

Screening of an FDA-approved Drug Library Reveals Mitogen-activated Protein Kinase Kinase Inhibitors Block Production of OA Mediators in Activated Human Chondrocytes and Synovial Fibroblasts and Enhance Chondrocyte Proteoglycan Production

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INTRODUCTION: There is a critical need for a therapeutic that halts the loss of joint tissue in OA. A central feature of OA is progressive cartilage degradation driven by increased production of matrix degrading enzymes including MMP-13. Secretion of MMPs by chondrocytes can be triggered by extracellular matrix fragments, such as fibronectin fragments (FN-fs). In previous studies, we showed FN-f (FN7-10) stimulation of normal chondrocytes models the OA chondrocyte phenotype. The goal of this project was to use a novel high-throughput screen (HTS) that uses primary human chondrocytes treated with FN7-10 to identify non-cytotoxic compounds that inhibit the catabolic signaling responsible for production of MMPs and proinflammatory mediators.

METHODS: We used FN7-10 as an OA relevant catabolic stimulus and MMP-13 as the readout for catabolic signaling to screen 2679 small molecules from a library of FDA-approved drugs. Primary human chondrocytes were isolated from cadaver donor ankle tissue (n = 6 males), plated on 384-well compound plates pre-stamped at 10 μM, and then treated with recombinant FN7-10 for 24 hrs. Calcein AM staining was used to determine viability, and MMP-13 was measured using a fluorogenic probe. Z' testing was used to determine statistical significance. The top 20 kinase inhibitors were used for a dose-response screen (n = 3; 2 females, 1 male) with 10 concentrations ranging from 5 nM - 100 μM. Follow-up testing included quantifying the effects on 18 OA factors in chondrocytes (n = 3; 2 males, 1 female) and OA synovial fibroblasts (n = 3; 2 males, 1 female) using Luminex, as well as high throughput alcian blue staining assay using micromass cultures (n = 6 males) to measure chondrocyte proteoglycan production. Sex of tissue donors was not controlled for every experiment due to limitations in donor availability when the experiment was conducted. Use of human tissue was IRB approved.

RESULTS: The HTS found 238/2679 compounds blocked chondrocyte FN7-10 induced MMP-13 production by >80% without cytotoxicity. The top 4 categories for hits were kinases. From these, mitogen-activated protein kinase kinase (MEK) inhibitors trametinib, selumetinib, and cobimetinib were found to be among the most potent inhibitors of MMP-13 production (Table 1). Alcian blue staining demonstrated that MEK inhibitors also enhanced chondrocyte proteoglycan production (Fig. 1). A Luminex assay analyzing 18 known OA-factors revealed trametinib blocked FN7-10 induced production of CCL20, CCL7, LIF, CLL4, CXCL5, MMP-3, and TNF-α in synovial fibroblasts, and CCL20, CCL7, LIF, CCL4, CXCL5, CCL5, TNF-α and MMP-13 in chondrocytes (Fig. 2). To test for the potential of off-target effects of the MEK inhibitors, we used a MEK1/2 Proteolysis Targeting Chimera (PROTAC). The PROTAC degraded MEK in chondrocytes, blocked phosphorylation of the MEK substrate ERK, and inhibited FN7-10 induced MMP-13 production by chondrocytes by 90% (p = 0.015; n = 4 males) and IL-6 production by synovial fibroblasts by 70% (p = 0.0025; n = 4; 2 females, 2 males).

DISCUSSION: A screen of FDA-approved drugs found MEK as a potential therapeutic target for OA. MEK inhibitors enhanced proteoglycan production in chondrocytes while also blocking production of multiple OA mediators in both chondrocytes and synovial fibroblasts.

SIGNIFICANCE/CLINICAL RELEVANCE: Given the lack of effective drugs on the market, the discovery of novel OA therapeutics that eliminate catabolic and inflammatory signaling in multiple joint tissue cell types would be a tremendous benefit to public health.

MMP13 IC ₅₀ (nM)	Product Name	Target
0.07786	Ingenol Mebutate	PKC
0.878	Fostamatinib	Syk
2.7410	Trametinib	MEK
5.731	Selumetinib	MEK
6.296	Palbociclib	CDK
10.37	Masitinib	RTKs
11.62	Cobimetinib	MEK
17.73	Nordihydroguaiaretic acid	IGF-1R
24.37	Emodin	Casein kinase
29.35	Afatinib	EGFR
32.98	Midostaurin	PKC
40.41	Pazopanib Hydrochloride	RTKs
47.42	Pexidartinib	c-Fms; c-Kit
128.90	Abemaciclib methanesulfonate	CDK
200.10	Upadacitinib	JAK
236.50	Peficitinib	JAK
284.60	Wedelolactone	IKK
345.80	Dabrafenib	Raf
679.20	Regorafenib	RTKs
818.30	Enzastaurin	PKCβ

Table 1. Top 20 kinase inhibitors that inhibited MMP-13 production with low cytotoxicity in normal human chondrocytes treated with FN7-10 for 24-hrs.

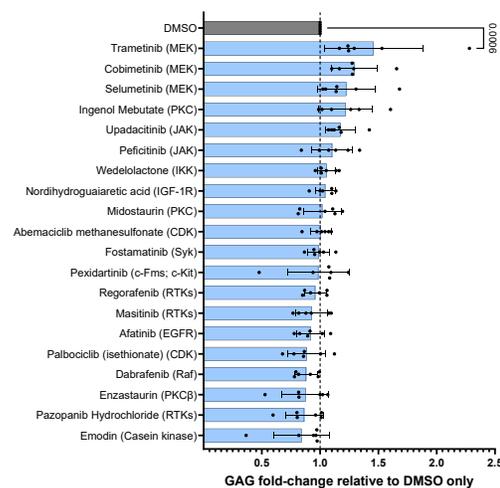


Fig. 1. Effects of selected kinase inhibitors on glycosaminoglycan (GAG) production in normal human chondrocytes. Chondrocytes from 6 independent donor samples were plated in micromass cultures and treated with 1 μM of the indicated compounds for 72-hrs. Cells were then stained with alcian blue and absorbance values were obtained via plate reader and compared to a 0.1% DMSO-treated control. P-values were calculated via one-way ANOVA with Dunnett's correction. Error bars are mean ± 95% CI.

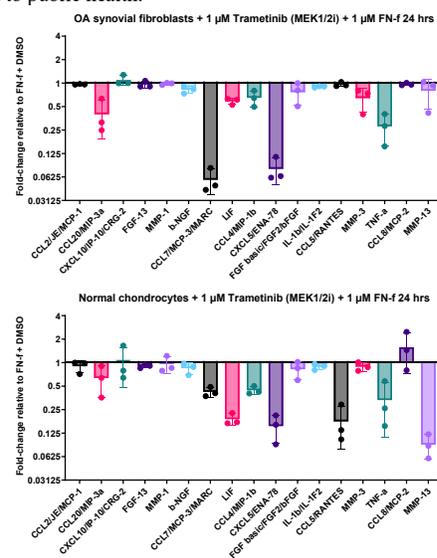


Fig. 2. Luminex assay of selected OA-factors in trametinib-treated joint tissue cells. Cells from 3 donor samples were treated with 1 μM trametinib for 2-hrs. before FN7-10 stimulation for 24-hrs. Media was then collected for analysis. Protein secretion relative to DMSO-treated controls were obtained for synovial fibroblasts (Top) and articular chondrocytes (Bottom). Error bars are mean ± SD.