Quantitative Immunohistochemistry of PDGFRα, PDGFRβ, c-KIT and CSF1R in Human PAH Lung Samples

PA4143

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BACKGROUND

- New therapies targeting inflammatory, proliferative, and fibrotic kinase-mediated signaling are under development for pulmonary arterial hypertension (PAH)
- Both preclinical and clinical studies suggest an important role of kinase signaling through PDGF receptors, c-KIT, and CSF1R
- CSF1R expression in human PAH samples has recently been reported[1]
- This study performed quantitative immunohistochemistry in human PAH lung samples for PDGFR, CSF1R, and c-KIT kinases to confirm relevance of these targets in PAH

METHODS

- Human lung samples were obtained from the Pulmonary Hypertension Breakthrough Initiative (PHBI) tissue repository (**Table 1**). Control samples consisted of donor lungs not used for transplant. Ten PAH and ten control samples were studied.
- Sections of formalin-fixed paraffin-embedded (FFPE) lung tissues were stained with the following primary antibodies: PDGF Receptor α, PDGF Receptor β, CD117 (c-KIT), and CSF1R. Antibody binding was visualized with DAKO polymer labeled, HRP-bound, secondary reagent.
- Quantitative analysis of the signals from the IHC samples was performed with MetaMorph® Image Analysis Software
- Statistical analysis was performed with GraphPad Prism. Linear regression was performed to compare integrated intensity of the signals to total area in which the signal was expressed. The unpaired t-test was used to compare signals between the PAH and control groups.

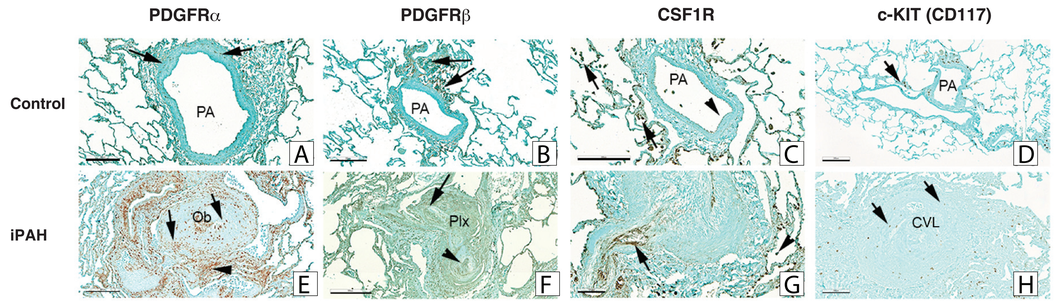
Table 1. PHBI Clinical Data

Subject	Age, years	Sex	Race	Ethnicity	mPAP [mmHg]	PCWP [mmHg]	CO [L/min]	PVR [Wood units]	PH MEDS
IPAH	28	F	White	Hispanic or Latino	59	9	3.8	14.53	sildenafil, bosentan, IV epoprostenol
FPAH	37	M	White	Non-Hispanic	77	14	4.43	16.38	sildenafil, ambrisentan, IV epoprostenol
IPAH	40	M	White	Hispanic or Latino	64	12	3.1	19.65	sildenafil, ambrisentan, SC treprostinil
IPAH	7	F	White	Non-Hispanic	102	11	NA	NA	sildenafil, bosentan, IV treprostinil
APAH (meth)	50	F	White	Hispanic or Latino	65	10	3.4	18.12	sildenafil, ambrisentan, IV epoprostenol
IPAH	13	M	White	Hispanic or Latino	82	12	NA	NA	tadalafil, bosentan, IV epoprostenol
APAH (CHD)	23	F	White	Non-Hispanic	NA	NA	NA	NA	sildenafil, IV treprostinil
IPAH	33	F	White	Non-Hispanic	59	NA	NA	NA	bosentan, IV epoprostenol
IPAH	27	F	White	Non-Hispanic	56	9	3.91	13.32	sildenafil, bosentan, IV treprostinil
IPAH	27	M	White	Non-Hispanic	53	13	2.9	17.28	sildenafil, bosentan, IV epoprostenol

Controls ranged from 25 – 56 years of age, included 5 females and 4 males, were white (6), Asian (1), or of unknown race (2), and either of non-hispanic (6) or hispanic or latino (3) ethnicity. CO, cardiac output; APAH (meth/CHD), methamphetamine-/congenital heart disease-associated PAH; FPAH, familial PAH; IPAH, idiopathic PAH; IV, intravenous; mPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance

RESULTS

Figure 1. Representative photomicrographs of expression of PDGFRα (A, E), PDGFRβ (B, F), CSF1R (C, G), and c-KIT (D, H) in control (n=10) and iPAH (n=10) human lungs. Scale bars: 100 μm (G), 200 μm (A-E, H), 300 μm (F).



- PDGFRα was expressed in media smooth muscle cells in normal pulmonary arteries (PA) in control lungs (arrows) (**A**), with marked expression in iPAH obliterative lesion (Ob; arrows) and perivascular tissue (arrowhead) (**E**)
- PDGFRβ was predominantly expressed in perivascular tissue in control lung (arrows) (**B**), while a complex pulmonary vascular lesion in iPAH shows intense expression in the intima (arrow) and within the incipient blood vessels and cell clusters (Plx; arrow) (**F**)
- CSF1R expression was noted in the intima of control pulmonary arteries (PA; arrowhead) (**C**), with stronger expression in alveolar macrophages (arrows). In iPAH lungs, there was marked expression in the intima of the obliterative lesion (arrow), with expression in macrophages (arrowhead) (**G**)
- Individual c-KIT positive cells were sparsely seen around normal pulmonary arteries in control lungs (arrow) (**D**), while iPAH lungs showed permeation of concentric vascular lesions (CVL) with positive cells (arrows) (**H**)

Figure 2. Quantitative IHC demonstrated a significant increase of integrated intensity for PDGFRα, PDGFRβ, CSF1R and c-KIT, in iPAH lung sections compared to controls (*P<0.05, **P<0.01, ****P<0.0001)

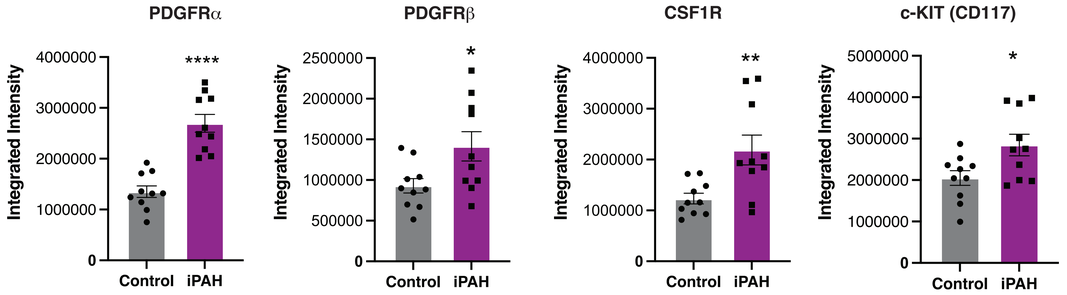
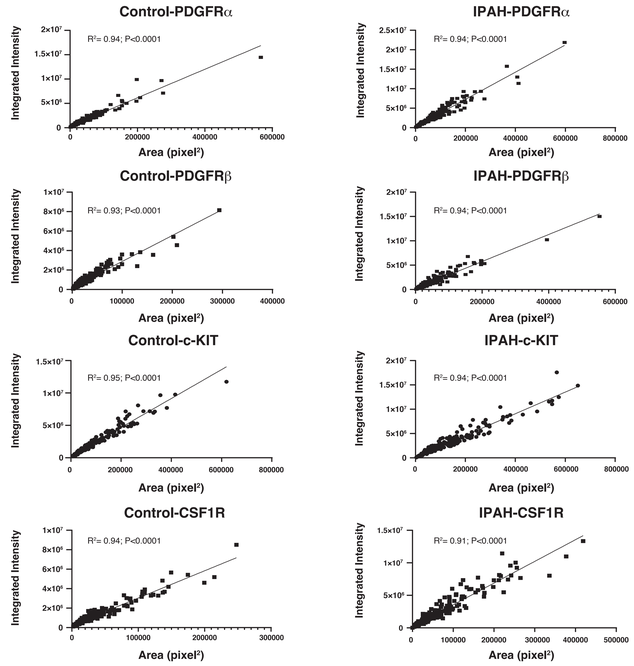


Figure 3. Correlation analysis between integrated intensity and area for PDGFRα, PDGFRβ, CSF1R and c-KIT, in iPAH lung sections compared to controls



SUMMARY AND CONCLUSIONS

- PDGFRα, PDGFRβ, c-KIT and CSF1R were significantly increased in pulmonary arteries of PAH vs. control lungs
- A high correlation between integrated intensity and area indicated that the signals were increased as a result of a greater en masse cell area expressing the target rather than an increased signal density within individual cells
- Targeting PDGFR, c-KIT and CSF1R may represent a therapeutic strategy for PAH

REFERENCES

1. Galkin A, Sitapara R, Clemons B, et al. Inhaled Seralutinib Exhibits Potent Efficacy in Models of Pulmonary Arterial Hypertension. *Eur Respir J* 2022; DOI: 10.1183/13993003.02356-2021. Online ahead of print.

Presented at the ERS International Congress, 4-6 September, 2022