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ARTICLE

Chondrocyte Phenotype and Ectopic Ossification in Collagenase-induced Tendon Degeneration

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SUMMARY We report chondrocyte phenotype and ectopic ossification in a collagenase-induced patellar tendon injury model. Collagenase or saline was injected intratendinously in one limb. The patella tendon was harvested for assessment at different times. There was an increase in cellularity, vascularity, and loss of matrix organization with time after collagenase injection. The tendon did not heal histologically until week 32. Ectopic mineralization as indicated by von Kossa staining started from week 8. Tendon calcification was mediated by endochondral ossification, as shown by expression of type X collagen. viva CT imaging and polarization microscopy showed characteristic bony porous structures and collagen fiber arrangement, respectively, in the calcific regions. Marrow-like cells and blood vessels were observed inside calcific deposits. Chondrocyte-like cells as indicated by morphology, expression of type II collagen, and sox 9 were seen around and embedded inside the calcific deposits. Fibroblast-like cells expressed type II collagen and sox 9 at earlier times, suggesting that erroneous differentiation of healing tendon fibroblasts may account for failed healing and ossification in collagenase-induced tendon degeneration. Because this animal model replicates key histopathological changes in calcific tendinopathy, it can be used as a model for the study of its pathogenesis at the patellar tendon.

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KEY WORDS
chondrogenesis
calcification
ossification
tendinopathy
animal model

CHRONIC INSERTIONAL TENDINOPATHY is a poorly characterized tendon degenerative disorder that is extremely common in athletes and in the general population. Despite its prevalence, its underlying pathogenesis is poorly understood, and treatment is usually symptomatic. Calcific tendinopathy is also a degenerative disorder of the tendon with calcium deposits in the mid-substance. It has a similar pattern of occurrence and is particularly common with some degenerative tendinopathy such as that in the patella (Lagier and Gerster 1991; Fenwick et al. 2002), Achilles (Fenwick et al. 2002), rotator cuff, and supraspinatus tendons (Faure and Daculsi 1983; Matsumoto et al. 2005).

Histologically, tendinopathic tissue showed a non-healing status characterized by increase in cellularity, proteoglycan deposition, particularly the oversulfated form, collagen matrix degradation, matrix metalloproteinase 1 (MMP1) and tissue inhibitor of metalloproteinase 1 (TIMP-1), and gelatinolytic activity (Jarvinen et al. 1997; Khan et al. 1999; Fu et al. 2002a,b,2007; Riley 2005). Recently, sprouting of substance P–positive nerve fibers was reported in Achilles tendinopathy (Schubert et al. 2005). Despite the lack of infiltration of inflammatory cells in tendinopathic tissues, disturbances in inflammatory response may still be associated with the development of tendinopathy. Expression of cyclooxygenase-2, prostaglandin E2 (PGE2), and transforming growth factor (TGF)-β1 was detected in tendinopathic specimens (Fu et al. 2002b; Fredberg and Stengaard-Pedersen 2008). Although deposits of calcium phosphate salts such as hydroxyapatite and calcium pyrophosphate have been reported in spontaneously ruptured tendons (Jozsa et al. 1980; Kannus and Jozsa 1991), the association of calcium deposits with the pathological process of tendon degeneration and rupture is not clear at present.

To facilitate the study of the pathogenesis of tendinopathy, animal models are needed. Current animal

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models are mainly established by overuse or injection of collagenase and cytokines such as PGE2 (Warden 2007). However, there has been no report on animal models for the study of the pathogenesis of calcific tendinopathy.

Recent studies reported that human and mouse tendons harbored a unique cell population with universal stem cell characteristics (tendon stem/progenitor cells), and they could differentiate into chondrocytes and osteoblasts (Salingcarnboriboon et al. 2003; Bi et al. 2007; de Mos et al. 2007). These isolated tendon stem/progenitor cells could regenerate tendon-like tissues after extended expansion in vitro and transplantation in vivo (Bi et al. 2007). Chondrocyte markers were expressed in the clinical samples of calcific insertional Achilles tendinopathy (Maffulli et al. 2006) and rotator cuff tendinopathy (Sharma and Maffulli 2005; Yokota et al. 2005). This implies that the chondral metaplasia in insertional tendinopathy and ectopic ossification in calcific tendinopathy may be caused by erroneous differentiation of tendon cells. However, it is not known whether it could be observed in the healing responses to degenerative tendon injuries in vivo. In this study, we report acquisition of chondrocyte phenotype and ectopic bone formation in a collagenase-induced tendon injury rat model. The association of cartilaginous metaplasia and ossification with tendon degeneration at different times and locations was examined.

Materials and Methods

Collagenase-induced Injury

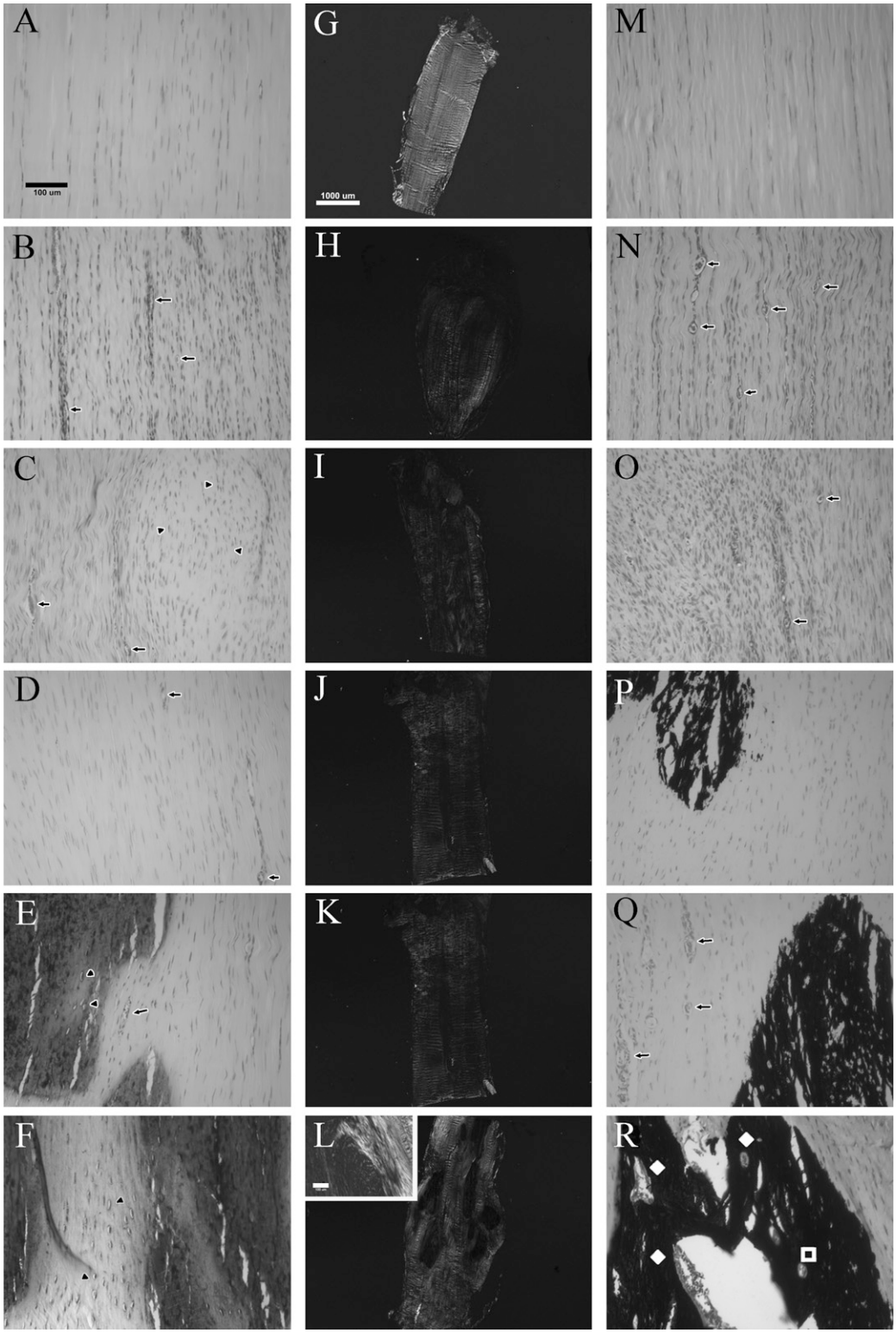
The use of rats for experiments in this study was approved by the animal research ethics committee of the authors' institution. Thirty-eight male Sprague Dawley rats (8 weeks, weight 200–250 g) were used in this study. After anesthesia with 2.5% pentobarbital (4.5 mg/kg body weight), hairs over the lower limb were shaved. The patellar tendon was located by positioning the knee at 90 degrees. Twenty microliters (0.015 mg/μl in 0.9% saline, i.e., 0.3 mg) of bacterial collagenase I (Sigma-Aldrich; St. Louis, MO) or saline was injected into the patellar tendon intratendinously with a 30-gauge needle in one limb, whereas the contralateral limb was left untreated (Chen et al. 2004). Free cage activity was allowed after injection. All the animals survived until they were scarified. At weeks 2, 4, 8, 12, and 16 for the collagenase-injected group and at week 16 for the saline group, the rats were killed, and the patellar tendon was harvested for routine histology, IHC, and von Kossa staining (n=6 for each time point). Two rats with collagenase injection were sacrificed at week 32 for viva CT imaging.

General Histology and IHC

The patellar tendon was washed in PBS, fixed in buffered formalin and 100% ethanol, embedded in paraffin, cut longitudinally to 5-μm-thick sections, and mounted on 3-aminopropyl-triethoxy-silane (Sigma-Aldrich)–coated slides. After deparaffination, the sections were stained with hematoxylin–eosin. IHC was done as described previously (Fu et al. 2003; Lui et al. 2007). Briefly, after removal of paraffin and rehydration, the sections were decalcified with 9% formic acid for 10 min and washed for 1 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 20 min at room temperature. Antigen retrieval was performed with 2 mg/ml protease (Calbiochem; Bie and Berntsen, Rødovre, Denmark) at 37C for 30 min for collagen type II detection; 2 mg/ml hyaluronidase (Sigma-Aldrich) at room temperature for 30 min, and digestion with 0.02 mg/ml protease at room temperature for 30 min for collagen type X detection; 1 mg/ml hyaluronidase at 37C for 45 min, followed by incubation with 10 mM citrate buffer at 74C for 20 min for sox 9 detection. After blocking with 5% normal goat serum, the sections were stained with specific antibodies against collagen type II (1:100, NeoMarkers; Lab Vision, Fremont, CA), collagen type X (1:100; Sigma-Aldrich), or sox 9 (1:30; Santa Cruz Biotechnology, Santa Cruz, CA) in a humid chamber at 4C overnight. The spatial and temporal localization of these proteins were visualized by incubating with goat anti-mouse/rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody (cat. no. AP124P and AP132P, respectively; Chemicon International, Temecula, CA) for an hour,

Figure 1 Representative images of histological, polarization, and von Kossa staining of patella tendon at different times after intratendinous collagenase injection. **(A–F)** Hematoxylin–eosin staining. After intratendinous collagenase injection, the cell density and vascularity increased with time, with a transient decrease at week 8. The condition deteriorated with an increase in cell density and vasculature at weeks 12 and 16. Chondrocyte-like cells first appeared at week 4, and they surrounded calcific deposits starting at week 8. Calcific deposits were present in all samples at week 12 and increased in size at week 16. There was no sign of infiltration of inflammatory cells. These changes were not observed in the saline injection control. **(G–L)** Polarization microscopy. There was progressive loss of collagen birefringence with time compared with that in the saline injection control. Focal loss of collagen birefringence occurred at weeks 12 and 16. Trabecular-like collagen fiber alignment was observed in the calcific deposits in some specimens at week 16 (inset). The extracellular matrix of the saline injection control remained intact as indicated by high collagen birefringence. **(M–R)** von Kossa staining. Mineralized matrix was observed starting from week 8. There was no staining in the saline injection control. Note the presence of marrow-like structures (diamond) and blood vessels (rectangle) within the calcific deposit at week 16. The saline injection control as follows: week 16, **A,G,M**; week 2, **B,H,N**; week 4, **C,I,O**; week 8, **D,J,P**; week 12, **E,K,Q**; week 16, **F,L,R**. Arrow, blood vessels; arrowhead, chondrocyte-like cells; diamond, marrow-like cells inside calcific deposit; rectangle, blood vessels inside calcific deposit. Bars: **A–F,M–R,inset** = 100 μm; **G–L** = 1000 μm.

followed by 3,3′ diaminobenzidine tetrahydrochloride (DAKO; Glostrup, Denmark) in the presence of H_2O_2. Afterward, the sections were rinsed, counterstained in hematoxylin, dehydrated with graded ethanol and xylene, and mounted with *p*-xylene-bis-pyridinium bromide (DPX) Permount (Sigma Aldrich). Primary antibody was replaced with blocking solution in the controls. For good reproducibility and comparability,



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all incubation times and conditions were strictly controlled. The sections were examined under light microscopy (Leica DMRXA2; Leica Microsystems, Wetzlar, Germany). Collagen fiber alignment was examined by polarization microscopy (Leica DMRB; Leica Microsystems).

von Kossa Staining

Sections were deparaffinized and rehydrated with xylene and graded ethanol. They were incubated with 1% silver nitrate solution under light bulb illumination for 1 hr, followed by incubation in 5% sodium thiosulfate solution for 5 min to remove unreacted silver. Slides were washed and counterstained with 0.2% nuclear fast red solution. Finally, stained slides were dehydrated and mounted with DPX. The calcium nodules appeared brown.

viva CT Scanning

A fan-beam viva CT system (VivaCT40; Scanco Medical AG, Bassersdorf, Switzerland) was used to locate the mineralized tissue in the tendon mid-substance and to study its three-dimensional structure. The patellar tendon was immersed in 70% ethanol and scanned transversely, with vertical displacement of 30 μm. The sections were three-dimensionally reconstructed using the built-in software.

Image Analysis

The whole histological section of tendon samples was imaged with a Leica DM5000B microscope and DFC 490 Digital camera (optical magnification: 16×) under standardized illumination and photographic conditions. Image analysis was performed with Image Pro Plus (Media Cybernetics; Bethesda, MD). Image segmentation was performed to include the whole tendon section by selecting the background and taking the inverse of the selection. A binary mask of the segmented image was created with the "Dilate" and "Close" filters. The processed binary image was thresholded to create a region of interest (ROI) inscribing the whole tendon section, and the total area of the tendon section was measured. The ROI was applied to the original image for measurement of percentage area of specific staining. The immunoreactivity of collagen type II was thresholded with a set of standardized color space parameters that selected the brown color of immunopositive signal. The positive von Kossa staining was thresholded as black color. For the evaluation of the extent of degenerative injuries with polarization macroscopic imaging, the darkened regions in tendon samples indicated the loss of collagen birefringence in contrast to the brightened collagen fibers. These darkened regions were thresholded as dark brown and black color with a standardized set of color space parameters for all samples. Because only the polarization images, von Kossa stain, and IHC staining of type II collagen showed spatial information with respect to the whole tendon section at a low magnification, image analyses to obtain percentage area of positive staining or birefringence were performed only on these imaged specimens. Because the immunopositivity of sox 9 and type X collagen was observable only at higher magnification, the percentage area of positive staining for the whole tendon section was not measured.

Data Analysis

The percentage area of loss of collagen birefringence and type II and type X collagen signals out of the total tendon area at different times is presented in a box plot. To compare the difference among different time points, the Kruskal-Wallis test was used, followed by post hoc comparison of different time points with saline control using the Mann-Whitney U test. $p<0.05$ was regarded as statistically significant.

Results

Hematoxylin–Eosin Staining

The cell density increased markedly at weeks 2 and 4 (Figures 1B and 1C). There was loss of matrix organization. Vascularity increased at week 2 and decreased slightly at week 4 (Figures 1B and 1C, arrow). Cells separated from the pericellular matrix by lacunar space, resembling chondrocytes, were first observed at week 4 (Figure 1C, arrowhead). The cell density and vascularity decreased at week 8 (Figure 1D), indicating that the tissue was trying to heal. However, chondrocyte-like cells still existed. The condition deteriorated at week 12, with increases in cell density and vasculature (Figure 1E). Calcific deposits were present in all samples. Many chondrocyte-like cells were observed, and some of them were embedded within the matrix surrounding the calcific deposits (Figure 1E, arrowhead). A larger area of calcification and more chondrocyte-like cells were observed at week 16 (Figure 1F). Pore structure with lining, marrow-like structures (Figure 1R, diamond), and blood vessels (Figure 1R, rectangle) were observed at weeks 12 and 16 inside the calcific deposits. Less vasculature was observed at week 16, but cellularity remained high. There was absence of infiltration of inflammatory cells up to week 16. These changes were not observed in the saline injection control (Figure 1A). Tendon cells were well aligned within the tightly packed and longitudinally arranged collagen fibrils.

Polarization Microscopy

Collagen fiber alignment was observed under polarization microscopy, and we measured the percentage area

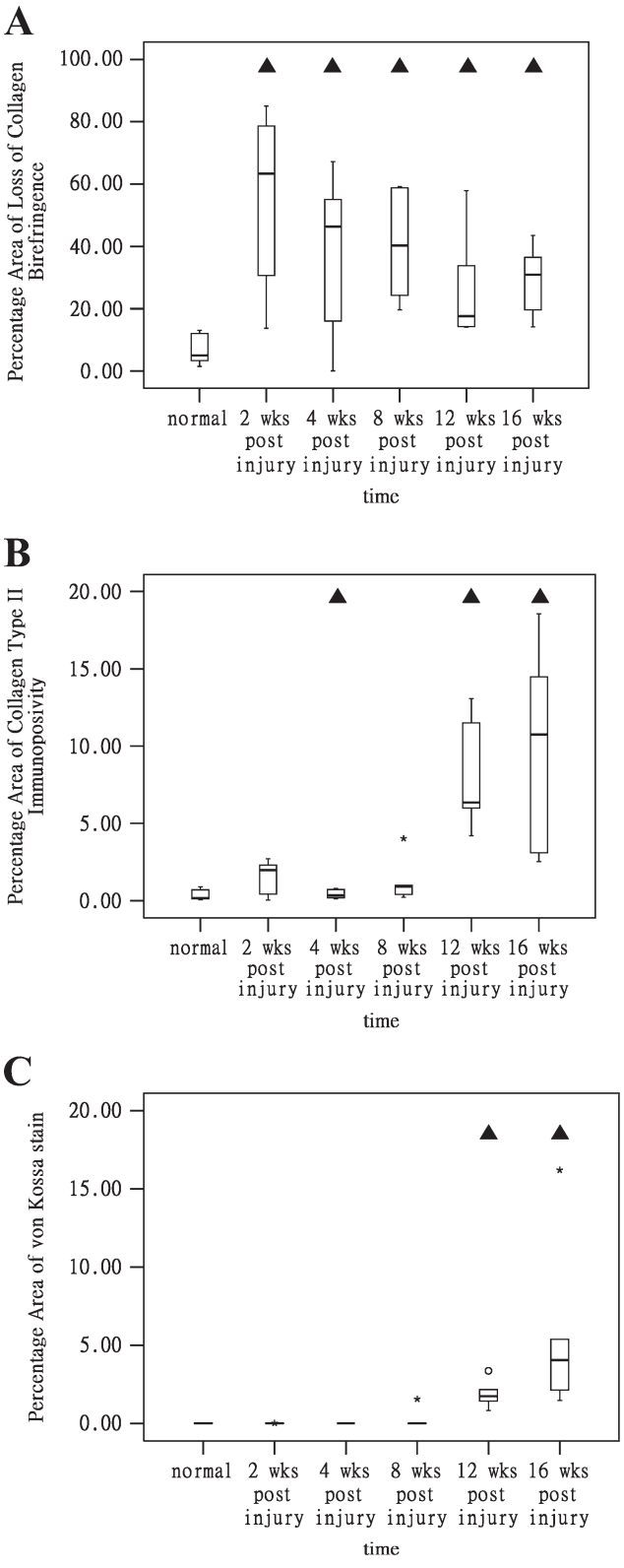

Figure 2 (**A**) Graph showing percentage area of loss of collagen birefringence at different times after intratendinous collagenase injection. There was significant loss of collagen birefringence at all time points compared with saline control. (**B**) Graph showing percentage area of type II collagen immunopositive stain at different times after intratendinous collagenase injection. There was significantly higher percentage area of type II collagen immunopositivity at weeks 12 and 16 compared with the saline control. (**C**) Graph showing percentage area of von Kossa stain at different times after intratendinous collagenase injection. There was a significantly higher percentage area of von Kossa staining at weeks 12 and 16 compared with that in the control. Triangle indicates $p<0.05$ in post hoc comparison compared with the saline injection normal control.

of loss of collagen birefringence (Figures 1G–1L and 2). There was significant loss of collagen birefringence at all time points compared with saline control ($p=0.012$ overall, $p=0.004$ for post hoc comparisons; Figure 2). Focal loss of collagen birefringence occurred at weeks 12 (Figure 1K) and 16 (Figure 1L). Characteristic collagen alignment as in trabecular bone was observed in the calcific deposits in some specimens at week 16 (Figure 1L, inset). There was no degenerative change of the extracellular matrix in the saline injection control as indicated by high collagen birefringence (Figure 1G).

von Kossa Staining

We examined the degree of matrix mineralization with von Kossa staining. Focal matrix mineralization was detected in one sample at week 8 (Figure 1P) and all samples with higher intensity at weeks 12 (Figure 1Q) and 16 (Figure 1R). Interestingly, a clear zone was observed in the center of some calcific deposits at weeks 12 and 16. There was no von Kossa staining in the saline injection control (Figure 1M). There was a significantly higher percentage area of von Kossa staining at weeks 12 and 16 compared with the saline control ($p<0.001$ overall and $p=0.004$ for post hoc comparisons; Figure 2).

viva CT

We measured the three-dimensional structure of the calcific deposits in the injured tendon at week 32 by viva CT (Figures 3A and 3B, arrow). The calcific deposits have porous structures (Figure 3C, arrowhead) and were distributed throughout the tendon.

Expression of Type II Collagen

We measured the expression of type II collagen by IHC. Immunopositivity was first observed at week 2, mainly in tendon cells, with some staining of its surrounding extracellular matrix (Figure 4B). The staining was mainly localized at the chondrocytes and the extracellular matrix of tendon cells at week 4 (Figure 4C). There was intense staining at week 8, and it occurred mainly at the chondrocyte-like cells and their surround-

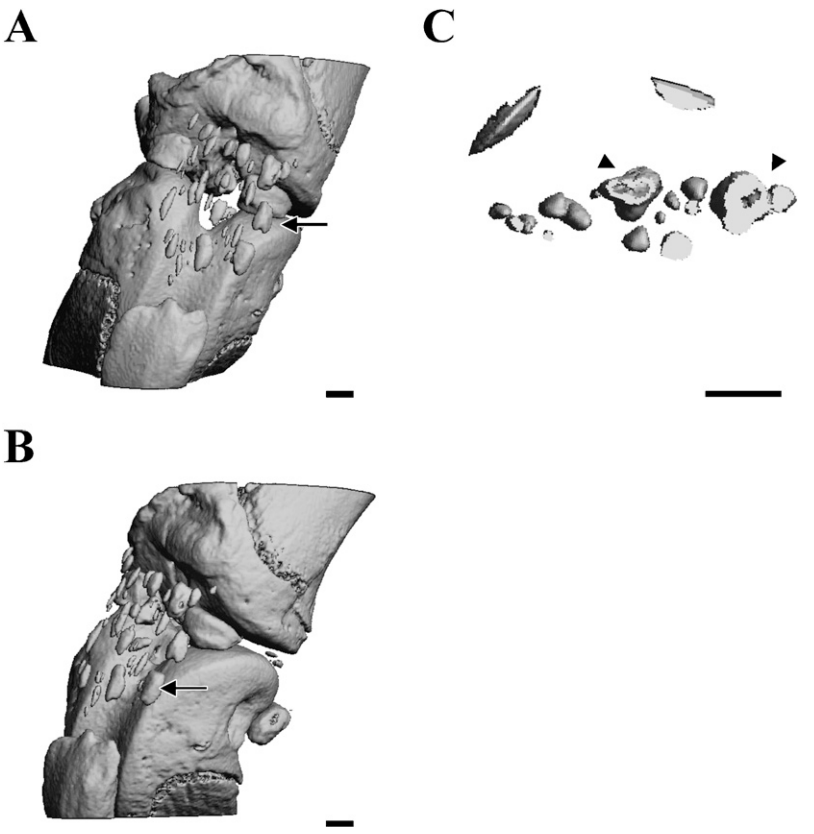

Figure 3 Representative viva CT images of calcific deposits inside the tendon at week 32 after intratendinous collagenase injection. Note the localization of calcific deposits (arrow) in the tendon. Note the porous structures of the calcific deposits (arrowhead). (**A**) Anterior-posterior view. (**B**) Oblique view. (**C**) Calcific deposit at higher magnification. Bar = 1 mm.

ing matrix at the calcific deposits. However, tendon cells were not stained (Figure 4D). Similar results were observed at weeks 12 (Figure 4E) and 16 (Figure 4F). However, the central part of the calcified deposit was not stained at week 16 (Figure 4F, star). There was no expression of type II collagen in the saline injection control (Figure 4A). There was a significantly higher percentage area of type II collagen immunopositivity at weeks 12 and 16 compared with that in the control ($p<0.001$ overall, $p=0.004$ for post hoc comparisons).

Expression of Type X Collagen

Type X collagen, a marker of hypertrophic chondrocytes and indication of endochondral ossification, was detected in chondrocyte-like cells at week 8 (Figure 4J), some calcific deposits and their surrounding chondrocyte-like cells at week 12 (Figure 4K), and all calcific deposits and their surrounding chondrocyte-like cells at week 16 (Figure 4L). There was no expression of type X collagen in the saline injection control (Figure 4G).

Expression of sox 9

Strong expression of sox 9 was observed in tendon cells in the high cell density region at week 2 (Figure 4N). At weeks 4 (Figure 4O, arrowhead) and 8 (Figure 4P, arrowhead), the staining intensity was reduced, and a weak signal was also observed in some chondrocyte-

Figure 4 Representative IHC images of the patella tendon at different times after intratendinous collagenase injection. (**A–F**) IHC of type II collagen. Immunopositivity was first observed at week 2 in tendon cells. There was intense staining of the chondrocyte-like cells and their surrounding matrix close to the calcific regions at weeks 8, 12, and 16. The tendon cells were not stained. The central part of the calcific deposit was not stained in some specimens at week 16 (star). There was no expression of type II collagen in the saline injection control. (**G–L**) IHC of type X collagen. Immunopositivity was observed in chondrocyte-like cells at week 8, some calcific deposits and their surrounding chondrocyte-like cells at week 12, and all calcific deposits and their surrounding chondrocyte-like cells at week 16. There was no expression of type X collagen in the saline injection control. (**M–R**) IHC of sox 9. There was strong expression of sox 9 in tendon cells in high cell density region at week 2. At weeks 4 and 8, a weak signal was also observed in some chondrocyte-like cells. At weeks 12 and 16, chondrocyte-like cells surrounding calcific deposits were stained, and the intensity was higher at week 12 compared with that at week 16. There was no expression of sox 9 in the saline injection control. Saline injection controls: week 16, **A,G,M**; week 2, **B,H,N**; week 4, **C,I,O**; week 8, **D,J,P**; week 12, **E,K,Q**; week 16, **F,L,R**. Arrowhead, chondrocyte-like cells; star, clear zone of calcific deposit; CR, calcific region.

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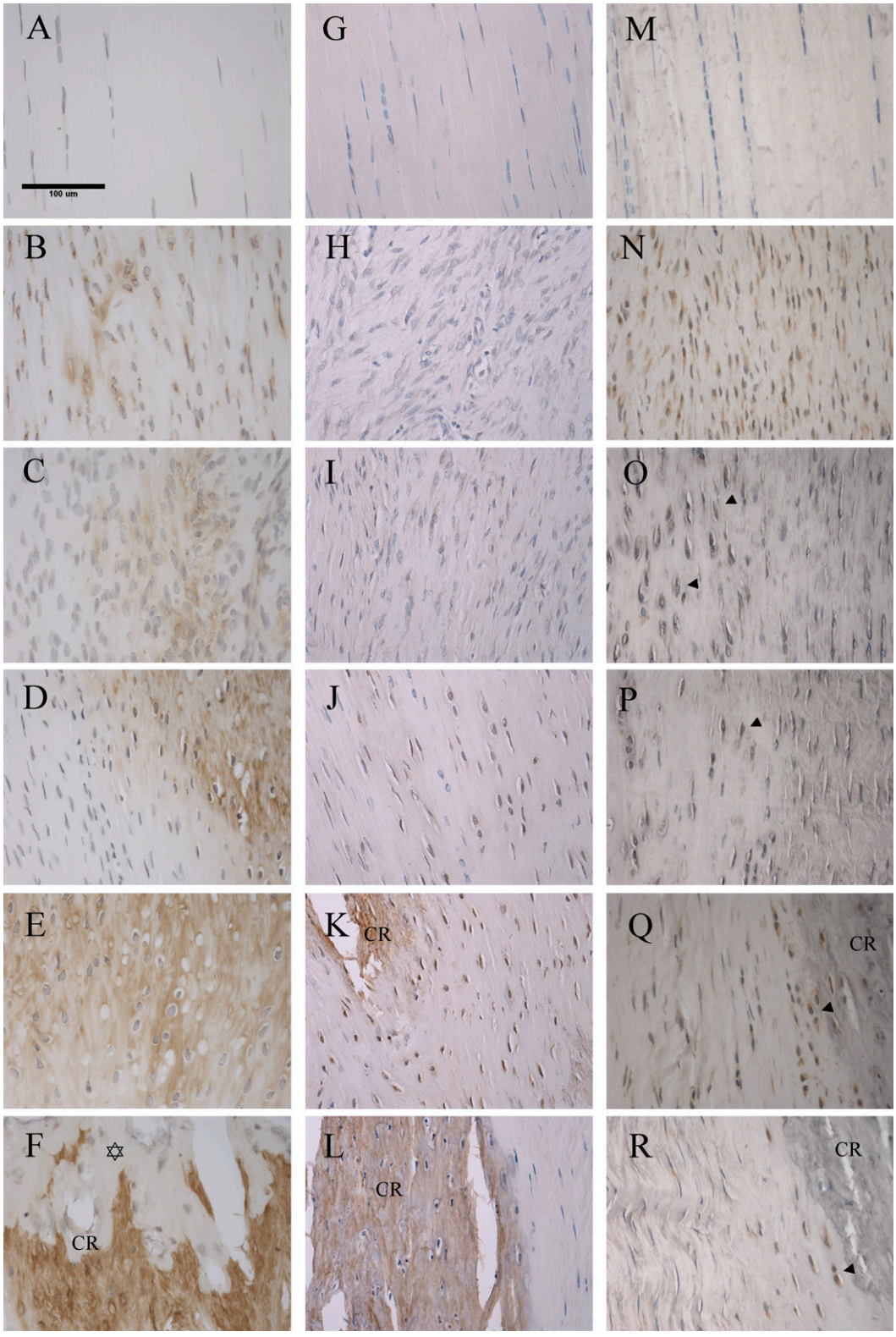

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like cells. At weeks 12 (Figure 4Q) and 16 (Figure 4R), chondrocyte-like cells surrounding the calcific deposits were stained. The staining intensity was higher at week 12 compared with that at week 16. There was no expression of sox 9 in the saline injection control (Figure 4M).

Discussion

Tendon degeneration is common in many patients with chronic tendon pain including insertional tendinopathy and calcific tendinopathy. Little is known about the pathogenesis, and consequently, few effective therapies are available. Better understanding of the pathogenesis is essential for development of effective treatment modalities. We reported acquisition of chondrocyte phenotype and ectopic bone formation and the association of different cell types with tendon degeneration after collagenase injection in tendon.

Our results showed that collagenase injection exhibited many key features of tendinopathy including hypercellularity, loss of matrix organization, increased vascularity, and absence of infiltration of inflammatory cells as reported in previous studies (Jarvinen et al. 1997; Khan et al. 1999; Riley 2005; Fu et al. 2002a, b,2007). The patellar tendon failed to heal histologically by the end of week 32. In addition, we observed consistent formation of calcific deposits in the mid-substances in all tendon specimens, and it persisted up to week 32. Current animal models for the study of pathogenesis of tendinopathy were mainly established by overuse or injection of collagenase and cytokines such as PGE2 (Warden 2007). No ectopic mineralization and ossification were reported in these studies using these models. Our study showed that acquisition of the chondrocyte phenotype and ectopic bone formation were consistently induced in the mid-substances of the patellar tendon after collagenase injection. Because this model replicates key histopathological changes of calcific tendinopathy, it can be used as a model for the study of its pathogenesis at the patellar tendon. The use of collagenase to induce degenerative tendon injury for the study of calcific tendinopathy was further substantiated by reports showing the increase of MMP-1—which also degrades collagen type I as bacterial collagenase used in this study—in human patellar tendinopathic specimens (Fu et al. 2002a), and the increase of MMP1 in cultured tendon fibroblasts after large magnitude repeated stretching (Yang et al. 2005). The use of MMP inhibitors in clinical trials was also reported to be associated with clinical tendinopathy, which rapidly resolved after cessation of therapy (Hutchinson et al. 1998; Jones et al. 1999; Tierney et al. 1999). Bone formation in the Achilles tendon after mid-point tenotomy has also been reported by Rooney et al. (1992,1993).

We observed transient tendon healing at week 8 after collagenase injection as indicated by the decrease in cellularity, although degeneration of the extracellular matrix was still observed. Extracellular matrix synthesis and remodeling should take a longer time to occur compared with changes in cellularity. However, the condition deteriorated afterward, and the tendon did not heal histologically with ectopic bone formation. This indicated that the tendon tissue was attempting to heal but failed in response to injury, consistent with the hypothesis of failed healing as the cause of tendinopathy (Cook et al. 2002; Riley 2005; Maffulli et al. 2006).

Our results showed that tendon calcification after collagenase-induced injury was mediated by endochondral ossification because type X collagen, a marker of endochondral ossification, was expressed. Ectopic bone formation was further supported by the presence of collagen matrix characteristic of trabecular bone under polarization microscopy, bony structures as shown by viva CT imaging, and the presence of bone marrow–like cells and vasculature inside the calcific deposits. The presence of a true bony deposit was also observed in clinical samples (Archer et al. 1993) and was suggested to be caused by a renewed blood supply to the previously calcified tendon (Uhthoff 1975). Our results were also consistent with a previous report that suggested endochondral ossification as the mechanism of calcification in calcific Achilles and patellar tendinopathy (Fenwick et al. 2002) and midpoint tenotomy of Achilles tendon (Rooney et al. 1992, 1993). The central portion of some calcific deposits was not stained by von Kossa stain. This might be because of spontaneous resorption of the calcific deposits. The regression of calcific deposits was also reported clinically. The presence of both a formative and a resorptive phase of the calcific deposits was suggested (Uhthoff et al. 1976).

Chondrocyte-like cells as indicated by cellular morphology and expression of sox 9 and type II collagen were observed around the calcific deposits in collagenase-induced degenerative tendon injury. This was consistent with a previous study reporting the presence of chondrocyte-like cells in the vicinity of mineralized nodules of a clinical specimen of tendinopathy at the rotator cuff tendon (Uhthoff et al. 1976; Archer et al. 1993), supraspinatus tendon (Chard et al. 1994), and Achilles tendon (Maffulli et al. 2006). There was an increase in the expression of aggrecan and biglycan mRNA in Achilles tendinopathy (Corps et al. 2006), as well as proteoglycans in patellar tendinopathy (Fu et al. 2007). Increased expression of type II collagen was also seen in human rotator cuff tendinopathy (Sharma and Maffulli 2005; Yokota et al. 2005). There was overexpression of chondrocyte markers in an overuse rat model (Archambault et al. 2007). Not all the

chondrocyte-like cells in our study expressed type II collagen and sox 9. A similar result was also reported by Archer et al. (1993).

We reported the expression of type II collagen and sox 9 by fibroblast-like cells that preceded the expression of these markers in chondrocyte-like cells. We propose that erroneous cell differentiation during healing might account for tendon degeneration and tendon calcification. This is corroborated with other studies that reported the upregulation of cartilage-associated genes and downregulation of tendon-associated genes in the rat supraspinous tendon (Archambault et al. 2007) and in the horse superficial digital flexor tendon (Clegg et al. 2007) after overuse injury. Differentiation of tendocytes into fibrocartilage has been suggested to be the necessary initial stage for mineralization (Uhthoff et al. 1976). Ectopic bone formation in the Achilles tendon after mid-point tenotomy has also been reported to be caused by the direct conversion of tendon tissue to cartilage (Rooney et al., 1993). Other studies also reported the presence of resident progenitor cells with multidifferentiation potential in the tendon (Salingcarnboriboon et al. 2003; Bi et al. 2007; de Mos et al. 2007). Injection of recombinant human bone morphogenetic protein -2 (rhBMP-2) into the tendon increased ectopic bone formation, indicating that the tendon consisted of cells that were responsive to BMP and were capable of differentiating along the chondro-osseous pathway (Hashimoto et al. 2007). The differentiation of tendon progenitor cells into chondrocytes and bone cells was reported to be modulated by the expression of small leucine-rich repeat proteoglycans such as biglycan and fibromodulin, which control the differentiation process associated with BMP-2 activities (Bi et al. 2007). Clinically, there was ectopic overexpression of BMPs in the subacromial bursa, and it was suggested to account for the chondrogenic transformation and ectopic mineralization of the rotator cuff tendon in patients (Neuwirth et al. 2006).

In conclusion, cartilaginous metaplasia and ossification were observed in a collagenase-induced degenerative tendon injury model. Because this model replicates key histopathological changes of calcific tendinopathy, it constitutes a valuable model for unraveling the common molecular pathways of its pathogenesis at the patellar tendon. The temporal and spatial relationship of degenerative injuries, chondrogenesis, and tissue ossification suggest a sequence of events that involves aberrant cell differentiation in damaged extracellular matrix that favors ectopic ossification.

Acknowledgments

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