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Review

New aspects of the pathogenesis of osteoarthritis: the role of fibroblast-like chondrocytes in late stages of the disease

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Summary. It is thought that the general increase in life expectancy will make osteoarthritis the fourth leading cause of disability by the year 2020. Even though the pathogenesis of idiopathic osteoarthritis has not been fully elucidated, the main features of the disease process are the altered interactions between the chondrocytes and their surrounding extracellular matrix. In the course of these disturbances, three types of chondrocytes are typically present in the pathologically altered extracellular matrix of the articular cartilage: healthy chondrocytes which are continually undergoing degeneration, degenerated cells which are continually being degraded and finally fibroblast-like chondrocytes which seem not to be influenced by this process and, therefore, are found in ever-increasing numbers. These fibroblast-like chondrocytes take part in tissue regeneration even in advanced stages of osteoarthritis, but only in as much as they form fibrocartilaginous or scar tissue, since, as we were able to show, they mainly synthesize collagen type I and not collagen type II, typical for healthy cartilage. However, we were further able to show that fibroblast-like chondrocytes also produce increasing amounts of the proteoglycans decorin and biglycan which physiologically are involved in the formation of collagen type II, as well as perlecan. These multifunctional fibroblast-like chondrocytes could present an ideal therapeutic starting point if they could be modified to synthesize the collagen type II typical for cartilage and to, thereby, contribute to reversing the damage of the joint cartilage that has occurred by the late stages of osteoarthritis.

Key words: Osteoarthritis, Fibroblast-like chondrocytes, Collagen type I, Collagen type II, Proteoglycans

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Introduction

Osteoarthritis (OA) is a chronic and mainly degenerative joint disease in which the degeneration is progressive and loss of articular cartilage finally leads to the eburnation of the subchondral bone. This process is accompanied by a limited inflammatory synovial reaction (Poole et al., 1993, Garnero and Delmas, 2003). OA is the most common musculoskeletal disease in the elderly with a worldwide distribution. As an example, according to Reginster (2002) up to 1.75 million people alone in England and Wales suffer from symptomatic OA. However, the number of asymptomatic cases is estimated to be much higher. There is a strong association between its prevalence and increasing age, since up to 20 % of the population over 60 years of age show signs of OA (Haq et al., 2003). The severity of OA also increases indefinitely with age and up to now the condition is not reversible (Woolf and Pfleger, 2003). As OA often remains asymptomatic until late in the disease progress and early markers as reliable tools of diagnosis are still lacking up to now, total knee replacement is the ultimate therapeutic intervention. This means that important parts of health care resources have to be spent on coping with this disease (Reginster, 2002). The general increase in life expectancy and the resulting aging populations are expected to make OA the fourth leading cause of disability by the year 2020 (Woolf and Pfleger, 2003). This warrants the further elucidation of the pathogenesis of OA with the final goal of gaining insight into the disease processes to render a cell biological therapy possible and within reach.

OA is classified into two general groups: Primary OA, of unknown cause, and secondary OA resulting from various conditions such as joint injury, infection, developmental and metabolic disorders. In the present review we will only discuss primary or idiopathic OA, focusing on our recent findings in patients with late stages of the disease (Fig. 1A). We would like to apologize for not being able to cite all the excellent

papers published in this field. Here, we will concentrate on those contributions with particular interest in changes in the chondrocyte phenotype, i.e. the appearance of different cell types and changes in their metabolisms in late stages of OA of the human knee joint.

Ultrastructure of chondrocytes and matrix composition in normal articular cartilage

Articular cartilage is a highly specialized and uniquely designed tissue, which covers the articulating ends of long bones (Kuettner, 1992, Morris et al., 2002). Macroscopically it appears glossy and almost translucent. Articular cartilage is an avascular, aneural and alymphatic tissue. Its nutrition is fully dependent on the diffusion of synovial fluid produced by the joint synovia (Muir, 1995). The resilience, integrity and function of articular cartilage all depend on the composition of the abundant extracellular matrix synthesized by the single cell type found in this tissue, the chondrocyte (Morris et al., 2002). In comparison, the proportion of the cells -the chondrocytes- to their surrounding extracellular matrix is lower in articular cartilage than in any other tissue. These few cells, lacking direct cell-cell contacts, are responsible for the production and maintenance of the extracellular matrix of articular cartilage which is composed of a network of proteoglycans embedded in a system of collagen fibrils (Poole, 1997; Kuettner, 1992; Muir, 1995; Fukui et al., 2001). The chondrocytes are organized into chondrons, which are the functional and mechanical units of articular cartilage. Their surrounding extracellular matrix can be classified as the pericellular space adjacent to the cells composed of a matrix with small fiber diameters, next to it, the territorial matrix and, finally, the interterritorial matrix between the different chondrons with the well known collagen fibers (Poole, 1997). Histologically, articular cartilage can be divided into four separate zones: a superfizial zone, which lies next to the joint space, a transitional zone, a radial zone and the zone of calcified cartilage adjacent to the subchondral bone (Fig. 1B). Whereas the chondrocytes of the superficial zone have a flat and ellipsoid shape and lie parallel to the articular surface, the chondrocytes of the transitional zone are rounder and larger. In the radial zone the round cells form columns, lying perpendicular to the cartilage surface, as do their surrounding collagen fibers (Buckwalter and Mankin, 1998).

The abundant extracellular matrix of articular cartilage is composed of two major elements: the collagens and the proteoglycans. Normal articular cartilage contains types II, III, VI, IX, X, XI, XII and XIV collagens, the most abundant being collagen type II (Mayne and Brewton, 1993; Eyre, 2002). Collagens type II, IX and XI form fibrillar alloys with type XI collagen as core and type IX collagen on the outside possibly limiting the fiber diameter (Kuettner, 1992; von der Mark, 1999; Hansen and Bruckner, 2003). In addition, the proteoglycans, a heterogeneous group of extracellular matrix proteins, consisting of a central core protein substituted with one or more glycosaminoglycan side chains constitute the other major extracellular matrix components (Hardingham and Fosang, 1992). A few good examples would be, first of all, aggrecan, the large cartilage matrix proteoglycan (Doege, 1999) responsible for cartilage tissue maintenance together with several other small proteoglycans (Iozzo, 1999) which are also important for its function, such as decorin (Heinegard et al., 1999, Knudson and Knudson, 2001), biglycan (Bianco et al., 1990; Miosge et al., 1994) and fibromodulin (Dourado et al., 1996).

Changes in the ultrastructure of the cells and the matrix composition in osteoarthritic articular cartilage

A disturbed cell-matrix relationship lies at the centre of the pathogenesis of OA (Poole, 1999). The primary

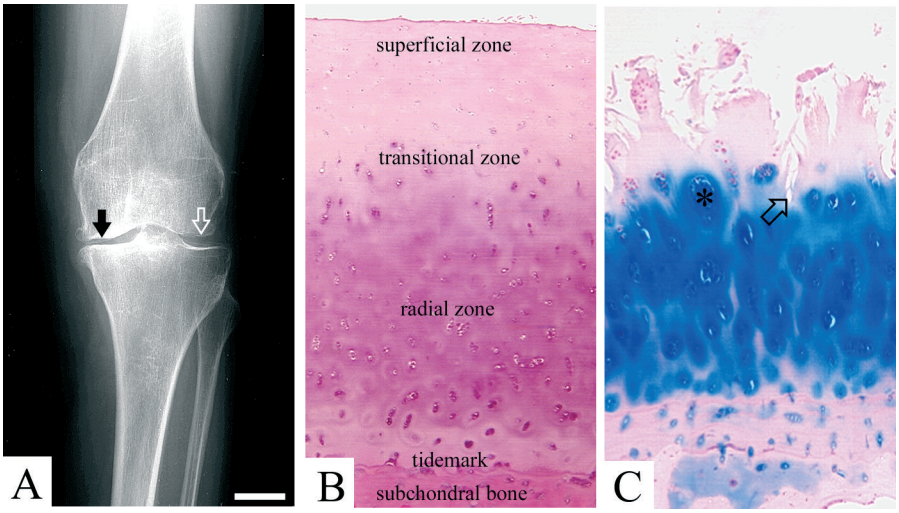

Fig. 1. Adapted from Tesche and Miosge 2004, Osteoarthritis and Cartilage. **A.** Radiograph of a patient suffering from late-stage OA. Tissue samples were taken from an area adjacent to the main defect (black arrow) and a macroscopically intact area (open arrow). Bar: 2 cm. **B.** Histological evaluation of a normal cartilage samples, H.E. Bar: 70 µm. **C.** Cartilage samples from the area adjacent to the main defect. Note the increasing number of chondrocytes arranged in clusters (asterisk), fissures (arrow), tidemark duplication, loss of the superficial zone, and the overall decreased Alcian blue staining. Bar: 70 µm.

lesion of the osteoarthritic process involves the chondrocytes as well as their surrounding matrix (von der Mark and Gluckert, 1990). In the progress of OA a disruption of the extracellular matrix framework and an increasing water content followed by a loss of matrix strength can be observed (Buckwalter and Mankin, 1998). The degradation of the tissue is underlined by a loss of the main proteoglycan, aggrecan, collagen fiber fibrillation and surface splits (Poole et al., 1993; Poole, 1999). Mainly in early stages of OA, the production of collagen type II is initially increased (Aigner et al., 1992). Furthermore, fissures extending deep into the cartilage substance and cell cluster formation occur (Fig. 1C). These stages of disease owe their progress to degenerative processes catalyzed by tissue proteases which ultimately lead to the destruction of the articular cartilage with denudation of the subchondral bone (Gardner, 1994; Martel-Pelletier, 1999). This tissue degeneration is intermingled with regeneration efforts (Sandell and Aigner, 2001; Aigner and McKenna, 2002), which might possibly be seen in the occurrence of chondrocyte clusters and are certainly seen in the appearance of fibrocartilaginous tissue with a more fibrillar matrix and a newly emerging cell type (Calandruccio and Gilmers, 1962; Miosge et al., 1998; Poole, 1999). These cells were initially identified and described at the ultrastructural level and named elongated secretory type 2 cells and had an irregular shape with a prominent rough endoplasmic reticulum (Kouri et al., 1996). We call them fibroblast-like chondrocytes. They are mainly found in the deep zones of osteoarthritic cartilage. The remaining normal chondrocytes, called type 1 cells (Kouri et al., 1996) show a typical round chondrocyte phenotype with numerous filopodia and may be arranged in clusters (Kouri et al., 1996; Miosge et al., 1998; Bock et al., 2001). At the more advanced stages of OA, the fibroblast-like chondrocytes express collagens, such as collagen type I and type III (Miosge et al., 1998; Sandell and Aigner, 2001) while the amounts of collagen type II are decreased (Poole, 1999). The levels of transcription and translation for decorin and biglycan are also up-regulated in these cells (Bock et al., 2001). It remains to be established whether these cells are trans-differentiated chondrocytes or derive from progenitor cells migrating through the fissured tidemark from the bone marrow into the diseased cartilage tissue.

In the following we will focus on the synthesis and tissue distribution of several matrix components and establish which type of cell, normal chondrocytes or fibroblast-like chondrocytes, produce these molecules in late stages of OA. Tissue samples for our investigations were obtained from human articular cartilage from the knee joints of patients suffering from OA who were undergoing total knee replacement operations. They were taken from a macroscopically intact area and an area adjacent to the main defect area of the osteoarthritic knee joint. The predominant cell type in the macroscopically intact area was the normal chondrocyte showing a typical chondrocyte phenotype (Fig. 2A). The

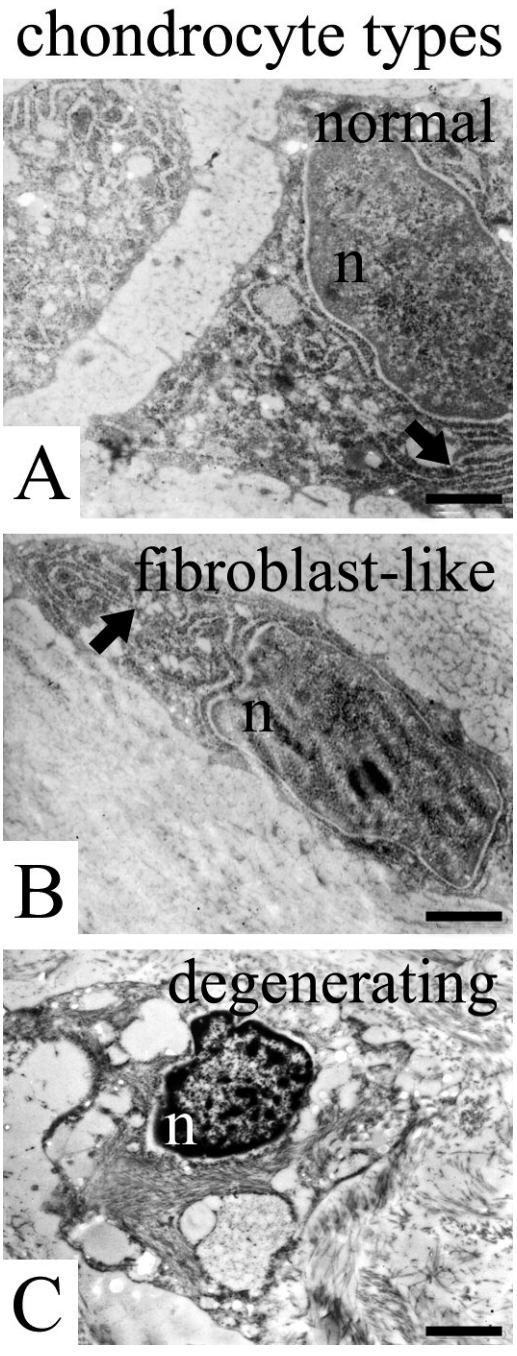

Fig. 2. Adapted from Bock et al. 2001, Osteoarthritis and Cartilage. Electron micrographs of the three chondrocyte types: **(A)** normal, **(B)** fibroblast-like, **(C)** degenerating. n: nucleus; black arrows: endoplasmic reticulum. Bars: 0.7µm.

deep zones of the area adjacent to the main defect predominantly revealed elongated secretory fibroblast-like chondrocytes (Fig. 2B) whereas chondrocytes, undergoing degeneration (Fig. 2C), were mainly found in the main defect area and were not investigated.

Decorin in late stages of OA

The small proteoglycan, decorin, has been localized in the interterritorial matrix of developing human articular cartilage (Bianco et al., 1990) as well as in normal adult articular cartilage of the human knee joint (Miosge et al., 1994). Investigations at the light microscopic level revealed the highest staining intensity for decorin in the superficial zone of the cartilage tissue as compared with the deep zone and the intermediate zone (Miosge et al., 1994). Also, the chondrocytes themselves showed a positive reaction for decorin throughout all zones of the hyaline articular cartilage. Investigations at the ultrastructural level showed that this staining pattern was restricted to cytoplasmic vesicles within the chondrocytes and that decorin is mainly located at the outside of the collagen fibers (Fig. 3A) in the interterritorial matrix (Miosge et al., 1994). The proteoglycan, decorin, takes part in the modulation of the metabolism of collagen type II in vitro possibly limiting the lateral growth of the collagen fibrils (Vogel et al., 1984). Furthermore, decorin is involved in the regulation of several important biological functions such as matrix organization, cell adhesion, migration and proliferation (Gallagher, 1989; Kresse et al., 1993; Iozzo, 1999). Because of these functions it was interesting to see whether decorin was also involved in the pathogenesis of OA. Investigations at the light microscopic level revealed reduced amounts of decorin protein in tissue samples taken from osteoarthritic cartilage (Poole et al., 1996; Witsch-Prehm et al., 1992) as well as increased levels of the corresponding mRNA of decorin when compared with physiological articular cartilage (Cs-Szabo et al., 1997). In late stages of OA, the levels of transcription as well as the levels of translation for both decorin and its corresponding mRNA are up-regulated (Fig. 3B) especially in tissue samples taken from the area adjacent to the main defect, i.e. the area of maximal mechanical load (Bock et al., 2001). These areas predominantly consist of fibroblast-like chondrocytes. We were able to show that these cells produce higher amounts of decorin mRNA which is also translated into the corresponding protein (Fig. 3C). Furthermore, an inverse relationship between the intracellular mRNA message level and the extracellular staining pattern for decorin protein was found, perhaps indicating that a low decorin content of the extracellular matrix of osteoarthritic cartilage might be a signal for an increased production of the corresponding mRNA which is than translated and secreted (Bock et al., 2001). In vivo, decorin has been described to stabilize the fibrillar matrix and to influence its assembly (Danielson et al., 1997). The increased amounts of decorin mRNA as well as the increased amounts of decorin protein might be seen as an effort on the part of the cartilage tissue to rebuild an orderly assembly of collagen fibrils, which, in turn, would affect the tensile strength of the extracellular matrix. In contrast, Vynios et al. (2001) described interactions between decorin and collagen type I but not type II. It is tempting to speculate that the increased amounts of collagen type I and decorin further each other in late stages of OA and could, therefore, be

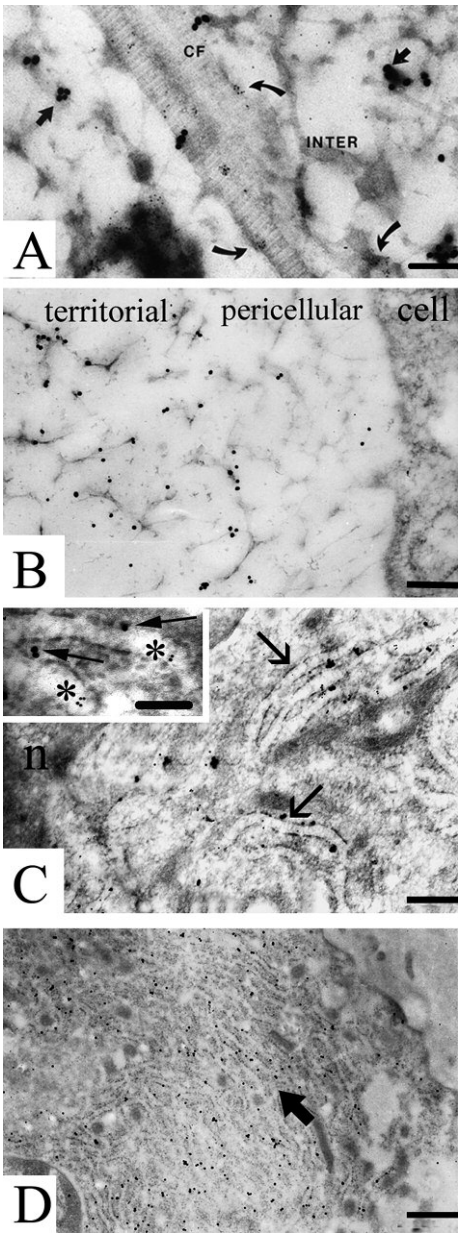

Fig. 3. A. Adapted from Miosge et al. 1994 Histochemical Journal. **B-D.** Adapted from Bock et al. 2001, Osteoarthritis and Cartilage. **A.** Double immunostaining for biglycan (small gold particles, bent arrows) and decorin (large gold particles, arrows) in the interterritorial matrix of the intermediate zone of normal human articular cartilage. Bar: 0.15 µm. **B.** Staining for decorin in the interterritorial matrix next to a fibroblast-like chondrocyte. Bar: 0.25 µm. **C.** Double labeling for decorin protein and the corresponding mRNA (arrows), small gold particles represent the protein in the lumen of the rough endoplasmic reticulum (asterisk in the inset) and large gold particles represent the corresponding mRNA at the border of the reticulum. n: nucleus. Bar: 0.25 µm. In the inset: small gold particles for decorin (asterisks) and large gold particles for decorin mRNA (fine black arrows). Bar: 0.13 µm. **D.** Ultrastructural in situ hybridization in a fibroblast-like chondrocyte for biglycan, mRNA adjacent to the rough endoplasmic reticulum (arrow). n: nucleus. Bars: 0.3 µm.

detrimental for the disease process.

Biglycan in late stages of OA

Biglycan is found in many connective tissues such as skin, bones and blood vessels (Fisher et al., 1989; Bianco et al., 1990). In articular hyaline cartilage, biglycan is mainly localized in the pericellular environment of the chondrocytes (Fisher, 1993; Miosge et al., 1994) and on the chondrocyte cell surface suggesting the involvement of this small proteoglycan in the control of chondrocyte proliferation (Bianco et al., 1990) and the modulation of morphogenesis and differentiation (Hunziker, 1992). Also, interactions of the biglycan protein with collagen type I have been described in vitro (Schönherr et al., 1995). Interestingly, biglycan is mainly localized in the superficial layer of articular cartilage. In contrast to decorin, biglycan is not found in the deep zones of articular cartilage whereas the zone adjacent to the tidemark revealed its presence (Miosge et al., 1994). Performing investigations at the ultrastructural level, Miosge et al. (1994) detected biglycan predominantly in the pericellular matrix of the chondrocytes but also at a lower concentration in the interterritorial matrix in all zones of normal adult articular cartilage where it was also attached to collagen fibrils. In osteoarthritic cartilage, reduced amounts of biglycan have been described (Poole et al., 1993) as well as higher levels of the corresponding mRNA when compared with physiological articular cartilage (Cs-Szabo et al., 1997). Our ultrastructural analysis revealed that biglycan is present in the pericellular matrix as well as in the interterritorial matrix of osteoarthritic cartilage. Again the fibroblast-like chondrocytes produced the highest amounts of biglycan protein as well as the corresponding mRNA (Fig. 3D) when compared with normal chondrocytes (Bock et al., 2001). In late stages of OA, atypical collagens, for example, collagen type I, are expressed in increasing amounts (Kuettner, 1992; Miosge et al., 1998, 2004) and interactions between biglycan and collagen type I have been described in vitro (Schönherr et al., 1995). Therefore, one might assume that biglycan, might also be involved in the regulation of collagen type I in late stages of OA (Bock et al., 2001).

Perlecan in late stages of OA

Perlecan, the large heparan sulfate proteoglycan from basement membranes (Iozzo, 1998), has lately been investigated in articular cartilage. In normal articular cartilage, perlecan seems to be enriched in the pericellular matrix of the chondrocytes (Arikawa-Hirasawa et al., 2001; Melrose et al., 2002), suggesting a role in the attachment of the cells to their own

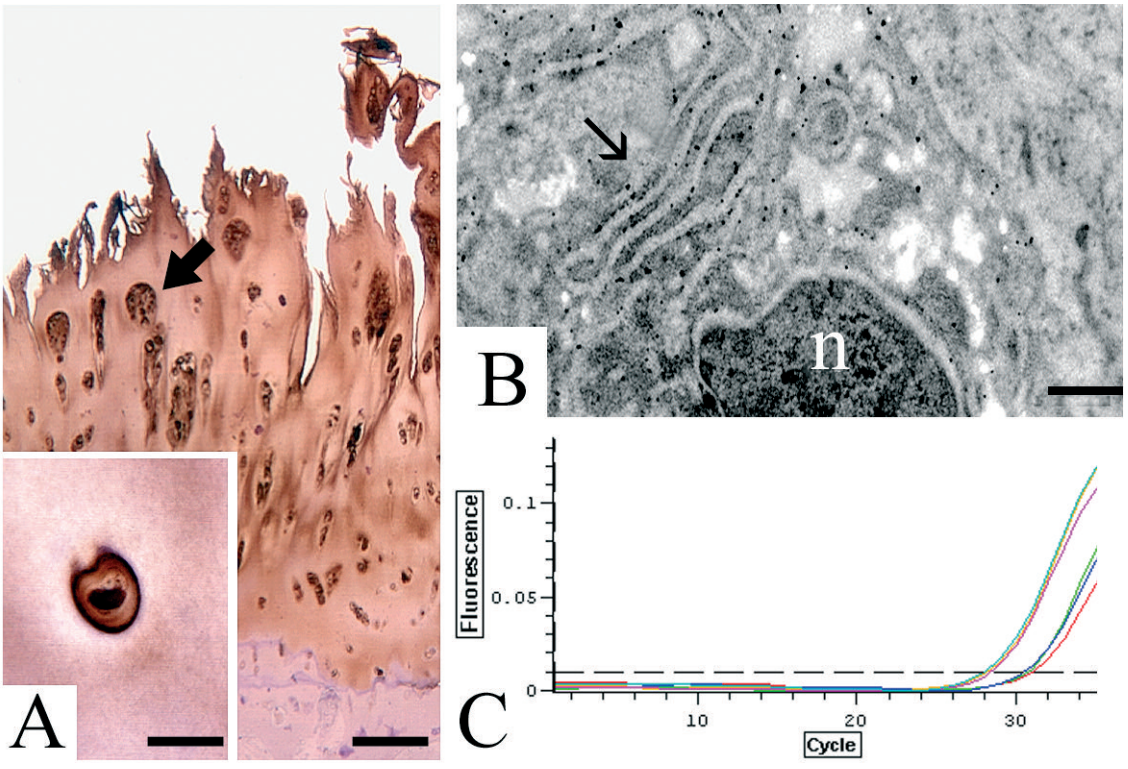

Fig. 4. Adapted from Tesche and Miosge, 2004 Osteoarthritis and Cartilage. **A.** Perlecan staining in the area adjacent to the main defect, especially in the surrounding matrix of chondrocytes arranged in clusters (arrow). Inset: higher magnification of a chondrocyte from the radial zone. Bars: 70 µm; insets bars: 10 µm. **B.** *In situ* hybridization for perlecan mRNA in a fibroblast-like chondrocyte. Bar: 0.3 µm. **C.** Increasing intensity of fluorescence per PCR cycle for native cartilage tissue, note that the slopes of the graphs, each color representing one PCR reaction, are similar and that the thresholds for RNA isolated from a the area adjacent to the main cartilage defect lie around 28 cycles (first group of curves) and from the macroscopically intact area lie around 30 cycles (second group of curves).

substratum (SundarRaj et al., 1995). The perlecan protein core consisting of five different subdomains is involved in several important biological functions, such as cell adhesion (Brown et al., 1997), maintenance of the extracellular matrix (Costell et al., 1999; Hassell et al., 2002), cartilage development (Arikawa-Hirasawa et al., 1999), regulation of chondrocyte differentiation (French et al., 1999) and binding to several matrix macromolecules (Hopf et al., 1999). Furthermore, mutations in the perlecan gene lead to skeletal disorders, such as the Schwartz-Jampel-Syndrome and the neonatal lethal dyssegmental dysplasia, Silverman-Handmaker-Type, both leading to severe disorganization in the structure of articular cartilage (Arikawa-Hirasawa et al., 2001, 2002). Also, perlecan-minus mice show disorganization in the columnar arrangement of chondrocytes especially in the deeper zones of hyaline cartilage, reduced glycosaminoglycan and reduced numbers of collagen fibrils as well as lack of the typical collagen network of the extracellular matrix (Arikawa-Hirasawa et al., 1999; Costell et al., 1999; Aszodi et al., 2000). We were the first to describe perlecan in late stages of OA and found it mainly enriched in the pericellular matrix of chondrocytes arranged in clusters (Fig. 4A) especially in the radial zone of articular cartilage (Tesche and Miosge, 2004). Investigations at the ultrastructural level using immunogold histochemistry, revealed a 30% higher amount of perlecan protein in the extracellular matrix of fibroblast-like chondrocytes, as compared with normal chondrocytes (Tesche and Miosge, 2004). Furthermore, these fibroblast-like chondrocytes described earlier as a sign of the regeneration efforts of the osteoarthritic cartilage tissue (Bock et al., 2001; Kouri et al., 1996; Miosge et al., 1998) revealed a 45 % rise in the synthesis rate of intracellular perlecan mRNA in comparison with normal chondrocytes in vivo (Fig. 4B). The rise in the amount of perlecan mRNA was confirmed with the help of quantitative real-time RT-PCR (Fig. 4C) which revealed a 50 % increase of perlecan (Tesche and Miosge, 2004). The higher level of perlecan mRNA especially in the fibroblast-like chondrocytes predominantly found in the areas adjacent to the main defect, where the main regeneration processes take place, and the increased level of perlecan protein indicate the involvement of this proteoglycan in the pathogenesis of OA and might reflect an attempt on the part of the cartilage tissue to stabilize the remaining extracellular matrix of late-stage osteoarthritic cartilage.

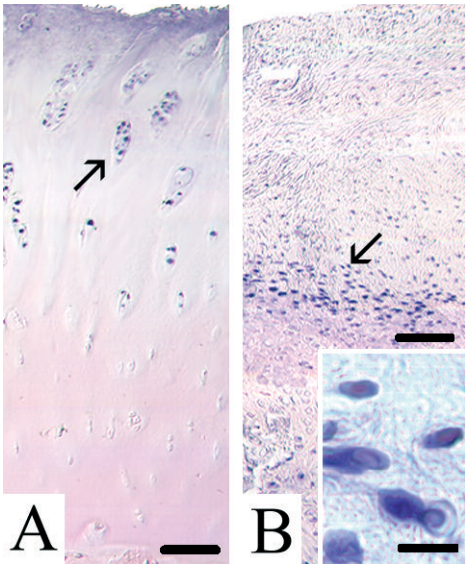

Fig. 5. Adapted from Miosge et al. 2004, Histochemistry and Cell Biology. **A**. Collagen type II mRNA is seen mainly in clusters (arrow) of chondrocytes of stage I disease. Bars: 20 µm. **B.** Strong staining is found in the fibrocartilaginous tissue of stage IV disease for collagen type I mRNA, especially in the area of the intermediate zone (arrow). Bar: 40 µm. Inset: depicts the elongated fibroblast-like cells of stage IV OA stained for collagen type I mRNA. Bar: 40 µm.

Collagen type I in late stages of OA

Collagen type I protein composed of two α1 (I) chains and one α2 (I) chain is produced by fibroblasts as a key component of the extracellular matrix of skin, tendon and ligaments (Holbrook and Smith, 1993; von der Mark, 1999) whereas physiological articular cartilage reveals only collagen type II. In contrast, collagen type I protein has been detected at the light microscopic level in osteoarthritic cartilage with the help of immunohistochemistry (Gay et al., 1976; Adam et al., 1984; Nerlich et al., 1993). A recent investigation of consecutive stages of OA revealed an increased amount of collagen type I mRNA in advanced disease stages (Miosge et al., 2004). Ultrastructurally, we also detected collagen type I mRNA predominantly in the cytoplasm of fibroblast-like chondrocytes of the deep zones of late stage osteoarthritic cartilage (Miosge et al., 1998). Furthermore, Goldwasser et al. (1982) detected collagen type I protein in tissue extracts obtained from fibrocartilaginous tissue. Later disease stages revealed collagen type I mRNA in fibroblast-like chondrocytes throughout all zones of the tissue (Fig. 5A). The strongest reaction was seen in the deep intermediate zone (Miosge et al., 2004). These findings support the notion that degenerative cartilage shows a switch in the collagen type from collagen type II to collagen type I with higher levels of collagen type I in late stages of OA as compared with healthy normal cartilage (Lipiello et al., 1977; Nerlich et al., 1993). The increased synthesis rate of collagen type I especially in later stages of OA, which cannot fulfill the physiological functions of collagen type II in articular cartilage, is an important factor in the disease process (Gay et al., 1976; Miosge et al., 2004).

Collagen type II in late stages of OA

Collagen type II is composed of three identical α1 (II) chains and belongs to the family of the fibrillar collagens as does collagen type I. It is the major collagen type of articular hyaline cartilage and acts in concert with other collagens (Mayne, 1989; von der Mark, 1999)

such as collagen type IX and XI and matrix proteoglycans such as aggrecan (Doege, 1999), decorin (Voss et al., 1986; Miosge et al., 1994) or biglycan (Bianco et al., 1990; Miosge et al., 1994). The collagen network, especially collagen type II, provides the tensile strength and stiffness of articular cartilage (Frenkel and Di Cesare, 1999). Immunohistochemistry revealed collagen type II protein in the interterritorial matrix of osteoarthritic cartilage (Gay et al., 1976; Nerlich et al., 1993; Miosge et al., 2004). Collagen type II mRNA produced by chondrocytes has been detected in vivo at the light microscopic level in normal human articular cartilage as well as in osteoarthritic human articular cartilage (Aigner et al., 1993, 1995, 1997; Lui et al., 1995; Poole, 1999). In early and intermediate stages of OA an enhanced expression of collagen type II was detected especially in the middle layers of osteoarthritic cartilage by in-situ hybridization at the light microscopic level (Aigner et al., 1992) which might be seen as an effort on the part of the cartilage tissue to restore the damaged extracellular matrix. In these stages of the disease, collagen type II mRNA was produced by normal chondrocytes mainly arranged in clusters which were found in the upper intermediate zone (Fig. 5B). In contrast, in late stage OA, collagen type II mRNA was located predominantly in the intermediate zone (Miosge et al., 2004). However, with the progression of the disease, less collagen type II, but increasing amounts of collagen type I were produced (Miosge et al., 2004). Furthermore, collagen type II mRNA expression was not detectable in the fibroblast-like chondrocytes of late stage osteoarthritic cartilage (Miosge et al., 1998). Therefore, the fibroblast-like chondrocytes do not contribute to a restoration of the normal collagen type found in healthy cartilage during the regeneration efforts of late disease stages.

Conclusions

In recent years much progress has been made in the understanding of the disease process of OA in human articular cartilage, but still important details of changes in the proteoglycan and collagen metabolism remain to be elucidated. Since multifactorial conditions with a complex pathogenesis and no simple linear progression lead to OA, a clear histological delineation of degenerative and regenerative changes is very difficult (Ostergaard et al., 1999). The osteoarthritic cartilage tissue which we investigated revealed signs of degeneration as well as signs of regeneration. A main obstacle in OA research lies in the fact that generalized hypotheses are made on the basis of results obtained from differing stages of OA derived from differing zones of tissue. We investigated human knee joint cartilage samples from patients with OA and classified them histopathologically according to the well established grading system developed by Collins and McElligott (1960) and made every effort to be aware of the histological zones from which the tissue sample was taken.

In summary, at the ultrastructural level, three major phenotypes of chondrocytes were identified. These are normal chondrocytes with a reduced synthesis of collagen type II, the fibroblast-like chondrocytes with a generally enhanced synthesis rate of matrix components and degenerative chondrocytes which undergo degradation. Especially the fibroblast-like chondrocytes from the deeper zones of articular cartilage predominantly occurring in the late stages of OA seem to have an enhanced synthesis rate for collagen type I, decorin, biglycan and perlecan (Miosge et al., 1998, 2004; Bock et al., 2001; Tesche and Miosge, 2004). The changes detected in the cell-matrix-interactions suggest a pivotal role of the fibroblast-like chondrocytes in the progress of this disease. As the amounts of aggrecan, the major proteoglycan and collagen type II, the major collagen type of articular cartilage, decrease in the osteoarthritic process, the increased synthesis rate for decorin and biglycan (Bock et al., 2001), collagen type I (Miosge et al., 1998, 2004) and perlecan (Tesche and Miosge, 2004) produced by the fibroblast-like chondrocytes might be seen as an attempt on the part of the osteoarthritic cartilage tissue to compensate for the general loss of matrix molecules and to stabilize and restore the remaining extracellular matrix in late stages of OA. Nevertheless, these regeneration efforts cannot delay the progress of cartilage degeneration. The physical and mechanical properties rely on the specific chemical composition and metabolic activity of the cartilage tissue, i.e. the extracellular matrix and the chondrocytes embedded in it, and it seems that the regenerated extracellular matrix with a different composition from the physiological cartilage matrix cannot compensate for the overall loss of aggrecan and collagen type II. From this point of departure, one attempt in the future might be to influence the production of matrix molecules by the manipulation especially of the fibroblast-like chondrocytes towards a matrix containing more collagen type II and aggrecan. This would contribute to the regeneration of the articular surface and establish the basis for a cell biological therapy in late stages of OA.

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References

Adam M., Novotna J. and Deyl Z. (1984). Changes in collagen metabolism-another look at osteoarthrosis. Acta Biol. Hung. 35, 181-187.

Aigner T. and McKenna L. (2002). Molecular pathology and pathobiology of osteoarthritic cartilage. Cell Mol. Life Sci. 59, 5-18.

Aigner T., Stoss H., Weseloh G., Zeiler G. and van der Mark K. (1992).

Activation of collagen type II expression in osteoarthritic and rheumatoid cartilage. Virchows Arch. (B) 62, 337-345.

Aigner T., Bertling W., Stoss H., Weseloh G. and von der Mark K. (1993). Independent expression of fibril forming collagens I, II and III in chondrocytes of human osteoarthritic cartilage. J. Clin. Invest. 91, 829-837.

Aigner T., Dietz U., Stoss H. and von der Mark K. (1995). Differential expression of collagen types I, II, III and X in human osteophytes. Lab. Invest. 73, 236-243.

Aigner T., Vornehm S.I., Zeiler G., Dudhia K., von der Mark K. and Bayliss M.T. (1997). Suppression of cartilage matrix gene expression in upper zone chondrocytes of osteoarthritic cartilage. Arthritis Rheum. 40, 562-569.

Arikawa-Hirasawa E., Watanabe H., Takami H., Hassell J.R. and Yamada Y. (1999). Perlecan is essential for cartilage and cephalic development. Nat. Genet. 23, 354-358.

Arikawa-Hirasawa E., Le A.H., Nishino I., Nonaka I., Ho N.C., Francoma C.A., Govindraj P., Hassell J.R., Devaney J.M., Spranger J., Stevenson R., Iannaccone S., Dalakas M.C. and Yamada Y. (2002). Structural and functional mutations of the perlecan gene cause Schwartz-Jampel syndrome, with myotonic myopathy and chondrodysplasia. Am. J. Hum. Genet. 70, 1368-1375.

Arikawa-Hirasawa E., Wilcox W.R. and Yamada Y. (2001). Dyssegmental dysplasia, silverman-handmaker type: Unexpected role of perlecan in cartilage development. Semin. Med. Genet. 106, 254-257.

Aszodi A., Bateman J.F., Gustafsson E., Boot-Handford R. and Fässler R. (2000). Mammalian skeletogenesis and extracellular matrix: what can we learn from knockout mice? Cell Struct. Funct. 25, 73-84.

Bianco P., Fisher L.W., Young M.F., Termine J.D. and Gehron R.P. (1990). Expression and localization of two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. J. Histochem. Cytochem. 38, 1549-1563.

Bock H.C., Michaeli P., Bode C., Schultz W., Kresse H., Herken R. and Miosge N. (2001). The small proteoglycans decorin and biglycan in human articular cartilage of late-stage osteoarthritis. Osteoarthritis Cartilage 9, 654-663.

Brown J.C., Sasaki T., Gohring W., Yamada Y. and Timpl R. (1997). The C-terminal domain V of perlecan promotes beta1 integrin-mediated cell adhesion, binds heparin, nidogen and fibulin-2 and can be modified by glycosaminoglycans. Eur. J. Biochem. 250, 39-46.

Buckwalter J.A. and Mankin H.J. (1998). Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr. Course Lect. 47, 487-504.

Calandruccio R.A. and Gilmers W.S. (1962). Proliferation, regeneration and repair of articular cartilage of immature animals. J. Bone Joint Surg. (Am) 44A, 431-455.

Collins D.H. and McElligott T.F. (1960). Sulphate (35SO4) uptake by chondrocytes in relation to histological changes in osteoarthritic human articular cartilage. Ann. Rheum. Dis. 19, 318-330.

Costell M., Gustafsson E., Aszodi A., Morgelin M., Bloch W., Hunziker E., Addicks K., Timpl R. and Fassler R. (1999). Perlecan maintains the integrity of cartilage and some basement membranes. J. Cell Biol. 147, 1109-1122.

Cs-Szabo G., Melching L.I., Roughley P.J. and Glant T.T. (1997). Changes in messenger RNA and protein levels of proteoglycans and link protein in human osteoarthritic cartilage samples. Arthritis Rheum. 40, 1037-1045.

Danielson K.G., Baribault H., Holmes D.F., Graham H., Kadler K.E. and Iozzo R.V. (1997). Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J. Cell Biol 136, 729-743.

Doege K.J. (1999). Aggrecan. In: Guidebook to the extracellular matrix, anchor and adhesion proteins. Kreis T. and Vale R. (eds). Oxford University Press. Oxford. pp 359-361.

Dourado G.S., Adams M.E., Matyas J.R. and Huang D. (1996). Expression of biglycan, decorin and fibromodulin in the hypertrophic phase of experimental osteoarthritis. Osteoarthritis Cartilage 4, 187-196.

Eyre D. (2002). Collagen of articular cartilage. Arthritis Res. 4, 30-45.

Fisher L.W. (1993) Biglycan. In: Guidebook to the extracellular matrix, anchor and adhesion proteins. Kreis T. and Vale R. (eds). Oxford University Press. Oxford. p 21.

Fisher L.W., Termine J.D. and Young M.F. (1989). Deduced protein sequence of bone small proteoglycan I (biglycan) shows homology with proteoglycan II (decorin) and several non- connective tissue proteins in a variety of species. J. Biol. Chem. 264, 4571-4576.

French M.M., Smith S.E., Akanabi K., Sanford T., Hecht J., Farach-Carson M.C. and Carson D.D. (1999). Expression of the heparan sulfate proteoglycan, perlecan, during mouse embryogenesis and perlecan chondrogenic activity in vitro. J. Cell Biol. 145, 1103-1115.

Frenkel S.R. and Di Cesare P.E. (1999). Degradation and repair of articular cartilage. Front Biosci. 4, D671-865.

Fukui N., Purple C.R. and Sandell L.J. (2001). Cell biology of osteoarthritis: the chondrocyte's response to injury. Curr. Rheumatol. Rep. 3, 496-505.

Gallagher J.T. (1989). The extended family of proteoglycans: social residents of the pericellular zone. Curr. Opin. Cell Biol. 1, 1201-1218.

Gardner D.L. (1994). Problems and paradigms in joint pathology. J. Anat. 184, 465-476.

Garnero P. and Delmas P.D. (2003) Biomarkers in osteoarthritis. Curr. Opin. Rheumatol. 15, 641-646.

Gay S., Müller P.K., Lemmen C., Remberger K., Matzen K. and Kuhn K. (1976). Immunohistological study on collagen cartilage-bone metamorphosis and degenerative osteoarthrosis. Klin. Wochenschr. 54, 969-976.

Goldwasser M., Astley T., van der Rest M. and Glorieux F.H. (1982). Analysis of the type of collagen present in osteoarthritic human cartilage. Clin. Orthop. 167, 296-302.

Hansen U. and Bruckner P. (2003). Macromolecular specificity of collagen fibrillogenesis: fibrils of collagens I and XI contain a heterotypic alloyed core and a collagen I sheath. J. Biol. Chem. 278, 37352-37359.

Hardingham T.E. and Fosang A.J. (1992). Proteoglycans: many forms and many functions. FASEB J. 6, 861-870.

Hassell J., Yamada Y. and Arikawa-Hirasawa E. (2002). Role of perlecan in skeletal development and diseases. Glycoconj. J. 19, 263-267.

Haq I., Murphy E. and Dacre J. (2003). Osteoarthritis. Postgrad. Med. J. 79, 377-383.

Heinegard D., Saxne T. and Lorenzo P. (1999). Noncollagenous proteins: glycoproteins and related proteins. In: Dynamics of bone and cartilage metabolism. Seibel M.J., Robins S.P. and Bilezikian J.P. (eds). Academic Press. pp 59-69.

Holbrook K.A. and Smith L.T. (2002). Morphology of connective tissue: Structure of the skin and tendon. In: Connective tissue and its

heritable disorders. Royce P.M. and Steinman B. (eds). Wiley-Liss. New York. pp 51-71.

Hopf M., Göhring W., Kohfeldt E., Yamada Y. and Timpl R. (1999). Recombinant domain IV of perlecan binds to nidogens, laminin, nidogen complex, fibronectin, fibulin-2 and heparin. Eur. J. Biochem. 259, 917-925.

Hunziker E.B. (1992). Articular cartilage structure in humans and experimental animals. In: Articular cartilage and osteoarthritis. Kuettner K.E., Schleyerbach R., Peyrou J.G. and Hascall V.C. (eds). Raven Press. New York. pp 183-199.

Iozzo R.V. (1998). Matrix proteoglycans: from molecular design to cellular function. Annu. Rev. Biochem. 67, 609-652.

Iozzo R.V. (1999). The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins. J. Biol. Chem. 274, 18843-18846.

Knudson C.B. and Knudson W. (2001). Cartilage proteoglycans. Semin. Cell Dev. Biol. 12, 69-78.

Kouri J.B., Jimenez S.A., Quintero M. and Chico A. (1996). Ultrastructural study of chondrocytes from fibrillated and non-fibrillated human osteoarthritic cartilage. Osteoarthritis Cartilage 4, 111-125.

Kresse H., Hausser H. and Schönherr E. (1993). Small proteoglycans. Experientia 49, 403-415.

Kuettner K.E. (1992). Biochemistry of articular cartilage in health and disease. Clin. Biochem. 25, 155-163.

Lipiello L., Hall D. and Mankin H.J. (1977). Collagen synthesis in normal and osteoarthritic human cartilage. J. Clin. Invest. 59, 593-600.

Lui V.C., Ng L.J., Nicholls J., Tam P.P. and Cheah K.S. (1995). Tissue-specific and differential expression of alternatively spliced alpha 1 (II) collagen mRNAs in early human embryos. Dev. Dyn. 203, 198-211.

Martel-Pelletier J. (1999). Pathophysiology of osteoarthritis. Osteoarthritis Cartilage 6, 371-783.

Mayne R. (1989). Cartilage collagens. Arthritis Rheum. 32, 241-246.

Mayne R. and Brewton R.G. (1993). New members of the collagen superfamily. Curr. Opin. Cell Biol. 5, 883-890.

Melrose J., Smith S., Knox S. and Whitelock J. (2002). Perlecan, the multidomain HS-proteoglycan of basement membranes, is a prominent pericellular component of ovine hypertrophic vertebral growth plate and cartilaginous endplate chondrocytes. Histochem. Cell Biol. 118, 269-280.

Miosge N., Flachsbart K., Goetz W., Schultz W., Kresse H. and Herken R. (1994). Light and electron microscopical immunohistochemical localization of the small proteoglycan core proteins decorin and biglycan in human knee joint cartilage. Histochem. J. 26, 939-945.

Miosge N., Waletzko K., Bode C., Quondamatteo F., Schultz W. and Herken R. (1998). Light and electron microscopic in-situ hybridization of collagen type I and type II mRNA in the fibrocartilaginous tissue of late-stage osteoarthritis. Osteoarthritis Cartilage 6, 278-285.

Miosge N., Hartmann M., Maelicke C. and Herken R. (2004). Expression of collagen type I and type II in consecutive stages of human osteoarthritis. Histochem. Cell Biol. 122, 229-236.

Morris N.Pl., Keene D.R. and Horton W.A. (2002). Morphology and chemical composition of connective tissue: Cartilage. In: Connective tissue and its heritable disorders. Molecular, genetic, and medical aspects. Royce P.M. and Steinmann B. (eds). Wiley -Liss. New York. pp 41-65.

Muir H. (1995). The chondrocyte, architect of cartilage. Biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. Bioessays 17, 1039-1048.

Nerlich A.G., Wiest I. and von der Mark K. (1993). Immunohistochemical analysis of interstitial collagens in cartilage of different stages of osteoarthrosis. Virchows Arch. (B) 63, 249-255.

Ostergaard K., Andersen C.B., Petersen J., Bendtzen K. and Salter D.M. (1999). Validity of histopathological grading of articular cartilage from osteoarthritic knee joints. Ann. Rheum. Dis. 58, 208-213.

Poole A.R. (1999). An introduction to the pathophysiology of osteoarthritis. Front Biosci. 4, D662-670.

Poole A.R., Rizkalla G., Ionescu M., Reiner A., Brooks E., Rorabeck C., Bourne R. and Bogoch E. (1993). Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and reorganization. Agents Actions Suppl. 39, 3-13.

Poole A.R., Rosenberg L.C., Reiner A., Ionescu M., Bogoch E. and Roughley P. (1996). Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. J. Orthop. Res. 14, 681-689.

Poole C.A. (1997) Articular cartilage chondrons: form, function and failure. J. Anat. 191, 1-13.

Reginster J.Y. (2002). The prevalence and burden of arthritis. Rheumatology 41 (Suppl. 1), 3-6.

Sandell L.J. and Aigner T. (2001). Articular cartilage and changes in arthritis. An Introduction: Cell biology of osteoarthritis. Arthritis Res. 3, 107-113.

Schönherr E., Witsch-Prehm P., Harrach B., Robenek H., Rauterberg J. and Kresse H. (1995). Interaction of biglycan with type I collagen. J. Biol. Chem. 270, 2776-2783.

SundarRaj N., Fite D., Ledbetter S., Chakravarti S. and Hassell J.R. (1995). Perlecan is a component of cartilage matrix and promotes chondrocyte attachment. J. Cell Sci. 108, 2663-2672.

Tesche F. and Miosge N. (2004). Perlecan in late stages of osteoarthritis in the human knee joint. Osteoarthritis Cartilage 12, 852-862.

Vogel K.G., Paulsson M. and Heinegard D. (1984). Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. Biochem. J. 223, 587-597.

von der Mark K. (1999). Structure, biosynthesis, and gene regulation of collagens in cartilage and bone. In: Dynamics of bone and cartilage metabolism. Seibel M.J., Robins S.P. and Bilezikian J.P. (eds). Academic Press. pp 3-29.

von der Mark K. and Gluckert K. (1990). Biochemical and molecular biological aspects for the early detection of human arthrosis. Orthopaede 19, 2-15.

Voss B., Glössl J., Cully Z. and Kresse H. (1986). Immunocytochemical investigation on the distribution of small chondroitin sulfate-dermatan sulfate proteoglycan in the human. J. Histochem. Cytochem. 34, 1013-1019.

Vynios D.H., Papageorgakopoulou N., Sazakli H. and Tsiganos C.P. (2001). The interactions of cartilage proteoglycans with collagens are determined by their structure. Biochimie 83, 899-906.

Witsch-Prehm P., Miehlke R. and Kresse H. (1992). Presence of small proteoglycan fragments in normal and arthritic human cartilage. Arthritis Rheum. 35, 1042-1052.

Woolf A.D. and Pfleger B. (2003). Burden of major musculoskeletal conditions. Bull. World Health Organ. 81, 646-656.

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