SM04690, a Potential Disease-Modifying Treatment for Knee Osteoarthritis, Functions Through Inhibition of CLK2 and DYRK1A, Novel Molecular Regulators of Wnt Signaling, Chondrogenesis, and Inflammation

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Background

- In synovial joints, upregulated Wnt signaling affects osteoarthritis (OA) pathogenesis by increasing inflammation, subchondral bone formation, and thinning cartilage[1]
- A novel small molecule, SM04690, was previously shown to exhibit OA disease-modifying properties through Wnt pathway inhibition *in vitro* and *in vivo*[1]
- Herein, we describe the novel mechanism of action of SM04690 leading to Wnt pathway inhibition, chondrocyte differentiation, and anti-inflammatory activity

Methods

- *In vitro*, Wnt pathway inhibition was assessed by luciferase reporter assay in SW480 colon cancer cells
- A kinome screen (318 kinases) was performed
- SM04690 effects on protein phosphorylation, serine and arginine rich splicing factor (SRSF) proteins, FoxO1, and Sirt1 in hMSCs, chondrocytes, and synovial fibroblasts were measured by Western blot
- SM04690 and siRNA knockdown effects on (1) chondrogenic and Wnt pathway gene expression in hMSCs were measured using nCounter® gene expression panels (NanoString Technologies) and (2) LPS-induced inflammatory cytokine expression (IL-6, IL-8, TNF-α) in BEAS-2B cells were measured by qPCR and ELISA
- *In vivo*, SM04690 effects were evaluated in rat knee OA models: (1) surgical: anterior cruciate ligament transection with partial medial meniscectomy (ACLT+pMMX) (2) inflammatory: monosodium iodoacetate (MIA) injection-induced knee OA model, followed by single intra-articular SM04690 or vehicle injections
- Knee cartilage was isolated on Days 10 and 35 and phosphorylation and expression of SRSF proteins, NFKB, STAT3, and Sirt1 were measured by Western blot
- Statistical analyses: One-way ANOVA for multiple group comparisons and t-tests for two group comparisons

Conclusions

- SM04690, a potent Wnt pathway inhibitor, appeared to inhibit intra-nuclear kinases CLK2 and DYRK1A
- Biochemical and pharmacological studies identified a primary role for CLK2 in the induction of early chondrocyte differentiation from hMSCs
- Inhibition of STAT3 phosphorylation and NFKB expression by SM04690 provided potent anti-inflammatory effects
- CLK2 and DYRK1A were validated as novel targets for inhibition of the Wnt pathway, induction of chondrogenesis, and anti-inflammatory activity

Significance

- Through inhibition of CLK2 and DYRK1A, SM04690 protected cartilage, induced chondrogenesis, and reduced inflammation, supporting its potential for modifying disease and improving signs and symptoms in knee OA
- Human studies are ongoing

Results

SM04690: A potent inhibitor of the Wnt pathway, CLK2 and DYRK1A *in vitro*

- Luciferase reporter assay identified SM04690 as an inhibitor of Wnt signaling (EC_{50} = 11 nM)
- Kinome screen identified cdc-like kinases (CLK2, EC_{50} = 5.8 nM) and dual-specificity tyrosine kinase (DYRK1A, EC_{50} = 26.9 nM) as molecular targets of SM04690

SM04690 inhibited SRSF proteins, Sirt 1, and FoxO1 phosphorylation compared to vehicle

- SM04690 inhibited phosphorylation of SRSF proteins and Sirt1 in hMSCs and chondrocytes
- SM04690 inhibited FoxO1 phosphorylation (increasing total and nuclear FoxO1 levels) in chondrocytes

Validation of SM04690 mechanism of action *in vivo*

- SM04690 inhibited phosphorylation of SRSF proteins, Sirt1, FoxO1, and STAT3 and expression of TCF7 and NF-KB in the ACLT+pMMX and MIA models compared to vehicle

Representation of dual mechanism of action of SM04690

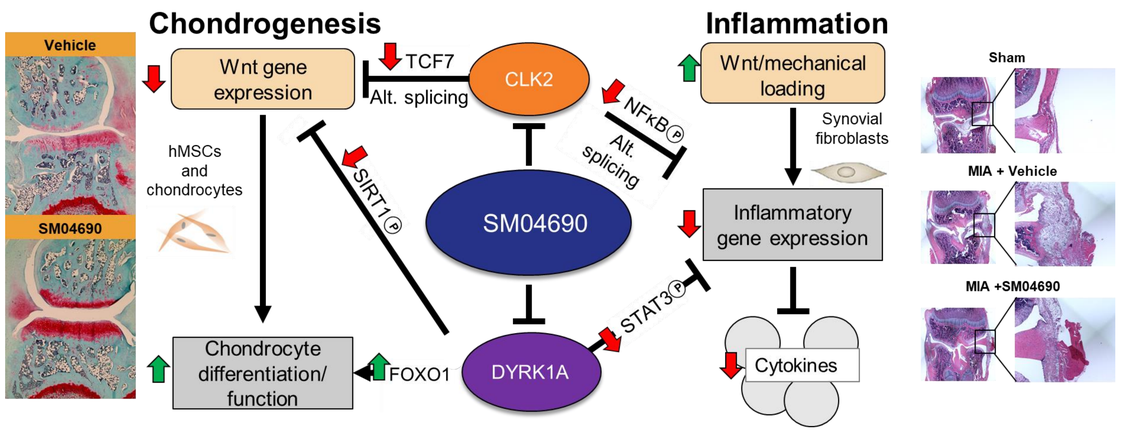

Figure 1. CLK2 and DYRK1A knockdowns inhibited the Wnt pathway

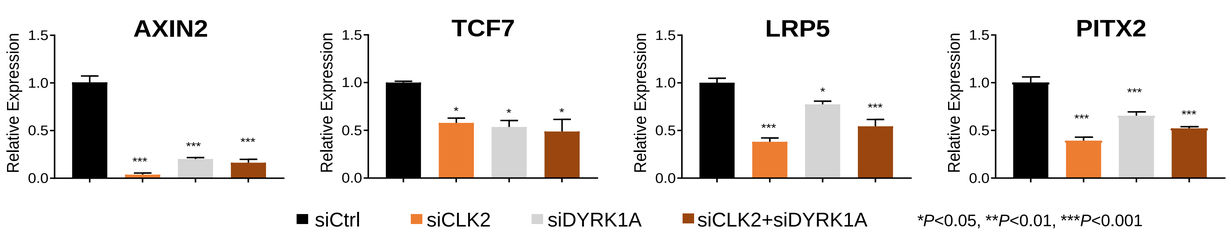

Figure 1. Knockdowns of CLK2 and DYRK1A led to inhibition of Wnt pathway genes including *AXIN2, TCF7, LRP5, and PITX2*

CLK2 and DYRK1A knockdowns inhibited the Wnt pathway (Data not shown)

- Knockdowns of CLK2 and DYRK1A led to upregulation of secreted Wnt inhibitors SFRP2 and DACT1 compared to siRNA controls

Figure 2. Combined DYRK1A/CLK2 knockdown induced chondrocyte differentiation

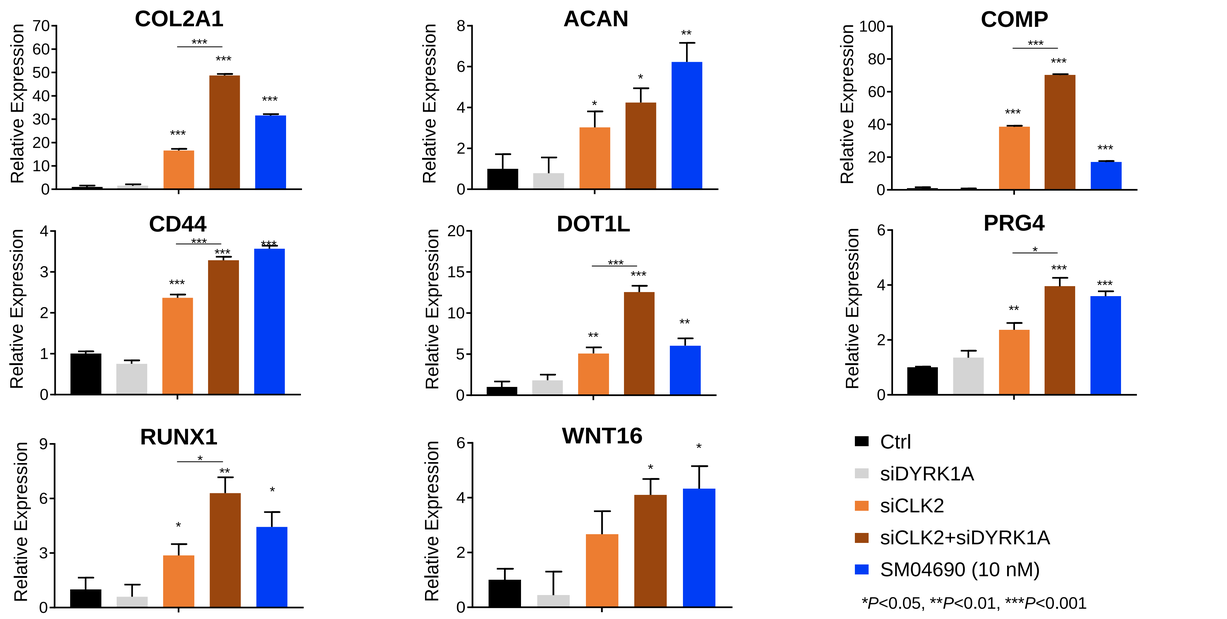

Figure 2. Combined DYRK1A/CLK2 knockdown demonstrated increased expression of several chondrocyte genes (*COL2A1, ACAN, COMP, CD44, DOT1L, PRG4, RUNX1, WNT16*) compared to siRNA control or siCLK2 alone

TCF7 induced chondrocyte differentiation (Data not shown)

- TCF7 knockdown increased chondrogenic genes (COMP, SOX9, RUNX1, but not RUNX2) compared to siRNA controls
- LEF1, TCF4, or β-catenin knockdowns did not lead to chondrocyte differentiation

Figure 3. SM04690 inhibited inflammation via inhibition of CLK2 and DYRK1A

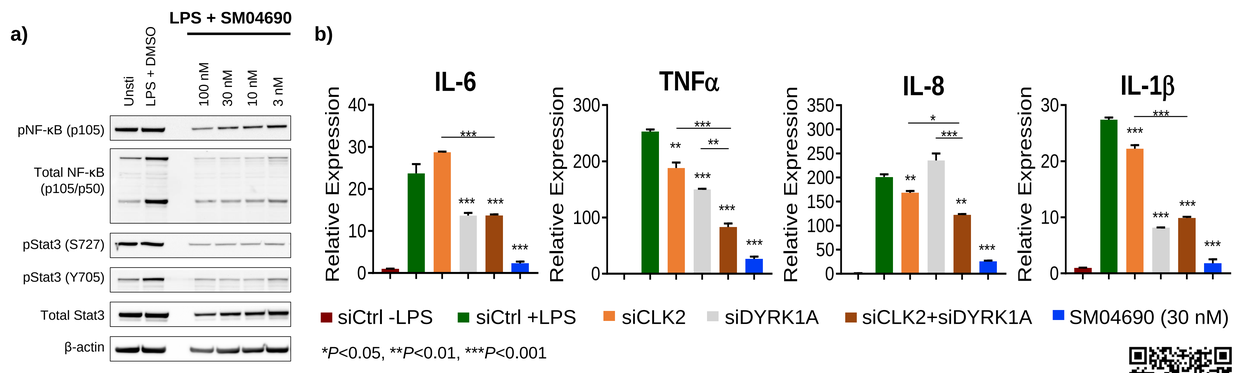

Figure 3. (a) SM04690 treatment of synovial fibroblasts resulted in decreased phosphorylation of NFkB and STAT3 compared to DMSO **(b)** Knockdown of DYRK1A and CLK2 inhibited production of inflammatory cytokines IL-6, TNF-α, IL-8, and IL-1β in LPS-stimulated BEAS-2B cells compared to siRNA control

Reference: 1. Deshmukh et al. OAC 2017

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