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Session: Bone, Cartilage and Connective Tissue Matrix: Cartilage and Chondrocytes

Presentation Number: M128

Title: Non-covalent Interaction of MMP13 and LTBP1 in a Unique TGFb Large Latent Complex Produced by Hypertrophic Chondrocytes

Presentation Start: 9/17/2007 11:30:00 AM

Presentation End: 9/17/2007 2:30:00 PM

Category: E - Growth Factors, Cytokines, Immunomodulators

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Our lab has shown that hypertrophic chondrocytes produce a unique TGFb2 large latent complex that contains collagenase 3 (MMP13) in non-covalent association with latent TGFb binding protein (LTBP1). In this study, we investigate hypertrophic chondrocyte production of the elements of this unique TGFb2 large latent complex. Hypertrophic chondrocytes from the avian sterna were cultured 5 days in serum-free alginate. RNA, conditioned media, cell-associated matrix (isolated after release of the chondrocytes from alginate), and cell extracts were analyzed for TGFb2, MMP13 and LTBP1. Immunoblot analysis revealed the presence of immunoreactive bands for TGFb2, MMP13 and LTBP1 in the cellular extracts. Assembly into the extracellular matrix and secretion of the components was indicated by the presence of immunoreactive bands for all three proteins in the cell-associated matrix and conditioned media fraction. Immunocytochemistry demonstrated the presence of TGFb2, MMP13 and LTBP1 in association with the cells and in a pericellular staining pattern. In order to determine whether MMP13 binds non-covalently to LTBP1 of the TGFb large latent complex, primary monolayer cultures of hypertrophic chondrocytes were double-labeled with antibodies against MMP13 and LTBP1. Optical serial sections were collected from these cells and the images deconvoluted into three-dimensional micrographs. LTBP1 and MMP13 were present both intracellularly and in association with the extracellular matrix between cells. In addition, the fluorescent signal indicated substantial co-localization of the two proteins within the extracellular matrix of the cultures. For additional confirmation, we produced a biotin-labeled peptide corresponding to the C-terminal hemopexin domain of MMP13 that bioinformatics indicated could interact non-covalently with the C-terminal EGF-calcium domains of LTBP1. Protein from hypertrophic chondrocyte tissue was extracted from day 17 avian upper sternum and immunoprecipitated with antibody to LTBP1. Dot blot analysis of the immunoprecipitate showed binding of the biotin-labeled MMP13 peptide with proteins in the tissue extracts. These data demonstrate that the components of the unique TGFb2 large latent complex are produced and secreted by hypertrophic chondrocytes and are present in compartments that are characteristic of extracellular matrix assembly. In addition, these data confirm that MMP13 can interact with LTBP1 and underscores the candidacy of MMP13 in the unique mechanism of activation of TGFb by hypertrophic chondrocytes.