Decreased chondrocyte hypertrophic differentiation by inhibition of cyclooxygenase-2

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INTRODUCTION:
Chondrocyte hypertrophic differentiation is an essential process during endochondral bone formation, however it hampers the application of many cartilage regenerative techniques and may play a role at the onset of osteoarthritis (1). Heterotopic bone formation after orthopaedic surgery is suppressed by non-steroidal anti-inflammatory drugs (NSAIDs) and recent studies point to an essential role of Cyclooxygenase-2 (COX-2) in osteocytes during endochondral ossification (2). COX-2 and its metabolite PGE2 are thought to regulate the function of bone morphogenic protein-2 (BMP-2) and viae VEGF, which might provide an explanation for the role of COX-2 during endochondral ossifi cation (3). It is largely unknown how and in which phase NSAIDs affect endochondral bone formation. In this study, we aim to determine the involvement of COX-2 in chondrocyte hypertrophy and provide an explanation for the suppressive effect of NSAIDs on heterotopic ossification and fracture healing.

METHODS:
To study the role of COX-2 during endochondral ossification, ATDC5 cells were differentiated in the chondrogenic lineage (4) in the presence of BMP-2 for 14 days. The NSAIDs indomethacin (COX-1/COX-2 inhibitor) or NS398 (COX-2 specific inhibitor) were used to inhibit COX-activity. Expression analysis was performed by RT-qPCR and western blot (Collagen II, Collagen X, RunX2, COX-2, BMP-2). Growth curves were obtained from increasing indomethacin- and NS398-concentrations. Cell numbers were determined by X-violet staining. Rabbit periosteal grafts were differentiated for 3 weeks in an agarose sandwich (5) in the presence of BMP-2 (30 ng/ml) and indomethacin (2 µM) or NS398 (2 µM). Differentiated grafts were harvested for RT-qPCR and (immuno-)histological analysis. PGE2 production in medium was measured using a specific ELISA.

RESULTS SECTION:
Hypertrophic differentiation of ATDC5 cells was obvious from day 14. Chondrogenesis was accompanied by upregulation of COX-2 expression and PGE2 in the media. Accordingly, expression of Collagen X was also significantly upregulated. COX-2 activity was completely inhibited by indomethacin or NS398. Surprisingly, specific inhibition of COX-2 by NS398 did not affect Collagen II expression whereas expression of RunX2 and Collagen X was significantly decreased in a concentration dependent way. COX-inhibition by indomethacin showed similar results, however had inhibitory effects on Collagen II expression at higher concentrations. Expression of BMP2 decreased upon increasing NSAID concentrations, confirming a functional connection between COX-2 and BMP2. To determine whether COX-inhibition specifically affects differentiation, the proliferation rate of ATDC5 cells under proliferating and differentiating conditions was determined. No significant effect on proliferation was detected. Chondrocyte hypertrophy of rabbit periosteal grafts was dependent on BMP2. This BMP2-mediated chondrocyte hypertrophy was suppressed by COX-inhibition as determined by ELISA, RT-qPCR and histological analysis, confirming our findings in ATDC5 cells (Fig 1).

DISCUSSION:
Our data show that COX-2 is upregulated during chondrogenesis and BMP2 induces chondrocyte hypertrophy and COX-2 expression, suggesting a functional connection. Using two independent systems we were able to show that inhibition of COX-activity decreases chondrocyte hypertrophy during endochondral bone formation via a yet unknown mechanism. Ongoing research focuses on a potential molecular pathway by which COX-2 might regulate chondrocyte hypertrophy. Insight into the molecular mechanism is essential to understand the effect of NSAIDs on fracture healing. In addition, our data may provide a novel strategy to improve the outcome of cartilage regenerative medicine by decreasing the level of unwanted chondrocyte hypertrophy.

REFERENCES
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