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Joint aging and chondrocyte cell death

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Abstract

Articular cartilage extracellular matrix and cell function change with age and are considered to be the most important factors in the development and progression of osteoarthritis. The multifaceted nature of joint disease indicates that the contribution of cell death can be an important factor at early and late stages of osteoarthritis. Therefore, the pharmacologic inhibition of cell death is likely to be clinically valuable at any stage of the disease. In this article, we will discuss the close association between diverse changes in cartilage aging, how altered conditions influence chondrocyte death, and the implications of preventing cell loss to retard osteoarthritis progression and preserve tissue homeostasis.

Keywords

aging; apoptosis; autophagy; cartilage; cell death; chondrocyte; necrosis; osteoarthritis

Aging and the development of cartilage degeneration involve many factors, which either alone or in combination may precipitate the onset of osteoarthritis (OA). Much evidence indicates that a single factor may induce a number of sequential responses and structural changes, which either affects the cartilage extracellular matrix (ECM) or cell function, or which makes the tissue more vulnerable to compressive loads or injury. These changes eventually lead to a disruption of tissue homeostasis and reduced capacity for regeneration, which manifest as OA and eventual tissue destruction. Cell-based or ECM-based factors identified to play a major role in the onset and progression of OA include cell senescence, accumulation of glycation end products, oxidative damage, reduced growth factor responsiveness, altered mitochondrial function and apoptosis [1–4].

Aging has been associated with progressively reduced cellularity in articular cartilage [5,6], probably a consequence of cell death over time. Cell death in the form of apoptosis has been linked with OA, yet the strength of this causal link has yet to be determined. The difficulty in establishing causality is partly owing to the fact that primary OA presumably develops over many years, which is contrary to some reports showing high numbers of dying cells in diseased tissue. Nevertheless, a number of proapoptotic stimuli have been associated with chondrocyte apoptosis and have been linked to OA development [7,8]. The major mechanisms of chondrocyte apoptosis include the involvement of Fas, TNF, TNF-related apoptosis-inducing ligand (TRAIL)-R1, TRAIL-R2 and nitric oxide (NO) exposure [4].

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Cartilage homeostasis is mediated by resident chondrocytes, and loss of cells due to death leads to the characteristic features of OA tissue, including loss of cartilage ECM and abnormal tissue remodeling; the latter most likely an attempt of the remaining cells to repair degenerating tissue [9,10]. Parallel changes in companion structures that make up the joint are also evident, such as the subchondral bone and inflammation of the synovium [11–15]. In this article, we outline significant associations between aging, cell death, and the initiation and progression of OA. A number of mechanisms have been proposed, each alone may be generally involved or may present as a secondary consequence of the diseased state over time (Figures 1 & 2).

Types of cell death

Various types of cell death have been described, primarily including apoptosis (type I), autophagy-associated cell death (type II) and necrosis (type III). Specific biochemical events, such as caspase activation, cytochrome c release and internucleosomal DNA fragmentation, have been used to identify cell death associated with apoptosis or morphological features such as double membrane vacuole formation in autophagy. Necrosis is classically distinguished by the absence of these events. Without phagocytosis, defining true necrosis can be difficult since apoptotic cells eventually become secondary necrotic cells sharing the morphological features of primary necrosis [16]. While the definitions of each type of cell death are distinct, in actuality a spectrum of modes of death exist with a variety of morphologic and molecular manifestations [17]. The latest review by the Nomenclature Committee on Cell Death (NCCD) outlines and defines these phenomena [18]. Apoptosis is classified as a form of programmed cell death (PCD) that is either physiologic (e.g., part of natural developmental processes) or pathologic. In most circumstances, necrosis represents cell death as a consequence of a pathological incident. In principle, necrosis represents the final stage of any form of cell death, including oncosis and apoptosis [3]. Criteria used to distinguish the distinct modalities of cell death are presented in Box 1.

Box 1

Criteria for classifying the major types of cell death

Morphological features

- Apoptosis (type I):
  - Rounding of cells
  - Plasma membrane blebbing
  - Nuclear fragmentation
  - Chromatin condensation
  - Reduction in cellular and nuclear volume
  - Apoptosis body formation
  - Mitochondrial swelling (rare)
- Autophagy-associated cell death (type II):
  - Accumulation of autophagic vacuoles (double membrane)
  - Lack of chromatin condensation
  - Late-stage mitochondrial swelling

- Necrosis (type III):
  - Plasma membrane rupture
  - Mitochondrial and cytoplasmic swelling (oncosis)
  - No vesicle formation
  - Moderate chromatin condensation

Biochemical features & molecular pathways

- Apoptosis (type I):
  - Activation of Bcl-2 proteins
  - Mitochondrial transmembrane permeabilization
  - Cytochrome c release
  - Caspase activation
  - PARP cleavage
  - DNA fragmentation
  - ATP dependent
  - Death-associated proteins
  - Reactive oxygen species overgeneration
- Autophagy-associated cell death (type II):
  - Cathepsin B activity (lysosomal)
  - Death-associated proteins
  - PI3K and mTOR
  - LC3-1 to LC3-II conversion
  - Beclin-1 dissociation from Bcl-2/X_L
  - Dependency on *atg* gene products
  - Degradation of $p62^{Lck}$
- Necrosis (type III):
  - PARP activation
  - Loss of ion homeostatsis
  - Drop in ATP levels
  - Death-associated proteins
  - Activation of calpains and cathepsins
  - HMGB1 release

PARP: Poly(ADP-ribose) polymerase.

Data taken from [18,197–199].

In cartilage tissue, the classical morphological features described in other tissue systems, for example cell blebbing, are often absent. Roach *et al*. coined the term 'chondroptosis' to

indicate a specific form of chondrocyte apoptosis [19]. This type of PCD involves altered protein synthesis as evidenced by increased endoplasmic reticulum (ER) and Golgi apparatus, distinct from typical receptor-mediated or mitochondrial pathways. The ER membranes segment the cytoplasm to produce autophagic vacuoles in the cytoplasm where organelles are digested and finally disposed into the lacunae. This divergent cell death process appears to be consistent with the avascular nature of cartilage, where chondrocytes are isolated within their lacunae and cannot rely on the phagocytotic removal typical in other tissues [20].

Structure-forming or developmental PCD can also be the consequence of autophagy: a type of cell death that is mechanistically distinct from apoptosis and is dependent on the lysosomal machinery of the cell. Autophagy has been investigated in yeast and some of the involved genes are found in higher vertebrates, including humans. Chondrocytes express autophagic proteins [21]. A recent study indicates that autophagy has a protective role for the maintenance of the homeostatic state in normal cartilage, but aging leads to reduced autophagic protein levels and increased apoptosis (see section on autophagy) [22].

An alternative process, termed 'oncosis', has been proposed as another distinct form of cell death that is principally regulated by changes in adhesion to ECM (see section on regulators of cell death: matrix components). This process displays some features associated with necrosis, such as increased membrane permeability or cell and organelle swelling, but is not associated with internucleosomal DNA fragmentation [23,24]. However, since oncosis involves distinct cellular processes, studies suggest that it is a form of PCD [25,26]. Some evidence suggests that failure of ionic pumps and ATP depletion may be among the causes of oncosis [25,27]. Cell death resembling oncosis has been observed in atherosclerotic lesions [28] and in ischemic heart disease [29] and may also occur in bone and cartilage [30].

Regulators of chondrocyte death

Matrix components

The cartilage ECM is a dynamic network of proteins secreted by chondrocytes, which serves as a structural support and as a reservoir for cytokines and growth factors to regulate cell behavior by modulating their proliferation and differentiation, thus providing cues that are critical for cell survival [31–33]. Changes in the structure of the chondrocyte environment during the aging process can alter the physical forces experienced by the cell, as well as the biochemical signals that regulate cell response [34]. As degeneration continues, the loss of matrix leads to the propagation of cell death and tissue degeneration (Figure 1). There are two major influences of ECM: adhesion changes and signaling through receptors. Either influence directly initiates apoptotic pathways (e.g., Fas and TNF-α receptor) or indirectly alters the cytoskeleton, which leads to induction of apoptosis (Figure 2) [35–38].

The Greek word anoikis, meaning 'homelessness', was used to describe apoptotic cell death as a result of lost, reduced or inappropriate cell adhesion in endothelial cells (review in [34]). Initiation and execution of this apoptotic process are mediated through various pathways that eventually converge to activate caspases. The signals may be intrinsic, usually mitochondrial based, or extrinsic through cell surface death receptors (Figure 2). Extrinsic pathways are initiated by extracellular death ligands, such as Fas ligand (FasL/CD95L) or TNF-α, through their respective cell surface death receptors, Fas and TNF-α receptor [39,40].

Among the different cartilage ECM components, collagen type II is critical in maintaining chondrocyte viability and preventing apoptosis, as demonstrated in transgenic mice lacking

this protein [41]. Integrin receptors bind many ECM proteins, including laminin, fibronectin and collagen types II and IV [32,42], and appear to be an important interface between the ECM and mediators of cell survival. Antibodies against the integrin α5-subunit (CD49e) induce death in human chondrocytes [43], and RGD peptides reduce cell viability in cultured chicken chondrocytes [44]. RGD peptides induced apoptosis in cultured chondrocytes and in cartilage explants, probably through direct activation of caspase-3 [45]. Type X collagen deposition and chondrocyte survival in chicken sterna were dependent on CD49b and CD49c integrin subunits [35]. These studies indicate a direct involvement of integrin–ligand interactions in chondrocyte death.

While intact ECM proteins modulate cell survival, ECM protein fragments can elicit different effects. For example, the 29-kDa fragment of fibronectin induces inflammatory responses [46], including an increase in catabolic proteases such as matrix metalloproteinase (MMP)-13 [47], although it does not directly induce cell death in cultured human chondrocytes. A 24-mer synthetic peptide of type II collagen (residues 195–218; CB12-II) lacking any RGD sequence has been demonstrated to induce apoptosis in bovine cartilage explants. This type of cell death may be related to chondrocyte hypertrophic events [48,49]. Blocking CD44 and hyaluronan interaction decreases chondrocyte survival [50] and fragments of hyaluronan may augment the production of NO in a CD44-dependent manner [51]. Hyaluronan oligosaccharides can induce MMP-13 production and cause further matrix breakdown [52]. Conversely, hyaluronan fragments of 500–730 kDa interact with CD44 and CD54 to inhibit Fas ligand-induced apoptosis [53].

Alteration in the ECM properties leads to tissues less able to bear normal load or withstand low-impact injuries, which leads to a chain reaction of events that damage and further drive disease progression. Changes in cartilage ECM due to aging include altered aggrecan sizes [54,55] and increased fibril crosslinking of collagen type II. The latter process increases the stiffness of cartilage [56,57], and increased stiffness of the matrix has been attributed to an accumulation of glycation end products (nonenzymatic protein modifications) over time [58,59]. The accumulation of glycation end products results in the activation of receptor for advanced glycation end products (RAGE) receptors [60] and induces reactive oxygen species (ROS) and catabolic pathways (Figure 2) [61]. ROS can be produced by chondrocytes or by the synovial lining [62], which impairs chondrocyte response to growth factors, such as IGF-1, and inhibits mitochondrial function [63–65] and DNA repair capacity [66].

Small calcium-binding S100 proteins have been implicated in various inflammatory conditions, including arthritis. S100A4 may play an important role in cartilage degradation by mediating ECM destruction and indirectly altering chondrocyte viability [67]. The interaction of S100A4 with RAGE increases MMP-13 production in cartilage (Figure 2) [67], and is also known to upregulate *MMP-13* and other *MMP*s in rheumatoid arthritis-derived synovial fibroblasts [68]. S100A4 was reported to bind p53 tumor suppressor and to regulate its function [69], possibly promoting apoptosis (see section on p53 and c-myc).

Mechanical stress & injury

As previously outlined, changes in ECM not only alter cellular response, but also modify the mechanical properties of the tissue, leaving the cell more vulnerable to normal loading. Mechanical injury has been demonstrated to induce cell death, and cartilage matrix degradation in response to mechanical injury has been reported in bovine [70–74] and human cartilage [74–77]. Loss of glycosaminoglycan may indirectly predispose cells to necrotic cell death following mechanical injury, which later precipitates PCD (Figure 1) [78].

Mechanical stimuli releases proteoglycans from cartilage explants [74,79,80] and induces the production of inflammatory or catabolic peptides, such as MMPs [81], NO [82], ADAMTS-5 [83] and IL-1β (Figure 2) [79]. A single episode of mechanical injury in human cartilage explants resulted in a time-dependent increase in apoptotic cells. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) was partially inhibited by incubating the impacted explants with the pan-caspase inhibitor z-VAD-fmk [74]. Apoptosis has also been demonstrated to be induced by repetitive trauma *in vitro* [84]. *In vivo* cartilage degeneration, generated by anterior cruciate ligament transaction in rabbit knees, was significantly reduced with caspase inhibition, supporting a potential therapeutic role [85]. Other studies also demonstrate the potential therapeutic role of caspase inhibitors [74,77], BMP7 [86] and P188 surfactant [76] for chondroprotection following impact trauma or tissue injury as a result of surgical procedure [87].

It has been hypothesized that mechanical injury releases ROS. This hypothesis has been supported by reports of antioxidants (*N*-acetylcysteine and a superoxide dismutase [SOD] mimetic) increasing cell viability after mechanical injury [88,89].

Nitric oxide

Nitric oxide is present in normal and young cartilage, yet its production is elevated in aged tissue [90,91]. NO mediates apoptosis through a mitochondria-dependent mechanism [92–94] and contributes to the breakdown of the ECM by enhancing the expression of proinflammatory cytokines [95–97]. High concentrations of the NO donor sodium nitroprusside (SNP) can induce apoptosis-like cell death in cultured human chondrocytes [98]. Incubation of human articular chondrocytes with SNP can induce events characteristic of apoptosis, including increased caspase-3 and caspase-7 gene expression and downregulating *Bcl-2* (an antiapoptotic molecule) mRNA levels (Figure 2) [93]. SNP induces apoptosis of human chondrocytes through sequential events, involving cytoskeletal remodeling (disruption and reduced polymerization of F-actin and microtubule cytoskeleton), MEKK1/JNK activation, Bax translocation, mitochondrial dysfunction (decreased complex I NADH dehydrogenase activity and cytochrome c release) and sequential caspase activation (caspase-9, -3 and -6), leading to DNA fragmentation [94].

IL-1β, an inducer of inducible NO synthase expression and production of NO in chondrocytes, did not induce chondrocyte apoptosis [98,99]. Nevertheless, combining IL-1β with dimethyl sulfoxide resulted in hypoploidy and DNA fragmentation. Use of a specific inhibitor of inducible NO synthase reduced apoptosis induced by IL-1β, indicating that cell death is dependent on endogenous NO generation. In addition, the proapoptotic effect of NO could be blocked by ROS [99]. The balance between intracellular NO and ROS has been proposed to determine whether chondrocytes die through apoptosis or necrosis, with a low concentration of ROS promoting apoptosis in the presence of NO and a high concentration of ROS promoting necrosis.

Interestingly, del Carlo and Loeser found that incubation with NO alone does not induce apoptotic cell death in chondrocytes [100]. They also demonstrated that both NO and ROS are required to induce apoptosis, suggesting that NO alone may have beneficial effects in chondrocytes. Other studies on the effect of NO on chondrocyte apoptosis have focused on the role of apoptosis in terminal differentiation, again illustrating an alternative, nonpathological role for NO in development. However, most reports indicate that NO is primarily a catabolic factor in OA that can induce cell death and further contribute to disease progression.

Death receptors

Members of the TNF-receptor family are transmembrane receptors that activate well-characterized apoptosis pathways. Fas (CD95) is expressed on the cell surface of cultured chondrocytes and is detected in cartilage from normal and OA donors [101]. Fas activation by agonistic antibody leads to apoptotic cell death in cultured chondrocytes, linking this receptor to functional apoptosis signaling components. In tissue, however, antibody to Fas has not been shown to induce cell death [102], which has been attributed to the barrier created by matrix proteins that prevents antibody interaction with the chondrocytes. In addition, chondrocytes in ECM may not respond to Fas stimulation, since they may be protected from Fas-dependent apoptosis through survival signals generated by the interaction of cell membrane receptors, such as integrins, with their respective ECM ligands [3]. However, in degraded tissues the Fas/FasL system may be more effective in inducing cell death. To date, TNF-α-mediated chondrocyte death has not been conclusively established. TNF-α stimulation (48 h) of monolayer chondrocytes led to a small increase in the number of TUNEL- or *in situ* nick-end labeling (ISNEL)-positive cells [103]. In another report, DNA fragmentation in response to TNF-α was detected with a sensitive ELISA-based technique when the chondrocytes were simultaneously stimulated with proteasome inhibitors [102]. Therefore, TNF-α alone may have no effect on apoptosis. However, TNF-α in combination with actinomycin-D or Ro 31–8220 induces an increase in caspase-1 and -8 mRNA and protein levels [104,105].

The sensitivity of T cells to TNF-α-induced apoptosis appears to be age dependent [106]. In aged lymphocytes, apoptosis is associated with increased expression of TRADD, FADD and Bax [107,108], and decreased expression of Bcl-2, TRAF2 and TNFRII. To date, such age-related changes have not been established in articular cartilage.

Mitochondria

The influence or involvement of mitochondria in apoptosis and cell necrosis has been extensively investigated (for reviews see [109–112]). Altered mitochondrial function has been associated with apoptosis, aging and a number of pathological conditions, including OA [4,113–116].

In addition to the mitochondrial involvement in NO-induced apoptosis [94], oxidative stress and mitochondrial dysregulation play an important role in OA development and progression [117,118]. Oxidation changes occur during the aging process, which illustrates a possible relationship between aging, chronic inflammation and cartilage degradation in OA [119].

Reactive oxygen species activity is balanced by enzymatic and nonenzymatic antioxidants, which act by inhibiting oxidative enzymes or scavenging free radicals [113]. Decreased mitochondrial SOD2 within OA chondrocytes affects chondrocyte intracellular metabolism [120]. Degenerating regions of OA cartilage [121] possessed lower anti-oxidative capacity and the resulting oxidative stress induced replicative senescence and telomere genomic instability. This result indicates that inadequate control of ROS is an essential factor in OA pathophysiology.

Mitochondria may be involved in epiphysial chondrocyte death during bone development. Mitochondria in the avian growth plate show a maturation-dependent reduction of oxidative phosphorylation [122] and changes in Bcl-2 protein levels [123]. Loss of mitochondrial function may be linked to NO production induced by phosphate ions [124]. A causal relationship between phosphate ions, NO production and mitochondrial dysfunction in avian growth plate chondrocytes has recently been established [125], although no evidence is available to directly link this relationship to OA.

p53 & c-myc

A majority of studies suggest that p53 is involved in increased cell death in aging cartilage. Experiments using mice with hyperactive p53 tumor suppressor gene show a senescence phenotype and signs of premature aging [126], although cartilage from p53-knockout mice shows no significant effect on cell death [127]. In degenerated lesions of arthritic cartilage, ISNEL, denoting apoptotic cells, correlated with the expression of p53 and c-myc [128]. Hydrostatic pressure induced apoptosis in cultured human chondrocytes, which was associated with increased p53 expression [129]. In aged rabbits, the expression of p53 was increased and was associated with decreased viable cell density [130]. NO was shown to cause cell death and induce p53 via p38 MAPK and NF-κB, indicating that p53 plays a role in chondrocyte survival in the presence of NO (Figure 2) [131].

Levels of c-myc increased in fully differentiated hypertrophic chondrocytes [132] and in hypertrophic chondrocytes in rat growth plates, indicating a role for c-myc in terminal chondrocyte differentiation [133]. Subcellular localization also changed with intranuclear concentration of c-myc, which decreased with the maturing of chondrocytes. Another study demonstrated that the s-myc protein was expressed in rat embryo cells committed to undergo differentiation into hypertrophic chondrocytes [134]. In rabbit growth plates, c-myc staining frequently colocalized with cells showing DNA strand breaks. In a canine model of OA, high levels of c-myc were found in areas of cartilage erosion [135]. Furthermore, c-myc expression also colocalized with apoptotic cells in human arthritic cartilage [128]. Similar to p53, apoptosis induced by hydrostatic loading was linked to c-myc [129]. These findings suggest that in addition to p53, c-myc may also regulate developmental and OA-related chondrocyte death.

Wnt/β-catenin signaling

Two recent studies reveal a close relationship between aging, Wnt/β-catenin signaling and apoptosis [136]. In these studies, Col2a1– inhibitor of β-catenin and T-cell factor (ICAT)-transgenic mice inhibited β-catenin signaling in chondrocytes and significantly increased cleaved PARP, caspase-3 and TUNEL-positive cells (Figure 2) expression. Conversely, Bcl-2 and Bcl-X_L were decreased and caspase-9 and caspase-3/7 activity were increased, suggesting that increased cell apoptosis may contribute significantly to the articular cartilage destruction observed in Col2a1–ICAT-transgenic mice.

In another study, both Wnt signaling and chromatin protein high mobility group box protein 2 (HMGB2) expression decreased with aging in mouse joints, and conditional deletion of β-catenin in cultured mouse chondrocytes induced apoptosis (Figure 2) [137]. The loss of HMGB2–Wnt signaling interaction represents a new mechanism in aging-related cartilage pathology.

Autophagy

Autophagy is an important cellular process involved in recycling of long-lived proteins and organelles and is upregulated in response to ischemia/reperfusion and pressure overload in the heart [138,139]. Therefore, autophagy may be similarly altered in mechanoresponsive tissues such as cartilage. The role of autophagy in cartilage biology has only recently received attention. Observations by Caramés *et al.* indicate that aged and OA articular cartilage are associated with reduced expression or loss of ULK1, Beclin1 and light chain 3, which was accompanied by an increase in apoptosis (Figure 2) [22]. Hypoxia-inducible factor (HIF) activity influences the expression of Beclin 1 (a major factor in autophagy) and regulates HIF's interaction with caspase-8 and members of the Bcl-2 family of proteins [21]. The induction of autophagy appears to delay chondrocyte death until completion of the maturation process; however, prolonged autophagy may play a role in PCD.

Evidence of crosstalk has been reported between autophagy and apoptosis [140,141]. The autophagy protein, Atg5, induces mitochondria-based apoptosis, while Bcl-2 overexpression protected against Atg5-mediated mitochondrial dysfunction. Beclin 1, an essential autophagy protein, is regulated by the Bcl-2 proteins in normal conditions. Bcl-2 and Bcl-X_L suppress autophagy by associating with Beclin 1 [142]. Reduced Beclin 1 heterozygous mice (Beclin $1^{+/-}$) have reduced autophagy and apoptosis and heart infarct size after ischemia/reperfusion injury [143], suggesting that Beclin 1 might activate apoptosis. Continued research into the relationship between age, apoptosis and autophagy may reveal alternative means to preserve cartilage viability.

Growth factor responsiveness

TGF-β and IGF-I are major growth factors regulating chondrocyte survival, proliferation, differentiation and matrix synthesis. IGF-I is essential in maintaining chondrocyte viability [144]. Studies demonstrate that as mice and men age, chondrocyte responses to growth factors are reduced (Figure 2) [6,145,146]. van der Kraan and van den Berg [147] propose that after the age of 40 years, chondrocytes lose the ability to maintain a normal phenotype or resist terminal differentiation. Disruption in normal TGF-β signaling (absence of ALK5 and or Smad2/3) appears to be the major underlying cause, as illustrated by knockout mice and human family studies [148–150]. While the proliferative response to TGF-β1, FGF2 and PDGFbb was not reduced in human articular chondrocytes, the cartilage-forming capacity following expansion with growth factors was lower in older individuals [6]. These age-related changes in growth factor response then shift cartilage tissue homeostasis toward tissue destruction and eventual cell death. Such age-related changes are thought to be significant factors in the increased susceptibility to injury and degeneration and to the reduced repair response with aging.

Apoptosis inhibitors

Mitochondria-associated proteins play key roles in activating apoptosis. The Bcl-2 family regulates the release of proteins (such as cytochrome c) from the space between the inner and outer mitochondrial membrane that, once in the cytosol, activate caspase proteases. Bcl-2 is an antiapoptotic protein and is expressed typically in the mid-zone of normal cartilage. Overexpression of Bcl-2 protects against apoptosis [151] and Bcl-2 expression appears to be regulated by IL-1β and NO (Figure 2) [99,152]. The overall expression of Bcl-2 is reduced in OA, although there is relatively greater expression in chondrocytes near arthritic defects [153,154]. Bcl-2 is also implicated in cell death associated with collagen type II deficiency [41]. In mice, Bcl-2 expression decreased with age, indicating a decline in antiapoptotic activity [155].

Cellular control of apoptosis is complex and several intracellular inhibitors of apoptotic signaling cascades have been characterized. Inhibitor of apoptosis (IAP) proteins inhibit caspases or block the pathways that activate them. The IAP proteins primarily function as ubiquitin E3 ligases and possess protein–protein interaction domains (reviewed in [156,157]). XIAP is a potent inhibitor of the catalytic self-activation of caspase-3. Fas activation in cultured chondrocytes often leads to incomplete caspase-3 processing [102]. This suggests potent apoptotic inhibitory mechanisms at or above the level of caspase-3 activation. The mediators of these mechanisms are still being elucidated. However, evidence implicates that low expression of caspase-8 and expression of FLICE inhibitory protein (FLIP) leads to suppression of the Fas signal [102,158].

Cytokines have also been implicated to prevent apoptosis. In human chondrocytes, TNF-α increases NF-κB activity, which alone does not induce apoptosis [102,104,105]. NF-κB has been shown to block TNF-α-induced apoptosis [159]. However, at least partial inhibition of

NF-κB activation protects chondrocytes against Fas- and NO-induced death [99,152]. IL-1β can also block Fas-induced apoptosis, which is thought to be dependent on tyrosine phosphorylation [99]. IL-4 downregulated cyclic tensile stress-induced inducible NO synthase mRNA expression and NO production by chondrocytes and reduced NO induced apoptosis [160]. Intra-articular injection of IL-4 into rat joints appeared to exert chondroprotective properties against mechanical stress-induced cartilage destruction, probably by inhibiting NO production by chondrocytes [161]. Cilostazol, a selective phosphodiesterase type III inhibitor, inhibited NO-induced apoptosis by preventing the upregulation of phosphorylated p53 and p38, reducing heme oxygenase 1 and caspase-3, -7 and -8 activation [162].

Cell death, aging & OA

Osteoarthritis is generally thought to be a slowly progressive disease. In humans, as well as in animal models, it is linked with chondrocyte death, which is assumed to be largely apoptotic in nature. Experiments with articular cartilage of C57BL/6 mice and Wistar rats demonstrated a significant age-dependent increase of the percentage of apoptotic cells (TUNEL-positive) for all joint surfaces in both species [163]. Electron microscopy of human OA cartilage reveals cytoplasmic and nuclear features consistent with apoptosis. In very early OA, when the superficial zone is still intact, empty lacunae, lysosome-like structures, matrix vesicle-like structures, fragmented chondrocytes and nuclear condensation are observed [7]. To illustrate the spectrum of cell death, Kuhn *et al.* showed evidence of chondrocytes with the ultrastructural features of apoptosis or necrosis in OA cartilage [3]. Chondrocyte death correlates strongly with age and severity of OA. Several reports have associated a significantly greater number of TUNEL- or ISNEL-positive cells in OA cartilage relative to normal [7,8,128,164]. The number of potentially apoptotic cells also correlated significantly with the OA grade [8]. Flow cytometric analysis of chondrocytes isolated from osteoarthritic tissue demonstrated increased rates of apoptosis (by TUNEL) when compared with cells from normal cartilage. The matrix surrounding TUNEL-positive cells contained lower proteoglycan concentrations [8]. Increased numbers of empty lacunae in cartilage were associated with higher arthritic grade when compared with age-matched normal cartilage [165,166]. TUNEL has been frequently used to quantify apoptosis and can sometimes label necrotic cells. This false-positive staining can lead to overestimation of the number of apoptotic cells (thus, a combination of techniques is recommended) [77,167]. However, the significant difference in TUNEL-positive cells between normal and OA cartilage indicates that increased cell death (regardless of mechanism) is an integral feature of OA pathology. These dead cells tend to persist in their lacunae owing to lack of vascularity or phagocytotic removal. Eventually, disintegration of apoptotic chondrocytes leads to formation of membrane-enclosed structures resembling matrix vesicles [7,164,168]. These structures may be responsible for the matrix mineralization often associated with OA.

Animal models of OA (such as the cartilage degeneration induced by anterior cruciate ligament transection) have linked the histologic severity of cartilage lesions with chondrocyte death, matrix loss, production of NO, increased intracellular caspase-3 activity and an increased frequency of TUNEL-positive cells [8,85,165]. More research is necessary to elucidate the precise role of conventional apoptosis pathways and the potential role of caspases other than caspase-3 in OA-linked chondrocyte death. In mice activation of caspase-12, which is located in the ER, led to apoptosis-like cell death [169]. Nonclassical pathways of PCD may also be useful candidates for future investigations.

Novel mechanisms of age-induced reduction in cellularity are being discovered. The nonhistone chromatin protein HMGB2 is a transcriptional regulator, which is specifically expressed in the superficial zone of human articular cartilage, and which gradually reduces

with aging [170]. Genetic deficiency of HMGB2 in mouse chondrocytes increases susceptibility to apoptosis induction *in vitro. In vivo*, a dramatic reduction in cellularity is followed by an accelerated and more severe form of OA. HMGB2 in human articular cartilage is therefore an important chondrocyte survival factor and directly links aging with OA.

Future perspective

The aging process is inevitable, yet our understanding of the consequences of this phenomenon is incomplete. The breakdown of cartilage tissue structure and aging leads to the development and progression of OA. OA and other rheumatic diseases are among the most common of all health conditions and are the number one cause of disability in the USA, affecting an estimated 27 million Americans [171]. At present, the most common treatment of advanced OA is joint replacement, which is estimated to reach 2 million knees and hips per year by 2015 in the USA alone [172]. The impact of arthritic conditions is also expected to grow as the population increases and ages in the coming decades. Current North American (American College of Rheumatology [ACR]) and European (European League Against Rheumatism [EULAR]) recommendations for the treatment of OA include only symptom-modifying therapies [173,174]. Unlike rheumatoid arthritis, presently there are no intervention therapies available for altering OA.

The multifaceted nature of joint disease indicates that the contribution of cell death can be an important factor at all stages of the disease. Matrix homeostasis relies on a balance between net anabolic and catabolic activities, which are directly influenced by the number of available chondrocytes. The weight of existing evidence offers chondrocyte death as an excellent target for therapeutic intervention in OA. To achieve prophylactic and therapeutic success, further research into chondrocyte death, cartilage degeneration and arthritic progression is required.

Within the next 5–10 years, it is envisaged that researchers and clinicians will develop more distinct pharmacological and/or cell-based methods to slow or reverse age-related tissue degeneration. A better resolution of the mechanistic links between aging changes in ECM, receptor and signaling pathways that instigate initial matrix degradation that leads to cell death, and OA progression will ensue. Therapies that preserve cell viability modulate and control cellular response *in vivo* will probably be the main focus of research over the next decade, which should lead to clinical application soon thereafter. The main areas that may evolve to clinical application include: inhibition of apoptosis; pharmacological approaches to retard overtly catabolic and ROS activities that lead to accelerated ECM degradation and cell death; and utilization of cartilage progenitor cells.

Inhibition of apoptosis

This could occur either through caspase inhibitors or other chondropreserving factors such as BMP7 [86]. The clinical efficacy of caspase inhibition in other diseases currently under investigation includes acute and chronic neurodegenerative diseases, myocardial infarction and liver apoptosis [175–177]. Application of these inhibitors should firstly translate into treatment of acute post-traumatic joint injuries. More precise identification of the intrinsic and extrinsic mechanisms leading to chondrocyte death will be established to provide novel targets for therapeutic interventions.

Pharmacological approaches to retard overtly catabolic & ROS activities that lead to accelerated ECM degradation & cell death

Such treatments may include use of antioxidants or enhancement of SOD to combat excess ROS [178,179]. Further development of aggrecanase (specifically ADAMTS-4 and

ADAMTS-5) [180–182] and matrix metalloproteinase inhibitors [183] will be better refined and employed to slow tissue degradation. Means to inhibit or reduce excessive matrix extracellular sulfatase activity, observed in OA cartilage [184], are currently being developed and will offer yet another means to control loss of tissue homeostasis and OA progression.

Utilization of cartilage progenitor cells

Over the last 20 years, cell-based methods to repair cartilage defects and diseased tissues using chondrocytes [185] and mesenchymal stem cells have been studied [186]. In fact, human chondrocytes have been used for almost 20 years with unclear clinical outcomes [187]. Nevertheless, over the past decade, mounting evidence shows that progenitor cell populations reside in articular cartilage [188–196], which are principally located in the articular cartilage superficial zone [189,190,196]. Loss of these progenitors during aging and resulting from injury may be an important factor that leads to loss of tissue homeostasis. These cells may be a better cell source to repair injured or degenerated tissue; thus, preserving this subpopulation may be critical. Furthermore, therapies that utilize these cells *in situ* would represent a more elegant means to restore joint function.

Executive summary

Cell death is a normal consequence of aging

- Cellularity in articular cartilage progressively reduces with age.
- Reduced cellularity is compounded by cell senescence, accumulation of glycation end products, oxidative damage and reduced growth factor responsiveness.

Chondrocyte death has many faces

- Three primary modes of chondrocyte death are apoptosis, autophagy-associated cell death and necrosis.
- These modes of cell death often have overlapping rather than distinct modes of morphologic features.

Cell–matrix interactions are critical to survival

- Collagen type II supports cell survival via integrin binding.
- Matrix protein fragments have inflammatory effects on cartilage matrix.
- Aging- or osteoarthritis (OA)-related matrix changes can alter the mechanical environment of the chondrocyte making it more susceptible to injury and death.

Stresses associated with cell death

- Mechanical injury induces cell death: a major component of which is apoptotic in nature.
- Mitochondrial dysfunction is associated with aging, OA and cell death.
- Exogenous nitric oxide induces cell death, while the balance between endogenous nitric oxide and reactive oxygen species determines potential for cell death.
- p53 and c-myc are implicated in cell death and OA.

OA is intimately associated with increased cell death

- Cell density is inversely correlated with the grade of OA.

- The matrix surrounding apoptotic cells has lower proteoglycan concentration.
- Apoptotic bodies resemble matrix vesicles found in OA tissues and can be sites of nucleation for calcific deposits.
- Animal models have linked OA with increased caspase activity, nitric oxide, matrix loss and cell death.

Prevention of cell death holds promise as a treatment of post-traumatic OA

- Caspase inhibitors and antixoxidants can prevent cell death induced by mechanical injury.
- Inhibition of caspases *in vivo* has significantly reduced the grade of OA.

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▪ of interest

▪▪ of considerable interest

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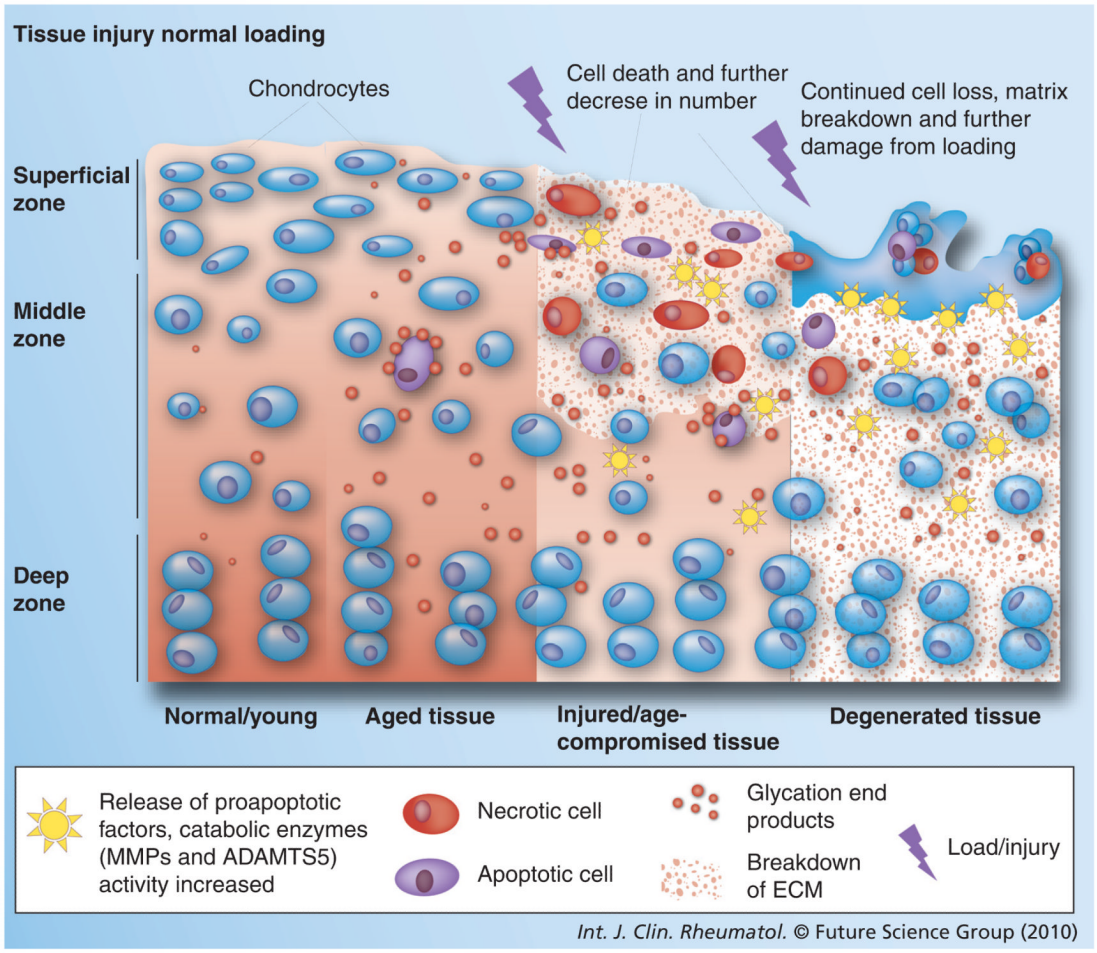

Figure 1. Cellular and extracellular matrix changes associated with age that lead to chondrocyte death and osteoarthritis development in articular cartilage

Cell density reduces with age and ECM properties are altered with age, resulting in reduced load-bearing capacity. All of these changes increase the tissue's vulnerability to loading/injury cell death (necrotic and apoptotic). Continued loading on compromised tissue leads to further cell death and matrix degradation.

ECM: Extracellular matrix; MMP: Matrix metalloproteinase.

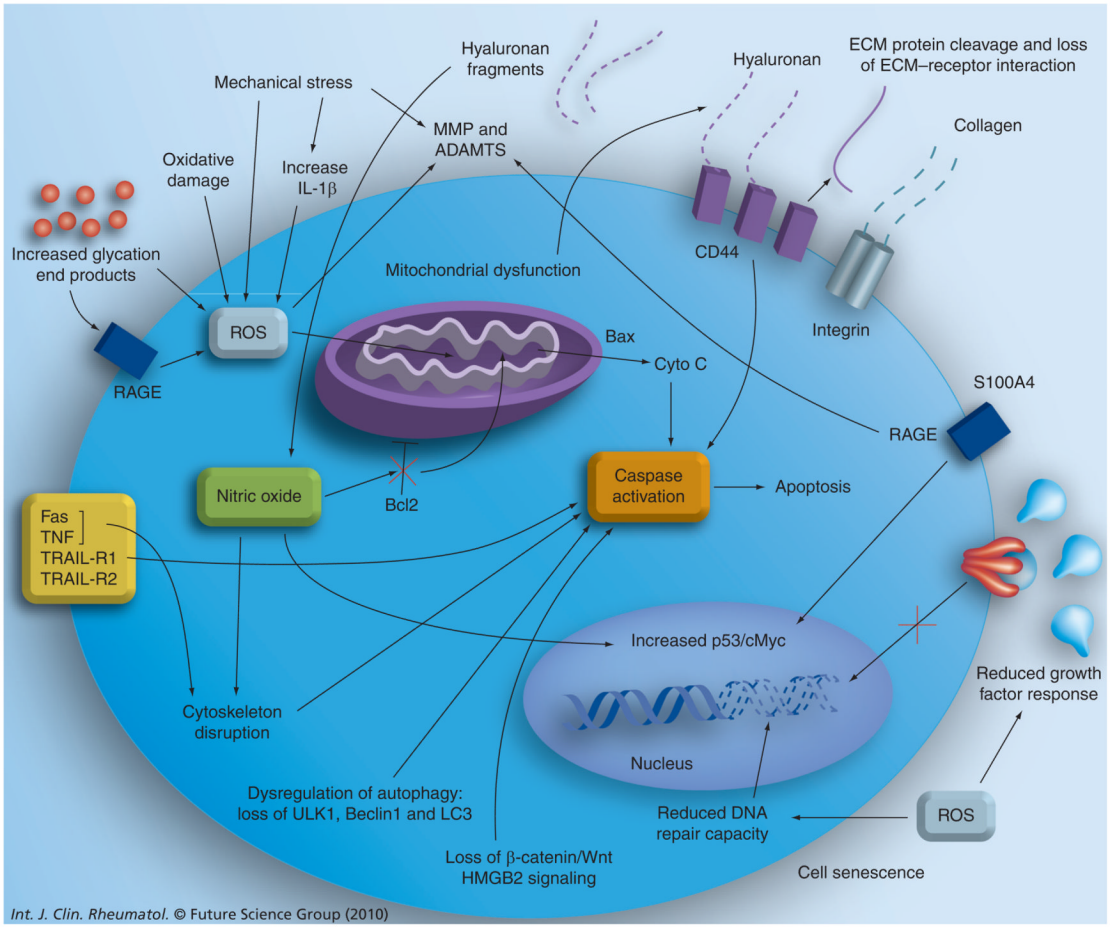

Figure 2. Cellular and extracellular matrix changes associated with age that lead to chondrocyte death and osteoarthritis development in articular cartilage

Summary of cell-based (intrinsic and extrinsic) factors known to change with age and alter cell viability (see text for details).

ECM: Extracellular matrix; MMP: Matrix metalloproteinase; RAGE: Receptor for advanced glycation end products; ROS: Reactive oxygen species.