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
- SM04690, a novel small molecule, was previously shown to exhibit OA disease-modifying properties through Wnt pathway inhibition in vitro and in vivo
- The novel mechanism of action of SM04690 leading to Wnt pathway inhibition, chondrogenesis 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 kinase screen (318 kinases) was performed
- **In vitro** SM04690 effects on protein phosphorylation of serine and arginine rich splicing factor (SRSF) proteins, FoxO1, and Src11 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)

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, Src11, and FoxO1 phosphorylation**

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

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

**Figure 4. SM04690 reduced inflammation via inhibition of CLK2 and DYRK1A**

**Conclusions**

**In vitro and in vivo**

- SM04690 inhibited intra-nuclear kinases CLK2 and DYRK1A, leading to Wnt pathway inhibition
- Inhibition of CLK2 induced early chondrogenic 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