Chondroid metaplasia is a long-term consequence of tendinopathy induced by both stress-deprivation and over-stress: temporal differences in molecular pathology

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Introduction
Degeneration and tearing of rotator cuff tendon causes significant morbidity in our ageing population. While conservative treatment is favored, there are no current drug therapies to specifically treat cuff or other tendon injuries so surgical repairs are common (over 14,000 annually in Australia). Unfortunately half of these repairs fail within 12 months. The poor management options and outcomes for rotator cuff disorders are driven by the degenerative changes in the tendon that both precede and lead to tearing, and result from altered loading of tendon after partial rupture. Pre- and post-rupture changes are difficult to study in humans due to unknown temporality of injury. An animal model of shoulder tendon injury induced by partial infraspinatus tendon transection, has been recently established in our laboratories [1]. In this model the effects of both overstress (OS) and stress deprivation (SD) can be simultaneously evaluated in different regions of the one tendon. At 4 weeks post stress alteration, spatial changes in collagen, proteoglycan and catabolic ADAMTS and MMP enzymes were demonstrated [1]. We now report the temporal changes in pathology, proteoglycan localisation and gene expression changes for up to a year after induction of tendinopathy. These studies aimed to determine whether the early pathology resolves or progresses and the molecular changes that underlie these temporal changes.

Methods
Twenty-four 2-year-old Merino wethers had the infraspinatus tendon of one forelimb partially transected on the cranial side, midway between the humoral attachment and the musculotendinous junction. Another 24 sheep had tendons exposed but not cut (sham) and 24 sheep were non-operated controls (NOC). After sacrifice of 6 animals in each group at 2, 13, 26 and 52 weeks post-transection, 2 regions of tensile tendon proximal to the transection (defined by finite element analysis to be SD cranially or OS caudally by the partial transection) were removed from each shoulder. Portions of tendon were formalin-fixed and processed into paraffin for histology. Sections (5µm) were stained with toluidine blue, picrosiris red, and H&E. All sections were scored by 2 observers (MS & TM) blinded to treatment, time and tendon region for: cell number, cell morphology, interfibrillar matrix accumulation, vascularity, collagen fibre alignment and proteoglycan content [1]. Other portions of tendon were snap-frozen for subsequent total RNA extraction and quantitative reverse transcription real-time PCR using validated ovine-specific primers for aggrecan, versican, decorin, lumican, fibromodulin, biglycan, MMP-13 and ADAMTS1, -4 and -5.

Results
There were no differences between NOC and sham-operated animals for any measured parameter at any time. All subsequent reported comparisons are between equivalent regions of partially-transacted versus sham-operated tendons. Two weeks after transection, significant histopathological changes were apparent in both SD and OS tensile tendon (p < 0.05). By 26 and 52 weeks, histopathology scores for cell number, cell morphology, interfibrillar matrix accumulation, vascularity and collagen fibre alignment had returned to sham levels in OS but not SD tensile tendon. Notably, in both OS and SD regions, proteoglycan staining within the tendon fascicles significantly increased by 2 weeks and was still elevated at 52 weeks (p < 0.02), when areas of distinct chondroid metaplasia had become evident (Fig 1).

Figure 1. Representative toluidine blue stained sections of SD (A & B) and OS (C) tendon from sham operated (A) and transected (B & C) sheep infraspinatus tendon after 52 weeks (bar = 100µm).

In SD tendon, expression ratios (fold change transected/sham) of versican, biglycan and lumican were significantly increased (all p < 0.05) and decorin and fibromodulin decreased (p < 0.005) at multiple time points. In OS tendons decorin, fibromodulin and lumican were not regulated at any time. Distinct temporal differences between SD and OS were noted, with a delayed increase and subsequent decrease in aggrecan expression, but an earlier and more sustained increase in MMP-13 mRNA expression observed in SD compared with OS tendon regions (Fig 2). Expression of the ADAMTS that would normally be implicated in aggrecan and versican proteolysis was not significantly changed in either SD or OS tendon at any time in this study.

Figure 2. Temporal differences in fold change (transected/sham, log scale) of aggrecan and MMP-13 gene expression in OS and SD regions of partially-transacted tendons.

Discussion
OS and SD following partial rupture lead to classical degenerative histopathological changes in tensile tendon. Despite spontaneous repair of the partial transection, degenerative histopathological change did not resolve in SD tendon regions even 1 year after injury. Proteoglycan accumulation and chondroid metaplasia is a major pathological finding in both SD and OS tensile tendon that does not resolve, but in fact progresses up to 1 year after injury in both tendon regions. However, the molecular mechanisms that underlie and are associated with the pathology are temporally different in SD compared with OS. Pathological accumulation of proteoglycan, especially aggrecan and versican, in tensile tendon may be associated with lack of coordinate upregulation of ADAMTS activity to accompany the increased proteoglycan expression. Excessive proteoglycan and chondroid metaplasia may alter tendon biomechanics, increase susceptibility to tearing and inhibit repair. This process appears to continue to develop for at least a year after tendon injury. These results suggest that therapies that decrease aggrecan and increase ADAMTS may be beneficial in treating tendinopathy and tears. Inhibition of ADAMTS to treat osteoarthritis may induce unwanted side effects in tendons.


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