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SM04755, a Potential Disease-Modifying Treatment for Tendinopathy, Modulates the Wnt Pathway via Inhibition of CLK2 and DYRK1A

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Background: Tendinopathy is an inflammatory and degenerative disorder of tendons caused by injuries and/or overuse. Left untreated, tendinopathy can lead to pain and rupture. Current therapeutic options only treat symptoms. Stem cell- and growth factor-based treatments are under investigation but have not established safety or efficacy, leaving considerable unmet need. The Wnt pathway is upregulated in chronic tendinopathy, affecting inflammation and tenocyte differentiation. SM04755, a novel, topical, small-molecule Wnt pathway inhibitor, has previously been shown to inhibit inflammation, protect tenocytes, and increase tenocyte differentiation in nonclinical models.[1] This is the first report of the molecular targets of SM04755 and its associated mechanism of action.

Methods: Wnt pathway inhibition was measured using a cell-based luciferase reporter assay controlled by a β-catenin/TCF-responsive promoter in SW480 colon cancer cells. A kinome screen (318 kinases) and kinase assays were performed. Effects of SM04755 on phosphorylation of proteins including serine/arginine-rich splicing factor (SRSF) proteins in human mesenchymal stem cells (hMSCs), tendon-derived stem cells (TDSCs), and peripheral blood mononuclear cells (PBMCs) were measured using Western blot. SiRNA-mediated CLK2 and DYRK1A knockdowns were performed in hMSCs and TDSCs. Effects of SM04755 and siRNA knockdowns on Wnt pathway gene expression and catabolic enzymes (MMPs) were measured using qPCR. Effects of SM04755 and siRNA knockdowns on LPS-induced expression of inflammatory cytokines (IL-6, IL-8, TNF-α) in BEAS-2B cells were measured by qPCR and ELISA. Statistical analyses used one-way ANOVA for multiple group comparisons and t-tests for comparison between two groups.

Results: SM04755 was a potent inhibitor (EC_{50}=156 nM) of Wnt signaling. Biochemical assays identified CDC-like kinases (CLKs) and dual-specificity tyrosine kinase (DYRK1A) as molecular targets of SM04755. SM04755 potently inhibited CLK-mediated phosphorylation of SRSF proteins compared with DMSO controls. Knockdowns of CLK2 and DYRK1A led to inhibition of Wnt pathway genes (AXIN2, TCF7, TCF7L2, LEF1, etc.) and had no effects on β-catenin levels compared with siRNA controls (siCtrl). Furthermore, SM04755 had no effect on CHIR- and WNT3A-stimulated active or total β-catenin levels in hMSCs and TDSCs. CLK2 and DYRK1A knockdowns also inhibited IL-1β-induced expression of catabolic enzymes (MMP1, 3, 9, 13) in tenocytes compared with siCtrl. SM04755 treatment of LPS-stimulated PBMCs resulted in decreased phosphorylation of NF-kB and STAT3 compared with DMSO. Knockdown of DYRK1A was sufficient to inhibit production of inflammatory cytokines (IL-6, IL-8, TNF-α) in LPS-stimulated BEAS-2B cells compared with siRNA control; combined knockdown of DYRK1A/CLK2 enhanced the anti-inflammatory effects of DYRK1A knockdown.

Conclusion: SM04755 was a potent Wnt pathway inhibitor that appeared to inhibit CLK2 and DYRK1A. SM04755 and knockdowns of CLK2 and DYRK1A, compared with control siRNA, identified a β-catenin-independent mechanism of Wnt pathway inhibition and potential for tenocyte protection from catabolism. This supports the potential disease modification of tendinopathy with SM04755. Furthermore, the anti-inflammatory effects of SM04755 are mechanistically supported by the decreased phosphorylation of STAT3 and NF-kB. These data support SM04755 as a single agent that may potentially benefit symptoms and provide disease modification in tendinopathy. SM04755 provides a novel mechanism for β-catenin-independent modulation of the Wnt pathway through its effects on two distinct molecular targets (CLK2 and DYRK1A). Human tendinopathy trials are planned.

References: 1. Deshmukh V, et al. *Arthritis and Rheum*. 2016.