Thyroid hormone is highly permissive in angioproliferative pulmonary hypertension in rats

Aysar Al Husseini MD¹, Gianluca Bagnato MD¹, Laszlo Farkas MD¹, Jose Gomez-Arroyo MD¹, Daniela Farkas BSc¹, Shiro Mizuno MD PhD¹, Donatas Kraskauskas DVM¹, Antonio Abbate MD PhD², Benjamin Van Tassel Pharm D², Norbert F Voelkel MD¹ and Harm Jan Bogaard MD PhD³

¹Pulmonary and Critical Care Medicine Division and Victoria Johnson for Lung Research;
²Division of Cardiology, Virginia Commonwealth University, Richmond, VA, USA;
³Dept of Pulmonary Medicine, VU University Medical Center, Amsterdam, the Netherlands

Corresponding Author/ Reprints: Harm Jan Bogaard, Dept of Pulmonary Medicine, VU University Medical Center, PO Box 7057, 1007 MB, Amsterdam, the Netherlands. Tel: 31.20.4444782, Fax: 31.20.4444328, hj.bogaard@vumc.nl

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Epidemiological evidence links pulmonary arterial hypertension (PAH) with thyroid disease, but a mechanistic explanation for this association is lacking. Because a central hallmark of vascular remodelling in pulmonary hypertension is lumen obliteration by endothelial cell growth and because thyroid hormones are known to be angiogenic, we hypothesized that thyroid hormones play a role in the control of endothelial cell proliferation in experimental PAH in rats. Hypothyroidism was induced by subtotal thyroidectomy and treatment with propylthiouracil (PTU) in rats with experimental PAH after combined exposure to VEGF-R inhibition and hypoxia (the SuHx model). Subtotal thyroidectomy prevented and PTU treatment reversed the development of severe experimental PAH. T4 repletion restored the PAH phenotype in thyroidectomised SuHx rats. The prevention of PAH by thyroidectomy was associated with a reduced rate of cell turnover, reduced Erk1/2 phosphorylation, and reduced expression of αvβ3 integrin, fibroblast growth factor (FGF)2 and FGF receptor. Thyroidectomy mitigated hypoxia-induced pulmonary hypertension, but this effect was not associated with a decreased pulmonary vascular resistance. These data suggest that thyroid hormone permits endothelial cell proliferation in PAH. A causal link between thyroid diseases and the onset or progression of vascular remodeling in PAH patients remains to be determined.

Key Words: Angiogenesis. Integrins. Pulmonary Circulation. Thyroid Hormone
INTRODUCTION

Chronic pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling involving the smooth muscle cells of the media and growth of phenotypically altered endothelial cells (1;2). Many forms of severe chronic PAH are refractory to treatment with vasodilator drugs (3) and the overall mortality remains high. It appears that in many PAH patients the pre-capillary arterioles are obliterated by apoptosis-resistant endothelial cells via a process resembling angiogenesis (4). Severe forms of pulmonary arterial hypertension (PAH) have been classified as idiopathic, heritable, drug- and toxin-induced and associated forms (5;6). Examples of associated forms are PAH in connective tissue disease, HIV infection, sarcoidosis and sickle cell disease (7-9). A large number of PAH patients also have thyroid disease (10), but it is unclear whether this association between PAH and thyroid disease is coincidental or relates to an etiological role of thyroid hormones in the development of PAH. Thyroid hormones stimulate endothelial cell growth (11-13) and enhance hypoxic pulmonary vasoconstriction (14), and we therefore hypothesized that thyroid disease can affect pulmonary vascular remodeling in the setting of chronic PAH. We made use of the Sugen-chronic hypoxia (SuHx) rat model of severe angioproliferative PAH (15-17), which model depends on two hits: the induction of initial lung endothelial cell apoptosis by the vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor SU5416 and the cell growth-promoting chronic exposure to hypoxia. Here we show for the first time that thyroid hormones are highly permissive for both pulmonary hypertension and formation of lumen obliterating lung vascular lesions.
METHODS

Animal models
Subtotal thyroidectomized Sprague-Dawley rats used in this study were purchased from Harlan Laboratories (Frederic, MD). 5 mg of thyroxin (T4) was prepared in 60-day release pellets (Innovative Research of America (IRA), Sarasota, FL). Severe angioproliferative pulmonary arterial hypertension and right heart failure was induced in male Sprague-Dawley rats (body weight 200g, age 6 weeks) through their exposure to the VEGF receptor antagonist SU5416 and chronic hypoxia (SuHx), as described previously (16;17). One subset of thyroidectomized rats was given the SuHx combination starting 2 weeks after the thyroidectomy surgery and another one was given the T4 pellet subcutaneously 2 days before starting with the SuHx exposure. In another subset of SuHx animals, propylthiouracil (PTU; 10 mg/kg/day, Sigma, St. Louis, MO) was administered subcutaneously 5 days/week for 4 weeks starting 2 weeks after establishing the PAH model. Finally, the effects were studied of thyroidectomy on pulmonary vascular remodeling and hemodynamics after 4 weeks of chronic exposure to hypoxia (FIO2 10%). Cardiac ultrasound, hemodynamic measurements, tissue harvest and processing were performed as described previously. Cardiac output (CO) and stroke volume were indexed for body surface area (BSA, calculated from body weight (18), yielding cardiac index (CI) and stroke index (SI), respectively). Pulmonary vascular resistance index (PVRI) was calculated as 80*(mPAP-LVEDP)/CI, where mPAP is mean pulmonary artery pressure and LVEDP is left ventricular end-diastolic pressure. In those instances where catheter advancement into the pulmonary artery was not possible (approximately 1 out of every 8 rats), mPAP was estimated from right ventricular systolic pressure (RVPS) as described previously (19). Further details for animal models, assessment of angioproliferative vascular lesions, immunohistochemistry, Western blot analysis and statistical analysis are available in the expanded Methods in the Online Data Supplement.
RESULTS

Pulmonary hypertension and pulmonary vascular remodeling in the SuHx rat model of severe pulmonary hypertension are prevented by thyroidectomy

To address the question of whether thyroid hormones contribute to the development of PAH and pulmonary vascular remodeling in the SuHx model, we assessed whether subtotal thyroidectomy prevented the development of hemodynamic and lung histological changes characteristic of this model. Euthyroid and thyroidectomized rats were exposed to a single dose of the VEGF receptor tyrosine kinase inhibitor SU5416 (25mg/kg s.c.) and hypoxia for 4 weeks, a sufficient exposure time to generate severe vaso-obliterative PAH (15). As can be seen from Figure 1, thyroidectomy prevented an increase in RVSP in response to SuHx. The reduction in RVSP after thyroidectomy of SuHx rats was not only related to a decreased CI -an expected finding in hypothyroid animals- but was also to a large extent attributed to a decreased PVRI. T4 levels were decreased, as expected, in the subtotal thyroidectomy group and T4 levels closely correlated with RVSP values. Development of pulmonary hypertension was associated with an abnormal Doppler signal in the pulmonary artery and a progressive shortening of the pulmonary artery acceleration time (Supplemental Figure 1). Thyroidectomy prevented these changes in Doppler signal. The prevention of pulmonary hypertension in the thyroidectomized SuHx rats was paralleled by prevention of right ventricular (RV) dilatation and hypertrophy. Supplemental T4 restored physiologic levels of T4 in the thyroidectomized SuHx rats, which led to restoration of RVSP and PVRI to the levels seen in the euthyroid SuHx rats. Whereas a large number of arterioles were either completely or partially occluded in the lungs from SuHx rats treated with SuHx (Figure 2A, B), thyroidectomy resulted in a lung histology characterized by patent but muscularized arterioles (Figure 2C, D). Supplementation of T4 in thyroidectomized SuHx animals permitted PAH-characteristic vessel obliteration (Figure 2E, F). The numbers of partially
patent and fully lumen obliterated vessels (in percent) is shown in Figure 2G and the percent of patent vessels was correlated with RVSP (Figure 2H). Because hypoxia can affect the secretion of thyroid hormones and can also cause changes in plasma levels of T4 (20-22), we tested whether the combination of SU5416 plus administration of T4 (however, without hypoxia) caused pulmonary vascular remodeling and pulmonary hypertension. Our data demonstrate that chronic administration of T4 alone only causes mild pulmonary hypertension in SU5416 treated rats (Supplemental Figure 2). Thus, chronic T4 supplementation without hypoxia does not reproduce the pulmonary vascular disease observed in the SuHx model.

The effect of thyroidectomy on hypoxic pulmonary vascular remodeling
Persistence of a certain degree of medial wall thickening after thyroidectomy (Figure 2) suggested that the lack of thyroid hormone mainly affected the obliteration of lung vessels in thyroidectomized SuHx rats. To determine whether thyroid hormones also affect the degree of muscularization, we subsequently exposed normal and thyroidectomized rats to chronic hypoxia alone (a model of medial wall thickening but not vascular occlusions), without concomitant SU5416 administration (see Figure 3). Thyroidectomy decreased media wall thickness and lowered RVSP after hypoxic exposure, but the reduction in PVRI was statistically not significant and not associated with a reduced RV hypertrophy. Chronic hypoxic exposure led to an expected increase in hematocrit of 20-30% and this increase was not affected by thyroidectomy.

Thyroidectomy decreased or inhibited the expression of cell proliferation and cell death markers in the lungs from SuHx animals
To assess whether thyroidectomy prevented lesion cell growth we employed immunohistochemistry (IHC) to localize the expression of proliferating cell nuclear antigen (PCNA; Supplemental Figure 3) and the same lung tissues protein lysates were analyzed by western blot (WB; Figure 4). Because the development of angio-obliterative lesions in the SuHx
model depends also on lung cell death (16), we assessed cleaved caspase 3 expression in lung tissue by IHC (Supplemental Figure 3) and WB (Figure 4). PCNA\textsuperscript{+ve} cells were found abundantly in the examined tissue sections of SuHx lungs and both IHC and WB showed that the expression of PCNA was reduced in the lungs from thyroidectomized SuHx animals when compared with lungs from euthyroid SuHx rats. In addition, thyroidectomy reduced the expression of cleaved caspase 3 in SuHx lungs and T4 supplementation in thyroidectomized SuHx rats was related to increased expression of PCNA and cleaved caspase 3 (Figure 4 and Supplemental Figure 3).

The permissive effect of thyroid hormone on angio-obliteration in SuHx rats is associated with altered integrin α\textsubscript{v}β\textsubscript{3} and FGF2 signaling

Because one of the signaling pathways distal to cell membrane thyroid hormone receptors involves the angiogenic fibroblast growth factor (FGF)2 and the integrin α\textsubscript{v}β\textsubscript{3} (11;12) -which is highly expressed in activated endothelial cells (23;24)-, and because FGF-2 has been associated with severe PAH (25-27), we assessed the expression of this growth factor and of integrin α\textsubscript{v}β\textsubscript{3} in the lungs from animals which had developed severe PAH. Thyroidectomy reduced in the lung from SuHx animals the protein expression of FGF2, α\textsubscript{v}β\textsubscript{3} integrins (Figure 4A, B, C, D) and p-Erk (Figure 4A, H). T4 supplementation in thyroidectomized SuHx animals restored the expression of FGF2 and of the α\textsubscript{v}β\textsubscript{3} integrin proteins to the levels observed in the SuHx animals (Figure 4). Figure 5 and Supplemental Figures 4-7 demonstrate that FGF2 and α\textsubscript{v}β\textsubscript{3} proteins are overexpressed in lungs from SuHx rats and that overexpression does not occur in thyroidectomized animals. Lung tissue expression of the FGF2 protein and the integrin α\textsubscript{v}, and of PCNA and α\textsubscript{v} integrin were correlated (Supplemental Figure 3I, J) suggesting that in the setting of severe PH cell proliferation, FGF2 expression and integrin expression are perhaps synchronized.
**PTU treatment of rats with established pulmonary hypertension**

Having established that thyroidectomy prevents the development of angioproliferative PAH in the SuHx model we next examined whether propylthiouracil (PTU) would affect established PAH in the SuHx model. Four weeks treatment with daily dosing of PTU reduced the RVSP, PVRI and RV hypertrophy (Figure 6). These results show that reduction in T4 levels is able not only to prevent but also reverses, at least in part, PH and its consequences on the RV. Figure 7 shows that PTU treatment reduced the number of completely or partially occluded arterioles in the lung. In fact the number of patent vessels correlated with the RVSP, and the number of obliterated vessels also related to the degree of RV hypertrophy. Figure 7G shows the percent of patent and obliterated arterioles. Similar to the thyroidectomized SuHx animals, PTU treatment resulted in a reduction in the number of PCNA^{+}ve cells (Supplemental Figure 8). The expression of FGF-2 but not of the αvβ3 protein was reduced in the lung tissues from the PTU treated animals (Supplemental Figure 9). Thus, PTU treatment of SuHx rats with established PAH and lung arteriolar obliteration altered the tissue protein expression of growth, and apoptosis related factors in a direction similar to thyroidectomy.
DISCUSSION

Based on the knowledge that the thyroid hormones stimulate endothelial cell growth (11-13) and enhance hypoxic pulmonary vasoconstriction (14), we hypothesized that pulmonary vascular remodeling during the development of severe PAH is thyroid hormone dependent. Here we show that induced hypothyroidism prevents and reverses vascular obliteration in the lungs of SuHx rats and mitigates hypoxic pulmonary vascular remodeling. Other important findings were that thyroidectomy and PTU treatment both reduced the high overexpression of the growth factor FGF2 and integrin αvβ3 proteins which characterizes the SuHx lungs and that prevention or treatment of PAH via modulating T4 leads to significantly less RV hypertrophy and dilatation.

Whereas the association of thyroid disorders with both idiopathic and non-idiopathic forms of pulmonary hypertension has been well recognized, there is presently no stated hypothesis which attempts to explain how thyroid hormones influence the pathogenesis of pulmonary vascular disorders. Several publications document a prevalence of hypothyroidism of about 30% in patients with PAH (28-30). Given the significant overlap between idiopathic PAH and autoimmune disorders, one hypothesis is that the cause of hypothyroidism in patients with idiopathic PAH is an autoimmune thyroiditis (31). There is also a strong association between hyperthyroidism and pulmonary hypertension (see supplemental Table 1). It is unclear whether pulmonary hypertension in hyperthyroid patients relates to left heart disease, to a hyperdynamic circulation or to a permissive effect of thyroid hormones on pulmonary vascular remodeling (11;12). The possible clinical translations of our findings are the following: 1) We found no evidence for a cause-effect relationship between hypothyroidism and PAH; 2) Thyroid hormone replacement in hypothyroid PAH patients should probably be done cautiously, to avoid enhanced vascular remodeling; 3) Hyperthyroidism may accelerate precapillary forms of pulmonary hypertension.
The mechanisms underlying pulmonary arterial muscularization and lumen occlusion in SuHx rats are undoubtedly complex and guided by multicellular interactions. Here we focused our attention on apoptosis and cell growth signals expressed by lumen-filling cells and in perivascular cellular clusters. In thyroidectomized SuHx rats only a few cells coating the luminal surface of the arterioles by IHC expressed PCNA and cleaved caspase 3 (Supplemental Figure 2) when compared to the lung sections from pulmonary hypertensive SuHx animals. These results were supported by analysis of whole lung tissue PCNA and cleaved caspase 3 protein expression (Figure 4). Taken together with the data derived from thyroidectomized SuHx rats which received T4 supplementation (Figure 2), our data indicate that indeed T4 drives lumen obliterating cell growth in the setting of severe PAH. The mitigation of hypoxic pulmonary vascular remodeling after thyroidectomy suggests that T4 is also permissive for pulmonary artery smooth muscle cell hypertrophy. Our studies were not designed to determine the exact nature of smooth muscle and endothelial cell interactions in the SuHx model of experimental PAH. The trend of a reduced PVRI in thyroidectomized hypoxic rats was not tracked by a reduction in RV hypertrophy, which was unexpected but perhaps related to an unpredictable effect of hypothyroidism on cardiomyocyte hypertrophy.

We had postulated that the angiogenesis factor FGF2 and signaling via Erk might be involved in the mechanism of thyroid hormone-related vascular remodeling (11) in our animal model. Examining random lung tissue samples, the expression of FGF2 and p-Erk was clearly thyroid hormone dependent. By IHC analysis we found that FGF2, FGFR1 and p-Erk were expressed in lung vascular lesion cells and that the expression of these proteins in the lesions was affected by thyroidectomy and T4 replacement treatment. In a similar fashion lung tissue expression of the integrin protein αv was decreased in thyroidectomized SuHx rat lungs and its expression was restored following T4 replacement (Figure 4C) while the expression changes for the β3
chain (Figure 4D) were less pronounced. Having observed the thyroid hormone-dependent pulmonary vascular remodeling changes in thyroidectomized rats, we assessed the effect of PTU treatment on established PAH and on lung vessel remodeling. We show that PTU treatment reduced the RVSP and PVR, thereby reducing RV hypertrophy, although the effects of PTU were substantially smaller than the preventative effects of thyroidectomy (Figure 5). PTU treatment reopened obliterated arterioles, the number of patent vessels in the lungs from PTU-treated SuHx lungs had increased and this greater degree of vessel patency correlated with the RVSP (Figure 6). Whereas PTU treatment clearly reduced the expression of the angiogenesis factor FGF2, in the SuHx lungs the effect of PTU treatment on αvβ3 expression was insignificant (Supplemental Figure 9) suggesting perhaps that in the SuHx lungs the FGF2 expression depends on the action of thyroid hormone. A potential pathogenetic role for FGF2 in PAH has been proposed by Izikki et al. and FGF2 expression in human lung vascular lesions has been shown (27). Here we show that in the setting of VEGF receptor blockade and chronic hypoxia FGF2 is highly expressed in the lung tissue samples and propose that FGF2 is angiogenic in the SuHx model of PAH. Measurement of plasma T4 concentrations revealed that both thyroidectomy and PTU treatment reduced the T4 levels, SU5416 combined with chronic hypoxia did not increase plasma T4 levels and that T4 replacement of thyroidectomized SuHx rats restored the T4 levels to normal or slightly more than normal. Thus in the SuHx animals, the plasma T4 levels correlated with the RVSP (Figure 1D).

The angiogenic actions of thyroid hormones in our SuHx rat model of angio-obliterative severe PAH may be explained via thyroid hormone cell membrane receptor signaling (32). Under conditions of chronic VEGF receptor blockade (caused by SU5416), FGF2 becomes over-expressed and integrin αvβ3 may function as a signaling modulator for both FGF2 (33) and the thyroid hormones (11). In cell experiments the growth promoting actions of FGF2 and of T4 have been inhibited by a MAP kinase inhibitor (PD -98059) (11). In particular, Bergh et al have
shown that integrin αvβ3 contains cell surface receptor binding sites for T4 which are linked to MAP-kinase activation (12), whereas a separate binding domain on the αvβ3 integrin links to PI3K and may contribute to cell growth signaling via activation of nuclear HIF-1α (32). Whether these cell signaling events of T4 which have been worked out in cultured endothelial cells (11;12;32) to explain the angiogenic actions of T4 are indeed applicable to the thyroid hormone dependent angioproliferation in our SuHx model is presently unknown and will have to be examined in the further experiments.

Study limitations

The descriptive nature of our studies precludes definite conclusions regarding the exact mechanism of thyroid hormone related angioproliferative remodeling. Because mouse models of pulmonary hypertension lack a consistent angioproliferative component (34), our research makes by necessity exclusively use of rat models, thereby limiting the possibility to use transgenic interventions. In contrast to the strong evidence provided by our study for a role of thyroid hormones in the initial development of angioproliferative remodeling, the role of thyroid hormones in disease maintenance is less clear. PTU partially reversed the increase in pulmonary artery pressures, but the effects was relatively small in comparison to the preventive effect of thyroidectomy. This study provides no explanation for the frequently reported association between hypothyroidism and pulmonary hypertension. Rather, the results suggest that this association is more likely the result of the link between both conditions and autoimmunity (31).

In conclusion, in this report we show for the first time that in experimental PAH, thyroid hormones are highly permissive to the development of severe angioobliterative PAH and contribute to the injury response of lung vessels when lung vascular endothelial cells undergo apoptosis (16). The thyroid hormone attributable angioproliferative component may not require
elevated plasma hormone levels but rather may depend on facilitated thyroid hormone cell membrane receptor (αvβ3 integrin) signaling (32).

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**STATEMENT OF INTEREST**: None.

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LEGENDS TO THE FIGURES

Figure 1: (a-h) Shows the effects of subtotal thyroidectomy and subtotal thyroidectomy with replacement of T4 in SuHx rats on plasma T4 level (a), right ventricular systolic pressure (RVSP; panel b), pulmonary vascular resistance index (PVRI; panel c), RV internal diameter (RVID; panel e), RV hypertrophy (right ventricular weight over left ventricular plus septal weight, or RV/LV+S; panel f), stroke index (panel g) and cardiac index (panel h). (d) Shows the relationship between plasma T4 levels with right ventricular systolic pressure (RVSP). Data are expressed as mean±SE (n=5-8). *P <0.05 versus control, #P <0.05 versus SuHx, †P <0.05 versus SuHx-THx.
Figure 2: (a-f) Representative photomicrograph of HE-stained lung sections of SuHx rat (a, b), SuHx rat with thyroidectomy (C, D) and SuHx rat with thyroidectomy plus T4 replacement (e, f). (a, c, e) are 2.5X magnification, scale bar 500µm and (b, d, f) are 10X magnification, scale bar
100µm. Percentage of small pulmonary arteries (< 80 um in diameter) classified as patent, partially obliterated and fully obliterated (g). Panel h shows the relationship between percentage of patent vessels and right ventricular systolic pressure (RVSP). **P < 0.05 versus % of patent vessels in SuHx rats’ lungs, †† P < 0.05 versus % of fully obliterated vessels in SuHx rats’ lungs, †P <0.05 versus % of partially obliterated vessels in SuHx rats’ lungs, *P < 0.05 versus % of patent vessels in SuHx with thyroidectomy rats’ lungs and #P < 0.05 versus % of partially obliterated vessels in SuHx with thyroidectomy rats’ lungs. Data expressed as mean ± SE (n = 3-5).

Figure 2: Subtotal thyroidectomy in rats exposed to chronic hypoxia led to a decreased right ventricular systolic pressure (RVSP; panel a) and a trend towards a lower pulmonary vascular resistance index (PVRI; panel b), whereas right ventricular weight (indexed for left ventricular plus septal weight, or RV/LV+S; panel c), stroke index (panel d) and cardiac index (panel e) remained the same. Media wall thickness (MWT) as percentage of external diameter (ED) of

Figure 3: Subtotal thyroidectomy in rats exposed to chronic hypoxia led to a decreased right ventricular systolic pressure (RVSP; panel a) and a trend towards a lower pulmonary vascular resistance index (PVRI; panel b), whereas right ventricular weight (indexed for left ventricular plus septal weight, or RV/LV+S; panel c), stroke index (panel d) and cardiac index (panel e) remained the same. Media wall thickness (MWT) as percentage of external diameter (ED) of
small and medium sized pulmonary vessels was reduced after thyroidectomy. Data are expressed as mean±SE (n=5). *P <0.05 versus Hx.

FIGURE 3

Figure 4: (a) Representative western blot analysis of Integrin αv, Integrin β3, FGF2, PCNA, Cleaved caspase-3, P-Erk, Erk, P-Akt, Akt, β-actin in lung protein extracts from control, SuHx, SuHx with thyroidectomy and SuHx with thyroidectomy and replaced with T4. (b-h) The bar
graphs show the ratios of FGF2, Integrin αv, Integrin β3, P-Akt/Akt ratio, PCNA, Cleaved caspase-3 and P-Erk/Erk ratio protein expression relative to controls. Data are expressed as mean ± SE (n=4). *P <0.05 versus control, #P <0.05 versus SuHx, †P <0.05 versus SuHx-THx.

FIGURE 4
**Figure 5:** Representative optical sections acquired by confocal microscopy of double immunofluorescence stainings for vWF/integrin αν, vWF/FGF2, VWF/FGFR1 and vWF/pErk1/2.  
(a-c): vWF/integrin αν double immunofluorescence staining shows increased integrin αν staining (green) in lumen occluding vWF⁺ (red) cells, media layer cells and surrounding cellular infiltrate and alveolar cells of SuHx animals (b), whereas less integrin αν staining is found in and around vessels of control (a) and SuHx/thyroidectomised (SuHxThx; c) animals.  
(d-f): vWF/FGF2 double immunofluorescence staining demonstrates elevated FGF2 staining (green) predominantly in lumen occluding vWF⁺ (red) cells, but also in media layer cells and surrounding cellular infiltrate and alveolar cells of SuHx animals (e), whereas FGF2 is found less in and around vessels of control (d) and SuHxThx (f) animals.  
(g-i): vWF/FGFR1 double immunofluorescence staining reveals extensive immunoreactivity for FGFR1 (green) in lumen occluding vWF⁺ (red) cells, media layer cells and surrounding cellular infiltrate and alveolar cells of SuHx animals (h), whereas less FGFR1 staining is found in and around vessels of control (g) and SuHxThx (i) animals.  
(j-l): vWF/pErk1/2 double immunofluorescence staining clearly demonstrates multiple lumen occluding vWF⁺ cells (red) exhibiting strong nuclear and cytoplasmatic pErk1/2 staining (green) in an angioproliferative lesion of SuHx animal (k), whereas less vWF⁺/pErk1/2⁺ cells are found in vessels of control (j) and SuHxThx (l) animals. Please note the highly increased pErk1/2 staining in media layer cells, infiltrating and alveolar cells of SuHx animals as compared to control and SuHxThx animals. Please note the difference in endothelial (vWF⁺, red) morphology between control animals (flat nucleus) and SuHxThx animals (round, activated nucleus) in images a-l. Original magnification: 630×, scale bar: 20 μm. Nuclear counterstaining with DAPI (blue).
Figure 6: (a-e) Show the effects of PTU treatment in SuHx rats on plasma T4 level (panel a), right ventricular systolic pressure (RVSP; panel b), pulmonary vascular resistance index (PVRI; panel c), RV hypertrophy (right ventricular weight over left ventricular plus septal weight, or RV/LV+S; panel d), cardiac index (CI; panel e) and stroke index (SI; panel f). Data are expressed as mean ± SE (n = 5-8). *P <0.05 versus control, #P <0.05 versus SuHx.
Figure 7: (a-d) Representative photomicrograph of HE-stained lung sections of SuHx rats (a, b) and SuHx rats treated with PTU (c, d). (a, c) are 2.5X magnification, scale bar 500µm and (b, d) are 10X magnification, scale bar 100µm. Relationship between percentage of patent vessels and right ventricular systolic pressure (RVSP) (e) and with RV hypertrophy (f). (g) Percentage of small pulmonary arteries (< 80 um in diameter) classified as patent, partially obliterated and fully obliterated. *P < 0.05 versus % of patent vessels in SuHx rats’ lung, #P < 0.05 versus fully obliterated vessels in SuHx rats’ lung. Data expressed as mean ± SE (n = 5-8).


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