INTRODUCTION:
Osteoarthritis (OA) is a degenerative disease and one of the major causes of disability in humans. An important feature of OA is the degradation of articular cartilage which is composed of abundant extracellular matrix rich in type II Collagen and Aggrecan. This process is believed to be due to an imbalance between the synthesis of matrix molecules and excess production of matrix degrading factors such as proinflammatory cytokines and matrix metalloproteinases (MMPs). To date, the accumulated findings show that selective inhibition of IL-1, MMPs, and Aggrecanases could reduce the progression of structural changes in experimental OA. Thus, the modulation of these catabolic factors may lead to the identification of new mechanisms for the treatment of OA in humans. The objective of the following study is to evaluate the expression and function of an orphan nuclear receptor, Rev-ErbAα in cartilage and in osteoarthritis (OA).

METHODS:
The expression of Rev-ErbAα was evaluated at both the RNA and protein level in cartilage and chondrocytes derived from human and bovine donors by real time PCR and immunocytochemical techniques. The effect of cartilage catabolic and anabolic agents on the expression of Rev-ErbAα was also evaluated by real time PCR assay. Overexpression of Rev-ErbAα was achieved by either adenoviral transduction or treatment with a PPARα agonist whereas its expression was suppressed using antisense oligonucleotides. The effect of overexpression and suppression on the expression of genes encoding matrix degrading enzymes and production of Aggrecanase was measured by real time PCR and a chondrocyte-based Aggrecanase assay, respectively. The presence of functional DR2 elements in SOX9 and Col2A promoters as predicted by bioinformatic tool (NuBiScan) was confirmed by chromatin immunoprecipitation (ChiP).

RESULTS:
In a survey examining the expression of all 48 nuclear receptors, Rev-ErbAα was found to be the most highly expressing nuclear receptor in chondrocytes. Rev-ErbAα is also expressed in several other tissue and cell types. Immunocytochemical assay revealed a restricted but prominent expression of Rev-ErbAα in the midzone of articular cartilage obtained from human donors. Treatment of isolated articular chondrocytes with known catabolic agents resulted in an induction of Rev-ErbAα, while stimulation with anabolic agents led to a decrease in its expression. Increased expression of Rev-ErbAα was associated with an increase in levels of mRNA encoding matrix degrading enzymes such MMP13 and ADAMTS-4 whereas a decrease in Rev-ErbAα expression led to a concomitant reduction in mRNA levels of matrix degrading enzymes and a corresponding decrease in IL-1-stimulated Aggrecanase enzyme production (Figure 1).

Unlike other liganded nuclear receptors Rev-ErbAα belongs to a subfamily of orphan receptors that are repressors of target gene transcription. It represses transcription by binding to DR2 elements on target genes promoters. Using a bioinformatics tool, NuBiScan, we identified putative DR2 elements on promoters of Col2A and SOX-9. The assumption that endogenous EAR1 interacts with the predicted DR2 elements in the SOX9 and Col2A promoter regions was confirmed by ChiP in chondrocytes. This suggested that EAR1 could directly repress Col2A and SOX9 transcriptionally.

Based on these findings we hypothesize that increase in levels of EAR1 by catabolic stimuli leads to imbalance in the cartilage synthesis and degradative process. This occurs because it directly represses genes like Col2a and SOX9 that are involved in cartilage repair and synthesis and perhaps indirectly stimulates expression of genes encoding matrix degrading enzymes.

DISCUSSION:
This is the first report demonstrating a key role of Rev-ErbAα in both catabolic and anabolic processes in cartilage and suggests that modulation of Rev-ErbAα may represent a novel therapeutic target for OA.

AFFILIATED INSTITUTION FOR CO-AUTHORS:
** Institute for Genome Sciences & Policy, Duke University, Durham, NC. 27708