1   **Short Research Communications**

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3   **PTPN1 deficiency modulates BMPR2 signaling and induces endothelial dysfunction in**

4   **Pulmonary Arterial Hypertension**

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Abstract

Bone morphogenic protein receptor 2 (BMPR2) expression and signaling are impaired in pulmonary arterial hypertension (PAH). How BMPR2 signaling is decreased in PAH is poorly understood. Protein tyrosine phosphatases (PTPs) play important roles in vascular remodeling in PAH. To identify whether PTPs modify BMPR2 signaling we used a siRNA-mediated high throughput screening of 22,124 murine genes in mouse myoblastoma reporter cells using ID1 expression as read-out for BMPR2 signaling. We further experimentally validated the top hit, PTPN1 (PTP1B), in human healthy pulmonary arterial endothelial cells (PAECs) either silenced by siRNA or exposed to hypoxia and confirmed its relevance to PAH by measuring PTPN1 levels in blood and PAECs collected from PAH patients. We identified PTPN1 as a novel regulator of BMPR2 signaling in PAECs, which is downregulated in the blood of PAH patients and documented that downregulation of PTPN1 is linked to endothelial dysfunction in PAECs. These findings point to a potential involvement for PTPN1 in PAH and will aid in our understanding of the molecular mechanisms involved in the disease.

Keywords: PTPN1, BMPR2 signaling, hypoxia, endothelial dysfunction, pulmonary hypertension

Introduction

Pulmonary Arterial Hypertension (PAH) is an obliterative disease of the pulmonary arteries that affects 50-100 individuals in 1 million people worldwide. The progressive increase in pulmonary vascular resistance ultimately leads to right heart failure, responsible for the high mortality in PAH. On a cellular level, pulmonary arterial endothelial cell (PAEC) dysfunction and apoptosis along with abnormal growth of smooth muscle cells (SMC) leading to medial thickening and neointima formation, causing the occlusive vasculopathy in PAH [1]. Understanding the molecular mechanisms that regulate the remodeling process of the vasculature is an area of intense study, yet no approved drug is available capable of reversing the remodeling, leaving PAH without a cure. Deficiencies of bone morphogenetic protein receptor 2 (BMPR2) expression and signaling are implicated in the development of PAH [2]. While BMPR2 mutations strongly predispose to PAH, only 20% of mutation carriers develop clinical disease,

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63   indicating that in addition to gene mutations, additional factors might be involved in the
64   pathogenesis of PAH. Moreover, in many non-familial PAH forms, BMPR2 protein and
65   signaling levels are reduced [2], suggesting that defective BMPR2 expression and signaling is a
66   common phenomenon in different types of PAH. However, how the BMPR2 signaling is
67   precisely regulated is largely unknown, especially in the non-genetic forms of PAH. Human
68   clinical PAH features were observed in pulmonary endothelial-specific conditional BMPR2
69   knockout mice [3], SMC-specific BMPR2 dominant-negative mice [4, 5], and BMPR2
70   heterozygous mutant rats [6]. Furthermore, defective BMPR2 signaling was shown to be linked
71   with abnormal vascular cell phenotypes, such as abnormal proliferation, apoptosis, and
72   angiogenesis of PAECs, and hyperproliferation and apoptosis resistance of pulmonary arterial
73   smooth muscle cells (PASMCs) [2]. Increasing BMPR2 expression and signaling has therefore
74   been proposed as an attractive solution with therapeutic potential for PAH treatment [2]. Indeed,
75   our group has previously demonstrated that increasing BMPR2 signaling with Tacrolimus
76   (FK506) [7] and BMPR2 expression with Enzastaurin [8] improved pulmonary vascular
77   remodeling and PH in murine models of experimental PH. Most recently Sotatercept has been
78   shown to effectively re-balance TGFb/BMPR2 signaling in preclinical models of PH as well as
79   in a phase II PAH trial and was able to improve pulmonary vascular resistance in PAH patient on
80   background PAH therapy (NEJM 2021), validating the approach of restoring normal BMPR2
81   signaling as a therapeutic approach in PAH.

82

83   Based on a high-throughput (HTS) siRNA screen of ~24000 genes, using a BRE-reporter mouse
84   cell line with ID1 expression as readout for increased BMPR2 signaling, in combination with an
85   analysis of publicly available PAH RNA expression data, our group previously identified
86   clinically relevant novel BMPR2 signaling modifier genes namely fragile histidine triad (FHIT)
87   [8] and Lymphocyte Cell-Specific Protein-Tyrosine Kinase (LCK) [9]. Using the same siRNA
88   HTS data set as well as subsequent experimental validation *in vitro*, *in vivo*, and PAH clinical
89   samples, we identified another novel BMPR2 signaling modifier gene, protein tyrosine
90   phosphatase non-receptor type 1 (PTPN1) with potential relevance to PAH. In general, protein
91   tyrosine phosphatases (PTPs) are involved in regulating signal transduction pathways and
92   maintaining phospho-tyrosine levels in cells. Thereby they modulate a range of cellular
93   processes, such as proliferation, differentiation, and apoptosis by working in concert with

94   protein tyrosine kinases (PTKs)[10]. A recent study shows that the EYA3 tyrosine phosphatase

95   activity promotes pulmonary vascular remodeling in PAH[11].

96

97   The PTPN1 encodes for PTP1b was the first discovered PTP. In a case-control study, PTP1b

98   Single Nucleotide Polymorphisms (SNPs) were shown to contribute to hypertension [12].

99   Chronic insulin-mediated inhibition of PTPN1 function was found to upregulate platelet-derived

100   growth factor (PDGF) signaling and to promote neointima formation in the balloon-injured rat

101   artery [13]. PTPN1 knockout mice had increased mean arterial pressures[14]. Endothelial

102   specific PTP1b inhibition was shown to promote neointima formation in obese mice[15].

103   Adenoviral mediated overexpression of dominant negative PTPN1 increased neointima

104   formation in injured rat carotid arteries [16]. Furthermore, following vascular injury, mice

105   deficient to PTPN1 in SMCs developed perivascular fibrosis in carotid arteries[17]. Using

106   hypoxic PASMCs and hypoxia-induced PH mouse models, Freyhaus *et al.,* demonstrated that

107   hypoxia promoted PDGFRB pathway signaling through inhibiting PTPs, such as SHP-2, TC-

108   PTP, DEP-1, and PTPN1 [18]. PTPN1 knockout mice showed exacerbated inflammation and

109   leukocyte trafficking following ovalbumin challenge [19]. These findings suggested that PTP1b

110   might play a significant role in pulmonary vascular remodeling and PAH.

111

112   As endothelial dysfunction plays an important role in PH pathogenesis and as the BMPR2

113   receptor is highly expressed in endothelial cells, we aimed to investigate the role of PTPN1 in

114   modulating BMPR2 signaling and its effect on PAEC function in PAH. We found that PTPN1

115   silencing with siRNA decreases BMPR2 signaling, which was associated with impaired

116   proliferation, angiogenesis, and induced apoptosis in PAECs. We also find that PTPN1 RNA

117   expression is downregulated in hypoxic PAECs, lung tissues of Sugen5416-hypoxia PH rat

118   models and in whole blood samples of PAH patients by RNA sequencing. These findings point

119   to a potential involvement for PTPN1 in endothelial dysfunction in PAH and will aid in our

120   understanding of the molecular mechanisms involved in the disease.

121

122   **Methods**

123   **High Throughput siRNA Screen (HTS):** To identify novel BMPR2 signaling modifiers, a high

124   throughput siRNA screen of > 22,124 genes was performed in an Id1-BRE luciferase containing

125   C2C12 mouse myoblastoma reporter cell line treated with or without BMP4, as previously

126   described [8, 9]. Following knockdown of the genes, viability of cells was assessed in cells

127   stained with tryptan-blue staining. Changes in ID1 expression and cell viability after knockdown

128   of the genes with siRNAs were calculated compared with non-targeting siRNA-treated cells.

129

130   **Cell culture:** Human healthy primary pulmonary arterial endothelial cells (PAECs) were

131   purchased from PromoCell GmbH, Heidelberg, Germany (Cat # C12281). Cells were grown in

132   standard endothelial growth medium (Cat # C-22120; PromoCell GmbH) with growth factors

133   supplementation and 100 U/mL Penicillin-Streptomycin Solution (Gibco) under standard

134   conditions (37°C, 5% CO_2, 21% O_2, 90% humidity). Cells were sub-cultured at 1:4 ratio and

135   passages between 4 and 6 were used for all PAECs experiments.

136

137   **RNAi.** Knockdown of PTPN1 and BMPR2 was performed in PAECs. Briefly, PAECs (1.5 x

138   10^5/well) were seeded onto 6-well plates and incubated at 37ºC in a humidified 5% CO_2

139   atmosphere. Next day, cells were transfected with 50nM siRNAs against PTPN1 (Cat # 4390824,

140   Thermofisher), BMPR2 (Cat # 4390824) and non-target (NT) controls (Cat # 4390843,

141   Invitrogen), with 2ul of Lipofectamine RNAimax (Cat # 13778-1.5, Invitrogen) in a total 1ml of

142   OPTIMEM media. Six hours after transfection, the medium was changed to complete growth

143   medium. After 48 hours, knockdown efficiency was assessed by qRT-PCR.

144

145   **Cell proliferation, apoptosis, and angiogenesis:** Cell viability was assessed by MTT assay (Cat

146   # V13154, Invitrogen, Waltham, MA), as described previously [8]. Apoptosis was assessed by

147   commercially available caspase 3/7 assay (Cat # G8090, Promega, Madison, WI) as per the

148   manufacturer's instructions. Matrigel Tube formation assay was performed to assess

149   angiogenesis, as described previously [9].

150

151   **Hypoxia induction:** PAECs were grown under 1% O_2 condition in a hypoxia chamber, as

152   described previously[20]. Hypoxia induction was verified by measuring expression of hypoxia

153   responsive gene, VEGFA mRNA expression by qRT-PCR.

154

155 **Sugen5416-Hypoxia-induced PH rat models:** Ptpn1 mRNA and protein expression in the lung
156 of Sugen5416-Hypoxia-induced PH experimental rat models was measured by qRT-PCR and
157 western blotting, respectively. Pulmonary vascular remodeling and hemodynamic parameters of
158 the rat samples chosen were previously reported in Dannewitz Prosseda et al., [8].

159

160 **Gene expression quantification:** Total RNA from PAECs was extracted and purified with
161 commercially available RNeasy® Plus Mini Kit (Cat # 74134, Qiagen, Hilden, Germany). Total
162 RNA from rat's lung tissues was isolated using Trizol extraction method as described
163 previously[21]. Total RNA was then converted to cDNA using high-capacity cDNA Reverse
164 Transcription Kit (Cat # 4368813, Applied Biosystems^{TM}, Foster City, CA) and gene expression
165 was assessed by TaqMan qRT-PCR with targeted TaqMan assay probes, human PTPN1
166 (Hs00942477), GAPDH (Hs01786624_g1), 18S (Hs99999901_s1), BMPR2 (Hs00176148_m1),
167 ID1 (Hs03676575_s1), rat Ptpn1 (Rn01423685_m1), rat Gapdh (Rn01775763_g1) and
168 TaqMan™ 2x Universal PCR Master Mix (Cat # 4304437).

169

170 **RNAseq analysis of whole blood and PAECs from PAH patients and healthy controls:** To
171 determine whether PTPN1 expression was downregulated in the blood and PAECs from PAH
172 patients, we analyzed a large RNAseq data of whole blood of patients with idiopathic, heritable,
173 and drug-induced PAH (n=359) compared to age- and sex-matched healthy controls (n=72) [22]
174 and a publicly available RNAseq data set comprising 9 healthy and 9 PAH PAECs (GSE126262,
175 [23]).

176

177 **Western blotting:** Western blotting was performed as described previously [9], with primary
178 antibodies PTP1b (Cat # CST5311, Cell Signaling, 1:1000), BMPR2 (Cat # MA5-15827,
179 Invitrogen, 1:800), pSMAD1/5/9 (Cat # 13820S, Cell Signaling, 1:1000), ID1 (Cat # sc-133104,
180 Santa Cruz, 1:100), b-Actin (Cat # SC4778, Santa Cruz, 1:600) and secondary antibodies goat
181 anti-rabbit IgG H&L HRP (Cat # ab6721, Abcam, 1:5000) and goat anti-mouse IgG H&L HRP
182 (Cat # ab205719, Abcam, 1:5000).

183

184 **Statistical analysis:** All data were analysed using GraphPad prism version 9.0. Data are
185 represented as mean ± standard error mean. Data comparing between two groups, non-parametric

186   student t-test was performed. P values <0.05 were considered as significantly changes. Number

187   of samples for each experimental conditions are presented in each figure and legend.

188   **Results**

189   **PTPN1, a novel BMPR2 signaling modifiers**

190   To identify novel modulators of BMPR2 signaling, we previously performed an unbiased

191   siRNA-mediated HTS of 22,124 genes in a BRE-ID1-LUC reporter C2C12 mouse myoblastoma

192   cell line. The screened hits were cross-validated in publicly available gene expression data sets

193   of PBMC and lung tissues of PAH patients. These screening approaches identified three

194   important BMPR2 modifying genes FHIT [8], LCK [8, 9] and Fyn [9], which play an important

195   role in PAH. LCK and Fyn are PTKs. The phosphorylation of protein tyrosine is crucial to

196   cellular signaling pathways. The level of protein tyrosine phosphorylation is regulated by PTKs

197   and PTPs [24]. Several human diseases, such as cancers, are linked to aberrant tyrosine

198   phosphorylation, and are associated with a dysbalance between PTK and PTP activity [24]. Here

199   we focused on the role of PTPs on BMPR2 signaling modulation. We assessed the select PTPs

200   included in the HTS of 22,124 siRNAs, for their potential to modulate ID1 expression (**Figure**

201   **1A-B**). There are over 100 of PTPs in humans [25], we here focused on the receptor and non-

202   receptor type PTPs, phosphatase of regenerating liver PTPs, Map kinase phosphatases and

203   atypical dual-specificity phosphatases PTPs, and Myotubularins PTPs, CDC14s and class III

204   PTPs in the HTS screening data set. Knockdown of the select PTPs showed inhibition of ID1

205   by several PTPs, such as Ptpn1, Pptn11, and Ptpru, while several PTPs, such as Pptprs and

206   Ptpn20 showed increased expression of ID1 (**Figure 1B**). After re-testing of those PTPs that

207   inhibited ID1 when knocked down (= ID1 stabilizing/activating PTPs) Ptpn1knockdown had the

208   strongest effect on ID1 inhibition (**Figure 1C**). We then concentrated on Ptpn1 on BMPR2

209   signaling modulation and validated these findings in PAECs. We found that PTPN1 knockdown

210   with siRNA resulted in downregulation of BMPR2 and ID1 expression in PAECs after 48 hours

211   (**Figures 1D-H**). To determine whether PTPN1 was the upstream of BMPR2 signaling, we

212   silenced BMPR2 with siRNA and found that PTPN1 expression was not altered (**Figure 1I and**

213   **J**). This indicated that PTPN1 was upstream of the BMPR2 signaling. Together, these findings

214   suggested that PTPN1 regulated BMPR2 expression and signaling in PAECs, as measured by

215   ID1.

216

PTPN1 inhibition induces endothelial dysfunction in hPAECs *in vitro*

Abnormal proliferation, apoptosis, and tube formation of PAECs is strongly linked with the pathogenesis of PAH [1, 2]. We investigated whether PTPN1 regulates endothelial function in PAECs. We found that knockdown of PTPN1 with siRNA decreased cell viability, as measured by MTT assay and hemocytometer cell counts (**Figure 2A**). Compared to non-target siRNA controls, PTPN1 siRNA-mediated knockdown induced apoptosis, as confirmed by increased level of caspase3/7 activity (**Figure 2B**). We also observed that siRNA-mediated knockdown of PTPN1 reduced tube formation 6 hours after cells were seeded in a Matrigel tube formation assay compared with cells treated with control non-target siRNA (**Figure 2C**). These data suggest that PTPN1 deficiency may lead to endothelial dysfunction in PAH.

PTPN1 is downregulated in hypoxic hPAECs and in the lung of the Sugen5416/hypoxia/normoxia rat model

Hypoxia is considered one of the contributing factors to Group 3 PH (PH linked with hypoxia and lung disease) and is used as an insult to produce experimental PH in rodents. Furthermore, a previous study described the regulation of PTP by hypoxia[18]. We therefore asked whether hypoxia exposure altered expression of PTPN1 in PAECs. PAECs were exposed to hypoxia for 72 hours and PTPN1 expression was measured by qRT-PCR. Consistent with the previous findings in PASMCs [18], we here found that hypoxia downregulated PTPN1 expression in PAECs (**Figure 3A**). Furthermore, we also measured Ptpn1 mRNA and protein expression in the lungs of a sugen5416/hypoxia/normoxia induced PH rat model by qRT-PCR and western blot, respectively. PH was induced in the rats that received a single subcutaneous dose of Sugen5416 (a VEGF receptor tyrosine kinase inhibitor), followed by exposure to chronic hypoxia (10% O2) for 3 weeks and normoxia for 5 weeks (21% O2). The PH model was confirmed by measuring RVSP, RV hypertrophy (Fulton Index: weight ratio of the RV/ left ventricle (LV) and septum (LV + S) (Please see the data in [8]). We found a trend towards lower Ptpn1 mRNA and protein expression in the lung of sugen5416-hypoxia (SuHx) treated rats compared to normoxia treated rats (**Figures 3B and C**), with a high variability between samples. Together, these findings suggest that Ptpn1 deficiency may be associated with experimental, hypoxia associated PH.

PTPN1 is downregulated in the blood of PAH patients

8

248   To determine whether PTPN1 expression was reduced in clinical PAH, first we measured

249   PTPN1 expression in whole blood of patients with idiopathic, heritable, and drug-induced PAH

250   (n=359) compared to age- and sex-matched healthy controls (n=72) by RNAseq. We observed a

251   significantly downregulated PTPN1 expression in PAH patients compared to healthy controls.

252   (**Figure 4A**, for subject characteristics, please see in [22]). Next, to determined whether PTPN1

253   expression was decreased in PAECs from PAH patients, we re-analyzed a publicly available

254   RNAseq data set comprising 9 healthy and 9 PAH PAECs (GSE126262, [23]).Although we did

255   not observe changes in PTPN1 expression between PAH and healthy control PAECs, we found a

256   significant correlation of PTPN1 expression with BMPR2, SMAD5 and SMAD9 expression

257   (**Figures 4C-G**), suggesting the link of PTPN1 with BMPR2 signaling in PAH.

258

259   **Discussion**

260   Previously several PTPs have been shown to be linked to the pathogenesis of PAH. For instance,

261   pharmacological inhibition of Shp2, also known as PTPN11, ameliorated monocrotaline-induced

262   PH related hemodynamic parameters (mPAP, RVSP, and RVH) and improved pulmonary

263   vascular remodeling in rats [26]. Xu *et al.*, showed that inhibition of PTPRD in human PASMCs

264   and rats resulted in PH through promoting PASMCs migration via the PDGFRB/PLCγ1 axis

265   [27]. DUSP5-mediated inhibition of PASMCs proliferation suppressed PH and RVH [28]. Here,

266   through a combined approach of HTS, in vitro validation and analysis of a large cohort of PAH

267   clinical samples, we identified another PTP that is associated with PAH. We found PTPN1 as a

268   novel modifier of BMPR2 signaling and showed that PTPN1 is decreased in the blood of PAH

269   patients and PTPN1 deficiency is associated with induction of markers of endothelial

270   dysfunction.

271

272   Loss of function mutation in BMPR2 occurs in 60-80% of familial cases of PAH patients, but the

273   disease penetrance rate is low [29, 30], suggesting that in addition to the gene mutations, other

274   unidentified genetic, epigenetic, or environmental factors may contribute to the pathogenesis of

275   PAH. Importantly, defective BMPR2 signaling is also a common phenomenon in PAH patients

276   regardless of their etiologies of the disease. However, the molecular mechanism of how BMPR2

277   signaling is precisely regulated remains unclear. Through a siRNA mediated HTS and further

278   experimental validation, we identified PTPN1 as a novel regulator of BMPR2 signaling.

279 However, it remains unknown how PTPN1 regulates BMPR2 signaling. While we did not find

280 relevant studies linking PTPN1 to BMP-SMAD signaling, a previous study performed in

281 hepatocytes from PTPN1 knockout mice showed that those cells were resistant to TGFβ-induced

282 downregulation of cell viability, and -upregulation of apoptosis [31]. The authors also revealed

283 that PTPN1 deficient hepatocytes were less responsive to TGFb as a significant decrease of

284 Smad2/3 phosphorylation and increased NF-κB pathway activation in Ptpn1 knockout

285 hepatocytes was observed upon TGFß treatment [31]. Matulka et al., showed that PTPN1 was

286 involved in the Activin/ALK4 signaling to modulate p-ERK1/2 signaling, which represents a

287 noncanonical Activin pathway in embryonic stem cells [32]. While these studies support the role

288 of PTPN1 in TGFß signaling, the precise mechanism by which PTPN1 might facilitate the

289 TGFb/BMPR2 disbalance observed in PAH needs to be explored in future studies.

290

291 We found a significant downregulation of PTPN1 in whole blood of a large cohort of PAH

292 patients by RNAseq analysis, yet the precise cell type or tissue responsible for the observed

293 PTPN1 downregulation is not known. Previous reports describe a link between PTPN1 SNPs and

294 systemic hypertension [12, 33]. Hypoxia downregulates select PTPs including PTPN1 in

295 PASMCs exposed to hypoxia and in the lung of hypoxia-induced PH mice [18]. Given that

296 BMPR2 is highly expressed in endothelial cells, we herein investigated the role of PTPN1 in

297 PAECs. In line with a previous report [18], we demonstrated a decreased expression of PTPN1

298 in PAECs exposed to hypoxia. While we observed a trend towards lower PTPN1 levels in the

299 lung of sugen5416/hypoxia/normoxia treated rats with PH, the results were not significant, most

300 likely due to the high variability of PTPN1 expressions in the lung samples. While hypoxia is

301 used as an injurious stimulus in experimental PH, hypoxemia is usually not observed in PAH

302 patients - in contrast to Group 3 PH patients. While the cause of PTPN1 downregulation remains

303 to be determined, its effect on BMPR2 signaling and endothelial health could be an important

304 contributor to PAH. We find that PTPN1 deficiency induces endothelial dysfunction by

305 reducing proliferation, causing apoptosis and reducing tube formation of PAECs, all features

306 linked to the development of PAH. Berdnikovs et al. furthermore demonstrated that PTP1B was

307 involved in inflammation as inducible endothelial cell-specific deletion of PTP1B showed a

308 significant increase in accumulation of eosinophils bound to the luminal surface of the

309 endothelium in the lung vasculature and had a decrease in leukocyte recruitment into the lung

310 tissue during ovalbumin-induced allergic lung inflammation[34]. Furthermore, in response to

311 arterial injury, vascular smooth muscle cells deficient for PTPN1 promoted perivascular fibrosis

312 [17]. During respiratory syncytial viral-induced exacerbation of chronic obstructive pulmonary

313 disease, PTPN1 deficiency was shown to promote disease symptoms partly through enhancing

314 S100A9 levels, a damage-associated molecular pattern molecule [35]. In contrast to these

315 findings, several other lung studies show that PTP1b deficiency protected against lung

316 inflammation. For instance, using polysaccharides (LPS)-induced acute lung injury (ARDS)

317 models, Song et al., showed PTP1b inhibition protected against lung injury potentially regulating

318 through the CXCR4/PI3Kγ/AKT/mTOR signaling axis [36]. Whether PTPN1 deficiency protects

319 or exacerbates lung remodeling is likely dependent on the context and animal disease model. As

320 endothelial dysfunction and perivascular inflammation are key players in PAH pathobiology[37],

321 maintaining adequate PTPN1 levels could be significant for pulmonary vascular remodeling in

322 PAH.

323

324 While we observe a significant downregulation of PTPN1 expression in the whole blood of PAH

325 patients, we did not find these changes in PAECs of PAH patients. This could be due to the fact

326 of relatively low expression of PTPN1 and variation in PAECs than the blood cells. Further

327 larger studies would require confirming these findings in PAECs of PAH patients. However, we

328 found a signification correlation between PTPN1 and BMPR2 signaling marker gene expression

329 in the data set (**Figures 4B-G**), indicating again the involvement of PTPN1 in BMPR2 signaling

330 in PAH.

331

332 This study has several limitations. First, we demonstrated that inhibition of PTPN1 decreases

333 BMPR2 expression and signaling, and induces endothelial dysfunction, however, it would be

334 important to the see whether opposite is true as well: Whether overexpression of PTPN1

335 activates BMPR2 signaling and reverses abnormal endothelial cell behaviors. Second, this study

336 lacks the direct causal link between PTPN1 and PAH *in vivo*. Further studies need to characterize

337 whether global or cell specific deletion of (PTPN1) causes experimental PH or alternatively

338 predisposes to a more severe PH phenotype in response to injurious agents used to induce

339 experimental PH such as hypoxia alone, sugen5416/hypoxia, or monocrotaline. Third, we were

340 not able to validate in this pilot study the PTPN1 PAH blood RNAseq expression data in a

341 second sample cohort. Fourth, mechanisms of how PTPN1 regulates BMPR2 signaling in PAH

342 remains to be explored.

343

344 In summary, we find PTPN1 expression is downregulated in whole blood of PAH patients and

345 PAECs exposed to hypoxia and that PTPN1 downregulation is associated with endothelial

346 dysfunction in PAECs. Furthermore, we discovered that PTPN1 is a novel modulator of the

347 BMPR2 signaling pathway. These findings will help investigate the precise role of PTPN1 in

348 PAH.

349

350 **Acknowledgment**

353

354 **Support statement**

358

359 **Author contributions**

360 Conceptualization: M.K.A., E.S.; Methodology: M.K.A; Data curation: M.K.A., X.T., A.A.,

361 L.Z., C.J.R.; Writing - original draft: M.K.A.; Writing - review & editing: M.K.A., E.S., K.S.;

362 C.J.R., M.R.W., M.R.N., Supervision: E.S., Funding acquisition: E.S.

363

364 **Conflict of Interest statement:**

365 The authors declare no competing or financial interests.

366

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470   **Figure legends**

471   **Figure 1. PTPN1 is a novel regulator of BMPR2 signaling pathway.** A siRNA-mediated HTS

472   (n=22,124) was performed to identify possible BMPR2 signaling modifiers in mouse

473   myoblastoma BRE-ID1-LUC incorporated reporter cells. After 48 hours knockdown of the

474   genes, cells were treated with BMP4 to activate the signaling for two hours and then measured

475   ID1-linked luciferase levels. A colorimetric tryptan-blue cell viability was also performed. A)

476   Changes in Id1-linked luciferin expression (n=22,124) (X-axis) versus cell viability (Y-axis)

477   were plotted. Red dots denote the pre-selected protein tyrosine kinases (PTPs) selected from all

478   major PTPs. B) Changes in Id1-linked luciferin expression of the selected PTPs (% changes from

479   NTi). C) Selected PTPs tested with individual siRNA (reconstructed from the secondary

480   screening data n=96 from [8]. Data represented as mean ± SEM (n=2-3). D-J) PTPN1, BMPR2

481   and ID1 expression was measured by qRT-PCR in PAECs silenced to either PTPN1 or BMPR2

482   for 48 hours. Data represented as mean ± SEM (n=3), student t-test. *P<0.05, **P<0.01,

483   ***P<0.001, ****P<000.1.

484

485   **Figure 2. PTPN1 deficiency induced endothelial dysfunction in PAECs.** About 10,000 cells

486   were seeded on 96 well plates and next day PTPN1 siRNA 10nM were transfected with

487   RNAimax in 100ul of optimum media. After 5-6 hours, the media was replaced with fresh

488   complete media. After 48 hours, cell viability and apoptosis were measured by MTT assay and

489   caspase 3/7 assay. For Matrigel Tube formation assay, 96-well plates were coated with 50 µl of

490   matrigel/well and after one hour, PTPN1 knock downed and controls cells were seeded. After 6

491   hours incubation at 37°C incubator and images were taken on light microscope. Angiogenesis

492   was quantified in the images using ImageJ software.

493

494   **Figure 3. PTPN1 is downregulated in healthy human PAECs exposed to hypoxia and in the**

495   **lung sugen5416/hypoxia-induced PH rats.** A) 150,000 PAECs were seeded onto 6 well-plates

496   and exposure to 72 hours of hypoxia. After that, PTPN1 expression was measured by qRT-PCR.

497   Induction of hypoxia was verified by measuring VEGF expression by qRT-PCR[20]. B) PTPN1

498   mRNA and protein expression was also measured in the lung of sugen5416/hypoxia rate models

499   by qRT-PCR and western blotting (please see the PH model description and phenotypes data in

500   [8]). Data are represented as mean ± standard error mean, n=3-5, student t-test was performed to

501   compare data between two samples. ****P<0.0001.

502   **Figure 4. PTPN1 is downregulated in the blood but not in the PAECs of PAH patients.** We

503   analysed RNAseq data of PTPN1 expression in the whole blood ((n=72 healthy and n=359

504   PAH), for subject characteristics, please see in [22]) (A) and in PAECs ((n=9/group,

505   (GSE0126262, [23])) of PAH patients (B). TPM values for PTPN1 expression was shown in the

506   graphs. PTPN1 was correlated with expression of BMPR2, ID1, SMAD1, SMAD5, and SMAD9

507   in healthy and PAH PAECs (GSE0126262) (C-G).

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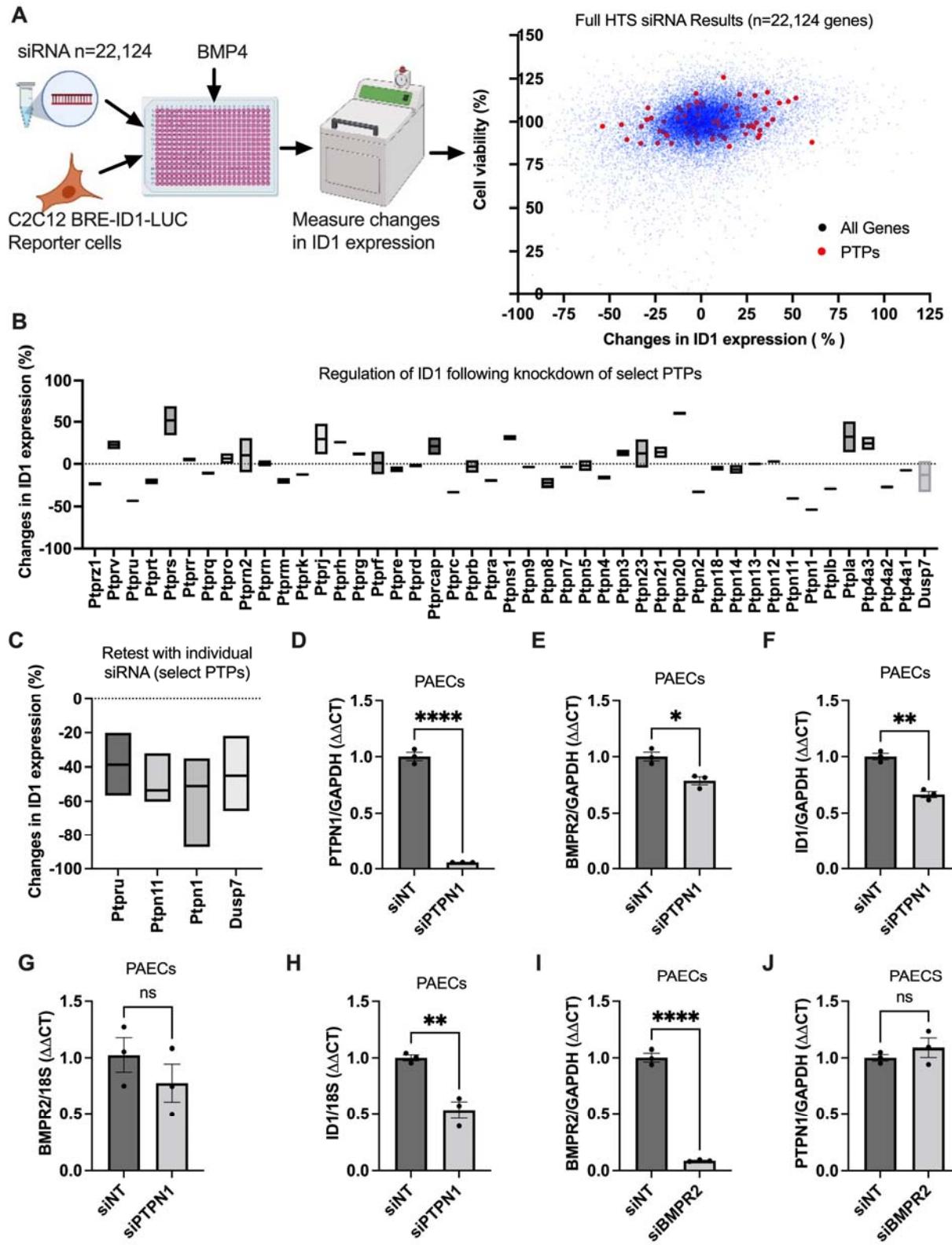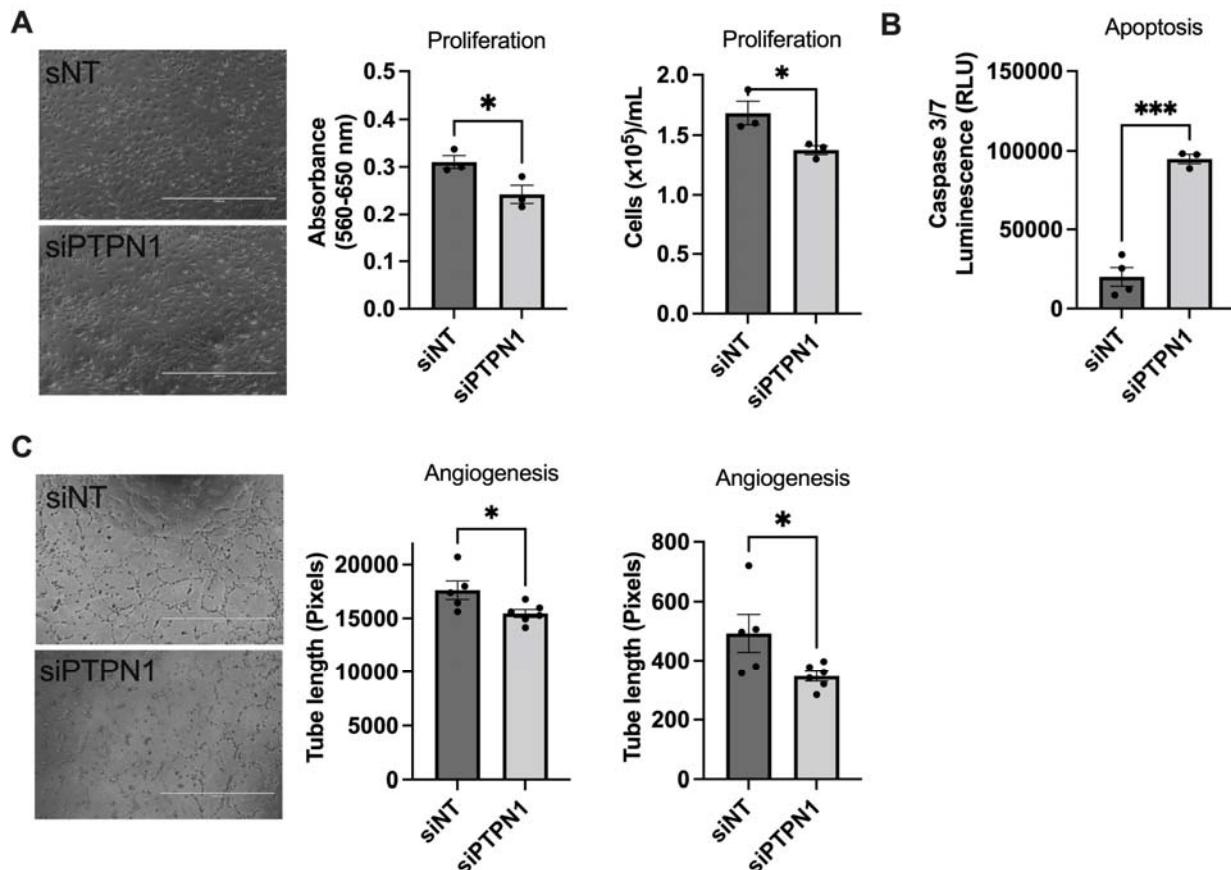
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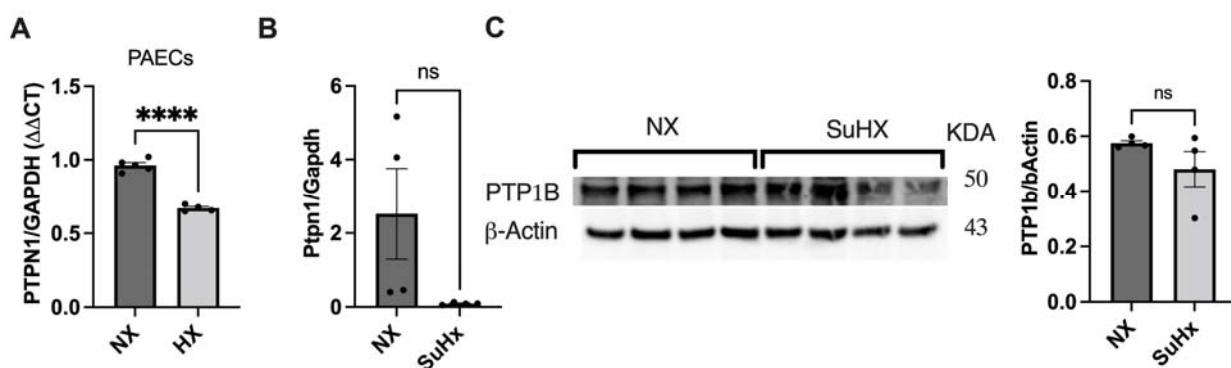
533   **Figure 1**

Figure 2

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Figure 3

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Figure 4

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