while being significant at D14 in GPR22+ (prior using mineralization medium). From D14 to D21, Runx2 mRNA remained elevated, while Ihh was decreased, in both controls and GPR22+. MMP13, Col10a1 and Alp mRNA were increased, and significantly higher in GPR22+ than in controls. Alizarin red was still more elevated in GPR22+. These data were consistent with the enhanced activation of ERK1/2 at D14–21 in GPR22+ compared with controls.

Conclusions: Overexpression of GPR22 blocks features from the proliferative phase of chondrogenesis, but enhances those from the hypertrophic and mineralization phases. This underlines a critical role for GPR22 in the fate of chondrogenic cells, and may be of interest regarding the hypertrophic switch of articular chondrocytes in the course of OA.

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TNFR1 DEFICIENCY FACILITATES ARTICULAR CARTILAGE OSSIFICATION AND OSTEOEPHYTE FORMATION
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Purpose: Proinflammatory cytokines, such as IL-1 and tumor necrosis factor α (TNFα), are suspected of causing damage to osteoarthritic (OA) cartilage. TNFα content is elevated in the synovial fluid of OA joints. There are 2 cell surface receptors for TNFα including p55 (TNFR1) and p75 (TNFR2). TNFα and TNFRs I and II are up-regulated in OA cartilage. For bone metabolism, mice null for TNFα have significantly increased peak bone mass, resulting from elevated bone formation. In vitro, TNFα inhibited mineralization of osteoblasts through regulation of NF-kB activity. Thus, whereas TNFR1 plays roles in osteoblast differentiation, its functions in chondrocyte metabolism is unknown. Endochondral ossification is an essential process for development of OA, which is characterized by cartilage degradation and osteophyte formation. The aim of this study is to investigate the role of TNFR1 in the maintenance of articular tissues.

Materials and Methods: Histological evaluation of aged TNFR1−/− mice: Knee joints of TNFR1−/− and their wild-type (WT) littermates (52–60 weeks of age) were evaluated immunohistologically. Whole knee joints were removed by dissection, fixed in 4% paraformaldehyde, and decalcified in EDTA. After dehydration and paraffin embedding, serial sections were stained with Safranin O-fast green and HE. Osteophyte area and articular cartilage thickness were quantified in each slide using Image-Pro Plus 4.1 software.

Surgical induction of OA in TNFR1−/− mice: TNFR1−/− and their wild-type (WT) littermates were surgically induced to develop OA by medial meniscectomy. OA cartilage was removed by dissection, fixed in 4% paraformaldehyde, and decalcified in EDTA. After dehydration and paraffin embedding, serial sections were stained with Safranin O-fast green and HE. Osteophyte area and articular cartilage thickness were quantified in each slide using Image-Pro Plus 4.1 software.

Results: At 52–60 weeks of age, whereas osteophyte formation was detected at the medio-anterior edge of tibial plateau regardless of genotype, osteophyte area was significantly increased in TNFR1−/− mice. Moreover, articular cartilage thickness was reduced at medial end region of tibia plateau in TNFR1−/− mice, suggesting ossification of articular chondrocyte was advanced by TNFR1 deficiency. We next compared osteoarthritis development between adult littermates of wild-type and TNFR1−/− mice by creating a surgical osteoarthritis model through induction of instability to the knee joints. Histological evaluation confirmed that the TNFR1 deficiency caused significant acceleration for osteophyte formation. Degree of cartilage destruction was almost comparable between TNFR1−/− and WT. Real time PCR analysis for epiphyseal chondrocyte from new born mice revealed mRNA expression of type X collagen, a marker for chondrocyte hypertrophy, was significantly elevated in TNFR1−/− mice compared to WT littermate. Currently, we are picking up a number of candidate genes for targets of TNFR1 in the course of chondrocyte hypertrophy using cDNA microarray analysis.

Conclusion: Endogenous TNFR1 had a protective effect against osteophyte formation through inhibition of type X collagen synthesis.