Age-Related Loss of the Transforming Growth Factor β Receptor ALK5 Precedes Osteoarthritis Development In Cartilage

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Introduction: Osteoarthritis (OA) is the most common joint disease and (old) age is its main risk factor. One of OA's main hallmarks is degradation of articular cartilage. TGFβ-superfamily signaling plays an important role in cartilage homeostasis and maintenance via induction of Smad phosphorylation (pSmad). pSmad2/3 is a potent inhibitor of chondrocyte terminal differentiation and cell death, whereas pSmad1/5/8 induces these processes. In cartilage, two TGFβ receptors that induce Smad phosphorylation are ALK for Smad1/5/8 and ALK5 for Smad2/3. Previously, we have shown an age-related shift in receptor balance towards dominant Alk1 expression compared to Alk5 expression, and linked elevated Alk1 levels to increased Mmp13 and ColX expression and cartilage damage. However, OA development and ageing are concomitant in mice, and possibly both processes interfere. To uncouple ageing and OA-related processes, we investigated if age-related changes in TGFβ-superfamily signaling occur without interference of the OA process, and if these changes result in an altered cellular response to growth factors. We chose healthy bovine cartilage explants as a model system because bovine samples can be obtained in a wide age range without concurrent OA development.

Methods: From the metacarpophalangeal joint of cows (Bos Taurus) (< 3 h post mortem), full thickness cartilage explants were collected. Macroscopically none of the animals showed any signs of OA. Samples were either immediately frozen in liquid nitrogen or, after 24 h equilibration in serum free medium, stimulated with growth factors for 24 h. Subsequently, mRNA was isolated and gene expression was measured by qPCR, using cDNA specific primers and SYBR-green.

Results: Gene expression analysis of bovine explants ranging from 0.5 up to 12 years old showed a very significant 128-fold (R² = 0.68, p < 0.0001) decrease in Col2a1 expression (fig. 1A) and a 3-fold decrease in Tgfb1 (R² = 0.3, p = 0.002) expression with increasing age compared to 4 reference genes; Gapdh, Rpl22, Rps14 and Gusb. Furthermore, Alk5 expression decreased 4-fold (R² = 0.3, p = 0.0004) whereas Alk1 expression remained unchanged. These changes in receptor level were reflected in cellular response to growth factors. Over the course of 8 years, expression of the Smad2/3p response gene Serpine1 (Pai1) became 11-fold less (p = 0.01) responsive to 1 ng/ml of the ALK5 ligand TGFβ1 (fig. 1B). In contrast, the response of 8 year old cartilage to the ALK1 ligand BMP9 (5 ng/ml) was not significantly different compared to 1 year old cartilage as measured by expression of the Smad1/5/8 response gene Id-1 (fig. 1C).

Discussion: The loss of Col2a1 and Tgfb1 expression are two characteristics of ageing cartilage, and confirm our system as valid to study age-related changes in cartilage. As no signs of OA on macroscopic level could be detected, changes in TGFβ-superfamily receptor expression occur independently of the OA process. The observed decrease in Alk5 expression results in loss of Smad2/3p signaling in response to TGFβ1, indicating that TGFβ1's cartilage protective function is lost with increasing age. We propose that this loss of response predisposes chondrocytes for the OA process.

Significance: This study helps with unraveling the interlinked processes of ageing and OA development.