Introduction: Ageing is the primary risk factor for osteoarthritis (OA). During OA, chondrocytes show deviant behavior resembling terminal differentiation of growth-plate chondrocytes, characterized by elevated MMP-13 expression. Previously we demonstrated a reduction in TGF-β signaling via the ALK5 receptor in aged mice and in experimental OA.

ALK5 activates the Smad2/3 route, which is known to suppress terminal differentiation.

Recently, it has been shown that in certain cell types TGF-β is also able to signal via the alternative TGF-β receptor ALK1, thereby activating the Smad1/5/8 route instead of the Smad2/3 route. The Smad1/5/8 route has been shown to induce chondrocyte terminal differentiation. Therefore, we investigated whether during ageing and OA, TGF-β signaling switched from ALK5 to ALK1, favoring progression of chondrocyte differentiation.

Materials and Methods: First we investigated whether TGF-β was able to signal via both ALK5 and ALK1 in chondrocytes. Therefore we analyzed phosphorylation of Smad2 (Smad2P) and Smad1/5/8 (Smad1/5/8P) upon TGF-β stimulation by Western Blotting. Furthermore, we assessed whether ALK5 and ALK1 signaled specifically via Smad2 or Smad1/5/8 respectively. To assay the latter we transfected chondrocytes with constitutive active TGF-β receptors, Ad-caALK5 or Ad-caALK1, after which a Western Blot was performed staining for Smad2P and Smad1/5/8P. RNA was isolated to evaluate the response to ALK5 or ALK1 signaling on expression of aggrecan, collagen type II and MMP-13 mRNA.

In addition, knee joints were isolated from naive C57BL/6 mice aged 1 or 2 years, OA prone STR/ORT mice (aged 3, 6 and 9 months and 1 year) and 3 months old C57BL/6 mice in which OA was induced by destabilizing the medial meniscus (DMM model) and prepared for histology in paraffin sections. Immunohistochemistry for ALK5, ALK1, PAI1 (marker Smad2/3 route) and Id1 (marker Smad1/5/8 route) was performed. The number of cells staining positive for each receptor in tibial cartilage was measured with a computerized imaging system and corrected for the cell number in HE-stained sections.

Results: In vitro, chondrocytes displayed both Smad2P and Smad1/5/8P upon stimulation with TGF-β. Thus both routes can be activated by TGF-β. Transfection of chondrocytes with caALK5 specifically led to Smad2P, whereas caALK1 specifically led to Smad1/5/8P. Chondrocytes that overexpressed caALK5 showed enhanced aggrecan expression and slightly reduced collagen type II expression. Chondrocytes overexpressing caALK1 had elevated expression of aggrecan, collagen type II and MMP-13, thereby displaying an OA-associated phenotype.

From 1 year old to 2 year old mice ALK5 decreased 88% in medial tibial cartilage and 76% in lateral tibial cartilage. ALK1 also was reduced, but only 51% in medial and and 33% in lateral tibial cartilage. PAI1 expression had been drastically reduced in old mice by 77% on the medial side and 61% on the lateral side of the tibia. Id1 expression had also decreased, but by 58% and 56% respectively. Thus, with age there was a stronger reduction in ALK5 than ALK1 expression and signaling via Smad2/3 (PAI1) appears to be more reduced than signaling via Smad1/5/8 (Id1).

In the DMM model no change was observed on the lateral side, but a reduction of 91% ALK5 positive cells compared to 75% ALK1 positive cells in the OA developing medial side. PAI and Id1 only showed a reduction in staining in the cartilage on the medial side with 79% and 70% respectively. This again shows a stronger reduction in ALK5 signaling than signaling via ALK1.

STR/ORT mice develop the most severe OA in the medial tibia. The number of cells staining positive for ALK5 was already reduced to 7% in the medial tibial cartilage by 3 months of age. This declined to less than 1% by 9 months. (Figure 1)

Discussion: Our data show a strong decrease in ALK5 expression in cartilage of ageing mice, whereas ALK1 expression only slightly diminished. In murine models of OA we showed a pronounced decrease of ALK5 expression in progressive OA and a less pronounced decrease of ALK1. In addition, PAI1 expression decreased more rapidly than Id1 expression, indicating that the change in receptor expression ratio results in an altered downstream signaling and protein expression. Thus, with age and OA progression, the balance between ALK1 and ALK5 expression is in favour of the ALK1 side. We have shown in vitro that ALK1 signaling in chondrocytes induces OA-like changes indicating a role for ALK1 signaling in OA development.

Overall our data suggest a role for decreased ALK5 and increased ALK1 signaling in deviant chondrocyte behavior in ageing and OA development.