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SMALL-MOLECULE SUPPRESSORS OF PROTEOGLYCAN CATABOLISM IN DEGENERATIVE DISC CELLS

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INTRODUCTION: Proteoglycans (PGs) occupy the predominant extracellular matrix (ECM) components in cartilages and intervertebral discs (IVDs). Large numbers of compositional glycosaminoglycans (GAGs) render PG highly hydrophilic for providing water retention capacity and swelling pressure to the connective tissues to neutralize the axial mechanical loads [1]. Its loss of content is tightly associated with the tissue degeneration. In the intervertebral disc degeneration (IDD), a reduction of PGs in central gelatinous nucleus pulposus (NP) was characterized as the first event, which consequently leads to a replacement of fibrocartilaginous matrix, and loss of compressive strength and shock-absorbing abilities. This process may be promoted by imbalance of anabolic synthesis and catabolic degradation of PG, of which the breakdown of PG is mainly mediated by matrix degradation enzymes, such as metalloproteinases (MMPs), as they are thought to be dysregulated by the combined changes of proinflammatory cytokines and growth factors.

We have previously performed a chemical genetics study aiming to identify novel synthetic compounds that may be of pharmaceutical value for the treatment of IDD. Via sequential multiple screens, lead molecules were found with bioactivities in up-regulating the production of GAGs in chondrocytes and bovine NP cells. In this study our goal is to determine the potent hits with specifically promoting PGs production in human degenerative disc cells and rat-tail discs with induced-degeneration, and reveal their molecular mechanisms to interfere the PGs' degradation.

METHODS: NP cells (D-hNP) isolated from patients with lumbar disc degeneration (n=8) were cultured in alginate for 7 days, and subjected to hits treatment. Production of GAG was measured by dimethylmethylene blue (DMMB) assay and normalized to DNA content. Compositional disaccharides from chondroitin sulfate (CS) were calculated by FACE. Expression of aggrecan PG was assessed at protein and mRNA level by ELISA and RT-PCR respectively. To investigate the role of hits in catabolic degradation of PG, IL-1 alpha pre-conditioning was utilized for activation of catabolic activity, followed by combined hits treatments. Production of NO and gelatinase MMPs was evaluated by greiss reagent assay and zymography.

RESULTS:

1. Hits identification

Through our customized high-throughput screen (HTS) in chondrocytes on a chemical library containing 50,240 diverse small molecules, two hits were filtered out with dose-dependently stimulating GAGs' production in bovine disc cells (Fig. 1A), and this specific effect was further indicated in human degenerative NP cells (D-hNP) (Fig. 1B). Moreover, it was evidenced of chondroitin-4 sulfate (C-4-S) to cause the dose-sensitivity of GAG to hits compounds (Fig. 1C).

2. Molecular mechanism of hits compounds

As aggrecan PG possessed most abundant CS-GAG, we firstly investigated the aggrecan

expression under hits treatment. Interestingly, neither protein nor gene expression of aggrecan was altered (Fig. 2). Moreover, transcription factor, SOX9, presented an unaffected expression level. This suggested an irrelevant role of hits compounds with aggrecan core protein synthesis. IL-1 has been reported to be capable to evoke the catabolic degradation of PG due to the promoted expression of matrix degrading enzymes, such as MMPs and ADAMTs [2, 3]. Subsequent combined treatment with hits compounds in our study presented a recovery of GAGs' production (Fig. 3A). Surprisingly, in addition to sGAG it was also observed of the reversed expression of aggrecan protein in pCSG1-treated to control level, while no substantial differences was found in pCSG2-treated (Fig. 3B). Both of these two hits could diminish the up-regulation of MMPs activities by IL-1 (Fig. 3C). Nitric oxide involvement was previously demonstrated in IL-1 enhanced MMPs expression [3]. Here the promotion of NO production by IL-1 could be fully suppressed by pCSG2 (Fig. 3D). Our data strongly support the notion that hits compounds can interfere the catabolic pathway of PG production in disc degeneration.

DISCUSSION: This is the first study to present small molecular structures to modulate PGs' production in disc degeneration. Two hits identified from HTS showed their potentiality to intervene the disc degeneration process. To fish out their targets can not only facilitate our interpretation of proteoglycans metabolism, but also benefit our understanding of defense mechanism to disc degeneration. Our compounds can further be utilized to drug development for other proteoglycan disorder disease, such as osteoarthritis, and inflammation disease for their capability to anti-IL-1.

SIGNIFICANCE:

Our hits compounds may in future advance our understanding to proteoglycan metabolism in the context of disc degeneration and provide chemical structures for developing drugs to alleviate disc degeneration.

Figure 1. Hits identification with dose-dependent enhancement in GAGs' production. (A) Chemical structures of the best-acting promoters (pCSG) in HTS. (B) Dose-responses of GAGs production in D-hNP by DMMB assay. (C) FACE analysis of chondroitin sulfate GAG.

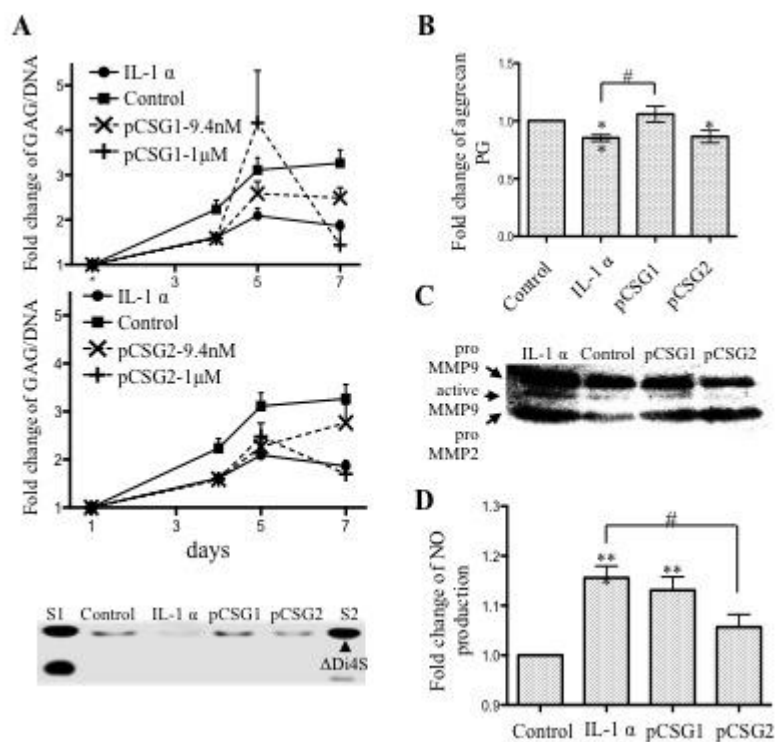
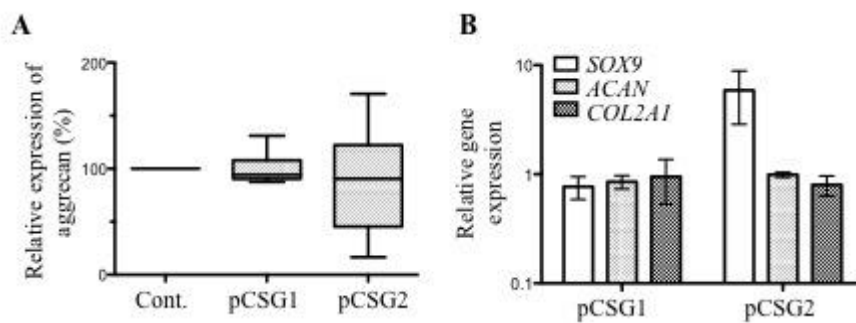
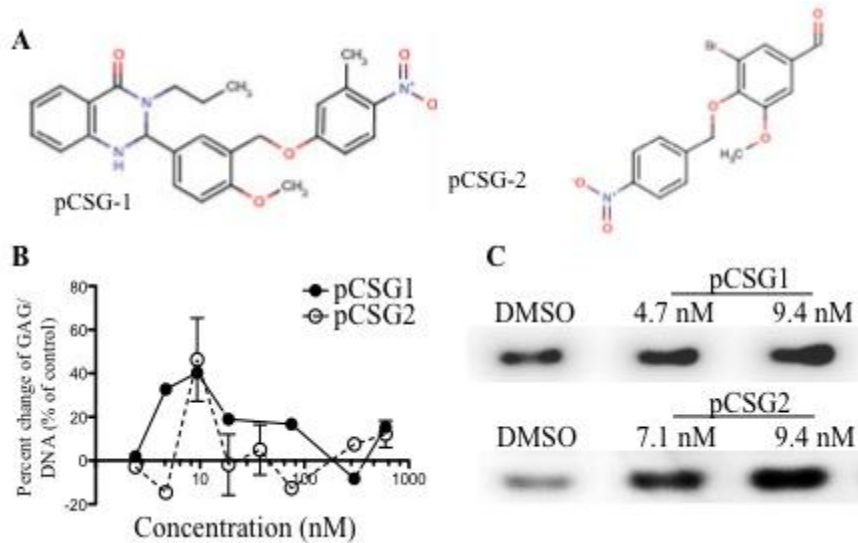
Expression of ?Di4S was predominantly detected in CS digests from D-hNP cells.

Figure 2. Effect of hits on aggrecan expression. Aggrecan PG expression was assessed via ELISA kit (A). (B) Gene expression of SOX9, aggrecan and collagen II.

Figure 3. Interference of hits to IL-1 induced-catabolic pathway. (A) Counterbalance from hits on IL-1 induced reduction of GAG by DMMB, specifically CS-GAG by FACE. Effect of hits on expression of aggrecan PG (B) and gelatinase MMPs (D), and NO production (E) was also assessed in IL-1 treated D-hNP. Statistic analysis was performed for the comparison of treatment groups to non-treated DMEM control (*), and compounds stimulated to IL-1 treatment group (#).

* p ? 0.05 versus DMEM; # p ? 0.05 versus IL-1; unpaired t test. S1 and S2 contain a mixture of ?disaccharides from top to bottom: S1 - ?Di6S and ?Di2, 6S; S2 - ?Di4S and ?Di4, 6S.

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