Chondroptosis: A variant of the Classical Apoptosis. A Confocal Microscopy Study

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Programmed cell death (PCD) by apoptosis has been studied in human osteoarthritis (OA) and experimental OA models (1-4). The occurrence of this type of death has been reported by a broad number of author using several methods, such as cellular ultrastructural studies, immunolocalization and western blot assays of pro-apoptotic proteins, mainly caspases, TUNEL histoenzimatic procedure for *in situ* labeling of nuclear DNA fragmentation, agarose DNA laddering and ELISA agarose gel electrophoresis for internucleosomal DNA fragmentation. Although TUNEL technique has been the most common practice used to detect apoptosis, however this procedure has been controversial due to the diversity in the results obtained by different authors. Therefore, the most likely approach to determine apoptosis is the use a combination of technical approaches.

Recently, the concept that apoptosis might have different characteristic from the classical apoptosis described by Kerr and Willies (5) has been strengthen. This might be the case for chondrocytes apoptosis within the articular cartilage and the growth plate cartilage. The term chondroptosis was proposed to reflect the fact that such cells are undergoing apoptosis in a non-classical manner that appears to be typical in chondrocytes death in vivo (6). Although chondroptosis has some features in common with classical apoptosis and to other types of cell death (Table1), such as cell shrinkage, chromatin condensation and the probable involvement of caspases, other features are different such as, an prominent Golgi and ER . Perhaps the one of the most significant difference of chondroptosis relates to not being dependent on phagocytosis, which may serve to eliminate cells detritus without inflammation in situations in which phagocytosis would be difficult.

Noteworthy, *in vitro* studies observed with the Confocal Microscope (Fig.1) we described the phagocytic capability of chondrocytes (7). This support the idea that phagocytosis by neighbours chondrocytes is possible, although it might be very rare. Furthermore, in osteoarthritic cartilage has been described small particles and fibers engulfed by chondrocytes in vacuoles, which may well be related to phagocytosis. Similar findings were observed in human osteoarthritic cartilage, predominantly those comparable to the features described in late stages of the rat OA model.

In more recent times, the ultrastructural observations were complemented with the immuno colocalization of Golgi 58K protein with caspase-2 within many TUNEL positive (Fig.2) as observed with a Confocal Microscopy of osteoarthritic human chondrocytes (8).

All these changes plausibly might be associated to the shift of OA chondrocyte synthesis activity, which shifts to a degradative pattern synthesizing metalloproteases over their inhibitors (9), which are involve in the extracellular matrix (ECM) degradation; this certainly alter the relationship ECM/chondrocytes.

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	Classical Apoptosis	Paraptosis	Necrosis-like	Necrosis	Chondroptosis
Nuclei and chromatin	-Condensed -Nuclear fragmentación	-Chromatin poorly condensed no nuclear fragmentation	-No chromatin condensation	-No nuclear fragmentation	-Nuclear Condensation
Cytoplasm y organelles	-Condensation + fragmenrtation -No vacuolzation	- Extense citoplásmic vacuolization - Mitochondrial swelling		-Cytoplasm and organelles swelling	-Prominent Golgi y ER - Autophagic vacuoles - Cytoplásmic Extrution -Empty Lacunes
Blebbing	-Present			-Cell membrane disruption	
Apoptotic bodies	-Present	-Absent			
Caspases	-Caspasa-3	-Independient	-Absent -Ocasionally caspases-1,8		-Present
Fosfatidilserina Translocation	-Present				
TUNEL	-Present	-Absent		-Absent	-Positive

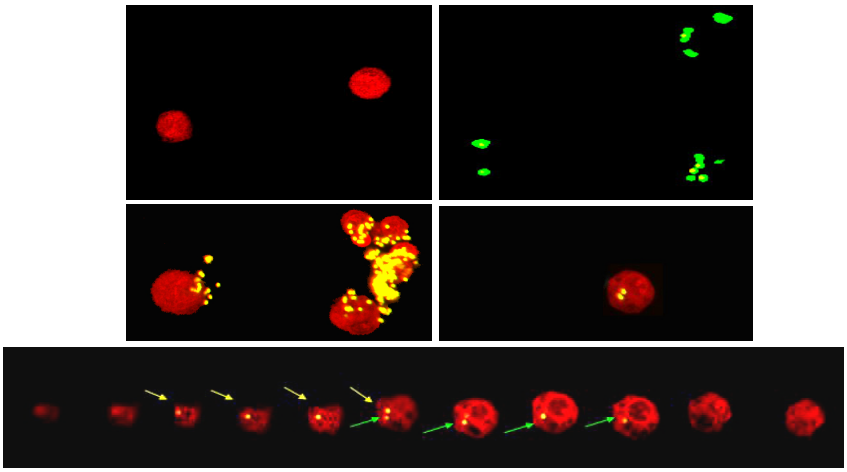

Figure 1. Confocal microscopy: A: Chondrocytes stained with propidium iodide. B: FITC-labelel latex particles. C: projection of several optical section from chondrocytes with latex particles inside and outside them. D: Projection of a chondrocyte with two latex particles engulfed. E: eleven optical sections of 0.5 mm in series z of a chondrocytes that showed 2 fluorescent latex particles (yellow and green arrows). Latex particles are observed inside the cells in the optical sections 3 or 9, and did not appear in the optical sections 1-2 and 10-11, indicating that they were inside the cells X 60

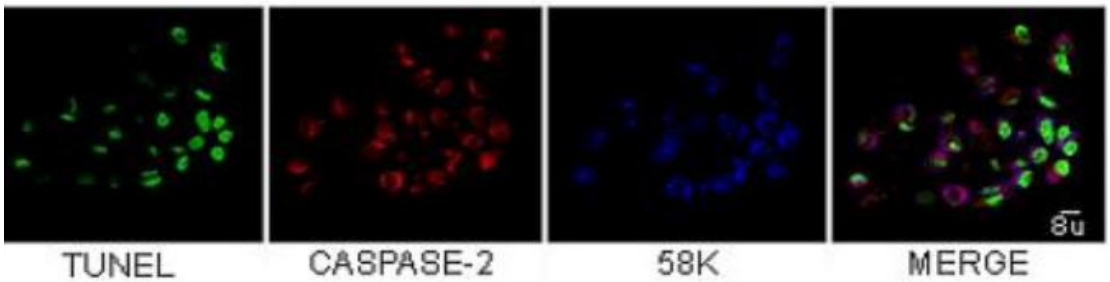

Figure 2 . Triple labelling of TUNEL (green), caspase-2 (red) and 58-K Golgi protein from clustered chondrocytes. The merge of the three labeling shows the co localization of caspase-2 and Golgi within many TUNEL positive chondrocytes. This show that in chondroptotic cells that the prominent Golgi complex caspase-2 synthesis was activated.