hypocellular, with decreased chondrocyte number. BrdU incorporation assays and immunohistochemical staining for proliferating cell nuclear antigen (PCNA) showed a marked reduction in replicating cells in mutant mice. In addition, proliferation assays revealed decreased chondrocyte numbers from mutant mice in culture, which is rescued by NO donor treatments (DATA-NO and SNAP). Organ culture of tibiae from E15.5 eNos mutant mice demonstrates reduced bone growth and chondrocyte proliferation compared with the wild type littermates, suggesting that the role of eNOS signaling in chondrocyte proliferation is cell-autonomous. Reduced chondrocyte number may in part be due to premature cell-cycle exit because of decreased cyclin D1 and increased p57 expression in mutants. Additional, reactive oxygen species were increased in mutant chondrocytes as indicated by a fluorescent dye (DCFH), in parallel to increased apoptosis as indicated by increased staining for cleaved caspase-3. Mutant mice also demonstrated increased total and phosphorylated p38 and GSK-3β, reduced total and phosphorylated Akt, and decreased SOX9 expression.
Conclusions: In summary, these data identify an essential role of eNOS in chondrocyte proliferation, endochondral bone growth and demonstrate that the eNOS gene regulates the coordination between cell-cycle exit and chondrocyte differentiation in cartilage.

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ESE-1 REGULATES MMP13 EXPRESSION IN CHONDROCYTES

M. Otero[1], D.A. Plumb[1], C. Dragomir[1], K. Tsuchimochi[1], J. Chen[1], D. Gluck[1], P. Roman[1], J. Harkess[1], L. Xu[2], Y. Li[2], M.B. Goldring[1]
[1]Hosp. for Special Surgery, New York, NY; [2]Harvard Sch. of Dental Med., Boston, MA

Purpose: Matrix metalloproteinase (MMP)-13 plays a major role in osteoarthritis (OA) due to its-rate limiting role in collagen degradation. MMP-13 expression is regulated by a variety of transcription factors including ESE-1, a member of the E26 transformation-specific sequence (ETS) family. ESE-1 is induced by inflammatory cytokines and is expressed in OA chondrocytes, where it represses type II collagen promoter activity. The aim of this study was to determine whether ESE-1 participates in MMP-13 transcriptional regulation in chondrocytes.
Methods: The binding of in vitro-translated ESE-1 to the MMP-13 promoter was analyzed by electrophoretic mobility shift assay (EMSA) and supershift analysis. To analyze the response of the MMP-13 promoter to ESE-1, luciferase reporter assays were performed using cell extracts of C-28/I2 and T/C-28a2 cells co-transfected with constructs of the human MMP-13 promoter and expression vectors encoding ESE-1, Runx2, cFos and cJun. For knockdown of ESE-1 expression, human primary chondrocytes and human immortalized chondrocytes were transfected with a pool of four individual siRNA oligonucleotides against ESE-1 or control nonspecific siRNA. After incubation for 96 h, the knockdown was confirmed by real time PCR and Western blotting, and MMP-13 mRNA was analyzed by real time PCR. ESE-1 and MMP-13 protein levels and type II collagen cleavage products were addressed by immunohistochemical staining in human OA cartilage, cho/+ mouse cartilage and Ese1 -/- mouse cartilage specimens.
Results: Immunohistochemical analysis revealed increased ESE-1 levels in both human and mouse OA cartilage specimens, and ESE-1 co-localization with MMP-13 protein and type II collagen cleavage products. EMSA and supershift analysis detected specific binding of ESE-1 to three ETS sites in the proximal MMP-13 promoter. Luciferase reporter assays showed that ESE-1 overexpression leads to the MMP-13 promoter activation in synergism with Runx2 and AP-1 (cFos/cJun). Deletion of the sequences containing the ESE-1 binding sites blocked activity. Real time PCR analysis revealed lower MMP-13 basal mRNA expression associated with ESE-1 knockdown and significant reduction of IL-1-induced MMP-13 mRNA levels. Immunohistochemical analysis of cartilage from Ese1 -/- mice revealed low or undetectable MMP-13 protein levels compared to wild type mice.
Conclusions: Our results in chondrocyte cultures suggest that this transcription factor participates in the cytokine-induced activation of MMP-13 gene expression, as well as in the maintenance of basal expression. Moreover, our finding that increased ESE-1 levels in OA cartilage correlate with increased MMP-13 protein and type II collagen cleavage products highlights its involvement in cartilage catabolic processes. Indeed, we are currently investigating the relative contribution of ESE-1 absence to the OA onset and progression in mouse OA models. Finally, the decreased MMP-13 protein levels in the cartilage of Ese1 -/- mice further reinforces the role of the ESE-1 in homeostatic cartilage remodeling. Altogether, our results show that ESE-1 is a key regulator of MMP-13 gene expression in chondrocytes.

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CELL DEATH IN CHONDROCYTES DURING THE PATHOGENESIS OF OSTEOARTHRITIS IS A COMBINATION OF APOPTOSIS AND AUTOPHAGY

M. Almonte Becerril[1], F. Navarro-García[1], A. González-Robles[1], M.A. Vega-López[1], C. Lavalle[2], J.B. Kouri[1]
[1]Centro de Investigación y de Estudios Avanzados del Inst. Politécnico Naciona (Cinvestav-IPN), México, D.F., Mexico; [2]Univ. Natl. Autónoma de México, División de Estudios de Posgrado, México, D.F., Mexico

Purpose: The aim of this study was to identify the type of death that chondrocytes undergo and to correlate it with changes in the endoplasmic reticulum (ER) and Golgi complex in the three zones of the cartilage during Osteoarthritis (OA) pathogenesis in an experimental model.
Methods: The experimentally OA-induced model was accomplished by unilateral knee menisectomy and post-surgery training; normal rats were used as a control. Animals were sacrificed by CO2 overdose and right femoral condyles were removed and processed for either electron microscopy (EM) or Immunohistochemistry (IHC). Chondrocytes death was identified with TUNEL and IHC for cleaved caspase 3 active and LC3II in cartilage from rats with 5, 10, 20 and 45 training days (td). At the same time development of ER and G membranes were monitored with markers to calnexin and 58k-9 protein respectively. EM was used as a complementary methodology to identify ER, Golgi complex (at 5 td) and autophagic vacuoles (20 td) in OA cartilage.
Results: Cell death analysis showed that throughout early stages of OA, chondrocytes death started by apoptosis in the superficial (SZ) and middle zones (MZ) of the cartilage. As the degenerative process progressed, autophagy began to be evident in the SZ. Finally, in late stages of OA, chondrocytes co-expressed both death markers in the SZ and MZ; while in the deep zone (DZ), just apoptosis was discernible. In agreement with cell death processes, ER and Golgi complex suffered changes during the first 5 td and showed a prominent ER with dilated cisterns and enhanced Golgi complex membranes; in late stages of OA these changes were maintained especially in the SZ and MZ.
Conclusions: Our results suggest that during the OA pathogenesis, chondrocytes undergo changes to increase their synthetic as well as catabolic activities at early stages and late stages of OA respectively. Afterward, at late stages of OA, when chondrocytes repair capacity is overwhelm, they activate its own program of cellular death, which includes autophagy, apoptosis and changes in ER and G, characteristics of chondroptosis.