

## Investigations using an Ovine Annular Lesion Model of Experimental Intervertebral Disc Degeneration

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### INTRODUCTION

Intervertebral disc (IVD) lesions have a very poor healing capacity and are problematic to treat clinically. Controlled experimental annular lesions [1] also have a limited repair potential. The outer margins of such defects heal spontaneously within 3 mth, however their innermost regions do not heal even after a 2 yr recovery period and may propagate to form circumferential and radial tears affecting the nucleus pulposus (NP) leading to proteoglycan loss, NP degeneration and loss of IVD function. One of the problems inhibiting repair processes in the annulus fibrosus (AF) is that granulation tissue in the defect site is not effectively resorbed and replaced by new functional annular lamellae. Hyaluronan oligosaccharides (HA oligos) have been shown to stimulate a number of cell types to produce elevated levels of active matrix metalloproteases (MMPs) capable of ECM remodelling. Furthermore, HA oligos also stimulate articular chondrocytes to up-regulate a number of anabolic ECM genes (collagen-II, aggrecan, HAS-2) [2]. HA oligos therefore have the potential not only to stimulate matrix removal but also its replacement by de-novo synthesis. The aim of this study was to assess HA oligos for the promotion of ECM remodeling and matrix repair in an established ovine model [1, 3].

### METHODS

Ovine AF and NP cells were established in monolayer and alginate bead culture in the absence and presence of HA oligos (10-16 mers, 0.05-1 g/ml). Conditioned media samples were collected for MMP analysis by gelatin zymography, cells were isolated and RNA extracted with TRIzol for RT-PCR to determine relative mRNA expression levels for MMP-2, 9; aggrecan and type I and type II collagen on days 2, 5 and 10 in bead culture. The HA oligos were also assessed in an ovine annular lesion model [1, 3]. The HA oligos (10 mg/ml) were administered to annular lesion sites via a gelatin sponge, no treatment and carrier plus sponge were also assessed for their abilities to promote repair of the annular lesion over a 3 mth recovery period. This was

determined using a histological scoring scheme which evaluated lesion depth, degree of AF re-integration, proteoglycan loss, extent of blood vessel in-growth and macromolecular annular collagen re-organisation.

### RESULTS

AF cells were poorly responsive to the HA oligos (0.05-1 g/ml) in monolayer culture with proMMP-2 levels only marginally elevated and MMP-9 unaffected. In contrast, proMMP-2 production by NP cells in monolayer culture displayed a strong dose dependant increase, MMP-9 was not affected. In alginate bead cultures the AF and NP cells were both responsive to the HA oligos which significantly elevated MMP-2 and MMP-9 activity in a dose dependant manner. Aggrecan, Type I and II collagen expression in the AF and NP cells however were differentially regulated by the HA oligos and were up-regulated in NP cells and down regulated in AF cells. The HA oligos did not significantly improve the healing response (as assessed by histological criteria) in the ovine AF lesion model [1, 3].

### DISCUSSION & CONCLUSIONS

MMP-2 and 9 activity were both up regulated in AF and NP cells by the HA oligos however this was not evident at the mRNA level indicating that the HA oligos did not act at the transcriptional level at least with these MMP genes. In contrast, the anabolic matrix genes (aggrecan, type I/II collagen) were differentially regulated by the HA oligos in the AF and NP cells. The effect on NP cells was similar to the stimulatory properties displayed by HA oligos on articular chondrocytes [2] but clear differences were evident between the regulation of these genes in AF and NP cells.

**REFERENCES:** <sup>1</sup>Osti OL, Vernon-Roberts B, Fraser RD (1990) *Spine* **15**:762-7. <sup>2</sup>Knudson W. et al (2000) *Arth Rheum* **42**: 1165-74. <sup>3</sup> Melrose J et al *J Orthop Res* **10**: 665-76.

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