IL-1 and TNF-α Stimulate Pregnancy-Associated Plasma Protein A Production in Human Annulus Cells in Three-Dimensional Culture.

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INTRODUCTION:
Pregnancy-associated Plasma Protein-A (PAPP-A) is a newly recognized metalloproteinase expressed by several cell types, including fibroblasts, osteoblasts and smooth muscle cells. PAPP-A has an extremely important role because it cleaves IGFBP-2, -4 and -5 in the extracellular matrix (ECM), thereby modulating local IGF bioavailability to nearby cells. We have recently identified the presence of this metalloproteinase in the intervertebral disc, and shown that it is significantly upregulated in cells in more degenerated discs (1). However, little is known about the regulation of PAPP-A gene expression in disc cells. We hypothesize that PAPP-A expression is regulated in the degenerating disc by cytokine stimulation, and report here our findings on the stimulation of PAPP-A production by IL-1 and TNF-α in human annulus cells in three-dimensional (3D) culture.

METHODS:
Following our Institutional Review Board approval, human annulus cells were derived from surgical disc specimens and cultured in 3D within a collagen sponge using previously published methods (2,3). Cells were cultured in 3D for 9 days with media changes in the presence of control (minimal essential medium supplemented with 20% FBS (MEM20) or treated media; cells then grew for 5 days without feeding. Upon termination, conditioned media were collected and frozen. IL-1 (Pierce Biotechnology, Rockford IL) or TNF-α (Fitzgerald Industries International, Concord, MA) were added to the cultures at concentrations of 10^-1 to 10^4 pM. Cytokines were tested in a total of 8 annulus cultures; cells were derived from one Thompson grade II, two grade III, and five grade IV surgical specimens. PAPP-A levels in cell-conditioned medium were measured with the Quantikine Human Pappalysin-1/PAPP-A kit (R&D Systems, Inc., Minneapolis, MN) following kit instructions. Assay sensitivity was 0.053 ng/mL. Each culture was tested in duplicate and results averaged. Standard statistical analyses included ANOVA (GraphPad Instat 3, San Diego, CA). Data are expressed as means ± s.e.m.

RESULTS:
A dose-dependent increase in PAPP-A production by 3D cultured human annulus cells was seen after treatment of cells with IL-1 (Figure 1) (p = <0.0001). IL-1 showed maximal effectiveness at 10^3 pM, with a 37 fold increase over control levels.

DISCUSSION:
The pro-inflammatory cytokines IL-1 and TNF-α were chosen for study here because of their high importance during disc degeneration; we now know that disc cells produce these cytokines in vivo and also respond to them (for reviews, see 4,5). Data presented here has further advanced our understanding of the interplay between PAPP-A and IL-1 and TNF-α by looking directly at accumulation of PAPP-A in cell-conditioned medium during cytokine exposure in 3D. Our findings that PAPP-A production is stimulated by IL-1 and TNF-α suggests a mechanism for the regulation of PAPP-A in response to the degenerative processes involved in development of disc degeneration.

REFERENCES:

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