192 ZONAL GENE EXPRESSION OF CHONDROCYTES IN OSTEOARTHRITIC CARTILAGE

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Purpose: In osteoarthritis (OA), chondrocyte metabolism changes dramatically. Since chondrocyte metabolism is known to differ by depth within articular cartilage, such metabolic change with OA could differ among cartilage zones. In this study, we investigated the characteristics of chondrocyte metabolism in respective cartilage zones of OA cartilage.
Methods: The study was performed under the approval of the ethical committees of the institutes. OA cartilage was obtained from macroscopically intact areas of 4 knee joints with end-stage disease. The cartilage was divided into three cartilage zones (superficial, middle and deep zones) and gene expression profiles were determined in the respective zones by a custom-designed microarray that focused on chondrocyte-related genes. For genes the expression of which was significantly different among the zones, the expression was compared between OA and control cartilage in respective zones by an analysis using laser capture microdissection (LCM) and real-time PCR. For several genes, correlation of expression among OA cartilage samples was investigated in specific cartilage zones.
Results: Approximately 40% of the genes investigated were found to be expressed at significantly different levels across the zones. Among them, the expression of 25 genes (12 genes for cartilage components, 8 bone-related genes, and 5 metalloproteinase genes) was compared between OA and control cartilage in respective zones by LCM and real-time PCR, which confirmed the validity of the microarray analysis.
The expression of 12 cartilage component genes was all enhanced in OA cartilage compared with that in the control. For these, the level of enhancement was considerably different among the genes (2- to 220-fold), suggesting that the balance of their synthesis might be considerably disturbed in OA cartilage. In contrast, for all 12 genes, the difference of expression levels among the three zones was very similar between the OA and control cartilage, indicating that for each gene, the increase of expression in OA cartilage occurred at a similar level throughout the zones.
The expression of 8 bone-related genes was most enhanced in either the superficial (*COL1A2*, *POSTN*, *TNA*) or deep zone (*COL10A1*, *IBSP*, *SPARC*, *SPP1*, *WNT5B*) within OA cartilage. Interestingly, the former three genes are known to be expressed by osteoblasts, while two of the latter are characteristically expressed by hypertrophic chondrocytes. Since the expression of those genes was mutually correlated in the superficial and deep zones, respectively, their expression in OA cartilage was assumed to be induced by the phenotypic change of the chondrocytes at those zones.
The expression of 5 proteinase genes (*MMP2*, *MMP3*, *MMP11*, *MMP13*, *ADAMTS5*) was all most enhanced in the superficial zone within OA cartilage. In that zone, their expression was mutually correlated, which might suggest the presence of a common regulatory mechanism(s) for their expression. Among the five genes, the expression of *MMP11* and *MMP13* was most enhanced in OA cartilage (up to 40-fold increase), implying that those proteinases could be deeply involved in the pathology of OA.
Conclusions: In OA cartilage, the metabolic activity of chondrocytes differed considerably among zones. Characteristic changes were observed in superficial and deep zones.

193 MOLECULAR CONTROL OF ARCTICULAR CARTILAGE DEGENERATION BY TRANSFORMING GROWTH FACTOR ALPHA

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Purpose: Articular cartilage degeneration is a hallmark of osteoarthritis (OA). We previously identified increased expression of transforming growth factor alpha (TGFα) and chemokine (C-C motif) ligand 2 (CCL2) in articular cartilage from a rat model of OA. We subsequently reported that TGFα signaling modified chondrocyte cytoskeletal organization, increased catabolic and decreased anabolic gene expression and suppressed Sox9. Due to other roles in chondrocytes, we hypothesized that the effects of TGFα on chondrocytes are mediated by Rho/ROCK and MEK/ERK signaling pathways.
Methods: Primary cultures of chondrocytes and articular osteochondral explants were treated with pharmacological inhibitors of MEK1/2 (U0126), ROCK (Y27632), Rho (C3), p38 MAPK (SB202190) and PI3K (LY294002) to elucidate pathway involvement.
Results: Using G-LISA we determined that stimulation of primary chondrocytes with TGFα activates RhoA. Reciprocally, inhibition of RhoA/ROCK but not other signaling pathways prevents modification of the actin cytoskeleton in response to TGFα. Inhibition of MEK/ERK signaling rescued suppression of anabolic gene expression by TGFα including SOX9 mRNA and protein levels. Inhibition of MEK/ERK, Rho/ROCK, p38 MAPK and PI3K signaling pathways differentially controlled the induction of MMP13 and TNFα gene expression. TGFα also induced expression of CCL2 specifically through MEK/ERK activation. In turn, CCL2 treatment induced the expression of MMP3 and TNFα. Finally, we assessed cartilage degradation by immunohistochemical detection of type II collagen cleavage fragments generated by MMPs. Blockade of RhoA/ROCK and MEK/ERK signaling pathways reduced the generation of type II collagen cleavage fragments in response to TGFα stimulation.
Conclusions: In conclusion, Rho/ROCK signaling mediates TGFα-induced changes in chondrocyte morphology, while MEK/ERK signaling mediates the suppression of Sox9 and its target genes, and CCL2 expression. CCL2, in turn, induces the expression of MMP3 and TNFα, two potent catabolic factors known to be involved in OA. These pathways may represent strategic targets for interventional approaches to treating cartilage degeneration in osteoarthritis.

194 TRANSFORMING GROWTH FACTOR BETA 1 MEDIATED COLLAGEN BIOSYNTHESIS IN ARTICULAR CHONDROCYTES IS DEPENDENT ON AN INTACT MICROTUBULAR NETWORK

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Purpose: One of the major cytoskeletal architectures in chondrocytes is the tubulin microtubules. Tubulin is essential for many fundamental cellular processes including mitosis and protein secretion. In chondrocytes, an intact tubulin network has previously been shown to be required for secretion of collagen and sulphated glycosaminoglycans (sGAG). Interestingly, the spatial organisation of these networks has been observed to be disrupted in human osteoarthritic chondrocytes or absent in a rat model of osteoarthrosis. Therefore the aim of this study was to characterise the effect of tubulin microtubule disassembly on articular chondrocyte matrix synthesis and elucidate the mechanism(s) involved in this differential regulation.
Methods: Chondrocytes from 7-day-old bovine articular cartilage were plated at 1×10^6cells/well of a 24-well plate. Tubulin microtubules were disrupted with 1 μM colchicine over a 1, 3 or 7 day period, and disassembly confirmed by confocal microscopy. The effect of microtubule disruption on *de novo* collagen and sGAG synthesis was measured by incubating chondrocytes with 1 μM colchicine for 7 days in the presence of 20 μCi/ml [^{3}H]-proline and 10 μCi/ml [^{35}S]-sulphate; untreated cultures served as controls. Unincorporated label was removed and counts performed. Expression levels of β-tubulin and type II collagen mRNA and protein were assessed by quantitative PCR and Western blotting respectively. Expression and activation of TGFβ1 was assessed using an ELISA. Chondrocytes were subsequently pretreated with 1 μM colchicine for an hour prior to the addition of 10 ng/ml TGFβ1 for 1, 3 or 7 days, and both Western blotting and quantitative PCR performed to determine expression levels of type II collagen, TGFβ1 and TGFβ1 receptor. Downstream signalling effects i.e. activation of the MAP kinases was monitored in tubulin-disrupted chondrocytes, in the presence or absence of TGFβ1. Restoration of matrix production was assessed by pre-treating chondrocytes with selective MAPK inhibitors prior to addition of colchicine.
Results: Disruption of the chondrocyte tubulin networks with 1 μM colchicine not only affected spatial organisation but also significantly inhibited the expression of both β-tubulin mRNA and protein over the 7 day culture period. *De novo* collagen and sGAG synthesis were inhibited by tubulin disruption (stabilisation of the networks with 1 μM taxol enhanced the chondrocytic phenotype). In the absence of intact tubulin networks, there was a significant reduction in TGFβ1. TGFβ1 is a