growth plate is similar to that of the controls, indicating that chondrocyte proliferation is not increased in these mice (Figure 3A; data not shown). This increase in growth plate width does not lead to longer limbs but rather to significantly shorter hind limbs (Figure 4). These results suggest that loss of type IX collagen has a more significant impact, compared with loss of any of the individual TSP proteins.

Loss of type IX collagen also affects MATN3 incorporation into the interterritorial matrix of the growth plate (Figure 5), a finding that is consistent with previous observations. The loss of MATN3 immunostaining does not obviously correlate with chondrocyte organization. For example, when comparing control with TSP1/3/5 growth plates, no appreciable differences in MATN3 immunostaining are observed despite a significant difference in growth plate width (Figure 3A; data not shown). This increase in growth plate width does not lead to longer limbs but rather to significantly shorter hind limbs (Figure 4). These results suggest that loss of type IX collagen has a more significant impact, compared with loss of any of the individual TSP proteins.

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<tr>
<td>Col9/TSP5</td>
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Table 3. Effects on Articular Cartilage from Hind Limbs from Knockout Mice Subjected to Exercise Regimen

TSP3 and TSP5 do not play completely redundant or compensatory roles. In contrast, the loss of both TSP5 and type IX collagen does not have a substantial impact on the growth plate parameters that is more than the loss of type IX collagen alone (Figures 1 to 3). These results suggest that type IX collagen plays a more significant role in the growth plate than members of the TSP family and that the latter proteins most likely function in same pathway.

With the exception of the TSP1/3/5-null strain, all triple- and quadruple-null strains have approximately 10% or less of their chondrocytes organized into columns. The TSP3/5/Col9 knockout strain showed the most significant growth plate disorganization and limb length reduction (20%; Figures 1 to 4). The loss of TSP1 has a minor impact on the organization of the growth plate and hind limb lengths. However, the TSP1/3/5/Col9 knockout strain has a much wider growth plate at 2 months when compared with the control or the TSP3/5/Col9 strain. This finding suggests that TSP1 could play a role in timing of growth plate closure when other ECM proteins are absent (Figure 3C). Whereas few hypertrophic chondrocytes are observed in the TSP5/Col9, TSP1/5/Col9, TSP3/5/Col9, and TSP1/3/5/Col9 strains at 1 month of age, significant numbers are present at 2 months of age (Figure 1). Type X collagen immunostaining was used to identify the hypertrophic zone (data not shown). This observation may be indicative of delay in chondrocyte maturation, despite the presence of TSP1 in some of the strains. Altogether, these observations suggest a role for TSP1 in the regulation of growth plate closure and a role for TSP5 and type IX collagen maturation of chondrocytes. These findings are consistent with recent work showing slow cartilage maturation and bone formation during fracture repair in type IX collagen knockout mice.

To evaluate the integrity of the articular cartilage matrix, TSP5, Col9, and TSP5/Col9 knockout strains were subjected to an exercise protocol from 6 weeks to 5 months of age. We found that exercise-induced flattening of the articular cartilage superficial zone cap was most pronounced in the hind limbs of the type IX collagen knockout mice but was also present, to a lesser extent, in the TSP5 knockout mice. Exercise had the most dramatic effect on the articular cartilage of Col9 knockout mice by exacerbating joint erosion. Interestingly, mice lacking both type IX collagen and TSP5 have a flattened articular cartilage even without exercise, and exercise caused fraying of the articular cartilage surface. Osteoarthritic changes were not observed in the wild-type mice with exercise (Figure 6). These results suggest that both TSP5 and type IX collagen play a role in maintaining homeostasis of the articular cartilage. These findings also have implications for the human skeletal dysplasias caused by mutations in TSP5 and type IX collagen genes that are associated with joint erosion and early-onset osteoarthritis. The abnormalities are likely related to diminished amounts of these proteins in the matrix. However, other factors, such as the presence of mutant protein could contribute to the disease phenotype.

The results of these studies indirectly suggest different and nonredundant roles for TSP1, TSP3, TSP5, and type IX collagen.
IX collagen in the growth plate. TSP1 appears to primarily affect the timing of growth plate closure. TSP3, TSP5, and type IX collagen all participate in growth plate organization that directly modulates linear growth. Additionally, TSP5 and type IX collagen affect chondrocyte maturation and articular cartilage homeostasis. These findings can be extrapolated to the growth plate pathology observed in skeletal dysplasias caused by mutations in TSP5, type IX collagen, and MATN3 genes. These skeletal dysplasias result from chondrocyte pathology related to cellular protein retention and to loss of one or more of these proteins in the ECM. The results of this study suggest that loss of these proteins from the matrix affects organizational regulation and may significantly contribute to the growth plate pathology observed in pseudoachondroplasia and MED. In summary, we suggest that TSP3, TSP5, and type IX collagen operate in concert and are important for proper growth plate organization and that TSP1 functions in a minor or accessory role in the growth plate.

Acknowledgments

TSP5 and collagen type IX knockout mice were generously provided by Drs. Ake Oldberg and Bjorn Olsen, respectively. We thank Elise Bales and Shawn Tang for technical assistance.

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