Introduction

Osteoarthritis (OA) is the most common chronic degenerative joint disorder that leads to severe disability in 25% of our senior population. The disease is characterized by progressive joint pain, deformity, and immobility. There are no current treatments to slow the progression of this disease and the only reliable pain-relieving treatment, total joint replacement surgery, is reserved for those with long-standing, severe pain. Despite the widespread health implications of this disease the basic mechanisms underlying it are incompletely understood. Articular chondrocytes remain a stable phenotype during adulthood. The main function of articular chondrocytes is to maintain articular cartilage homeostasis. Recent findings, including findings from our laboratory suggested that articular chondrocytes might lose their phenotype during OA progression and that the loss of articular chondrocyte phenotype may be a major contributor to OA progression and cartilage destruction. Our and other laboratories have shown that hypertrophic and terminal differentiation events of articular chondrocytes occur in OA cartilage. (1-3) In addition, a previous study has shown that the progression of surgically-induced OA in runx2 haplosufficient mice was delayed compared to the progression of surgically-induced OA in wild type littermates. (4) Runx2 is a major transcription factor regulating hypertrophic and terminal differentiation events of growth plate chondrocytes. (5) These findings suggest that hypertrophic and terminal differentiation events may play a major role in progression of OA and that interfering with these events may prevent or slow down the progression of OA. Previous studies have shown that annexin V, annexin VI, and the progressive ankylosis protein (ANK) are highly upregulated during the progression of OA. (6-7) Annexin V and VI have been shown to stimulate terminal differentiation and mineralization events of growth plate chondrocytes and interfering with annexin functions delays terminal differentiation and mineralization of growth plate chondrocytes. (8-9) ANK, a transmembrane protein that transports intracellular pyrophosphate (PPi) to the extracellular milieu, has been shown to stimulate mineralization of growth plate chondrocytes. (10) Therefore, the annexins and ANK play major roles in the regulation of terminal differentiation and mineralization events of growth plate chondrocytes. Based on these findings we tested the hypothesis that annexin V and VI, and ANK may play important roles in the stimulation of terminal differentiation and mineralization events in OA articular chondrocytes and that interfering with these proteins’ functions may prevent terminal differentiation in OA cartilage and ultimately may slow down the progression of OA.

Methods

Human OA Chondrocyte Cell Culture – Human OA cartilage was obtained from patients undergoing total knee arthroplasty at NYU Hospital for Joint Diseases under IRB approval. Cells were grown in monolayer cultures at high density in DMEM containing 5% FCS, 2 mM L-glutamine, and 50 units/ml penicillin and streptomycin. When cells reached semi-confluency, they were transfected with empty pcDNA expression vector, pcDNA expression vector containing full length annexin V, annexin VI or ank cDNA. After transfection, cells were cultured for up to 6 days. Real time PCR analysis - Total RNA was isolated from cell cultures using the RNeasy Minikit (Qiagen) at 2, 4, and 6 days after transfection. Gene expression was quantified by real-time PCR analysis using SYBR Green as described previously. (10,11)

Results

Transfection of human OA articular chondrocytes with pcDNA expression vector containing full-length annexin V, annexin VI, or ank cDNA resulted in a 3-4 fold increase of annexin V, annexin VI and ANK protein expression. Overexpression of annexin V, annexin VI, or ANK in OA articular chondrocytes mimics the in vivo increase of the expression of these proteins during OA progression. Under our culture conditions the mRNA levels of hypertrophic and terminal differentiation markers decreased during the 6-day culture period in untransfected or empty vector-transfected human OA articular chondrocytes, whereas the mRNA levels of type II collagen and aggrecan increased in these cultures. Overexpression of annexin VI resulted in a significant increase of the mRNA levels of hypertrophic and terminal differentiation markers, including alkaline phosphatase (APase), matrix metalloproteinase (MMP)-13, runx2, and type X collagen, whereas annexin V overexpression did not change the mRNA levels of these genes. However, overexpression of annexin V resulted in an increase of the mRNA levels of annexin VI. Overexpression of ANK resulted in the increase of the mRNA levels of APase, MMP-13 and runx2, whereas mRNA levels of type X collagen were not increased compared to mRNA levels of empty vector transfected cells. In addition, overexpression of annexin V, annexin VI, or ANK resulted in decreased mRNA levels of type II collagen and aggrecan compared to the levels of empty vector-transfected cells.

Discussion

The findings of this study show that overexpression of annexin VI or ANK in human OA articular chondrocytes, which mimics the in vivo increase of the expression of these proteins during OA progression, stimulated hypertrophic and terminal differentiation events as indicated by increased expression of hypertrophic and terminal differentiation markers, including APase, MMP-13, and runx2, and decreased expression of articular cartilage markers, including type II collagen and aggrecan. Type X collagen mRNA levels were increased in annexin VI-overexpressing, but not in ANK-overexpressing human OA chondrocytes. A previous study has shown that different signaling pathways activate or stimulate the expression of different hypertrophic and terminal differentiation marker genes. For example, retinoic acid-mediated upregulation of type X collagen via stimulating p38 MAP kinase signaling, whereas increase of osteopontin expression in RA-stimulated growth plate chondrocytes was mediated via activation of the ERK MAP kinase signaling pathway. (12) Annexin VI stimulates terminal differentiation and mineralization events of growth plate chondrocytes via mediating Ca2+ influx into these cells, whereas ANK affects terminal differentiation and mineralization of growth plate chondrocytes via the regulation of extracellular PPi/phosphate (Pi) homeostasis. (13) Therefore, these proteins use different signaling pathways to stimulate terminal differentiation events of chondrocytes, and therefore it is possible that these proteins stimulate the expression of different hypertrophic and terminal differentiation marker genes. In conclusion, our findings demonstrate that hypertrophic differentiation events are major contributors to the progression of OA, and that annexin VI and ANK, two proteins, which regulate these events during development, also stimulate terminal differentiation events of OA human articular chondrocytes. Current experiments are underway to determine whether inhibiting the expression or function of annexin VI and/or ANK will prevent or slow down the progression of OA.

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References