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

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Background: Tendinopathy is associated with inflammation, tendon degeneration, and failed healing. Despite the high prevalence of tendinopathy, its underlying pathogenesis is not fully understood. The Wnt pathway is upregulated in tendinopathy, affecting inflammation and tenocyte differentiation. SM04755, a topical, small-molecule Wnt pathway inhibitor, has previously been shown to inhibit inflammation and increase tenocyte differentiation in nonclinical models.[1] The objective of this study was to identify molecular targets of SM04755 and its associated mechanism of action.

Methods: Wnt pathway inhibition was measured using a luciferase reporter assay in SW480 cells. A kinome screen (318 kinases) and kinase assays were performed. The effects of SM04755 on protein phosphorylation in rat tendon-derived stem cells (rTDSCs) and peripheral blood mononuclear cells (PBMCs) were measured using western blot. SiRNA-mediated knockdown of CDC-like kinases (CLKs) and dual-specificity tyrosine kinase (DYRK1A) were performed in human mesenchymal stem cells (hMSCs), rTDSCs, and rat tenocytes. Wnt pathway and catabolic enzyme (MMP) gene expression were measured using qPCR. Tenocyte marker expression was assessed by qPCR and immunostaining. Inflammatory cytokine expression in PBMCs was measured by qPCR and ELISA.

Results: SM04755 was a potent inhibitor (EC_{50}=156 nM) of Wnt signaling. Biochemical assays identified CLKs and DYRK1A as molecular targets of SM04755. SM04755 potently inhibited CLK-mediated phosphorylation of serine/arginine-rich splicing factor proteins compared with DMSO control. Knockdowns of CLKs and DYRK1A led to inhibition of Wnt pathway genes (*AXIN2*, *LEF1*, *TCF4*, *TCF7L*) compared with siRNA controls (siCtrl). CLK and DYRK1A knockdowns also induced expression of tenocyte markers in rTDSCs and inhibited expression of MMP1, 3, 9, and 13 in tenocytes compared with siCtrl. SM04755 treatment of LPS-stimulated PBMCs reduced NF-κB and STAT3 phosphorylation and inhibited inflammatory cytokine production compared with DMSO.

Conclusion: SM04755 provides a novel mechanism for modulation of the Wnt pathway through its effects on two distinct molecular targets, CLKs and DYRK1A, and thus has potential as a treatment for tendinopathy. Human tendinopathy trials are planned.

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