Relation between TGF-β-induced LH2b and TGF-β-induced synovial fibrosis during OA

INTRODUCTION
In knee joints with OA, fibrosis is a major contributor to both joint pain and stiffness. We found that TGF-β overexpression in murine knee joints leads to persistent fibrosis of the joint capsule while overexpression of CTGF, induced transient fibrosis. Strikingly, TGF-β overexpression gave a major increase in lysyl hydroxylase 2b (LH2b) expression, whereas CTGF did not change LH2b gene expression. It has been shown that hydroxylysine-derived cross links, formed by LH2b, are much more resistant to degradation than lysine-associated cross links. Therefore a causal relationship between LH2b and irreversible fibrosis after TGF-β exposure is plausible.

We investigated whether TGF-β can also induce LH2b in human synovial fibroblasts similar to our findings in mice. In addition we investigated which TGF-β route ALK1 (Smad1/5/8) or ALK5 (Smad2/3) is responsible for LH2b regulation in human fibroblasts.

METHODS
Human synovial fibroblasts were isolated from OA synovial tissue, obtained from knee joints arthroplasties. Primary cell cultures derived from five different donors were used to carry out the experiments. The fibroblasts were transduced with either Ad-TGF-β or Ad-CTGF or Ad-Luc to determine their effects on LH2b gene expression. To evaluate whether the effects on LH2b are ALK1 and ALK5 dependent, the fibroblasts were transduced with an adenovirus overexpressing constitutive active ALK1 (Ad-caALK1) or ALK5 (Ad-caALK5). The fibroblasts were stimulated with TGF-β to determine the effects on LH1, LH2b, LH3, lysyl oxidase (LOX), collagen type 1A1 (COL 1A1), and CTGF gene expression. RNA was isolated and the gene expression was measured with RT-PCR. ALK1 (Smad1/5/8) or ALK5 (Smad2/3) signaling were blocked with SB505124 (SB5) and dorsomorphin (DM) respectively. Changes in Smad 2/3 and Smad 1/5/8 phosphorylation were determined with Western Blotting.

RESULTS
Transduction with Ad-TGF-β induced LH2b gene expression whereas Ad-CTGF did not alter the LH2b gene regulation (Fig 1A). Transduction with Ad-caALK5 induced LH2b in contrast to Ad-caALK1 which induced down regulation of LH2b (Fig 1B).

DISCUSSION
CTGF and LOX gene expression were fully blocked by DM while LH2b gene expression was only decreased by DM and not completely blocked. Identical results were observed for all five primary fibroblast cell cultures.

Fig 2. mRNA expression of LH1, LH2b, LH3, LOX and COL1A1 in human synovial fibroblasts 24 h after TGF-β stimulation with and without 4 h pre-incubation with SB-5 or DM.

Fig 3. Hypothetical working mechanism LH2b induction.