EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

IL-33 Initiates Vascular Remodelling in Hypoxic Pulmonary Hypertension by up-Regulating HIF-1α and VEGF Expression in Vascular Endothelial Cells

Jie Liu [a,b], Wang Wang [b], Lei Wang [b], Shihao Chen [a], Bo Tian [c], Kewu Huang [d], Chris J. Corrigan [e], Sun Ying [a], Wei Wang [a,*], Chen Wang [f]

[a] The Department of Immunology, School of Basic Medical Sciences, Capital Medical University, Beijing, China
[b] The Department of Physiology and Pathological Physiology, School of Basic Medical Sciences, Capital Medical University, Beijing, China
[c] Department of Thoracic Surgery, Beijing Chao-Yang Hospital, Capital Medical University & Beijing Institute of Respiratory Medicine, Beijing, China
[d] Department of Respiratory and Critical Care Medicine, Beijing Chao-Yang Hospital, Capital Medical University & Beijing Institute of Respiratory Medicine, Beijing, China
[e] Faculty of Life Sciences & Medicine, School of Immunology & Microbial Sciences, Department of Inflammation Biology, Asthma UK Centre in Allergic Mechanisms of Asthma, King's College London, London, UK
[f] The Department of Respirology, Capital Medical University, Beijing, China

ARTICLE INFO

Article history:
Received 11 May 2018
Received in revised form 3 June 2018
Accepted 6 June 2018
Available online xxxx

Keywords:
IL-33
Hypoxic pulmonary hypertension
Hypoxia inducible factor 1
Vascular endothelial growth factor

ABSTRACT

IL-33 may play a role in the vascular remodelling of hypoxic pulmonary hypertension (PH) but the precise mechanisms are still unclear. We hypothesized that hypoxia promotes expression of IL-33 and its receptor ST2 on vascular endothelial cells, which in turn leads to dysfunction of vascular endothelial cells and smooth muscle cells contributing to PH. Immunohistochemistry showed that immunoreactivity for IL-33 and ST2 was significantly increased in lung tissue of murine model of hypoxia-induced PH (HPH) and of subjects with bronchiectasis-PH. *trans*-Thoracic echocardiography showed that haemodynamic changes and right ventricular hypertrophy associated with HPH were significantly abrogated in *St2*$^{-/-}$ compared with WT mice. Administration of IL-33 further exacerbated these changes in the hypoxia-exposed WT mice. *In vitro*, hypoxia significantly increased IL-33/ST2 expression by human pulmonary arterial endothelial cells (HPAECs), while exogenous IL-33 enhanced proliferation, adhesiveness and spontaneous angiogenesis of HPAECs. Knockdown of endogenous *Il33* or *St2* using siRNA transfection significantly suppressed these effects in both normoxic and hypoxic culture-conditions. Deletion of the *St2* gene attenuated hypoxia-induced, elevated lung expression of HIF-1α/VEGFA/VEGFR-2/ICAM-1, while administration of exogenous VEGFA partially reversed the attenuation of the haemodynamic indices of PH. Correspondingly, knockdown of the *St2* or *Hif1α* genes almost completely abrogated IL-33-induced expression of HIF-1α/VEGFA/VEGFR-2 by HPAECs *in vitro*. Further, IL-33-induced angiogenesis by HPAECs was extensively abrogated by knockdown of the *Hif1α*/*Vegfa* or *Vegfr2* genes. These data suggest that hypoxia induces elevated expression of IL-33/ST2 by HPAECs which, at least partly by increasing downstream expression of HIF-1α and VEGF initiates vascular remodelling resulting in HPH.

1. Introduction

Hypoxic pulmonary hypertension (HPH) is commonly observed in the context of chronic hypoxia caused by a variety of lung diseases [1, 2]. Although the precise mechanisms of HPH are largely unknown, hypoxia enhanced pulmonary vasoconstriction and vascular remodelling are considered to be the two major pathogenic processes of HPH, resulting in elevated pulmonary vascular resistance and consequent pulmonary artery pressure with eventual cardiac hypertrophy and failure. The process of vascular remodelling involves many factors including local inflammation, dysfunction and abnormal proliferation of vascular endothelial cells, smooth muscle cells and fibroblasts, although hypoxia is regarded as the key driver [3, 4].

IL-33, a member of the IL-1 family of cytokines expressed most prominently at epithelial and endothelial surfaces, acts by binding to the membrane receptors ST2 and IL-1RAcP (IL-1 receptor accessory protein), in a number of lung diseases involving pulmonary and pulmonary vascular remodelling [5–9]. It is therefore reasonable to hypothesize that IL-33 also contributes to the vascular remodelling processes of HPH through actions on vascular endothelial cells. In addition, vascular endothelial cell growth factor (VEGF) is a key "end effector" molecule in the vascular remodelling

* Corresponding author at: The Department of Immunology, School of Basic Medical Sciences, Capital Medical University, 10# Xi Tou Tiao, You An Men Wai, Fengtai District, Beijing 100069, China.
E-mail address: wy_robin@ccmu.edu.cn (W. Wang).

https://doi.org/10.1016/j.ebiom.2018.06.003

of HPH, which promotes angiogenesis by regulating the proliferation, migration and differentiation of endothelial cells [1–3]. The expression of VEGF is in turn regulated by the "molecular switch" molecule hypoxia-induced factor 1-alpha (HIF-1α) [10, 11]. A recent study has reported that HIF-1α may interact with the IL-33 promoter region, resulting in induction by HIF-1α of IL-33 expression in intestinal epithelial cells [12]. Other studies have shown that IL-33 promotes VEGF production by skin mast cells and the human keratinocyte cell line HaCaT [13, 14]. However, possible interactions between the IL-33/ST2 and HIF-1α/VEGF axes in the pathogenesis of HPH have not so far been addressed. We, here aimed to explore the role of both axes in the pathogenesis of the hypoxic pulmonary vascular remodelling which results in HPH.

2. Methods

2.1. Pulmonary Surgical Specimens

Collection of human lung tissues was approved by the Research Ethics Committee of Beijing Chao-yang Hospital. Prior written, informed consent was obtained from all patients to donate lung tissues. Surgical specimens of lung tissue were collected from patients with bronchiectasis-induced pulmonary hypertension (PH) ($n = 3$) and normal donor lungs removed following accidental death for 3 patients undergoing lung transplantation in the Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China. Lung tissues were fixed in 10% formaldehyde then embedded in paraffin.

2.2. Animal Models

Male C57BL/6 and BALB/c mice (8 weeks) were purchased from the Vital River Laboratory Animal Technology Company of Beijing in China. $St2^{-/-}$ mice (BALB/c background) were a kind gift from Professor Andrew N.J. McKenzie of the Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom [15]. All experiments were undertaken with approval of the Institutional Animal Care and Use Committee (IACUC). Mice were randomly divided into two groups which were exposed to normal air or mixed air containing 10% oxygen in a normobaric chamber (BioSpherix, USA) for 4 weeks. The chamber was partially ventilated and equipped with an external oxygen controller which sensed the ambient oxygen concentration and replaced it with nitrogen when necessary. Cages were opened for cleaning every 3 days for 30 min.

2.3. Measurements of Haemodynamics in the Murine Model of HPH

The measurements were performed as previously described [16, 17]. Following anaesthesia of the mice with 2% sodium pentobarbital (50 mg/kg i.p.), right ventricular systolic pressure (RVSP), conventionally used as an indicator of mean pulmonary arterial pressure, was measured by closed-chest puncture of the right ventricle (RV) with a transducer attached to the PowerLab system (ADInstruments, Australia) as previously described [16]. A blood sample was then taken by cardiac apex puncture for haematocrit analysis (Radiometer, Denmark). Serum was stored in aliquots at −80 °C. Following euthanisation, the murine lungs and hearts were collected and the weights of the right ventricles (RV) and left ventricles including the interventricular septa (LV + S) measured separately to evaluate the right ventricular hypertrophy index (RVHI), which reflects right ventricular remodelling, as calculated by the formula: RVHI (%) = RV/(LV + S) × 100. The ratio of RV weight to body weight was calculated as RV/Body weight [16]. The diameters of individual cardiomyocytes in histological sections of the left and right ventricular walls were measured as previously described [17].

2.4. Analysis of Lung Morphometrics in the Murine Model of HPH

The left lungs of the mice were embedded in paraffin, serially sectioned at a thickness of 4 μm then stained using haematoxylin and eosin (H&E) and immunostaining. With the aid of the H&E stain, all pulmonary vessels with external diameters <100 μm in entire left lung were selected and the medial thickness of these vessels quantified by immunofluorescent labelling with anti-α-SMA (1:200 dilution, Sigma-Aldrich, USA), visualised with second layer Fluor (AF) 488-labelled goat anti-mouse IgG (ZsBio, China). Medial thickness was calculated as a percentage of the external diameter of the vessels as follows: percentage medial thickness (%MT) = (external diameter − internal diameter)/external diameter × 100 [17]. The data were segregated into four groups based on the external diameters of the vessels: 0–25, 26–50, 51–75 and 76–100 μm. Images of pulmonary vessels were captured with a Nikon microscope digital camera system and circumferences measured using its image analysis software as previously described [17].

2.5. Immunohistochemical Analysis

Immunoreactivity for IL-33 and ST2 was measured using a rabbit anti-IL-33 monoclonal antibody (1/200, Santa Cruz, CA) or rabbit anti-ST2 monoclonal antibody (1/200, Abcam, UK) and analyzed as described previously [16].

2.6. Cell Culture and Proliferation Assay

Human pulmonary arterial endothelial cells (HPAECs) and human pulmonary arterial smooth muscle cells (HPASMCs) (ScienCell Research Laboratories, USA) were cultured according to the instruction of the manufactures. Human pulmonary arterial endothelial cells (HPAECs) (3–6 passages, ScienCell Research Laboratories, USA) were cultured in complete Endothelial Cell Medium (ECM). Cells were maintained in a CO_2 incubator (5%, Thermo, USA) at 37 °C and used for experiments at 80%–90% confluence. After starvation by culture in ECM containing 2% foetal bovine serum (FBS) to arrest growth for 4–6 h, cells were pretreated with either human recombinant IL-33 (rhIL-33) (10 ng/mL, R&D systems, MN, USA) or vehicle (PBS) in 5% FBS-ECM, followed by stimulation under conditions of hypoxia (3% oxygen) or normoxia (21% oxygen) for a further 24 h. Human pulmonary arterial smooth muscle cells (HPASMCs) (4–8 passages, ScienCell Research Laboratories) were cultured in complete smooth muscle cell medium (SMCM). Cells were maintained in a CO_2 incubator (5%, Thermo, USA) at 37°C and used for experiments at 80%–90% confluence.

Proliferation of HPAECs was assayed using a bromodeoxyuridine (BrdU) flow kit purchased from BD Pharmingen (BD Pharmingen, USA) according to the manufacturer's instructions. Briefly, HPAECs were seeded at 5×10^5cells per culture bottle and cultured for 24 h under different conditions. For analysis of DNA synthesis, cells were incubated with BrdU (10 μM) at 37 °C for 1 h. After collection, cells were treated with cytopermeabilisation/fixation medium (BD) then exposed to DNase (300 μg/mL dissolved in Dulbecco's Phosphate Buffered Saline, D-PBS) at 37 °C for a further 1 h. The incorporated BrdU was stained with specific anti-BrdU fluorescent antibody at room temperature for 20 min. A dye (7-aminoactinomycin D, 20 μL per sample) was added for staining of total DNA. Cells were analyzed using an LSRFortessa flow cytometer (Becton Dickinson, USA) which discriminated cells in S phase (P4), G0/G1 phase (P3), G2 phase (P5) and cells undergoing apoptosis (P6). The percentage of cells in S phase was calculated as P4/(P3 + P4 + P5) × 100%.

Proliferation of human pulmonary artery smooth muscle cells (HPASMCs) was measured using a commercial kit (Cell Counting Kit-8, Dojindo, Japan). Briefly, HPASMCs were seeded at 5×10^3cells per well in a 96-well plate, then incubated at 37 °C for 24 h before adding rhIL-33 (R&D Systems) at final concentrations of 10 ng/mL or normal saline control in a total volume of 200 μL in culture medium without foetal bovine serum (FBS). Cells were cultured with platelet-derived growth

Please cite this article as: Liu J, et al, IL-33 Initiates Vascular Remodelling in Hypoxic Pulmonary Hypertension by up-Regulating HIF-1α and VEGF Expression in Vascular End..., EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.06.003