Intervening Intervertebral Disc Degeneration By Small Molecules That Positively Modulate Proteoglycan Production

Yi Sun¹, Yuen-Kee Tsui², Koichi Masuda², Richard Kao¹, Danny Chan³, Kenneth Cheung¹; Victor YL Leung¹.
¹The University of Hong Kong, Hong Kong, China; ²University of California San Diego, La Jolla, CA, USA.


Introduction: Intervertebral discs are proteoglycan-rich cartilaginous joints of the spine and have limited capacity to self-repair from injury or degeneration. Degeneration of the intervertebral disc is associated with low back pain [1]. As the major matrix macromolecules, proteoglycans provide compressive strength and viscoelastic properties to the cartilaginous tissues, and their loss has been indicated in the process of intervertebral disc degeneration. Nucleus pulposus (NP) cells are the active proteoglycan producing cells in the disc. In this study, we hypothesized that synthetic chemical compounds have potential in enhancing proteoglycan production and are competent to alleviate disc degeneration. We aimed to screen a chemical library to identify small molecules that have a function in upregulating proteoglycan production in NP cells and investigate if they can promote intervertebral disc repair.

Methods: We conducted a high-throughput screen of 50,000 diverse small molecules from a chemical library [2] to test their effects on glycosaminoglycan (GAG) production in primary porcine chondrocytes (n=3) using an optimized DMMB assay. MTT assay was used to monitor their effects on the global metabolic activity. The positive hits were subsequently tested in alginate-encapsulated bovine NP cells (n=4) and human degenerated NP cells (Schneiderman's scale grade III/IV, n=8) to validate their action in stimulating glycosaminoglycan (GAG) production and counteracting interleukin-1 induced catabolism (by DMMB assay, Q-PCR of aggrecan gene, fluorophore assisted carbohydrate electrophoresis, and gelatin zymography), and to reveal their dosage kinetics and cell specificity (by DMMB assay). The in vivo action of the compounds was tested in an adult Lewis rat tail intervertebral disc degeneration model. Rat disc degeneration was induced at C6/7 level by 19G needle puncture, followed by intra-discal injection of 2ul compound at EC50 on day 14 with 31G needle (n=4). Non-operated level (C4/5) and carrier injection (C5/6, 0.1% DMSO) were used as controls. GAG content and disc height index were determined by 14 days of compound injection (n=4).

Results: Through conducting multiple screening, 4 lead molecules were identified from the library that can upregulate GAG accumulation in a cell type-specific and dose-dependent manner (with EC50 below 10nM) without significantly effects on global cell activity. Carbohydrate analysis showed that they induce up to 2.5 fold increase in chondroitin-4-sulfate accumulation after 7-day treatment of the degenerated NP cell culture (Fig. 1). Two small molecules could upregulate aggrecan mRNA expression and suppress the IL-1 induced proteoglycan degradation and activity of MMP2 and/or MMP9 in degenerated NP cells. Interestingly, injection of the two compounds into degenerated rat discs significantly enhanced GAG content in the NP (up to 126% relative to degenerative control) and induced a recovery of disc space (up to 80% of normal) (Fig. 2), indicating their capacity of the compounds in alleviating or reversing the degeneration progression in vivo.

Discussion: Our study showed that specific synthetic small molecules in the chemical library are potent in enhancing proteoglycan production in NP cells and in promoting intervertebral disc repair. Their capacity in inhibiting IL-1 mediated proteoglycan catabolism and intervening the loss of disc function in the animal model suggests the compounds may possibly target key regulators involved in the pathophysiology of disc degeneration. These molecules may provide new tools for treating disc degeneration and possibly other related disorders such as osteoarthritis. Moreover, they may be utilized to better understand the pathway of proteoglycan metabolism, especially in cartilaginous tissues.

Significance: Intervertebral disc has limited capacity to self-repair. Our findings may facilitate the dissection of regulatory pathways of proteoglycan metabolism in the context of disc degeneration and potentiate the development of therapeutics to alleviate the disorder.

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Figure 1. Effects of 4 lead small molecules (C3,4,5,7) on chondroitin-4-sulfate (C4S) expression. C4S accumulation in alginate-encapsulated degenerated human NP cells was analyzed by FACE assay after 7-day treatment with the small molecules.

Figure 2. Capacity of small molecule in alleviating disc degeneration. Rat tail disc degeneration was induced by needle puncture for 14 days, followed by injection of one of the small molecules (C5). Disc height index was assessed with reference to Non-
operated (Normal) and punctured (Deg) discs (n=4). * and # indicate P < 0.05 in Mann-Whitney nonparametric test.

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