International Journal of
Rheumatic Diseases

International Journal of Rheumatic Diseases 2011; **14**: 159–166

REVIEW ARTICLE

Chondrocyte apoptosis: a cause or consequence of osteoarthritis?

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Abstract

Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation and changes in the subchondral bone. Over the last two decades, there has been increasing evidence showing association between cartilage degradation and chondrocyte death, and different types of cell death in cartilage have been reported, including apoptosis and chondroptosis as well as necrosis, but which of these types of cell death predominate in OA is debatable. There are also some methodological difficulties in detecting the specific form of cell death in articular cartilage. Current 'gold standard' for detecting chondrocyte death is electron microscopy which suggests that the morphological changes of chondrocytes in OA cartilage are attributed to apoptosis and/or chondroptosis. However, the current literature appears to suggest that classic apoptosis plays an important role in OA; but whether chondrocyte apoptosis is a cause or a result of cartilage degeneration in OA is hotly contested. Studies of suitable animal models, especially longitudinal studies, are needed to address the cause-and-effect relationship.

Key words: articular cartilage degradation, chondrocyte apoptosis, osteoarthritis.

INTRODUCTION

Osteoarthritis (OA) is a common degenerative joint disease characterized by cartilage loss, subchondral bone changes, low-grade synovitis and other joint tissue alterations. For a summary of changes in the major joint tissues and other pathological features of OA see Table 1. OA largely affects weight-bearing joints like knee and hip[1] and is known to be a leading cause of disability among elderly populations worldwide. Patients with OA usually experience joint pain, stiffness, tenderness, mobility difficulties and cracking noise with joint movement.[2–4] The pathogenesis of OA is complex and not fully understood. The causes of OA are not known but over the years a number of important risk factors for developing OA have been identified. These risk factors include age, genetic predisposition, obesity, anatomical abnormalities, excessive load and joint injury.

Bone remodelling and loss/degeneration of cartilage are hallmarks of OA and historically bone had been the major focus of research. However, during the Bone and Joint Decade, studies of cartilage biology have also provided some insight into cartilage matrix biology and further impetus for research into cartilage degradation and pathogenesis of OA.[5] Recent studies suggest that chondrocyte (the only cell present in cartilage) death is a key player in cartilage degeneration.[6–8] Chondrocyte death by apoptosis,[6,7,9–12] necrosis,[13] chondroptosis,[14] or combination of these processes[15,16] has been implicated in the pathogenesis of OA. However, it remains to be established what role these process(es) play in cartilage damage and development of OA, and an important current research question is whether chondrocyte death is a cause or consequence of OA? Either way, understanding mechanisms involved and the precise role of cell death in cartilage is crucial in our search for an effective

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Table 1 Summary of pathological features of OA

Joint tissues	Tissue changes	References
Cartilage	Fibrillation, degeneration and fragmentation	2,3,8,10,11,21,24,34,48–56
	Hypocellularity	8,21,48,51–54,56
	Empty and/or debris in lacuna	7,8,19
	Chondrocyte clustering/death	9,19,22,24,34,48,50,51,53,54
	Proteoglycan loss	3,8,10,21,24,55,56
	Collagen type II loss	8,9,19,22,49,51,53,56,57
	Cartilage ossification	19,27,56
Subchondral bone	Sclerosis	2,3,10,27,34,48,51–53,55,56,58,59
	Marginal osteophyte formation	2,3,21,27,49,51,53,58
	↑ BMD	48,52,59
	↑ Bone turnover	2,3,55,58,59
	Cyst formation	2,3,27,50,51
	Joint space narrowing	3
	Alteration of subchondral trabeculae architecture	2,34,52,53
Synovium	Capsular fibrosis	3,51,55,56
	Synovial hyperplasia	3,21,24,50,55,56,60
	Synovitis	2,3,21,56,60
	Production of MMPs and ROS	56
Synovial fluid	↑ MMPs	54,56
	↑ IL-1β	56
	↑ TNF-α	56
	↑ ROS	56,61
	↑ PGE	56
	↑ Aggrecan fragments	27,62
	Decreased viscosity	61

BMD, bone mineral density; MMP, matrix metalloproteinase; ROS, reactive oxygen species; IL-1β, interleukin-1 β; TNF-α, tumour necrosis factor α; PGE, prostaglandin E.

therapeutic agent for this common degenerative disease. This article will discuss the recent evidence on chondrocyte death in articular cartilage and its role in pathogenesis of OA.

Mechanisms of cell death

Cells usually die by one of the two processes, apoptosis or necrosis. Apoptosis, or programmed cell death, was first described by Kerr *et al.*[17] This term was used to describe a physiological cell death mechanism with distinctive morphological manifestations, which include nuclear fragmentation, chromatin condensation, membrane blebbing, cell shrinkage and presence of apoptotic bodies. Apoptosis plays an important role in normal physiological processes (e.g. endochondral ossification and cell turnover) as well as in pathology (e.g. autoimmunity and cancer). There are two classical pathways for apoptosis, namely, the death receptor pathway and mitochondrial pathway. Both possess different cascades of cystein proteases, or caspases, that cleave specifically at aspartate residue. In the death receptor pathway, the death receptors such as tumor necrosis factor (TNF) or Fas receptors are activated by specific death ligands, TNF-α and Fas ligand respectively. While in the mitochondrial pathway it is initiated by stimuli that change mitochondrial membrane permeability toward pro-apoptotic proteins.[18]

Necrosis, on the other hand, is a pathological form of cell death; it is a non-programmed, caspase- and energy-independent form of cell death.[18,19] Cells die by necrosis when there is tissue damage as a result of exposure to highly toxic substances or extreme physiological conditions.[20] The main difference between apoptotic and necrotic cells is that the latter is always accompanied by inflammatory reaction in response to accumulation of cytoplasmic contents in intercellular regions due to the loss of cell membrane integrity. In addition, necrotic cells also show other morphological changes, such as formation of cytoplasmic vacuoles, swelling of mitochondria and other organelles which eventually lead to total cell lysis.

Chondrocyte death and OA

Both, apoptotic and non-apoptotic,[6,10,11,14] forms of cell death have been reported in OA cartilage. Chondrocyte death in osteoarthritic cartilage is supported by the presence of large numbers of empty lacunae and hypocellularity[8,19] and correlated with mechanical injury, increased production of reactive oxygen species (ROS), disruption of extracellular matrix integrity and loss of production of growth factor by the cells.[20] Many studies have demonstrated significant correlations between increasing numbers of chondrocyte apoptosis and severity of OA in both *in vitro* and *in vivo* studies in animals[10,11,21–23] and in human.[6–9,12,24,25] These studies used a wide range of analysis, such as histology, terminal deoxynucleotidyl trasferase dUTP nick end labelling (TUNEL), expression of caspase-3, enzyme-linked immunosorbent assay (ELISA), anti-poly (ADP-ribose) polymerase (anti-PARP) p85 and fluorescence-activated cell sorter analysis (FACS) to demonstrate the relationship between apoptosis and OA. In several of these studies, electron microscopy was used to identify the ultrastructural changes of chondrocytes in osteoarthritic cartilage attributable to apoptosis, in order to confirm the increased level of chondrocyte apoptosis in OA cartilage.[6,7,9,12] If chondrocyte apoptosis is indeed a cause of OA, then future development and use of intra-articular pharmacological inhibitors of apoptosis would be an interesting new treatment option for patients with OA.

In a recent study Roach *et al.*[15] proposed a variant of classical apoptosis for cartilage cells known as chondroptosis. The authors noted in their study that some of the published micrographs of chondrocyte apoptosis[6,7,9,12] show morphological changes which deviate from established apoptotic features (Fig. 1). Co-localization between Golgi 58 K protein and caspase-2L in TUNEL-positive cells in human osteoarthritic cartilage support this contention that cell death in cartilage involves the process of chondroptosis rather than apoptosis.[26] Another study suggests that chondrocyte death in cartilage may also occur by a combination of apoptosis and autophagy (destruction mechanism in chondroptosis) depending on the stage and zone of degenerative cartilage.[16] Thus, although the concept of increased cell death in OA is generally accepted, the precise mechanism of cell death is yet to be established. Many of the studies in this area have shown increased chondrocyte dying by classic apoptosis in OA and highlight the important role chondrocyte apoptosis could play in cartilage damage and development of OA, but whether chondrocyte apoptosis is a cause or as a result of cartilage degeneration in OA remains to be answered.

Chondrocyte apoptosis is a cause of OA?

During the last decade many studies demonstrated independently that there is significant decrease in chondrocyte numbers in articular cartilage with aging,[27–31] while others reported moderate to strong

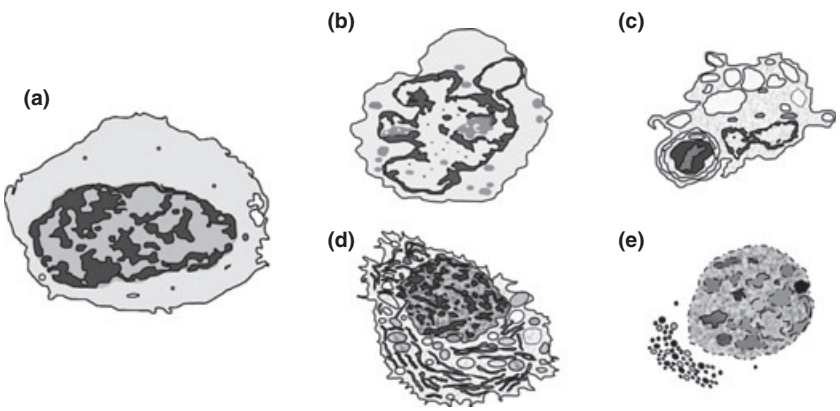

Figure 1 (a) A normal chondrocyte. Slightly round and large nucleus. Re-drawn after Kühn *et al.*[37] (b) Early stage of chondrocyte apoptosis with peripheral chromatin condensation and nuclear budding. (c) Late stage of chondrocyte apoptosis. Note the presence of vacuoles and an apoptotic body within the degenerative chondrocyte. (d) Early stage of chondroptosis. Note the patchy and unorganized chromatin condensation and increased numbers of Golgi apparatuses and endoplasmic reticulum in cytoplasm. (e) Late stage of chondroptosis. Note the presence of autophagic vacuoles in the degenerative chondrocyte and accumulation of vesicles in extracellular matrix. (b–e) are re-drawn after Roach *et al.*[15]

positive correlations between degree of cartilage damage and chondrocyte death by apoptosis.[6,9,11,24,32] Hashimoto *et al.*[6] specifically examined the strength of correlation between chondrocyte apoptosis and cartilage degeneration in human OA, and found that in some of the 40–60-year-old donors' cartilages there were unusually high numbers of apoptotic chondrocytes in macroscopically normal cartilage. These observations, together with data from epidemiological studies showing high prevalence of OA among elderly, led to the theory that chondrocyte apoptosis may be a possible cause of OA. More direct evidence implicating apoptosis in initiation of OA comes from studies of animal tissues. For example, Allen and co-workers[28] reported that the viable cell density in aged rabbit articular cartilage decreased by about 50–70% compared to skeletally mature cartilage. The older rabbits also had significantly higher expression of pro-apoptotic genes like Fas, Fas ligand (FasL), caspase-8, inducible nitric oxide synthase (iNOS) and p53. Moreover, mechanical compression on bovine articular cartilage explants had shown that chondrocyte apoptosis could be induced even at stresses lower than the threshold level of cartilage degradation and biochemical changes.[33] These studies using articular cartilage from animals imply that changes in chondrocyte number/death is an early process in OA and this deduction is also supported by studies of human osteoarthritic cartilage. For example, Bobinac *et al.*[34] has shown that in knee joints with histomorphologically normal subchondral bone and macroscopically normal cartilage, there were changes in the chondrocyte number, position and proteoglycan contents. Likewise, Aigner *et al.*[35] had found that relatively normal-looking human cartilage had severely altered gene expression in cartilage, including genes that related to programmed cell death. Our recent studies of cartilage from equine joints have shown that chondrocyte apoptosis is positively correlated with early stages of OA and severity of cartilage damage, suggesting that this process is intrinsically linked to cartilage damage and may be associated with the initiation of cartilage degradation in OA.[36] In addition, we have also demonstrated recently that equine joints which frequently develop OA are more susceptible to apoptosis induction using TNF than those joint that rarely develop the disease.[36] The results of this study not only suggest that apoptosis is important in the pathogenesis of OA, but also provide a possible explanation for the joint-specific nature of the disease.

There may be a number of possible mechanisms involved in chondrocyte apoptosis-mediated cartilage damage and development of OA. First, during aging chondrocytes may undergo phenotypic changes which make them more vulnerable to pro-apoptotic and other catabolic stimuli and also less responsive to anti-apoptotic and anabolic factors. As a result, small but increased numbers of chondrocytes die by apoptosis, leading to hypocellular cartilage. Reduced cellularity means that the chondrocytes are no longer able to maintain the vast extracellular matrix and therefore there is a net degradation and loss of cartilage in these joints, resulting in OA. More direct damage to cartilage may be caused by the apoptotic bodies, the end product of apoptosis. Cartilage is avascular, there are no phagocytic cells in cartilage, therefore apoptotic bodies in cartilage are not cleared quickly and accumulation of these bodies in pericellular or interterritorial matrices, especially in advanced OA, lead to cartilage matrix damage.[29] In addition, the apoptotic bodies may also produce alkaline phosphatase and induce precipitation of calcium, which results in abnormal calcification in the subchondral bone, and subsequent cartilage degradation.[29]

The studies discussed above support the theory that apoptosis may be an important cause of OA, but fall far short of providing any direct evidence of chondrocyte apoptosis leading to OA. To address the issue of causality, one needs to determine the temporal sequence between apoptosis and specific cartilage matrix changes in OA joints. This would require longitudinal studies of a suitable animal model and controls. Finally, to obtain 'proof of concept' that chondrocyte apoptosis can indeed cause OA, apoptosis needs to be induced in an animal free of disease, and cartilage damage/subchondral bone changes need to be monitored carefully from very early stages of OA.

Chondrocyte apoptosis is a consequence of OA?

The concept that chondrocyte apoptosis could be secondary to cartilage degradation is supported by the fact that cell–matrix interaction is vital for chondrocyte survivability. The phenomenon of 'anchorage dependence' states that cells need to attach to the extracellular matrix or to each other for survival, and therefore when the extracellular matrix is damaged by either mechanical load or inadequate synthesis and/or expression of extracellular matrix molecules, chondrocytes may undergo apoptosis and exacerbate existing cartilage matrix breakdown. One of the most convincing evidence for this theory comes from the study of Zemmyo *et al.*[21] who have shown that cartilage from

alpha-1 integrin knockout mice contains increased numbers of apoptotic chondrocytes and they develop OA-like lesions characterised by severe glycosaminoglycan (GAG) loss, synovial proliferation and presence of osteophytes.

Chondrocyte survivability is thought to be mediated by integrins,[37] α/β-heterodimeric receptors that connect the extracellular matrix components like collagen, laminin and fibronectin to various intracellular cytoskeletal proteins.[21,38] Loss of this adhesion may trigger chondrocytes to endure apoptosis. The above study by Zemmyo *et al.*[21] clearly demonstrated that integrin α1 knockout mice had higher percentage of apoptotic chondrocytes, hypocellularity, GAGs loss and, matrix metalloproteinase (MMP)-2 and MMP-3 expression compared to the wild-type mice. Similarly, another study by Yang *et al.*[39] found increased chondrocyte apoptosis and decreased expression of Bcl-2 protein in transgenic mice lacking type II collagen. These data are in agreement with our own recent studies which show that the extent of chondrocyte apoptosis is positively correlated with expression of fibronectin, one of the key extracellular matrix molecules involved in communication between the cartilage cells and surrounding matrix, and up-regulation of expression of which is associated with the severity of articular cartilage damage (Z. Zamli and M. Sharif, unpubl. data). Furthermore, as expression of fibronectin is known to occur early in the development of OA,[40,41] the positive association with apoptosis means that both expression of fibronectin and chondrocyte apoptosis are early events and could be involved in initiation of cartilage degradation in OA. Other cross-sectional studies looking at severity of cartilage damage and rate of apoptosis support these observations.[6,7,11,12] Taken together these studies suggest that decreased expression or availability of important matrix macromolecules in cartilage is sufficient to induce chondrocyte apoptosis and cause exacerbation of matrix damage.

Other evidence supporting the theory that apoptosis is a consequence of OA comes from studies of cartilage following mechanical loading/damage. Abnormal mechanical loading is a major risk factor for developing OA and vigorous cyclic loading of normal cartilage can cause collagen denaturation,[42] expel GAGs from articular cartilage,[43] and induce cell death,[44] possibly by apoptosis.[25] Loening *et al.*[33] reported that injurious mechanical loading on bovine cartilage explants induced chondrocyte apoptosis, which coincided with collagen degradation, tissue swelling and GAGs release, especially in the central region of loaded area in a dose-dependent manner. Moreover, the apoptotic chondrocyte can be detected when the loading stress is as low as 4.5 MPa and reached the optimum level at 24 h after loading.[33] Similarly, injurious mechanical loading at 14 MPa on human cartilage explants had been shown to induce chondrocyte apoptosis by TUNEL analysis, and which was confirmed by electron microscopy.[45] This study also reported a significant increase in GAGs release compared to a non-loading control and 50% reduction in apoptosis when mechanically damaged cartilage was cultured with z.VAD.fmk, a non-specific caspase inhibitor.[45] In another study using cartilage from fractured human tibia it was found that there were significantly higher numbers of TUNEL-positive chondrocytes relative to controls and that these TUNEL-positive cells were localized adjacent to OA lesions.[46] Collectively these findings suggest that abnormal mechanical stress on normal cartilage can lead to chondrocyte apoptosis, and degradation and loss of cartilage.

The precise mechanism of injury-induced chondrocyte apoptosis and cartilage loss remains to be worked out. However, it is likely that disruption in chondrocyte–matrix interaction is either due to direct injury to the cartilage, causing biochemical changes (e.g. increased MMPs messenger RNA expression) or loss of extracellular matrix components (e.g. denaturation of type II collagen, changes in fibronectin and other matrix protein expressions). Cyclic mechanical loading can expel GAGs from articular cartilage, and that the rate of expulsion increases rapidly when the cartilage surface is damaged (fissured) by mechanical overload.[43] This could result in loss of anchorage-survival signals from the surrounding matrix, and trigger the chondrocyte to undergo apoptosis. In addition, early cartilage fibrillation may also expose the chondrocyte to catabolic factors such as nitric oxide and cytokines secreted by synoviocytes as well as chondrocytes. These mediators are likely to induce further chondrocyte death by apoptosis and cause progression of cartilage damage.

Other studies suggest that presence of collagenases and other degradative enzymes in joints may predispose chondrocytes to apoptosis. For example, the results of experiments carried out by Lo and Kim[47] have shown that collagenase treatment of primary human chondrocyte induces chondrocyte apoptosis in a dose-dependent manner and the process can be inhibited by caspase inhibitors as well as insulin-like growth factor-1 (IGF-1). Later, a study by D'Lima *et al.*[23] confirmed that caspase inhibitors can reduce

severity of cartilage lesions in rabbit anterior cruciate ligament transaction-induced OA. These are very interesting and important studies and clearly support the notion that chondrocyte apoptosis is a consequence of OA. However, further studies are needed to determine whether apoptosis follows onset of OA.

CONCLUSION

The question of whether chondrocyte apoptosis is a cause or consequence of OA is at present finally balanced. As reviewed above, there are some convincing studies supporting both concepts, but direct evidence demonstrating a causal link between chondrocyte apoptosis and OA remains to be established. Clearly, longitudinal studies of animal models of OA and/or pharmacological induction of chondrocyte apoptosis, possibly in an animal that is normally free of OA, are needed to address the question of causality. In either case, careful analyses of apoptosis in cartilage sections from longitudinal studies are needed to establish how chondrocyte apoptosis fits in the sequence of primary and induced OA pathogenesis. Moreover, the method of detection of apoptosis is also important and as no one method seems to be adequate, definitive studies should use electron microscopy to confirm the rate of apoptosis measured by other means. We are currently engaged in these types of study and we are making use of several spontaneous animal models of OA to clarify how chondrocyte apoptosis and cartilage matrix destruction are spatially and temporally linked in the context of development of early OA. Preliminary results of our studies should be available in early 2011.

CONFLICT OF INTEREST

There is no competing of interests in this paper.

ACKNOWLEDGMENT

Zaitunnatakhin Zamli is recipient of a scholarship from the Ministry of Higher Education in Malaysia.

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