SMO4646 inhibited Wnt pathway gene expression stimulated in response to Transforming Growth Factor-β and was effective in a chronic model of bleomycin-induced pulmonary fibrosis

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Rationale

• The Wnt/β-catenin pathway is associated with Transforming Growth Factor-β (TGF-β) mediated fibrosis, and aberrant activation is implicated in idiopathic pulmonary fibrosis (IPF) pathophysiology.
• TGF-β1 modulates expression of Wnt pathway genes Frizzled-8 (FZD8) and WNT1 inducible signaling protein 1 (WISP1).
• Endoplasmic reticulum (ER) stress can also induce TGF-β-mediated fibrosis.
• SMO4646, a novel small-molecule Wnt pathway inhibitor in development as an inhaled IPF therapy, inhibited TGF-β1-stimulated fibrogenic gene expression in vitro, and attenuated pulmonary fibrosis in an acute bleomycin mouse model.
• The ability of SMO4646 to inhibit TGF-β-stimulated fibrotic and Wnt pathway gene expression in combination with anti-fibrotics (nintedanib [NIN] and pirfenidone [PIRF]) was investigated.

A chronic bleomycin-induced pulmonary fibrosis model, more closely resembling IPF than the acute model, was used to investigate SMO4646 efficacy and expression of fibrosis and ER stress genes.

Methods

Wnt pathway and fibrotic gene expression

• Normal primary human lung fibroblasts (NHLF) and a normal lung fibroblast cell line (MRC-5) were treated for 24 hours with DMSO (vehicle) or TGF-β1 (10 ng/ml). TGF-β1-stimulated cells were treated with SMO4646 (1 μM), PIRF (1600 μM), or NIN (1 μM) alone and in combinations. Effects on extracellular matrix (smooth muscle cell-α-actin [ACTA2], collagen type 1 α1 [COL1A1], and fibronectin [FN1]) and Wnt pathway (FZD8, WISP1) gene expression were evaluated by TaqMan RT-qPCR and normalized to the housekeeping gene GAPDH.

Chronic bleomycin-induced pulmonary fibrosis mouse model

• Bleomycin (2U/kg) or PBS was instilled in the lungs of C57BL6 mice 7 days before initiation of intermittent “2 weeks on 2 weeks off” aerosol dosing for 16 weeks with vehicle or SMO4646 (0.1 mg/kg QD x 5 days/week during 2-week-on period) (n=12/group). (Oro-Nasal and Respiratory Exposure System [CH Technologies]). Lung fibrosis was blindly evaluated by Ashcroft scoring post-mortem (n=8/group). Mouse lung expression of fibrotic, Wnt and ER stress (heat shock protein 5 [Hsp5] and 1 [Hspd1]) genes were measured by RT-qPCR.

Results

SMO4646 alone or combined with PIRF or NIN inhibited fibrotic gene expression in human lung fibroblasts

• SMO4646 alone or combined with PIRF or NIN inhibited fibrotic gene expression stimulated by TGF-β1, demonstrating potential crosstalk reduction between these pathways.
• Compared with vehicle, SMO4646 reduced ER stress markers, showed trends (ns) in reducing fibrotic and Wnt markers, and attenuated pulmonary fibrosis in a chronic bleomycin model.
• These data show potential for SMO4646 as a therapy for IPF.
• A phase 1 study of SMO4646 in IPF subjects is ongoing.

Conclusions

References

2. Saperfar et al. FASEB J. 2016/2019

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Figure 1. Expression of ACTA2, COL1A1 and FN1 in (a) NHLF, and (b) MRC-5 cells treated for 24 hours with monotherapies or combinations as indicated. Fold changes are relative to DMSO control. Y-axes adjusted to fold-change degree. *p<0.05; **p<0.01; ***p<0.001 vs. DMSO. p<0.05; p<0.01 vs SMO4646; p<0.05; p<0.01 vs SMO4646; p<0.05; p<0.01, p<0.001, p<0.0001; n=3-4 experiments; mean ± SEM

Figure 2. Expression of FZD8 and WISP1 in (a) NHLF and (b) MRC-5 cells treated for 24 hours with monotherapies or combinations as indicated. Fold changes are relative to DMSO control. Y-axes adjusted to fold-change degree. *p<0.05; **p<0.01; ***p<0.001 vs. DMSO. p<0.05; p<0.01 vs SMO4646; p<0.05; p<0.01, p<0.001, p<0.0001; n=3-4 experiments; mean ± SEM

Figure 3. Expression of Hsp5, Hspd1, Coll1a1, Fst, and Wisp1 in mouse lungs following 16-week chronic bleomycin treatment. Fold changes are relative to 0. Percentage values are degree of decline of SMO4646 vs. vehicle. Y-axes adjusted to fold-change degree. *p<0.05; **p<0.01; ***p<0.001; ns = not significant; mean ± SEM

Figure 4. (a) Representative H&E staining images of bleomycin-induced pulmonary fibrosis in C57BL6 mice lungs (n=5/group). (10x objective) (b) Blinded Ashcroft scoring of fibrosis (n=25-34 sections from 8 mice/group) (mean ± SEM; **p<0.01; t-test vs. vehicle)

SMO4646 attenuated bleomycin-induced upregulation of ER stress vs. vehicle and showed trends in reducing fibrotic and Wnt markers

• SMO4646 attenuated bleomycin-induced upregulation of ER stress vs. vehicle and showed trends in reducing fibrotic and Wnt markers