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Downregulation of PGI_2 pathway in Pulmonary Hypertension Group-III patients

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ABSTRACT

Pulmonary hypertension (PH) is a progressive and life-threating lung disorder characterized by elevated pulmonary artery pressure and vascular remodeling. PH is classified into five groups, and one of the most common and lethal forms, PH Group-III is defined as PH due to lung diseases and/or hypoxia. Due to the lack of studies in this group, PH-specific drug therapies including prostacyclin (PGI_2) analogues have not been approved or recommended for use in these patients. PGI_2 is synthesized by the PGI_2 synthase (PGIS) enzyme, and its production is determined by measuring its stable metabolite, 6-keto-$PGF_{1\alpha}$. An impaired PGI_2 pathway has been observed in PH animal models and in PH Group-I patients; however, there are contradictory results. The aim of this study is to determine whether PH Group-III is associated with altered expression of PGIS and production of PGI_2 in humans. To explore this hypothesis, we measured PGIS expression (by western blot) and PGI_2 production (by ELISA) in a large variety of preparations from the pulmonary circulation including human pulmonary artery, pulmonary vein, distal lung tissue, pulmonary artery smooth muscle cells (hPASMC), and bronchi in PH Group-III (n = 35) and control patients (n = 32). Our results showed decreased PGIS expression and/or 6-keto-$PGF_{1\alpha}$ levels in human pulmonary artery, hPASMC, and distal lung tissue derived from PH Group-III patients. Moreover, the production of 6-keto-$PGF_{1\alpha}$ from hPASMC positively correlated with PGIS expression and was inversely correlated with mean pulmonary artery pressure. On the other hand, PH Group-III pulmonary veins and bronchi did not show altered PGI_2 production compared to controls. The deficit in PGIS expression and/or PGI_2 production observed in pulmonary artery and distal lung tissue in PH Group-III patients may have important implications in the pathogenesis and treatment of PH Group-III.

1. Introduction

Pulmonary hypertension (PH) is characterized by a progressive increase in mean pulmonary artery pressure (mPAP > 20 mmHg) and vascular resistance due to sustained pulmonary artery vasoconstriction [1,2]. The clinical classification of PH is intended to categorize multiple clinical conditions into five groups. Depending on this classification, PH Group-III is defined as PH due to lung diseases and/or hypoxia. Common lung diseases associated with PH are chronic obstructive pulmonary disease (COPD), interstitial lung disease and combined pulmonary fibrosis and emphysema [3].

Most of the studies examining the pathogenesis of PH are performed in PH Group-I (idiopathic/heritable), which is more commonly referred to as Pulmonary Arterial Hypertension (PAH). In these patients, few studies have shown that the increased production of vasoconstrictor/proliferative mediators [such as endothelin-1 (ET-1), thromboxane (TxA_2)] and reduced production of vasodilator/anti-proliferative mediators [such as prostacyclin (PGI_2), nitric oxide (NO)] lead to

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pulmonary endothelial dysfunction and the development of PH [4–7]. Among these mediators, more attention is focused on PGI_2 because of the widespread use of it and its stable mimetics for treating PH. PGI_2, a lipid mediator derived from arachidonic acid, is a powerful vasodilator and a potent inhibitor of platelet adhesion/aggregation and smooth muscle cell proliferation and growth. Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), which is then converted to PGI_2 by PGI_2 synthase (PGIS), an enzyme constitutively expressed, among others, in pulmonary vascular endothelial cells [8,9], smooth muscle cells [10], and bronchial epithelial cells [11]. Once PGI_2 is released from cells, it binds to the prostanoid IP receptor, a G_s-coupled receptor, and elevates cAMP levels [12–15]. These mechanisms are responsible for vasodilation in human lung [16].

Epoprostenol (PGI_2) was the first drug approved by the FDA in 1984 for the treatment of PH [17], and then other PGI_2 analogues with better stability profiles, such as iloprost and treprostinil, were developed. PGI_2 therapy improves the functional capacity and decreases the mortality rate of PH Group-I patients [18,19]. Until now, these treatments have not been recommended for PH Group-III patients by European Guidelines due to the lack of randomized controlled trials [20].

The importance of PGIS in the development of PH has been demonstrated in several studies conducted in PH animal models and PH patients (mostly Group-I). However, there are differing results regarding PGIS expression and PGI_2 production between PH and non-PH preparations [8,9,21–27]. Many of the PH animal models have limitations, and some do not have complex arterial occlusive remodeling which is the characteristic of human PH diseases [28]. Therefore, studies conducted using PH patients are of great importance. To our knowledge, there are no studies evaluating the PGIS and PGI_2 levels in PH Group-III patients, which is one of the most common and lethal form of PH [29].

The aim of this study is to determine whether PH Group-III is associated with alterations in the expression of PGIS and the formation of PGI_2 in humans. To explore this hypothesis, we measured PGIS expression and PGI_2 production in large variety of preparations from various locations in the pulmonary circulation, including human pulmonary artery (HPA), pulmonary vein (HPV), distal lung tissue, pulmonary artery smooth muscle cells (hPASMC), and bronchi in PH Group-III and control patients. Our results studying physiological alterations in PH Group-III patients provide insight into disease pathogenesis and its treatments.

2. Materials and methods

2.1. Human pulmonary and bronchi preparations

Human pulmonary and bronchial preparations were collected in Bichat Hospital (Paris) after obtaining patients' informed consent with Ethics Committee approval from INSERM and AP-HP (CEERB du GHU Nord) Institutional Review Board (n° IRB00006477). These investigations conform to the principles outlined in the Declaration of Helsinki. Control preparations were obtained from patients (20 males, 4 females and 8 anonymous donors) who underwent surgery, mostly for lung carcinoma, while PH preparations were obtained from patients (26 males, 9 females) who had undergone surgery for lung transplantation. Patient categories are PH due to lung diseases and/or hypoxia [Group-III of PH classification [3]], with detailed characteristics presented in supplemental data (Table S1), and control patient characteristics were presented in Table S2. PH lungs used in our study were from patients having catheter-measured mPAP ≥ 20 mmHg. All preparations were dissected in the anatomical pathology laboratory and used within 1–12 h post-surgery. HPA and HPV preparations (6–2 mm internal diameter) were dissected from pulmonary lobes. The "distal lung tissue" was cut at the periphery of the pulmonary lobes and contained vessels or bronchioles (< 1 mm internal diameter) and parenchyma.

2.2. Culture of human pulmonary artery smooth muscle cells

The culture of hPASMC was carried out in a biosafety level 2 laboratory in a vertical laminar flow hood. The hPASMC were obtained from pulmonary arteries derived from control and PH Group-III patients. These arteries were removed after dissection of lung lobes or whole lungs. First, proximal arteries were opened, cleaned of any connective tissue (parenchyma) and then rinsed with phosphate--buffered saline (PBS) containing 1/20 penicillin, streptomycin, amphotericin B (PSA). After rinsing, the artery media was isolated and cut with a scalpel into small 1-2 mm pieces. This preparation was placed in a medium containing collagenase (Type 1) and elastase and then incubated for 30 to 40 min at 37 °C. After incubation and enzymatic digestion of the extracellular matrix, the preparation was filtered (40 µm filter) and centrifuged at 1000 rpm for 10 min at 20 °C. After centrifugation, the supernatant was aspirated, and the pellet was resuspended in a T25 flask containing Smooth Muscle Cell Growth Medium 2 supplemented with 20% fetal calf serum (FCS), PSA and growth factors [human EGF (epidermal growth factor), human bFGF (fibroblast growth factor), IGF (insulin-like growth factor)] to allow the proliferation of smooth muscle cells (SMC). The cells were cultured in an incubator at 37 °C in humid atmosphere containing 5% CO_2. When confluence was reached, the hPASMC were detached from the T25 flask using 2 ml trypsin. In this first passage, the cells were resuspended in 12 ml of culture medium (10% FCS) and transferred to a T75 flask. Depending on the extent of cell confluence, passages were performed approximately every 2 weeks. The SMC were confirmed morphologically; we obtained spindle shaped cells forming the "hill and valley" configuration which is typical of SMC (see Fig. 3). In passage 3–4, the hPASMC were washed twice with PBS. Subsequently, hPASMC were detached as described earlier and diluted in culture medium with 10% FCS in order to have a concentration of 3×10^6 cells per 100 ml. For one cell culture derived from one individual, the cells in homogeneous suspension were then seeded (2 mL /well) in 6 well plates. After proliferation of the cells in the 6-well plates (90% confluence), the culture medium (10% FCS) was aspirated and replaced with a 0% FCS culture medium. Cells were then kept in an incubator at 37 °C in a humid atmosphere containing 5% CO_2 for 48 h.

2.3. Western blot analysis

Human pulmonary arteries, veins, bronchi, and distal lung tissue samples were homogenized in liquid nitrogen, using a porcelain mortar. Homogenates were diluted (100 mg/ml) in lysis buffer (RIPA buffer supplemented with protease and phosphatase inhibitors). hPASMC in 6 well plates were washed twice with PBS and lysed with lysis buffer. Proteins were quantified by BCA protein assay kit, and a 12.5–50 µg of protein sample were loaded on a 12% polyacrylamide gel. Proteins were electroblotted onto nitrocellulose membranes. Membranes were blocked (TBS, 0.05% Tween 20, 5% non-fat dry milk) and incubated overnight at 4 °C with a primary antibody specifically targeting PGIS in TBS (0.05%) Tween-20 (1%) non-fat dry milk. Subsequently, the membranes were incubated with an appropriate peroxidase-conjugated secondary antibody. Bands were visualized using the ECL prime luminescence system. For quantification, the film was scanned, and the integrated optical density of the bands was estimated with Scion Image (Scion Corporation, NIH, Frederick, MD, USA) and normalized to β-actin. For densitometrical analysis, no brightness/contrast adjustments have been performed, except in Fig. 3, where automatic contrast adjustments were performed on the area (about 2 cm x 2 cm) around the band of interest, prior to blank and band density calculations. For PGIS western blot bands, the inferior band corresponding to 56 kDa was quantified.

2.4. ELISA measurements

Samples of pulmonary arteries, pulmonary veins and distal lung tissue were dissected and cut into small pieces and placed into 12-well plates (100-200 mg tissue/well) containing RPMI (pH 7.4) supplemented with antibiotics (penicillin, 1000 IU/mL; streptomycin, 100 μg/mL) and an antimycotic agent (amphotericin, 0.25 μg/mL) for 12 h. The volume of the RPMI was adjusted to 1 mL for 70 mg of tissue. In addition, hPASMC in 6 well plates were incubated with culture medium for 48 h. All cell/tissue incubations were conducted at 37 °C in a humidified atmosphere of 5% CO_2 in air using a culture incubator. 6-keto-$PGF_{1\alpha}$ concentrations were measured in supernatants/culture medium from incubation of pulmonary arteries, pulmonary veins, distal lung tissue and hPASMC. For bronchi, 6-keto-$PGF_{1\alpha}$ levels were measured in tissue homogenates. All measurements were conducted using an enzyme immunoassay kit.

2.5. Statistical analysis

All results obtained from different patients (n) were expressed as mean ± standard error of the mean (SEM). After testing normality and equal variances of the data, we performed Student or Mann Whitney-Rank Sum tests and calculated Pearson or Spearman correlation coefficients (r). For all statistical tests, P-values less than 0.05 were considered statistically significant. Statistical analyses were performed using SigmaStat version 3.5 (Systat Software, Point Richmond, CA, USA). ELISA measurements were performed in duplicate and an average taken in each sample to calculate the final mean data.

2.6. Compounds and materials

Protease inhibitor cocktail, antibiotics, antimycotic, and β-actin antibody were from Sigma-Aldrich (St. Louis, MO, USA). 6-keto $PGF_{1\alpha}$ ELISA kit and antibody against PGIS were from Cayman Chemical (Ann Arbor, MI, USA; Reference number: 515211, 160640 respectively) [30,31]. Polyclonal rabbit antibody used as secondary antibody was from Jackson ImmunoResearch 73120, (Pensylvannia, USA). RPMI, trypsin and collagenase were from Gibco Invitrogen (Paisley, UK). Elastase was from Worthington (Lakewood, NJ, USA). BCA protein assay kit was from Thermo (Rockford, USA). Nitrocellulose membranes and ECL Plus® system were from Amersham Biosciences (Buckinghamshire, UK). Smooth Muscle Cell Growth Medium 2 was from PromoCell (Heidelberg, Germany). Phosphatase inhibitor (phosSTOP) was from Roche (Basel, Switzerland).

3. Results

3.1. PGIS expression and 6-keto-$PGF_{1\alpha}$ levels in human pulmonary arteries derived from control and PH Group-III patients

The expression of PGIS enzyme was significantly decreased in HPA preparations derived from PH patients versus control patients (Fig. 1A, C, $P = 0.02$). Consistent with this finding, 6-keto-$PGF_{1\alpha}$ levels were significantly lower in HPA preparations derived from PH patients as compared to control patients (Fig. 1B, $P < 0.0001$).

3.2. PGIS expression and 6-keto-$PGF_{1\alpha}$ levels in human pulmonary veins derived from control and PH Group-III patients

The expression of PGIS enzyme was not significantly different between HPV preparations derived from PH patients and control patients (Fig. 2A, C, $P = 0.89$). Consistent with this finding, 6-keto-$PGF_{1\alpha}$ levels, while decreased, were not statistically significantly different between HPV preparations derived from PH patients and control patients (Fig. 2B, $P = 0.18$).

3.3. PGIS expression and 6-keto-PGF1α levels in human pulmonary artery smooth muscle cells derived from control and PH Group-III patients

The expression of PGIS enzyme was significantly decreased in hPASMC derived from PH patients versus control patients (Fig. 3A, C, $P = 0.004$). Consistent with this finding, 6-keto-$PGF_{1\alpha}$ levels were significantly lower in hPASMC derived from PH patients as compared to control patients (Fig. 3B, $P = 0.008$). Furthermore, the production of 6-keto-$PGF_{1\alpha}$ from these cells positively correlated with PGIS expression (Fig. 3D) and was inversely correlated with mPAP (Fig. 3E).

3.4. PGIS expression and 6-keto-$PGF_{1\alpha}$ levels in distal lung tissue derived from control and PH Group-III patients

The expression of PGIS enzyme was not significantly different in distal lung tissue derived from PH patients versus control patients (Fig. 4A, C; $P = 0.27$); whereas, the 6-keto-$PGF_{1\alpha}$ levels were significantly lower in distal lung tissue derived from PH patients as compared to control patients (Fig. 4B, $P = 0.03$).

3.5. PGIS expression and 6-keto-$PGF_{1\alpha}$ levels in human bronchi derived from control and PH Group-III patients

The levels of PGIS enzyme expression and 6-keto-$PGF_{1\alpha}$ were similar in human bronchi derived from PH patients and control patients (Fig. 5A–C; $P = 0.91$ and $P = 0.37$, respectively).

4. Discussion and conclusions

The current study demonstrates for the first time that the expression of PGIS and/or 6-keto-$PGF_{1\alpha}$ levels are significantly decreased in human pulmonary arteries, hPASMC, and distal lung tissue derived from PH Group-III patients versus control patients. On the other hand, human pulmonary veins and bronchi preparations showed no differences. These results suggest that the deficiency of the PGIS pathway is restricted to pulmonary arteries.

The therapeutic importance of PGI_2 therapy in PH may be related to the deficiency of PGIS in pulmonary tissue, however this has not been investigated in PH Group-III patients. In the present study, the significant decrease of PGIS expressions in arterial preparations derived from PH Group-III patients (Figs. 1A and 3A) is consistent with previous immunohistochemical results from Tuder *et al.* These authors showed a lower PGIS expression in small/medium-size, but not in large, pulmonary arteries derived from PH Group-I patients [9]. Similarly, studies performed on PH rats, lambs or piglets demonstrated that PGIS expression was significantly decreased in the pulmonary arteries [8,24,25]. In accordance, PGIS overexpression/gene transfer in animal models protected against PH [32,33]. It is worth mentioning that decreased PGIS expression not only contributes to the pathophysiology of PH by reducing PGI_2 production but likely also by shifting the PGH_2 metabolism towards TxA_2. The latter effect was demonstrated by increased TxA_2 levels in urine or plasma derived from PH patients [5,6,34,35]. This shift towards TxA_2 may lead to vasoconstriction, an increase of SMC proliferation and matrix protein synthesis, an exaggerated response to growth factors, and endothelial dysfunction. Taken altogether, the level of PGIS expression is a key factor in PH development. [32,33].

Our results showed no change in PGIS expression in distal lung tissue derived from PH Group-III patients (Fig. 4A), an observation that is consistent with previous findings in whole lung homogenates derived from pigs with elevated pulmonary blood flow and PH [23]. In contrast, studies using whole lung homogenates from chronic hypoxia or monocrotaline (MCT)-induced PH rats showed an increased PGIS expression [22,26]. Consistent with this finding, PGIS mRNA expression in human whole lung tissue derived from PH Group-I patients showed a trend toward a positive correlation with mPAP [27]. When considering

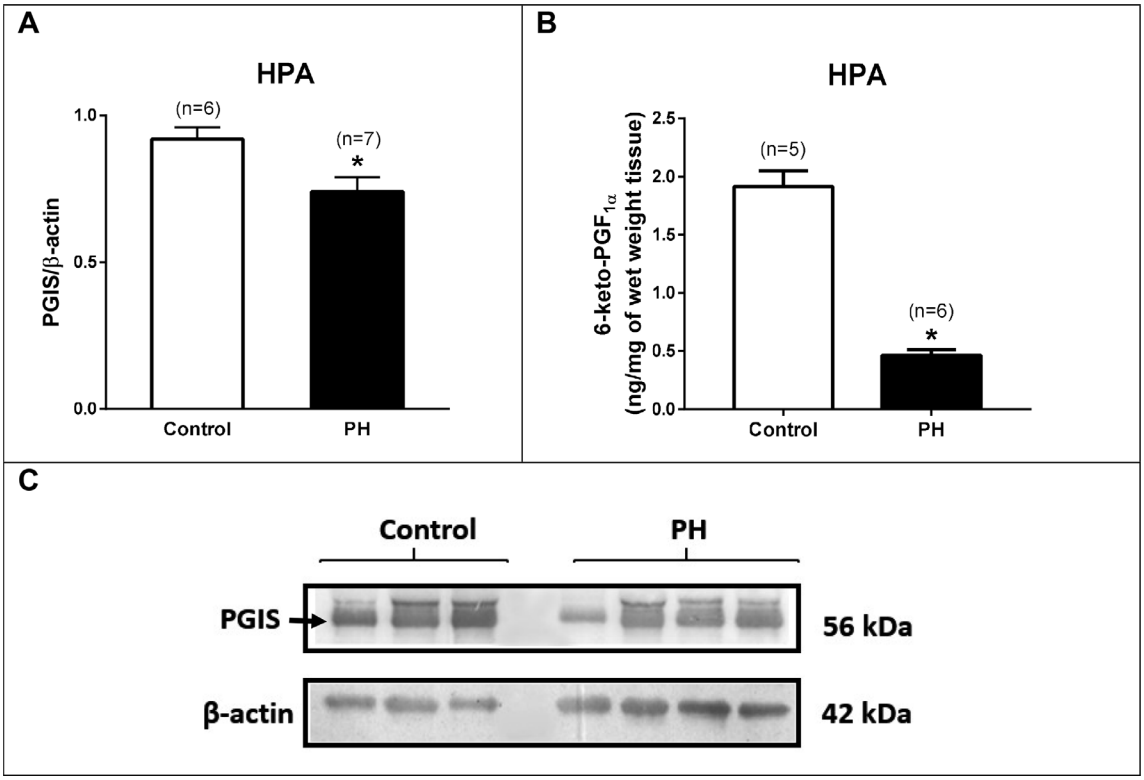

Fig. 1. PGIS expression (A) and 6-keto-$PGF_{1\alpha}$ levels (B) in human pulmonary artery (HPA) derived from control and pulmonary hypertension (PH) Group-III patients. Western blot analyses for PGIS were normalized to β–actin in human preparations. Values are means ± SEM, derived from n patients indicated in parenthesis. *Data significantly different (Student's *t*-test) between control and PH patient groups, $P = 0.02$ in panel A and $P < 0.001$ in panel B. A representative image of one PGIS blot having three Control and four PH patient samples is presented in panel C.

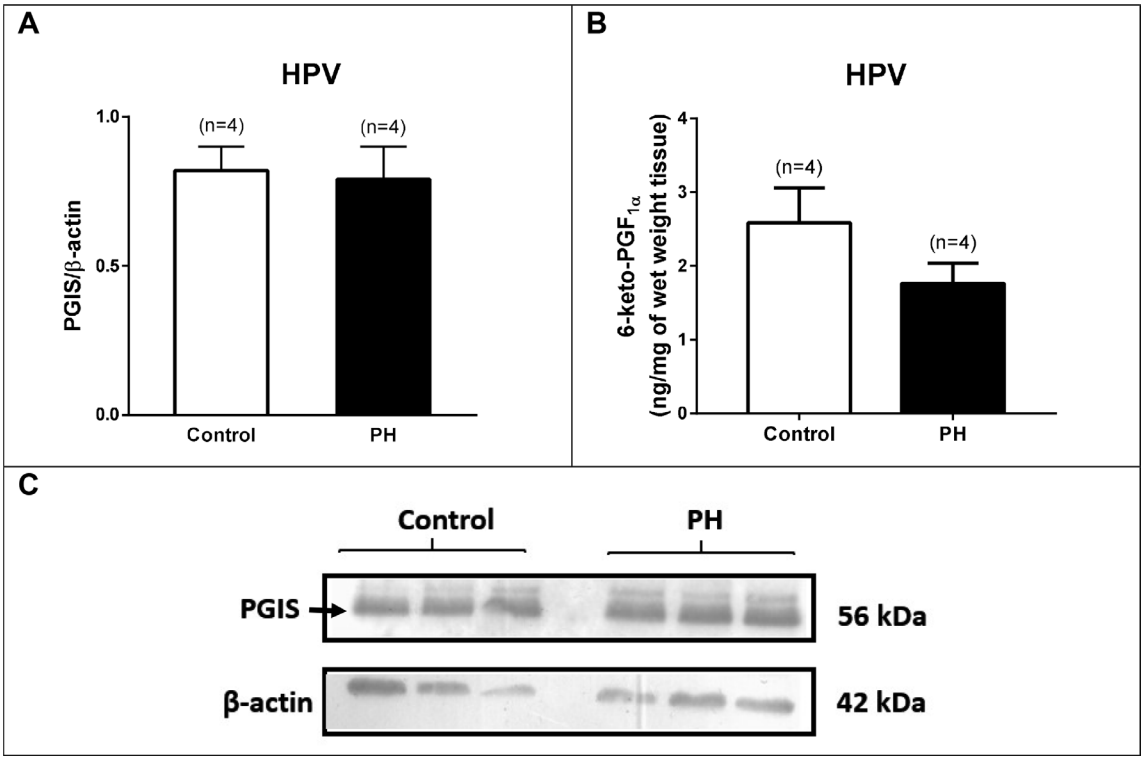

Fig. 2. PGIS expression (A) and 6-keto-$PGF_{1\alpha}$ levels (B) in human pulmonary vein (HPV) derived from control and pulmonary hypertension (PH) Group-III patients. Western blot analyses for PGIS were normalized to β–actin in human preparations. Values are means ± SEM, derived from n patients indicated in parenthesis. No statistically significant differences (Student's *t*-test) were found between Control and PH values in panels A and B. A representative image of one blot having three Control and three PH patient samples is presented in panel C.

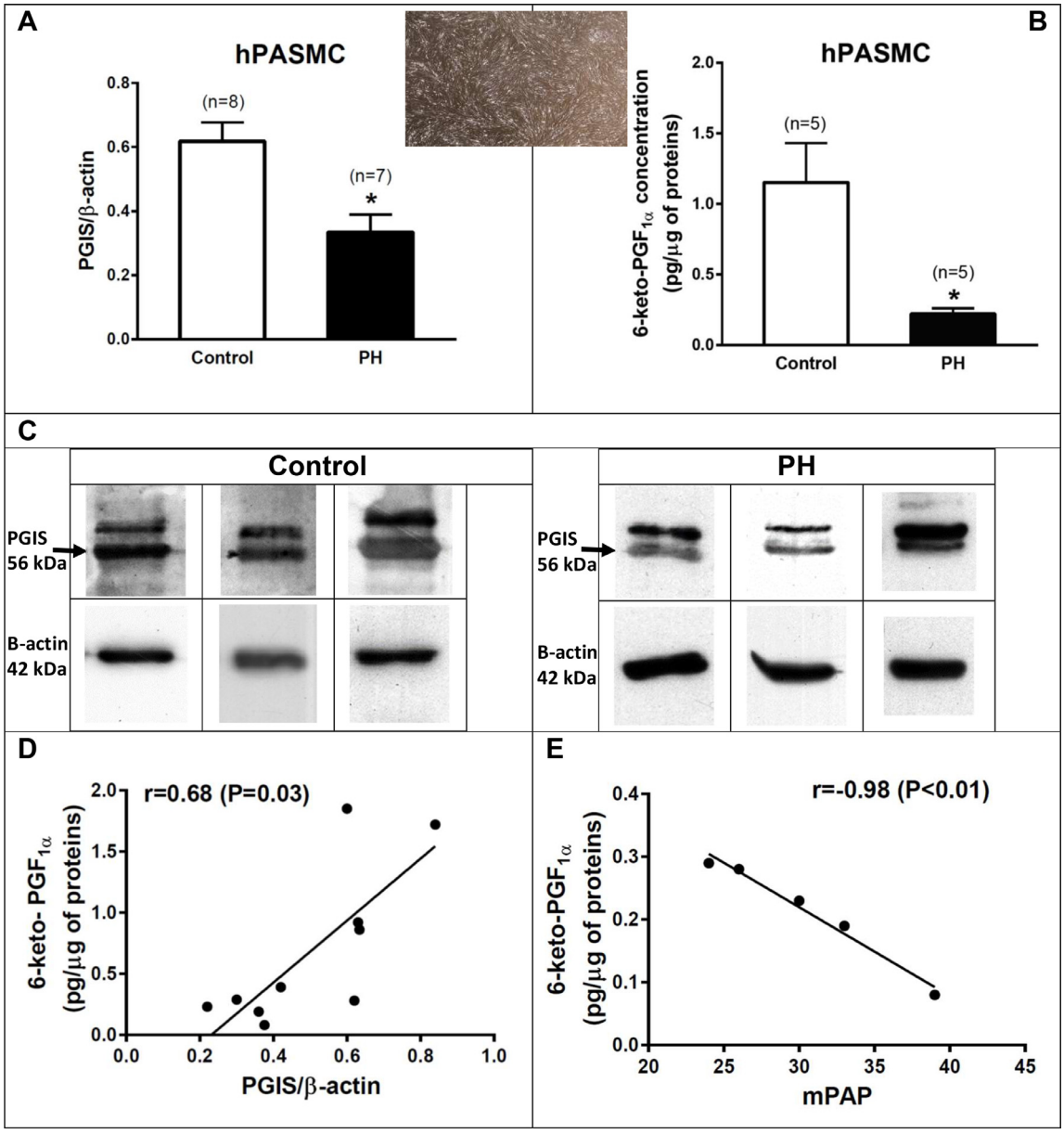

Fig. 3. PGIS expression (A) and 6-keto-$PGF_{1\alpha}$ levels (B) in human pulmonary artery smooth muscle cells (hPASMC) derived from control and pulmonary hypertension (PH) Group-III patients. A representative picture of hPASMC culture is presented at the top. Western blot analyses for PGIS were normalized to β-actin levels in hPASMC. Values are means ± SEM derived from n patients indicated in parenthesis. *Data significantly different ($P < 0.01$) between control and PH patient groups in Figs. 3A (Student's *t*-test) and Fig. 3B (Mann-Whitney rank sum test). Representative bands (with their respective β-actin loading control) derived from different western blots are presented in panel C. The significant correlations between 6-keto-$PGF_{1\alpha}$ levels released from PASMC and PGIS expression ($n = 10$ control and PH patients, Spearman correlation) or mean pulmonary artery pressure (mPAP from $n = 5$ PH patients, Pearson correlation) are presented in panel D and E, respectively. Samples used for ELISA measurements have corresponding patient samples used for western blot analyses.

these sometimes consistent and sometimes contradictory observations in lung tissue, it appears that the changes in endogenous PGIS expression and activity may be dependent on the samples analyzed (pulmonary artery versus whole lung) or the model/ PH group used.

Previous studies using PH animal models demonstrated that decreased PGIS expression was associated with lower PGI_2 production in pulmonary arterial preparations [8,21,24,25]. This is consistent with our current study in human pulmonary artery (Fig. 1A and B) and even more so in hPASMC where we show a significant positive correlation between PGIS expression and 6-keto-$PGF_{1\alpha}$ levels (Fig. 3D). In addition, our results demonstrated a significant inverse correlation between mPAP and the PGI_2 metabolite released by hPASMC of the same patients (Fig. 3E), a result suggesting that PGI_2 levels are an important determinant for the severity of disease. Consistent with our results, 24 h urinary excretion of a stable metabolite of PGI_2, 2,3-dinor-6-keto-$PGF_{1\alpha}$, was reduced in patients with primary and secondary PH [6]. Our results from HPA could not be explained solely by the endothelial dysfunction observed in PH. In our previous work using the same fresh "control" pulmonary arteries, the production of PGI_2 induced by arachidonic acid was significantly reduced by 68% and 56% in endothelium-stripped HPA and HPV, respectively [16]. This previous work and current data demonstrating attenuated PGI_2 synthesis in PH hPASMC (Fig. 3B) suggest that both endothelial and smooth muscle cells dysfunction are responsible for the decreased PGI_2 levels measured in HPA from PH patients (Fig. 1B). Furthermore, published work performed in PH Group-I patients [36–38] and our recent studies in PH Group-III patients [31] demonstrated that IP receptor expression was also decreased in pulmonary preparations. These data support the

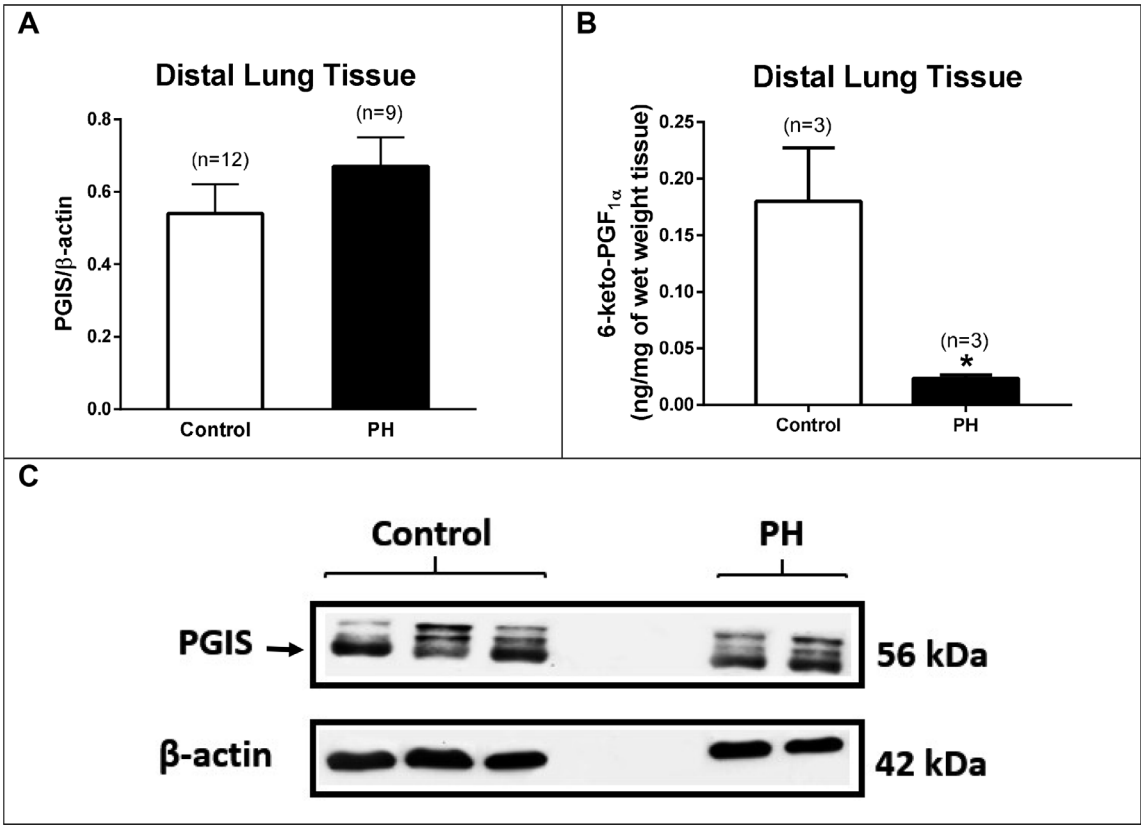

Fig. 4. PGIS expression (A) and 6-keto-PGF$_{1\alpha}$ levels (B) in human distal lung tissue derived from control and pulmonary hypertension (PH) Group-III patients. Western blot analyses for PGIS were normalized to β–actin in human preparations. Values are means ± SEM derived from n patients indicated in parenthesis. *Data significantly different between control and PH patient groups ($P < 0.05$, Student's *t*-test). A representative image of one blot with three Control and two PH patient samples is presented in panel C. Most samples used for ELISA measurements have corresponding patient samples used for western blot analyses.

whole PGI_2 pathway plays a role in the development of PH. Importantly, our data contribute to understanding the beneficial role of PGI_2-mimetic treatments in PH Group-III patients (in particular with severe PH) that were previously suggested [39–45].

Interestingly, in PH distal lung tissue preparations, although there was no significant change in PGIS expression, 6-keto-PGF$_{1\alpha}$ production was significantly attenuated (Fig. 4B). One mechanism that could explain this discrepancy is the tyrosine nitration of PGIS by oxidative stress and/or inflammation, which can be observed in PH. Nitration of PGIS could result in its inactivation, an effect that was described in pulmonary artery endothelial cells derived from a model of persistent PH in new-born lambs [8]. This discrepancy between PGIS expression and PGI_2 levels in distal lung tissue from PH Group-III patients should be further investigated. In addition, the level of 6-keto-PGF$_{1\alpha}$ released in supernatants from distal lung tissue was approximately 10-fold less when compared with supernatants from artery or vein preparations, an observation that may be explained by the complex structure of distal lung tissue which could trap the PGI_2 metabolite.

Although it has been suggested that pulmonary veins and bronchi [31] play an important role in normal lung physiology and the pathophysiology of PH, there is a lack of studies exploring the prevalence of venous/bronchial dysfunction in the various forms of PH. In our study, dysregulation of the PGIS expression and activity was not observed in either pulmonary vein (Fig. 2) or bronchi (Fig. 5) derived from PH Group-III patients. Consistent with our results, Badesh *et al.* demonstrated that PGI_2 release was not changed in pulmonary veins derived from calves with hypoxia-induced severe PH. The authors suggested that pulmonary veins are not exposed to increased wall tension or shear stress and are protected from the hemodynamic stress of PH [21].

Most of our knowledge about PH and the development of pharmacological/therapeutic strategies come from studies using PH animal models. Unfortunately, most of these models do not fully mirror the pathophysiological features of this disease. For instance, chronic hypoxia is defined as a model of PH Group-III; however, it is species-dependent and is not reflective of the challenges that exist for improving the vascular bed, haemodynamic status or mortality rate in humans [28]. For all of these reasons, *in vitro* studies using samples isolated from PH patients, especially Group-III which is one of the most common and lethal form of PH [29], are indispensable.

While these data are compelling, there are some limitations for these types of assessments. Obtaining fresh lung samples after lung transplantation of PH Group-III is quite difficult, and while the n value of experiments is acceptable for statistical and scientific interpretation, it is not robust. This low availability of tissue also hindered assessing the cellular localization of PGIS by immunohistochemistry in these studies. In the literature, two studies have described PGIS localization in human pulmonary endothelial [9] and epithelial cells [11]. Our results demonstrate that PGIS is expressed in hPASMC, an observation only previously described in rat PASMC [10]. Further experiments are needed to evaluate PGIS expression in fibroblasts or alveolar type I/II cells in control and PH patients.

5. Conclusion

Our results demonstrate significant decreases of PGIS expression and/or 6-keto-PGF$_{1\alpha}$ levels in human pulmonary artery, smooth muscle cells, and distal lung tissue derived from PH Group-III patients. More importantly, our study suggests for the first time that PH Group-I and Group-III share a common pathophysiological decrease of PGIS pathway expression and function. Despite the fact that inhaled PGI_2 therapy in PH Group-III patients seems to be a promising treatment [39-45], current guidelines for PH management indicated that published

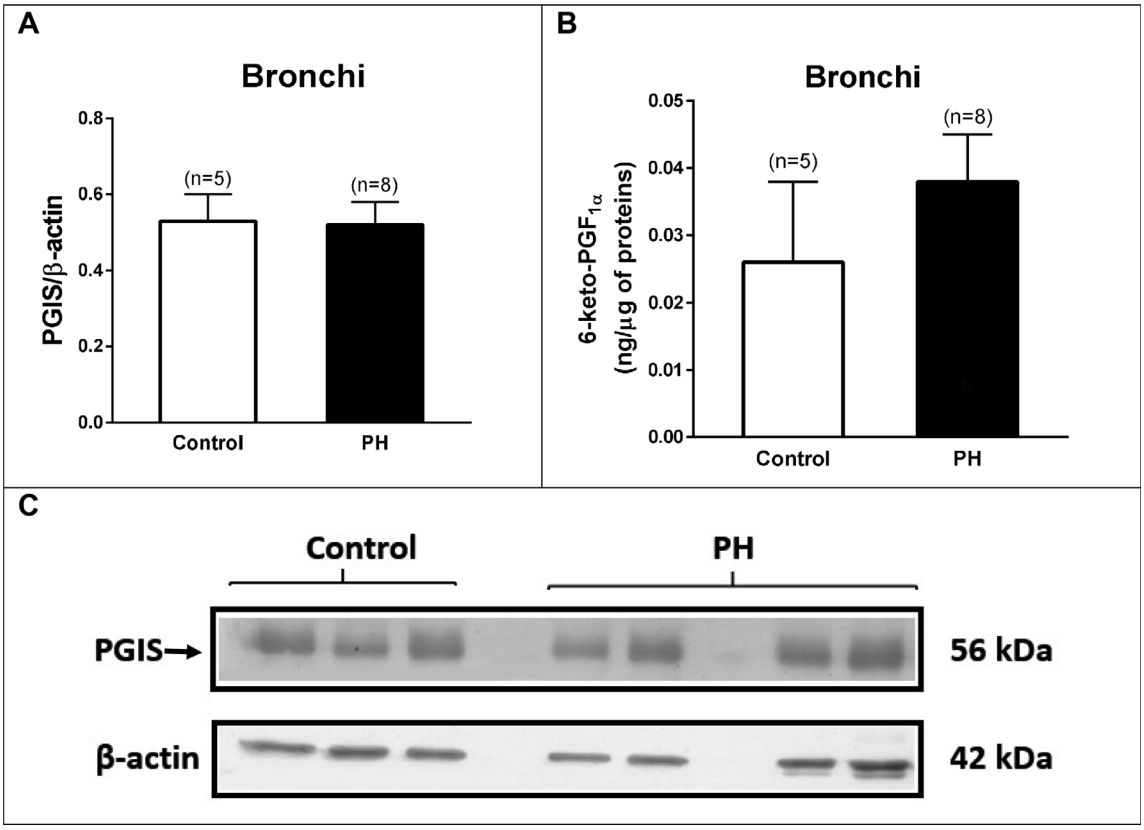

Fig. 5. PGIS expression (A) and 6-keto-$PGF_{1\alpha}$ levels (B) in human bronchi derived from control and pulmonary hypertension (PH) Group-III patients. Western blot analyses for PGIS were normalized to β–actin in human preparations. No significant differences (Student's t-test) were found between Control and PH values in panels A and B. A representative image of one blot with three Control and four PH patient samples is presented in panel C. Samples used for ELISA measurements have corresponding patient samples used for western blot analyses.

experience with PGI_2 therapy is scarce in PH Group-III, and it is not currently recommended for these patients [3]. Our findings provide additional support for considering the use of PGI_2 mimetics to treat PH Group-III.

CRediT authorship contribution statement

Gulsev Ozen: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Yasmine Amgoud:** Investigation, Formal analysis, Writing - review & editing. **Heba Abdelazeem:** Investigation, Formal analysis, Writing - review & editing. **Salma Mani:** Resources, Writing - review & editing. **Chabha Benyahia:** Investigation, Formal analysis. **Amel Bouhadoun:** Writing - review & editing. **Alexy Tran-Dinh:** Resources, Writing - review & editing. **Yves Castier:** Resources. **Alice Guyard:** Resources. **Dan Longrois:** Resources, Writing - review & editing. **Adam M. Silverstein:** Writing - review & editing. **Xavier Norel:** Conceptualization, Formal analysis, Writing - original draft, Supervision.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2020.102158.

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