View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by HKU Scholars Hub

Title	Identifying therapeutic chemical agents for osteoarthritis by high throughput screening
Author(s)	Tsui, YK; Leung, VY; Kao, RY; Masuda, K; Chan, D; Cheung, KM
Citation	The 55th Annual Meeting of the Orthopaedic Research Society, Las Vegas, NV., 22-25 February 2009.
Issued Date	2009
URL	http://hdl.handle.net/10722/61554
Rights	Creative Commons: Attribution 3.0 Hong Kong License

Identifying Therapeutic Chemical Agents for Osteoarthritis by High Throughput Screening

[1]Tsui, Y K; [1]Leung, V Y; [2]Kao, R Y; [4]Masuda, K; [3]Chan, D; +[1]Cheung, K M
+[1]Departments of Orthopaedics & Traumatology, [2]Microbiology, and [3]Biochemistry, The University of Hong Kong, HKSAR, China
[4]Department 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 administrated 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 aseptic digestion using pronase and collagenase II were then added to each well (30, 000 chondrocytes/cm^2). Assay plates were incubated at 37ºC in 5% CO_2 for 72 hours. The glycosaminoglycan (GAG) content in the media was measured by the dimethylmethylene 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.

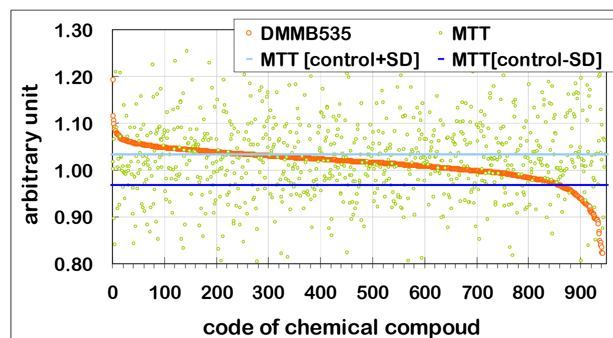

Fig. 1. Normalized assay readouts of 950 chemical compounds. ○ and ◊ represent the result of DMMB and MTT assay vs. unperturbed control; - and - refer to one standard deviation above and below (±) the MTT control, respectively.)

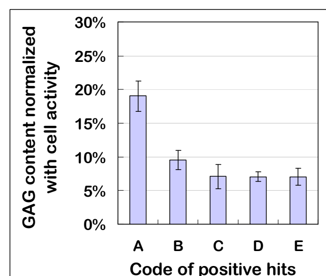

Fig. 2. The percentage increase of GAG content, normalized with cell activity (i.e., DMMB readouts normalized with the MTT readouts), of positive hits compared with the unperturbed control.

The *secondary HTS* suggests that there are chemical compounds capable of modulating the proteoglycan metabolism of chondrocytes without significant alteration of global metabolism or cell proliferation. In the future, the top 5 hits will be validated using human chondrocytes and kinetic studies will be carried out to determine the median effective concentrations to evaluate their efficacies. Gene expression profiling will be followed to investigate the genes involved for their action.

Our data suggest that chemical compounds can be potentially effective in maintaining or arresting the reduction of the proteoglycan content in cartilage. A further understanding of the affect of these compounds on chondrocytes will facilitate the selection of candidates for animal model studies, as well as targets for future development of orally-administrated drugs for treating OA. These compounds may also help to identify interacting proteins that will facilitate the dissection of the molecular pathways relevant to GAG and/or proteoglycan metabolism, as well as the lesion process of cartilage.

REFERENCES:

[1] Hunziker EB. Osteoarthr Cartilage 10, 432-63, 2001. [2] McLaren AC et al. Can J Surg 34, 595-8, 1991. [3] Brent B. Plast Reconstr Surg 90, 355-74, 1992. [4] Bobic V. Orthopade 28, 19-25, 1999. [5] Stockwell BR. Nat Rev Genet 1, 116-125, 2000. [6] Farndale RW et al. Biochim Biophys acta. 883, 173-177, 1986.

ACKNOWLEDGEMENTS:

This study is supported by grants from the Hong Kong RGC (HKU7658/06M) and CRCG of HKU (200511159167), and the Research Center of Infection and Immunology of The University of Hong Kong that provided the HTS facilities.