P38-ATF2 Signaling Regulates Chondrocyte Maturation: the potential novel therapeutics for osteoarthritis

+1Li TF; 1Lin G; 1Zuscik MJ; 1Chen D; 1Schwarz EM; 1Hilton MJ; 1’O’Keefe RJ
1University of Rochester, Rochester, NY
Senior author: tianfang_li@urmc.rochester.edu

INTRODUCTION
During limb development, a cartilage template is formed from mesenchymal condensations. Subsequent longitudinal growth depends on endochondral ossification whereby chondrocytes sequentially proliferate and differentiate. Chondrocyte maturation is marked by profound physical and biochemical changes, including the increase of alkaline phosphatase and type X collagen (Col-X).

In contrast to epiphyseal growth plate, articular cartilage remains in a prehypertrophic state. In some pathologic conditions, however, the articular chondrocytes may recapitulate the differentiation process seen in growth plate cartilage, which leads to abnormal maturation of articular chondrocytes and ossification of articular cartilage, culminating in osteoarthritis (OA).

TGF-β superfamily members are closely involved in the OA process. While it is widely accepted that BMP-2 accelerates chondrocyte maturation and induces ossification of articular cartilage, TGF-β antagonizes BMP-2 effect. Imbalance of these molecules results in different pathological change in articular cartilage. A typical example is that loss of smad3, an important mediator of TGF-β signaling pathway, results in premature OA, eventually leading to total joint destruction.

In addition to the classic smad3/4 pathway, TAK1-MKK3/6-p38-ATF2 pathway can also transduce TGF-β effect. In the present study, we investigated the role of this pathway in chondrocyte maturation. Our long-term goal is to identify a molecule related to this pathway for potential OA treatment.

METHODS
Histological examination: The knee joint samples were harvested from the 13-month-old mice of wild type (smad3+) and smad3 null mutant (smad3–/–). Hematoxylin and eosin staining was done for histological observation of the joints.

Retroviral infection: Primary sternal chondrocytes isolated from smad3–/– and smad3+ mice were infected with retrovirus overexpressing either ATF2 or dominant negative ATF2 from 3 days and the RNA samples were harvested for Real-Time quantitative PCR (qPCR) using the primers for Col-X.

P38 agonist anisomycin treatment: The primary mouse sternal chondrocytes were treated with anisomycin for 12 hours. The cell viability was examined using cell-titer blue method. Col-X mRNA level was then monitored with qPCR.

Immunohistochemical staining for p38: The knee joint samples from one-month-old wild type mice were fixed in paraformaldehyde and embedded in paraffin. Tissue sections were used for immunostaining with the antibody against p38.

Western blotting: Mouse sternal chondrocytes were harvested and western blot was performed using antibodies against p38 isoforms α, β, γ and δ.

RESULTS
Histology: although it has been reported that smad3 mutant mice develop premature OA (1), the progression of the disease process is not clear. In this study, we examined the knee samples of 13-month-old mice. Histological study showed that Smad3–/– mice developed the end-stage arthritis similar to that in human with total joint destruction. A large portion of articular cartilage was replaced by osseous tissue.

ATF2 inhibits abnormal chondrocyte maturation: Overexpressing ATF2 in Smad3–/– chondrocytes lowered the mRNA expression of Col-X to a level similar to the wild type chondrocytes. In contrast, overexpressing dominant negative ATF2 further increased of Col-X expression in Smad3+ chondrocytes, which was already significantly higher than wild type cells (2).

Anisomycin had similar effect as ATF2: Primary mouse chondrocytes treated with p38 MAPK activator anisomycin showed a significant reduction in Col-X mRNA expression.

P38 is expressed in joint tissue: Immunohistochemical staining verified the existence of p38 in both articular cartilage and perichondrium.

Mouse primary chondrocytes isolated from sternae express p38 isoforms α, β, and δ, but not γ. Western blot results demonstrated that the protein level of the p38α is the highest, followed by isoforms δ and β.

DISCUSSION
We first demonstrate that the premature OA lesion in smad3 mutant mice progresses gradually till total joint destruction. These findings further support that TGF-β signaling is not only involved in the initiation but also progression of OA. Modulation of TGF-β signaling would help us identify new therapeutics for OA. Although TGF-Smad pathway seems very simple, modification of this pathway is difficult to apply. TAK1-MK3/6-p38-ATF2 pathway consists of a series of kinases and chemical modification of one or more kinases would be more practical.

Our results show that ATF2 inhibits chondrocyte maturation as evidenced by a significant reduction of Col-X mRNA level in the chondrocytes infected with ATF2 virus. The result was further supported by the observation that dominant negative ATF2 increased the already very high level of Col-X in smad3 deficient chondrocytes. Our next experiment is to verify the role of the ATF2 upstream activator p38 MAPK. Chondrocytes treated with p38 activator anisomycin showed a decreased level of Col-X. These findings indicate that activation of p38 chemically may have some potential use in the OA treatment. The major obstacle is that p38 activation causes local inflammation. Therefore, identification of a specific p38 isoform being able to inhibit chondrocyte maturation but not to induce joint inflammation is of highly clinical importance.

Immunohistochemical staining shows that p38 is detectible in the articular cartilage and perichondrium. However, the antibodies against different p38 isoforms did not very well for immunostaining. We instead did western blot with these antibodies using the protein samples isolated from primary mouse sternal chondrocytes and demonstrated that p38 isoforms α, β, and δ were expressed in these cells. Once the p38 isoforms are identified in articular cartilage, chemically synthesized activator for certain p38 isoform may be used for OA treatment.

REFERENCE