MEK-1 REGULATES AGGRECAN GENE EXPRESSION THROUGH EXON 1 (5' UTR)

*Palmer GD, Ho JY, Hung CT, Ratcliffe A and +Valhmu WB; *Orthopaedic Research Laboratory, Columbia University, New York, NY

Introduction: Stimulation of chondrocytes by environmental factors including growth factors, cytokines, and mechanical stresses activates signal transduction pathways that can modulate gene expression. However the mechanisms by which chondrocytes transduce intracellular signals to the nucleus are poorly understood. Mitogen Activated Protein Kinases (MAPK) have been found to be stimulated in rabbit articular chondrocytes in response to cytokines IL-1 and TNFα [1]. We have previously shown that these cytokines regulate human aggrecan gene expression through specific regions of the promoter [2]. In this study we investigate the role of MAPK kinase (MEK-1), on aggrecan gene expression. We also perform transient transfection studies with promoter deletion clones in order to identify regions of the aggrecan promoter subject to regulation by the MAPK pathway.

Methods: Cell culture: Cartilage was harvested from bovine calf joints within 4 hours after slaughter. Chondrocytes were isolated by collagenase and hyaluronidase digestion and plated in monolayer culture on 30mm dishes at $4x10^5$ cells per dish. Plated cells were then either transfected with reporter constructs or treated with PD 098059 and later assayed for aggrecan mRNA levels.

RNA extraction ,Reverse Transcription and Quantitative PCR: Total cellular RNA was extracted by the acid gaunidinium method of Chomczynski and Sacchi [3]. Aggrecan cDNA was amplified using the method of non-competitive QPCR [4]. PCR products were quantified using computer image analysis of agarose gels, and standardized to total RNA (quantified by spectrophotometry).

Transfection of promoter constructs: Luciferase reporter constructs containing various portions of the human aggrecan promoter were made as described previously [2]. Constructs were transiently transfected in equimolar amounts with a β-galactosidase cotransfecting plasmid by calcium phosphate coprecipitation for 48 hrs in the presence of 10% FBS. Cell extracts were assayed using the Luciferase Assay System (Promega) and GalactoLight Assay Kit (Tropix).

Addition of MEK-1 inhibitor, PD 098059: PD 098059 was added to monolayer cultures 48 hrs after plating for both the RT-PCR and transfection experiments to a final concentration of 25μM (unless otherwise stated) in DMEM containing 10% FBS. Cells were incubated for a further 24 hrs in the presence of inhibitor, before harvesting. Data was analysed using student's t-test ($p=0.05$).

Results: Treatment with PD 098059 caused a 4-fold increase in aggrecan mRNA levels ($p<0.03$) (fig. 1). Treatment with PD 098059 increased the luciferase activity of the full promoter construct, pAGC1(-2368)/5'UTR, dose dependantly from 5μM to 25μM. A maximum 2.5 fold increase was observed at 25 and 50 μM concentrations (data not shown). The luciferase activity of all promoter deletion constructs containing the 5' UTR were significantly ($p<0.001$) increased after PD 098059 treatment (fig. 2). A maximal 4-fold increase was observed with the -1508 deletion clone and further reductions in promoter length to -52 bp did not significantly reduce the stimulatory effect of the inhibitor. However, PD 098059 only increased luciferase activity 1.2-fold when the 5' UTR was removed from the full promoter construct, pAGC1(-2368) ($p<0.05$). PD 098059 failed to stimulate a minimal thymidine kinase promoter construct, pTKLuc, and also a promoter construct containing 1.6 kb of the aggrecan promoter upstream region, p1671TK.

Discussion: We have shown that inhibition of MAPK kinase (MEK-1) by addition of PD 098059 increases aggrecan mRNA levels and promoter activity in primary chondrocytes. Subsequent deletional analysis of the human aggrecan promoter region has shown that induction of promoter activity via the MEK-1/MAPK pathway is primarily mediated through the 5' UTR. This result is consistent with previous studies where it has been shown that the 5' UTR has an essential role in controlling basal aggrecan gene expression. It is possible that the MAPK pathway regulates aggrecan synthesis by modulating general transcriptional factors that associate with the 5' UTR.

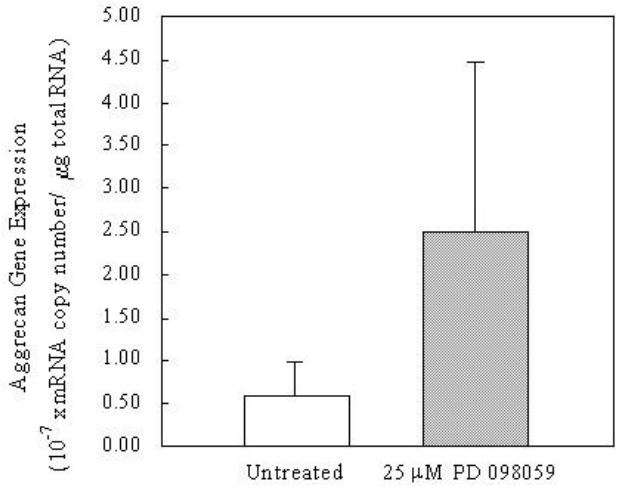

Fig 1. Effect of PD 098059 on aggrecan mRNA levels.

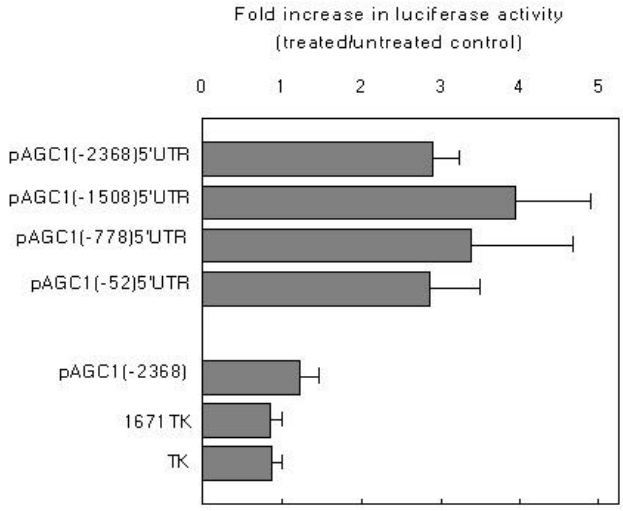

Fig 2. Activities of promoter constructs after PD 098059 treatment.

Acknowledegements: This work was funded in part by OREF grant #95-024 (WBV), and NIH grants AR43597(WBV) and AR42003(AR).

References:

[1] Scherle et al. Biochem.Biophys.Res.Comm. **230(3)**: 573-577, 1997
[2] Palmer et al. Trans.Orthop. Res.Soc **23**:107, 1998
[3] Chomcynski et al. Anal.Biochem **162**:156, 1987
[4] Re et al. Anal Biochem **225**:357, 1995

Corresponding Author:

+Wilmot B. Valhmu, PhD., Orthopaedic Research Laboratory, 630W 168 st., BB-1412, New York, NY 10032; Phone: (212) 305-6446; FAX: (212) 305 2741; e-mail: valhmu@cuorma.orl.columbia.edu.

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

☒ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.