Endochondral ossification in Achilles and patella tendinopathy

Sir, A relatively common occurrence in tendon pathology is the intratendinous accumulation of calcific deposits [1]. During dissection and processing of tendons obtained from 86 patients with chronic tendinopathy, small hard mineralized deposits were detected macroscopically in four specimens. Three specimens were taken from the mid-zone (4–6 cm from the bone insertion) of Achilles tendons (aged 31, 41 and 48 years respectively) and one specimen was taken approximately 1 cm from the inferior pole of a patella tendon (aged 36 years). Due to the bony nature of the deposits, we investigated whether these had formed by endochondral ossification. The tissue was snap-frozen and mounted in Optimal Cutting Temperature (OCT) compound (Tissue-Tek®, Agar Scientific Ltd, Stansted, Essex, UK). Sections were cut at 5 μm, mounted on slides coated with 3-aminopropyltriethoxysilane (Sigma, Poole, UK) and allowed to air-dry before brief fixation in acetone. Tissue sections were stained with Von Kossa stain to show the presence of mineralized tissue. Sections were also stained chemically for alkaline phosphatase (AP) and tartrate-resistant AP (TRAP) according to published protocols [2, 3]. Type II collagen was immunolocalized using a commercially available antibody (monoclonal CIIC1; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA).

Phase-contrast microscopy of the specimens showed a clear distinction between the tendon matrix and the mineralized nodule (Fig. 1A). Von Kossa staining confirmed the presence of a core of mineralized matrix surrounded by a region of osteoid tissue (Fig. 1B). Rounded chondrocyte-like cells around the deposit lacked organization, the zone of columnar chondrocytes appearing short (Fig. 1B). The histological appearance was consistent with a truncated process of chondrocyte maturation at the rim of the mineralized region, similar to that described for heterotopic ossification in osteo-physes by Bord et al. [4]. Tissue surrounding the deposit was hypercellular and had lost its normal architecture, and was similar to that described in studies of Achilles tendinopathy [5]. Type II collagen was localized to regions surrounding the mineralized nodule, demonstrating a cartilaginous matrix and chondrocytic cell phenotype (Fig. 1C). AP-positive cells were detected in close apposition to this bony core, indicating the presence of osteoblasts (Fig. 1D), while TRAP-positive cells (osteoclasts) were located within the mineralized tissue (Fig. 1E).

Few previous studies have attempted to study the pathological mechanism of tendon calcification. Uhthoff and Sarkar proposed that calcifying tendinitis developed via a fibrocartilage intermediate and that the condition resolved spontaneously [6]. We are aware of two studies that have attempted to investigate the cellular mechanisms involved. One study showed no evidence for endochondral ossification in the calcifying supraspinatus tendon [7]. There was an absence of type II collagen, matrix vesicles and alkaline phosphatase, and the histological evidence was consistent with a degenerative process [7]. In contrast, a recent study by Nakase et al. [8] has shown osteoclasts in calcifying supraspinatus tendon, and these are presumably involved in the resorption of the deposit. Our study demonstrates that at least some mineralized deposits in Achilles and patella tendons are formed by a process resembling endochondral ossification, with bone formation and remodelling mediated by populations of osteoblasts and osteoclasts.

The origin of the cells participating in tendon ossification is unknown. Several cellular sources have been proposed, including the bone marrow, precursor cells within the tendon and the tendon cells themselves [9]. In a number of diseases, including myositis ossificans and diffuse idiopathic skeletal hyperostosis, it is thought that ectopic bone is derived as a result of the metaplasia of tendon cells into osteogenic cells [9]. Recently, vascular pericytes have been shown to have the ability to differentiate into both chondrocytes and osteoblasts [10]. With the increased vascularity associated with chronic tendinopathy [5], this is a likely source of the cells participating in the calcification process.
Fig. 1. (A) Phase-contrast micrograph of a mineralized deposit (M) and surrounding tendon tissue (T) ($\times$200). (B) Von Kossa staining showing a black mineralized area (M) surrounded by a deep red zone of osteoid (Os) encompassed by (purple) tendon tissue (T) ($\times$400). (C) Fluorescent type II collagen staining (II) at the edge of a mineralized region (M) ($\times$200). (D) AP-positive cells (arrows) at the edge of a mineralized region (M). T is the tendon ($\times$400). (E) TRAP-positive cells (arrows) within a mineralized region (M). Tendon is marked with a T ($\times$400).
Further investigation is required into why a small percentage of degenerate tendon lesions appear to undergo endochondral ossification.

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