Differential Responses of Human Nucleus Pulposus Cells with Varying Degenerative Levels to TGFβ3, CTGF, and Link-N

INTRODUCTION

Matricellular proteins offer promise to slow progression of intervertebral disc (IVD) degeneration\(^1\), yet little is known of their potential to modify the pathogenesis of human degenerated IVD cells, and how that compares to growth factor therapies. It also remains unknown if degenerative grade is an important factor in establishing treatment efficacy. This study evaluated regenerative potential of matricellular proteins CTGF and Link-N as compared to TGFβ3+Dexamethasone, on nucleus pulposus (NP) cells isolated from grade IV (moderately) and grade V (severely) degenerated IVDs from symptomatic patients.

METHODS

Isolation and culture of human NP cells from surgical samples:

NP tissue was obtained from patients undergoing anterior interbody fusions for low back pain secondary to degenerative disc disease with institutional review board approval and graded as either moderately or severely degenerated. NP cells were released from tissue by sequential enzymatic digestion (0.2% pronase, 0.2% collagenase), filtered through a 70-μm mesh, washed with PBS, and expanded in culture. Cells were trypsinised and either used for qPCR analysis (day 0) or resuspended in alginate beads at a density of 2x10^6 cells/mL alginate. Cells were cultured for 21 days in Basal containing TGFβ3-Dex (10 ng/mL TGFβ3, 10% Dex), CTGF (100 ng/mL), or Link-N (100 ng/mL). Cell constructs were assayed for metabolic activity by the MTT assay, DNA content by the Picogreen assay, glycosaminoglycan (GAG) content by the DMMB assay, and gene expression with a custom SYBR green PCR array of 42 genes.

Dependent Variables:

- **MTT**: Metabolic activity of the cells was assessed by incubating alginate beads in a 2 mg/mL solution of MTT for 4 hours. Alginate beads were then dissolved (55mM Sodium citrate, 30mM EDTA, 0.15M NaCl for 10 minutes), and read at an optical density of 570 nm and normalized to the control basal reading.

- **DMMB assay**: To determine the amount of GAG produced during culture, alginate constructs were dissociated, centrifuged, and 400μl of a digestion buffer (100 mM NaOAc, 10mM EDTA, 0.1 mM L-cystine, 300 μg / mL Papain stock, in distilled water) was added to each pellet and supernatant and incubated for 20 hours at 60°C. The media collected over the culture period, as well as the solutions containing digestion buffer were assessed using separate standard curves corresponding to the digestion buffer and basal media. Results were summed for each group.

- **DNA content**: Cell Pellets were analyzed with the Picogreen dsDNA quantitation Kit (Invitrogen) according to their instructions.

- **Gene expression**: RNA was isolated, cDNA synthesized and a custom RT profiler PCR array (SA Biosciences: CAPH-0817A) was run. Relative gene expression was calculated using the comparative Ct method normalized to 3 housekeeping genes and values of cells at day 0.

RESULTS

MTT demonstrated a decreased metabolic activity with CTGF and Link-N in severe patients, while GAG amounts were similar for all groups (Figure 1). Moderately degenerated cells showed greater regenerative potential by responding to stimulation with increased proliferative capability (Picogreen), decreased IL1β and MMP9 expression, and ability to downregulate COL1A1, and up-regulate TGFβ receptors (Figure 2). When treating moderately degenerated NP cells, TGFβ3+Dex up-regulated anabolic gene expression (ACAN, COL2A1, SOX9), did not alter pro-inflammatory cytokine expression (IL1β and TNFα), and decreased COL1A1 and ADAMT5. Both matricellular proteins Link-N and CTGF had similar effects on NP cells, and were less able to alter the degenerated phenotype or reduce IL1β than cells treated with TGFβ3+Dex.

DISCUSSION

Moderately degenerated cells had a greater response to therapies than degenerated cells, and this is likely associated with higher TGFβ receptor expression. Matricellular proteins Link-N and CTGF were less able to reduce pro-inflammatory cytokines than TGFβ3+Dex and exhibited a more catabolic response, suggesting matricellular proteins may require anti-inflammatory supplementation. Based on the comparison of the these three treatment groups, we infer that growth factors may be more effective at stimulating cell proliferation and increasing metabolic rates than matricellular proteins alone.

SIGNIFICANCE

This human IVD cell culture model screens potential therapeutic targets that show promise to regenerate or slow the degeneration of IVD tissue. This study suggests, even on a cell level, that targeting moderate degeneration shows more promise than severe degeneration, and that pivotal treatments should include an anti-inflammatory agent.

REFERENCES:


Figure 1. ANOVA was performed with a p values for degeneration (Dp), media (Mp) and their interaction (Ip) indicated. (A) MTT absorbance at 570 nm (B) GAG measured by DMMB.

Figure 2. ANOVA was performed with p-values for degeneration (Dp), media (Mp) and their interaction (Ip) indicated. Significant effects of treatment groups and degenerative grade are represented by different capital and lower case letters, respectively. ~ indicates the gene was not expressed by that group. * represents significant differences from day 0 (p<0.05).