TYPE II COLLAGEN PEPTIDE INTERACTIONS WITH ANNEXIN V RECEPTOR ON HUMAN ANKLE CHONDROCYTES

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INTRODUCTION: Type II collagen, a major component of cartilage, consists of a triple helical domain along with N and C non-helical domains. A variety of naturally occurring fragments can be generated by various enzymes. Matrix metalloproteinase – 13 is known to cleave type II collagen within the N-telopeptide; this cleavage releases N-telopeptide and in the process disturbs the fibrous network. Treatment of ankle explants at physiological concentrations (1000ug/ml) with collagen fragments (enriched in N-telopeptide) resulted in an increase in proteoglycan synthesis at 8 days, however there were no significant changes in the release of proteoglycans. It is unclear which receptors are involved in collagen type II peptide (N, C, and helical peptides) binding. The objective of this work was to characterize receptor specific interactions.

METHODS: In order to define the effect that collagen telopeptides have on surface binding, synthetic peptides (synthesized by Dr. Lee (UIC) ) were generated to correspond to the N, C, and helical peptides of human type II collagen. The sequences were obtained through the NIH molecular biology database. Synthetic type II collagen peptides were conjugated to rhodamine according to manufacturer’s directions.

Cell culture: For monolayer or cell suspension cultures, the chondrocytes were obtained from human ankle joints from Regional Organ Bank of Illinois (ROBI) and released by pronase and collagenase P. The tali were graded according to a scale of Collins. Grades 0-2 were used to acquire the data. Flow cytometry: Chondrocytes were suspended for 2h at 37°C in DMEM/F12 and 5% Fetal Bovine Serum (FBS) and then incubated with either 1 mg/ml synthetic N-terminal peptide, 1 mg/ml synthetic C-terminal peptide or 1 mg/ml synthetic helical peptide for 1h at 4°C. Cells were analyzed by FACScan. Flow cytometry profiles were also used to assess binding to the annexin V receptor. Chondrocytes were incubated with antibodies to annexin V to block the receptor and subsequently incubated with 1 mg/ml rhodamine synthetic N-terminal peptide, 1 mg/ml rhodamine synthetic C-terminal peptide or 1 mg/ml rhodamine synthetic helical peptide. All blocking experiments were performed at 4°C.

Immunofluorescence: Chondrocytes were cultured in monolayer for 24h in DMEM/F12 and 5% FBS with 1 mg/ml synthetic N-terminal peptide, 1 mg/ml synthetic C-terminal peptide or 1 mg/ml synthetic helical peptide.

RESULTS

Labeled synthetic peptides bound chondrocytes in monolayer cultures or in suspension.

Rhodamine peptides were added to cell suspensions or to monolayer culture. Figure 1 (a, b, c) demonstrates rhodamine binding to cells in monolayer.

DISCUSSION

These data demonstrate that N-telopeptide interacts with annexin V, while C-telopeptide and helical peptides do not. Also, due to the punctate pattern seen around the cells treated with C-telopeptide, we postulate that C-telopeptide interacts with chondrocytes through integrin receptors.

REFERENCES


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