Annexin A6 Accelerates Osteoarthritis Progression by Stimulating NF-κB Activity

INTRODUCTION:

Annexin A6 (AnxA6) belongs to the annexin protein family. The annexins are a family of proteins, which all bind to membranes in the presence of Ca²⁺. Recently, changes of annexin expression and/or localization have been shown to be involved in disease pathology. In particular, changes in AnxA6 expression levels have been shown to play crucial roles in several diseases, including cancer and end stage heart failure. For example, high expression of AnxA6 in EGFR-positive breast cancer cells makes these cells less metastatic because AnxA6 inhibits epithelial growth factor receptor (EGFR)/Ras signaling activity. Inhibition of this pathway results in reduced proliferation and oncogenic events in EGFR-positive breast cancer cells. Contrary, low expression of AnxA6 in EGFR-positive breast cancer cells results in high metastatic and oncogenic activity because of the increased activity of EGFR/Ras signaling. These anti-angiogenic properties of AnxA6 are a consequence of its ability to bind to p120GAP, an important signaling component of the EGFR/Ras signaling pathway, thereby altering EGFR/Ras signaling activity. AnxA6 is not expressed in normal healthy articular cartilage, but is highly expressed in OA cartilage. AnxA6 expression increases with OA progression. However, nothing is known about the role of AnxA6 in OA pathogenesis. NF-κB transcription factors play a major role in OA pathogenesis. The activity of NF-κB transcription factors is being stimulated by a host of stress-related stimuli including pro-inflammatory cytokines such as interleukin-1 (IL-1), excessive mechanical stress, and ECM degradation products. Activated NF-κB regulates the expression of many cytokines and chemokines, adhesion molecules, inflammatory mediators, and several matrix-degrading enzymes, including MMP-13. NF-κB also has indirect positive effects on downstream regulators of terminal chondrocyte differentiation (including beta-catenin and Runx2).

Therefore, we tested the hypothesis that AnxA6 plays a major role in OA pathogenesis by modulating NF-κB activity, and that interfering with AnxA6 function may provide a novel therapeutic strategy to stop or slow down the progression of OA.

METHODS:

Animals – Post-traumatic OA was generated in five 10-week-old AnxA6−/− mice and five wild type littermates using the transection of the medial collateral ligament and partial medial meniscectomy (PMM) joint instability model or sham surgery.

Cell Culture – Human articular chondrocytes were isolated from leftover tissue obtained from patients undergoing total knee arthroplasty at NYU Hospital for Joint Diseases under IRB approval. Mouse articular chondrocytes were isolated from articular cartilage caps from 2-month-old AnxA6 knockout (−/−) mice and wild type littermates. Cells were grown in monolayer cultures at high density. When cells reached semi-confluency, they were transfected with empty pcDNA expression vector or pcDNA expression vector containing AnxA6 cDNA. Cells were cultured for up to 3 days in the absence or presence of IL-1 (10ng/ml). Femoral heads from 16-18-week old wild type or AnxA6−/− mice were cultured for 4 days in the absence or presence of IL-1 (10ng/ml).

Histology – Knee joints were isolated 10 weeks after surgery. Safranin O-stained slides were used to quantify OA-related cartilage damage using the histological scoring system of OA (0 to 6) in the mouse recommended by OARSI. Cartilage explants were processed for histology. Sections were stained with safranin O. Safranin O-stained samples were graded from 0 to 3 with 0 being normal staining; 1 being slightly reduced staining; 2 being moderately reduced staining; and 3 being no or close to no staining. In addition, sections were immunostained with antibodies specific for MMP-13.

Real time PCR analysis - Total RNA was isolated from cell cultures 72 h after transfection. Gene expression was quantified by real-time PCR analysis using SYBR Green.

Luciferase assays - Human chondrocytes were co-transfected with empty pcDNA or AnxA6pcDNA, the pNF-κB-MetLuc2-reporter vector (NF-κB-Luc; Clontech), and a pSEAP2 control vector. Luciferase activity in the medium was measured 48 h after transfection.

RESULTS:

The treatment of AnxA6−/− femoral head explants with IL-1 for 3 days resulted in a markedly reduced proteoglycan loss and MMP-13 immunostaining compared with IL-1−treated wild type femoral head explants. OA severity 10 weeks after PMX surgery was significantly reduced in AnxA6−/− mice compared with wild type littermates (OA score for AnxA6−/− mice of 2.11 ± 0.51 versus 4.68 ± 0.82 for wild type littermates; n=7; p<0.01). In addition, AnxA6−/− articular chondrocytes transfected with the NF-κB luciferase reporter showed markedly reduced luciferase activity after 3-day treatment with IL-1 compared with IL-1−treated wild type cells. Western blot analysis revealed that the lack of AnxA6 in AnxA6−/− cells decreased the phosphorylation of the p65 subunit of NF-κB and resulted in a decreased amount of the p65 subunit in the nucleus after IL-1 treatment compared with wild type cells. The mRNA levels of catabolic factors, including IL-6, iNOS, MMP-13 and ADAMTS-5, and hypertrophic markers, including alkaline phosphatase (APase), runx2, and type X collagen, were reduced, whereas mRNA levels of articular cartilage markers (aggrecan, Sox-9, type II collagen) were increased in IL-1−treated AnxA6−/− chondrocytes compared with wild type cells. Contrary, overexpression of AnxA6 in human articular chondrocytes, which mimics the in vivo increase of AnxA6 expression during OA progression, resulted in increases of catabolic and hypertrophic differentiation marker mRNA levels, whereas articular cartilage marker mRNA levels were decreased compared with the levels of empty vector-transfected cells. In addition, AnxA6 overexpression in human articular chondrocytes resulted in increased NF-κB activity as indicated by increased luciferase activity of the NF-κB-Luc reporter compared with the luciferase activity of empty vector-transfected cells. AnxA6 overexpression also resulted in a further marked increase of luciferase activity of the NF-κB-Luc reporter in IL-1−treated chondrocytes compared with the activity of IL-1−treated, empty vector-transfected cells. Consequently, MMP-13 mRNA levels were further increased in IL-1−treated, AnxA6 overexpressing articular chondrocytes compared with the MMP-13 mRNA levels in IL-1−treated, empty vector-transfected cells.

DISCUSSION:

In this study, we provide evidence that AnxA6 plays a critical role in OA pathogenesis. Loss of annexin function resulted in less severe OA progression after PMX surgery compared with wild type littermates. In addition, IL-1 treatment resulted in reduced mRNA levels of catabolic factors and hypertrophic factors in AnxA6−/− articular chondrocytes compared with wild type cells. More importantly, our findings show that AnxA6 directly affects NF-κB activity. Contrary to AnxA1, which has been shown to interact with the IKK complex and accelerates the release of active NF-κB from IκB resulting in the constitutive activation of NF-κB in cancer cells, AnxA6 appears to stimulate the phosphorylation of the p65 subunit of NF-κB and the nuclear translocation of NF-κB. The overexpression of AnxA6 in articular chondrocytes resulted in increased NF-κB activation in the absence or presence of IL-1. Consequently, increased expression of AnxA6 as detected in OA articular cartilage resulted in a further increase of MMP-13 levels in IL-1−treated chondrocytes compared with IL-1−treated, empty vector-transfected cells or AnxA6 overexpressing cells.

SIGNIFICANCE:

We present for the first time evidence that changes in AnxA6 expression play an important role in OA pathogenesis and that interfering with the AnxA6 function may provide a novel therapeutic strategy for the treatment of OA.

REFERENCES:


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