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.
- A novel small molecule, SM04690, was previously shown to exhibit OA disease-modifying properties through Wnt pathway inhibition in vitro and in vivo.
- 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 kinase 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 transaction 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.

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

- Luciferase reporter assay identified SM04690 as an inhibitor of Wnt signaling (EC50 = 11 nM).
- Kinome screen identified ccdc-like kinases (CLK2, EC50 = 5.8 nM) and dual-specificity tyrosine kinase (DYRK1A, EC50 = 26.9 nM) as molecular targets of SM04690.

SM04690 inhibited SRSF proteins, Sirt1, 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-κB in the ACLT+pMMX and MIA models compared to vehicle.

Results

Figure 1. CLK2 and DYRK1A knockdowns inhibited the Wnt pathway

- 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

- 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.

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

- 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.