Upregulation of IAP Proteins by Cartilage Oligomeric Matrix Protein (COMP)/Thrombospondin 5 Protects Cells from Apoptosis

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Introduction. Cartilage oligomeric matrix protein (COMP) or Thrombospondin 5 is an extracellular matrix (ECM) protein expressed primarily in cartilage, tendon, ligament and synovium. COMP may play a role in the structural integrity of cartilage by interacting with other ECM proteins (aggrecan, collagen and fibronectin). Increased concentration of COMP and its fragments in human serum and synovial fluid correlates with the severity of cartilage degradation and apoptosis in osteoarthritis (OA), rheumatoid arthritis and bone fracture. Since chondrocyte death and COMP degradation are a hallmark of arthritic cartilage and since many ECM protein serve important biological functions other than structural, such as in cell survival, we wondered whether COMP plays a role in cell viability. Here we report that COMP induces the expression of the Inhibitors of Apoptosis Proteins (IAP) family of antiapoptotic proteins and provides a direct protective mechanism against cell death. Taken together, our data provide a novel anti-apoptotic function of COMP.

Methods. The 293 and HeLa cell line were purchased from the ATCC. Human chondrocytes were isolated from the cartilage of OA patients. Cells were grown in monolayer culture in Dulbecco’s modified Eagle’s medium and were transfected with DNA constructs using calcium phosphate transfection (293 cells) or AMAXA nucleofector solution (HeLa, chondrocytes), according to the manufacturer’s procedures. Cells harvested from cultures were processed for RNA extraction followed by RT-PCR or for cell cycle analysis. Cell extracts were generated and immunoblotted by standard procedures. Cell death was assessed by TUNEL test analysis.

Results. To explore the function of COMP we have expressed the gene in a variety of cell types. We found that COMP overexpression in 293 (Fig.1 A) and chondrocytes (data not shown) increased the viable cell number by 3-4 fold. Our data show that the survival effect was due to a decrease in apoptosis that was confirmed by TUNEL test and cell cycle analysis (Fig.1 B). We found a 3-fold decrease in apoptosis in COMP expressing cultures. Furthermore the apoptotic pathway is caspase-dependent. As seen in the immunoblot (Fig.1 C), active caspase 3 is present in the control transfected cells but its level is reduced in the COMP transfected cells.

To identify the mechanism by which COMP protects cells from apoptosis we examined a variety of antiapoptotic and antiapoptotic proteins. We find that COMP elevates the IAPs such as cIAP2, Survivin and XIAP (Fig.1 D). The levels of these proteins are increased by COMP but their corresponding RNA levels are not increased suggesting that COMP regulates these proteins at the posttranslational level.

We have also recently assessed the levels of XIAP in human OA chondrocytes transfected with the COMP expressing plasmid. Our data show that XIAP is significantly upregulated in COMP expressing chondrocytes (Fig.2).

Figure 2. COMP induces XIAP in human OA chondrocytes. Extracts were generated from human OA chondrocytes transfected with a vector control or COMP expressing plasmid. These extracts were used in immunoblot assays for the indicated antibodies.

To demonstrate that XIAP mediates survival in COMP-expressing cells, we performed siRNA experiments. 293 cells were transfected with either a COMP expression plasmid or a vector control, in the presence or absence of an XIAP siRNA. Cell number was assessed 48 hours post-transfection. As seen in Fig. 3A, COMP was no longer able to enhance survival in cells expressing the XIAPsiRNA. Thus reducing XIAP levels in these cells blocks the survival effect of COMP.

Since arthritis is a disease that can be initiated by cytokines such as TNF alpha, it was important to determine if COMP could block cell death mediated by this apoptotic inducer. We first assessed the ability of COMP to block apoptosis in Hela cells treated with TNF alpha, since these cells are very sensitive to this cytokine and show a dramatic apoptotic response to low dose TNF alpha (10ng/ml). As seen in Fig. 3B, TNF alpha is able to induce death in these cells resulting in a five-fold reduction in cell numbers. Importantly COMP expression in these cells was able to very efficiently block induction of death by TNF alpha As shown in Figure 2B. When cell extracts were generated and assayed for XIAP levels, we found that XIAP was dramatically upregulated in the COMP transfected Hela cells, both in the presence and absence of TNF alpha (Fig.3 C). These data indicate that COMP can block death receptor signaling, and it is likely due to an effect on XIAP induction.

Figure 3. XIAP mediates the survival effect of COMP. (A) The COMP expression plasmid was transfected into 293 cells with either a control siRNA (COMP) or with an XIAPsiRNA (COMP+XIAPsiRNA). Shown are the total viable cell numbers at two days posttransfection. (B) COMP expression blocks the apoptotic effect of TNF alpha. Hela cells were Amaxa transfected with either a vector control or the COMP expressing plasmid. At one day after transfection, the cells were treated with or without 10ng/ml TNF alpha. The cells were cultured for an additional 24 hours and either viable cell numbers were assayed (B) or extracts were generated and used in an immunoblot with the indicated antibodies (C).

Discussion. These data indicate a novel function for the COMP protein in that it blocks apoptosis by increasing the levels of the IAP proteins in 293 cells and human chondrocytes. It is known that COMP undergoes cleavage during cartilage degenerative diseases, resulting in fragments in serum and synovial fluid. Since evidence indicates that COMP is cleaved in arthritic cartilage in part due to the action of the TNF alpha, it suggests the survival function of COMP may be lost in this disease, leading to increased chondrocyte apoptosis, a hallmark of arthritis. Our data point to a novel role of COMP in a human cartilage protective mechanism.

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