Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04690) as a Potential Treatment for Degenerative Disc Disease

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Methods

To identify Wnt signaling inhibitors, a small molecule chemical library was screened in a cellular Wnt pathway-based β-catenin/TCF-responsive reporter assay in SW480 colon cancer cells.

In vitro proliferation of NP cells isolated from rat coccygeal discs, treated with vehicle or SM04690 for 5 days, was measured by cell doubling index (CDI=cell number/initial cell number/days).

Differentiation of NP progenitor cells into chondrocyte-like NP cells with 12 days of vehicle or SM04690 treatment was measured by Alcian blue staining and absorbance based quantification.

Pharmacokinetics were evaluated by intradiscal injection in rats and rabbits, followed by LC-MS analysis of compound concentrations in the disc and plasma.

Results

SM04690 demonstrated specific and potent inhibition of Wnt signaling

CDI for primary NP-derived progenitor cells was ~2-fold higher in cells treated with SM04690 compared to DMSO.

Increased Alcian blue staining indicated the presence of chondrocyte-like cells after 12 days of treatment with SM04690.

SM04690 stimulated NP-derived progenitor cell proliferation

A single intradiscal injection of SM04690 maintained disc health and shape in a rat in vivo model of degenerative disc disease

Significantly higher percent DHI observed in the SM04690-treated group compared to vehicle, 5 weeks post-treatment.

Conclusion

SM04690 induced the proliferation and differentiation of NP-derived progenitor cells in vitro and in vivo (Figure 2 and 4). Single intradiscal injection of SM04690 had sustained residence time in the disc and minimal systemic exposure in rats (Figure 3).

Single intradiscal injection of SM04690 improved disc health, height, and shape after injury in vivo in a rat model of DDD (Figure 4 and 5) compared to vehicle controls.

SM04690 regenerated the NP areas and IVDs in this in vivo model of DDD.

An Investigational New Drug application for SM04690 in DDD is open and human trials are planned for 2017.

References


Figure 3. Pharmacokinetics of SM04690 in the rat disc following a single intradiscal injection of SM04690 at various doses.

Figure 4. (a) Images from IVDs treated with vehicle or 33 µM SM04690 (C9/1 and C9/10); 8 weeks post-injury and stained with Safranin O/Fast green (top panels) or Masson’s Trichrome (lower panels). (B) Histology scores 8 weeks post-injury for the vehicle and 33 µM SM04690-treated discs as determined by measuring AF, AF and NP border, cellularity of the NP, and content of matrix of the NPs were significantly lower for SM04690-treated discs compared to vehicle.

Figure 5. (a) Radiographic images of discs from rats pre-injury, and following intradiscal needle puncture and treatment with either vehicle or 33 µM SM04690 (red arrows indicate C9/8 and C9/10 discs). (B) Percent DHI calculated based on radiographic images at 1 and 6 weeks post-injury and compared to preoperative (pre-injury) radiographic images. (n=9, Mean ± SD, *p<0.05, student’s t-test).

Figure 1. (a) Dose response of SM04690 treatment of SW480 cells transduced with TCF/LEF promoter-driven luciferase reporter. (b) Expression of genes in the Wnt pathway in hMSCs following treatment with SM04690 or DMSO for 24hrs as measured by qRT-PCR. (n=3, Mean ± SEM, *p<0.05, **p<0.01).

Figure 2. (a) NP-derived progenitor cells treated with SM04690 or DMSO control for 12 days and stained with Alcian blue. (b) CDI for NP-derived progenitor cells treated with various doses of SM04690 or DMSO control for 5 days. (c) Quantification of Alcian blue staining for the cells in (a). (n=3, Mean ± SD, *p<0.05, **p<0.01, one-way ANOVA).