Pferdeheilkunde 26 (2010) 4 (Juli/August) 588-590

Type V Collagen in human and equine articular cartilage

Yvette S. Bland[1], Michael T. Bayliss[2], Brigitte von Rechenberg[3] and Doreen E. Ashhurst[1]

Department of Anatomy, St. George's Hospital Medical School, Tooting, London, UK[1], Department of Veterinary Basic Sciences, The Royal Veterinary College, London, UK[2] and Musculoskeletal Research Unit (MSRU), Equine Hospital, Vetsuisse Faculty Zurich, University of Zurich, Switzerland[3]

Summary

A striking feature of developing rabbit joints is the presence of a pericellular ring of type V collagen around the cells of the chondrogenous layers before cavitation and this persists around the articular chondrocytes throughtout life. To investigate whether type V collagen is found with a similar distribution in the articular cartilage of other species, equine and human articular cartilage was examined. A pericellular ring of type V collagen was found around the articular chondrocytes of both species in cartilage from young and old animals. Type XI collagen is closely related to type V collagen and hybrid molecules of types V and XI α-chains are known to exist. A significant feature of type V collagen is that it may be used as a marker of articular chondrocytes in a variety of mammalian species.

Keywords: articular cartilage, equine, human, type V collagen.

Typ 5-Kollagen im Gelenkknorpel von Mensch und Pferd

Artikulärer Knorpel entwickelt sich anders als andere Knorpelgewebe. Er entwickelt sich aus der Interzone der Knorpelschicht. Eine herausstechende Eigenschaft bei jungen Kaninchen ist die Präsenz einer perizellulären Ringes vom Kollagen Typ V, der um die Zellen der knorpeligen Schicht vor der Kavitation und bleibt für den Rest des Lebens bestehen. Equiner und humaner Knorpel wurden untersucht um herauszufinden, ob das in anderen Spezies auch der Fall und eine ähnliche Verteilung vorhanden sein würden. Ein perizellulärer Ring von Kollagen Typ V wurde sowohl bei jungen wie auch alten Individuen gefunden. Kollagen vom Typ XI ist dem Typ V sehr ähnlich und Hybrid-Moleküle von Typ V und XI -Ketten existieren. Die Signifikanz von Kollagen Typ V ist, dass es als Biomarker für artikuläre Chondrozyten in verschiedenen Spezies gebraucht werden kann.

Schlüsselwörter: Gelenkknorpel, Pferd, Mensch, Typ 5-Kollagen, Kollagen

Introduction

Articular cartilage is distinct from other cartilages. It develops from the highly cellular chondrogenous layers of the interzone. A striking feature of the fetal rabbit knee joint before cavitation is the pericellular distribution of type V collagen in the matrix of the chondrogenous layers; there is none in the underlying epiphyseal cartilage. The pericellular distribution of type V collagen around articular chondrocytes persists throughout life in the rabbit (*Bland* and *Ashhurst* 1996 and 2001). Other unique features of articular cartilage are that the matrix does not contain type II collagen until after cavitation and matrilin-1 is never found in normal articular cartilage; both are present in epiphyseal cartilage (*Bland* and *Ashhurst* 1996 and 2001, *Kavanagh* and *Ashhurst* 1999, *Murphy* et al. 1999, *Segat* et al. 2000, *Hyde* et al. 2007). It seems unlikely that this pericellular distribution of type V collagen is unique to the rabbit.

Type V collagen is very closely related to type XI collagen and some fibrils may be heterotypic associations of α-chains from both collagens (*Fichard* et al. 1994). Type XI collagen has not been localized in articular cartilage, although it is known to be a constituent of the matrix (*Eyre* et al. 1987, *Furuto* et al. 1991, *Thomas* et al. 1994). In epiphyseal cartilage it is found throughout the matrix (*Petit* et al 1993).

The aim of the present investigations was to determine the distribution of type V collagen in human articular cartilage from 7 to 70 years and in horse articular cartilage from 2 weeks to 17 years.

Materials and Methods

Tissue preparation

Normal human articular cartilage was obtained with consent from the femoral condyles of knee joints of patients aged between 7 and 70 years undergoing amputation as a result of osteo- or soft tissue sarcocomas; it was macroscopically normal. It was fixed within 2 to 6 hours after surgery. The horse articular cartilage was from the tarsal joints of 2 neonatal foals (1-3 days old) and a 2-week-old foal, from the fetlock (metacarpophalangeal) joints of two 7-year-old geldings, and the tarsal joint of a 17-year-old gelding. The tissue was fixed in 4% buffered formaldehyde and embedded in wax. Sections were cut at 7 μm.

Immunohistochemistry

A goat anti-rabbit type V collagen antibody was used (*Page* et al. 1986). This antibody was affinity purified. Its primary reactivity is against the α2 chain of type V collagen, but it shows low reactivity against the α2 chain of type XI collagen (*Bland* and *Ashhurst* 1996).

The immunohistochemical procedure used was described in detail by *Bland* and *Ashhurst* (1996). Briefly, the sections were pretreated with trypsin, hyaluronidase , l-lysine, and undiluted heat-inactivated normal rabbit serum. The sections were exposed to the anti-collagen antibody at optimal dilution in 1% BSA in PBS overnight at 4°C. The bound antibodies were

located with rabbit anti-goat IgG antibody conjugated to alkaline phosphatase.

Two controls were performed. The specific antibody was omitted or was substituted by pre-immune goat serum at appropriate dilution.

Results

In both the human and horse articular cartilage at all the ages examined strong binding of the antibody to type V collagen was observed as a pericellular ring around the chondrocytes (Figs 1, 2 and 3). Some antibody-binding occurred in the interterritorial matrix, especially in the surface layer of the cartilage. Antibody-binding in the human interterritorial matrix gradually decreases with age so that it is much reduced at 35 years, but absent at 70 years. The results were negative when the sections were exposed to pre-immune goat serum or the primary antibody was omitted; there was no coloration.

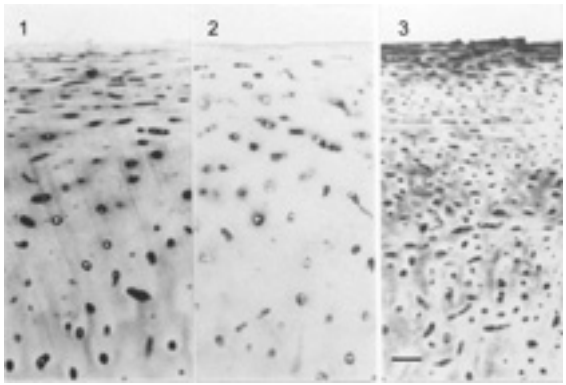

Fig. 1 Immunohistochemical localization of type V collagen around the chondrocytes in articular cartilage - cartilage from a 35-year-old man.

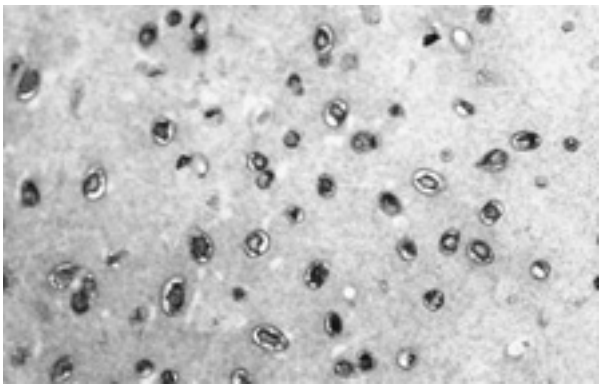

Fig. 2 Immunohistochemical localization of type V collagen around the chondrocytes in articular cartilage - cartilage from a 70-year-old man.

Discussion

These results indicate that type V collagen is present in the pericellular matrix of articular chondrocytes of man and horses from after birth to old age. This agrees with the observations of rabbit articular cartilage (*Bland* and *Ashhurst* 1996, 2001). Type V collagen is very closely related to type XI collagen and hybrid molecules of both type V and type XI α-chains may occur (*Fichard* et al. 1994). An attempt to locate type XI collagen using a rabbit anti-bovine type XI collagen antibody failed because the control experiment substituting pre-immune rabbit serum for the specific antibody gave an identical result. Non-specific binding of rabbit immunoglobulins to type XI collagen was observed by *Eyre* (personal communication), which may explain this finding. Thus, the specific antibody may be simply trapped by the steric properties of the matrix. It is noteworthy that there are few accounts of the localization of type XI collagen in articular cartilage (see below).

The few observations of the distributions of types V and XI collagens in developing limbs and articular cartilage indicate that they have different localizations. Type V collagen is present in rabbit articular cartilage throughout life, but there are no similar studies of type XI collagen. Type V collagen was located in the matrix of the developing articular cartilage, but not in that of the underlying epiphyseal cartilage, of developing chicken long bones (*von der Mark* and *Ocalan* 1982). Type XI collagen is evenly distributed throughout the matrix of bovine and human fetal epiphyseal cartilage (*Swoboda* et al.

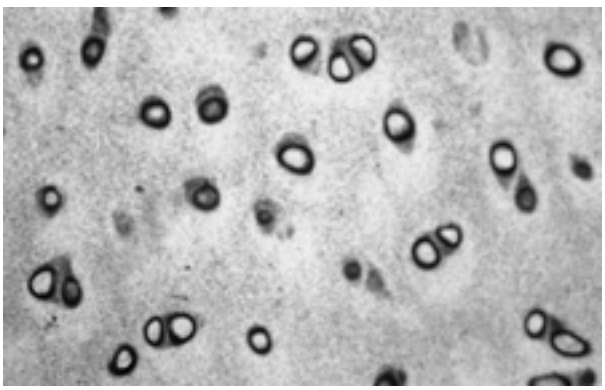

Fig. 3 Immunohistochemical localization of type V collagen around the chondrocytes in articular cartilage - cartilage from a 7-year-old gelding. Magnifications of Figs 1 to 3 are the same; bar on Fig. 1 equals 20 μm.

1989, *Petit* et al. 1993). Of the antibodies raised to specific regions of the N-terminal region of the α1(XI) chain only one antibody, p8, to a 20 amino-acid region, was bound by developing articular cartilage in fetal rats (*Morris* et al. 2000); the other antibodies were bound by diaphyseal and epiphyseal cartilage. A brief report suggests that both types V and XI collagens are located in the chondrocytes of articular cartilage of mature rats (*Furuto* et al. 1991). Identification of the cells expressing the mRNAs for these collagens supports the immunohistochemical observations. Type V collagen mRNAs, α1(V) and α3(V), are expressed by cells lining developing joint cavities, presumably putative articular chondrocytes, but not by epiphyseal chondrocytes of 15.5 and 16.5 day fetal mice, whereas type XI collagen mRNAs, α1(XI) and α2(XI), are expressed only by epiphyseal chondrocytes of 15.5-day fetuses (*Andrikopoulis* et al. 1992, *Sugimoto* et al. 1998, *Imamura* et al. 2000).

Types V and XI collagen molecules provide a template around which types I and II molecules aggregate to form fibrils (*Eyre* 2002, *Hansen* and *Bruckner* 2003, *Wenstrup* et al. 2004, *Wu* et al. 2009). Type V collagen is associated with type I collagen and type XI collagen with type II, but, as was mentioned above, some of the molecules are hybrid. Such molecules

occur in articular cartilage. Recent investigations show that the α1(V) chain gradually replaces the α2(XI) chain in the fibrils of mature bovine articular cartilage (*Wu* et al., 2009). Various permutations of the molecular associations of types V and XI α-chains in forming fibrils have been detected in different tissues which suggests that type V/XI molecules may be important in determining the type of fibrils appropriate to each tissue.

The identification of type V collagen in the matrix around the putative chondrocytes in the chondrogenous layers of developing joints (*Bland* and *Ashhurst* 1996 and 2001) showed that these cells are unique and distinct from the chondrocytes of epiphyseal cartilage. Since that time further distinctive features have emerged. Matrilin-1 is not present in mouse or rabbit articular cartilage at any stage, whereas it is present in epiphyseal cartilage (*Kavanagh* and *Ashhurst* 1999, *Murphy* et al. 1999, *Segat* et al. 2000, *Hyde* at al. 2007). In contrast, versican is present in articular, but not epiphyseal cartilage (*Snow* et al. 2005). More recently, *Koyama* et al. (2008) traced Gdf5-expressing cells in developing mouse interzones and provided further evidence that they form a cohort distinct from the epiphyseal chondrocytes. While these investigations are restricted to mice and rabbits, the identification of type V collagen around human and horse articular chondrocytes suggests that these cells are also distinct from the earliest developmental stages. In contrast to most other chondrocytes, articular chondrocytes persist for long periods; their lifespan is not known. It would be of interest to investigate the distribution of type V collagen and the molecules mentioned above in other persistent cartilages, such as the rings in the trachea.

Acknowledgements

This research was supported by the Arthritis and Rheumatism Campaign, UK. We should like to thank Dr. David Eyre for his interest and suggestions during the preparation of this paper. We regret to report that Michael Bayliss died during the preparation of this paper.

References

Andrikopoulos K., Suzuki H. R., Solursch M. and *Ramirez F.* (1992) Localization of pro-α2(V) collagen transcripts in the tissues of the developing mouse embryo. Dev. Dyn. 195,113-120

Bland Y. S. and *Ashhurst D. E.* (1996) Development and ageing of the articular cartilage of the rabbit knee joint: distribution of the fibrillar collagens. Anat. Embryol. 194, 607-619

Bland Y. S. and *Ashhurst D. E.* (2001) The hip joint: the fibrillar collagens associated with development and ageing in the rabbit. J. Anat. 198, 17-27

Eyre D. R. (2002) Collagen of articular cartilage. Arthritis Res. 4, 30-35

Eyre D. R., Wu J. J. and *Apone S.* (1987) A growing family of collagens in articular cartilage; identification of 5 genetically distinct types. J. Rheumatol. 14, 515-531

Fichard A., Kleman J.-P. and *Ruggiero F.* 1994 Another look at collagen V and XI molecules. Matrix Biol. 14, 515-531

Furuto D. K., Gay R. E., Stewart T. E., Miller and *Gay S.* (1991) Immunolocalization of types V and XI collagen in cartilage using monoclonal antibodies. Matrix 11, 144-149

Hansen U. and *Bruckner P.* (2003) Macromolecular specificity of collagen fibrillogenesis. J. Biol. Chem. 278, 37352-37359

Hyde G., Dover S., Aszodi A., Wallis G. A. and *Boot-Handford R. P.* (2007) Lineage tracing using matrilin-1 gene expression reveals that articular chondrocytes exist as the joint interzone forms. Dev. Biol. 304, 825-833

Imamura Y., Scott I. C. and *Greenspan D. S.* (2000) The pro-α3(V) collagen chain. J. Biol. Chem. 275, 8749-8759

Kavanagh E. and *Ashhurst D. E.* (1999) Development and ageing of the articular cartilage of the rabbit knee joint: distribution of biglycan, decorin, and matrilin-1. J. Histochem. Cytochem. 47, 1603-1615

Koyama E., Shibukawa Y., Nagayama M., Sugito H., Young B., Yuasa T., Okabe T., Ochiai T., Kamiya N., Rountree R. B., Kingsley D. M., Iwamoto M., Enomoto M. and *Pacifici M.* (2008) A distinct cohort of progenitor cells participates in synovial joint and articular cartilage formation during mouse limb skeletalogenesis. Dev. Biol. 316, 62-73

Morris N. P., Oxford J. T., Davies G. B. M., Smoody B. F. and *Keene D. R.* (2000) Developmentally regulated alternative splicing of the α1(XI) collagen chain: spatial and temporal segregation of isoforms in the cartilage of fetal rat long bones. J. Histochem. Cytochem. 48, 725-741

Murphy J. M., Heinegård D., McIntosh A., Syerchi D. and *Barry F. P.* (1999) Distribution of cartilage molecules in the developing mouse joint. Matrix Biology 18, 487-497

Page M., Hogg J. and *Ashhurst D. E.* (1986) The effects of mechanical stability on the macromolecules of the connective tissue matrices produced during fracture healing. I. The collagens. Histochem. J. 18, 251265

Petit B., Ronzière M. C., Hartmann D. J. and *Herbage D.* (1993) Ultrastructural organization of type XI collagen in fetal bovine epiphyseal cartilage. Histochemistry 100, 231-239

Segat D., Frie C., Nitsche P. D., Klatt A. R., Piecha D., Korpos E., Deák F., Wagener R., Paulsson M. and *Smyth N.* 2000 Expression of matrilin-1, -2 and -3 in developing mouse limbs and heart. Matrix biology 19, 649-655

Snow H. E., Riccio L. M., Mjaatvedt C. H., Hoffman S. and *Capehart A. A.* (2005) Versican expression during skeletal/joint morphogenesis and patterning of muscle and nerve in the embryo mouse limb. Anat. Rec. A Discov. Mol. Cell Evol. Biol. 282, 95-105

Sugimoto M., Kimura T., Tsumaki N., Matsui Y., Nakata K., Kawahata H., Yasui N., Kitamura Y., Nomura S. and *Ochi T.* (1998) Differential in situ expression of α2(XI) collagen mRNA isoforms in the developing mouse. Cell Tissue Res. 292, 325-332

Swoboda B., Holmdahl R., Stöß H. and *von der Mark K.* (1989) Cellular heterogeneity in cultured human chondrocytes identified by antibodies specific for α2(XI) collagen chains. J. Cell Biol. 109, 1363-1369

Thomas J. T., Ayad S. and *Grant M. E.* (1994) Cartilage collagens: strategies for the study of their organization and expression in the extracellular matrix. Ann. Rheum. Dis. 53, 488-496

Von der Mark K. and *Öcalan M.* (1982) Immunofluorescent localization of type V collagen in the chick embryo with monoclonal antibodies. Collagen Rel. Res. 2, 541-555

Wenstrup R. J., Florer J. B., Brunskill E. W., Bell S. M., Chervoneva I. and *Birk D. E.* (2004) Type V collagen controls the initiation of collagen fibril assembly. J. Biol. Chem. 279, 53331-53337

Wu J.-J., Weis M. A., Kim L. S., Carter B. G. and *Eyre D. R.* (2009) Differences in chain usage and cross-linking specificities of cartilage type V/XI collagen isoforms with age and tissue. J. Biol. Chem. 284, 5539-5545

Dr. D. E.Ashhurst
Department of Anatomy
St. George's Hospital Medical School Tooting
London SW17 0RE
dashhurs@sghms.ac.uk