Pulmonary Arteriole Gene Expression Signature in Idiopathic Pulmonary Fibrosis

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Abstract:
A third of patients with idiopathic pulmonary fibrosis (IPF) develop pulmonary hypertension (PH-IPF), which is associated with increased mortality. Whether an altered gene expression profile in the pulmonary vasculature precedes the clinical onset of PH-IPF is unknown. We compared gene expression in the pulmonary vasculature of IPF patients with and without PH to controls.

Pulmonary arterioles were isolated using laser capture microdissection (LCM) from 16 IPF patients, 8 with PH (PH-IPF) and 8 with no PH (NPH-IPF), and 7 controls. Probe was prepared from extracted RNA, and hybridized to Affymetrix Hu133 2.0 Plus genechips. BRB array tools and Ingenuity Pathway Analysis software were used for analysis of the microarray data.

Univariate analysis revealed 255 genes that distinguished IPF arterioles from controls (p<0.001). Mediators of vascular smooth muscle and endothelial cell proliferation, Wnt signaling and apoptosis were differentially expressed in IPF arterioles. Unsupervised and supervised clustering analyses revealed similar gene expression in PH-IPF and NPH-IPF arterioles.

The pulmonary arteriolar gene expression profile is similar in IPF patients with and without coexistent PH. Pathways involved in vascular proliferation and aberrant apoptosis that may contribute to pulmonary vascular remodeling are activated in IPF patients without clinical PH.
Keywords:

DNA microarray

Laser capture microdissection

Pulmonary arterial hypertension

Usual interstitial pneumonia
Introduction

Idiopathic pulmonary fibrosis (IPF) is a debilitating and fatal diffuse parenchymal lung disease with a median survival of less than 3 years. More than a third of patients with IPF already have pulmonary hypertension (PH-IPF) at the time of initial evaluation for lung transplantation, with an overwhelming majority developing clinically overt PH by the time of transplantation. [1],[2] It is unclear if the subset of IPF patients with PH have a distinct vascular molecular phenotype, or whether all patients with IPF have molecular differences in pulmonary vascular gene expression compared to normals. The mechanism of pulmonary vascular dysfunction in IPF is valuable to discern, as PH-IPF is associated with decreased functional status and an increased risk of death.[1, 3]

Pulmonary arterioles from patients with PH-IPF exhibit marked intimal hypertrophy, medial smooth muscle cell proliferation, and adventitial thickening due to fibroblast accumulation and extracellular matrix deposition.[1] Previous studies have utilized DNA microarrays to evaluate gene expression in idiopathic pulmonary arterial hypertension (PAH) and PH-IPF. Genes associated with TGF-β, ERK/MAP kinase, PDGF and cAMP signaling are differentially expressed in whole lung homogenates from patients with PAH compared to patients with PH-IPF.[4] Decreased expression of angiogenic genes and upregulation of genes associated with vascular remodeling have been observed in whole lung homogenates in PH-IPF.[5] Considering that the pulmonary vasculature accounts for a relatively small portion of gene expression in whole lung homogenates, differences in gene expression observed in these studies may predominantly reflect changes in the lung parenchyma rather than the pulmonary vasculature. Ascertaining directly whether gene expression is altered in the pulmonary vasculature of IPF patients with and without PH is clinically relevant as differences between the two groups would
imply distinct vascular phenotypes. Differences in vascular gene expression in IPF compared to controls would suggest specific pathologic processes in IPF, which might lead to pulmonary vascular remodeling.

Thus, we assessed the gene expression of laser capture microdissected pulmonary arterioles from IPF patients free of PH and those with coexistent PH and compared it with controls. We sought to identify genes and/or biological pathways that could potentially mediate the development of vascular remodeling in IPF. Some of the results have previously been reported as an abstract.[6]

**Methods**

**Study Population**

Please see online supplement for full details. We identified potentially eligible study subjects by querying the Columbia University Tissue Bank database for the terms “lung” and “usual interstitial pneumonia” and/or “pulmonary hypertension”. We included IPF patients who met American Thoracic Society criteria (previous and current) for a diagnosis of IPF.[7, 8] Patients with IPF were considered in the PH-IPF group if they had a mean pulmonary artery pressure (mPAP) > 25 mm Hg with a pulmonary capillary wedge pressure (PCWP) ≤ 15 mm Hg by right heart catheterization, meeting WHO Group 3 criteria.[9, 10] IPF patients were included in the NPH-IPF group if they had a mPAP ≤ 25 mm Hg.

We excluded patients with clinical evidence of collagen vascular disease, another known etiology for pulmonary hypertension (e.g., COPD, obstructive sleep apnea, thromboembolic disease), left ventricular dysfunction or left-sided valvular disease, missing hemodynamic or pulmonary function data, and age < 18 years old.
Fifteen IPF samples were obtained from lung explant and one from surgical lung biopsy. Specimens from four unused donor lung shavings and three normal lung sections obtained during resection of pulmonary carcinoid tumors were used as controls. Approval and a waiver of consent for clinical data collection were obtained from the Columbia University Institutional Review Board (IRB-AAAC0695).

**Processing & Laser Capture Microdissection (LCM) of Tissue Samples**

All lung tissues were obtained peri-operatively, snap-frozen in liquid nitrogen and stored at -80°C. At the time of microdissection, the tissue samples were sliced into 5 µm sections, fixed with 95% ethanol and air-dried on P.A.L.M. membrane slides (P.A.L.M. Microlaser Technologies, Germany). The sections were stained using the LCM Staining Kit (Ambion, Foster City, CA). The Zeiss Axiovert Microscope (Carl Zeiss Microimaging, Inc., Thornwood) with the P.A.L.M. Microbeam LCM System (P.A.L.M. Microlaser Technologies) was utilized for vessel dissection. All pulmonary arterioles (100-450 µm) which could be visualized within a bronchovascular bundle were isolated using LCM.

**RNA Isolation, Microarray Processing and Analysis, and Immunohistochemical Staining**

The RNAqueous-Micro Kit (Ambion) was utilized to isolate RNA. The modified Eberwine procedure was used to amplify and biotinylate RNA for a minimum goal of 15 µg RNA for hybridization. Target cRNA was hybridized to Affymetrix Hu133 2.0 Plus oligonucleotide arrays. BRB array tools software v3.7.2. (National Cancer Institute, Dr. Richard Simon and Dr. Amy Lam) was used for normalization, filtering and initial quality control assessment of the genechip data. Supervised analyses of differential gene expression between class assignments was performed using univariate analyses with a p < 0.001. The false discovery rate for all
comparisons was set at a q < 10%. A permutation analysis was also performed utilizing 10,000 random permutations, with a global p < 0.01. Gene lists were further categorized into biological pathways using Ingenuity Pathways Analysis (IPA, Ingenuity Systems, www.ingenuity.com).

Paraffinized tissue blocks of 10 IPF cases (5 PH-IPF, 5 NPH-IPF) and 5 normal donor lungs were sectioned and stained for antibodies against SFRP2 (Sigma, monoclonal Rabbit anti-human), SMOC2 (Lifespan Biosciences, polyclonal Rabbit anti human) and STAT1 (Sigma rabbit anti human, monoclonal) using the DAKO Envision Dual Link kit (Dako, Denmark). Examination for statistical significance was performed using the Fisher’s Exact Test (SPSS, v16.0).

Results

We included 8 patients with PH-IPF, 8 with NPH-IPF and 7 Controls (Table 1). IPF patients were older than the controls and predominantly male. Patients with PH-IPF and NPH-IPF were similar with regards to age, sex, race, smoking history, and lung function. Pulmonary function data revealed the presence of a restrictive impairment and the absence of obstructive physiology in both groups. One patient had mild upper lobe emphysema by CT; no patients had significant emphysema histologically or by chest CT. Notably, the degree of pulmonary hypertension present in the PH-IPF group was mild with a mPAP of 29 mm Hg.

The raw gene expression values for 4 commonly expressed endothelial and epithelial genes were examined to confirm the isolation of pulmonary arterioles while minimizing inclusion of lung parenchyma or airways. Endothelial gene expression was elevated across all samples, while epithelial gene expression was minimal (e-Table 1), confirming the successful isolation of pulmonary arterioles.
We first compared PH-IPF arterioles to NPH-IPF arterioles. Unsupervised hierarchical clustering is a method of grouping gene microarray data according to similarity of content between groups. The dendogram resulting from unsupervised hierarchical clustering analysis of PH-IPF and NPH-IPF arterioles revealed no separation between the two groups (Figure 1), and there were no significant differences in gene expression by univariate analysis (e-Table 2).

We then analyzed the gene expression of the combined IPF samples (PH-IPF + NPH-IPF) versus controls. Unsupervised hierarchical clustering demonstrated a significant separation between the IPF samples and the controls (Figure 2). Two hundred and fifty five genes were differentially expressed in IPF arterioles as compared to control vessels using univariate analysis (p<0.001, q<10% [Figure 3]), with select results shown in Table 2 (full list in e-Table 4). Two mediators of Wnt signaling, SFRP2 and CCND1, were up regulated in IPF arterioles, along with members of the complement family (C4A, C4B and CFH). Expression of STAT1 and SMOC2, a transcription factor and extracellular protein associated with vascular proliferation, was increased in IPF arterioles.

Conversely, members of the Kruppel like family of transcription factors (KLF6 and 10), which modulate endothelial function, were down regulated in IPF arterioles compared with controls. S1PR1, whose ligand sphingosine 1 phosphate is an effector of endothelial cell barrier integrity, was also down regulated. Expression of arginase II, a mediator of nitric oxide signaling,[11] was decreased in IPF arterioles. The effectors of apoptosis, including IGFBP3 and TIMP3, were down regulated in IPF arterioles.

Lastly, we analyzed the gene expression of the PH-IPF group versus controls and the NPH-IPF group versus controls. The analysis of PH-IPF versus controls revealed no statistically
significant genes. Results of analysis of the NPH-IPF group versus controls are displayed in e-
Table 3.

*Biological pathways analysis*

We utilized Ingenuity Pathway Analysis (IPA) software to determine if particular biological
pathways and/or canonical signaling pathway gene expression was more highly represented in
IPF arterioles versus controls (Table 3). The top biological function for IPF arterioles was
“Cancer”, while the top molecular and cellular function was “Cellular growth and proliferation”.
The top canonical signaling pathways included amino acid metabolism, AMPK signaling, CNTF
signaling and the complement system. Genes involved in VEGF signaling (ACTB, EIF1, PIM1,
PIK3CA) and hypoxia related signaling (KLF10, ARG2, ARHGAP26) were down regulated in
IPF arterioles, as were markers of nitric oxide signaling (ITPR2, PIK3CA and IGFBP3). There
was both up and down regulation of genes involved in ERK/MAP Kinase and TGF-β signaling.
STAT1 and PLAU, both mediators of smooth muscle proliferation, were up regulated in IPF
arterioles. A number of genes impacting cancer and apoptosis were differentially expressed in
IPF arterioles (Table 3).

*Validation of Gene Array Data with Immunohistochemical staining*

Immunohistochemical staining of selected gene products of biologic interest was performed
to confirm the gene expression data (Figure 4). STAT1 and SMOC2 protein expression was
significantly up regulated in IPF arterioles compared to controls (p < 0.05). SFRP2 protein
expression also tended to be more highly expressed in IPF arterioles (p=0.10). These results
corroborate the gene expression data.
Discussion

Our findings provide direct evidence that genes involved in vascular proliferation and aberrant apoptosis are differentially expressed in pulmonary arterioles of IPF patients with or without PH compared with controls. Furthermore, patients with IPF who were free of PH and those with coexistent PH had similar gene expression in their pulmonary arterioles, suggesting that preclinical pulmonary vascular disease may be present in IPF patients with normal hemodynamics.

The mechanisms that mediate pulmonary vascular remodeling in IPF are unknown. To our knowledge, this is the first study to utilize gene expression profiling of laser capture microdissected pulmonary arterioles, a technically demanding process in fibrotic lung disease, to examine vascular gene expression in IPF. The patients in our study also underwent extensive and precise clinical phenotyping.

Two previous studies of gene expression profiling in PH-IPF have utilized whole lung homogenates. Gagermeier et al. reported gene expression data from 13 patients with PH-IPF.[5] They found decreased expression of VEGF, ET-1 and PECAM, while phospholipase A2 and other inflammatory genes were up regulated. We also found that genes associated with VEGF signaling were down regulated, though inflammatory genes were not highly represented in our IPF samples. Rajkumar et al. compared whole lung gene expression from 18 subjects with PAH to that of 8 subjects with PH-IPF.[4] Rho kinases and mediators of cAMP signaling were up regulated in PAH as compared to PH-IPF. It is difficult to make a direct comparison between this study and ours, as we did not examine patients with PAH. More recently, Mura et al. examined lung gene expression in patients with varying etiologies of pulmonary fibrosis (UIP and non-UIP) with and without severe PH (mPAP > 40 mm Hg).[12] Cellular proliferation and fibroblast
migration pathways were highly expressed in the PH group. While the former pathway was also highly represented in the vasculature of IPF patients in our study, there was no overlap in the most highly up-regulated genes. There were significant phenotypic differences between the patients in this study and ours. Differences in results may further be explained by our direct focus on isolated pulmonary arterioles rather than the mix of parenchyma, airways, and vessels in prior studies. Investigation of isolated pulmonary arterioles enabled us to determine gene expression directly in the pulmonary vasculature, without potential artifact of lung parenchyma affected by IPF.

Genes that promote cellular proliferation were upregulated in IPF pulmonary arterioles regardless of the presence of coexistent PH. SMOC2 promotes angiogenesis and endothelial proliferation,[13] and SMOC2 ablation inhibits DNA synthesis in mouse fibroblasts exposed to PDGF-β and other growth factors implicated in the pathogenesis of IPF.[14] Additionally, SMOC2 promotes cell cycle progression via cyclinD1, which was also upregulated in our samples.[15] Given these findings, it is plausible that SMOC2 contributes to vascular remodeling in IPF. In addition, gene and protein expression of STAT1 was increased in IPF pulmonary arterioles regardless of the presence of coexistent PH. STAT1 increases vascular smooth muscle cell proliferation in response to angiotensin II and serotonin, proposed mediators of PAH.[16, 17] Woods et al. demonstrated that STAT1 mediates increased endothelin-1 (ET-1) production in human vascular smooth muscle cells (HVSMCs).[18] Additionally, PLAU, which was overexpressed in our IPF samples, has been shown to activate STAT1 in both human vascular endothelial cells and HVSMCs.[19, 20] These data suggest that PLAU and other mediators may activate STAT1 in IPF, leading to vascular proliferation. Our finding of increased SFRP2 expression in IPF arterioles may indicate a role for Wnt signaling in promoting the development
of PH-IPF. Secreted frizzle-related protein 2, a mediator of both canonical and noncanonical Wnt signaling, promotes angiogenesis and inhibits hypoxia-induced endothelial cell apoptosis in experimental models.[21] Moreover, noncanonical Wnt signaling has recently been established to be active in the development of idiopathic PAH.[22] Lastly, genes that inhibit proliferation were down regulated in in pulmonary arterioles of IPF patients with and without coexistent PH. KLF10 mediates anti-proliferative and anti-apoptotic effects via TGFβ Smad signaling.[23] KLF6 functions as a tumor suppressor that is expressed in endothelial cells, and is induced by hypoxia and other forms of cellular stress.[24] Down regulation of KLF10 and KLF6 may contribute to cellular proliferation and deregulation of apoptosis in the vasculature in IPF. YY1, a zinc-finger transcription factor that inhibits vascular smooth muscle cell growth and functions downstream from heme oxygenase 1(HO-1), was down regulated in our samples.[25, 26] Reduced HO-1 expression characterizes severe PAH, suggesting a potential association between the pathobiology of PAH and vascular change in IPF.[26, 27] Similar alterations in expression of genes that promote and inhibit cellular proliferation in pulmonary arterioles of IPF patients with and without coexistent PH strongly suggest that vascular function is altered early in the course of IPF. Such findings concur with previous reports indicating the development of clinical PH in the vast majority of IPF patients over time.[2] Alternatively, similar gene expression in pulmonary arterioles of IPF patients with or without PH may indicate that the biologic processes which account for pulmonary vascular changes in IPF may be a manifestation of IPF as a disease rather than PH.

Interestingly, the genes that were differentially expressed in IPF vessels compared to controls largely fell into categories of cellular proliferation and cancer. Shared pathogenic ties between cancer biology and IPF, including aberrant cellular proliferation and impaired apoptosis, have
been increasingly noted.[28] In PAH, STAT3 activation contributes to the propagation of hyper proliferative, apoptosis resistant endothelial cells.[29] Several modulators of apoptosis were under-expressed in our samples. Conversely, mediators of proliferation, such as STAT1 and SMOC2, were up regulated.[19, 20] These findings suggest that uncontrolled vascular proliferation and impaired apoptosis may contribute to vascular remodeling in IPF as well. Lastly, increased SFRP2 expression in our samples suggests an active role for Wnt signaling in the development of vascular changes in IPF.[22]

There were several limitations in our study. We studied a small number of patients, albeit a typical sample size for gene microarray studies. We used technically demanding LCM to isolate vessels for analysis, a technologically sophisticated approach that is novel in studies of the vasculature in IPF. RT-PCR was not utilized for validation as accurate dissection of pulmonary arteriolar tissue from architecturally distorted, fibrotic parenchyma precluded extraction of a sufficient quantity of tissue for both expression microarray analysis and RT-PCR. Patients with PH-IPF had only mild elevations in pulmonary artery pressure, but fulfilled the WHO criteria and are typical of PH-IPF.[3, 30] Patients with more severe PH-IPF might have shown significant differences in gene expression compared to the NPH-IPF group. Combined pulmonary fibrosis with emphysema (CPFE) is associated with PH in IPF,[31] however only one patient in our study had evidence of mild upper-lobe emphysema.[31] Moreover, spirometry and lung volumes were similar between the groups. We used specimens obtained from normal lung in patients undergoing carcinoid resection for a portion of control samples, and acknowledge that while the tissue appeared normal architecturally, it may have been abnormal at the molecular level. Lastly, while high-throughput gene expression analysis may be subject to Type 1 error, we constrained our false-positive rate and confirmed selected gene expression findings using IHC.
In conclusion, the pulmonary vascular gene expression signature is similar in patients with coexistent IPF and PH and those with IPF alone and is characterized by aberrant cellular growth and deregulated apoptosis. Characterization of preclinical pulmonary vascular alterations in IPF may lead to development of novel therapeutic strategies for coexistent PH in these patients.
Acknowledgements:


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References


Figure Legends:

Figure 1: PH-IPF vessels vs. NPH-IPF vessels

Unsupervised hierarchical clustering analysis, the dendogram shows no separation of gene expression between PH-IPF and NPH-IPF samples.
Figure 2: IPF vessels vs. Control vessels

Unsupervised hierarchical clustering analysis, the dendogram demonstrates clustering of gene expression of IPF samples separately from controls.

Figure 3: Heat map of differentially expressed genes in IPF vessels vs. Controls,
259 genes differentially expressed at a p < 0.001 with FDR < 10%; red= up regulated genes, green=down regulated genes
Figure 4:

- Blood vessel from UIP tissue showing marked nuclear and cytoplasmic staining for STAT1 in endothelium and medial smooth muscle cells (Panel A), while normal vessel (Panel B) shows only endothelial staining.
- Blood vessel from UIP tissue shows intense cytoplasmic for SMOC2 in medial smooth muscle cells and endothelial cells (Panel C), while normal vessel (Panel D) is negative.
- Blood vessel wall from UIP tissue shows cytoplasmic staining for SFRP2 in medial smooth muscle cells (Panel E), but no staining in normal vessel (Panel F).
Table 1: Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th>Study Group</th>
<th>PH-IPF (n=8)</th>
<th>NPH-IPF (n=8)</th>
<th>Normals (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic Information</td>
<td>Age, yrs.</td>
<td>59 ± 5</td>
<td>63 ± 4</td>
</tr>
<tr>
<td></td>
<td>Female gender</td>
<td>38%</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Non-Hispanic white 88% 75% 86%
Hispanic 12% 25% 14%

Body Mass Index, kg/m2 27 ± 5  (n = 7) --

Ever smoker 50% 43%*

Median duration of IPF [IQR] (yrs.) 3 [1, 4] 1.5 [1.4] --

FVC (% predicted) 55 ± 20 48 ± 14 --
FVC absolute (liters) 2.2 ± 1.0 1.9 ± 0.8 --
FEV1 (% predicted) 59 ± 17 54 ± 18 --
FEV1 absolute (liters) 1.8 ± 0.6 1.6 ± 0.7 --
FEV1/FVC% 87 ± 9 81 ± 6 --

TLC (% predicted) 53 ± 20 48 ± 13* --

DLCO (% predicted) 21 ± 10 34 ± 9 --

Six minute walk distance (m) 273 ± 75 468 ± 87 --

Hemodynamics

RA (mm Hg) 3 ± 2* 1 ± 2* --
mPAP (mm Hg) 29 ± 4 12 ± 2 --
PCWP, mm Hg 9 ± 3 2 ± 2 --
Cardiac Index (liters/min/m²) 3.4 ± 1.5 2.4 ± 0.5* --
PVR (dyne/sec/cm⁵) 415 ± 304 214 ± 65* --

*1 subject missing. Values are presented as means ± standard deviation, unless otherwise indicated.

Definition of abbreviations: Forced vital capacity (FVC), Forced expiratory volume in 1 s (FEV1), Total lung capacity (TLC), Diffusing capacity for carbon monoxide (DLCO), Right atrial pressure (RA), Mean pulmonary artery pressure (mPAP), Pulmonary capillary wedge pressure (PCWP), Pulmonary vascular resistance (PVR)

Table 2: Select Top Up and Down Regulated Genes in IPF Vessels Compared to Controls

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Probe set ID</th>
<th>Gene Description</th>
<th>Parametric p-value</th>
<th>FDR</th>
<th>Fold change</th>
<th>Direction in IPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT1</td>
<td>200887_s_at</td>
<td>signal transducer and activator of transcription 1, 91kDa</td>
<td>0.0001192</td>
<td>0.03</td>
<td>1.8</td>
<td>Up</td>
</tr>
</tbody>
</table>

23
<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Probe set ID</th>
<th>Gene Description</th>
<th>Parametric p-value</th>
<th>FDR</th>
<th>Fold-change</th>
<th>Direction in IPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMO2</td>
<td>223235_s_at</td>
<td>SPARC related modular calcium binding 2</td>
<td>1.83E-05</td>
<td>0.01</td>
<td>2.34</td>
<td>Up</td>
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<tr>
<td>YY1</td>
<td>224718_at</td>
<td>YY1 transcription factor</td>
<td>0.0003999</td>
<td>0.05</td>
<td>0.58</td>
<td>Down</td>
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<tr>
<td>KLF10</td>
<td>202393_s_at</td>
<td>Kruppel-like factor 10</td>
<td>0.0001628</td>
<td>0.03</td>
<td>0.44</td>
<td>Down</td>
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<tr>
<td>TGFB1</td>
<td>201506_at</td>
<td>transforming growth factor, beta-induced, 68kDa</td>
<td>1.41E-05</td>
<td>0.01</td>
<td>2.41</td>
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<td>THY1</td>
<td>213869_x_at</td>
<td>Thy-1 cell surface antigen</td>
<td>0.0001963</td>
<td>0.03</td>
<td>3.53</td>
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<td>SFRP2</td>
<td>223122_s_at</td>
<td>secreted frizzled-related protein 2</td>
<td>0.0004054</td>
<td>0.05</td>
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<td>IGFBP3</td>
<td>212143_s_at</td>
<td>insulin-like growth factor binding protein 3</td>
<td>0.0001555</td>
<td>0.03</td>
<td>0.29</td>
<td>Down</td>
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<tr>
<td>HIPK2</td>
<td>225368_at</td>
<td>homeodomain interacting protein kinase 2</td>
<td>4.39E-05</td>
<td>0.02</td>
<td>0.31</td>
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<td>PIM1</td>
<td>209193_at</td>
<td>pim-1 oncogene</td>
<td>9.92E-05</td>
<td>0.03</td>
<td>0.53</td>
<td>Down</td>
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<td>PLAU</td>
<td>205479_s_at</td>
<td>plasminogen activator, urokinase</td>
<td>0.0001612</td>
<td>0.03</td>
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<tr>
<td>S1PR1</td>
<td>239401_at</td>
<td>sphingosine-1-phosphate receptor 1</td>
<td>0.000134</td>
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<td>0.45</td>
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<tr>
<td>KLF6</td>
<td>224606_at</td>
<td>Kruppel-like factor 6</td>
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<tr>
<td>ARG2</td>
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<td>arginase, type II</td>
<td>0.000145</td>
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<tr>
<td>HIF3A</td>
<td>219319_at</td>
<td>hypoxia inducible factor 3, alpha subunit</td>
<td>0.0006036</td>
<td>0.05</td>
<td>0.34</td>
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<tr>
<td>ARHGAP26</td>
<td>205068_s_at</td>
<td>Rho GTPase activating protein 26</td>
<td>8.69E-05</td>
<td>0.03</td>
<td>0.47</td>
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<td>C4A</td>
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<td>complement component 4A</td>
<td>5.8e-06</td>
<td>0.01</td>
<td>2.93</td>
<td>Up</td>
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<td>C4B</td>
<td>208451_s_at</td>
<td>complement component 4B</td>
<td>2.07e-05</td>
<td>0.01</td>
<td>2.71</td>
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<tr>
<td>CFH</td>
<td>213800_at</td>
<td>complement factor H</td>
<td>0.0002801</td>
<td>0.04</td>
<td>3.61</td>
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<tr>
<td>KCNM64</td>
<td>219287_at</td>
<td>Potassium large conductance calcium-activated channel</td>
<td>0.0006496</td>
<td>0.05</td>
<td>0.42</td>
<td>Down</td>
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<tr>
<td>PTGFRN</td>
<td>224937_at</td>
<td>prostaglandin F2 receptor negative regulator</td>
<td>3.50E-05</td>
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<td>AGTR2</td>
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<td>angiotensin II receptor, type 2</td>
<td>0.0009539</td>
<td>0.06</td>
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</tr>
</tbody>
</table>

Univariate analysis demonstrated 255 differentially expressed genes between IPF arterioles and controls (p < 0.001, q < 10). A selection of the most highly up or down regulated genes is displayed (full list available in the online supplement).

Table 3: Differentially expressed genes in IPF arterioles vs. Normals
<table>
<thead>
<tr>
<th>Biological Pathway</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>ABAT, ADRA2C, AMD1, ARG2, ARHGAP26, ARIH2, C5, C4A, CAPN5, CCNL1, CFH, COL15A1, DUSP5, EIF1, HIF3A, HNRPD1, IGFBP3, INSR, KLF6, KLF10, LIFR, MAOA, MXRA5, PIM1, PLAU, SRFP2, STAT1, RIMP3</td>
</tr>
<tr>
<td>Apoptosis &amp; Cell Death</td>
<td>BAG5, HIPK2, IGFBP3, KLF10</td>
</tr>
<tr>
<td>Jak-STAT signaling</td>
<td>STAT1, PIM1, LIFR, PIK3CA</td>
</tr>
<tr>
<td>Complement &amp; Coagulation Cascade</td>
<td>C4A, C4B, PLAU, C5, CFH</td>
</tr>
<tr>
<td>Cellular Growth &amp; Proliferation</td>
<td>ANXA1, KLF10, PLAU, THY1, TGFBI, S1PR1, SMOC2, YY1</td>
</tr>
</tbody>
</table>

Ingenuity Pathways Analysis of differentially expressed genes in IPF arterioles vs. Controls displayed according to classification by biological pathway.