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TYPE II COLLAGEN PEPTIDE ACTIVATES AKT LEADING TO NF-κB UP-REGULATION IN OSTEOARTHRITIC CHONDROCYTES

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Background of this study: In addition to the proinflammatory cytokines, degradation products of cartilage matrix are thought to contribute to joint destruction in osteoarthritis (OA). Excessive degradation of cartilage matrix in OA involves enhanced cleavage of type II collagen by collagenases, resulting in denaturation of the triple helix of this collagen. Denatured and degraded type II collagen leads to an increase in proteolytic products of type II collagen. Recently, we have found that a 24-mer synthetic peptide of type II collagen named CB12-II stimulates type II collagen cleavage with induction of matrix metalloproteinase (MMP)-13 in association with nuclear factor-κB (NF-κB) activation. However, intracellular upstream events that cause NF-κB up-regulation in response to CB12-II remain unclear.
The aim of this study: Akt is a serine/threonine protein kinase that regulates cell survival signals in response to growth factors and cytokines. Akt is activated via the phosphoinositide-3-OH kinase (PI3K) pathway, an important pathway regulating immunity and inflammation. Accumulating evidence indicates that Akt could stimulate signaling pathways that up-regulate the activity of NF-κB. Therefore, this study was aimed to elucidate the involvement of PI3K/Akt pathway in NF-κB activation by CB12-II in OA chondrocytes.
Methods: OA cartilage specimens were obtained from the distal femur from patients undergoing total knee replacement surgery. Chondrocytes isolated from the OA articular cartilage were cultured in monolayer with CB12-II. Secreted levels of MMP-13 in conditioned media were determined with enzyme-linked immunosorbent assay (ELISA). The cell lysates were used to detect endogenous levels of phosphorylated Akt and phosphorylated p65 NF-κB by ELISA.
Results: In OA chondrocyte monolayer cultures, CB12-II stimulated MMP-13 production in association with up-regulation of NF-κB and Akt. Inhibition studies using BAY11-7085 confirmed that MMP-13 production by CB12-II was dependent on NF-κB pathway. Similarly, inhibition studies using LY294002 revealed that MMP-13 production by CB12-II was dependent on PI3K/Akt pathway. When OA chondrocytes were preincubated with LY294002, CB12-II-induced levels of phosphorylated p65 NF-κB were significantly decreased. Thus, NF-κB activation leading to MMP-13 production requires PI3K/Akt pathway in CB12-II-stimulated chondrocytes.
Conclusions: This is the first study demonstrating that the type II collagen-derived peptide, CB12-II, activates PI3K/Akt pathway leading to up-regulation of NF-κB. Elucidation of intracellular pathways activated by type II collagen fragments may be helpful to understand the pathological process in OA cartilage destruction.

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HYPERCHOLESTEROLEMIA IS A METABOLIC RISK FACTOR FOR OSTEOARTHRITIS

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Purpose: A growing body of evidence suggests that osteoarthritis (OA) is rather a "metabolic disorder" in which various interrelated metabolic mediators contribute to the initiation and progression of the disease process. One such metabolic risk factor could be high cholesterol levels in the body. The primary objective of this study is to delineate how abnormal cholesterol levels effects the cartilage biology and its relation to OA development. Further, in this study we tested whether hypercholesterolemia -induced mitochondrial DNA (mtDNA) damage contributed to increased oxidative stress, mitochondrial dysfunction and chondrocyte apoptosis.
Methods: We investigate the expression of genes regulating cholesterol efflux (NR1H3 and ABCA1) and influx (SREBF-2 and NPC1L1) in human chondrocytes that were isolated and graded according to the disease severity from the patients who were undergoing knee replacement surgeries. The effect of mtDNA damage on the mRNA expression of respiratory chain subunits, change of mitochondrial membrane potential ($\Delta\psi m$), overproduction of ROS, and apoptosis were assessed in high cholesterol-treated ACCs by qRT-PCR, flow cytometry, and confocal microscopy. The effects of high cholesterol treatment on the human and bovine cartilage explants were assessed by proteoglycan synthesis and various biochemical assays.
Results: In OA ACCs intracellular levels of cholesterol were higher compared to control group. Furthermore, OA ACCs showed dysregulation of the balance between cholesterol influx and efflux genes. Expression of cholesterol influx genes (SREBF-2 and NPC1L1) were increased in OA ACCs compared to control group. On the other hand, expression of cholesterol efflux genes, ABCA1 and NR1H3 were decreased in OA ACCs (Figure 1A). We then demonstrated that mtDNA oxidative damage increased rapidly after high cholesterol (300μM). Accordingly, treatment with high cholesterol resulted in decreased membrane potential and overproduction of ROS in ACCs ($P < 0.05$) and apoptosis (Figure 1B). Interestingly, cholesterol (300μM) treatment of cartilage explants culture showed an extensive proteoglycan loss (Figure 1C). MTT analysis showed that metabolic activity of ACCs was gradually decreased with ascending dose of cholesterol (50, 300, 600μM) stimulation. Protein and mRNA expression level of MMP-13 were increased in cholesterol-stimulated group compared to control; however, COL2A1 showed different expression trend after cholesterol treatment. Both protein and mRNA expression level of COL2A1 were increased in treatment group compared to control (Figure 1C).
Conclusion: mtDNA oxidative damage seems to be the "trigger" for cell dysfunction in high cholesterol-challenged ACCs by setting in motion the vicious circle of mtDNA damage leading to ROS overproduction and further apoptosis resulting in the development of abnormalities that resemble characteristic features associated with OA.

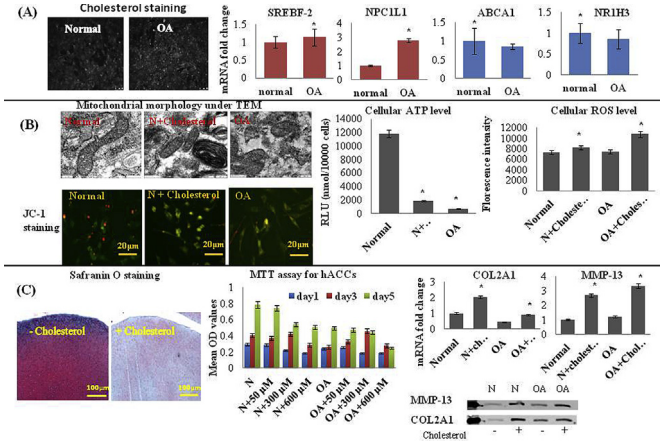

Fig.1 (A) cholesterol distribution in OA ACCs is higher compared to normal. **(B)** Cholesterol treatment induce oxidative stress environment derived from abnormal mitochondrial function. **(C)** Cholesterol treatment challenge cartilage haemostasis.

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RESVERATROL REGULATES THE WNT/ß-CATENIN PATHWAY IN HUMAN OSTEOARTHRITIS OSTEOBLASTS

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Purpose: Clinical and *in vitro* studies suggest that subchondral bone sclerosis due to abnormal osteoblasts (Ob) is involved in the progression and/or onset of osteoarthritis (OA). Human Ob isolated from sclerotic subchondral OA bone tissue show an altered phenotype, a decreased canonical Wnt/ß-catenin signaling pathway (cWnt), and a reduced mineralization *in vitro*, alterations linked with an abnormal response to BMP-2. Recent studies have shown an association between dietary polyphenols and the prevention of OA. Resveratrol (RSV) is a polyphenolic phytoestrogen that activates Sirtuin 1, which we previously showed to be reduced in OA Ob. RSV also stimulates Ob differentiation and may have a positive effect on cartilage protection. RSV regulates the cWnt pathway in different cell systems and stimulates BMP-2 expression in human Ob, however, the role of RSV and its effect in OA Ob remains unknown. Here we investigated the role of RSV in OA Ob and if it is responsible for their altered response to BMP-2.
Methods: We prepared primary human subchondral Ob using the sclerotic medial portion of the tibial plateaus of OA patients undergoing total knee arthroplasty, or from tibial plateaus of normal individuals at autopsy. The expression of genes was evaluated by qRT-PCR and the protein production by Western blot analysis. Alkaline phosphatase activity (ALPase) and osteocalcin release (OC) were measured by