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Review

Do chondrocytes undergo "activation" and "transdifferentiation" during the pathogenesis of osteoarthritis? A review of the ultrastructural and immunohistochemical evidence

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Summary. Chondrocytes, which are the only cell type in the articular cartilage, show substantial morphological and functional differences, depending on their location within the tissue. In OA cartilage, outstanding modifications have been reported concerning their structure and functions. Based on the principle that both structure and function run in a parallel manner, new concepts are arising related to morphological observations. Observations on OA chondrocytes, such as cytoskeleton disruption, development of the secretory machinery (rough endoplasmic reticulum and Golgi complex), and cell death by apoptosis, among others, certainly must be related to the role of chondrocytes in OA pathogenesis. In this degradative process, it has been acknowledged that cell death, matrix degradation and subchondral bone remodelling are the main causes of cartilage breakdown in osteoarthritis. The aim of this review was to correlate and integrate in a logical manner the modifications of chondrocytes with cartilage breakdown during osteoarthritis pathogenesis. Furthermore, we intend to open a debate on cell cycle and mitosis, as well as on signalling molecules that might be involved in the morphofunctional changes in OA chondrocytes, which we propose to name "activation" and "transdifferentiation" of chondrocytes. We expect this analysis to be useful for studying OA pathogenesis in depth, with the aim of finding new strategies for the early diagnosis and therapeutic procedures for this invalidating disease, which is already an important public health problem.

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Introduction

Osteoarthritis (OA) is a chronic disease which affects the entire joint, though most studies have been concentrated on the focal degeneration of the articular cartilage, associated with altered subchondral bone remodelling, sclerosis, bone hypertrophy, and the presence of osteophytes (Mankin et al., 1994). OA is a multifactorial disease that shows significantly higher incidence and prevalence in the elderly and, more recently, in young people and even in children. Even though OA is a high-morbidity disease (Abyad and Boyer, 1992; Badley and Tennant, 1993; Levy et al., 1993), its pathogenesis is still poorly understood and the diagnostic methods and current treatments do not satisfy the patient's expectations.

OA is considered a multifactorial disease, with mechanical stress and genetic and immunological influences playing an important role in its pathogenesis. In this context, not only the cartilage is involved in articular tissue damage; in recent years, it has become clear that synovial membrane also plays an important role in joint destruction, as indicated by joint swelling, synovial effusion and mild or, occasionally, heavy mononuclear cell infiltration of synovial membrane. Inflammation in OA is considered a secondary process due to joint space narrowing and cellular debris that induce synovial membrane damage, angiogenesis, leukocyte infiltration and autoimmunity even in the early stages of the osteoarthritic process (Yuan et al., 2003; Brenner et al., 2004; Malemud 2004; Benito et al., 2005; Bonnet and Walsh, 2005; Cecil et al., 2005).

Articular cartilage

Most of the advances made during the 70's, 80's and early 90's have been related to the structural and biochemical characterization of the extracellular matrix (ECM) of the cartilage. Moreover, the histological description of the cartilage has been mainly focused on describing different zones in the tissue (Hunziker, 1992). Furthermore, the histopathology of human OA has been assessed by several authors who described the degenerative stages and cell density of the different zones of the articular human cartilage (Vignon and Arlot, 1981; Mitrovic et al., 1983; Quintero et al., 1984). However, the study of chondrocyte ultrastructure, cell cycle, synthesis activity and death has only been conducted over the last few years. Therefore, we will focus this review on chondrocytes and their relationship to the extracellular matrix.

Chondrocytes

Mitosis and cell cycle

This is an important subject that needs further analysis, mainly because of its association with cartilage repair. We believe that mitosis within normal and OA chondrocytes is still a controversial question. In order to broaden this knowledge, we studied normal chondrocytes proliferation in depth and subsequently compared it with OA chondrocytes (Gomez-Camarillo and Kouri, 2005). Our results suggest that cell division is scarce if not absent in the OA cartilage studied. Nevertheless, the existence of factors essential for cell division leaves open the question concerning chondrocytes proliferation in OA cartilage, which is likely to be present in the early stages of the disease.

Phenotype variability

Phenotype variability within normal and OA chondrocytes has been reported exploring several extracellular macromolecules such as collagen, aggrecan, fibronectin, perlecan, decorin, byglican, as well as molecules associated with the catabolic signalling pathway within OA pathogenesis (von der Mark et al., 1992; Wang et al., 2003; Tesch and Miosge, 2004). In addition, morphological changes have also been reported by several authors (Kouri et al., 1996a,b, 1998, 2002a; Galois et al., 2004; Patwari et al., 2004; Tesch and Miosge, 2004; Todd-Allen et al., 2004) as indicative of phenotypic variability within normal and OA cartilage. This evidence might open new and important trails that might broaden the knowledge of OA pathogenesis.

It is well established that chondrocytes are the only cell type within the articular cartilage; their ultrastructure was first analyzed using transmission electron microscopy (TEM) in young and old people by Weiss and colleagues (1968, 1971), and also on experimental models (Huzinker, 1992). Later, we described different sub-populations of chondrocytes according to their ultrastructure and location within the cartilage (Kouri et al., 1996a,b 1998, 2002a).

The ultrastructural characteristics of OA chondrocytes led us to classify them into three types according to their location and phenotypic changes (Table 1). Type 1 was normal chondrocytes, observed in non-fibrillated regions distant from the damaged cartilage. However, Types 2 and 3, observed within the most damaged region of OA cartilage, were mostly clustered chondrocytes, which showed dissimilar morphological phenotypes that fluctuated between the typical secretory phenotype and osmiophilic contracted cells analogous to apoptotic cells (Kouri et al., 1996a,b; 1998, 2002a) (Fig. 1). The remarkable ultrastructural variability of the chondrocyte phenotype was found in both human and rat OA cartilages (Figs. 2-5). Although these observations were initially accomplished in human cartilage, we were able to determine the predominant cell type present in our rat model 5, 10, 20 and 45 days after OA induction (Abbud-Lozoya and Kouri, 2000; Kouri et al., 2002a). In addition to the study of ultrastructural phenotypic variability of upper zone chondrocytes a different scenario was found in deep zone chondrocytes. These cells showed abundant alkaline phosphatase (ALP)-rich vesicles budding from their surface (Kouri et al. 2000). Therefore, the morphological variability of chondrocytes from the entire depth of the cartilage should certainly be

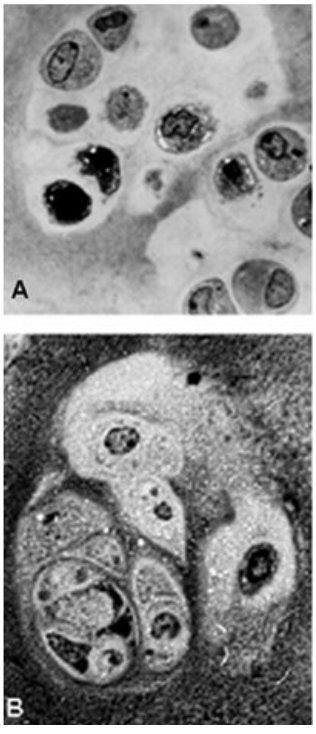

Fig. 1. A, B. Clustered chondrocytes from human OA cartilage observed from two semi-thin sections with a bright field microscope. Notice the phenotype variability of chondrocytes. Magnification. A, x 1,200; B, x 1,600

associated with different stages of tissue breakdown.

The fact that cartilage disintegration is a focal and not synchronic process, could explain the great diversity of OA chondrocyte phenotypes described at the ultrastructural level.

As follows, changes in chondrocyte shape, size and mainly ultrastructure needs to be associated with their synthetic activity, with the aim of finding a correlation between chondrocyte modifications and their pathological performance within OA pathogenesis.

Certainly, immunogold localization at the ultrastructural level is essential to be accomplished for correlating chondrocyte morphological characteristics with their synthesis activity.

Cytoskeleton

The cytoskeleton, which establishes a close

Table 1. Ultrastructural characteristic of normal and OA chondrocytes.

SUBPOPULATION	CELL PHENOTYPE	TISSUE LOCATION	SPECIFIC CHARACTERISTIC
Type 1	Normal	Superficial zone	Spindle. Like shape, perinuclear filaments scarce rer and golgi chromatin rejected toward the nuclear anvelope, light cytosol.
		Middle zone	Spheroide shape, rer with narrow cisterns and scarce Golgi membranes scant chromatin repelled toward the nuclear envelope, light cytosol.
		Deep zone	Large rounded, scarce rer with narrow cisterns and golgi. Scant chromatin distribuited throughout the carioplasm, light cytosol.
Type 2	OA Chondrocytes	Upper zone within the fibrillated cartilage.	
	Secretory 2a		Rounded or elongated shape, prominent rer with dilated cisterns showing abundant visible ribosomes. Enhanced Golgi, condense chromatin organized forming patches, light cytosol.
	2b		Irregular shape, prominent rer with extremely dilated cisterns, ribosomes visible, abundant golgi membranes. Dense cytosol. Irregular nucleus and dense chromatin throught the nucleoplasm.
		Only in the deep zone	Abundant vesicle budding from the cell membrane.
Type 3	Chondroptotic	All zones	Irregular and contracted cells. Prominent rer showing hardly visible ribosomes, enhanced, fragmented and expanded golgi membranes. Dense cytosol. Dense chromatin, Cell fragmentation and disintegration.

The different ultrastructural phenotypes of chondrocytes from normal and OA cartilage, according to their location within the tissue.

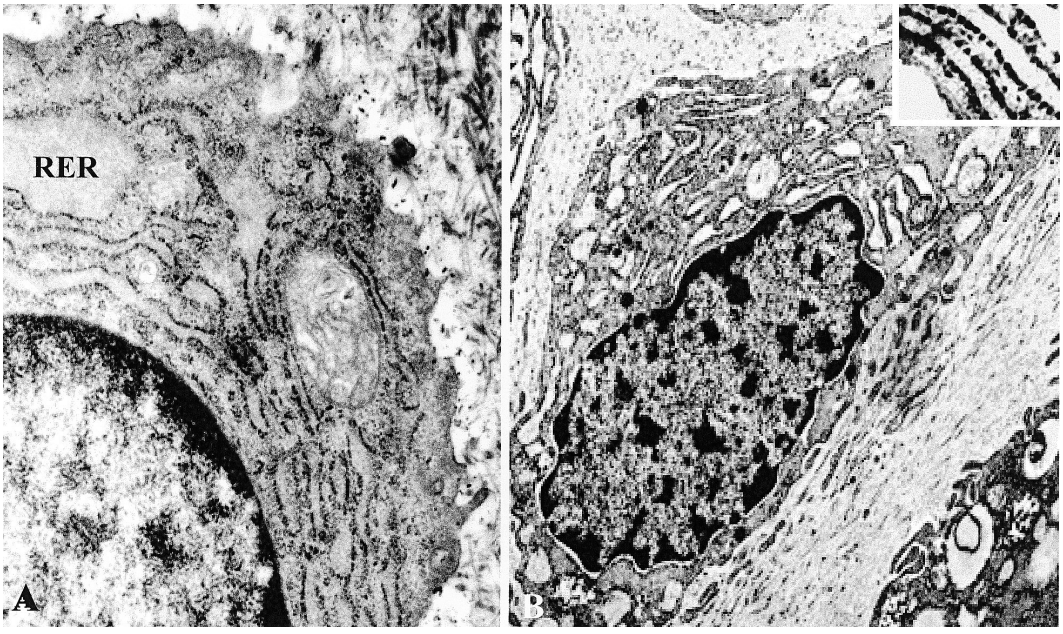

Fig. 2. A. Micrograph from OA-induced rat model (5 days after surgery) of superficial zone chondrocytes. Notice the rounded cell shape and the development of rough endoplasmic reticulum (RER); ribosomes associated with the membranes are clearly observed. **B.** Micrograph form OA human cartilage. Clustered chondrocyte is observed with a prominent RER showing dilated cisterns. This chondrocyte display a fibroblast-like shape. Notice in both micrographs the light appearance of the cytosol. Ribosomes are clearly observed bounded to the RER membranes (Inset). These cells might correspond to Type 2a chondrocytes. A, x 27,000; B, x 12,000; inset, x 35,000

relationship with the ECM, plays an important role in cell shape and function. Studies on the structure and organization of actin, vimentin, and tubulin in normal chondrocytes have been reported (Zanetti and Solursh, 1984; Hirsch and Hartford-Svoboda, 1993; Benjamin et al., 1994; Idowu et al., 2000; Langelier et al., 2000). Changes in the organization of these proteins are linked to several pathological states (Pena et al., 1983; Takeda et al., 1992; Mashima et al., 1999; Bajo et al., 2001). It seems this is also the case for OA chondrocytes.

By means of ultrastructural and immunohistochemical procedures, we showed that upper zone chondrocytes, mainly of clustered cells from OA patients, displayed a disrupted distribution of actin, vimentin, and tubulin (Kouri et al., 1998) (Fig. 5). Later we found similar results in chondrocytes from an OA rat model (Capin et al., 2004)

Golgi complex and endoplasmic reticulum (ER)

Prominent Golgi and RER in chondrocytes have been associated with the secretion of extracellular matrix components (Wong and Plaas, 1995; Martinez et al., 1977), drug effects (Moskalewski et al., 1975; Annefeld 1985; Stevens et al., 1985), development (Segawa et al., 1988), and clustered chondrocytes from papain-induced cartilage defect in an experimental model (Scheck et al 1975). More recently, our group found that human and OA-induced rat cartilages also show prominent Golgi and ER (Kouri et al., 1998, 2002a,b), suggesting increasing synthetic activity of the OA chondrocytes (Fig. 2-4). The latter might be related to the strong tendency of osteoarthritic articular chondrocytes to repair eroded cartilage matrix with type II collagen, as previously reported (von der Mark et al., 1992). Moreover, the relationship between chondrocyte ultrastructure and the synthesis of extracellular matrix molecules has been determined. In a recent review, Tseche and Miosge (2004) reported that fibroblast-like chondrocytes, synthesize perlecan (molecule involve in the development and maintenance of the cartilage), decorin and biglycan, though not collagen type II, in areas adjacent to the main defect of the cartilage. These authors suggest that these fibroblast-like chondrocytes, which might correspond to Type 2a chondrocytes reported among upper zone OA chondrocytes (Kouri et al., 1996a), are involved in the regeneration of the extracellular matrix of the damaged tissue. Astonishingly, upper zone chondrocytes have also been described to synthesize MMP-3 over their inhibitor (Pelletier et al., 1994; Abbud-Lozoya and Kouri, 2000), a cysteine protease that disrupts the ECM. This strongly suggests that during the pathogenesis of osteoarthritis, chondrocytes shift their synthesis pattern from reparative to degradative mode, which leads to cartilage breakdown.

Recently, we assessed MMP-3 immunolabels in clustered human OA chondrocytes and found a substantial increase of this enzyme compared to normal cells (Fig. 7); moreover, to determine the possible relationship between MMP-3 synthesis and apoptosis we immunolabelled MMP-3 in human OA cartilage previously labelled for the TUNEL procedure (unpublished data). We found that some chondrocytes showed only MMP-3 labelling, while others showed only TUNEL staining. Confocal merged images showed some cells with both markers.

These results and the ultrastructural evidence that several morphological phenotypes were observed within clustered chondrocytes, including apoptosis, suggest that chondrocyte modifications are asynchronic or is not a synchronic event. This also poses the question of the role of chondrocyte synthesis of MMP-3 and its involvement in cartilage degradation and cell death.

On the other hand, during cartilage breakdown, chondrocytes from the lower zones of the tissue increase their alkaline phosphatase synthesis (Kouri et al., 2000). Both upper and lower zone chondrocytes increase and modify their synthetic activity within OA pathogenesis. Upper zone chondrocytes are involved in matrix degradation, while lower zone chondrocytes are

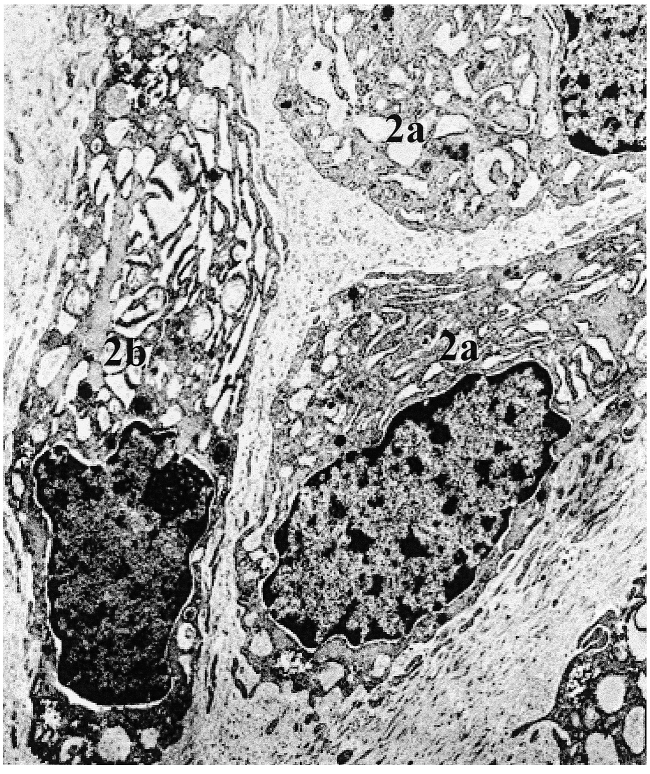

Fig. 3. Electron micrograph from OA human cartilage of three chondrocytes showing a rough endoplasmic reticulum (RER), displaying an abundant and dilated cisterns containing a material inside. These chondrocytes might correspond to 2a and 2b Type. The latter exhibited a more dilated RER, the cytosol is denser and the nucleus was contracted and denser compared to 2a Type chondrocyte. x 8,000

associated with subchondral bone remodelling.

In addition, modifications in the synthetic pattern of OA chondrocytes, which must certainly be linked to the development of ER and Golgi, have been related to programmed cell death by apoptosis. Lane et al. (2002) reported that the Golgi ribbon is fragmented into clusters

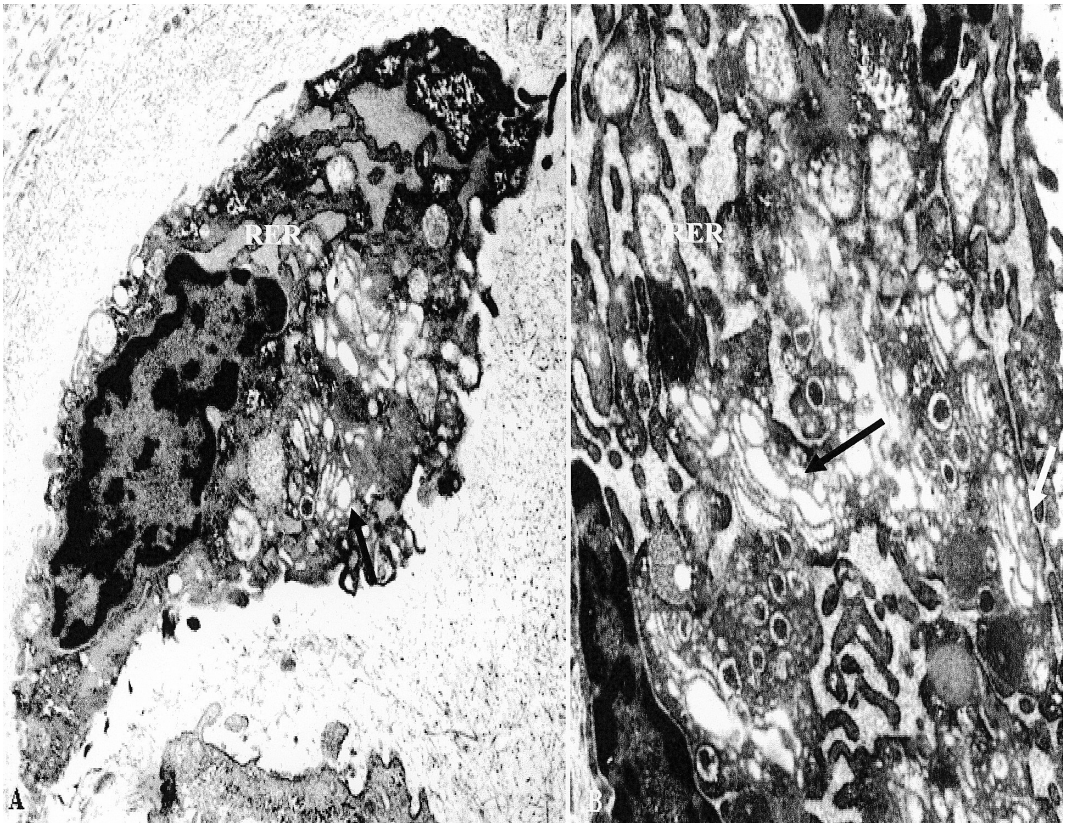

Fig. 4.A, B. Electon micrograph from OA human cartilage of two chondrocytes showing a prominent, fragmented and expanded Golgi Complex membranes (arrows). The rough endoplasmic reticulum (RER) displayed abundant and dilated cistens contanin a material inside. Cytosol was extremely dense. These might corresponde to the initial changes of the Type 3 chondrocytes (chondroptosis). Magnifications: 8 000 X and 20 000 X, repectively.

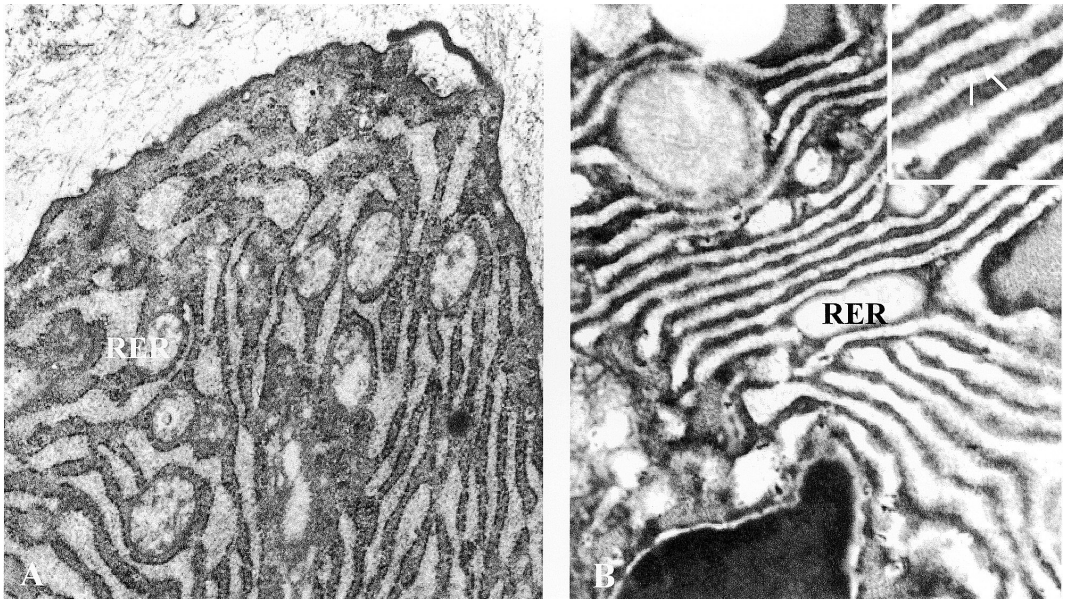

Fig. 5. Two chondrocytes from human OA cartilage. **A)** Notice the presence of abundant rough endoplasmic reticulum (RER) dilated cisterns containing osmiophilic material. Cytosol displays a dense and contracted appearance, although ribosomes are still noticeable. **B)** A chondrocyte showing a extremely dense nucleus with a very irregular morphology (down-left). The cytosol is also particularly dense. This chondrocyte displays abundant RER where ribosomes are hardly observed (Inset arrow) . The cell displays a nebulous appearance. These cells (A and B) might correspond to Type 3 chondrocyte (chondroptosis). A, x 14,000; x 12,000; inset, x 20,000

of tubulovesicular membranes and that fragmentation is caspase-dependant. They further identified a 65kDa stacking protein (GRASP65) as a substrate of caspase. Furthermore, it has been demonstrated that caspase-2 was localized at the Golgi complex, in addition to its nuclear distribution (Mancini et al., 2000). Therefore, chondrocyte cell death is another important issue, which requires examination within OA pathogenesis.

Chondrocyte Cell Death

In normal cartilage, some chondrocytes show morphological characteristics of death by necrosis (Kouri et al., 1996a) and others die by apoptosis (Kouri et al., 1997; Blanco et al., 1998; Hashimoto et al. 1998a,b; Kim et al., 2000), which is increased in osteoarthritis (Sharif et al., 2004).

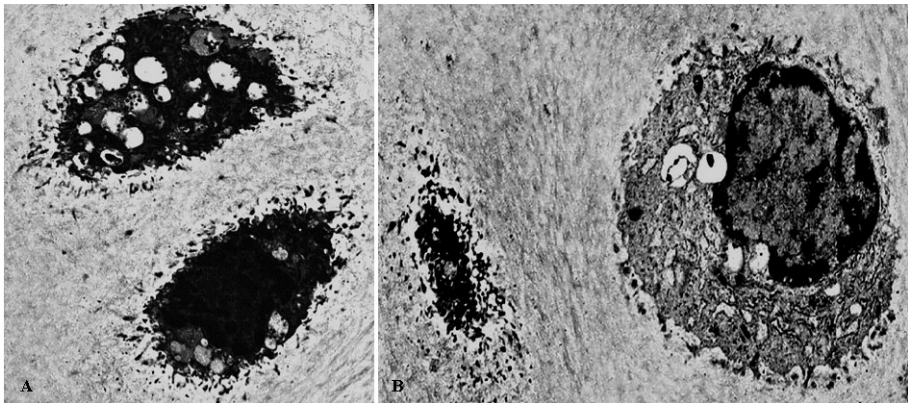

Fig. 6. Clustered human OA chondrocytes. **A.** Notice the extreme condensation of the cells, which are disintegrating. These might correspond to the final stage of chondroptosis. **B.** Two clustered chondrocytes from a human OA cartilage. In the right part of the micrograph, a Type 2a chondrocyte is observed. To the left, a chondroptotic disintegrating chondrocyte is observed. x 10,000

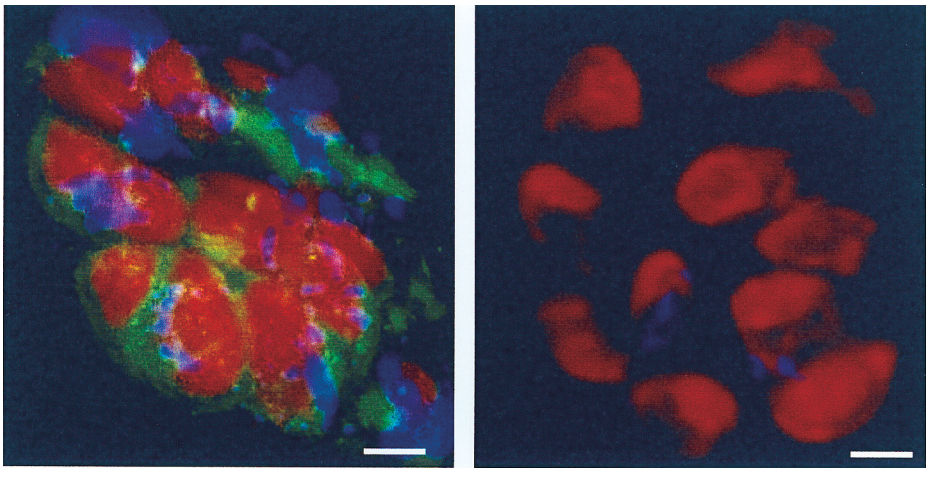

Fig. 7. Merging of confocal microscopy micrographs from OA human cartilage, showing two different clustered chondrocytes from the same tissue. **A.** Notice the disruption of the cytoskeleton of actin (green) and vimentin (blue). In **B**, the clustered cells show practically no immunolabels for these two cytoskeletal components. Nuclei were counterstained with propidium iodide (red). Magnification: Bars: 10 µm.

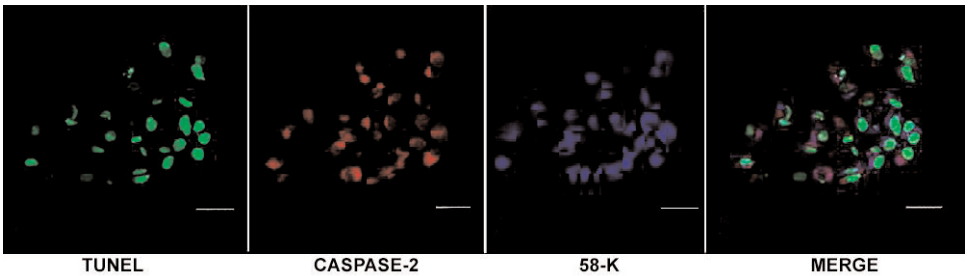

Fig. 8. Confocal micrograph of human OA clustered chondrocytes showing the relationship between TUNEL labelling (green), caspase-2 (red) and Golgi 58-K protein. The merged image (extreme right) shows the coincidence of these three markers in most of the chondrocytes. Bars: 10 µm.

In the past years, a different ultrastructural pattern for dying chondrocytes in the growth plate (Roach and Clark, 1999, 2000) and articular cartilage (Kouri et al., 1997, 2000, 2002) has been documented, displaying characteristics different to the classical apoptosis described by Kerr et al. (1972). The term chondroptosis was proposed to reflect the fact that such cells were undergoing apoptosis in a non-classical manner (Roach et al., 2004). Although chondroptosis has some features in common with apoptosis it differs from it by prominent Golgi and rough endoplasmic reticulum (Fig. 6). Recently, we co-localized Golgi complex protein 58K with caspase-2L immunolabeling, in apoptotic TUNEL positive chondrocytes from human osteoarthritic cartilage (Perez et al., 2005). As a result, two markers for apoptotic chondrocytes were found to be related to a prominent Golgi complex, suggesting that the development of the secretory pathway was involved with apoptosis within OA chondrocytes (Fig. 8). This result agrees with the present tendency to emphasize the role of stress of the cell secretory machinery as an important apoptosis inductor (Maag et al., 2003) and suggests that this might be an important pathway in chondroptosis induction.

Perhaps the characteristic that differentiates chondroptosis the most is its ability to eliminate cell detritus without inflammation in situations in which phagocytosis could be difficult (Roach et al., 2004). Nevertheless, we have recently shown that cultured chondrocytes ingest cell debris and latex particles (Castillo and Kouri, 2004), suggesting that these cells may undergo phagocytosis in vivo. This is also suggested by frequent findings in rat OA chondrocytes of cytoplasmic vacuoles filled with small particles and fibres. Similar material has also been observed in human OA cartilage. Phagocytosis may be present, although in a limited manner, in the removal of tissue detritus in OA cartilage, especially in regions where the ECM has been disrupted, where phagocytosis could easily occur.

Chondrocyte death results in cell disintegration

This is associated with: cytoplasmic budding (Fig. 5), release of membrane-bounded vesicles (frequently observed within the deep zone of the OA cartilage) (Kouri et al., 2000) and, finally, fragmentation of dead cells, with some remnants containing portions of the nucleus (Kouri et al., 2002a). These cell-derived fragments might be considered apoptotic bodies; however, there is also the preference to restrict the term of "apoptotic bodies" to those structures seen in the final stages of classical apoptosis (Roach et al., 2004).

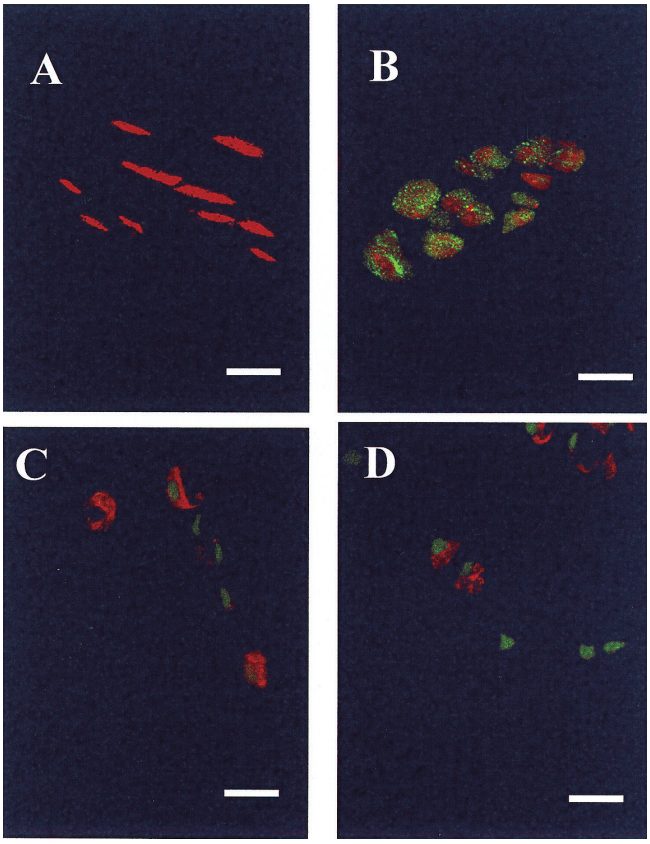

Fig. 9.A, B. Confocal microscope image of normal **(A)** and OA human cartilage **(B)** showing MMP-3 immunolabelling (green) in clustered chondrocytes, while normal chondrocytes did not show any immunolabelling. Nuclei were counterstained with propidium iodide (red). **C and D.** Confocal microscopy images of human OA cartilage. MMP-3 (red) and TUNEL (green) labellings were performed. Some cells show labelling for only one marker, while few other display both labellings. Bars : 10 µm.

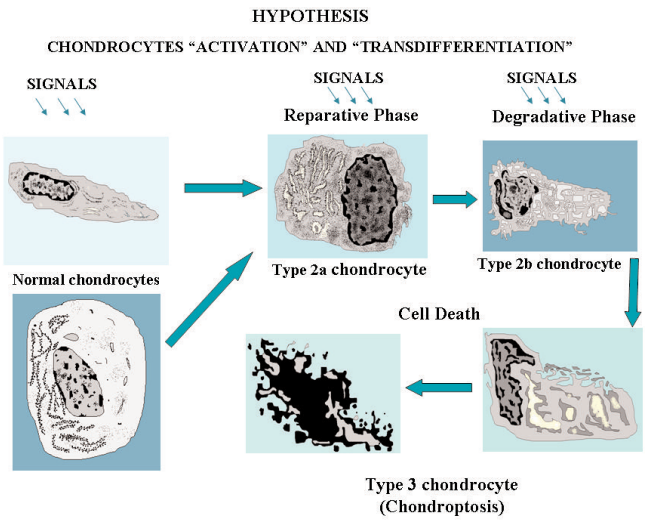

Fig. 10. Schematic representation of the different chondrocyte phenotypes described in OA cartilage

Hypothesis regarding the role of chondrocytes in OA pathogenesis:

The remarkable morphological changes in OA chondrocytes, which include cytoskeleton disruption, prominent Golgi/ER, chondroptosis and cell/matrix interactions, found in the most damaged tissue region, might certainly correspond to different physio-pathological stages of OA cartilage, shifting from a reparative to a degradative pattern. This major modification might be related to ECM disruption and cell death. Based on these findings, we hypothesize that normal chondrocytes undergo "activation" and go through major changes leading to cells with a secretory phenotype ("transdifferentiation"), which trigger a recovery mechanism followed by ECM degradation and chondroptosis when repair is unsuccessful (Fig. 9).

A number of reports might support the current hypothesis, as assessed in the following paragraphs.

It has been assumed that any factor capable of modifying cell shape and the cytoskeleton, would lead to lytic enzyme expression at the genomic level (Lombardi et al., 1993), supporting the reported relationship between cytoskeleton disruption and induction of modifications in expression genes (Nanette and Solurch, 1984; Lot et al., 1995). This supports the idea that chondrocyte cytoskeleton changes are probably related to the modifications of their synthetic activity.

In addition, cytoskeleton disruption and the proteolytic breakdown of the ECM should certainly alter the ECM/chondrocytes relationship, which has been documented to activate apoptosis. Also, it has been recognized that apoptosis might be mediated by a reorganization of the cytoskeleton, which leads to the release of the pro-apoptotic signal (Puthalakath et al., 2001). Also, it has been acknowledged that ß1-integrin serves as a link between the cell cytoskeleton and the ECM, and that it also mediates cell/matrix interactions that provide survival signals for chondrocytes. The loss of such interactions and the inability to respond to IGF-1 stimulation may be partly responsible for the hypocellularity and matrix degradation that characterizes OA (Hirsch et al., 1997; Gogg et al., 2003). Therefore, the modifications of chondrocyte structure and functions might be associated with apoptotic chondrocyte cell death.

Further studies are required to determine which signals are involved in the different steps leading to matrix degradation and chondroptosis.

Abnormal calcification of the subchondral bone

Bone remodelling is another subject related to OA pathogenesis. It is recognized that during the endochondral ossification of the growth plate, lower zone chondrocytes release matrix vesicles, which are involved in physiological matrix mineralization and later on by apoptosis (Hashimoto et al., 1998a; Kirsch et al., 2003). However, the budding of cellular detritus, extrusions, vesicles or any structure (Kouri et al., 2000) irregularly considered an apoptotic body were found increased in human OA cartilage around deep zone chondrocytes of the articular cartilage. These structures were released from the deep zone chondrocytes through cytoplasm budding. They displayed abundant ALP activity and increasingly showed hydroxyapatite crystals as they came closer to the subchondral bone. The subchondral bone, underlying the normal cartilage, showed that the Ca^{++}/P ratio was similar to other bones, however this ratio was found to be remarkably variable in OA cartilage. This might contribute to focal variability in the hardness and elasticity of the subchondral bone, which might determine an uneven distribution of the loads causing a mechanical distress within the cartilage/bone interphase. This mechanical distress may possibly be involved in the onset and progression of cartilage loss and substitution by a subchondral bone, which shows an abnormal Ca^{++}/P ratio. The role of apoptosis in this process has been proposed (Kouri et al., 2000); further studies are being carried out related to this subject.

Conclusions

An overall assessment in this review might be that all the processes described herein, such as cell cycle and chondrocyte proliferation, chondrocyte synthetic activity, chondroptosis, and subchondral bone remodelling, are important processes that need to be dissected within the mechanism of OA pathogenesis and are therefore open to debate. New approaches, like the study of inflammatory molecules, will certainly help scrutinize OA pathogenesis signalling, with the hope of finding new therapeutic strategies in this invalidating disease entity.

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