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Dasatinib inhibits TGFβ-induced myofibroblast differentiation through Src-SRF Pathway

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ABSTRACT

Persistent myofibroblast differentiation is a hallmark of fibrotic diseases. Myofibroblasts are characterized by *de novo* expression of alpha smooth muscle actin (αSMA) and excess fibronectin assembly. Recent studies provide conflicting reports on the effects of tyrosine kinase inhibitor dasatinib on myofibroblast differentiation and fibrosis. Also, it is not fully understood whether dasatinib modulates myofibroblast differentiation by targeting Src kinase. Herein, we investigated the effect of dasatinib on cSrc and transforming growth factor-β (TGFβ)-induced myofibroblast differentiation *in vitro*. Our results indicated that selective Src kinase inhibition using PP2 mimicked the effect of dasatinib in attenuating myofibroblast differentiation as evident by blunted αSMA expression and modest, but significant inhibition of fibronectin assembly in both NIH 3T3 and fibrotic human lung fibroblasts. Mechanistically, our data showed that dasatinib modulates αSMA synthesis through Src kinase-mediated modulation of serum response factor expression. Collectively, our results demonstrate that dasatinib modulates myofibroblast differentiation through Src-SRF pathway. Thus, dasatinib could potentially be a therapeutic option in fibrotic diseases.

1. Introduction

Over the past three decades, myofibroblasts have emerged as the central effector cells in wound healing and tissue fibrosis. Myofibroblasts promote abnormal hypertrophic scar formation process, which is characteristic of tissue fibrosis (Hinz and Gabbiani, 2010). A hallmark of fibroblast activation into myofibroblasts is the *de novo* expression of alpha smooth muscle actin (αSMA) and persistent extracellular matrix (ECM) accumulation (Hinz et al., 2012; Tomasek et al., 2002). Finding effective therapeutics remains a challenge due to the evolving paradigm in the pathogenesis of fibrotic diseases. While the mechanisms of the pathologic activation of fibroblasts are not completely understood, transforming growth factor β (TGFβ), pro-fibrotic cytokine, is a well-established trigger and promoter of persistent myofibroblast differentiation (Hinz and Gabbiani, 2010; Tomasek et al., 2002). Previous studies have also implicated a pro-fibrotic role of Src kinases in the non-canonical signaling of TGFβ and in mediating fibroblast adhesion, migration, and myofibroblast-mediated ECM assembly (Hu et al., 2014; Schlaepfer and Hunter, 1997; Skhirtladze et al., 2008). Src kinases are involved in fibroblast adhesion to the ECM via regulation of adhesion proteins such as FAK (Okutani et al., 2006; Vittal et al., 2005). This discrepancy on the effects of dasatinib and Src kinases on pulmonary fibrosis raises questions if dasatinib indeed mediates its effects on pulmonary fibrosis through activity modulation of Src kinases.

Dasatinib selectively targets Src family of kinases, Bcr-Abl and PDGF receptors, and is currently approved for the treatment of a variety of neoplasias (Kantarjian et al., 2006; Montero et al., 2011; Roskoski, 2015). Dasatinib has shown beneficial effects on reducing ECM production in systemic sclerosis through c-abl and Src modulation (Skhirtladze et al., 2008). However, the role of dasatinib in myofibroblast differentiation is not fully understood. Since TGFβ has been shown to activate Src signaling in fibroblasts and

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Src is a major regulator of profibrotic adhesion proteins, we hypothesized that targeting Src kinases using dasatinib may ameliorate myofibroblast differentiation and ECM fibronectin accumulation. In this study, the regulatory role of dasatinib on myofibroblast differentiation and ECM accumulation relevant to fibrosis were studied in mouse embryonic fibroblasts (NIH 3T3 cells), human primary lung fibroblasts (HLFs) and human fibrotic lung fibroblasts (HFLFs). We found that dasatanib significantly decreased αSMA expression and mimicked the effects of selective Src family kinases inhibitor, PP2. Additionally, similar to PP2, dasatinib decreased fibronectin matrix assembly by NIH 3T3 and HFLFs. We also found that dasatinib mediated these effects via modulation of Src signaling and the expression of transcription factor serum response factor (SRF). Our results indicate that targeted Src kinase inhibition using dasatinib could potentially be a therapeutic option in patients with organ fibrosis including IPF.

2. Material and methods

2.1. Cell lines and cell culture

NIH 3T3, HLFs, and HFLFs were obtained from ATCC (Manassas, VA). To examine optimal time for myofibroblast differentiation, NIH 3T3 and HLFs were cultured on 6-well plates, after reaching 70% confluence, they were subjected to serum starvation in the presence or absence of 100 pM recombinant TGFβ (R&D Systems, Minneapolis, MN), a pre-determined dose (Abdalla et al., 2013;

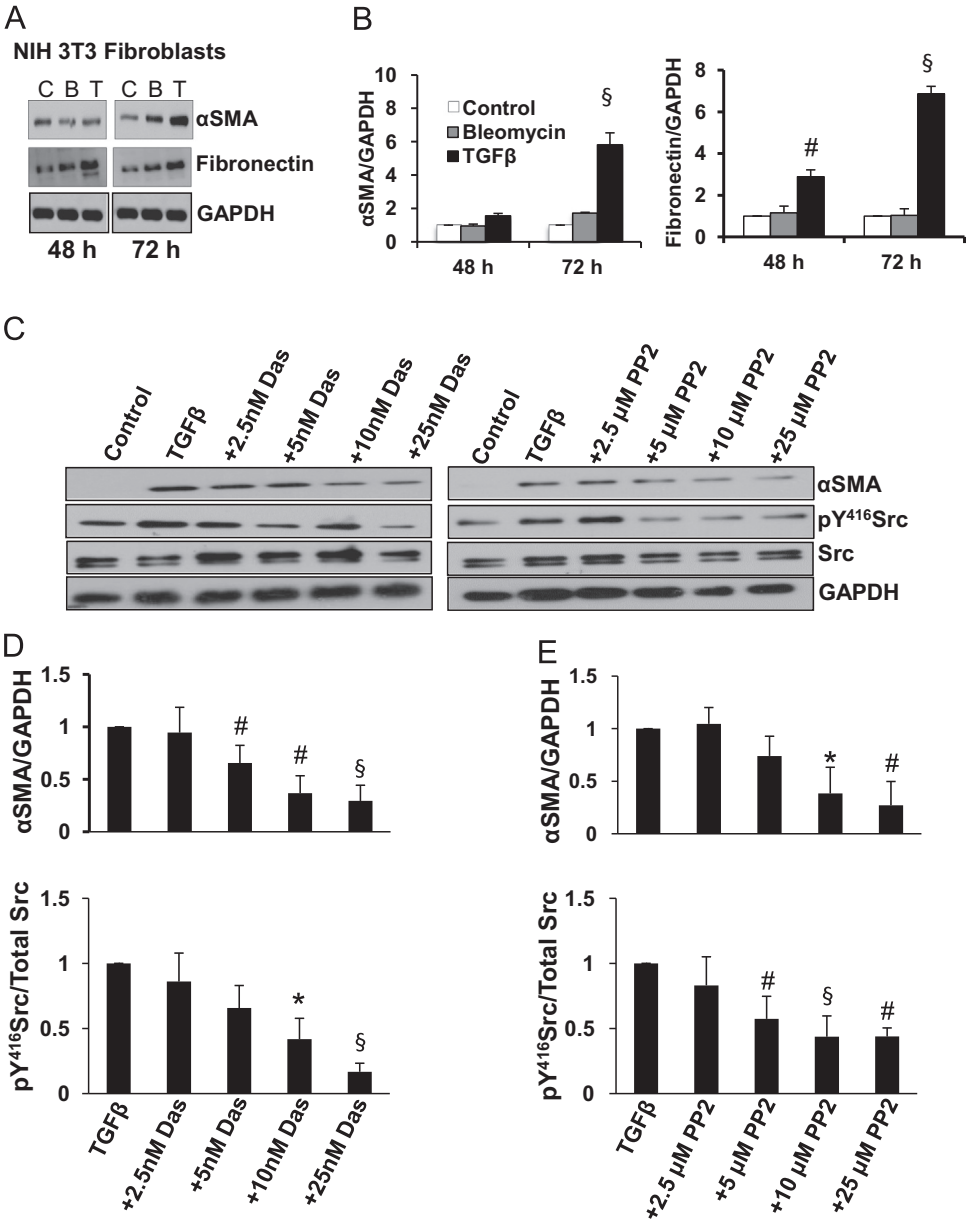

Fig. 1. Dasatinib inhibits TGFβ-induced myofibroblast differentiation and αSMA expression. A. Western blot images of NIH 3T3 lysates treated in the presence and absence of 100 pM TGFβ or 2.5 mU bleomycin for 48, and 72 h, probed for αSMA and fibronectin. **B.** Densitometry analysis of the Western bands showing expression changes in αSMA and fibronectin with 100 pM TGFβ and 2.5 mU bleomycin for 48, and 72 h and normalized to GAPDH ($n=3$–6). **C.** Western blot images of NIH 3T3 lysates treated in the presence and absence of 100 pM TGFβ (48 h) and in combination of TGFβ (48 h total) with various doses of dasatinib or Src inhibitor PP2 (24 h), probed for αSMA, pY^{416}Src and total Src. **D.** Densitometry analysis of the Western bands showing expression changes in αSMA and pY^{416}Src with 100 pM TGFβ combined with various doses of dasatinib, and normalized to GAPDH and total Src, respectively. ($n=3$). **E.** Densitometry analysis of the Western bands showing expression changes in αSMA and pY^{416}Src with 100 pM TGFβ combined with various doses of Src inhibitor PP2 and normalized to GAPDH and total Src, respectively ($n=3$). Data presented as mean ± S.D. $^{*}P<0.05$; $\#P<0.01$; $\S P<0.001$.