Hypoxia induces different genes in the lungs of rats compared with mice

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Methods

Hypobaric Hypoxic Exposure

For exposure to hypobaric hypoxia, 8-wk-old male C57BL/6 mice (n = 3) and 7-wk-old male SD rats (n = 3) were housed in a hypobaric chamber for 1 and 3 wk at a simulated altitude of 17,000 ft. (Pb = 410 mmHg; inspired PO2 = 76 mmHg). Three mice and three rats were kept at DA (5,280 ft.; Pb = 630 mmHg, inspired PO2 = 122 mmHg) for more than 3 wk. Evaluation at 1 wk of hypoxia was included to determine whether gene expression was altered at an earlier time point and to investigate the resultant changes in pulmonary artery pressure.

Measurement of Right Ventricular Pressure of Mice and Pulmonary Artery Pressure of Rats

Hemodynamic assessment was performed as previously described by direct measurement of right ventricular systolic pressure (RVSP) for mice (12, 13) and by measurement of mean pulmonary artery pressure (mPAP) utilizing a catheter inserted through the jugular vein for rats (23, 37). After RVSP or mPAP measurement, lung tissue was inflated with low-melt agarose and fixed with 10% buffered formalin.

Details for mice. Briefly, the mice were anesthetized with ketamine and xylazine (100 mg/kg and 15 mg/kg, respectively) by way of a hindquarter intramuscular injection. After adequate sedation, the animals were placed in a supine position, breathing room air. The pressure transducer was calibrated to a zero point at the mid-anteroposterior diameter of the chest. A 26-gauge needle was introduced percutaneously into the thorax through a subxyphoid approach. Right ventricular pressure was measured using a pressure transducer (Gulton-Statham, Costa Mesa, CA) and recorded on a multichannel recorder (Grass Instruments, Quincy, MA). Approximately 10 measurements per animal were made, and the average pressure value was recorded.

Details for rats. The rats were anesthetized with ketamine and xylazine (60 mg/kg and 8 mg/kg, respectively) intramuscularly. The pulmonary arterial pressure was measured after a polyethylene catheter (PE-10; Intramedic, Clay Adams, Parsippany, NJ) had been inserted into the main pulmonary artery through the right jugular vein. The shape of the pressure tracing displayed on an oscilloscope guided the placement of the catheter. The pressure was measured using a pressure transducer (Gulton-Statham); the zero reference point was the level of the midchest. Approximately 10 measurements per animal were made, and the average pressure value was recorded.

Histology and Morphometric Analysis

A representative hematoxylin and eosin-stained lung section through the hilum was coded and blindly evaluated for remodeling of pulmonary arteries in the range of 30–50 μm for mice and 10–150 μm for rats as previously described (13, 23, 39). Pulmonary arteries were differentiated from pulmonary veins by the identification of the double elastic lamina in the vascular walls of the arteries using phase contrast. The wall thickness of each artery (VWT) was expressed as percentage of the vessel diameter by the formula VWT = (Ed – Id/Ed × 100, where Ed is external diameter and Id is internal diameter. Between 10 and 20 measurements were made per animal (3 animals for each condition), and the average was calculated.

Gene Microarray Analysis

Three C57BL/6 mice and three SD rats were exposed to hypobaric hypoxia for 1 and 3 wk. Three mice and three rats were kept at DA. The animals were euthanized by lethal injection of sodium pentobarbital (Abbott Laboratories, North Chicago, IL). Lungs were excised, frozen in liquid nitrogen, and stored at −70°C. Total RNA was extracted from the lung tissues by using the RNeasy Total RNA Isolation Kit (Qiagen, Valencia, CA). Fifteen micrograms of total RNA was collected from each sample and evaluated individually. Twenty micrograms of the labeled cRNA mixture was applied to the GeneChip microarray analysis (Affymetrix, Santa Clara, CA) as described previously (14, 15, 34). Rat samples were hybridized to the Affymetrix Rat Genome U34A arrays, whereas murine samples were hybridized to the Murine Genome Array U74Av2 for these studies, unless otherwise stated. Detailed protocols for data analysis of Affymetrix microarrays and extensive documentation of the sensitivity and quantitative aspects of the method have been described (16, 28, 31).

Fig. 1. Mean pulmonary artery pressure (mPAP) of rats (A) and right ventricular systolic pressure (RVSP) of C57BL/6 mice (B) at Denver altitude (DA) and after exposure to hypobaric hypoxia at both 1 and 3 wk. A: after 1 wk of hypoxia, PAP values rose from 18.3 ± 0.3 to 24.8 ± 2.8 mmHg (P< 0.01). At 3 wk of hypoxia, pressures rose even higher compared with control (n = 3 vs. control, n = 3; 33.3 ± 1.3 vs. 18.3 ± 0.3 mmHg; *P < 0.001). B: C57BL/6 mice displayed higher RVSP after exposure to 3 wk of hypobaric hypoxia compared with control animals kept at DA (28.0 ± 1.0 at DA vs. 35.6 ± 1.5 mmHg at 3 wk; **P = 0.3 × 10−4). Although there was a trend toward higher RVSP after the 1 wk exposure, this did not reach statistical significance (control, n = 3; 28.0 ± 1.0 at DA vs. 30.7 ± 0.5 mmHg at 1 wk; P = NS).
Quantitative RT-PCR Analysis for the Rat Osteopontin Gene

To corroborate findings by microarray analysis, quantitative RT-PCR was performed on the gene encoding for osteopontin as described previously (15). The primers used were forward 5'-CAAGGCCTCTGGGAAGCA-3' and reverse 5'-CGATTCCGCAGCTCAGTTC-3' (63-bp band product). For the internal control, we quantitated ribosomal RNA content in each template using TaqMan ribosomal RNA control reagents (Perkin-Elmer) and normalized the copy number of each gene.

Statistical Analysis

Analysis of gene expression was conducted by previously reported Affymetrix software. All values were expressed as means ± SE. Means of several groups were compared by three methods including unpaired t-test, analysis of variance (ANOVA), and a matrix analysis of all possible combinations of baseline (DA) to each of the hypoxic conditions (1 wk and 3 wk). In this matrix analysis, a very stringent requirement was that all nine of nine possible comparisons (a 3 x 3 table created for each condition) must show concordance in the "increase" or "decrease" call generated by the Affymetrix Microarray Suite 5.0 (MAS 5.0) algorithm.

Prior to analysis by any of the statistical tests, variability filters were applied to the dataset to minimize multiple testing errors. The first filter uses the Affymetrix mRNA detection "call" (MAS version 5.0) to exclude all genes with an "absolute call" of "absent" in all experiments. The second filter identifies genes with at least moderate variance over the entire experiment; genes that do not vary at all cannot possibly be related to the system under study, which in this case is the hypoxia exposure. Since we can assume that most genes are not related to the system under study (hypoxia exposure), then the gene with the median variance is a reasonable model of null variation, that is, the variation due to other factors. We calculate the variances for each gene. The null hypothesis is that these variances represent random noise. We can then compute the statistic W = (N - 1)s²/median(s²), where N is the number of observations of the gene, which is approximately chi-square distributed with N - 1 degree of freedom (22), and s² is the variance of each gene, and median(s²) is the median over all the gene variances. We calculate a P value for rejecting the null hypothesis that the gene did not vary, and then perform the "false discovery rate" multiple testing correction (5), setting the false discovery rate to be 10%. This results in a list of genes with significantly greater variation than the median variation gene, with at most 10% of that list including genes having true variation less than or equal to median variation. These preprocessing steps screen out genes with low variance and low levels of mRNA expression. The remainder of the genes is then fed to the Kruskal-Wallis or ANOVA test and FDR correction (10% in this case), producing a final list of genes whose expression levels are significantly different between at least two time points.
Two-tailed parametric analysis was considered significant when \( P < 0.01 \). Analysis by the three separate methods produced multiple genes that were altered in the setting of hypoxia. A subset of genes that was significantly altered by two of the three statistical methods is reported. In all cases, every attempt was made to compare the same genes that were statistically different between the two species. This was accomplished by utilizing the NetAFFX website (http://www.affymetrix.com/analysis/index.affx) to cross reference the genes represented on the different arrays. If there was representation on both the rat and the murine arrays, this is noted in the tables. If the genes were statistically changed in one species, but the corresponding gene was not represented on the array from the other species, then it is noted in the tables; a symbol “‡” represents genes which are not represented on the array, and therefore their expression is undetermined.

**RESULTS**

*Both Rats and Mice Develop Pulmonary Hypertension After Exposure to Hypobaric Hypoxia*

Both 1 and 3 wk of hypobaric hypoxia caused a significant increase in mPAP in SD rats. After 1 wk of hypoxia, PAP values rose from 18.3 \( \pm \) 0.3 to 24.8 \( \pm \) 2.8.

<table>
<thead>
<tr>
<th>Upregulated Rat Genes</th>
<th>Rat Accession No.</th>
<th>Mouse Accession No.</th>
<th>Ratio 1 wk to DA</th>
<th>Ratio 3 wk to DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remodeling, proliferation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrin-( \alpha )</td>
<td>X52140</td>
<td>AV292400</td>
<td>2.73</td>
<td>5.97</td>
</tr>
<tr>
<td>Platelet phospholipase ( \alpha ), group 2*</td>
<td>X51529</td>
<td>X74266</td>
<td>-1.04</td>
<td>2.42</td>
</tr>
<tr>
<td>Phosphoinositide 3 kinase*</td>
<td>U50412</td>
<td>U50413†</td>
<td>6.91</td>
<td>5.23</td>
</tr>
<tr>
<td>CXC chemokine</td>
<td>U90448</td>
<td>U27267</td>
<td>1.71</td>
<td>1.47</td>
</tr>
<tr>
<td>Vascular tone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC class 2 antigen</td>
<td>U65217</td>
<td></td>
<td>7.08</td>
<td>6.52</td>
</tr>
<tr>
<td>Prostacyclin synthase</td>
<td>U53855</td>
<td>AB001607</td>
<td>1.74</td>
<td>1.52</td>
</tr>
<tr>
<td>Repression of apoptosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteopontin*</td>
<td>M14656</td>
<td>X16151†</td>
<td>3.85</td>
<td>7.44</td>
</tr>
<tr>
<td>Calponin</td>
<td>D14437</td>
<td>U28932</td>
<td>2.18</td>
<td>1.53</td>
</tr>
<tr>
<td>Response to oxidative stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clathrin light chain</td>
<td>M15883</td>
<td>AI849152</td>
<td>2.22</td>
<td>2.02</td>
</tr>
</tbody>
</table>

Graphic representation of these 9 genes is shown in Fig. 3. Unless otherwise marked, all changes in gene expression are significant by all three methods, as described in METHODS. *Gene changes that are significant by two of the three methods described. †Genes that were unaltered by examination of the murine Mu6500 array, but are not represented on the Mu74Av2 array. ‡Genes that are not represented on the Mu74Av2 array, and therefore their expression in the mouse is undetermined. The ratios are calculated by using the mean of the average difference values for each of the three samples and using the Denver altitude (DA) as the baseline value. Bold ratio values indicate the comparison that achieves statistical significance. Minus symbol (−) indicates genes whose expression is less than the DA control.
mmHg ($P < 0.01$). At 3 wk of hypoxia, pressures rose even higher compared with control ($n = 3$ vs. control, $n = 3$; $33.3 \pm 1.3$ vs. $18.3 \pm 0.3$ mmHg; $P < 0.001$ (Fig. 1A)). C57BL/6 mice displayed higher RVSP after exposure to 3 wk of hypobaric hypoxia compared with control animals kept at DA ($28.0 \pm 1.0$ mmHg at DA vs. $35.6 \pm 1.5$ at 3 wk, $P = 0.3 \times 10^{-3}$). Although there was a trend toward higher RVSP after the 1 wk exposure, this did not reach statistical significance ($control, n = 3; 28.0 \pm 1.0$ mmHg at DA; $30.7 \pm 0.5$, at 1 wk; $P =$ not significant (NS)) (Fig. 1B).

**Rats But Not Mice Developed Pulmonary Vessel Wall Thickening After Chronic Hypobaric Hypoxia**

In mice, chronically hypoxic and control animals showed no morphological differences in their pulmonary vessel wall thickness (Fig. 2B-c, a C57BL/6 mouse at DA; Fig. 2B-d, a mouse exposed to chronic hypoxia), whereas SD rats had significant pulmonary vessel wall thickening after chronic hypoxia (Fig. 2B-a, a SD rat, DA; Fig. 2B-b, a rat, hypoxia). The statistical analysis of the lung vascular morphometry also revealed that chronic hypobaric hypoxia caused significant lung vessel wall thickening of the rat pulmonary arteries with an external diameter of 10–150 μm (chronic hypoxia, $n = 3$ vs. control, $n = 3$; $39.5 \pm 1.0$ vs. $29.9 \pm 1.0\%$; $P = 0.9 \times 10^{-3}$), whereas chronically hypoxic ($n = 7$) and control ($n = 10$) mice did not differ in percent vessel wall thickness of the pulmonary arteries in the size range of 30–50 μm ($16.3 \pm 0.26$ vs. $16.3 \pm 0.24\%$; NS) (Fig. 2A).

**Gene Microarray Analysis Revealed That, in Both Rats and Mice, the Expression of Several Genes is Altered in the Lung Tissue as a Consequence of Chronic Hypoxia**

Between the animals kept at DA and those exposed to hypoxia, the expression profiles of 8,800 rat genes and 12,000 mouse genes were compared using the GeneChip Suite. To assess the observed changes in gene expression, results were subjected to stringent statistical analysis by ANOVA, $t$-test, and $9 \times 9$ analysis, as described in the METHODS. Comparison of the lung gene expression pattern between DA and 1 wk as well as 3-wk exposure hypoxic rats showed that 9 genes had an increased expression and 19 genes had a decreased expression. In the mouse lungs, chronic hypoxia exposure resulted in the increased expression of five genes and decreased the expression of seven genes. These results are shown graphically in Figs. 3–6 and the corresponding Tables 1–4.

**Several Genes Were Differentially Expressed During Chronic Hypoxia in Rat Lungs Compared with Mouse Lungs**

The genes that had an altered expression in the hypoxia-exposed rat lungs but did not change in the mouse lungs are shown in Figs. 3 and 4, with corresponding genes listed in Tables 1 and 2. In the tables, the mean average difference values for significantly altered genes are presented as ratio differences from the control condition at DA for a frame of reference. Genes altered in the hypoxic mouse but not hypoxic rat...
are presented in Figs. 5 and 6. The genes are further enumerated in Tables 3 and 4, with the corresponding ratios compared with DA. Genes were broadly categorized into those encoding proteins involved in cell proliferation, remodeling or migration of vascular smooth muscle cells, proteins which affect vascular tone, involved in transcription regulation, repression of apoptosis, structural proteins and proteins representing adaptation to chronic hypoxia.

**Quantitative RT-PCR of the Osteopontin Gene in Rat Lungs**

The osteopontin gene in the hypoxic rat lungs had a 7.4-fold increased expression compared with that in the rat lungs kept at DA. When we assessed the lung osteopontin gene expression using quantitative RT-PCR, we confirmed the microarray expression data (Fig. 7).

**DISCUSSION**

The results of our study that compared the effect of 1 and 3 wk of hypobaric hypoxic exposure of SD rats and C57BL/6 mice on lung vascular remodeling and lung tissue gene expression by microarray technology demonstrate 1) lack of lung vascular muscularization in response to chronic hypoxia in the C57BL/6 mice compared with rats and 2) a distinct profile of gene expression between mice and rats, suggesting that genes encoding proteins involved in endothelial cell proliferation are expressed on a higher level in rat lungs. On the other hand, genes encoding proteins involved in vasodilation and cell proliferation are expressed on a lower level in murine lungs under hypoxic conditions. We postulate that this species difference in gene expression may explain the species variation in pulmonary vascular remodeling in response to hypoxia.

We expected that the stimulus of chronic hypoxia would result in an altered lung tissue gene expression pattern when compared with the control lung tissue, since hypoxia-inducible factor 1α (HIF-1α) and egr-1 have been shown to be involved in the transcriptional regulation of a large number of genes (33, 46). Surprisingly, a relatively small number of genes were altered in their expression in the lungs after both 1 and 3 wk of hypoxia. Our novel approach of lung tissue gene expression profiling makes use of the absent lung vascular remodeling in the mouse and compares lung tissue gene expression patterns in chronically hypoxic mice and rats which do show a robust pulmonary arterial muscularization response. Based on our gene expression data, new specific hypotheses can be developed and strategies to test causal relationships.

Although descriptive, our study expands the spectrum of factors that may need consideration and functional analysis. Previous work by a number of investigators has focused on the role of extracellular matrix proteins (7), matrix metalloproteinases (35), mediators of inflammation (38), and growth factors (41), which all
very likely contribute to the process of vascular remodeling in chronically hypoxic lungs. We have recently used the gene microarray technique to analyze lungs from patients with primary PH (PPH) (14) and lungs from prostacyclin receptor (PGI-R) knockout (KO) and wild-type mice exposed to chronic hypobaric hypoxia. We see the tissue gene expression data as a first step of exploration, as a survey of candidate genes that are frequently “syn”-expressed in a pattern that may suggest a mechanism. In this context, it is of interest that
chronic hypoxia affects C57BL/6 mice and SD rats differently and that the gene expression pattern in the lungs from patients with PPH, which carry plexiform lesions composed of proliferating endothelial cells (38), is very different from that in pulmonary hypertensive lungs where plexiform lesions do not form. It is also of interest that genes varied in their time response to hypoxia. Some exhibited significant alterations at 1 wk, whereas others exhibited alterations at 3 wk.

Integrin-α, secretory phospholipase A2, CXC chemokine, and phosphoinositide 3-kinase, which all have roles in proliferation and remodeling, were significantly elevated in the rat but unchanged in the mouse. In addition, osteopontin, which may have a role in reducing vascular smooth muscle cell apoptosis, was also elevated. In contrast, mice had less upregulation of genes responsible for proliferation and remodeling and demonstrated a downregulation in genes encoding for proteins responsible for vasodilation. It has been reported that vascular smooth muscle cells of the synthetic phenotype but not of the contractile phenotype express osteopontin mRNA (45) and that osteopontin reduces vascular smooth muscle cell apoptosis.

The phosphoinositide 3-kinase gene had upregulated expression in the hypoxia-exposed rat lungs but not in the mouse lungs. This kinase has been shown in vascular smooth muscle cells to regulate the expression of p27KIP1 leading to an enhanced G1/S transition of vascular smooth muscle cells (4); p21 also plays a major role in IGF-I-stimulated migration and proliferation of vascular smooth muscle cells. The PP2A gene was upregulated in the hypoxia-exposed rat lungs but not in the mouse lungs. PP2A is a phosphatase with a role in inflammation, lipid mediator production, and proliferation of smooth muscle cells (2). Integrin-α was significantly upregulated in the hypoxic rat lungs but not in the mouse lungs. Authors have recognized that the class of integrins along with endothelins may play a role in remodeling via proliferation, migration, and apoptosis of smooth muscle cells (30).

Mice exposed to hypoxic conditions exhibited a very different pattern of gene expression that favored down-

### Table 3. Genes upregulated in the hypoxic mouse, but unaltered or not assessed in the rat

<table>
<thead>
<tr>
<th>Atherosclerosis</th>
<th>Upregulated Mouse Genes</th>
<th>Accession No.</th>
<th>Rat Accession No.</th>
<th>Ratio 1 wk to DA</th>
<th>Ratio 3 wk to DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-density lipoprotein related protein 1*</td>
<td>X67469</td>
<td>AI235282</td>
<td>-1.66</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Remodeling, proliferation</td>
<td>Tenascin C*</td>
<td>X56304</td>
<td>U15550</td>
<td>13.17</td>
<td>1.68</td>
</tr>
<tr>
<td>Fibronectin*</td>
<td>M18194</td>
<td>‡</td>
<td>-1.13</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td>Transcription regulation</td>
<td>SRY box containing gene 3*</td>
<td>AA866668</td>
<td>‡</td>
<td>20.84</td>
<td>1.36</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Metalloprotease disintegrin MDC 15</td>
<td>AF006196</td>
<td>‡</td>
<td>1.12</td>
<td>2.11</td>
</tr>
</tbody>
</table>

*Graphic representation of these 5 genes is shown in Fig. 5. Unless otherwise marked, all changes in gene expression are significant by all three methods, as described in METHODS. ‡Gene changes that are significant by two of the three methods described. Genes that are not represented on the Rat Genome U34A array, and therefore their expression in the rat is undetermined. The ratios are calculated by using the mean of the average difference values for each of the three samples and using the Denver altitude (DA) as the baseline value. Bold ratio values indicate the comparison that achieves statistical significance. Minus symbol (−) indicates genes whose expression is less than the DA control.

### Table 4. Genes downregulated in the hypoxic mouse, but unaltered or not assessed in the rat

<table>
<thead>
<tr>
<th>Downregulated Mouse Genes</th>
<th>Accession No.</th>
<th>Rat Accession No.</th>
<th>Ratio 1 wk to DA</th>
<th>Ratio 3 wk to DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remodeling, proliferation</td>
<td>Insulin-like growth factor binding protein*</td>
<td>X81581</td>
<td>‡</td>
<td>-1.82</td>
</tr>
<tr>
<td></td>
<td>Leptin receptor</td>
<td>U42467</td>
<td>U67207</td>
<td>-4.00</td>
</tr>
<tr>
<td></td>
<td>Endoglin/TGF binding protein</td>
<td>X77952</td>
<td>AA978551</td>
<td>-2.76</td>
</tr>
<tr>
<td>Vascular tone</td>
<td>Cytochrome P450 1A1</td>
<td>K02588</td>
<td>E00717UTR</td>
<td>-5.56</td>
</tr>
<tr>
<td>Response to hypoxic stress</td>
<td>Aminolevulinic acid synthase</td>
<td>M15268</td>
<td>D86297</td>
<td>-1.41</td>
</tr>
<tr>
<td></td>
<td>2,3-Bisphosphoglycerate mutase</td>
<td>X13586</td>
<td>‡</td>
<td>1.3</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Flavin containing monoxygenase</td>
<td>D16215</td>
<td>M84719</td>
<td>-2.94</td>
</tr>
</tbody>
</table>

*Graphic representation of these 7 genes is shown in Fig. 6. Unless otherwise marked, all changes in gene expression are significant by all three methods, as described in METHODS. ‡Gene changes that are significant by two of the three methods described. Genes that are not represented on the Rat Genome U34A array, and therefore their expression in the rat is undetermined. The ratios are calculated by using the mean of the average difference values for each of the three samples and using the Denver altitude (DA) as the baseline value. Bold ratio values indicate the comparison that achieves statistical significance. Minus symbol (−) indicates genes whose expression is less than the DA control.
regulation of genes responsible for vascular smooth muscle proliferation and vasodilation. In contrast, up-regulated genes in the hypoxic rat lungs were unchanged in the mouse. Downregulated genes included IGF binding protein, leptin receptor, and TGF-β binding protein. In animal models, IGF has been recognized as likely having a role in stimulating smooth muscle hyperplasia (8).

Leptin is an endocrine hormone that has recently received a great deal of attention in the field of research in obesity. Studies in leptin-deficient mice suggest that leptin has a role in inducing vascular permeability and stimulating angiogenesis in adipose tissue (6). Furthermore, cellular studies have concluded that hypoxia may regulate pro-angiogenic properties of leptin (1). Whether leptin is regulated by hypoxic conditions in other vascular beds is unclear but likely warrants further investigation. TGF-β binding protein appears to have a role in rats in smooth muscle cell migration and subsequent arteriole intimal thickening (26).

Data from our microarray experiments can be compared with previously published work examining gene expression changes during exposure to hypoxia. One surprising finding of our gene profiling study is that neither the genes encoding tenasin nor the serotonin transporter were upregulated in their expression in the hypoxic rats, in light of recent publications which assigned an important role for this matrix protein (25) and the serotonin transporter in hypoxia-induced pulmonary vascular remodeling in the rat (9–11). We have previously demonstrated that overexpression of the prostacyclin synthase gene in transgenic mice confers complete protection from the development of PH (13). Furthermore, patients with PH have decreased expression of the prostacyclin synthase gene (37). In the current expression analysis, prostacyclin synthase was elevated only in rats and was significant at the 1-wk time point. Other investigators have demonstrated that the endothelin receptors are important and involved in pulmonary vascular remodeling during chronic hypoxia. Li et al. (29) described the increased level of expression of endothelin and ET-A as well as ET-B receptors in rats exposed to chronic hypoxia. We could not detect a statistical difference in gene expression using our stringent criteria for change. Le Cras et al. (27) have investigated the regulation of iNOS and eNOS in chronic hypoxia in the rat lung and have demonstrated modest increases (1.4- to 2.1-fold change). The expressions of these transcripts are below the level of detection and are called “absent” using our technology. These findings underscore some of the differences between our approach and previous detection strategies. In the lung, there are more than 40 distinct cell types that contribute to the transcriptome of a whole lung preparation. Determining the specific cell type responsible for the overexpression would require further analysis using different techniques, such as in situ hybridization. Western analysis and immunohistochemistry could be used for the evaluation of protein expression.

In summary, the identical stimulus, chronic hypoxia, produces a very different gene expression pattern in rat lungs when compared with mouse lungs. Whether this difference is the cause or the consequence of the lack of hypoxia-induced pulmonary vascular remodeling in the mouse should be a focus of future investigation. In addition, since there were only two time points examined (1 and 3 wk), it is difficult to determine the timing of sequential genetic events. We compared our microarray results to those of other investigators who examined hypoxic response. In some instances, there were discrepancies that can be explained by several factors. First, genes were considered statistically significant if at least two of three statistical tests indicated significance, and, as a result, some genes of interest were only significant by one method. Secondly, some genes of interest were not present on our arrays. This underscores some of the present limitations of this technology. However, we propose that a group of genes, rather than a single gene, is critically involved in pulmonary vascular remodeling and also that the genes encoding proteins involved in vascular tone regulation are likely different from those involved in lung vessel cell growth.

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