Osteoarthritis and Cartilage xxx (2016) 1–9

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Early cathepsin K degradation of type II collagen *in vitro* and *in vivo* in articular cartilage

J.S. Mort † ‡ *, F. Beaudry §, K. Théroux ||, A.A. Emmott †, H. Richard ||, W.D. Fisher ‡, E.R. Lee † ‡, A.R. Poole ‡, S. Laverty ||

† *Genetics Unit, Shriners Hospitals for Children, Montreal, Québec, Canada*
‡ *Department of Surgery, McGill University, Montreal, Québec, Canada*
§ *Groupe de Recherche en Pharmacologie Animal du Québec (GREPAQ), Département de Biomédecine Vétérinaire, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada*
|| *Comparative Orthopaedic Research Laboratory, Département de Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada*

ARTICLE INFO

Article history:
Received 11 July 2015
Accepted 25 March 2016

Keywords:
Type II collagen
Cathepsin K
Neoepitope
Horse
Cartilage
Protease

SUMMARY

Objective: To characterize the initial events in the cleavage of type II collagen mediated by cathepsin K and demonstrate the presence of the resulting products in human and equine articular osteoarthritic cartilage.

Design: Equine type II collagen was digested with cathepsin K and the cleavage products characterized by mass spectrometry. Anti-neoepitope antibodies were raised against the most N-terminal cleavage products and used to investigate the progress of collagen cleavage, *in vitro*, and the presence of cathepsin K-derived products in equine and human osteoarthritic cartilage.

Results: Six cathepsin K cleavage sites distributed throughout the triple helical region were identified in equine type II collagen. Most of the cleavages occurred following a hydroxyproline residue. The most N-terminal site was within three residues of the previously identified site in bovine type II collagen. Western blotting using anti-neoepitope antibodies showed that the initial cleavages occurred at the N-terminal sites and this was followed by more extensive degradation resulting in products too small to be resolved by SDS gel electrophoresis. Immunohistochemical staining of cartilage sections from equine or human osteoarthritic joints showed staining in lesional areas which was not observed in non-arthritic sites.

Conclusions: Cathepsin K cleaves triple helical collagen by erosion from the N-terminus and with subsequent progressive cleavages. The liberated fragments can be detected in osteoarthritic cartilage and may represent useful biomarkers for disease activity.

Introduction

Osteoarthritis (OA) is characterized by the degradation of articular cartilage and remodeling of the underlying subchondral bone associated with mild to moderate joint inflammation, chronic pain and loss of joint function. Cartilage loss is a manifestation of complex pathological cellular and molecular processes that result in a final common pathway of enzymatic destruction of the matrix molecules, mainly aggrecan and type II collagen. The triple helical type II collagen molecule is the essential major structural protein of the articular cartilage extracellular matrix which determines the tissue's tensile strength. Excessive proteolytic degradation of this component is believed to be a key irreversible event in the process of cartilage degradation and leads to a progressive loss of both the structural and functional integrity of the matrix[1].

In addition to the well-documented involvement of the matrix metalloproteinase (MMP) and "a disintegrin and metalloproteinase with thrombospondin motifs" (ADAMTS) family members in cartilage breakdown associated with degenerative diseases such as arthritis, the contribution of other proteases has also become evident[2]. In particular, cysteine cathepsins have been

* Address correspondence and reprint requests to: J.S. Mort, Genetics Unit, Shriners Hospital of Children, 1003, boul. Décarie, Montreal, Quebec H4A 0A9, Canada.
E-mail address: jmort@shriners.mcgill.ca (J.S. Mort).

http://dx.doi.org/10.1016/j.joca.2016.03.016
1063-4584/

implicated in several aspects of joint pathology[3]. Of the members of this family, cathepsin K is of special importance since it is one of the few proteases, other than the MMP collagenases, that are able to degrade intact triple helical collagen at and near neutral pH[4]. While originally believed to be unique to the osteoclast, where it plays an important role in bone resorption (principally demineralization and degradation of type I collagen molecules) in health and pathology, cathepsin K is now known to be produced by many other cell types[5,6]. Its increased abundance in chondrocytes close to the articular surface[3] suggests that its action may contribute to cartilage fibrillation seen with aging and joint OA. This view is strengthened by the finding that OA changes are delayed in mouse models of the disease in cathepsin K null animals[7] and that in mouse, rabbit and dog OA models treatment with a specific cathepsin K inhibitor reduced cartilage degeneration[8,9].

Previous studies have demonstrated that cathepsin K degrades triple helical collagen but not by the canonical approach employed by MMP collagenases where the collagen chain is cleaved at a specific three quarter site [Fig. 1(A)] where the helix appears to be less stable[10,11]. In contrast, cathepsin K has been shown to cleave at a variety of sites throughout the molecule[12]. This process liberates a series of peptides as the cathepsin K action proceeds. One such cleavage site in type II collagen close to the N-terminal triple helix was identified several years ago[13]. Using a specific anti-neoepitope antibody which recognizes the small collagen fragment released by this cleavage event, we demonstrated the contribution of this cathepsin K to cartilage degeneration in human and equine OA[14–17].

In contrast to type I collagen, cleavage of type II collagen by cathepsin K has received much less attention. We hypothesized that a more comprehensive analysis of the cleavage sites generated by cathepsin K, which can be best realized using mass spectrometric methods, would identify a much more diverse set of epitopes that would be more sensitive and specific for the action of this enzyme. These could then be used for the development of assays to monitor disease activity and potentially the progress of therapeutic intervention. While the type II collagen protein sequence is highly conserved between mammalian species, there are substantial residue substitutions and the level and locations of post-translational modification (proline and lysine modification) is variable. In addition to the study of equine arthritis in its own right, the horse represents a useful arthritis model since cartilage is available from animals decades old and at varying degrees of disease severity. A detailed investigation of equine type II collagen was therefore undertaken. The comparative study of equine and human

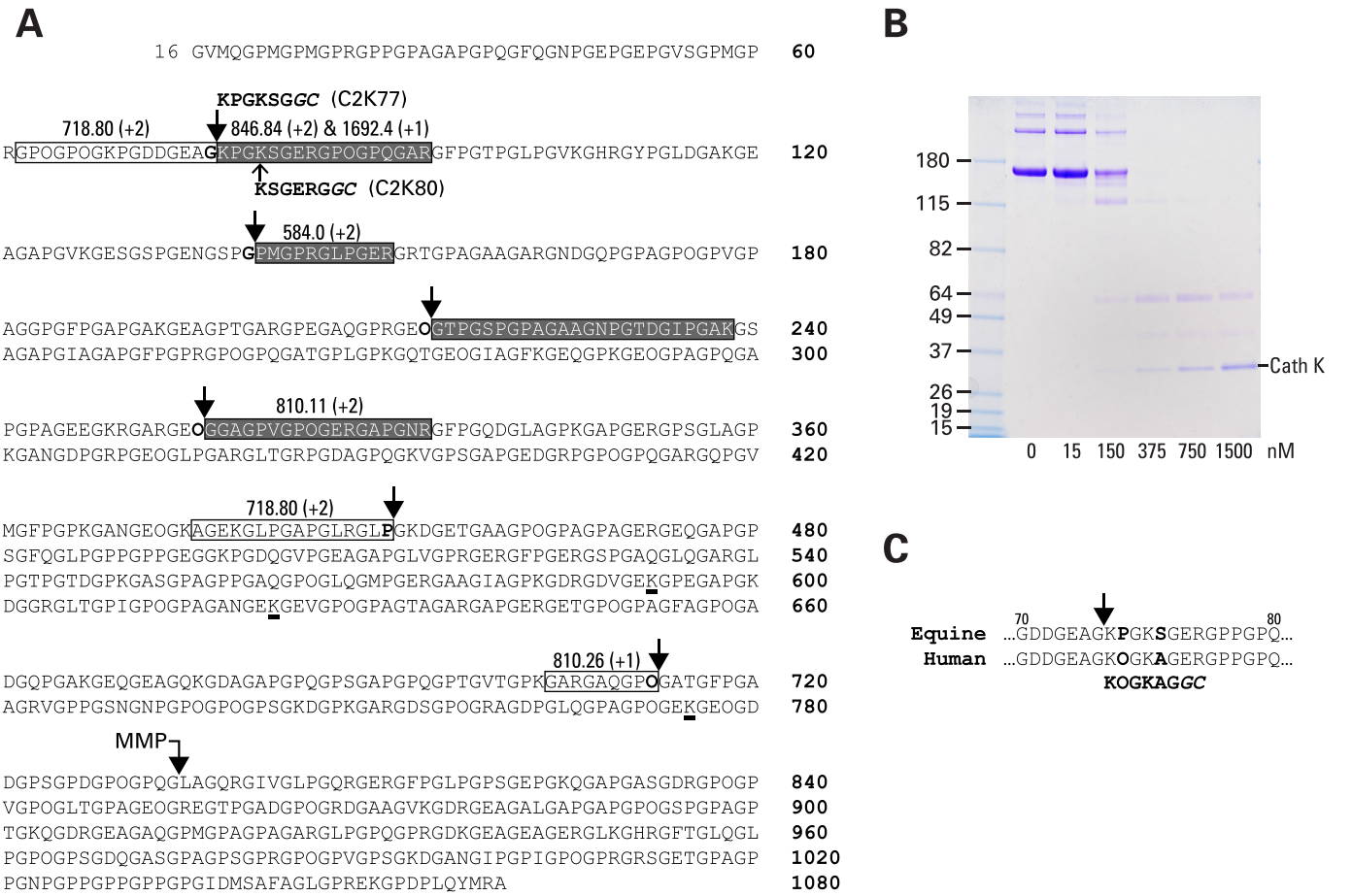

Fig. 1. Post-translational modification of equine type II collagen and its cleavage by cathepsin K. (A) Sequence of the equine collagen α_1(II) chain beginning at the pepsin cleavage site in the *N*-telopeptide[40] and including the triple-helical region followed by the *C*-telopeptide. Residue numbering represents the complete mature collagen sequence (Uniprot entry Q28396)[41] starting at the *N*-telopeptide. Proline residues identified as hydroxylated are indicated as O, and glycated lysine residues are underlined. The cathepsin K cleavage positions, indicated by triangular arrowheads, are based on the tryptic fragments outlined in white (N-terminal fragment) and black (C-terminal fragment) with the associated mass and charge. The P1 residues are indicated in bold. Also indicated is the previously determined cathepsin K cleavage position (open arrow)[13] and the classical collagenase ¾/¼ site (MMP). Peptide sequences used for antibody production (C2K77 and C2K80) are shown adjacent to the corresponding cleavage sites. (B) SDS/PAGE gradient gel analysis of equine type II collagen products following cathepsin K cleavage for 2 h using increasing enzyme concentrations. The cathepsin K band is indicated on the right. The migration positions of the pre-stained standards are indicated on the left for reference. However, as is well established, the anomalous migration of collagen α chains precludes correlative calculation of the molecular size of the fragments[42,43]. (C) Alignment of the sequences of human and equine type II collagen in the region of the C2K77 neoepitope and the sequence used to produce the antibody to the equivalent human epitope.

Please cite this article in press as: Mort JS, et al., Early cathepsin K degradation of type II collagen *in vitro* and *in vivo* in articular cartilage, Osteoarthritis and Cartilage (2016), http://dx.doi.org/10.1016/j.joca.2016.03.016