Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04755) as a Potential Topical Treatment for Tendinopathy

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Background

- Tendinopathy is an inflammatory, degenerative, and fibrotic condition of the tendon, caused by injuries or overuse. It is characterized clinically by pain, swelling, and impaired performance.[1-3] It can commonly present in man as Achilles' heel, tennis elbow, and jumper's knee.
- Current therapeutic options alleviate symptoms rather than treating underlying pathology.[4]
- The Wnt pathway plays an important role in tenocyte differentiation and is upregulated in tendinopathy. Altered Wnt signaling may contribute to tissue metaplasia and failed healing in some cases of tendinopathy.[5]
- Samumed is developing SM04755, a potent small molecule Wnt signaling pathway inhibitor, as a potential topical therapeutic for tendinopathy.

Methods

- Wnt pathway inhibition was measured by a cellular Wnt pathway-based reporter assay in SW480 colon cancer cells and was further confirmed by qRT-PCR for Wnt target genes.
- Effects on fibrosis were assessed in TGF-β-stimulated human dermal fibroblasts (HDFα) by measuring smooth muscle actin (αSMA), plasminogen activator inhibitor (PAI-1), collagen (Col2a), and connective tissue growth factor (CTGF) expression by qRT-PCR.
- *In vitro* and *in vivo* tendon regeneration were evaluated by differentiation of human mesenchymal stem cells (hMSCs) into tenocytes and assessment of), tenomodulin, and tenascin C expression by high-content imaging and qRT-PCR in rat tendons.
- Pharmacokinetics were evaluated by topical application on rats, followed by analysis of compound concentrations in tendon and plasma by LC-MS.
- *In vivo* efficacy of topical SM04755 was evaluated in single or repeat intra-tendon collagenase injection-induced rodent tendinopathy models by scoring (range 5-20) histological indicators of tendon health.
- *In vivo* inflammation was measured by chemokine ligand 1 (CXCL1) levels in plasma by ELISA and other inflammatory markers (IL-1β, TNF-α, IFN-γ, IL-6 and IL-8) in the tendon by qRT-PCR.
- Function was assessed via weight bearing as measured by an incapacitance meter after a single collagenase injection, followed by daily topical application of SM04755.

Results

SM04755 demonstrated specific and potent inhibition of Wnt signaling

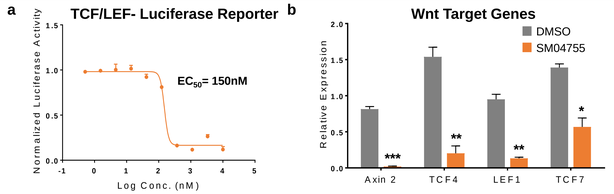

Figure 1. (**a**) Dose response of SM04755 treatment of SW480 cells transduced with the TCF/LEF promoter-driven luciferase reporter. (**b**) Wnt signaling pathway gene expression following treatment with SM04755 (1 μM) or DMSO for 24hrs as measured by qRT-PCR. n=3, Mean ± SD, *p<0.05, **p<0.01, ***p<0.001, t-test.

SM04755 prevented and reversed fibrosis *in vitro*

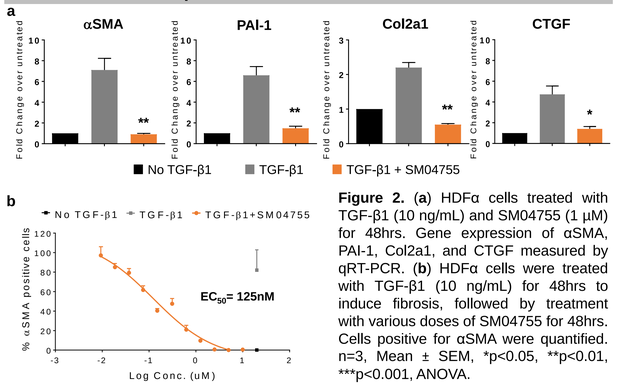

Figure 2. (**a**) HDFα cells treated with TGF-β1 (10 ng/mL) and SM04755 (1 μM) for 48hrs. Gene expression of αSMA, PAI-1, Col2a1, and CTGF measured by qRT-PCR. (**b**) HDFα cells were treated with TGF-β1 (10 ng/mL) for 48hrs to induce fibrosis, followed by treatment with various doses of SM04755 for 48hrs. Cells positive for αSMA were quantified. n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001, ANOVA.

SM04755 induced tenocyte differentiation from hMSCs *in vitro*

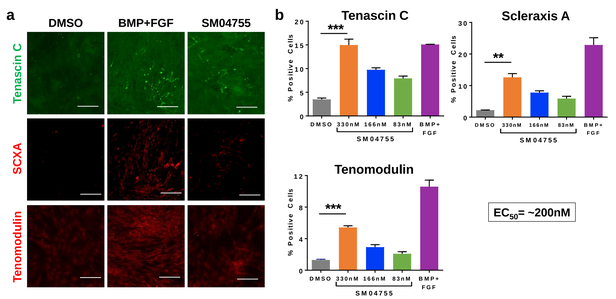

Figure 3. (**a**) hMSCs treated with either DMSO or SM04755 (330 nM) for 7 days and stained for tenascin C, SCXA, and tenomodulin. BMP-12 + FGF-2 was used as a positive control. Bars=50 μm. (**b**) Quantification of the number of tenocytes in (**a**). n=9, Mean ± SEM, **p<0.01, ***p<0.001, ANOVA.

SM04755 demonstrated sustained local and minimal systemic exposure

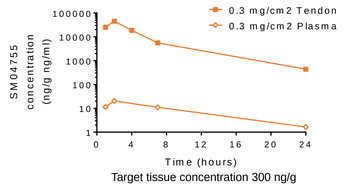

Figure 4. Pharmacokinetics of SM04755 in rat tendon and plasma following a single topical application. Target concentration achieved and retained in the tendon for up to 24hrs with minimal systemic exposure.

Results

SM04755 promoted *in vivo* tendon healing in single and repeat collagenase-induced tendinopathy models in rats

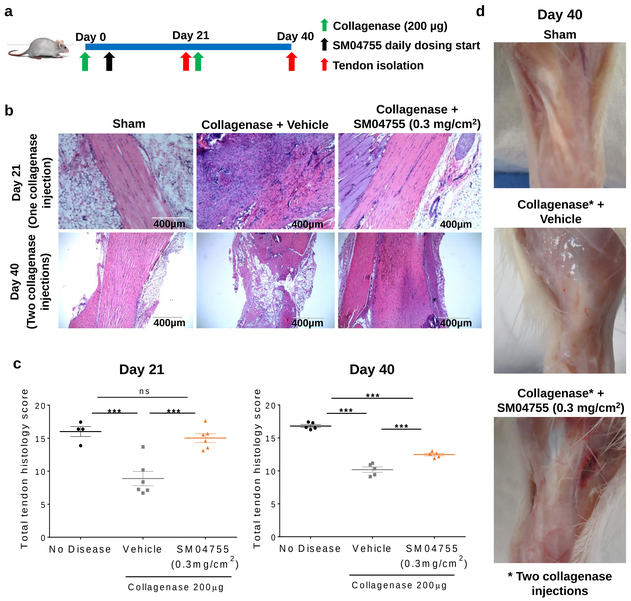

Figure 5. (**a**) Collagenase-induced rat tendinopathy model. (**b**) Images of rat tendons stained with H&E from sham or collagenase-injected and vehicle- or SM04755 (0.3 mg/cm^2)-treated rats on day 21 and day 40. (**c**) Histological score of inflammation, linearity and density of tendon fibers, shape of tenocytes, and hemorrhage for the rat tendons. Mean ± SEM, day 21: n=4 sham, n=6 vehicle & SM04755; day 40: n=5, ***p<0.001, ns = not significant, ANOVA. (**d**) Images of rat tendons on day 40.

SM04755 promoted *in vivo* tendon regeneration in single injection collagenase-induced tendinopathy model in rats

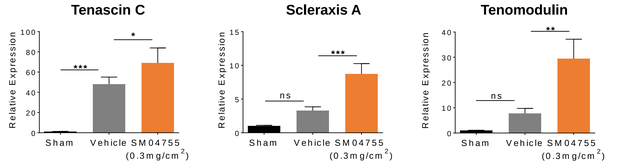

Figure 6. Expression of tenocyte markers in the tendon following sham or collagenase injection and treatment with either vehicle or SM04755 (0.3 mg/cm^2) for 21 days as measured by qRT-PCR. Fold change is relative to sham control. n=6, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001, ns = not significant, ANOVA.

SM04755 inhibited *in vivo* inflammation in single injection collagenase-induced tendinopathy model in rats

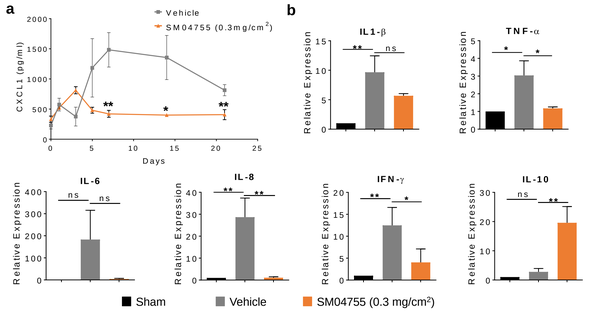

Figure 7. (**a**) Levels of circulating CXCL1 in peripheral blood following treatment as measured by ELISA. (**b**) Expression of pro- and anti-inflammatory genes in the tendon following sham or collagenase injection and treatment with either vehicle or SM04755 (0.3 mg/cm^2) for 21 days as measured by qRT-PCR. Fold change relative to sham control is shown. n=6, Mean ± SEM, *p<0.05, **p<0.01, ns = not significant, ANOVA.

SM04755 improved weight bearing after collagenase injection in *in vivo* collagenase model in rats

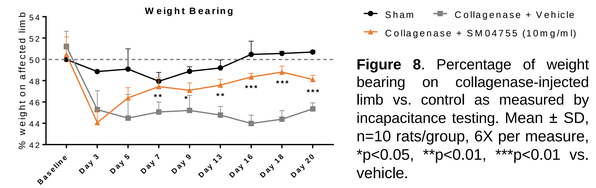

Figure 8. Percentage of weight bearing on collagenase-injected limb vs. control as measured by incapacitance testing. Mean ± SD, n=10 rats/group, 6X per measure, *p<0.05, **p<0.01, ***p<0.01 vs. vehicle.

Discussion

- In preclinical tendinopathy models, topical SM04755 reduced inflammation, differentiated progenitor cells into tenocytes, inhibited fibrotic markers, increased tendon regeneration markers, and improved tendon structure micro- and macroscopically. Function improved as measured by weight bearing in the affected limb by day 7.
- SM04755 demonstrated sustained tendon exposure, with minimal systemic exposure, in rats.
- SM04755 has potential as a therapeutic for chronic tendinopathy.
- A Phase 1 trial with healthy volunteers is ongoing.

References

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