Identifying Therapeutic Chemical Agents for Intervertebral Disc Degeneration by High Throughput Screening

INTRODUCTION:
Intervertebral disc (IVD) degeneration may manifest as pain or disturbed segment mechanics that result in the production of proteoglycan (PG) content in the disc matrix. Strategies that can restore the PG content would be of therapeutic benefit to prevent, delay, or even reverse the progression of the lesion. Various clinical and experimental approaches have been adopted for pain relief or healing of the lesion. However, all these approaches require surgical intervention. Orally administered drugs, if available, could revolutionize the treatment of IVD degeneration by allowing non-invasive and repeatable treatments. Small chemical compounds are able to interact and alter the function of specific proteins that are involved in physiological or pathogenic pathways. With the advance in chemical synthesis and robotic technologies, novel drugs can be swiftly discovered from high throughput screening (HTS) of chemical libraries. We hypothesize that chemical compounds are capable of interfering with the proteoglycan metabolic pathway. This study aims to use a chemical genetics approach to identify potent chemical compounds that can modulate the PG content produced by IVD cells with the aid of a HTS platform and functional assays.

MATERIALS AND METHODS:
A systematic HTS process was carried out using a chemical library consisting of 50,240 diverse compounds (ChemBridge Co). The strategy of the HTS (Fig. 1) was to progressively narrow the number of positive candidates that could stimulate the production of glycosaminoglycans (GAGs) at the end of the process. The GAG accumulation was measured by the dimethylmethane blue (DMMB) assay, and the corresponding mitochondrial activity was measured by the MTT assay (n=3). The results from the assays are represented as percentage change in absorbance normalized with unperturbed controls. Primary porcine costal chondrocytes were used for the 1st and 2nd HTS, whereas primary bovine tail annulus fibrosis (AF) and nucleus pulposus (NP) cells were used for the 3rd HTS.

Validation was carried out using 3D culture of primary bovine tail NP cells in alginate beads, where the GAG accumulation after 7 and 14 days of treatment with the hit compounds was measured. The cell specificity of the effect of hit compounds was investigated by comparing the GAG accumulation from primary bovine tail NP and AF cells, with that from primary porcine chondrocytes, the human osteosarcoma cell line SaOs-2, and the mouse fetal fibroblast cell line BAL/Bc 3T3. The sulfation patterns of chondroitin sulfate (CS) in the GAG produced by NP cells after 14 days of hit compound treatment was investigated by fluorophore-assisted carbohydrate electrophoresis (FACE). The mean concentration of CS (n=3) produced by NP cells was determined semi-quantitatively using the calibration curves constructed using a series of concentrations of DAl-4S standard.

RESULTS:
The HTS primary and secondary screenings narrowed a chemical library containing 50,240 compounds to 960 and 25 positive candidates that were shown to stimulate PG production, respectively. The tertiary screening identified seven hit compounds from the 25 candidates, where the maximum percentage increase of GAG in NP cells was 8.11 ± 0.94% (compound concentration at 0.5 μM) and that in AF cells was 10.99 ± 0.01% (compound concentration at 5 μM). Validation tests indicated that after seven days of treatment in 3D culture, two of the seven hit compounds resulted in a significant increase of GAG accumulation compared with the medium control (242.2% & 135.7% for compounds F and U, respectively) (Fig.2), whereas, the corresponding cellular activities were similar to the medium control. After 14 days of treatment, one of them (compound U) remained effective in stimulating GAG production up to 57.2% and concomitantly elevates the cellular activity (9.3%). Cell specificity tests showed that this compound could induce a significant increase in GAG production from disc cells and osteosarcoma cells at 5 μM, but not from chondrocytes or fibroblasts at this concentration. FACE analysis showed that the NP cells only expressed chondroitin-4-sulfate (C4S), and treatment with the hit compounds did not induce expression of other types of CS (Fig. 3).

Fig. 1. Progressive high throughput screening (HTS) process. The number of hit compounds was narrowed from 50,240 to approximately 10 after the screenings at various concentrations of the chemical compounds.

DISCUSSION:
This study demonstrated the effectiveness of seven hit compounds from a chemical library that are capable of enhancing the production of PGs in disc cells. Three of them could regulate the PG content in long term 3D culture, and specifically modulated the level of CS4 in NP cells. In addition to their action on disc cells, all of the three candidates could also promote the GAG content in other cell types of the skeletal system including chondrocytes and osteoblasts. The most potent compound was able to stimulate an increase in GAG production in NP cells after both 7 and 14 days of treatment, and specifically stimulated GAG production in disc cells and osteoblasts at 5 μM. Future study will investigate possible mechanisms that may be associated with the metabolism of C4S to further dissect the molecular pathways involved in the compound-mediated up-regulation of PG content. This will facilitate the development of the compounds into orally-administered therapeutics for treating IVD degeneration.

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REFERENCES: