Identifying Therapeutic Chemical Agents for Osteoarthritis by High Throughput Screening

Tsui, Y K; Leung, V Y; Kao, R Y; Masada, K; Chan, D; Cheung, K M
+1Departments of Orthopaedics & Traumatology, 2Microbiology, and 3Biochemistry, The University of Hong Kong, HKSAR, China
1Department of Orthopaedic Surgery, University of California, San Diego, USA

Senior author cheungmc@hku.hk

INTRODUCTION:
An articular cartilage lesion, notably generated by osteoarthritis (OA), is initiated partly by the loss of proteoglycan content from the extracellular matrix and manifests as pain or disturbed joint function [1]. Strategies that restore the proteoglycan content would be of therapeutic benefit to prevent, delay, or even reverse the progression of the lesion. Numerous clinical and experimental approaches have been widely applied [2-4] to relieve the pain or induce healing of the lesion; however these require surgical intervention. Orally administered drugs, if available, may revolutionize the treatment of osteoarthritis by allowing non-invasive and repeatable treatment. Small chemical compounds are able to interact and alter the function of specific proteins that are involved in physiological or pathogenic pathways [5]. With the advancements in chemical synthesis and high-throughput technologies, novel drugs can be swiftly discovered from high throughput screening (HTS) of chemical libraries. We hypothesize that chemical compounds that are capable of modulating the metabolic pathway of proteoglycans would thus affect the proteoglycan level in cartilage. Using progressive high-throughput screening processes, this study aims to use a chemical genetics approach to identify chemical compounds that can promote the proteoglycan production by chondrocytes.

MATERIALS AND METHODS:
The primary HTS started with 50,240 diverse compounds (20 µg/ml) from a synthetic chemical library (ChemBridge). Each compound was plated into individual wells of 384-well plates with the automatic liquid handling station (Biomek 2000, Beckman Coulter). Primary chondrocytes isolated from porcine costal cartilage by aspetic dissection using pronase and collagenase II were then added to each well (30,000 chondrocytes/cm²). Assay plates were incubated at 37°C in 5% CO₂ for 72 hours. The glycosaminoglycan (GAG) content in the media was measured by the dimethylmethylen blue (DMMB) assay at 535 nm (reference to 450 nm) [6]. The corresponding metabolic activity was measured in parallel under similar conditions by the MTT assay at 570 nm (reference to 640 nm). The screening was performed in triplicate. The DMMB and MTT readouts were normalized with unperturbed control (media without addition of any chemical compound). The top 950 compounds prioritized by the DMMB readouts over the medians were validated with the secondary HTS. The compounds were re-arrayed in 96-well plates at a lower concentration (5 µg/ml) and similar assays were performed. Positive hits were finally identified according to the capability of the compound to promote the GAG content of the media (DMMB readouts) normalized by the cellular activity (MTT readouts).

RESULTS:
From the primary HTS, the top 950 chemical compounds enhanced DMMB readouts from 4.5 to 50%, compared with the unperturbed control. In the secondary HTS, 125 out of these 950 compounds retained the ability to produce over a 4.5% increment in DMMB readouts. Figure 1 shows the prioritized DMMB result, together with the corresponding MTT result. Negligible changes in the MTT assay were observed with 297 chemical compounds; the MTT readouts were within ± one standard deviation from the medium control. Out of these 297 compounds, the top 5 gave DMMB readouts, which were normalized to their corresponding MTT values, ranging from 7.0 ± 1.3 % to 19.0 ± 2.2 % (Fig. 2); they are regarded as positive hits for further investigation.

DISCUSSION:
The primary HTS was performed to exclude compounds that do not promote GAG production or may reduce cellular metabolic activities. However, inherent inaccuracies in compound transfer by the liquid handling platform in the primary screening may result in false-positives. Instead of identifying positive hits from the primary HTS, a secondary screening with manual pipetting was performed to validate the effects of the compounds.

REFERENCES: