Modulation of the Wnt Pathway through Inhibition of CLK2 and DYRK1A by SM04690, a Novel Potential Disease-Modifying Treatment for Knee Osteoarthritis

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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]
- SM04690, a novel small molecule, was previously shown to exhibit OA disease-modifying properties through Wnt pathway inhibition *in vitro* and *in vivo*[1]
- The novel mechanism of action of SM04690 leading to Wnt pathway inhibition, chondrocyte differentiation, and anti-inflammatory activity is described

Methods

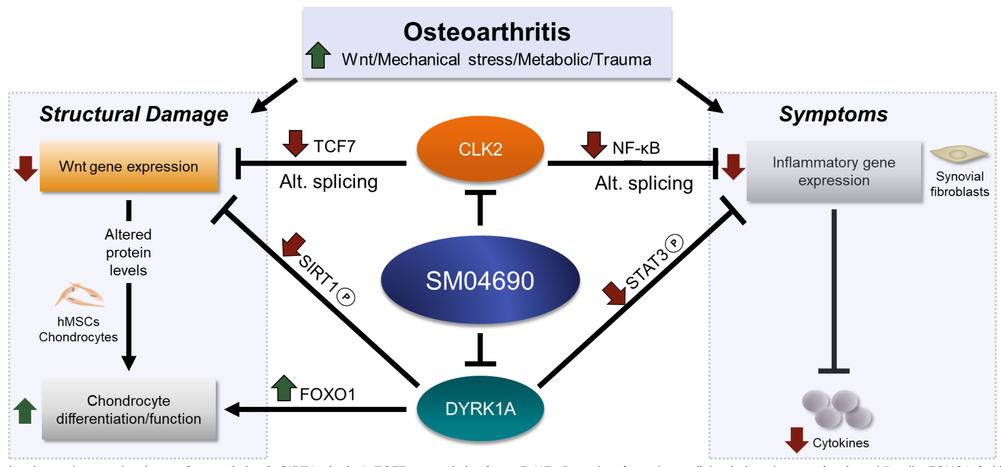
- ***In vitro*** Wnt pathway inhibition was assessed by luciferase reporter assay in SW480 colon cancer cells
- A kinome screen (318 kinases) was performed
- ***In vitro*** SM04690 effects on protein phosphorylation of serine and arginine rich splicing factor (SRSF) proteins, FoxO1, and Sirt1 in hMSCs, chondrocytes, and synovial fibroblasts were measured by Western blot
- ***In vitro*** SM04690 and siRNA knockdown effects on (1) Wnt pathway and chondrogenic gene expression in hMSCs were measured using nCounter® gene expression panels (NanoString Technologies) and (2) LPS-induced inflammatory cytokines (IL-6, IL-8, TNF-α) in BEAS-2B cells were measured by qPCR and ELISA
- ***In vivo*** SM04690 effects were confirmed in rat knee OA models: **(1) surgical:** anterior cruciate ligament transection with partial medial meniscectomy (ACLT+pMMX) and **(2) inflammatory:** monosodium iodoacetate (MIA) injection-induced knee OA model (data not shown)
- Statistical analyses: One-way ANOVA (multiple groups); t-tests (two groups)

Conclusions

***In vitro* and *in vivo*:**

- SM04690 inhibited intra-nuclear kinases CLK2 and DYRK1A, leading to Wnt pathway inhibition
- Inhibition of CLK2 induced early chondrocyte differentiation from hMSCs and inhibition of DYRK1A enhanced chondrocyte function
- Inhibition of STAT3 phosphorylation and NF-κB expression by SM04690 provided potent anti-inflammatory effects
- Through dual 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

Representation of dual mechanism of action of SM04690



STAT3: signal transducer and activator of transcription 3, SIRT1: sirtuin 1, TCF7: transcription factor 7, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, FOXO1: forkhead Box O1

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 (IC_{50} = 11 nM)
- Kinome screen identified cdc-like kinases (CLK2, IC_{50} = 5.8 nM) and dual-specificity tyrosine kinase (DYRK1A, IC_{50} = 26.9 nM) as molecular targets of SM04690

Figure 1. SM04690 inhibited SRSF proteins, Sirt1, and FoxO1 phosphorylation

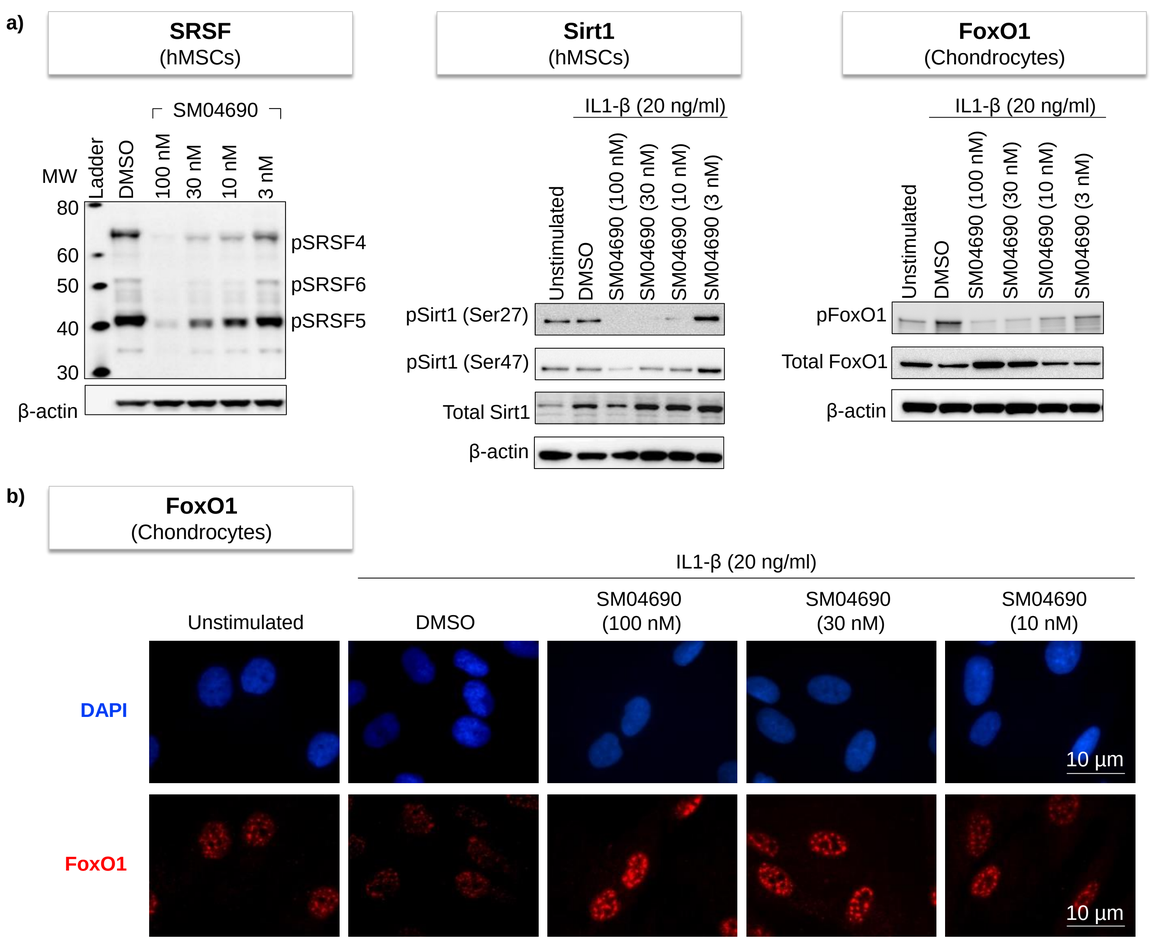


Figure 1. (a) SM04690 treatment of hMSCs and chondrocytes resulted in decreased phosphorylation of SRSF proteins, Sirt1, and FoxO1 compared to DMSO **(b)** SM04690 treatment of chondrocytes resulted in increased nuclear FoxO1 levels compared to DMSO

Figure 2. Inhibition of CLK2 and DYRK1A reduced Wnt pathway gene expression

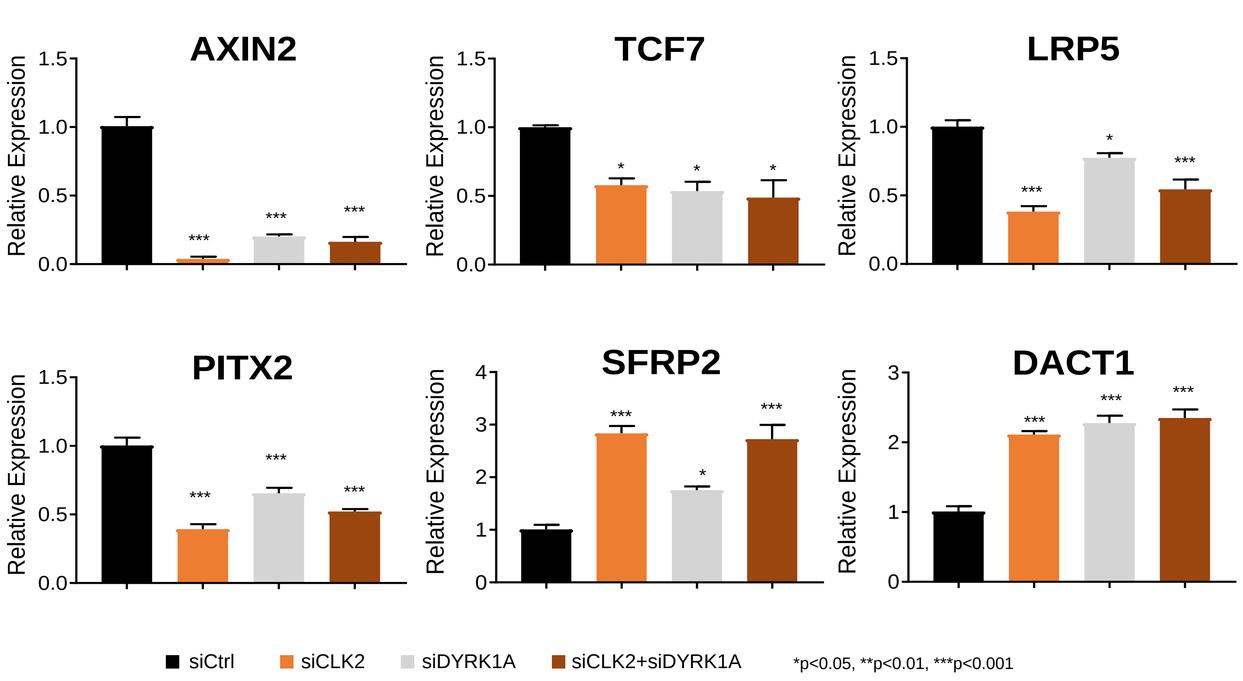


Figure 2. Knockdowns of CLK2 and DYRK1A led to inhibition of Wnt pathway genes including *AXIN2, TCF7, LRP5,* and *PITX2* and upregulation of secreted Wnt inhibitors *SFRP2* and *DACT1* compared to siRNA controls

Figure 3. Inhibition of both DYRK1A/CLK2 induced chondrocyte differentiation

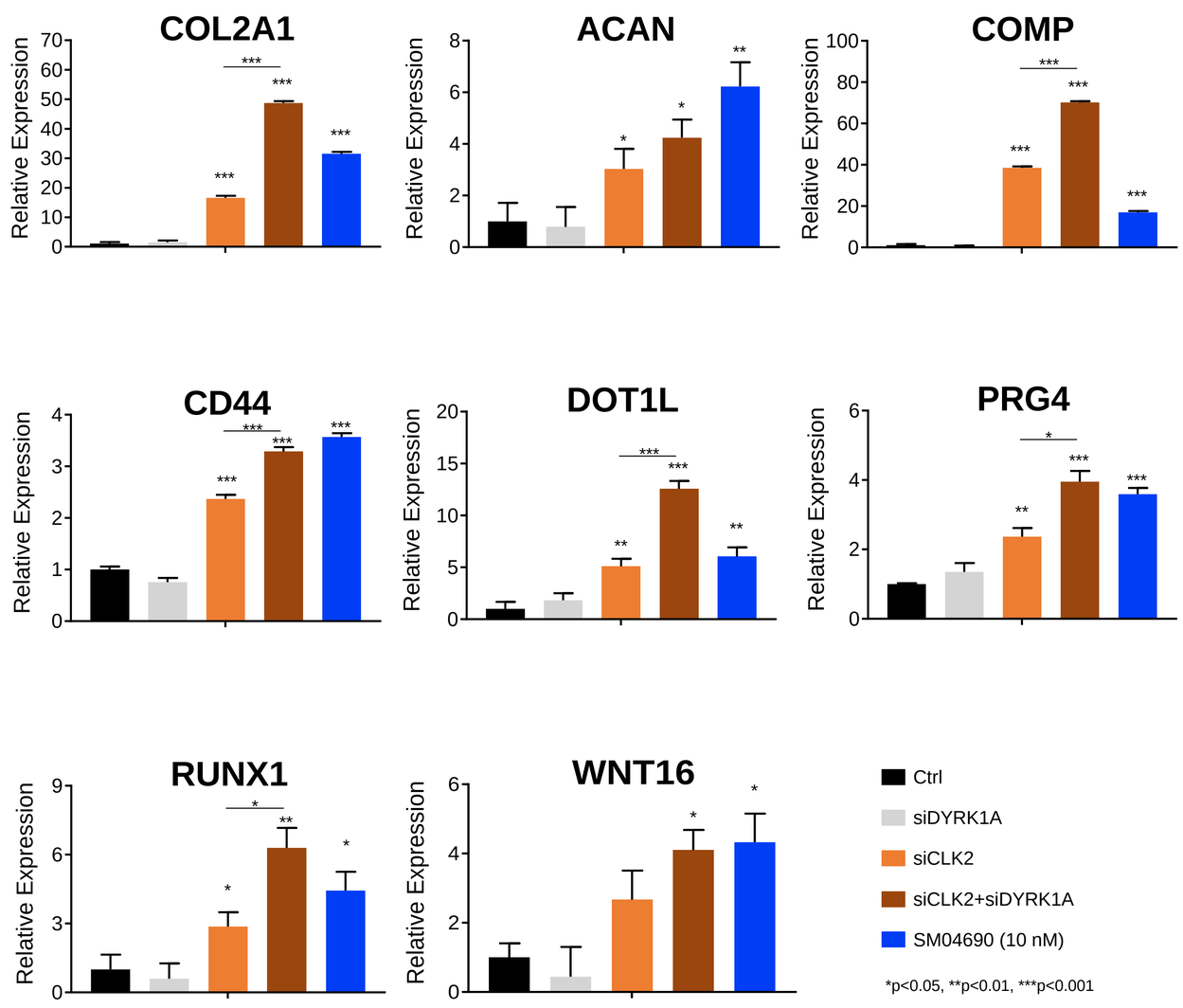


Figure 3. Combined DYRK1A/CLK2 knockdown and SM04690 demonstrated increased expression of several chondrocyte genes (*COL2A1, ACAN, COMP, CD44, DOT1L, PRG4, RUNX1, WNT16*) compared to siRNA control or siCLK2 alone

Inhibition of 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 4. SM04690 reduced inflammation via inhibition of CLK2 and DYRK1A

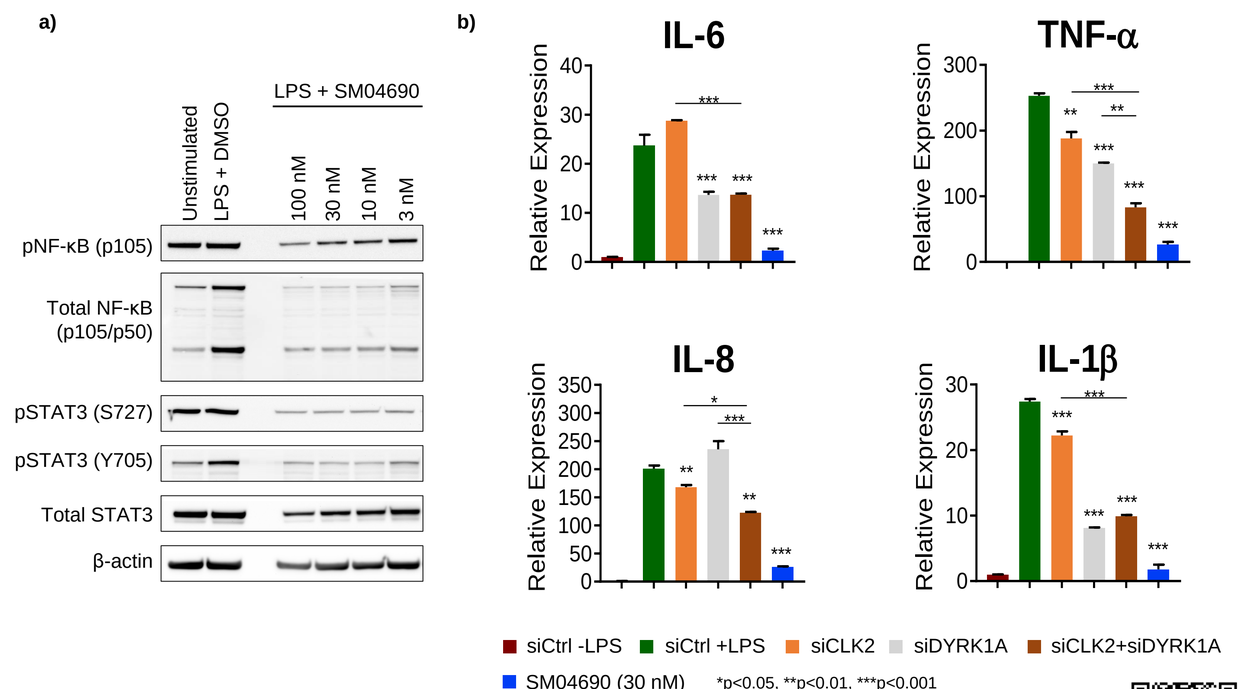


Figure 4. (a) SM04690 treatment of synovial fibroblasts resulted in decreased phosphorylation of NF-κB and STAT3 compared to DMSO **(b)** Knockdown of DYRK1A and CLK2, and SM04690 inhibited production of inflammatory cytokines *IL-6, TNF-α, IL-8,* and *IL-1β* in LPS-stimulated BEAS-2B cells compared to siRNA control

Reference: 1. Deshmukh V, et al. *OAC.* 2017

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