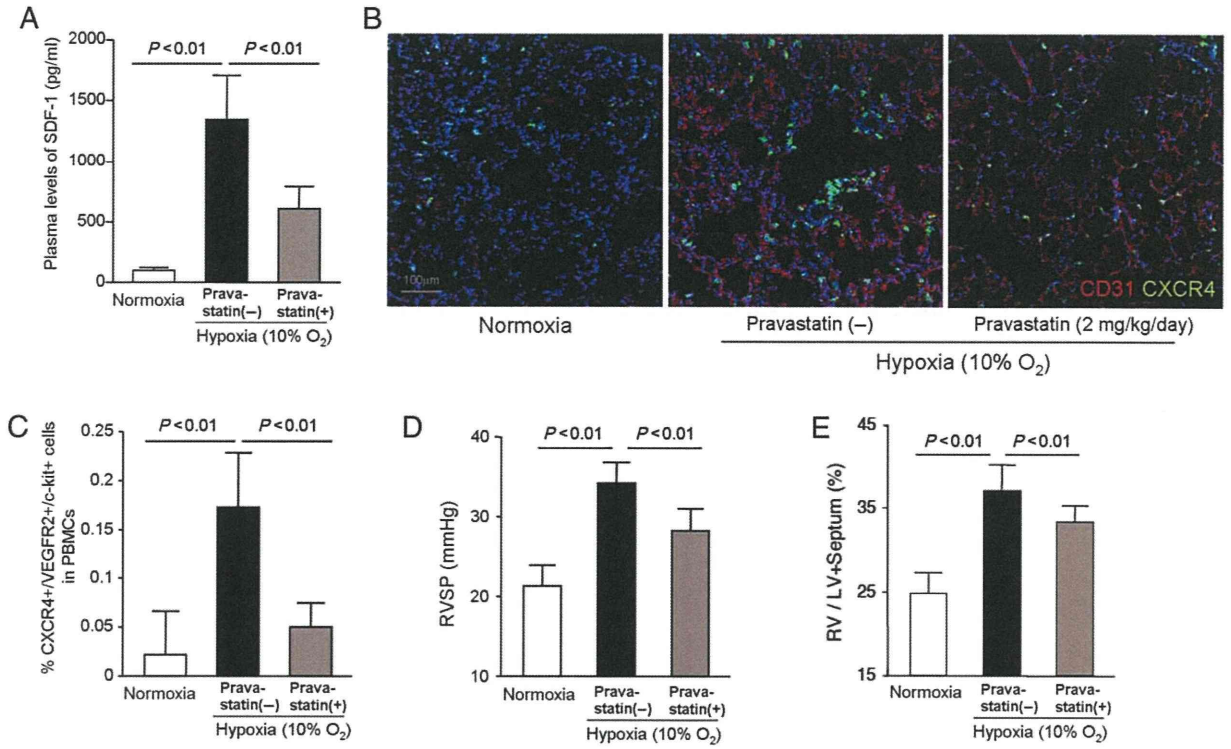

Figure 4 Reduction in plasma levels of stromal cell-derived factor (SDF)-1, mobilization of CXC chemokine receptor (CXCR)4$^+$/vascular endothelial growth factor receptor (VEGFR)2$^+$/c-kit$^+$cells, and ameliorated pulmonary arterial hypertension (PAH) by pravastatin. (*A*) Plasma levels of SDF-1 in hypoxic mice with or without pravastatin ($n = 11$ each). (*B*) Immunostaining of lung sections with CXCR4 (green signals) and CD31 (red signals) revealed the accumulation of CXCR4$^+$ cells in hypoxic lung, which was reduced by pravastatin. (*C*) Hypoxia significantly increased the number of CXCR4$^+$/VEGFR2$^+$/c-kit$^+$ cells in the peripheral blood, which was significantly suppressed by pravastatin ($n = 5$ each). (*D*,*E*) Pravastatin suppressed the development of hypoxia-induced PAH, as assessed by RV systolic pressure (RVSP) (*D*) and RV hypertrophy (*E*) in mice ($n = 10$ each). The extent of RV hypertrophy is expressed as a ratio of RV mass to LV plus septal mass (RV/LV + Septum). Results are expressed as means ± SD.

BM-derived cells, resulting in the inhibition of hypoxia-induced pulmonary artery remodelling and PAH (*Figure 6*).

4.3 Limitations of the study

There are several limitations should be mentioned for the present study.

First, hypoxia-induced PAH model may not fully represent the primary PAH in humans because this model shows considerably high plasma levels of cytokines/chemokines.[39] It has been shown that hypoxia increases the expression of SDF-1 through HIF-1-dependent mechanisms.[40] Consistently, in the present study, plasma level of SDF-1 was significantly increased in WT mice in response to hypoxia. Additionally, pravastatin significantly reduced the plasma levels of SDF-1 and ameliorated hypoxia-induced PAH, although the precise mechanism remains to be elucidated in future studies. On the other hand, blockade of SDF-1 by specific antibody has been shown to reduce neointimal formation in ApoE$^{-/-}$ mice by regulating neointimal smooth muscle content.[41] Taken together, it is possible that SDF-1 also plays a crucial role in the development of pulmonary vascular remodelling in PH.

Second, the role of EPCs in the development of PAH in humans remains to be elucidated. It was demonstrated that the number of circulating endothelial cells was significantly increased in patients with PAH.[11] The number of circulating EPCs is regulated not only by their recruitment or mobilization but also by their activity and consumption in the peripheral vasculature.[1–4] Therefore, when considering a therapy focusing on BM-derived progenitors, it would be important to evaluate their mobilization and homing as well as the character of each progenitor.

Third, it remains to be examined whether statins could ameliorate PH in humans through down-regulation of SDF-1. However, this important issue is beyond the scope of the present study, and we would like to address this issue in future studies.

Fourth, in the present study, we have demonstrated that the number of progenitor cells is decreased by co-treatment with pravastatin in which finding is different from that in atherosclerotic model.[42] Thus, future studies are needed to determine the role of progenitor cells, especially at the adventitia.

Fifth, the present study is a preventive study in nature. Because oxidative stress contributes to the initiation and the development of hypoxia-induced tissue injury and PAH, statins may be expected to reduce initial oxidative and subsequent events that lead to PAH. In this regard, statins have direct antioxidant effects: inhibit NAD[P]H activity and up-regulate the activity of antioxidant enzymes, such as catalase and paraoxonase, and also reduce circulating oxidized low-density lipoprotein (ox-LDL), inhibit ox-LDL uptake by macrophages, and reduce circulating markers of oxidation, such as F2-isoprostane and nitrotyrosine. Thus, it remains to be examined in future studies whether

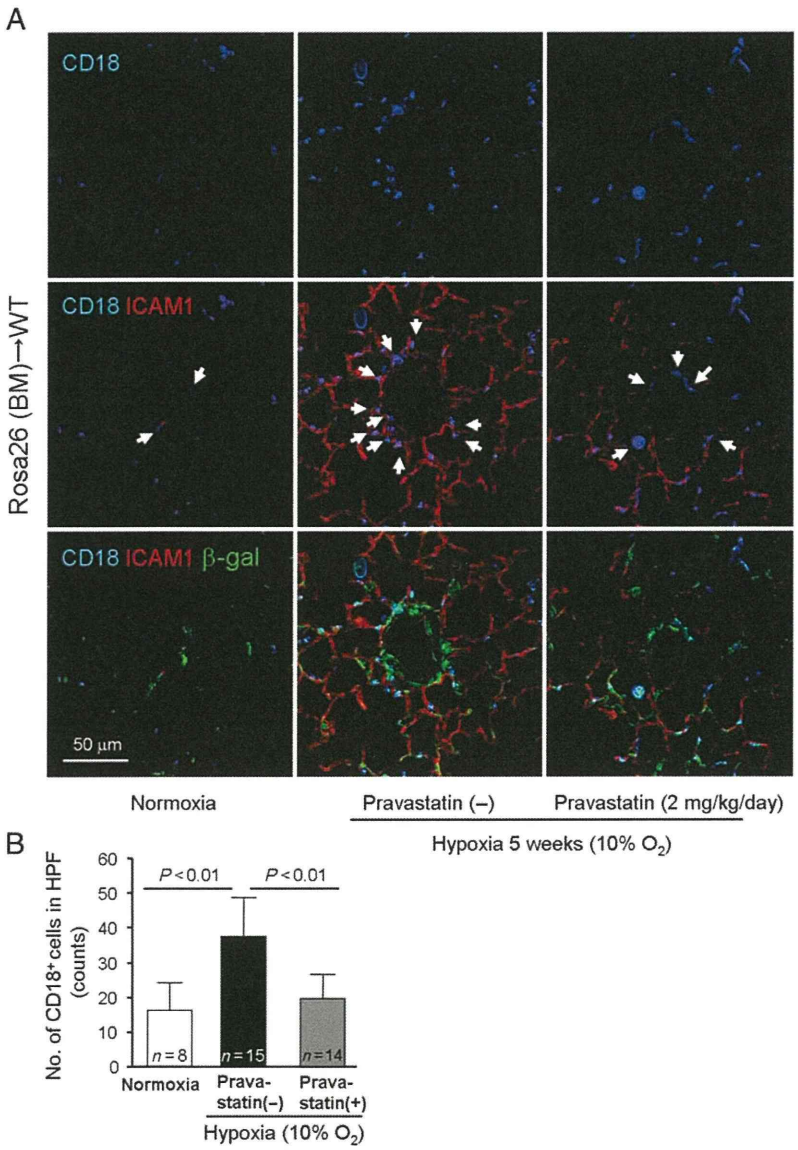

Figure 5 Bone marrow (BM)-derived cells in the adventitia of pulmonary arteries of chimeric mice through the suppression of intercellular adhesion molecule (ICAM)-1/CD18 interaction. (*A*) Immunostaining revealed that the expression of ICAM-1 (red signals) is enhanced in the lung and $CD18^+$ cells (blue signals) are accumulated to the pulmonary artery adventitia (arrowheads) in hypoxic mice. Pravastatin reduced the number of $CD18^+/\beta$-galactosidase $(\beta\text{-gal})^+$ cells that accumulated to the pulmonary artery adventitia (CD18/ICAM-1/β-gal). β-gal is expressed as green signals. (*B*) The number of $CD18^+$ cells was significantly increased in hypoxic lung, which was significantly suppressed by pravastatin. Results are expressed as means ± SD. Normoxia, normoxic mice; hypoxia, mice exposed to 5 weeks of hypoxia (10% O_2) with or without pravastatin (2 mg/kg/day). HPF, high-power field (×400).

statins exerts beneficial effects in animals with fully developed disease.

4.4 Clinical implications

We have previously demonstrated that statins alter smooth muscle cell accumulation and collagen content in established atheroma in rabbits.[43] It has recently been demonstrated that statins ameliorate congestive heart failure,[44] ischaemia/reperfusion injury,[45] and PAH[46] in humans. In the present study, we were able to demonstrate that pravastatin ameliorates PAH in mice associated with a marked reduction in the number of BM-derived progenitors at the pulmonary artery adventitia. Indeed, it was shown that *in vivo* depletion of circulating progenitors for α-SMA^+ cells resulted in marked attenuation of hypoxia-induced pulmonary vascular remodelling, such as adventitial thickening, perivascular fibrosis, and myofibroblast accumulation.[27] Therefore, modulation of mobilization and homing of BM-derived cells by statins could be a new therapeutic strategy for the treatment of PAH in humans.

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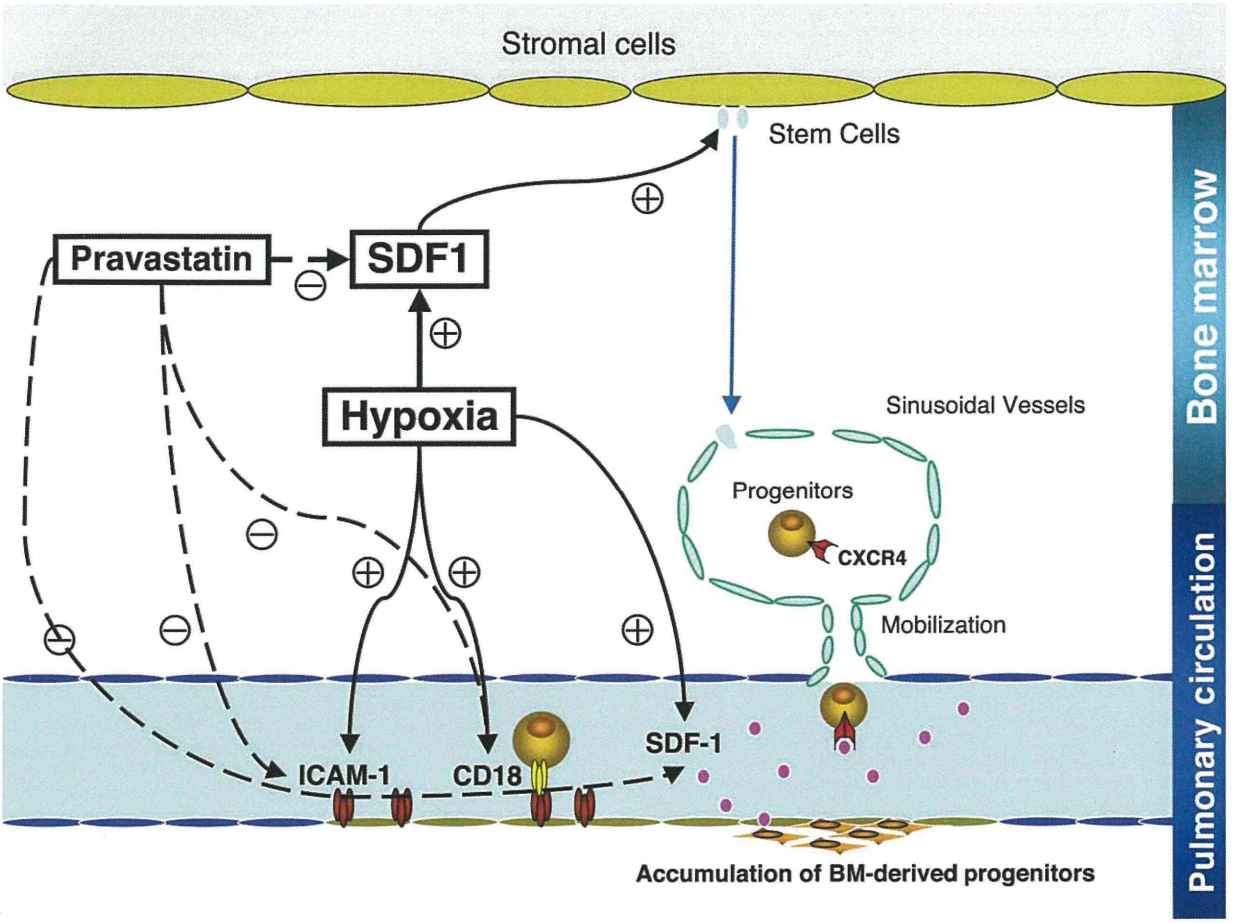

Figure 6 Summary of the present study. Stromal cell-derived factor (SDF)-1 mediates the mobilization and chemotaxis of bone marrow (BM)-derived progenitors in response to hypoxia. During the development of hypoxia-induced pulmonary vascular remodelling, pravastatin reduces the plasma levels of SDF-1 and the expression of intercellular adhesion molecule (ICAM)-1 in the lung, resulting in the reduced number of BM-derived progenitors in the adventitia and the amelioration of pulmonary arterial hypertension. Solid line, proven mechanism; dashed line, proposed mechanism; plus, stimulation; minus, inhibition.

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Conflict of interest: none declared.

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