Journal of Pathology
J Pathol 2013; **230:** 205–214
Published online 3 April 2013 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.4177

ORIGINAL PAPER

Essential role of stem cell factor–c-Kit signalling pathway in bleomycin-induced pulmonary fibrosis

Lin Ding,Vladilsav Dolgachev,[#] Zhuang Wu,[#,†] Tianju Liu,Taku Nakashima, Zhe Wu, Matthew Ullenbruch, Nicholas W Lukacs, Zidi Chen and Sem H Phan*

Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA

**Correspondence to: SH Phan, Department of Pathology, University of Michigan Medical School, 109 Zina Pitcher Pl, Ann Arbor, MI 48109-2200, USA. e-mail: shphan@umich.edu*

[†]Current address: Biomedical Research Center, University of Texas Health Science Center at Tyler, Texas, 75708, USA.
[#]These authors contributed equally to this study.

Abstract

Stem cell factor (SCF) and its receptor c-Kit have been implicated in tissue remodelling and fibrosis. Alveolar fibroblasts from patients with diffuse interstitial fibrosis secrete more SCF. However, its precise role remains unclear. In this study the potential role of the SCF–c-Kit axis in pulmonary fibrosis was examined. Fibrosis was induced by intratracheal instillation of bleomycin (BLM), which caused increased SCF levels in plasma, bronchoalveolar lavage fluid (BALF) and lung tissue, as well as increased expression by lung fibroblasts. These changes were accompanied by increased numbers of bone marrow-derived c-Kit$^+$ cells in the lung, with corresponding depletion in bone marrow. Both recombinant SCF and lung extracts from BLM-treated animals induced bone-marrow cell migration, which was blocked by c-Kit inhibitor. The migrated cells promoted myofibroblast differentiation when co-cultured with fibroblasts, suggesting a paracrine pathogenic role. Interestingly, lung fibroblast cultures contained a subpopulation of cells that expressed functionally active c-Kit, which were significantly greater and more responsive to SCF induction when isolated from fibrotic lungs, including those from patients with idiopathic pulmonary fibrosis (IPF). This c-Kit$^+$ subpopulation was αSMA-negative and expressed lower levels of collagen I but significantly higher levels of TGFβ than c-Kit-negative cells. SCF deficiency achieved by intratracheal treatment with neutralizing anti-SCF antibody or by use of *Kitl*^{Sl}/*Kitl*$^{Sl-d}$ mutant mice *in vivo* resulted in significant reduction in pulmonary fibrosis. Taken together, the SCF–c-Kit pathway was activated in BLM-injured lung and might play a direct role in pulmonary fibrosis by the recruitment of bone marrow progenitor cells capable of promoting lung myofibroblast differentiation.

Keywords: pulmonary fibrosis; stem cell factor; c-kit; bone marrow cells

Received 13 September 2012; Revised 22 January 2013; Accepted 1 February 2013

No conflicts of interest were declared.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, usually fatal lung disease of unknown aetiology, characterized by the accumulation of fibroblasts/myofibroblasts with aberrant remodelling of the lung parenchyma. Fibroblasts represent the key source of interstitial collagens; however, the mechanisms underlying the emergence of these cells and their differentiation into myofibroblasts are not fully understood. There is mounting evidence that bone marrow (BM)-derived cells are chemotactically recruited to the lung in response to signals released in reaction to lung injury and may play important roles in the process of repair and fibrosis [1]. Some of these cells have been implicated as extrapulmonary sources of fibroblasts or fibroblast-like cells capable of elaborating factors to promote myofibroblast differentiation from endogenous lung fibroblasts. Hence, improved understanding of the signals responsible for the recruitment and activation of bone marrow derived cells would be of great benefit to current understanding of the fibrotic process.

Stem cell factor (SCF) is expressed as soluble or membrane-bound forms and promotes survival, proliferation, mobilization from the bone marrow and adhesion of haematopoietic stem cells and other progenitor cells through binding with its cognate receptor, c-Kit [2–6]. Recent studies of multiple tissues, including the liver, kidney, heart and brain, indicate that the SCF–c-Kit axis is important for tissue remodelling and fibrosis [7–12]. In the lung their potential importance in IPF is suggested by a study showing that alveolar fibroblasts from patients with diffuse interstitial fibrosis secrete more SCF than cells from sarcoidosis patients [13]. In a

murine model of asthma, neutralization of SCF *in vivo* significantly attenuated airway remodelling and collagen deposition, and strongly suppressed the recruitment of BM-derived collagen I-expressing cells to the lung [14]. This may be dependent on fibrogenic factor production by SCF-responsive cells, such as eosinophils and mast cells, but a direct role for SCF–c-Kit in recruitment and activation of BM-derived fibroblast-like cells has not been excluded. These findings provide a compelling argument for further examination of the essential role of the SCF–c-Kit axis in pulmonary fibrosis.

In this study, these findings were extended to lung parenchymal fibrosis, using the bleomycin (BLM)-induced model to provide evidence that the SCF–c-Kit pathway was activated and might play a direct role in pulmonary fibrosis by the recruitment of c-Kit$^+$ bone marrow cells to sites of lung. The essential role of this pathway was demonstrated by reduced fibrosis in mice with SCF deficiency, due either to neutralizing antibody treatment or a genetic defect.

Materials and methods

Detailed methods are described in Supplementary materials and methods (see Supplementary material).

Animals

Female C57BL/6, FVB/NJ and *GFP* transgenic mice (C57BL/6 or FVB/NJ background) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and female Fisher 344 rats were purchased from Charles River Breeding Laboratories (Wilmington, MA, USA). *GFP* bone marrow chimera mice were prepared as before [15]. C57BL/6J-Tg[COL1α2–CreER(T)] mice, carrying a Cre-recombinase under control of a regulatory sequence from the α2(I) collagen gene, were a generous gift from Dr Benoit de Crombrugghe of the University of Texas MD Andersen Cancer Center [16]. WCB6F1/J *Kitl*^{Sl} *Kitl*^{Sld} mice were purchased from the Jackson Laboratory. All animal studies were reviewed and approved by the University Committee on Use and Care of Animals at the University of Michigan.

Induction of pulmonary fibrosis

Animals were intratracheally injected with bleomycin (Blenoxane; Mead Johnson, Princeton, NJ, USA) at a dose of 2.5 U/kg body weight for mice and 7.5 U/kg body weight for rats, as before [17]. The control group received the same volume of phosphate-buffered saline (PBS) only. Where indicated, SCF-treated mice received recombinant SCF (R&D systems, Minneapolis, MN, USA) intratracheally at a dose of 0.1 μg/g body weight.

Human lung fibroblasts

Human lung fibroblasts from IPF patients and control subjects were generously provided by Dr Antonio Xaubet (Universitat de Barcelona, Spain) and Dr Carol Feghali-Bostwick (University of Pittsburgh, PA, USA).

Cell migration assay

Assays were performed using a 24-well cell migration kit (Corning, Lowell, MA, USA), according to the instruction manual. Freshly isolated whole BM cells were incubated with Calcein-AM (BD Biosciences) for 30 min at 37°C before assay. After 4 h at 37°C, the migrated cells were collected for fluorescence measurements using a Gemini EM (Molecular Devices) plate reader.

Statistics

Differences between means of various treatments were assessed for statistical significance by ANOVA, followed by *post hoc* analysis using Scheffé's test for comparison between any two groups; $p < 0.05$ was considered significant.

Results

SCF expression was induced in BLM-induced pulmonary fibrosis

The rodent pulmonary fibrosis model was induced as before [17] and at the indicated time points bronchoalveolar lavage fluid (BALF), plasma and lung tissue samples were collected for analysis of SCF expression. The results showed that SCF protein was undetectable in BALF from control mice, as determined by ELISA, but was highly induced in samples from BLM-treated mice at all the indicated time points (Figure 1A). Similar assay of serum samples indicated significantly elevated levels of SCF in BLM-treated animals relative to controls (Figure 1B). These determinations detected only soluble SCF; however, for the lung tissue samples both soluble and membrane-bound forms were analysed by western blotting. In control and BLM-treated lung samples both membrane-bound and soluble forms were detectable (Figure 1C). The membrane-bound product contains a proteolytic cleavage site encoded by exon 6 and post-translational processing at this site results in the soluble form. While membrane-bound SCF was slightly reduced in BLM-treated lung samples, the soluble form was increased relative to the control samples at all time points, with the highest increase at days 3–7 (Figure 1C). However, these differences in lung SCF protein levels in response to BLM treatment were not reflected in *SCF* mRNA levels, which were not significantly different between the BLM-treated versus control samples (data not shown), suggesting that the increase in lung soluble SCF was predominantly due to post-translational processing.

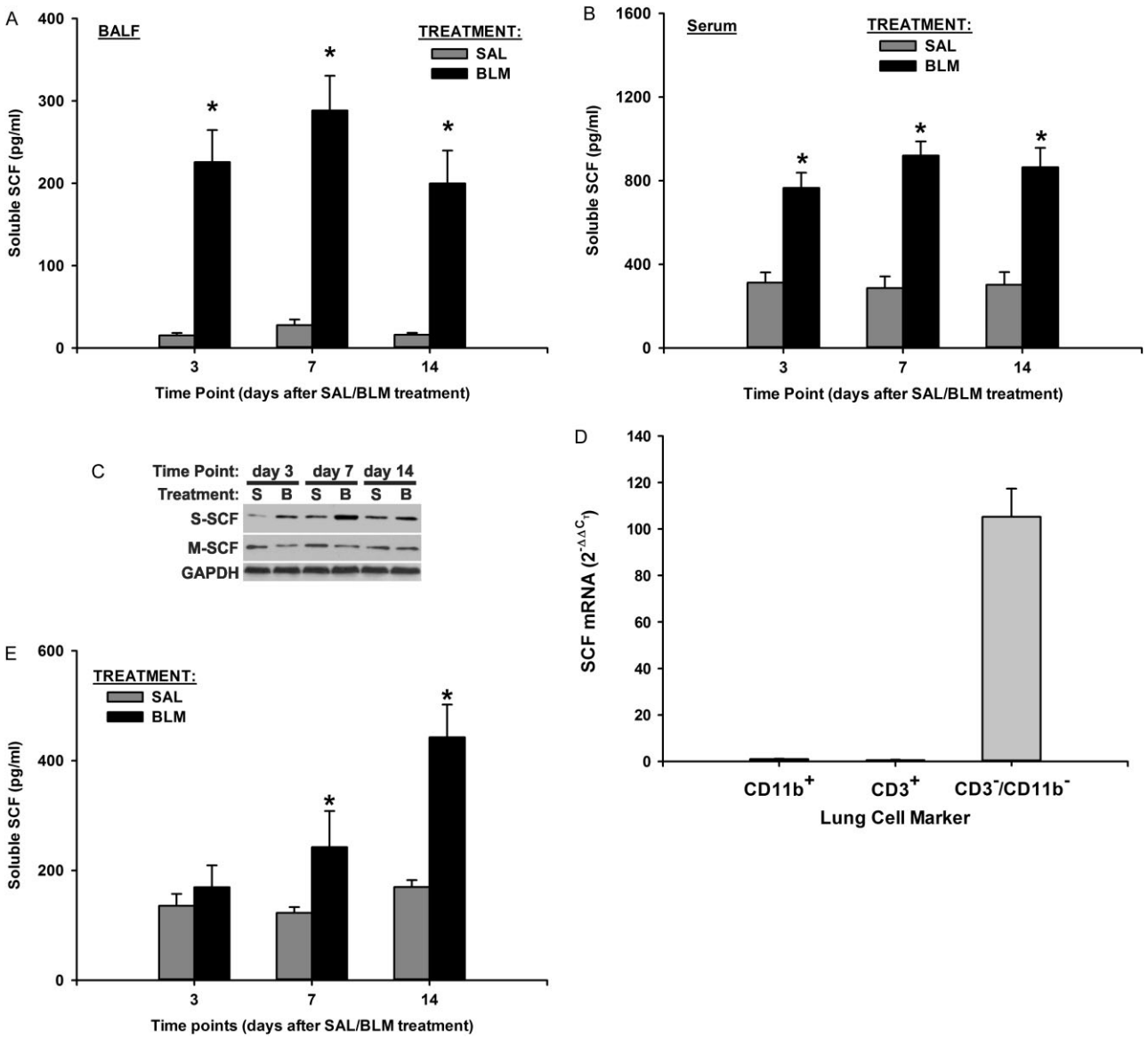

Figure 1. SCF expression in BLM-induced pulmonary fibrosis. BALF- (A), serum- (B), lung tissue- (C) and lung fibroblast-conditioned media (E) samples were obtained from mice at days 3, 7 and 14 after saline (SAL) or BLM treatment. Flow-sorted cells on the basis of the indicated marker expression from single-cell suspensions of lung tissue (D) were obtained from mice at day 21 after BLM treatment. Samples were analysed for SCF by ELISA (A, B, E), western blotting (C) or real-time PCR (D). Values represent mean ± SE ($n = 5$); *statistical significance ($p < 0.05$) when compared to respective saline controls.

To determine the cellular source of lung SCF expression, whole-lung, single-cell suspensions from BLM-treated mice were sorted to purify cells on the basis of CD3 (T-cells) or CD11b (inflammatory cells) expression, or absence of both markers ($CD3^-/CD11b^-$). When the sorted cells were analysed for *SCF* mRNA by real-time PCR, expression was essentially limited to the double-negative cells, which was over 100-fold higher than in CD3- or CD11b-positive cells (Figure 1D). These findings suggested that primarily structural cells, instead of inflammatory/immune cells, were the main cellular source of SCF in BLM-induced pulmonary fibrosis. Fibroblasts are known to express SCF [13,14]. The ELISA results showed that soluble SCF was significantly increased in conditioned media of lung fibroblasts from day 7 and day 14 BLM-treated animals, relative to those from their respective control animals (Figure 1E), suggesting that lung fibroblasts were an important source of SCF in the BLM model.

SCF-induced migration of c-Kit^+ BM cells to the lung

Immunostaining for c-Kit showed infiltration by c-Kit^+ cells in lungs of BLM treated mice, whereas only rare c-Kit^+ cells were observed in those from saline or BLM-treated $Kitl^{Sl}/Kitl^{Sl\text{-}d}$ mutant mice (Figure 2A). These mutant mice are compound heterozygotes of a null SCF mutation (Sl) and the Steel-Dickie (Sld) mutation, which lacks membrane-bound SCF and exhibit reduced serum levels of soluble SCF [12] (see Supplementary material, Figure S1). Thus c-Kit^+ cell

infiltration in this fibrosis model depended on an intact SCF–c-Kit axis.

Given the increased levels of SCF and c-Kit$^+$ cells in BLM-injured lung, a direct role of the SCF–c-Kit axis in homing of BM cells to the injured lung was examined. After intratracheal treatment with saline, BLM or recombinant SCF, BM cells and whole lung cells were analysed for c-Kit$^+$ cells by flow cytometry. Notably, the results showed increased numbers of c-Kit$^+$ cells in the lung, which correlated with a concomitant decrease of such cells in the BM after BLM or SCF treatment (Figure 2B). This pattern of changes in the percentage of c-Kit$^+$ cells in the two compartments would be consistent with induced migration of these cells from the BM to the lung as a result of treatment with either BLM or SCF. We further evaluated this possibility by using *GFP* BM chimeric mice. After stable engraftment the mice were treated with saline or BLM, and 7 days later the lung cells were analysed for GFP and c-Kit expression by flow cytometry. The results confirmed that the increased portion of c-Kit$^+$ cells in the injured lung was derived from BM on the basis of GFP expression (Figure 2C).

An *in vitro* assay using modified Boyden chambers was undertaken to see whether SCF was chemotactic for bone marrow cells. As shown in Figure 3, SCF exerted a robust effect on migration of freshly isolated rat BM cells. This effect was abolished by suppression of c-Kit expression, using c-Kit siRNA (Figure E2) or c-Kit kinase inhibitor, imatinib. Interestingly, lung tissue extracts from control animals did not affect BM cell migration, whereas extracts from the BLM group mimicked the effect of SCF, which was also suppressed by c-Kit siRNA pretreatment, suggesting the effect in a SCF-dependent manner. These findings confirmed the ability of pure SCF and SCF in lung extracts of BLM-treated animals to directly increase the c-Kit-dependent migratory activity of BM cells.

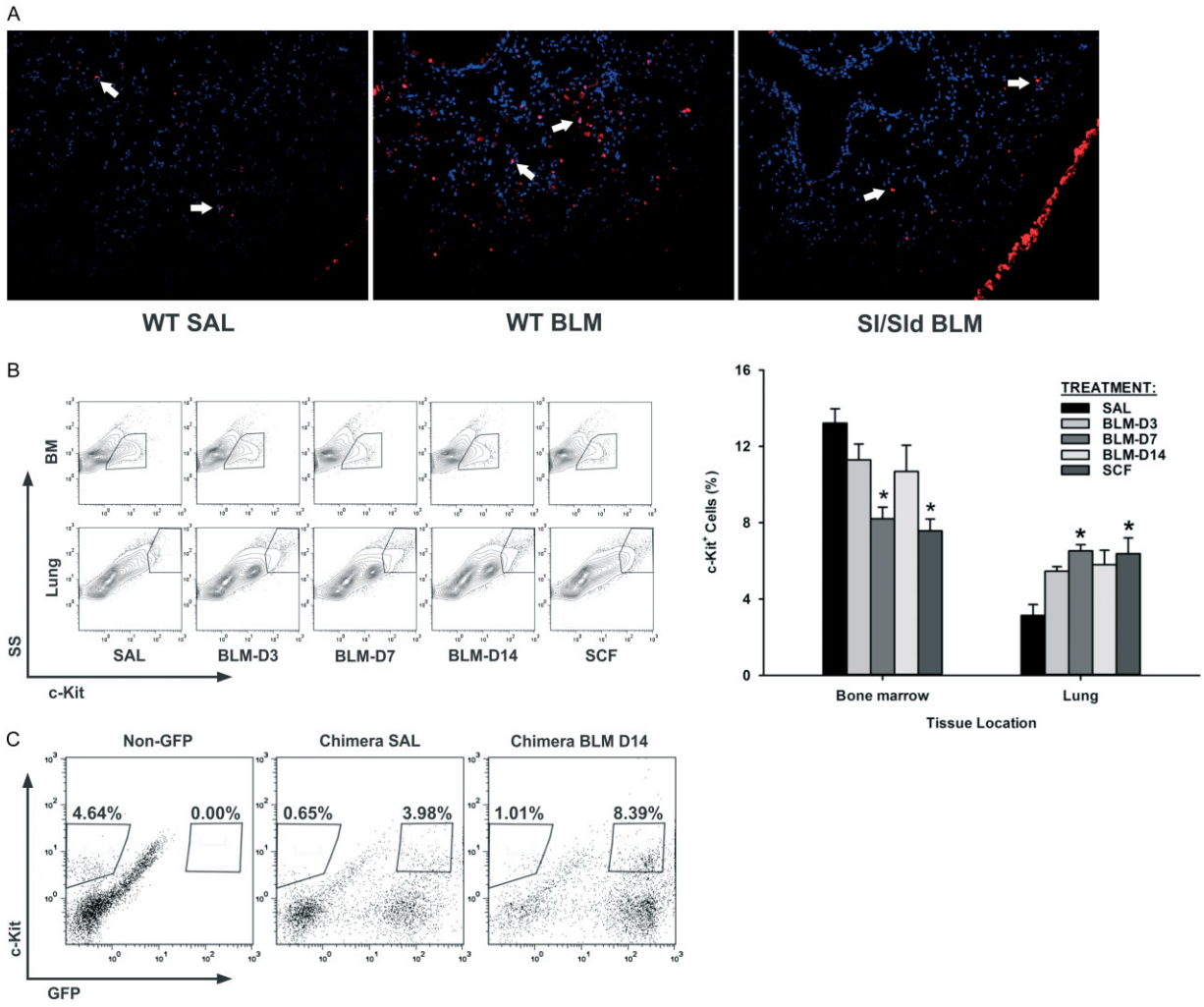

Figure 2. Qualitative and quantitative analyses of c-Kit$^+$ cells. (A) Immunofluorescent staining of c-Kit (red) in a representative lung tissue from WT and Sl/Sld mice at day 21 after SAL/BLM treatment, with DAPI (blue) counterstain. Arrows indicate c-Kit$^+$ cells; ×20 magnification. (B) Flow-cytometry analysis of c-Kit$^+$ cells in bone marrow and lungs from WT mice at days 3, 7 and 14 after BLM treatment or 1 day after recombinant SCF treatment. The percentage of c-Kit$^+$ cells in each group are shown as mean ± SE in the lower panel bar graph ($n = 5$). *Statistical significance ($p < 0.05$) when compared to corresponding SAL-treated mice. (C) Cells from lungs of GFP–BM chimeric and non-chimeric (Non-GFP) mice at day 7 after SAL/BLM treatment were similarly analysed for GFP and c-Kit expression. Flow-cytometry data were generated using FlowJo software.

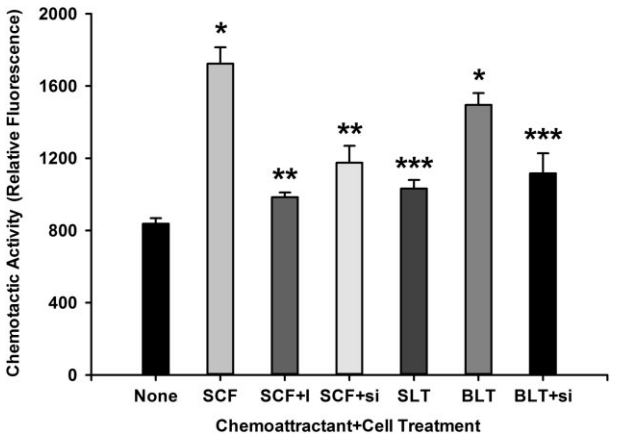

Figure 3. Chemotactic activity of rSCF on BM cells. Rat whole bone marrow cells were placed in the upper wells of modified Boyden chambers (pore size 5.0 μm) and the indicated test substance was placed in the bottom well – buffer only (None), SCF (100 ng/ml). Cells were transfected with control siRNA or c-Kit siRNA the day before assay (+si) or pretreated with c-Kit inhibitor, imatinib (5 μM; +I). SLT and BLT, lung tissue extracts from day 7 saline or BLM-treated rats, respectively. Values represent mean ± SE ($n = 5$). *Statistical significance ($p < 0.05$) when compared to the respective 'None' control; **statistical significance when compared to the respective SCF treatment group; ***significance when compared to BLT group.

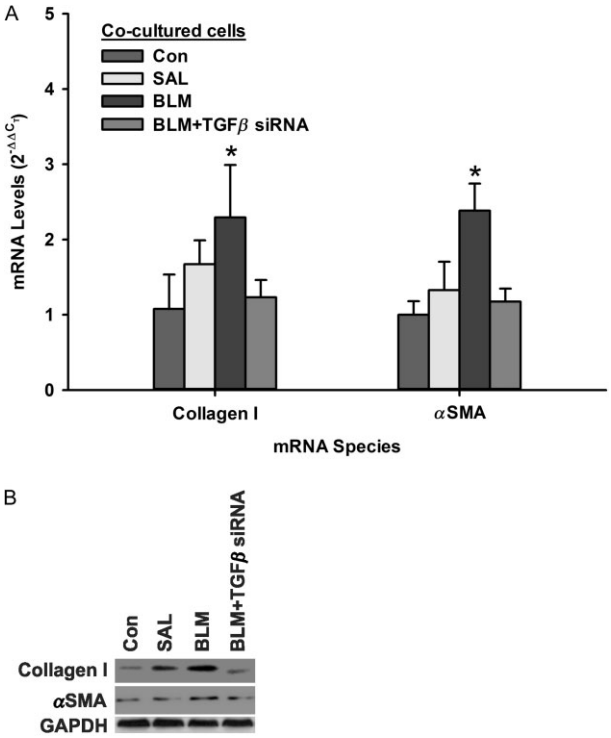

Figure 4. Effect of BM-derived lung c-Kit$^+$ cells on collagen I and αSMA expression in lung fibroblasts. c-Kit$^+$ GFP$^+$ cells sorted from lungs of GFP bone marrow chimera mice at day 7 after SAL or BLM treatment were placed in the upper transwell (pore size 0.4 μm) and fibroblasts were seeded at the bottom of the plastic wells. As a control (Con), lung fibroblasts were placed in inserts. Some sorted cells from BLM-treated mice were transfected with TGFβ siRNA the day before assay (BLM + TGFβ siRNA). After 16 h of co-culture, fibroblasts at the bottom of the wells were collected and analysed for *collagen I* and *αSMA* mRNA by real-time PCR (A) and protein by western blotting (B). Values represent mean ± SE ($n = 3$). *Statistically significant ($p < 0.05$) difference from the control cells. The western blot is representative of three independent experiments.

BM-derived c-Kit$^+$ cells promoted myofibroblast differentiation

c-Kit$^+$ GFP$^+$ cells sorted from lungs of *GFP* BM chimera mice were co-cultured *in vitro* with mouse lung fibroblasts in a Corning transwell cell culture system. The pore size of the insert membrane (0.4 μm) would allow for the diffusion of soluble factors between two cell populations, but prevent the transfer of any cells or cellular organelles. After 16 h, fibroblasts cultured with c-Kit$^+$ GFP$^+$ cells sorted from BLM-treated mice expressed greater amounts of mRNA for both collagen I and α-smooth muscle actin (αSMA) than did fibroblasts cultured with cells from saline-treated mice (Figure 4). Pretreatment of sorted cells with TGFβ siRNA abolished this induction. Thus these BLM-induced BM-derived c-Kit$^+$ cells represented a source of paracrine factor(s) capable of promoting myofibroblast differentiation, such as TGFβ.

Increased c-Kit$^+$ collagen I$^+$ cells in the lung

SCF is reported to recruit c-Kit$^+$ collagen I$^+$ BM-derived cells [14]. We performed immunostaining for c-Kit and Cre on lung sections of BLM-treated Col–Cre mice, which carry a Cre-recombinase under the control of a regulatory sequence from the α2(I) collagen gene [16]. Co-localization of c-Kit and Cre showed the presence of c-Kit$^+$ collagen I$^+$ cells in the injured lung (Figure 5A, upper panel). Flow-cytometric analysis confirmed infiltration of c-Kit$^+$ collagen I$^+$ cells in the BLM-treated lung, which was suppressed in $Kitl^{Sl}/Kitl^{Sl\text{-}d}$ mutant mice (Figure 5B). Interestingly we could detect the presence of c-Kit$^+$ collagen I$^+$ cells in lung fibroblast cultures (Figure 5A, lower panel, and C) and the percentage of these cells was significantly higher in cultures from BLM-treated versus SAL-treated lungs (2.69% versus 0.96%, respectively; $p < 0.05$, $n = 5$).

Expression of c-Kit in lung fibroblast cultures was evaluated. Cultures from BLM-treated or control animals revealed detectable expression of c-Kit by real time PCR (Figure 6A). Treatment with SCF caused a significantly higher level (3.2-fold versus 1.5-fold) in *c-Kit* mRNA in cells from BLM-treated animals when compared to the response in cells from controls. Moreover, higher induction of c-Kit expression by SCF was noted in lung fibroblast cultures isolated from IPF patients compared to cells from control subjects (Figure 6B), suggesting that this might be a property of fibroblast cultures from fibrotic lungs. The expressed c-Kit was functionally active, since SCF treatment caused rapid phosphorylation of this receptor, peaking at about 5 min and sustained for at least 10 min (Figure 6C). Notably, when compared to the predominantly c-Kit$^-$

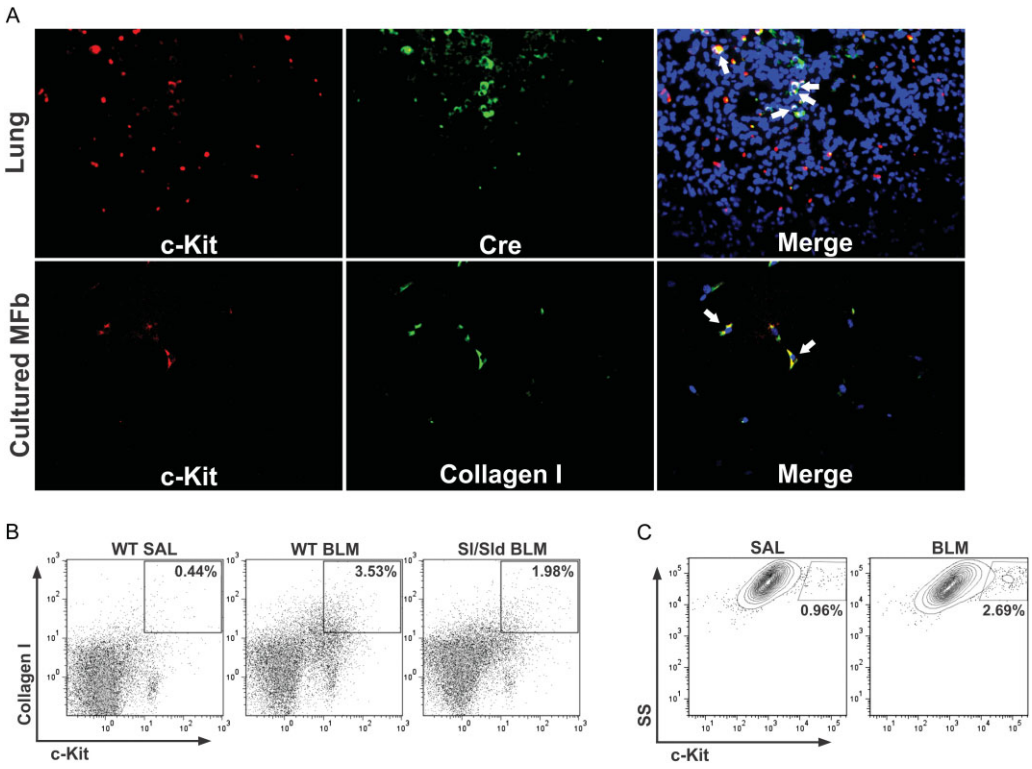

Figure 5. Analyses of c-Kit$^+$ Collagen I$^+$ cells. (A) Double staining of c-Kit (red) and Cre (green) on lung sections of Col–Cre mice, which carry a Cre-recombinase under the control of a regulatory sequence from the α2(I) collagen gene (upper panel, ×40 magnification) and double staining of c-Kit (red) and collagen I (green) on slides of cultured mouse lung fibroblasts (MFb; lower panel, ×20 magnification). Arrows indicate double-positive cells. DAPI (blue) counterstain. Flow-cytometry analysis of c-Kit and collagen I in (B) lung cells from wild-type (WT) and Sl/Sld mice and (C) passage 6-cultured lung fibroblasts. All mice were at day 21 after BLM treatment, with a saline control group (SAL) (B, C).

population, this c-Kit$^+$ subpopulation was essentially αSMA negative and expressed lower levels of collagen I but significantly higher levels of TGFβ (Figure 7A, B). This would be consistent with a paracrine role for these BM-derived c-Kit$^+$ collagen I$^+$ cells, as suggested above.

SCF deficiency attenuated BLM-induced lung fibrosis

The importance of this pathway in BLM-induced lung fibrosis was evaluated in mice made deficient in *SCF* by intratracheal treatment with anti-SCF antibodies or by use of $Kitl^{Sl}/Kitl^{Sl\text{-}d}$ mutant mice. When subjected to BLM-induced lung injury, the antibody-treated groups at doses of antibody ≥150 μg/animal and $Kitl^{Sl}/Kitl^{Sl\text{-}d}$ mutant mice showed significantly reduced fibrosis, both morphologically [haematoxylin and eosin (H&E) or Masson's trichrome stain] and biochemically by hydroxyproline analysis (see Supplementary material, Figures S3A, B, S8A, B, respectively). Notably, the reduction in fibrosis by anti-SCF antibody treatment was accompanied with a significant reduction in BLM-induced increase in BM-derived cells in the lung (see Supplementary material, Figure S3C). These data, taken together, indicated an essential role for the SCF–c-Kit signalling pathway in BLM-induced pulmonary fibrosis.

Discussion

Recent studies suggest that organ injury is 'sensed' distally in the bone marrow by cells that can migrate to the site of damage and undergo differentiation to aid in repair or promotion of fibrosis [18]. However, the nature of the signal(s) from the injured distal organ and the mechanism of sensing, as well as recruitment from the bone marrow, have not been fully elucidated in the case of pulmonary fibrosis. Induced lung SCF expression plays an essential role in airway remodelling in an allergic airway disease model [14]. In addition, alveolar fibroblasts from IPF patients secrete more SCF [13]. Thus, ample evidence is available to suggest the potential importance of the SCF–c-Kit pathway in pulmonary fibrosis. In this study the significance of the SCF–c-Kit signal pathway was investigated with respect to recruitment of BM-derived cells in the context of BLM-induced lung injury and fibrosis.

The findings revealed a coincident increase in expression of SCF at the site of lung injury and in the level of circulating SCF as early as day 3 after bleomycin treatment. This is comparable to observations in brain injury, myocardial infarction, serum nephrotoxic nephritis and wire-induced vascular injury, wherein SCF expression is also increased during injury and repair [8–10,12]. It is noteworthy

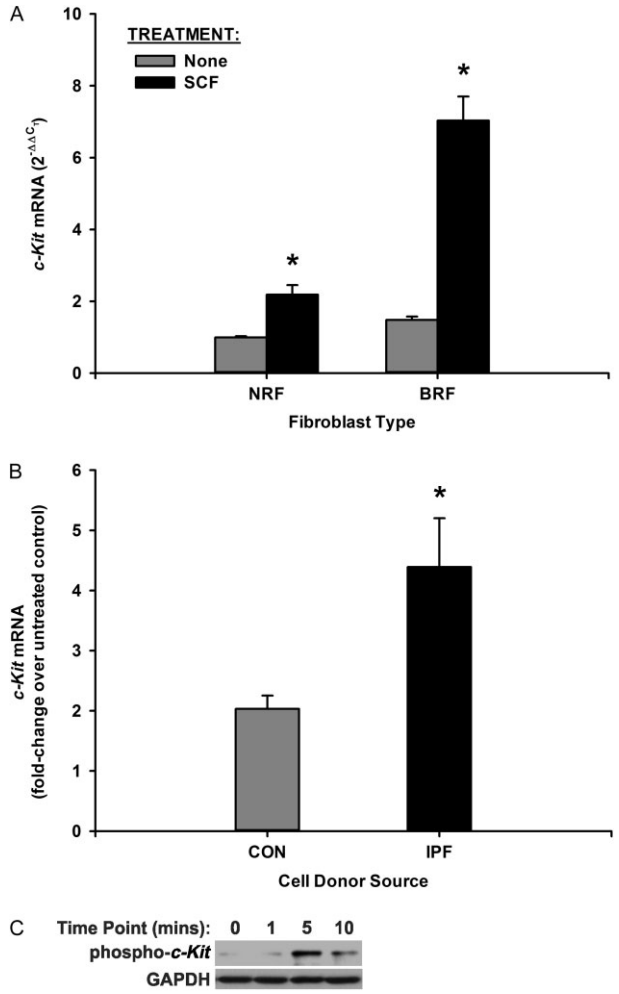

Figure 6. c-Kit expression and phosphorylation induced by SCF in lung fibroblasts. (A) Lung fibroblasts from saline (NRF) or BLM-treated rats were treated with or without SCF (100 ng/ml) and then analysed for *c-Kit* mRNA by real-time PCR. In (B) human lung fibroblasts from controls (CON) or IPF patients were similarly treated and analysed. The effect of SCF on c-Kit phosphorylation in BRFs was analysed by western blotting (C). Values represent mean ± SE ($n = 5$). *Statistically significant ($p < 0.05$) difference from the respective untreated control cells.

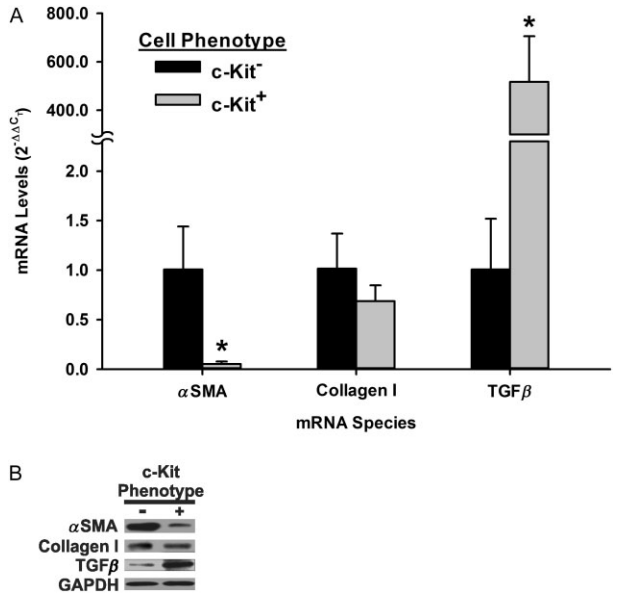

Figure 7. Gene expression analysis of c-Kit$^+$ versus c-Kit$^-$ cells in cultured lung fibroblasts. Lung fibroblast cultures from BLM-treated mice were flow-sorted into c-Kit$^+$ and c-Kit$^-$ subpopulations. The separated cells were then analysed for expression of the indicated genes by qRT–PCR (A) and western blotting (B). Values represent mean ± SE ($n = 3$). *Statistically significant ($p < 0.05$) difference from the mean value for the respective c-kit$^-$ cells. The western blot was representative of three independent experiments.

that increased soluble SCF was mainly regulated at the level of post-translational processing, consistent with results reported on the role of SCF in the remodelling process associated with nephrotoxic nephritis [8]. BLM-induced responses are known to include induction of some proteinases, such as MMP-2 and MMP-9 [19], which has the capacity to mediate cleavage of membrane-bound SCF with release into the interstitium and blood circulation as a soluble mediator, as observed in the BM niche [20] and vascular injury [12].

Current concepts of tissue repair and fibrotic process implicate the recruitment of BM-derived fibroblast-like cells to sites of injury in response to chemokine signals released from the injured tissue [21]. Here our evidence suggested that BLM-induced release of soluble SCF from the structural cells of the lung resulted in elevated levels of circulating SCF in plasma, which played a significant role in the recruitment of c-Kit$^+$ cells from BM to the injured lung. The concomitantly decreased c-Kit$^+$ cells in the BM suggested that normal homing of circulating progenitor cells is disrupted. C-Kit, considered as a marker for BM-derived haematopoietic stem cells, is also expressed on mast cells and dendritic cells [22–24]. However, mast cell participation was limited or negligible in our BLM-induced pulmonary fibrosis model, according to expression of the mast cell marker, tryptase (data not shown). The role of dendritic cells remains to be specifically elucidated. We showed here that BM-derived c-Kit$^+$ cells are capable of elaborating factors to promote myofibroblast differentiation and thus play a paracrine pro-fibrotic role. Some of these cells appear to be the c-Kit$^+$ collagen I$^+$ cells that were isolated along with lung fibroblasts, whose numbers were increased when isolated from fibrotic lungs. While they expressed type I collagen, these BM-derived cells, unlike lung fibroblasts, cannot undergo myofibroblast differentiation, as previously shown [14,25]. In contrast, fibrocytes are not known to express c-Kit but can undergo myofibroblast differentiation and thus are capable of contributing to matrix deposition directly [26]. While exogenous BM-derived mesenchymal stem cells (MSCs) protect against lung injury [27,28], endogenous recruitment of BM-derived cells, such as fibrocytes, mediated by the SDF-1/CXCR4 axis is reported to promote pulmonary fibrosis [26,29,30]. BM cell trafficking by SDF-1–CXCR4 is modulated by c-Kit and its phosphorylation [31]. This crosstalk between the SDF-1–CXCR4 and SCF–c-Kit signalling pathways favours the profibrotic role of the recruited endogenous BM c-Kit$^+$ cells, as observed previously

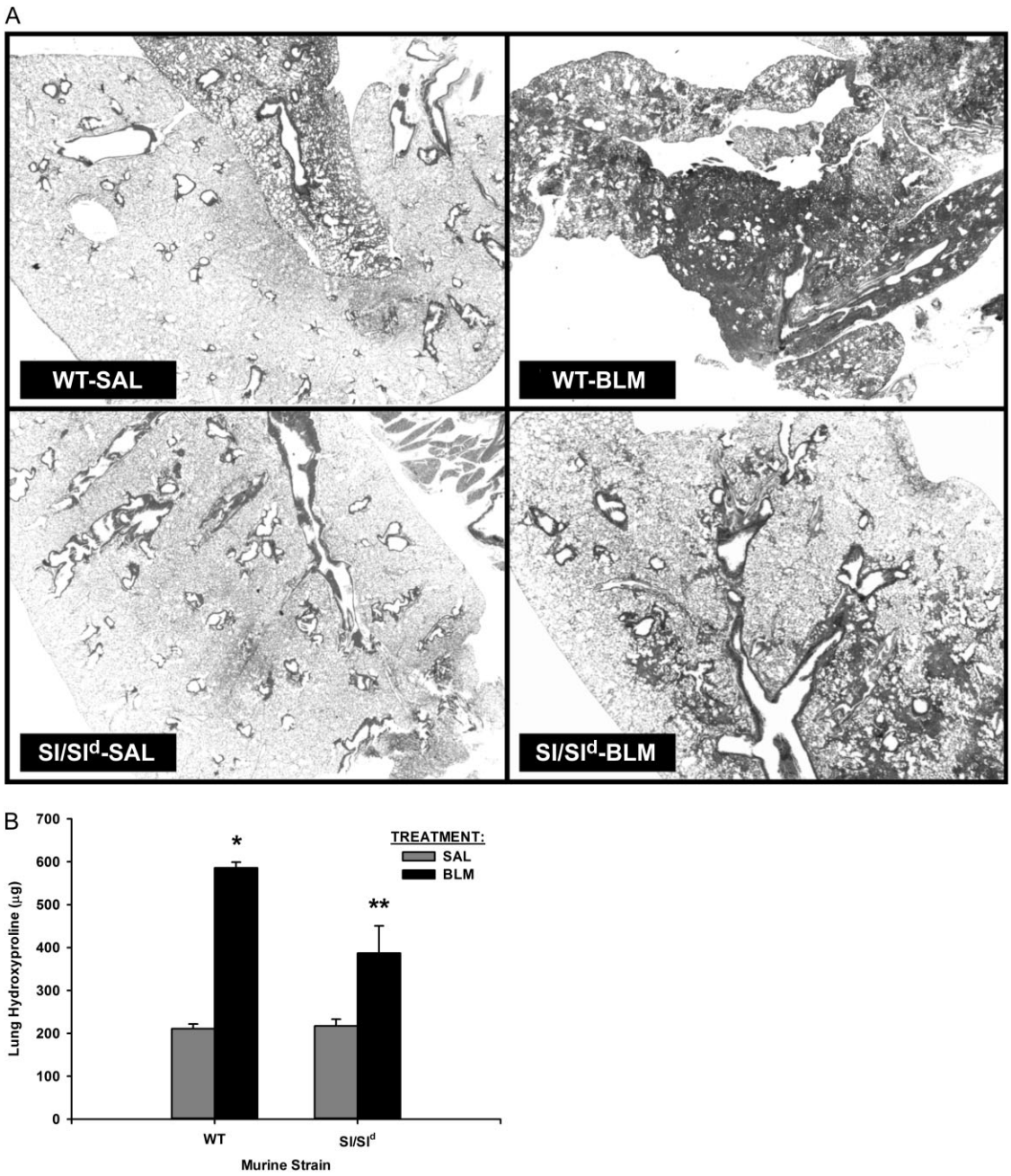

Figure 8. Effect of SCF deficiency on BLM-induced pulmonary fibrosis. Wild-type (WT) and SCF-deficient (SI/SI^d) mice were treated with saline (SAL) or BLM on day 0 and the lungs were harvested 21 days later. A representative Masson's trichrome-stained lung tissue section from each treatment group is included in the composite in (A) (×20 magnification). Fibrosis was quantitated biochemically as lung hydroxyproline content (B); mean ± SD, $n = 3$; *$p < 0.05$ compared to the WT saline-treated control mean; **$p < 0.05$ compared to the WT BLM-treated group.

for fibrocytes [26,29,30]. In this study, expression of c-Kit on some cells in cultured fibroblasts was significantly greater and more responsive to SCF induction in cells from fibrotic lungs, including those from IPF patients, which has been previously reported [32]. This would be consistent with the paracrine role of these c-Kit$^+$ cells in promoting the elevated myofibroblast differentiation in fibroblasts isolated from fibrotic lung tissue. Moreover, recruitment of haematopoietic stem cells and endothelial progenitor cells via activation of the SCF-c-Kit signalling pathway is shown to be essential for osteogenesis [11], a remodelling process akin to fibrosis, wherein the key participating cell type, the osteoblast, is viewed as the counterpart of a fibroblast [33]. In this regard, the role of SCF–c-Kit in osteogenesis is highly reminiscent of that in pulmonary fibrosis in the BLM model.

Direct *in vivo* confirmation of the role of SCF–c-Kit in pulmonary fibrosis was provided by animal models. Imatinib was found to have anti-fibrotic activity in the BLM-induced lung fibrosis model [34,35]. Although imatinib is a tyrosine kinase inhibitor capable of suppressing the effect of SCF, it is not specific for c-Kit. To attain greater specificity, anti-SCF antibodies

as well as SCF deficiency in $Kitl^{Sl}/Kitl^{Sl\text{-}d}$ mutant mice were used, and both strategies resulted in significant attenuation of BLM-induced pulmonary fibrosis. Taken together, the findings directly implicate the SCF–c-Kit axis in the pathogenesis of BLM-induced lung fibrosis, with activation of the SCF–c-Kit pathway resulting in the recruitment of cells from the BM compartment to sites of lung injury, with consequent paracrine activation of fibroblasts. Elevated c-Kit expression in IPF lung fibroblast cultures suggests potential relevance to human pulmonary fibrosis. Thus, attenuation of SCF expression and function may be an effective therapeutic modality for treatment of pulmonary fibrosis.

Acknowledgements

Human lung fibroblasts from normal controls and IPF patients were gifts from Dr Antonio Xaubet (Universitat de Barcelona, Spain) and Dr Carol Feghali-Bostwick (University of Pittsburgh, USA). C57BL/6J-Tg[COL1α2–CreER(T)] mice were gifts from Dr Benoit de Crombrugghe (University of Texas MD Anderson Cancer Center, Houston, TX, USA). This study was supported by the National Institutes of Health (NIH; Grant Nos HL28737, HL 52285, HL77297 and HL91775).

Author contributions

LD, VD, NWL and SHP conceived and designed experiments; LD, VD, MU, ZC, ZheW and ZhuangW performed experiments; LD, VD, TL and TN analysed data; and LD and SHP wrote the manuscript.

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*Cited in Supplementary material only.

SUPPORTING INFORMATION ON THE INTERNET

The following supplementary material may be found in the online version of this article:

Supplementary materials and methods

Figure S1. Effect of SCF deficiency approaches on BLM model

Figure S2. Decreased c-Kit expression by c-Kit siRNA transfection

Figure S3. Effects of anti-SCF antibody treatment on BLM model