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Matrix metalloproteinase-12 immunolocalization in the degenerating human intervertebral disc and sand rat spine: Biologic implications

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ABSTRACT

Matrix metalloproteinase-12 (MMP-12; macrophage metalloelastase) degrades a number of extracellular matrix components which are present in the intervertebral disc, including type IV collagen, fibronectin, laminin, chondroitin sulfates, elastin and fibrinogen. MMP-12 has recently discovered relationships with cytokines and chemokines which also relate to disc cell biology. To date, no study has assessed immunolocalization of MMP-12 in degenerating human intervertebral disc tissue. Immunocytochemical localization was performed on 18 human disc specimens and on lumbar spines of the sand rat, a small animal model with well-recognized age-related disc degeneration. In the human disc, intracellular localization was present in both the annulus and nucleus portions of the disc. The sand rat degenerating disc also showed MMP-12 disc localization, with additional presence in chondrocytes of the vertebral endplate of older animals. This is the initial characterization of the presence of MMP-12 in the human and sand rat disc, and in chondrocytes of the vertebral endplate in older sand rats with degenerating discs. Findings are important because they document the presence of an additional MMP-12 in disc tissue, thus expanding our understanding of disc extracellular matrix remodeling, and because they provide novel information on the presence of MMP-12 in the cartilage endplate as it undergoes sclerosis during disc degeneration in the aging sand rat.

Introduction

Matrix metalloproteinase 12 (MMP-12), also called macrophage metalloelastase, is an MMP involved with elastin degradation, and degradation of other extracellular matrix (ECM) components of the intervertebral disc such as type IV collagen, fibronectin, laminin, vitronectin, cartilage proteoglycan, and chondroitin sulfates (Chandler et al., 1996; Gronski et al., 1997; Janusz et al., 1999; Kerkelä et al., 2001). In 1993 Shapiro et al. showed that MMP-12 is produced as a secreted 54 kDa pro-enzyme which is then processed into 45- and 22-kDA active forms (Shapiro et al., 1993). Gene function of MMP-12 in matrix was elucidated when Curci et al. recovered MMP-12 from abdominal aortic aneurysm tissue and detected a 7-fold increase in MMP 12 compared to normal tissue (Folkman, 2006). This study showed localization of MMP-12 to elastic fiber fragments.

There appear to be few published studies on MMP-12 in the human disc or animal disc literature. A recent differential gene expression profiling work compared alginate bead-cultured bovine nucleus pulposus cells and bovine articular chondrocytes (Cui et al., 2010). MMP-12 was reported to be expressed in vitro in chondrocytes, but not by the nucleus cells from the disc.

Early biochemical work on the intervertebral disc focused upon enzymatic mechanisms for extracellular matrix degradation and recognized the presence of elastase, attributing potential sources to granulocytes and activated macrophages (Woessner, 1982). Studies have reported that with aging, the elastin to collagen ratio decreases slightly in the annulus, and strongly in the nucleus, with aging, and that the elastin content decreases with age (Olczyk, 1994). The elastic fiber network of the annulus is highly complex as revealed recently by the works of Yu et al. (Smith and Fazzalari, 2009; Yu et al., 2002, 2005, 2007).

MMP-12 now has a well-recognized role in asthma, chronic obstructive pulmonary disease (COPD) and smoking-induced pulmonary emphysema. In these conditions, MMP-12 acts not only via its degradation of the extracellular matrix (which can result in loss of elastic recoil), but also via its control of cytokine and chemokine activity (Parks et al., 2004).

The relationship of MMP-12 with interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) has been elucidated in cartilage, where elevated MMP-12 has been experimentally found to increase cartilage degradation and play a role in inflammatory joint disease (Milner et al., 2006; van Lent et al., 2005; Wang et al., 2004). A considerable body of knowledge present from the cartilage literature helps us understand how MMP-12 may be mediated by local cytokines via a number of pathways. MMP-12 has been found to be efficient at cleaving within

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both the intraglobular domain and the C-terminus of aggrecan in bovine articular cartilage (Durigova et al., 2011). Hypoxia-inducible factor 1α is known to be a catabolic regulator of MMP12 during cartilage destruction in osteoarthritis (Yang et al., 2010).

Of great relevance to disc degeneration, which shows increased IL-1 and TNF-α proinflammatory cytokine upregulation (LeMaitre et al., 2007), is the finding by Oh et al. that IL-1 induces MMP-12 expression and activation in chondrocytes via the mitogen-activated protein kinase signaling pathway and that MMP12 upregulates MMP-9, another MMP prominent in disc degeneration (Oh et al., 2008). Milner et al. have also studied MMP-12 and found it upregulated during application of IL-1 and oncostatin M to bovine nasal cartilage (Milner et al., 2006). Osteoarthritic synovium studied by Davidson et al. revealed that MMP-12 was among the four most significantly upregulated genes (Davidson et al., 2006). Other studies have shown that MMP-12 expressed is induced in chondrocytes during fetal development as well as malignant transformation (Kerkelä et al., 2001).

In light of the potential importance of MMP-12 activity in the healthy and degenerating disc, and since MMP-12 has received little attention in the human intervertebral disc literature, and has been not reported in the major recent human disc MMP studies (Crean et al., 1997; Cui et al., 2010; Le Maitre et al., 2004; Millward-Sadler et al., 2009; Weiler et al., 2002), we performed an immunohistochemical localization study in human discs. We also questioned whether the vertebral endplate, which shows sclerosis during both human and sand rat disc degeneration, might show the presence of MMP-12 in chondrocyes; we therefore performed an examination of lumbar spines of young and old sand rats, an animal model of spontaneous, age-related disc degeneration. Radiologic characterizations of degenerating discs in both cross-sectional and prospective groups of sand rats have been previously performed (Gruber et al., 2002a, 2007). Cross-sectional studies showed significant age-related changes comprising irregular disc margins, disc wedging, disc narrowing, endplate and ligament calcification and osteophyte formation which mimic changes in the human spine related to disc degeneration (Fraser et al., 1997).

Methods

Clinical study population

Study of human disc specimens was approved prospectively by the authors' Human Subjects Institutional Review Board at Carolinas Medical Center. The need for informed consent was waived by the ethical board since disc tissue was removed as part of routine surgical practice (and discarded). Scoring of disc degeneration utilized a modification of the Thompson scoring system (Thompson et al., 1990) incorporating ENH's radiologic, MRI and surgical findings. The Thompson system scores disc degeneration over the spectrum from a healthy disc (Thompson grade I) to discs with advanced degeneration (grade V, the most advanced stage of degeneration) (Thompson et al., 1990). Patient specimens were derived from surgical disc procedures performed on individuals with herniated discs and degenerative disc disease. Surgical specimens were transported to the laboratory in sterile tissue culture medium. Non-surgical control donor disc specimens were obtained via the National Cancer Institute Cooperative Human Tissue Network (CHTN); they were shipped overnight to the laboratory in sterile tissue culture medium and processed as described below.

Sand rat disc tissue

Animal studies were performed following approval by the Institutional Animal Care and Use Committee. *Psammomys obesus obesus*, the sand rat, is used in our laboratory in studies of disc degeneration. Colony housing and animal diet descriptions have been published previously (Gruber et al., 2002a, 2002b). Lumbar spines from six animals were used in the present study (ages 2, 6.3, 10, 10, 28 and 29 months). Spines were removed immediately following animal euthanization, and lumbar spine regions were harvested, fixed in 10% neutral buffered formalin, decalcified, and embedded in paraffin, and mid-sagittal sections of the discs, endplates and vertebrae obtained. Sections were processed for immunohistochemical localization of MMP-12 as described below.

Immunohistochemical localization of MMP-12

Paraffin sections were cut at 4 μm, collected on PLUS slides (Cardinal Health, Dublin, OH) and dried at 60 °C. Sections were deparaffinized in xylene (Cardinal) and rehydrated through graded alcohols (AAPER, Shelbyville, KY) to distilled water. Antigen retrieval was performed using Antigen Decloaker Solution, pH 6.0 (Biocare Medical, Concord CA) for 20 min at 95 °C followed by cooling for 20 min. The remainder of the procedure was performed using the Dako Autostainer Plus (Dako, Carpenteria, CA) automated stainer. Endogenous peroxidase was blocked using 3% H_2O_2 (Sigma, St Louis, MO). Slides were incubated for one hour with anti-MMP-12 (rabbit monoclonal EP1261Y) (Abcam, Cambridge, MA) at a 1:100 dilution, or for 2 h with anti-MMP-12 H-300:sc-30072 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a 1:25 . The rabbit monoclonal EP1261Y is an antibody raised against a synthetic peptide corresponding to residues on the C-terminus of human MMP-12. 12 H-300:sc-30072 is a rabbit antibody raised against amino acids 171-470 mapping at the C-terminus of MMP-12 of human origin. The Secondary antibody was 4 + Biotinylated Universal Goat Link (Biocare) for 10 min followed by 4+ streptavidin HRP Label (Biocare) for 10 min and DAB (Dako) for 5 min. Slides were removed from the stainer, rinsed in water, counterstained with light green, dehydrated, cleared and mounted with resinous mounting media. Human breast tumor was used as a positive control. Universal Rabbit Negative (Dako) was used as a negative control.

Results

Immunolocalization of MMP-12 with antigen retrieval was studied in clinical disc specimens of 18 subjects (Table 1) using a commercially available rabbit monoclonal antibody to the carboxyterminal end of MMP-12. Although some of the discs utilized here were from herniated discs, no regions with frank herniation were present in the these disc specimens.

MMP-12 cellular localization was identified using both of the anti-MMP12 antibodies employed here. Regions of outer annulus in healthy Thompson grade I discs showed that the majority of annulus cells were positive for MMP-12 localization (Fig. 1A–C). In the inner annulus of more degenerated specimens, many cells showed localization (Fig. 1D) in sites with nearby matrix loss. Few MMP-12 positive cells were present in the nucleus pulposus regions of the disc of older subjects (Fig. 1E).

Cells showing MMP-12 localization were found when cells were surrounded by concentric layers of matrix (Fig. 2A) and when cells were present in clusters (Fig. 2B).

Examination of sand rat spines included younger animals and older animals which exhibit spontaneous age-related disc degeneration. Small animal spines provide the advantage that adjacent vertebral mid-sagittal sections can be histologically examined in lumbar segments, allowing visualization of both the disc and its two adjacent end plates (Gruber and Hanley, 2014). MMP-12 was seen to be sparse in chondrocytes in endplates of younger animals with healthier discs (Fig. 3A). In older animals with more degenerated discs, however, MMP-12 was present in the majority of endplate chondrocytes (Fig. 3B). MMP-12 was also localized in some of the nucleus (Fig. 3C) and annulus (Fig. 3D) cells in the sand rat disc.

Discussion

The present work utilized immunohistochemistry to show the first documentation of the presence of MMP-12 in the human disc at the