Expression of Notch Signaling Components in Osteoarthritis and In Vitro Chondrogenesis
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Introduction: The aim of the present study was to investigate the role of Notch signaling in the pathogenesis of osteoarthritis (OA). OA is a slowly progressive degenerative disease characterized by gradual loss of articular cartilage accompanied by aberrant repair, resulting in remodeling of the subchondral bone and osteophyte formation. Alsalamah, et al. and Dowthwaite, et al. identified multipotential mesenchymal progenitor cells in adult human articular cartilage, specifically on the surface of the cartilage (2003 and 2004). The Notch signaling pathway is a highly conserved signaling system that plays a critical role in cell proliferation, differentiation, and apoptosis in the context of stem cell differentiation. Thus, aberrant regulation of Notch signaling may adversely affect the chondrogenesis of articular cartilage mesenchymal stem cells (MSCs) in diseased tissue.

Materials and Methods: Tissue: Normal human articular cartilage was isolated from tissue purchased from the National Disease Research Interchange, OA articular cartilage was obtained from patients (age 53-79 years) undergoing elective total knee arthroplasty, and bone marrow aspirate was obtained according to an approved IRB protocol.

Human cartilage Isolation and Cell Culture: Cartilage was digested with 1mg/ml collagenase II in a volume of 10 ml/g wet weight cartilage in serum-free medium overnight at 37°C under agitation. The chondrocyte (hCh) suspension was filtered, washed, and plated in DMEM containing 10% FBS and antibiotic-antimycotic solutions. Cells were allowed to adhere, spread and proliferate for 7-10 days. Normal and OA hChs were used for experiments between P1 and P2. Bone marrow aspirate was washed with DMEM and pelleted at 1000 g’s for 5 min. repeating twice and resuspending in DMEM. Cells were plated DMEM containing 10% FBS and antibiotic-antimycotic solutions. 24 h later, the cells were washed. The remaining adherent cells (MSCs) were then proliferated.

RNA processing: Total RNA was isolated using a Qiagen RNeasy kit. cDNA was generated using Invitrogen Superscript First-Strand synthesis. A Bio-Rad iCycler iQ qRT-PCR system was utilized to analyze transcript levels of Notch components in OA (Mankin score of 6) and normal tissue and monolayer hChs and MSCs. GeNorm 3.5 was used to create normalization factors for comparing normal cartilage, OA cartilage, monolayer hChs, and monolayer MSCs utilizing GAPDH, β-Actin, HMBS, RPL13A, SDHA, and UBC as housekeeping genes.

Differentiation: MSCs and hChs were cultured in high-density pellets in serum-free chondrogenic media. Pellets were cultured in the presence of 0.1, 1, and 10 ng/ml IL-1beta to mimic osteoarthritic conditions for 21 days.

Histo- and Immuno-chemistry: Histological staining was performed on primary tissue and harvested pellets with H&E, alcian blue (pH 1.0), alizarin red, fast green, and safrinin O. Cartilage was evaluated for morphology, cellular abnormality, matrix staining, and tidemark integrity using the Mankin scale (Mankin 1971). Sections were pre-digested with hyaluronidase in 10 mM Tris-HCl, pH 7.5, and Alsalameh et al. (2004) Arthritis and Rheumatism 50(5): 1522-32.

Discussion: The down-regulation of Notch components in OA cartilage supports the idea that the poor regenerative capacity of articular cartilage may be attributed to inhibition of chondrogenic differentiation by articular cartilage MSCs. In vitro chondrogenesis of MSCs supports also the hypothesis: under OA conditions, Notch expression is down-regulated and chondrogenesis is impaired. In vitro, chondrogenesis of hChs increases notch component expression and decreases chondrogenic gene expression under OA conditions. Therefore, hChs are seemingly dedifferentiating to MSCs, which are then prevented from undergoing chondrogenesis, resulting in a decrease of hChs and MSCs able to undergo chondrogenesis. Instead, the MSCs may be free to commit, and differentiate into alternative lineages, contributing to the inappropriate remodeling in OA. Our results suggest the Notch signaling pathway plays an important role in the pathogenesis of osteoarthritis via potentially impairing the repair capacity of diseased tissue.


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