Osteoarthritis and Cartilage (1999) **7,** 389–391

Article No. joca.1998.0220, available online at http://www.idealibrary.com on IDEAL®
1063–4584/99/040389+03 $12.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Mechanisms of chondrocyte apoptosis

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Summary

This study addresses the occurrence and significance of chondrocyte apoptosis in the pathogenesis of cartilage destruction. Chondrocyte apoptosis can be induced *in vitro* by nitric oxide donors, but not by pro-inflammatory cytokines, such as IL-1 or TNF. A subset of chondrocytes, located in the superficial zone of cartilage, expresses the Fas antigen. Activation of the Fas receptor triggers apoptosis in these cells. In human and experimental osteoarthritis (OA) induced in rabbits by anterior cruciate ligament transection increased numbers of chondrocytes were undergoing apoptosis. Cartilage areas that contained apoptotic cells showed proteoglycan depletion and the number of apoptotic cells was significantly correlated with the levels of nitric oxide production and with the severity of OA. Articular cartilage is not vascularized and does not contain mononuclear phagocytes. There is, thus, no apparent mechanism for the clearance of apoptotic bodies. Chondrocyte-derived apoptotic bodies produced pyrophosphate and precipitated calcium. These results suggest that chondrocyte-derived apoptotic bodies express functional properties that may contribute to the pathologic cartilage degradation and calcification. Inhibition of chondrocyte apoptosis may be of therapeutic value after cartilage injury and in arthritis.

Key words: Apoptosis, Chondrocytes, Nitric oxide.

Introduction

Chondrocyte death may be of pathogenetic significance during cartilage repair in response to traumatic injury, in the development of osteoarthritis and possibly also in the inflammatory arthropathies. Two features of cartilage in skeletally mature individuals are important in considering the consequences of chondrocyte death. Mononuclear phagocytes are not present in cartilage. This absence of phagocytic cells implies that remnants of dead cells will remain in the cartilage matrix and potentially affect matrix structure and the function of viable chondrocytes. Articular cartilage also does not contain mesenchymal stem cells and, as it is not vascularized, precursor cells can not be recruited. Chondrocytes are surrounded by a pericellular matrix and individual chondrocytes are separated by territorial and interterritorial matrix. This suggests that areas where cells have died will not or will only slowly be repopulated and this can impair the ability of cartilage to maintain and repair extracellular matrix. The fact that cartilage defects that exceed a certain size will not be repaired and the correlation between reduced cartilage cellularity and increased prevalence of osteoarthritic changes support the pathogenetic significance of chondrocyte death.

Understanding the occurrence of cell death, the forms of cell death and the regulation of chondrocyte death and survival are thus important in advancing concepts in cartilage biology and pathology.

Regulators of chondrocyte survival

Chondrocytes produce survival-promoting factors that act as autocrine or paracrine regulators of cell survival.[1] Most cells require intercellular communication for survival. Loss of cell–matrix interactions has been shown in several cell systems to initiate apoptosis.[2] Chondrocytes are a cell type which is dependent on adherence for survival. Adhesion to tissue culture surfaces promotes chondrocyte survival. If this adhesion is prevented such as in methylmethacrylate coated plates, cells will adhere to each other and survive. If under these conditions cell–cell adhesion is prevented, cells will undergo apoptosis. This suggests that cell adhesion independent of the adhesive substrate provides a survival signal (Kühn *et al.*, in preparation). Additional survival signals appear to be provided by specific components of cartilage extracellular matrix. When chondrocytes are challenged with inducers of apoptosis, a greater percentage of cells undergo apoptosis in monolayer culture as compared to cartilage slices.

Indirect evidence suggests that type II collagen influences chondrocyte survival. In transgenic mice homozygous for targeted inactivation of the collagen II gene collagen fibrils are not detectable in the cartilage. In the absence of collagen II, the chondrocytes had condensed nuclei and fragmentation of nuclear DNA.[3]

Inducers of apoptosis

FAS

A subpopulation of articular chondrocytes expresses the Fas antigen which upon cross-linking by antibodies induces apoptosis. Fas antigen expression is predominantly a feature of chondrocytes in the superficial and upper midzone of articular cartilage.[4] It is presently not known under which conditions chondrocytes express Fas ligand. The only known source of Fas ligand in the joint are inflammatory cells in the synovial tissue and fluid.

Table I
Regulators of chondrocyte apoptosis

Inducers of apoptosis	Survival factors
NO donors	Growth factors
Staurosporine	Collagen
Fas ligand	Hyaluronan
Hypertrophy	Adhesion
Lack of ECM*	
Lack of adhesion	

*Extracellular matrix.

NITRIC OXIDE

The role of nitric oxide (NO) in regulating cell survival and death is cell type-specific. Induction of apoptosis by exogenous NO derived from NO donors or endogenous cytokine induced NO has been demonstrated in macrophages, thymocytes, osteoblasts and pancreatic islet cells. In primary rat hepatocytes, pretreatment with the NO donor SNAP was associated with cytoprotection from TNF and actinomycin D-induced apoptosis.[5]

Human articular chondrocytes stimulated with cytokines such as IL-1 or TNF produce high levels of NO. NO generated from exogenous donors induced chondrocyte apoptosis. In contrast, oxygen radicals generated by hypoxanthine/xanthine oxidase caused necrosis but did not induce chondrocyte apoptosis. To analyze whether endogenously generated NO induces apoptosis, chondrocytes were stimulated with IL-1, but there was no evidence for apoptotic changes. IL-1-stimulated chondrocytes are known to produce oxygen radicals that react with NO to form products that can induce cell death in other systems. In the presence of oxygen radical scavengers, IL-1 was able to induce apoptosis which was inhibited by the NO synthase inhibitor N-monomethyl L-arginine.[6]

In bovine chondrocytes a mixture of IL-1 and TNF induced NO release but did not alter cell viability. However, there was evidence of NO-dependent oxidative responses and the formation of peroxynitrite. Chondrocyte viability was reduced by H_2O_2. The sensitivity to oxygen radical mediated cell death was increased by IL-l/TNF. The enhanced sensitivity was completely reversed when cells were incubated with an NO synthase inhibitor. Cell death under these conditions was caused by compromising the ability of cells to detoxify extracellular oxidants. These findings suggest that endogenous NO mediates cytokine-dependent susceptibility to oxidant injury.[7]

An additional mechanism by which NO may contribute to cell death is through interference with survival signals from extracellular matrix. NO inhibits chondrocyte attachment to fibronectin.[8] Adherence of chondrocytes to fibronectin enhances proteoglycan synthesis. The stimulatory effects of fibroblast growth factor and insulin growth factor (IGF-1) on proteoglycan synthesis were dependent on adherence to fibronectin. Although its role in chondrocyte survival has not been examined, IGF-1 is known to inhibit apoptosis in other cell types. Through interference with chondrocyte-matrix interactions NO may compromise cell survival.[9] Factors regulating chondrocyte survival and apoptosis are summarized in Table I.

Chondrocyte apoptosis in human and experimentally induced osteoarthritis

Fibrillated cartilage from human OA joints contained apoptotic cells in the superficial and midzones.[10] In contrast, very low numbers of apoptotic cells were detected in normal cartilage and they were almost exclusively located in the surface layer. Additional areas with a high frequency of apoptotic cells in OA cartilage were clusters that contained proliferating chondrocytes. Electron microscopy of apoptotic chondrocytes in OA cartilage showed that the cytoplasmic membrane is irregular, with multiple blebs and the cell remnant is surrounded by membrane-enclosed units, probably representing apoptotic bodies. The pericellular matrix is absent and the cell remnants appear withdrawn from the extracellular matrix. Chondrocyte apoptosis and cartilage degradation are related. Areas that contained apoptotic cells showed a reduced intensity of proteoglycan staining. Grading of the degree of histological–histochemical changes in OA cartilage and analysis of apoptosis revealed significant correlations between OA grade and chondrocyte apoptosis.[11] In experimental osteoarthritis induced in rabbits by anterior cruciate ligament transection (ACLT) a close correlation was observed between the levels of chondrocyte apoptosis, NO production and the severity of extracellular matrix degradation.[11] In-situ staining demonstrated apoptotic cells in superficial and middle zones of ACLT cartilage. A high number of apoptotic cells was present at the pannus-cartilage junction. Increased frequencies of apoptotic chondrocytes were also observed in the tidemark region. In control cartilage, the superficial zone contained a small number of cells in apoptosis. These changes were observed within a short time interval of four weeks after ligament transection. Possible implications for the pathogenesis of osteoarthritis are that NO production may lead to chondrocyte apoptosis and that both events contribute to the pathogenesis of cartilage degradation. Inhibitors of NO synthesis and chondrocyte apoptosis thus may be of therapeutic value after cartilage injury and in osteoarthritis.

Role of apoptotic bodies in cartilage

Electron microscopy of apoptotic chondrocytes suggested a similarity between apoptotic bodies and matrix vesicles. In the late 1960s, Bonucci and Anderson identified matrix vesicles in epiphyseal growth plates by electron microscopy.[12,13] Matrix vesicles were described as cell-derived, membrane enclosed units which are associated with hydroxyapatite deposition. It was also suggested that matrix vesicles may derive from budding or disintegrating cells in the upper portion of the epiphyseal plate. Numerous studies have demonstrated the presence of matrix vesicles in the areas of epiphyseal cartilage that eventually will calcify. The biochemical composition of matrix vesicles has been characterized. Alkaline phosphatase activity is abundant in matrix vesicles and is used as a marker for their identification. Matrix vesicles also contain pyrophosphate-generating nucleoside triphosphate pyrophosphohydrolase (NTPPH) activities. Matrix vesicles can be isolated from collagenase-digested articular cartilage and separated from chondrocytes by differential centrifugation and used for functional, biochemical and ultrastructural studies. Functionally, isolated matrix vesicles incorporate ^{45}Ca, hydrolyze ATP or other nucleoside triphosphates, and form orthophosphate. Matrix vesicles are thus able to raise the

Table II
Significance of chondrocyte apoptosis

- Chondrocyte apoptosis occurs in human and experimentally induced OA
- Apoptosis is positively correlated with the severity of cartilage destruction
- Apoptotic bodies produce pyrophosphate and precipitate calcium
- Extracellular matrix degradation and chondrocyte apoptosis are linked events in the pathogenesis of osteoarthritis

Ca×P product above solubility and promote precipitation of crystalline calcium phosphates. Matrix vesicles are found in articular cartilage as well as growth plate cartilage. On the basis of this information it was possible that expression of matrix vesicle-like activities in apoptotic bodies may contribute to the abnormal calcification of articular cartilage. Electron microscopy showed that apoptotic bodies derived from primary cultured human chondrocytes are membrane-enclosed structures of varying size. Chondrocyte apoptosis within articular cartilage was associated with several unique changes. The pericellular matrix which normally surrounds the chondrocytes and provides a transition to the interterritorial matrix is degraded and within the territorial matrix adjacent to the chondrocyte lacunae, accumulation of apoptotic bodies can be observed.[14] This indicates that in association with chondrocyte apoptosis, there is pericellular matrix degradation and this allows apoptotic bodies to enter the interterritorial space. The chondrocyte-derived apoptotic bodies produced pyrophosphate and contained NTPPH activity. One of the most important roles of matrix vesicles in epiphyseal cartilage is the formation of calcium-containing crystals. Apoptotic bodies derived from chondrocytes treated with the NO donor SNP or anti-Fas can precipitate calcium.[14] These observations suggest a role of chondrocyte apoptosis in the pathogenesis of osteoarthritis. Apoptosis of chondrocytes in articular cartilage is associated with the destruction of the pericellular matrix and accumulation of apoptotic bodies in chondrocyte lacunae and in the interterritorial space. Apoptotic bodies are likely to remain at these sites since articular cartilage does not contain phagocytic cells.

Summary and conclusions

Chondrocyte apoptosis as a feature of OA cartilage may contribute to OA pathogenesis (Table II). Chondrocyte proliferation occurs in osteoarthritis and clusters of proliferating cells contain cells in apoptosis. Chondrocyte-derived apoptotic bodies may share functional properties with matrix vesicles and contain enzymatic activities that are involved in the deposition of calcium. Cartilage does not contain mononuclear phagocytes and apoptotic bodies are thus likely to exert pathogenic effects on this tissue. Chondrocyte apoptosis in osteoarthritis may be the consequence of aberrant hypertrophic chondrocyte differentiation or induced by extracellular stimuli such as Fas ligand and other cytokines. The degradation or absence of extracellular matrix may predispose chondrocytes to undergo apoptosis.

This information characterizes cartilage matrix degradation and chondrocyte apoptosis as phenomena which are mechanistically related. As the relationship between these phenomena is more clearly defined, new concepts and molecular targets for the treatment of cartilage injury and osteoarthritis are likely to emerge.

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