REGULATORY MECHANISMS FOR HYALURONAN SYNTHESIS AND DEGRADATION IN ARTICULAR CARTILAGE

Introduction
The glycosaminoglycan hyaluronan (HA) is an essential component molecule of articular cartilage, contributing to both the structural and functional integrity of this highly specialized tissue. Recently, a family of enzymes (designated HAS for HA Synthase) responsible for the synthesis of HA have been identified (1), as has a group of at least six paralogue hyaluronidase (HYAL) genes (2) as well as one unrelated hyaluronidase designated MGEA5 (3). In the current study, we examined the mRNA expression profiles of three mammalian HAS enzymes (HAS1, HAS2 and HAS3), four hyaluronidases (HYAL1, HYAL2, HYAL4 and MGEA5) and the HA receptor CD44 in chondrocytes from normal (undiseased) animal and human cartilage and osteoarthritic human cartilage maintained in experimental culture systems exposed to catabolic or anabolic stimuli provided by cytokines, retinoic acid and growth factors.

Materials and Methods
Human articular cartilage was from the femoral condyles of 68- to 79-year old patients obtained following arthroplasty for advanced osteoarthritis of the knee or from undiseased distal humerus of a 74-year old patient. Bovine, ovine and porcine cartilage was obtained from the metacarpophalangeal joints of immature (<10 days old) or mature (>18 months old) cattle and mature (3-6 month old) sheep and pigs. Chondrocytes from full-depth cartilage slices were isolated by pronase/collagenase digestion and maintained in monolayer or agarose cultures as described (4). Cultures were maintained for 4 days in DMEM containing the ascorbate analogue ascorbinic acid (25 mg/ml) in the presence or absence of 10 ng/ml IL-1alpha, 0.001 mM all-trans retinoic acid, 40 ng/ml TNF-alpha, 2 ng/ml TGF-beta1 or 10 ng/ml IGF-1. For some experiments, bovine chondrocytes were isolated from the superficial or deep zones of articular cartilage from immature or mature animals and maintained in monolayer or agarose cultures. Explants of human distal humerus cartilage slices were cultured in DMEM/10% FBS for 5 days, then maintained for four days in DMEM (without FBS) ± 10 ng/ml recombinant human IL-1, 100 ng/ml recombinant human TNF-alpha, or 0.001 mM all-trans retinoic acid (RA). RNA was extracted from chondrocyte cultures and from freshly excised cartilage and cultured explants as described (4). RNA samples were subsequently treated with RNase-free DNase I and re-purified by phenol extraction. RT-PCR was performed on the RNA samples using oligonucleotide primers specific for HAS, hyaluronidase, CD44 or GAPDH cDNAs. All PCR product sequences were validated.

Results and Discussion
In order to determine which of the HAS enzymes were being expressed in vivo in articular cartilages, we first examined RNA samples extracted directly from freshly excised human, bovine and porcine tissues. Of the three mammalian HAS enzymes identified to date, only HAS2 and HAS3 mRNAs were expressed in native bovine or porcine cartilage (Fig. 1A). Similar results were obtained using RNA harvested from mature bovine, porcine and ovine chondrocytes that were maintained in monolayer culture. All RNA preparations from such cartilage slices contained mRNA for HAS2, however HAS3 mRNA was detected in bovine and ovine cultures but not in porcine chondrocytes grown under the same culture conditions. Chondrocytes from immature or mature bovine monolayer cultures expressed approximately equal levels of HAS2 mRNA, whereas the expression of HAS3 mRNA was significantly diminished in immature bovine chondrocytes treated with cytokines and growth factors (Fig. 1B). Additionally, HAS2 and HAS3 mRNA expression was observed in bovine chondrocytes derived from both the superficial and deep cartilage zones, although HAS3 mRNA levels were higher in superficial zone samples. When human chondrocytes were maintained in monolayer cultures, they also expressed HAS2 and HAS3 mRNAs (Fig. 1C). Human chondrocytes also expressed both HAS2 and HAS3 when cultured in the presence of two different catabolic stimuli, IL-1 or retinoic acid (Fig. 1C). As for the other animal species, the human chondrocytes did not express HAS1 mRNA under any of the culture conditions, a phenomenon which has also recently been observed by other investigators (5). Collectively, these data indicate that only HAS2 and HAS3 mRNAs are expressed in several mammalian cartilages. HAS2 mRNA appears to be constitutively expressed while HAS3 mRNA expression may be differentially regulated in morphological zones within the cartilage tissue, and may be affected by local and/or systemic catabolic or anabolic stimuli.

In parallel studies, hyaluronidase and CD44 expression was examined in cartilage derived from undiseased human articular cartilage. Expression of mRNA for HYAL1, HYAL2, HYAL3 and MGEA5 was readily detected in direct tissue extracts (Fig. 2). For the explant cultures, HYAL3 mRNA levels were upregulated by cytokine (IL-1) treatment, while HYAL1, HYAL2 and MGEA5 transcripts remained constant. Furthermore, the observed upregulation of CD44 mRNA expression in response to IL-1 stimulation (Fig. 2) may implicate a coordinate mechanism of HA degradation involving receptor mediated endocytosis (6) and intracellular degradation by acid-active hyaluronidases (7). In conclusion, these current investigations into HA metabolism compliment other insightful studies which are furthering our understanding of the mechanisms which regulate and modulate the steady state balance between expression of synthetic enzymes such as HAS2 [which appears to play an essential role in chondrocyte matrix assembly and retention (5)], and degradative enzymes such as HYAL3. Elucidation of such events will provide critical new insights into these important aspects of articular joint metabolism. [Fig. 1. RT-PCR analyses of chondrocyte HAS mRNAs. A, direct extracts of bovine (B) and porcine (P) cartilage; B, extracts from monolayer cultures of normal bovine and mature bovine chondrocytes; C, extracts from monolayer cultures of human chondrocytes. M: DNA Size Markers (bp).] [Fig. 2. RT-PCR analyses of hyaluronidases and CD44 in human explant cultures. A=direct tissue extract, B=explant cultures; B: control, C:+IL-1, D: +TNF, E: +retinoic acid. M: DNA Size Markers.]


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