Triciribine, a Selective Akt Inhibitor, Ameliorates Idiopathic Pulmonary Fibrosis and Pulmonary Hypertension

Maha Abdalla, Pharm.D., Ph.D. Candidate, 1,2,3 Alanna Pruitt, B.S., 1,2 Anna Goc, Ph.D., 1,2 Lakshman Segar, Ph.D., 1,3 Adviye Ergul, M.D., Ph.D., 1,2,3
Susan C. Fagan, Pharm.D., 1,2,3 and Somanath P.R. Shenyo, Ph.D. 1,2,3

1Clinical and Experimental Therapeutics, College of Pharmacy, University of Georgia, 2Charlie Norwood VA Medical Center, Augusta, GA, 3Department of Medicine, Georgia Regents University, Augusta, GA

Abstract

Purpose: Idiopathic pulmonary fibrosis (IPF) is an incurable, chronic and progressive disease with severe prognosis and often leads to pulmonary hypertension (PH). Persistent myofibroblast (MF) differentiation, marked by de novo expression of αSMA dress fibers, is the central orchestrator of tissue fibrosis and vascular remodeling that occurs in IPF and PH. Here we investigated the role of protein kinase B (Akt) in mediating MF differentiation and the efficacy of Triciribine (TCBN), a selective Akt inhibitor, currently in clinical trials for cancer therapy, as a potential therapeutic option for IPF and PH.

Methods: Mouse embryonic fibroblasts (MEFs), primary human lung fibroblasts (HLF) and HLFs transfected with hyperactive and inactive Akt variants (Myr-Akt and DN-Akt, respectively) were used in vitro. To evaluate the severity of IPF and PH, in vivo. Wild-type mice were subjected to the following results: Intratracheal adenovirus TGFβ (adTGFβ) gene delivery or chronic hypoxia, respectively. Mice were treated with placebo, insulin, alone, and insulin plus TCBN or Rapamycin.

Results: Hyperactivation of Akt resulted in a 6-fold increase in αSMA expression, an effect that was blunted in dominant negative (DN)-Akt cells despite TGFβ stimulation. In TGFβ-stimulated MEFs, HLFs, and primary human lung fibroblasts (HLF), Akt suppressed αSMA expression. Additionally, TCGN markedly attenuated MCA formation as evident by a marked decrease in collagen gel contraction assay. Furthermore, in vivo, TCBN (0.5 mg/kg/day) reversed adTGFβ- and hypoxia-induced IPF and PH, respectively, compared to Rapamycin. Mice treated with TCBN had markedly lower tissue dense infiltration and fibrosis, lower arterial and medial remodeling, lower αSMA, fibronectin and collagen assembly, and marked vasodilation.

Conclusions and translational impact: We are the first group to demonstrate the antifibrotic and anti-remodeling effects of Triciribine at a dose 50% lower than that utilized in preclinical cancer studies. Triciribine could potentially be a therapeutic option for IPF and PH.

Triciribine ameliorates idiopathic pulmonary fibrosis; Rapamycin induces severe vascular abnormalities

Figure 1. A schematic representation of our hypothesis that Akt mediates the fibrogenic switch to persistent myofibroblast differentiation leading to hypertrophic scar formation resulting in IPF and progressive vascular remodeling of peripheral pulmonary arteries in PH. Further, we investigate the therapeutic potential of Triciribine, a selective Akt inhibitor, in IPF and PH mouse models.

Figure 2. Sustained Akt activation results in a 6-fold increase in αSMA synthesis and assembly; an effect that is blunted in DN-Akt. Cells were subjected to (A) western analyses (n=3-4) and (B) immunofluorescence staining scale bar 20 μ m (I, p<0.0001 and II, p<0.02 compared to control untreated NH 373 ; compared to Myr-Akt control; † compared to TGFβ treated NH 373 )

Triciribine reverses MF differentiation by down-regulating both αSMA synthesis and ECM secretion in vitro

Figure 3. TCBN inhibits TGFβ-induced αSMA synthesis through SRF, independent of mTOR. Western analysis of αSMA synthesis SRF-selected active myr-Akt and DN-Akt (B) VP (C) Cells were subjected to (A) western analyses (n=3-4) and (B) immunofluorescence staining scale bar 20 μ m (I, p<0.0001 and II, p<0.02 compared to control untreated NH 373 ; compared to Myr-Akt control; † compared to TGFβ treated NH 373 )

Triciribine reverses chronic hypoxia-induced pulmonary hypertension; Rapamycin induces severe vascular abnormalities

Figure 5. TCBN reverses adenovirus TGFβ-induced pulmonary fibrosis; Rapamycin induces severe vascular abnormalities. WT mice were subjected to intratracheal injection of placebo or adenovirus TGFβ and treated as described in Methods (1). Lungs were isolated and subjected to western analysis and left lung subjected to immunofluorescence staining (Representative finding shown; n=6-8 per group).

Conclusions and Translational Impact

Importance of this problem: IPF is a progressive and incurable disease with severely poor prognosis that is complicated by PH and obliterator remodeling. Lung transplant remains the only effective option.

Gap in knowledge: From the 1980s until now, no substantial therapeutic interventions have been developed to reverse established fibrosis or even halt the chronic progression to respiratory failure. Despite the observation that Akt is upregulated in patients, the role of Akt in IPF/PH remains unclear.

Conclusions and Translational Impact: Our results shed light on the pathogenesis of these diseases and provide a potential therapeutic option through drug repurposing.

- We identify Akt as a novel target as it is crucial in mediating myofibroblasts differentiation, which is the central orchestrator of IPF and PH.
- We are the first group to demonstrate the novel anti-fibrotic and anti-remodeling properties of Triciribine, a selective Akt inhibitor, at a dose 50% less than that utilized in preclinical cancer studies. It can potentially be the first effective therapeutic option for IPF and PH.
- The unexpected finding that rapamycin does not transcriptionally regulate myofibroblasts holds an unanswered therapeutic implication and could further the detrimental effects of everolimus and sirolimus, rapamycin derivatives, observed in IPF patients. We demonstrate that while rapamycin regulates ECM proteins, IPF lung fibroblasts were treated with TCBN or Rap for 4h in the presence of 2% fetal bovine serum. Cell lysates were subjected to western analyses. (*p<0.012) (n=4; 4 h).

Future Direction

We will further investigate the mechanisms by which Triciribine modulates the transcriptional regulation governing αSMA synthesis in vitro. We will also examine the potential role of Triciribine in modulating vascular integrity in both IPF and PH mice models in vivo.

Program in Clinical and Experimental Therapeutics
College of Pharmacy, University of Georgia