ROLE OF TRANSGLUTAMINASE 2 IN APOPTOSIS OF HUMAN CHONDROCYTES INDUCED BY HYDROGEN PEROXIDE

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INTRODUCTION
Chondrocyte viability is essential for the maintenance of articular cartilage as they constitute the only cell type in articular cartilage. Apoptosis is a form of programmed cell death that is processed by specific intracellular signaling cascades and thus has been an attractive therapeutic target for diseases in which apoptosis is an integral part of its pathogenesis. Apoptosis of chondrocytes has been suggested as an important part of the pathogenesis in osteoarthritis.

Transglutaminase 2 (TGase 2) is a member of the transglutaminase family that serves as a bifunctional enzyme for catalyzing both Ca²⁺-dependent protein cross-linking and Ca²⁺-independent GTP and ATP hydrolyses. In several experimental models, TGase 2 has been shown to be induced and activated during apoptosis. However, the role of TGase 2 in apoptosis is not clear as different experimental systems exhibit both anti- and proapoptotic features.

In this study, we sought to determine the expression of TGase 2 in human chondrocytes undergoing apoptosis induced by hydrogen peroxide (H₂O₂) and to explore the role of TGase 2 in chondrocyte apoptosis.

METHODS
Human chondrocytes were obtained from the knee articular cartilage of patients undergoing total joint arthroplasty and cultured in monolayer. Chondrocyte apoptosis was induced by H₂O₂ and apoptosis was assessed by a combination of three methods, biochemically by nuclease enzyme-linked immunosorbent assay (ELISA) and Annexin-V/propidium iodide flow cytometry, and morphologically by nuclear staining for 4'6-Diamidine-2'-phenyllindole (DAPI). The expression of TGase 2 was examined with quantitative PCR, Western blot, in situ enzyme activity assay and immunocytochemistry. In situ enzyme activity was evaluated by determining the incorporated biotinylated pentylamine using horseradish peroxidase-conjugated streptavidin. The role of TGase 2 was evaluated by quantifying the difference in the amount of apoptosis before and after treating the cells with monodansylcadaverine (MDC), a competitive substrate of TGase 2.

RESULTS
1. H₂O₂ induced apoptosis of human chondrocytes in a dose-dependent manner. After 24 hours of treatment with H₂O₂, chondrocyte apoptosis was detected at concentrations of H₂O₂ as low as 0.25mM (Fig 1A). At a concentration of 1 mM of H₂O₂, time-dependent apoptosis of chondrocytes was observed. Apoptosis was detected as early as 3 hours and increased over 72 hour period (Fig 1B).

Figure 1. Induction of apoptosis of human articular chondrocytes cultured in monolayer induced by H₂O₂. A: Apoptosis was quantified using Annexin V FACS analysis. Numbers in the lower right quadrant represent percentage of apoptotic cells. A: Dose-dependent induction of apoptosis B: Time-dependent induction of apoptosis

2. The expression of TGase 2 in human chondrocytes in response to H₂O₂ treatment was examined. TGase 2 mRNA and was detected in all controls to determine the possible direct cytotoxic effects of MDC.

Figure 2. Increased expression of TGase 2 in chondrocytes treated with H₂O₂ A: RT-PCR B: Quantitative real-time PCR C: In situ enzyme activity

3. The role of TGase 2 in apoptosis of human chondrocytes was examined using MDC, a competitive inhibitor of protein-cross linking activity of TGase 2. Chondrocytes treated with only MDC were used as controls to determine the possible direct cytotoxic effects of MDC.

Figure 3. Immunocytochemical staining of increased expression of TGase 2 in apoptotic chondrocytes induced by H₂O₂. Human articular chondrocytes were treated with 0mM (A, B) or 1mM (C, D) of H₂O₂ for 12 hours. Immunocytochemical staining of TGase 2 protein (green fluorescence) and TGase 2 activity (red fluorescence; proteins incorporated with BP) were carried out. Concomitant staining of nuclei with DAPI was performed for identification of apoptotic cells. Note the increased red fluorescence in apoptotic cells with condensed (C) and fragmented (D) nuclei (Original magnification x 200 (A) C, x 630 (B) D).

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MDM increased the percentage of apoptosis in H₂O₂-treated cells in a dose-dependent manner (Fig 4A&B). The percentage of apoptotic cells was greater in H₂O₂-treated cells than in control cells, thereby excluding the possibility of cytotoxic effects of MDC.

Figure 4. Increased apoptosis in chondrocytes treated with MDC, a TGase 2 inhibitor. The chondrocytes were incubated with indicated concentrations of MDC in the presence (1mM) or absence of H₂O₂ for 24 hours. A: Representative Annexin V FACs analysis. B: Quantification of apoptotic cells

DISCUSSION
We demonstrated that TGase 2 expression is increased in human chondrocytes undergoing apoptosis and inhibition of TGase 2 promotes apoptosis. These results suggest a possible protective role of TGase 2 against apoptosis in human chondrocytes and may raise the possibility of TGase 2 as a modulator of cartilage damage in osteoarthritis by protecting against chondrocyte apoptosis.

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