HUMAN CARTILAGE GLYCOPROTEIN 39 ([HC GP-39]) IS EXPRESSED IN ADULT AND FETAL BONE AND CARTILAGE: AN IN SITU HYBRIDIZATION STUDY

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INTRODUCTION. Human cartilage glycoprotein 39 (HC gp-39), a member of the mammalian protein family related in sequence to the bacterial chitinases, has been reported as a major secretory glycoprotein of cultured human articular chondrocytes and synovial fibroblasts [1]. Although HC gp-39 was undetectable in normal cartilage by Northern and Western blot analyses, it was reported to be highly expressed in rheumatoid arthritis (RA) cartilage and synovium [1] and increased levels of protein have been detected in the serum and synovial fluid of patients with osteoarthritis (OA) or RA [2-3]. Additionally, it was detected as a secreted protein from the human osteoblast cell line, MG63 [4], in response to 1.25-(OH)2D3 treatment or extended time in culture. It was not detected in cultures of normal primary osteoblasts isolated from fetal and adult human bone and cultured under equivalent conditions [4]. The hallmark characteristic for the expression of HC gp-39, which has been detected in a variety of tissues, has been high level expression at sites of tissue remodelling [5-10].

Although HC gp-39 contains regions of homology with a group of bacterial and fungal chitinases, glycosidic activity against chitinase substrates has never been demonstrated [1]. Recently, Renkema et al [11] have shown that HC gp-39 is a chitin-binding lectin; however, its true physiological role remains unclear. The purpose of this study was to examine the distribution of expression of HC gp-39 mRNA in human cartilage and bone by in situ hybridization.

MATERIALS AND METHODS. OA cartilage from knees and osteophytes from osteoarthritic femoral heads were obtained from patients undergoing joint replacement surgery (Rothman Institute, Philadelphia PA). Normal adult cartilage and fetal bone was obtained from the Anatomical Gift Foundation (Laurel MD). All human tissue was obtained with informed consent and was used under the approval of the institutional review board. A total of ten different adult articular cartilage samples were analyzed. Cryostat sections (5 micron) of unfixed, undecalcified bone and cartilage were prepared as detailed previously [12]. Osteoblast populations were identified by the simultaneous naphthol phosphate method, with Fast Red TR as the coupler, which results in a red precipitate denoting sites positive for alkaline phosphatase activity. In situ hybridization was performed by a method detailed previously [12]. pBluescript SK containing the coding region of human HC gp-39 was obtained from Human Genome Sciences (Rockville MD). Riboprobes were prepared using the Promega (Madison WI) In Vitro transcription kit with [35S]thio CTP (Amersham). Cryostat sections were collected onto 3-aminopropilytriethoxylamine coated slides, fixed in 4% paraformaldehyde, dehydrated and frozen at –20°C prior to hybridization. For hybridization, sections were hydrated, demineralized and acetylated then hybridized at 42°C for 4 hr. Post-hybridization washes were to a final stringency of 0.1XSSC. Hybridized sections were coated in LM-1 film emulsion (Amersham), exposed at 4°C for 2 weeks, developed in Kodak developer and counterstained with methylene blue. The extent of hybridization signal was assessed by the autoradiographic grain density over the cell.

RESULTS. HC gp-39 mRNA was undetectable in the chondrocytes of normal articular cartilage (data not shown). In cartilage from patients with mild OA cartilage degeneration, characterized by mild surface fissuring and loss of matrix proteoglycan in the mid- and deep zones (Fig. 1A), intense HC gp-39 mRNA levels were expressed in the chondrocytes of the superficial zone (Fig. 1B, arrows) with moderate levels in the mid-zone chondrocytes (Fig. 1B, arrowheads). In advanced OA cartilage, chondrocytes of the mid-zone (Fig. 1C, upper bracket) expressed high levels of HC gp-39 mRNA, with lower expression in the deep zone chondrocytes (Fig 1C, lower bracket); cloning chondrocytes of the superficial zone expressed high levels of HC gp-39 (Fig. 1D).

In osteoarthritic tissue, the expression of HC gp-39 mRNA was intense in flattened, end-stage osteoblasts and in primary osteocytes at sites of both endochondral and intramembranous bone formation. Proliferating osteoblasts expressed low to moderate levels. Notably, mature osteocytes were negative for HC gp-39 expression. Chondrocytes in the prehypotrophic zone (site of future secondary ossification center) developing fetal cartilage demonstrated high expression, while hypertrophic and mineralized cartilage chondrocytes had lower expression. Osteoblasts at sites of endochondral and intramembranous fetal bone formation demonstrated high HC gp-39 expression.

DISCUSSION. The stage specific expression of HC gp-39 in fetal bone, osteoarthritic osteoblasts and osteocytes, and adult osteoarthritic cartilage provides evidence for a specific functional or structural role for HC gp-39 in bone and cartilage tissue. HC gp-39 is expressed in diseased human osteoarthritic cartilage and developing osteophyte, but not in non-diseased tissue, and its distribution within the tissue changes as disease progresses. OA is characterized not only by cartilage degeneration, but by increased subchondral bone formation and osteophytosis. The results from this study indicate that the increased HC gp-39 expression in OA serum and synovial fluid may reflect not only cartilage degeneration but increased osteogenesis.


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