Hypoxic pulmonary hypertension in mice with constitutively active platelet-derived growth factor receptor-β

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ABSTRACT

Platelet-derived growth factor (PDGF) has been implicated in the pathobiology of vascular remodeling. The multi-kinase inhibitor imatinib that targets PDGF receptor (PDGFR), c-kit and Abl kinases, shows therapeutic efficacy against experimental pulmonary hypertension (PH); however, the role of PDGFR-b in experimental PH has not been examined by genetic approach. We investigated the chronic hypoxia-induced PH in mice carrying an activating point mutation of PDGFR-b (D849N) and evaluated the therapeutic efficacy of imatinib. In addition, we studied pulmonary global gene expression and confirmed the expression of identified genes by immunohistochemistry. Chronically hypoxic D849N mice developed PH and strong pulmonary vascular remodeling that was improved by imatinib (100 mg/kg/day) as evident from the significantly reduced right ventricular systolic pressure, right ventricular hypertrophy and muscularization of peripheral pulmonary arteries. Global gene expression analysis revealed that stromal cell derived factor SDF-1α was significantly upregulated, which was confirmed by immunohistochemistry. Moreover, an enhanced immunoreactivity for SDF-1α, PDGFR-b and CXCR4, the receptor for SDF-1α was localized to the α-smooth muscle cell (SMC) actin positive pulmonary vascular cells in hypoxic mice and patients with idiopathic pulmonary arterial hypertension (IPAH). In conclusion, our findings substantiate the major role of PDGFR activation in pulmonary vascular remodeling by a genetic approach. Immunohistochemistry findings suggest a role for SDF-1α/CXCR4 axis in pulmonary vascular remodeling and point to a potential interaction between the chemokine SDF-1 and the growth factor PDGF signaling. Future studies designed to elucidate an interaction between the chemokine SDF-1 and the PDGF system may uncover novel therapeutic targets.

Key Words: hypoxia, remodeling, PDGFR, SDF-1α, imatinib

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive and fatal disease for which no cure is yet available. Pulmonary vascular remodeling that involves abnormal vascular cell proliferation, survival and migration is the key feature of PAH pathology.1,2 Moreover, PAH shares some mechanistic similarities with cancer.3 Growth factors and inflammatory mediators have been implicated in the abnormal cellular events;4 however, the precise molecular mechanisms is as yet incompletely understood.

Platelet-derived growth factor (PDGF) has been extensively studied over the past years. Upon ligand binding, the transmembrane PDGF receptor (PDGFR) monomers undergo hetero- and homodimerization, followed by increased intracellular tyrosine kinase (TK) activity and initiation of downstream signaling cascades that result in survival, proliferation and migration of cells.5-8 Activation of the PDGFRs thus plays a crucial role during development, normal cellular homeostasis as well as pathophysiological
conditions. Perturbed TK activation including the PDGFR is implicated in many malignant and benign proliferative disorders. Oncogenic PDGFR activation arising from gain-of-function mutations in the activation loop of PDGFR-α has been found in gastrostromal intestinal tumors. The altered regulation of PDGFR signaling has consistently been reported both in experimental and clinical PH. In line with this, the multikinase inhibitor imatinib has been demonstrated to provide therapeutic benefit in experimental pulmonary vascular remodeling. Yet, the pharmacological inhibition study does not rule out the role of the other imatinib targets such as c-kit and thus requires an investigation by genetic approach.

In addition to the PDGF system, the chemokine SDF-1 signaling through its cognate receptor CXCR4 is involved in the growth and progression of cancers. Interestingly, functional links between growth factors and chemokines are gradually emerging. The cross-talk between SDF-1/CXCR4 signaling and epidermal growth factor receptor (EGFR) has been described in cancer cells. Recently, a coexpression of the SDF-1 with PDGFR has been demonstrated in human glioblastoma, suggesting a possible cross-talk between SDF-1 and PDGFR signaling. In the context that a growing number of studies implicate SDF-1 in vascular remodeling, it is not unlikely that SDF-1 may colocalize with PDGFR in the pulmonary vasculature during structural remodeling. However, the chemokine SDF-1 has not been investigated along with the PDGFR in remodeled pulmonary vessels in experimental and clinical PH.

In the current study, we therefore employed transgenic mice with a point mutation in the activation loop of PDGFR-β (D849N) that confers ligand-independent receptor autophosphorylation resulting in increased cell motility and ant apoptotic signaling. We assessed the development of PH and vascular remodeling in chronically hypoxic D849N mice and their response to imatinib therapy. We further investigated the chemokine SDF-1α, one of the differentially expressed genes under chronic hypoxia as revealed by global gene expression study. We analyzed the pulmonary expression/localization of SDF-1α, its receptor CXCR4 and PDGFR-β. In addition, we investigated their localization in lung tissues from patients with idiopathic pulmonary arterial hypertension (IPAH). Some of the results of this study have been previously reported in the form of an abstract.

**MATERIALS AND METHODS**

**Chronic hypoxic exposure and imatinib therapy of mice**

Adult age- and sex-matched mice carrying an activating point mutation in PDGFRβ (D849N) and their corresponding wild type (WT) control were used in the study. Pulmonary vascular remodeling was induced in mice by hypoxic exposure (10% O2) for 35 days as described. After 21 days, both WT and D849N mice were randomized to receive either imatinib (100mg/kg day) or placebo orally by gavage. Control mice were kept in identical chambers under normoxic condition (21% O2). All studies were approved by the local authority (Regierungspräsidium Giessen) and were performed according to the guidelines of the University of Giessen that comply with national and international regulations.

**Hemodynamic and right ventricular hypertrophy**

At the end of therapy, hemodynamic and right ventricular hypertrophy (RVH) measurements were done as described. Briefly, right ventricular systolic pressure (RVSP) was measured by a catheter inserted into the RV via the right jugular vein and systemic arterial pressure (SAP) was measured by catheterization of the carotid artery. The right ventricle (RV) was separated from the left ventricle plus septum (LV+S). The ratio of RV to LV plus septum [RV/(LV+S)] as well as the ratio of RV to body weight (BW) [RV/BW] was calculated as a measurement for RVH.

**Histology and pulmonary vascular morphometry**

Lung tissue preparation, sectioning, staining and vascular morphometry were done as described. The degree of muscularization of peripheral pulmonary arteries was assessed by double-immunostaining the sections with an anti-α-smooth muscle actin antibody (dilution 1:900, clone 1A4, Sigma) and anti-human von Willebrand factor antibody (vWF, dilution 1:900, Dako). In each mouse, 80 to 100 intra-acinar arteries at a size between 20 and 70μm accompanying either alveolar ducts or alveoli were categorized as non-muscularized, partially muscularized or fully muscularized to assess the degree of muscularization.

**Microarray experiments**

RNA extraction and purification from murine lungs was performed as described. RNA quality was assessed by capillary electrophoresis using the Bioanalyzer 2100 (Agilent Technologies, Calif.). Purified total RNA was amplified and Cy-labeled using the dual-color LIRAK kit (Agilent) following the kit instructions. Per reaction, 1μg of total RNA was used. The samples were labeled with either Cy3 or Cy5 to match a balanced dye-swap design. Cy3- and Cy5-labeled RNA were hybridized at 60°C overnight to 4x44K 60mer oligonucleotide spotted microarray slides (Mouse Whole Genome 4x44K; Agilent). Hybridization and subsequent washing and drying of the slides were performed following the Agilent hybridization protocol. The dried slides were scanned using the GenePix 4100A scanner (Axon Instruments, Downingtown, Penn.). Image analysis was performed with GenePix Pro 5.0 software,
and calculated values for all spots were saved as GenePix results files. Stored data were evaluated using the R software and the Limma package from BioConductor. The spots were weighted for subsequent analyses according to the spot intensity, homogeneity, and saturation. The spot intensities were corrected for the local background using the method of Edwards with an offset of 64 to stabilize the variance of low-intensity spots. The M/A data were LOESS normalized before averaging. Genes were ranked for differential expression using a moderated t-statistic. Candidate lists were created by adjusting the false-discovery rate to 1% separately for each contrast.

Patient characteristics
Human lung tissues were obtained from donors and patients with IPAH undergoing lung transplantation. After explanation, lung tissues were formalin-fixed and paraffin-embedded according to common tissue processing protocol. The study protocol for tissue donation was approved by the ethics committee of the University Hospital Giessen in accordance with national law and international guidelines. Written informed consent was obtained from each individual patient or the patient’s next kin.

Immunohistochemistry
Paraffin-embedded lung tissue sections (3 µm thickness) from chronic hypoxic mice and IPAH patients were immunostained for SDF-1α, CXCR4 and PDGFR-β. Following antigen retrieval the sections were pretreated with hydrogen peroxide (15%) to quench endogenous peroxidase activity. After the blocking steps with BSA (10%) for 1 hour and then with blocking serum (Impress kit, Vector Laboratories) for 20 minutes, the sections were incubated with primary antibodies at 4°C overnight. Rabbit anti-mouse SDF-1α (1:300, eBioscience), rabbit monoclonal anti-PDGFR-β (1:600, Y92, Abcam), rabbit polyclonal anti-CXCR4 (1:300, ab2074, Abcam) and rabbit polyclonal anti-SDF-1α (1:600, ab9797, Abcam) were used as primary antibodies. Development of the dye was carried out with peroxidase and substrate (NovaRed kit) according to manufacturer’s instructions (Vector laboratories). Finally, sections were counterstained with hematoxylin (Zymed laboratory) and coverslipped using mounting medium.

Immunoprecipitation and immunoblotting
Cell culture, Immunoprecipitation (IP) and immunoblotting (IB) were performed as described. Briefly, wild type and mutant mouse embryonic fibroblast cells were pre-incubated with or without imatinib (3µM) for 3 hours and stimulated with PDGF-BB (20ng/ml, 10 min. 37°C). Rabbit polyclonal antibody that is isoform-specific for PDGFR-β (CTβ) was used for IP. Phosphorylated PDGFR-β in the precipitate was detected by immunoblotting using anti-phosphotyrosine monoclonal antibody (sc-7020, Santa Cruz, Calif.).

Data analysis
Data were expressed as mean±SEM. The different groups were compared by one-way analysis of variance (ANOVA) and subsequent Newman-Keuls test. A value of P<0.05 was considered as statistically significant.

RESULTS

Mutant PDGFR-β (D849N) is sensitive to imatinib
PDGF-BB stimulation resulted in an increase in phosphorylation of PDGFR-β in WT and mutant mouse embryonic fibroblasts (MEFs). Imatinib largely abrogated the phosphorylation of PDGFR-β in both WT and mutant cells, demonstrating that imatinib was effective to inhibit both the WT and mutant receptor activation (Fig. 1). The inhibition of phosphorylation was not associated with decreased protein content as evident from the absence of alteration in the total PDGFR-β upon imatinib treatment.

Right ventricular systolic pressure (RVSP) of hypoxic mutant (D849N) mice
The presence of the gain-of-function mutation in PDGFR-β did not confer a significant increase in RVSP in the D849N mice (29.5±1.2 mmHg) as compared to that of WT (29.8±1.3 mmHg) mice under normoxic condition. However, chronic hypoxic exposure did result in a
significantly higher RVSP in the D849N (38.9±1.8 mmHg) and WT mice (36.4±1.9 mmHg) compared to normoxic control, suggesting that the development of PH in hypoxic mutant and WT mice was comparable (Fig. 2). After PH was fully established, the treatment with imatinib for two weeks significantly reduced the RVSP in D849N and WT mice (33.6±0.7 and 32.4±0.4 mmHg, respectively) as compared to the placebo groups (Fig. 2). Both D849N and their WT control mice displayed similar systemic response to hypoxia and to the imatinib treatment as revealed by the comparable systemic arterial pressure (SAP) (Table 1).

**Right ventricular hypertrophy (RVH) in hypoxic mutant (D849N) mice**

The increased RVSP was accompanied by RVH as evidenced by a significantly higher RV/(LV+S) ratio (0.42 ±.01) in the hypoxic D849N mice as compared to normoxic control mice (0.27±0.02) (Fig. 3a). The RVH of the D849N mice was comparable to that of WT mice (RV/(LV+S), 0.42±0.02) under chronic hypoxia. Corroborating the RVSP data, imatinib treatment significantly improved RVH as evident from reduced RV/(LV+S) in hypoxic D849N (0.33±0.01) and WT (0.32±0.01) mice compared to placebo group (Fig. 3a). We also analyzed RV/BW ratio and found that chronic hypoxic exposure led to an enhanced RV/BW in D849N and WT mice (0.37±0.02 and 0.34±0.03 mg/g, respectively), whereas imatinib significantly reduced the RV/BW (0.28±0.02 and 0.26±0.02 mg/g respectively) (Fig. 3b).

**Chronic hypoxia-induced pulmonary vascular remodeling in mutant (D849N) mice**

We then investigated the effect of chronic hypoxia on vascular remodeling by assessing the degree of muscularization of peripheral pulmonary arteries. An increased muscularization was observed in the chronically hypoxic mice as reflected by an enhanced immunoreactivity for α-smooth muscle cell actin (Fig. 4a). Pulmonary vascular morphometry of hypoxic D849N and WT mice revealed a significant increase in partially (52.1±1.7% and 51.5±1.8%, respectively) and fully muscularized (16.8±2.1% and 10.6±1.6%, respectively) vessels and a decrease in non-muscularized vessels (23.1 ± 2.1% and 26.5±3.8%, respectively) as compared with normoxic control. Notably, D849N mice displayed a more severe degree of remodeling as evident from the higher percentage of fully muscularized vessels in comparison to WT mice (Fig. 4b). Consistent with the beneficial effects on RVSP and RVH, treatment of hypoxic D849N and WT mice with imatinib significantly decreased the proportions of partially (52.1±1.7% and 51.5±1.8%, respectively) and fully muscularized (7.4±0.6% and 8.5 ± 1.1%, respectively) vessels and increased the proportion of non-muscularized vessels (40.5±2.1% and 40±2.4%, respectively) (Fig. 4b).

**Global gene expression study and localization of SDF-1α, CXCR4 and PDGFR-β in the lungs of mutant (D849N) mice**

In order to investigate the genes and the biological pathways influenced by chronic hypoxia, global gene expression study of the lung homogenates was performed. Gene set enrichment analysis was employed to identify the differentially active pathways from the Kyoto Encyclopedia of Genes and Genomes database (KEGG). The analysis revealed that various biological pathways were differentially active in the D849N mice under hypoxia (Table 2). Majority of the identified pathways

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**Figure 2:** Right ventricular systolic pressure (RVSP) of hypoxic mutant (D849N) mice receiving imatinib. Wild type and D849N mice were exposed to hypoxia for 35 days or remained in normoxia throughout (normoxic control). Hypoxic mice (n=10) received imatinib orally by gavage from day 21 to day 35 at a dose of 100 mg· kg\(^{-1}\) BW. Hypoxic control animals (n=10) received placebo. RVSP (in mmHg) of different experimental groups are shown. Each bar represents mean±SEM. \(*P<0.05\) vs. normoxic control; \(\dagger P<0.05\) vs. corresponding hypoxic control.

**Table 1: Systemic arterial pressure, hematocrit and body weight of wild type and mutant (D849N) mice**

| Number (n) | SAP (mmHg) | Hematocrit (%) | BW (g) |
|------------|------------|----------------|-------|
| Normoxia   |            |                |       |
| WT         | 10         | 88.7±5.0       | 37.3±0.0 | 27.6±1.2 |
| D849N      | 10         | 79.8±6.0       | 38.3±1.0 | 29.3±2.1 |
| Hypoxia    |            |                |       |
| WT         | 10         | 64.4±1.5       | 61.8±0.9 | 25.1±1.2 |
| D849N      | 10         | 63.6±3.4       | 62.0±1.8 | 26.0±1.7 |
| Hypoxia +  |            |                |       |
| Imatinib   |            |                |       |
| WT         | 10         | 66.7±1.1       | 61.8±0.0 | 24.1±0.0 |
| D849N      | 10         | 76.1±4.1       | 60.0±1.0 | 25.3±1.3 |

Mean±SEM is given; SAP: systemic arterial pressure; BW: body weight; WT: wild type

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differentially influenced by chronic hypoxia were those involved in cellular processes and metabolism such as cell growth, division and immune response. Notably, the VEGF pathway was among the pathways with significantly altered activity (Table 2). Based on the gene expression data and the literature as outlined in the introduction, we further investigated the chemokine SDF-1α, one of the differentially regulated genes under hypoxia, by immunohistochemistry. Enhanced SDF-1α was detected predominantly in the smooth muscle cells (SMCs) in the hypoxic lungs as evident from the immunoreactivity for α-SMC actin (Fig. 5). Under normoxia, SDF-1α immunoreactivity was observed in peribronchial SMCs and to a lesser extent in airway epithelial cells. However, the immunoreactivity was intense in vascular SMCs under hypoxia (Fig. 5). Similarly, hypoxia resulted in enhanced expression of PDGFR-β which was predominantly localized in vascular SMCs and airway epithelial cells. Staining for CXCR4, the receptor for SDF-1α was present in the peribronchial SMCs and mildly also in mononuclear and airway epithelial cells. However, stronger immunoreactivity for CXCR4 was detected largely on vascular SMCs under hypoxia (Fig. 5). Overall, the data revealed an increased expression of SDF-1α, CXCR4 and PDGFR-β in hypoxic pulmonary vascular SMCs. However, we did not detect any remarkable qualitative difference in the immunohistochemistry findings between WT and D849N mice.

**Table 2: KEGG biological pathways for differentially expressed genes in chronic hypoxic D849N mice**

| ID   | Name                                      | Genes | P value     | Adj. P |
|------|-------------------------------------------|-------|-------------|--------|
| 3010 | Ribosome                                  | 98    | 9.25E-08    | 0.0000 |
| 4650 | Natural killer cell mediated cytotoxicity | 141   | 8.42E-08    | 0.0000 |
| 4060 | Cytokine-cytokine receptor interaction    | 253   | 2.83E-05    | 0.0018 |
| 4640 | Hematopoietic cell lineage                | 88    | 6.43E-05    | 0.0031 |
| 4070 | Phosphatidylinositol signaling system     | 90    | 3.00E-04    | 0.0115 |
| 5219 | Bladder cancer                            | 48    | 4.49E-04    | 0.0144 |
| 5052 | Inositol phosphate metabolism             | 60    | 6.72E-04    | 0.0148 |
| 4662 | C cell receptor signaling pathway         | 81    | 6.92E-04    | 0.0148 |
| 4670 | Leukocyte transendothelial migration      | 133   | 6.59E-04    | 0.0148 |
| 4115 | p53 signaling pathway                     | 74    | 8.24E-04    | 0.0158 |
| 4610 | Complement and coagulation cascades       | 75    | 1.23E-03    | 0.0215 |
| 5223 | Non-small cell lung cancer                | 66    | 1.85E-03    | 0.0296 |
| 0230 | Purine metabolism                         | 165   | 2.34E-03    | 0.0339 |
| 5216 | Thyroid cancer                            | 33    | 2.47E-03    | 0.0339 |
| 0520 | Nucleotide sugars metabolism              | 7     | 2.88E-03    | 0.0369 |
| 4370 | VEGF signaling pathway                    | 87    | 3.10E-03    | 0.0372 |

**Figure 3:** Right ventricular hypertrophy in hypoxic mutant (D849N) mice receiving imatinib. Wild type and D849N mice were exposed to hypoxia for 35 days or remained in normoxia throughout (normoxic control). Hypoxic mice (n=10) received imatinib orally by gavage from day 21 to day 35 at a dose of 100 mg·kg⁻¹·BW. Hypoxic control animals received placebo (n=10). (a) RV/(LV+S) and (b) RV/BW (in mg/g) of different experimental groups are shown. Each bar represents mean±SEM. *P<0.05 vs. normoxic control; †P<0.05 vs. corresponding hypoxic control.
Localization of SDF-1α, CXCR4 and PDGFR-β in the lungs of IPAH patients

To investigate if the localization of SDF-1, CXCR4 and PDGFR-β in experimental PH mimics that of clinical PH, immunohistochemistry was performed on the lung tissues of patients with IPAH. In addition, we also stained for α-SMC actin to determine if the positive immunoreactivity was present in SMCs. Clearly, a robust vascular remodeling was present in the lungs from IPAH patients as evident from the thickened vascular wall comprising majority of α-SMC actin positive cells (Fig. 6). Immunoreactivity for SDF-1α, CXCR4 and PDGFR-β was observed mainly in the α-SMC actin positive pulmonary vascular cells and it was considerably stronger in the lungs from IPAH patients compared to the donor lungs (Fig. 6). Overall, the data showed higher expression of SDF-1α, CXCR4 and PDGFR-β in SMCs of the remodeled pulmonary vessels in IPAH patients.

DISCUSSION

In the present study, we demonstrated: (1) that chronic hypoxic exposure resulted in strong PH and vascular remodeling in the mice with a gain-of-function mutation

**Figure 4:** Muscularization of pulmonary vessels in hypoxic mutant (D849N) mice receiving imatinib. Wild type and D849N mice were exposed to hypoxia for 35 days or remained in normoxia throughout (normoxic control). Hypoxic mice (n=10) received imatinib orally by gavage from day 21 to day 35 at a dose of 100 mg·kg⁻¹ BW. Hypoxic control animals received placebo (n=10). Lung sections were immunostained for α-SMC actin (arrow) and vWF (arrow head) followed by pulmonary vascular morphometry. A total of 80 to 100 intra-acinar vessels were analyzed in each lung. (a) Representative photomicrographs are shown. (b) Proportions of non-muscularized (N), partially muscularized (P), or fully muscularized (M) pulmonary vessels, as percentage is given. Bar represents mean±SEM. #1.6 times higher than the fully muscularized vessels in hypoxic WT. *P<0.05 vs corresponding normoxic control; †P<0.05 vs corresponding hypoxic control. Scale bar=20 mm.
in PDGFR-β; (2) that imatinib exerted significant therapeutic benefit by reducing RVSP, RVH and pulmonary artery muscularization; and (3) that an enhanced immunoreactivity for SDF-1α, its receptor CXCR4 and PDGFR-β, was detected largely in pulmonary vascular SMCs in experimental as well as in clinical PH.

PDGF system is believed to play a key role in the pathogenesis of pulmonary vascular remodeling;[11-13] however, in vivo investigation by a genetic approach has been missing. Because knocking out PDGF and their receptors in mice is lethal at embryonic stages,[34] a gain-of-function strategy would serve as an alternative genetic tool. We therefore investigated mice carrying a gain-of-function mutation of PDGFR-β (D849N).[23] We found that chronic hypoxic exposure resulted in the development of PH and vascular remodeling in the D849N mice. The hypoxia-induced RVSP and RVH in D849N mice were comparable to the WT, whereas the pulmonary vascular remodeling was stronger as evident from the higher proportion of fully muscularized vessels in the D849N mice. In general, the pulmonary vascular muscularization associated with chronic hypoxia is attributable to the PDGF-mediated proliferation and migration of vascular SMCs.[35-37] The ligand-independent receptor autophosphorylation and/or the increased sensitivity of the mutant PDGFR-β tyrosine kinase (TK) towards lower local concentration of PDGF[23] may have amplified the vascular SMC proliferation and/or shifted the proliferation towards earlier time points, leading to an increased muscularization in the hypoxic D849N mice. The remodeling in D849N mice, nevertheless, did not turn out to be as severe as was anticipated. Our data may be explained by previous studies.[38,39] In a chronic liver injury model, the D849N mice showed an enhanced proliferative response in the initial stage of disease with only a weak influence on the chronic disease stage.[39] In line with this, a syngenic and orthotopic tumor model study revealed that the tumor growth was faster in the D849N mice during the early establishment phase, whereas the tumor growth rate was similar between WT

**Figure 5:** Localization of stromal cell derived factor (SDF)-1α, CXCR4 and PDGFR-β in hypoxic murine lungs. The localization of SDF-1α, CXCR4 and PDGFR-β was performed by immunohistochemistry on lung tissues from normoxic and hypoxic mutant mice (n=6). The brown staining represents the positive immunoreactivity for the SDF-1α, CXCR4 and PDGFR-β as indicated (arrow) in the figure. Lung sections were also stained for α-SMC actin (purple staining, Arrow head). Representative photomicrographs of immunostained lung sections from mutant mice are shown. V- vessel, B- bronchiole, Scale=20 μm.
The enhanced early tumor growth may be attributable to higher basal activation and to higher sensitivity of mutant PDGFR-b toward lower ligand concentrations. However, in the current study, factors such as hypoxia and increased shear stress strongly induce the PDGF and PDGFR expression in vascular cells. Thus, the availability of the ligand and the receptor may be attributed to yield a comparable PDGFR activation in hypoxic pulmonary vessels of WT and D849N mice. This notion is supported by the experimental evidence that the ligand-dimerized WT and D849N receptors elicit a comparable kinetics of TK autoactivation. Moreover, study of a murine model of liver fibrosis has indicated that PDGFR signaling is not solely dependent on pure ligand activation and other factors such as reactive oxygen species can activate the receptor TK.

Corroborating the previous finding, we observed that imatinib significantly improved PH, RVH and pulmonary vascular remodeling in hypoxic WT and D849N mice. Moreover, we observed that imatinib inhibited the activation of mutant PDGFR-β (D849N) as effectively as that of WT receptor in vitro, suggesting that this could be the predominant mode of action involved in the therapeutic benefit. On the other hand, Gambaryan et al. report that imatinib prevents hypoxia-induced PH and vascular remodeling in mice by reducing the accumulation of perivascular BM-derived c-kit+ progenitor cells. However, the authors did not investigate the effects of imatinib after PH was established. We recently demonstrated that pharmacological inhibition of c-kit in a preventive approach ameliorated monocrotaline-induced PH, RVH and pulmonary vascular remodeling, but did not provide therapeutic benefit when we started the inhibition after the PH was established. Taken together, it may be deduced that PDGFR signaling plays a major pathogenic role and imatinib provides therapeutic benefits by targeting PDGFR activation in experimental PH; whereas c-kit may be involved in the early development of experimental PH.

We performed global gene expression studies and analyzed the data by pathway analysis database (KEGG). We found that various biological pathways were differentially active in D849N mice under hypoxia and as expected, the majority of the identified pathways were those involved in cellular processes and metabolism such as cell growth, division and immune response. Based on the gene expression data and the literature as outlined in the background, we investigated the chemokine SDF-1α, one of the differentially regulated genes under hypoxia. SDF-1α has been implicated in a range of pathological conditions including cancers and cardiovascular diseases. The inflammatory and progenitor cell...
recruitments are described as the modes of SDF-1 function by activating its cognate receptor CXCR4. However, the concordance as to the progenitor cell types recruited and their consequences in cardiovascular pathology is lacking. Some studies attribute a beneficial function to SDF-1α in therapeutic vascularization/angiogenesis by recruiting endothelial progenitor cells (EPC), whereas others describe a detrimental role in neointima formation by recruiting smooth muscle progenitor cells (SPC), suggesting that SDF-1α may have disease- or model specific functions. We found an enhanced immunoreactivity for SDF-1α and its receptor CXCR4 localized largely to the α-SMC actin positive cells in remodeled vessels. The higher SDF-1α/CXCR4 expression may be attributable to HIF-1 induction under hypoxic condition. In line with the recent studies, our data suggest a role for SDF-1 in the process of hypoxic pulmonary vascular remodeling. Moreover, SDF-1α expressed in neointimal SMCs has been proposed to play an essential role in local SPC recruitment, suggesting a paracrine function for SDF-1α. Subsequently, both autocrine and paracrine modes of actions have been proposed. The involvement of SDF-1α in growth and progression of cancer implies that it may act both in autocrine and paracrine fashion in cancer cells.

In conclusion, our findings substantiate the major role of PDGFR in pulmonary vascular remodeling by a genetic approach. The immunohistochemistry findings suggest a role for SDF-1α/CXCR4 axis in pulmonary vascular remodeling and point to a potential interaction between the chemokine SDF-1 and the growth factor PDGF signaling. Future studies designed to elucidate an interaction between the chemokine SDF-1 and the PDGF system may uncover novel therapeutic targets.

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