Functional and molecular factors associated with TAPSE in hypoxic pulmonary hypertension

**Biomarkers in Lung Diseases: from Pathogenesis to Prediction to New Therapies**

Slaven Crnkovic,1,6* Albrecht Schmidt,2* Bakytbek Egemenazarov,1 Jochen Wilhelm,3 Leigh M. Marsh,1 Bahil Ghanim,1,4 Walter Klepetko,4 Andrea Olschewski,1,6 Horst Olschewski,5 and Grazyna Kwapiszewska1,6

1Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria; 2Division of Cardiology, Department of Internal Medicine, Medical University of Graz, Graz, Austria; 3Department of Internal Medicine, Justus-Liebig-University Giessen, Universities of Giessen and Marburg Lung Center, German Center for Lung Research, Giessen, Germany; 4Department of Thoracic Surgery, Medical University of Vienna/Vienna General Hospital, Vienna, Austria; 5Division of Pulmonology, Department of Internal Medicine, Medical University of Graz; Graz, Austria; and 6Department of Experimental Anaesthesiology, Medical University of Graz, Graz, Austria

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PULMONARY HYPERTENSION (PH) may be associated with cardio-pulmonary disease (48) or it manifests as idiopathic disease of the pulmonary arteries (24). Right ventricular (RV) failure contributes to symptoms and mortality; however, a reduction in pulmonary vascular resistance does not always translate into improved RV function (55).

Right heart catheterization is the gold standard for detecting and monitoring PH progression (14). Noninvasive techniques such as echocardiography are suitable for screening but they currently offer few parameters with prognostic value. Interestingly, measures such as the left ventricular (LV) eccentricity index (45) and improved LV filling profiles with targeted pulmonary arterial hypertension (PAH) therapy (13) were among the first prognostic markers identified for PAH. More recently, RV parameters such as RV diameter (17) and tricuspid annulus plane excision (TAPSE) (12) have been associated with PH prognosis. TAPSE has been discussed as a measure of RV function but it may not be a reliable measure of RV contractility (1, 27).

It takes tremendous technical efforts to obtain a comprehensive description of RV function in humans with PH (31). Small rodent models have been used to study the RV function and signaling pathways involved in the remodeling of the right ventricle exposed to acute or chronic pressure overload (54). In animals, right heart catheterization is a terminal procedure and provides no insight into geometrical changes, whereas echocardiography as a complementary approach allows longitudinal investigations in the same animal (11). In this study, we sought to better understand the TAPSE changes in relation to other functional and molecular parameters in a mouse model of hypoxia-induced PH.

**METHODS**

Hypoxia-induced pulmonary hypertension. CD1 male mice (Harlan Laboratories, Udine, Italy), 7–8 wk old, were maintained in ventilated chambers (OxyCycler system, BioSpherix, Lacona, NY) under nor-
moxia or 10% normobaric hypoxia for specified times. Food and water were provided ad libitum, and cages were opened for maintenance and cleaning once a week. The animal experiments were approved by the Austrian Federal Ministry of Science, Research and Economy (approvals BMWF-66.010/0128-II/3b/2011 and BMWF-66.010/0105-II/3b/2013). Every effort was made to minimize animal suffering. All experiments were blinded with respect to the experimental group.

**Echocardiography.** Transthoracic echocardiographic measurements were taken with a Vevo 770 High Resolution Imaging System with a 30-MHz RMV-707B scan head (VisualSonics, Toronto, ON, Canada) as previously reported (11). Briefly, mice were mounted on a heated pad, kept under superficial anesthesia with isoflurane (0.8–1.2%), and fixed in position with continuous monitoring of heart rate and temperature. Chest hair was depilated and a layer of sonographic coupling gel was applied to the thorax. RV and free wall thickness as well as LV dimensions from a left parasternal long-axis view were measured in M-mode and two-dimensional modalities. Fractional shortening as a surrogate parameter of LV function and LV mass were calculated as described (11). The left parasternal short-axis view at the level of the papillary muscles was used to determine the LV eccentricity index (D1, D2, and D1/D2) and the LV area, RV area, and LV/RV area. Measurements of the RV outflow tract were obtained from the parasternal short-axis view at the level of the aortic valve during end diastole. From the same view, pulse wave Doppler flow recordings of the pulmonary artery were obtained with the sample volume positioned at the tip of the pulmonary valve leaflets and peak velocity, acceleration time (i.e., pulmonary artery acceleration time, PAAT), and RV ejection time were measured. From a modified apical four-chamber view, the inflow part of the RV was quantified as the maximal short-axis dimension in the basal one-third of the RV (RVEDD). Also using the four-chamber view, a sensor in M-mode was inserted through the lateral part of the tricuspid annulus with as good alignment as possible with longitudinal motion of the annulus to assess TAPSE. Finally, we used pulsed tissue Doppler sonography of the tricuspid annulus to assess RV diastolic function (RV-E′). All measurements were performed blinded with respect to the experimental group (hypoxic vs. normoxic) and averaged over five cardiac cycles. Representative video clips of the echocardiographic measurements are provided (see Supplemental material).

**Hemodynamic measurements.** RV systolic pressure (RVSP), LV systolic pressure (LVSP), and blood pressure were measured using a high-fidelity 1.4-F pressure catheter (SPR-671; Millar Instruments, Houston, TX) with a closed chest technique under isoflurane anesthesia as reported previously (9). Briefly, the catheter was inserted into the right jugular vein and directed to the right ventricle to measure RVSP. Systemic blood pressure measurements were obtained via the tricuspid annulus to assess RV diastolic function (RV-E′). All hemodynamic pressures were recorded and analyzed with Labchart 7 software (ADInstruments, Tarzana, CA). Representative video clips of the hemodynamic measurements are provided (see Supplemental material).

**Tissue and blood collection.** Animals were euthanized by cervical dislocation at the end of experiment for gene expression and protein analysis; the chest was opened and heart and lungs were flushed with ice-cold PBS. The lungs and heart were excised, atria were removed, and the right ventricle was separated from the left ventricle and septum. Tissue specimens were weighed, then snap-frozen in liquid nitrogen and stored at −80°C until analysis. For immunohistochemical (IHC) analysis, the heart and lungs were removed en block, fixed in 5% formalin, and embedded in paraffin. The tissue was collected into EDTA-coated tubes and centrifuged by pipetting 10 min at 2,000 g; the supernatant was aliquoted and stored at −80°C.

**Morphological analysis.** To assess fibrosis, formalin-fixed, paraffin-embedded heart tissue was cut into 2-μm-thick sections and stained for collagen content using Masson’s trichrome stain according to standard protocol. The slides were scanned and the level of fibrosis was determined with semiautomated image analysis (Visiopharm DP software; Visiopharm, Horsholm, Denmark) as described previously (11). Immunofluorescent (IF) staining against laminin (Abcam, Cambridge, UK) or IHC staining against endothelial cell marker thrombomodulin (R&D Systems, Minneapolis, MN) and interleukin-22 receptor alpha 2 (IL22RA2) (Abcam) was performed on deparaffinized heart sections after heat-induced antigen retrieval (pH 6). IHC sections were blocked with 3% H2O2 in methanol to quench endogenous peroxidases. Nonspecific binding was blocked using 5% donkey serum, and primary antibodies were diluted 100-fold in 0.1% BSA and incubated overnight at 4°C. After washing, IHC sections were incubated with ImmPress detection reagent (Vector Laboratories, Burlingame, CA) and signals were developed using diaminobenzidine chromogenic substrate (Vector Laboratories), followed by hematoxylin counterstaining. IF sections were incubated with fluorescently labeled secondary antibody Alexa Fluor 594-conjugated goat anti-rabbit antibody (Invitrogen, Eugene, OR). Mounting was performed with DAPI containing mounting medium (Vector Laboratories). Sections were scanned and RV capillary density was calculated as a fraction of thrombomodulin-stained area to total RV tissue area using semiautomated image analysis (Visiophorph). Cardiomyocyte diameter determination was performed using ImageJ software (National Institutes of Health, Bethesda, MD).

**Gene expression analysis.** RNA was isolated from snap-frozen tissue and reverse transcribed with the iScript kit (BioRad, Hercules, CA). Real-time quantitative PCR was performed with the QuantiFast SYBR PCR kit (Qiagen, Hilden, Germany) using a LightCycler 480 (Roche, Mannheim, Germany). The primer sequences are listed in Table 1. Mouse β2-microglobulin and porphobilinogen deaminase (PBGD) were used as reference genes. Expression levels were calculated by the ∆∆Ct method (∆∆Ct = Ct[mean Ct of reference genes] – Ct[target]; ∆∆Ct = ∆Ct[mean Ct] – mean ∆Ct(normoxia)) (34).

**Genome-wide expression profiling and microarray data analysis.** Purified total RNA (n = 4 per group, 200 ng per sample) was amplified and Cy5-labeled using the LIRAK kit (Agilent Technologies, Waldbronn, Germany) following the kit instructions. Cy5-labeled amplified RNA was hybridized to 60mer oligonucleotide-spotted microarray slides (Mouse Whole Genome, design ID 028005; SurePrint G3 Mouse GE 8 × 60K Microarray, Agilent Technologies). Images were analyzed with Mapix 6.5.0 software, and calculated values for all spots were saved as GenePix files. Stored data were evaluated with R software (43) and the limma package (50) from BioConductor (16). Log2 mean spot signals were taken for further analysis. Data were quantile normalized (52) before averaging. Genes were ranked for differential expression using a moderated t-statistic (51). Candidate lists were created by adjusting the false discovery rate to 5% and selecting genes with at least twofold differential expression. For pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) the false discovery rate was adjusted to 10%.

**Proliferation assay.** Primary human cardiac fibroblasts (PromoCell, Heidelberg, Germany) were seeded in 96-well plates, and growth was arrested by overnight incubation in serum-free medium (Thermo Fischer/Invitrogen, Carlsbad, CA). Proliferation upon 30 ng/ml IL-22 stimulation (PeproTech, Vienna, Austria) was determined by 3H-thymidine (Biotrend Chemikalien, Cologne, Germany) incorporation as an index of DNA synthesis and measured as radioactivity by a scintillation counter (Perkin Elmer/Wallac, Vienna, Austria).
a significant elevation in augmentation pressure at 5 wk (Fig. 1, RVEDP (Fig. 2) and left ventricular end-diastolic pressure (LVEDP, Fig. 2), but not with the contractility index (Fig. 2). Interestingly, aMhc correlated with RVSP (Fig. 2), consistent with RV hypertrophy attributable to increased afterload (Fig. 1, A–F). TAPSE was significantly decreased from 1.37 to 0.84 mm at 3 and 5 wk of hypoxia (Fig. 1, G), and donor cohorts used in this study were reported previously (25). Patient characteristics are shown in Table 2.

**RESULTS**

Chronic hypoxia-induced pulmonary hypertension is characterized by adaptive RV remodeling. Mice exposed to chronic hypoxia developed pulmonary hypertension. Significantly increased RVSP, PAAT, RV-to-LV weight ratio, RV-to-septum weight ratio (Fulton index), and RV free wall thickness were consistent with RV hypertrophy attributable to increased afterload (Fig. 1, A–F). TAPSE was significantly decreased from 1.37 to 0.84 mm at 3 and 5 wk of hypoxia (Fig. 1B and Table 3). In addition, vessel stiffness was assessed by calculating augmentation pressure (Fig. 1G). Chronic hypoxia resulted in a significant elevation in augmentation pressure at 5 wk (Fig. 1, G and H). The significant decrease in TAPSE and RV-E' was not associated with RV functional impairment (Fig. 1B, Table 3, and Supplementary online video clips) because hemodynamic measurements revealed increased max dP/dt, unchanged contractility index, and RV end-diastolic pressure (RVEDP, Fig. 1F and Table 4). In fact, TAPSE was strongly negatively correlated with RVSP (Fig. 2A) and RV max dP/dt (Fig. 2B), but not with the contractility index (Fig. 2C). Interestingly, TAPSE was moderately negatively correlated with both RVEDP (Fig. 2D) and left ventricular end-diastolic pressure (LVEDP, Fig. 2E), suggesting a positive relationship with diastolic function. Furthermore, normalized PAAT (Fig. 2F) and RV free wall thickness (Fig. 2G) were strongly correlated with hemodynamic changes.

Echocardiography revealed compression of the left ventricle due to the enlarged right ventricle as reflected by an elevated D1/D2 ratio and reduced LV area (Table 3 and Supplementary online video clips). Invasive hemodynamics demonstrated an unchanged LV contractility index. LVEDP was elevated at 3 wk and normalized at 5 wk under hypoxia (Table 4).

Morphological examination was consistent with adaptive RV hypertrophy (Fig. 3, A and B) without increased collagen deposition (Fig. 3, C and D) and preserved RV capillary density (Fig. 3, E and F) as assessed by laminin, Masson’s trichrome, and thrombomodulin staining, respectively.

**Molecular profile of chronic hypoxia-driven RV remodeling.** RV hypertrophy upon chronic hypoxia was associated with unaltered expression levels of the cardiac wall stress markers atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (Fig. 4, A and B). Increased expression of ANP and BNP was observed after only 3 days under hypoxia (Fig. 4, A and B). After 5 wk of hypoxia we observed downregulation of sarcoplasmic reticulum Ca$^{2+}$-ATPase (Serc2a2, Fig. 4C), but no fetal gene program reactivation as was suggested by normal expression ratios of adult (aMHC) to fetal (bMHC) isoforms of the cardiac contractile protein myosin heavy chain (MHC) (Fig. 4, D and E). Furthermore, there was upregulation of endothelin-1 (ET-1) and apelin (Apln) in the right ventricle (Fig. 4, F and G). Similarly, the tissue remodeling marker tenascin C (Tnc) was upregulated in the right ventricle (Fig. 4H), whereas expression of the profibrotic marker connective tissue growth factor (CTGF) was unchanged (Fig. 4I).

**Table 2. Clinical characteristics of donors and patients with idiopathic pulmonary arterial hypertension**

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<tr>
<th>Donors, n = 30</th>
<th>IPAH, n = 30</th>
</tr>
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<td>Age (yr)</td>
<td>50 ± 6</td>
</tr>
<tr>
<td>Sex, female/male</td>
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<tr>
<td>Mean pulmonary artery pressure, mmHg</td>
<td>53 ± 8</td>
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<tr>
<td>Pulmonary vascular resistance, dyn·s·cm$^{-5}$</td>
<td>834 ± 277</td>
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<tr>
<td>Cardiac index, l·min$^{-1}$·m$^{-2}$</td>
<td>2.41 ± 0.38</td>
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</tbody>
</table>

IPAH, idiopathic pulmonary arterial hypertension. Data are presented as means ± 95% confidence interval, except for sex.

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**Table 1. Primers used and corresponding sequences**

<table>
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<th>Accession Number</th>
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<th>Reverse (5′→3′)</th>
<th>Size, bp</th>
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<td>CACCGGAAGCGGCGTTCACAG</td>
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<tr>
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<td>TGGTTCGCACTGCTGTACATT</td>
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<tr>
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<td>TTGCCTGGATGCTGTCATCAACA</td>
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<td>B2 mg</td>
<td>NM_010217</td>
<td>ATCCAGCTGGCTGCTGTCATCAACA</td>
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<tr>
<td>CTgf</td>
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<td>ET-1</td>
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<td>Phn</td>
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<td>RnY</td>
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<td>VegfA</td>
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tissue growth factor (Ctgf) was downregulated at 3 wk of hypoxia (Fig. 4I). Gene expression levels of vascular endothelial growth factor A (VegfA) were unaltered in the right ventricle at both 3 and 5 wk of hypoxia (Fig. 4I).

Identification of ventricle-specific gene expression changes. To obtain a comprehensive understanding of gene expression changes associated with this type of RV remodeling, we performed microarray analysis of the right ventricle and left ventricle samples after 5 wk of normoxia or hypoxia. Gene ontology analysis revealed that angiogenesis is the most significantly differentially regulated biological process in RV hypoxia vs. normoxia samples (Fig. 5A). KEGG pathway subcategory analysis showed signaling molecules and interaction as the most prominently regulated pathway in the right ventricle upon chronic hypoxia (Fig. 5B). Using a 5% false discovery rate, we observed a similar number of significantly up- and downregulated genes in both right ventricle (215 vs. 159, respectively) and left ventricle (196 vs. 164) from hypoxia.
ia-exposed mice (Fig. 5, C and D). A large number of genes were regulated in common in both ventricles (267 upregulated and 229 downregulated). Transcription factor c-Fos and several members of the serine protease inhibitor family known as serpins (6 out of the top 10 upregulated genes) showed the highest levels of upregulation among annotated genes in both ventricles upon hypoxia (Table 5). Similarly, most downregulated genes were common to both ventricles and included the developmental regulator molecule notch 1, the neuronal anti- graft-vs.-host disease, and viral myocarditis. The top 30 differentially regulated genes between ventricles upon hypoxia according to log fold change, albeit with weak association reflected by high *P* values, are presented in Table 6. Among those, two soluble factors, IL22RA2 and FGF5 (Table 6), have already been investigated in the cardiovascular system (15, 39). IHC staining revealed that IL22RA2 protein expression in mouse RV was localized primarily to cardiomyocytes, both in the normoxic and hypoxic condition (Fig. 5H).

**Patients with IPAH have higher plasma levels of IL22RA2.**

In an attempt to test the possible relevance of the observations from the murine model, we determined the levels of IL22RA2 and FGF5 in plasma samples from patients with IPAH and matched controls (Table 2). There was no difference in FGF5 plasma levels between IPAH and controls (Fig. 6A). In contrast, circulating levels of IL22RA2 were significantly elevated in tissues from IPAH compared with controls (Fig. 6B). The accuracy of plasma IL22RA2 measurements for discriminating IPAH and control samples was assessed with ROC curve analysis (Fig. 6C), giving an area under the curve value of 0.818. Because IL22RA2 acts as a neutralizing factor for IL-22, a proinflammatory cytokine with involvement in fibrosis (7, 35), we investigated IL-22 trophic effects on human cardiac fibroblasts (hCF). IL-22 caused a mild but significant increase in human cardiac fibroblast proliferation (Fig. 6D).

**DISCUSSION**

This study used an animal model that permitted a comprehensive description of changes in echocardiographic correlates, hemodynamics, morphology, and molecular markers of hypoxia-induced pulmonary hypertension. Echocardiography has become established as a suitable mechanism for monitoring prognostically relevant changes associated with PH (12, 13, 23,
Table 4. Invasive assessment of right and left ventricular function in mice exposed to chronic hypoxia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normoxic Control</th>
<th>Hypoxic 3 wk</th>
<th>Hypoxic 5 wk</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>472</td>
<td>478</td>
<td>32</td>
<td>0.732</td>
</tr>
<tr>
<td>MaxSP, mmHg</td>
<td>23.91</td>
<td>15.11</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Pressure-time index, mmHg/s</td>
<td>1.11</td>
<td>0.619</td>
<td>0.001</td>
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<tr>
<td>Max dP/dt, mmHg/s</td>
<td>1,647</td>
<td>1,496</td>
<td>0.001</td>
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<tr>
<td>Contractility index,* 1/s</td>
<td>75.46</td>
<td>70.22</td>
<td>0.640</td>
<td></td>
</tr>
<tr>
<td>Tau, ms</td>
<td>15.46</td>
<td>14.96</td>
<td>0.691</td>
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</table>

Mice were exposed to normobaric hypoxia (3 or 5 wk) or normoxia. Ventricular function was invasively measured by Millar catheter in a blinded fashion. Data are presented as means ± SD. MaxSP, maximum systolic pressure; EDP, enddiastolic pressure; max dP/dt, velocity of pressure increase during contraction; max dP/dt/pmax, maximum systolic pressure increase during contraction normalized to maximal systolic pressure. Invasive assessment of the right ventricle in the hypoxic mouse. We cannot exclude the possibility that ventricular arterial coupling and pericardial restraint, evidenced by decreased LV/RV area ratio, could provide an alternative explanation for increased LVEDP after 3 wk of hypoxia (36).

In patients with PH, a few echocardiographic measurements have been shown to be predictive for survival, among which LV eccentricity index and TAPSE play an important role (12, 45). To investigate the relationship of echocardiographic measurements and RV functional and molecular changes upon pressure overload, we used a mouse model of chronic hypoxia exposure. In this model, TAPSE was significantly reduced in hypoxia, whereas our invasive measurements demonstrated significantly increased RV max dP/dt, unchanged RV contractility index, and signs of only mild biventricular diastolic dysfunction. The greater augmentation pressure in the RV pressure waveform indicates an excess of RV work and might be due to a wave reflection in the pulmonary artery, which in turn is due to a reduced pulmonary vascular compliance and increased vascular stiffening, as has been already suggested in a previous study (28).

There was a strong inverse correlation between invasively measured RVSP and noninvasively measured PAA, which is in agreement with results reported in a previous study (54). Interestingly, TAPSE was strongly negatively correlated with RV afterload as well as RV contractility assessed invasively by max dP/dt. One possible explanation for this finding is the linear increase in max dP/dt with increasing pulmonary pressure as shown in a human study (19). This pressure dependency makes max dP/dt an unreliable marker of RV contractile function and even patients with PH and RV failure can have increased max dP/dt (53). However, normalized max dP/dt (contractility index = max dP/dt/pmax) is less afterload dependent. Significant RV hypertrophy, unchanged contractility index, lack of RV fibrosis, unchanged natriuretic peptide expression, and MHC expression profile argue against impaired RV contractile function. This suggests that in our experimental setup, hypoxia caused PH with excellent RV adaptation and no overt failure.

Independently, Kolb et al. (29) have shown preservation of stroke volume and cardiac output for 3 wk and conclude that RV contractility is preserved in this model. As shown by Pettersen et al. (42), the right ventricle exposed to near systemic circulation pressure levels shifts its contraction pattern from longitudinal to circumferential shortening, reflecting an adaptive response rather than systolic RV dysfunction. This would imply that the longitudinal wall motion of the right ventricle, as assessed by TAPSE, might not be as sensitive to contractility as to increased afterload. In another study, TAPSE was only loosely correlated with RV pump function (27), and noninvasive indices of right ventricular function, including TAPSE, have recently been associated with ventricular-arterial coupling rather than ventricular contractility (21). This corresponds to our finding that the RV contractility index (max dP/dt/pmax) was not significantly correlated with TAPSE, although there was a moderate but significant negative correlation with LVEDP and RV EDP. This suggests that TAPSE might indicate diastolic rather than systolic function of the right ventricle in the hypoxic mouse. We cannot exclude the possibility that ventricular arterial coupling and pericardial restraint, evidenced by decreased LV/RV area ratio, could provide an alternative explanation for increased LVEDP after 3 wk of hypoxia (36).

Long-term observations suggest that RV hypertrophy precedes failure (2), although the underlying molecular mechanisms have not yet been identified (10). In contrast to studies...
that have examined end stage LV hypertrophy (47) and rats exposed to chronic hypoxia (10), we did not observe reactivation of the fetal gene program (βMHC and αMHC gene expression data) Furthermore, we observed concomitant activation of both adaptive and maladaptive gene expression responses without morphological signs of RV fibrosis. For instance, upregulation of the myocardial ET-1 could represent a compensatory mechanism to preserve RV contractility (38), which could work in concert with increased levels of apelin, known to increase cardiac contractility without causing hypertrophy (3). The downregulation of Serc2a2 could explain mild RV diastolic dysfunction (32). Finally, increased circulating tenasin C levels mirrored RV hypertrophy (data not shown), potentially serving as an early and sensitive marker of RV remodeling in response to increased afterload (46).

Previous studies of global gene expression changes in the rat chronic hypoxia model have identified angiogenesis, cell growth, and glycolysis as key pathways that differentiate RV hypertrophy from RV failure (5, 10). Although the gene expression of VegfA, a major proangiogenic factor (8, 29), was not changed in our study, we identified angiogenesis as the most regulated pathway in the right ventricle exposed to chronic hypoxia compared with normoxia. This could imply
Fig. 3. RV morphological changes in mice exposed to chronic hypoxia. Mice were exposed to normobaric hypoxia (3 or 5 wk) or normoxia. A: representative immunofluorescent images of laminin staining. B: hypoxia-induced change in cross-sectional myocyte diameter in the right ventricle. Scale bar = 20 μm. C: representative photomicrographs of Masson’s trichrome stain. Smaller inserts show high-power magnification. Scale bar = 1 mm. D: quantification of fibrosis in the right ventricle. E: RV representative images of thrombomodulin immunohistochemical staining of capillary endothelial layer (brown). Scale bar = 50 μm. F: quantification of capillary density in the right ventricle fraction of thrombomodulin-stained area to total RV area. Data (n = 4–8 per group) are presented as a box-and-whisker plot depicting median with interquartile range (box) ± min/max (whiskers). §P < 0.05.
Fig. 4. Chronic hypoxia-induced gene expression changes. Hypoxia-induced gene expression changes in the right ventricle and left ventricle with septum (LV+S) for atrial natriuretic peptide (ANP, A), brain natriuretic peptide (BNP, B), sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (Serc2a, C), α-myosin heavy chain (αMHC, D), and β-myosin heavy chain (βMHC, E), endothelin-1 (ET-1, F), apelin (Apln, G), tenascin C (TnC, H), connective tissue growth factor (Ctgf, I), and vascular endothelial growth factor A (VegfA, J). Data (n = 6–8 per group) are presented as box-and-whisker plots depicting median with interquartile range (box) ± min/max (whiskers). Statistically significant changes are marked as exact P values.
Fig. 5. Gene expression profiles of right and left ventricles after 5 wk of normoxia or hypoxia. A: top 5 out of 182 significantly differentially regulated biological processes, including the corresponding number of regulated genes in each pathway, between hypoxic and normoxic RV, based on \( P < 0.05 \). B: top 5 out of 15 significantly differentially regulated KEGG pathway subcategories, including the corresponding number of regulated genes in each pathway, between hypoxic and normoxic RV, based on \( P < 0.05 \). Venn diagrams of upregulated (C) and downregulated (D) genes in ventricles from mice exposed to 5 wk of normoxia or hypoxia. Diagrams are based on the top 1,000 regulated genes (up and down) with the lowest \( P \) values in each contrast, whereas numbers in diagrams correspond to the number of genes that are regulated either in only one comparison or contained within the top 1,000 genes in two, three, or all four comparisons. E: correlation of log2-fold changes between ventricles in hypoxia. F: volcano plots for comparison of hypoxia vs. normoxia in right ventricle (left) and left ventricle (right). G: volcano plot for comparison of genes based on interaction between ventricles upon hypoxia. H: representative images of interleukin-22 receptor alpha-2 (IL22RA2) immunohistochemical staining (brown) in right ventricles from normoxia and 5 wk hypoxia-exposed mice. Smaller insert shows negative control stain. Black bar = 50 \( \mu \)m.
that capillary density is maintained by other angiogenic factors. Furthermore, we observed an increase in the cell growth regulating factors c-Fos and Egr-1. Expression of these factors is mediated by Erk MAP kinase and Elk-1 transcription factor (37, 56), pointing to activation of an HIF-1-independent hypoxia-responsive pathway. Finally, in line with previous studies (5, 10), pathway analysis has identified fatty acid metabolism and absence of the glycolytic pathway among 182 significantly regulated pathways in the right ventricle exposed to chronic hypoxia (data not shown).

Going beyond known factors associated with maladaptive RV remodeling, we aimed to clarify molecular changes associated with compensated RV remodeling and the potential differences compared with LV adaptation. We applied microarray analysis of both ventricles because hypoxia acts as a confounding factor in this model and could mask the changes caused by RV afterload. Surprisingly, we saw no pronounced differences in single gene expression between the two ventricles. Subsequent pathway analysis revealed 13 differentially regulated pathways generally associated with intracellular signaling and immune processes. Among those, the hedgehog pathway is crucial for early heart development (20) and maintenance of adult coronary vasculature (33). Interestingly, within the group of genes showing the highest log fold change for interaction, we identified a soluble product FGF5. FGF5 acts as a proangiogenic factor, and in vivo gene transfer of FGF5 improves myocardial blood flow and function (18). Because decreased capillary density is one of key features of decompensated right ventricle (6), local upregulation of proangiogenic FGF5 in the right ventricle might preserve the capill-
Mice were exposed to normobaric hypoxia (5 wk) or normoxia. Genome-wide expression profiling was performed in right and left ventricles and compared for interaction under hypoxia. *Log2 fold change (differential expression in the respective contrast), means ± 95% confidence interval. †For differential expression, Benjamini-Hochberg adjusted (controlling the false discovery rate).

Fig. 6. Plasma levels of IL22RA2 and fibroblast growth factor 5 (FGF5) in samples from a patient with idiopathic pulmonary arterial hypertension (IPAH) and matched controls. FGF5 (A) and IL22RA2 (B) were determined in 30 IPAH and 30 matched control plasma samples (individual values and median depicted as a horizontal line). C: receiver operating characteristic (ROC) curve analysis for IL22RA2 measurements. D: IL-22 induced proliferation of human cardiac fibroblasts (two different donors) measured as 3H-thymidine incorporation. Data are represented as means ± SD of three independent experiments. §P < 0.05.
form of IL22RA2 might represent a compensatory protective mechanism. IL22RA2 is a decoy receptor for the proinflammatory cytokine IL-22 (30). Serum concentrations of IL-22 are increased in patients with chronic heart failure (15). The higher IL22RA2 levels measured in our patients with IPAH by neutralizing proinflammatory IL-22 could dampen the inflammatory response and potentially tip the balance toward compensated RV remodeling. Additionally, IL22RA2 could neutralize IL-22 profibrotic activity (7, 35). Accordingly, Overbeek et al. (40) did not observe a significant increase in either inflammatory cell count or fibrosis in the right ventricle from patients with IPAH compared with normal controls, although a different cohort of patients had increased fibrosis scores (44).

**Study limitations.** Because our investigations focused on the fully developed stage of hypoxia-induced PH, we cannot exclude that other genes may play a role in the RV adaptation process at an early stage. Furthermore, the limited number of samples used in the microarray study might not be sensitive enough to detect subtle changes between ventricles or changes potentially masked by sample intervariability. This could explain the differences observed between ventricles in expression of ET-1, TnC, and apelin using quantitative PCR but not with microarray analysis. Furthermore, because of the limited amount of tissue we used for different molecular and morphological analysis, capillary density measurements could not be performed using the gold standard stereology approach, which likely explains the differences between our results and those in another recently published report (29). Finally, because of the lack of available IPAH heart tissue samples at our research centers, most of the results are based on myocardial tissue from the murine chronic hypoxia model.

In conclusion, chronic hypoxia exposure in mice leads to pulmonary hypertension and RV hypertrophy with decreased TAPSE and mild diastolic dysfunction but no systolic dysfunction, normal natriuretic peptides, and no increase in fibrosis. Genome-wide expression profiling shows a similar gene expression pattern in both ventricles upon chronic hypoxia and identified differentially regulated IL22RA2 and higher plasma levels of the latter in patients with IPAH compared with controls.

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**AUTHOR CONTRIBUTIONS**


**REFERENCES**


