

CHONDROPTOSIS: AN IMMUNOHISTOCHEMICAL STUDY OF APOPTOSIS AND GOLGI COMPLEX IN CHONDROCYTES FROM HUMAN OSTEOARTHRITIC CARTILAGE.

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The Golgi complex in addition to participate in the protein traffic has been related to programmed cell death by apoptosis. Experimental evidences demonstrated the localization of caspase-2L in the Golgi (1). Also, has been reported the fragmentation caspase dependent of the organelle into clusters of tubulovesicular membranes (2). Previously, we have detected modifications of the Golgi complex in chondrocytes from an osteoarthritis induced rat model (3). Additionally, apoptosis is known to be involved in the pathogenesis in human osteoarthritis (4, 5, 6, 7). Recently, it has been suggested the term chondroptosis as a variant of the classical apoptosis in chondrocytes (8). Here, we colocalized the immunolabeling of Golgi complex protein 58K with caspase-2L immunolabeling, in apoptotic TUNEL positive chondrocytes from human osteoarthritic (OA) cartilage. Condyles were removed from patients with grade IV osteoarthritis during knee total replacement surgery, without prior trauma or any metabolic disease and without steroid treatment. Condyles from non-osteoarthritic donor cadavers were used as controls. Samples were fixed with 4% PBS-paraformaldehyde and were incubated with monoclonal anti-Golgi 58K or polyclonal antibody anti-caspase-2L followed by FITC-tagged antimouse IgG or FITC-tagged anti-rabbit IgG. Nuclei were counterstained with propidium iodide. For negative controls the primary antibody was omitted. When performing double labeling, sections were incubated with a mixture of both primary antibodies, followed by a mixture of both secondary. For double labeling and DNA fragmentation, sections were stained first for immunofluorescence, simultaneously, with a mixture of anti-Golgi 58K and anticaspase-2L, with CY5-tagged anti-mouse IgG and TRITC-tagged anti-rabbit IgG, respectively. This was followed by the histoenzimatic TUNEL technique for DNA fragmentation, using an in situ cell death detection kit with fluorescein. Samples were observed with Confocal System (Bio-Rad MRC 600) and a Confocal Leica Microscope (TCSP2 Leica). In control specimens, labeling with the 58K Golgi protein was scarce and compact, located in the paranuclear region in some chondrocytes (Fig. 1A). In contrast, in OA chondrocytes the Golgi labeling appeared as numerous granules finely dispersed in great part of the cytoplasm, predominantly within the upper zones clustered chondrocytes (Fig. 1B). Quantification of the Golgi labeling intensity from normal and OA cartilage showed a remarkable difference between both groups. Caspase-2L in normal cartilage, appeared as finely punctuate immunolabeling and extremely scarce restricted to the yuxtannuclear region cytoplasm of some chondrocytes (Fig. 1C). By contrast, OA chondrocytes display abundant caspase-2L labeling, which showed a granular pattern throughout the cytoplasm, also located predominantly within the upper zones clustered chondrocytes (Fig. 1D). Furthermore, when the average fluorescence intensities were assessed, a significant difference was found between the normal and OA cartilage. Co-localization between caspase-2L with 58K immunolabeling was observed in most clustered OA chondrocytes, suggesting an association between caspases-2L and the Golgi complex (Fig. 1 E-F). Nearly half of clustered chondrocytes displayed Golgi 58K/caspase-2L co-localization, within cells with positive TUNEL nuclei. However, others showed chondrocytes that displayed only 58K labeling, or caspase-2L labeling, or TUNEL labeling alone cells (Fig. 1 G-H). Our results made us speculate that within the damaged region of OA cartilage most cells develop the secretory pathway, which might first trigger a supposed recovery mechanisms followed by chondroptosis since repair was unsuccessful. The different combinations of the three labelings (58K, caspase-2L and TUNEL) described here, might well be associated to dissimilar stages of the catabolic metabolism from clustered chondrocytes during the progression of cartilage degradation.

References

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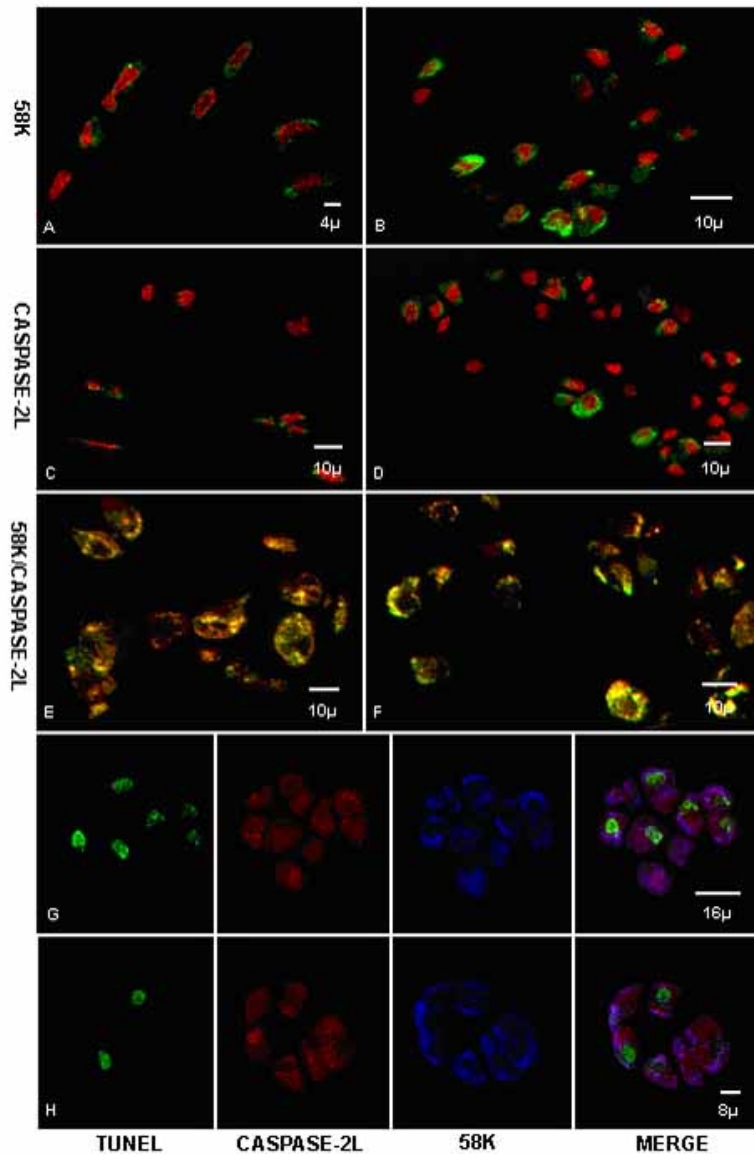


Figure 1. Golgi complex and apoptosis by immunofluorescence microscopy. Chondrocytes of normal (A) and OA cartilage (B) stained with anti-Golgi 58K (green); normal (C) and OA cartilage (D) stained with anti-caspase-2L (green). Nuclei were counterstained with propidium iodide (red). Double immunolabeling (E, F). Regions of overlap between both proteins are shown in the merge yellow. Double immunolabeling and DNA fragmentation (G, H).