Changes in TGF-beta Superfamily Signaling Balance from Smad2/3 to Smad1/5/8 are associated with Chondrocyte MMP-13 Expression

INTRODUCTION
Osteoarthritis (OA) is characterized by cartilage degradation. Changes in TGF-beta superfamily signaling regulate chondrocyte differentiation and play a role in OA development. The major TGF-beta signaling route is via Smads, Smad1/5/8 represent the so-called BMP route and Smad2/3 the TGF-beta route. We have shown that Smad2/3 signaling was reduced in OA (Figure 1 C,D). In contrast, BMP2, known to stimulate Smad1/5/8 signaling, was elevated nearby OA lesions (Figure 1 A,B). We studied whether BMP-2 expression is related to diminished Smad2/3 signaling and explored the potential biological significance of BMP-2 neighboring OA lesions.

RESULTS
Overexpression of BMP-2 in murine knee joints, thereby stimulating the Smad1/5/8 signaling route, decreased Smad2/3P expression in cartilage (Figure 2) and increased staining of VDIPEN and NITEGE epitopes (MMP- and ADAMTS-mediated cartilage breakdown respectively).

In vitro, chondrocyte exposure to BMP-2 resulted in up regulation of aggrecan, collagen type II, collagen type X and MMP13 expression. Thus, elevating Smad1/5/8 signaling is associated with concomitant reduction of Smad2/3 signaling and OA-like alterations.

After in vivo exposure to TGF-beta, a mild decrease in PG content (8%) in tibial cartilage was found. However, combining TGF-beta with BMP-2 resulted in 21% decrease in PG content. PG staining was lost in the deep zones of tibial cartilage, just above the tide-mark.

To investigate the effect of reduced Smad2/3 activation on overall Smad signaling we blocked ALK5 activity (Smad2/3 route) in TGF-beta activated chondrocytes with SB-505124. Besides the expected decrease in Smad2/3P, Smad1/5/8P was enhanced, thereby contributing to a shift in Smad signaling balance.

Human OA cartilage exposure to TGF-beta inhibited MMP-13 expression in 8 out of 10 patients. This inhibition was totally blocked by the ALK5 inhibitor SB-505124 in 7 out of 8 patients. This indicates that in chondrocytes inhibition of Smad2/3P activation enhances MMP-13 expression.

DISCUSSION
Our data indicate that the Smad2/3 and Smad1/5/8 balance determines chondrocyte function. Either up regulation of the Smad1/5/8 pathway or down regulation of the Smad2/3 route, both we have observed in OA cartilage, appears associated with increased chondrocyte MMP-13 expression. This fits with published observations that Smad1/5/8 stimulates, while Smad2/3 signaling blocks chondrocyte terminal differentiation. These results indicate that factors changing the balance to dominant Smad1/5/8 signaling, for instance changes in TGF beta superfamily ligands or receptors, will affect chondrocyte differentiation and MMP-13 expression and might play a role in OA development.