ORIGINAL ARTICLE

Hypoxic Gene Expression of Donor Bronchi Linked to Airway Complications after Lung Transplantation

Bryan D. Kraft[1], Hagir B. Suliman[2], Eli C. Colman[1], Kamran Mahmood[1], Matthew G. Hartwig[3], Claude A. Piantadosi[1,2], and Scott L. Shofer[1]

[1]Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, [2]Department of Anesthesiology, and [3]Division of Cardiovascular and Thoracic Surgery, Department of Surgery, Duke University Medical Center, Durham, North Carolina

Abstract

Rationale: Central airway stenosis (CAS) after lung transplantation has been attributed in part to chronic airway ischemia; however, little is known about the time course or significance of large airway hypoxia early after transplantation.

Objectives: To evaluate large airway oxygenation and hypoxic gene expression during the first month after lung transplantation and their relation to airway complications.

Methods: Subjects who underwent lung transplantation underwent endobronchial tissue oximetry of native and donor bronchi at 0, 3, and 30 days after transplantation (n = 11) and/or endobronchial biopsies (n = 14) at 30 days for real-time polymerase chain reaction of hypoxia-inducible genes. Patients were monitored for 6 months for the development of transplant-related complications.

Measurements and Main Results: Compared with native endobronchial tissues, donor tissue oxygen saturations (Sto_2) were reduced in the upper lobes (74.1 $\pm$ 1.8% vs. 68.8 $\pm$ 1.7%; $P < 0.05$) and lower lobes (75.6 $\pm$ 1.6% vs. 71.5 $\pm$ 1.8%; $P = 0.065$) at 30 days post-transplantation. Donor upper lobe and subcarina Sto_2 levels were also lower than the main carina (difference of -3.9 ± 1.5 and -4.8 ± 2.1, respectively; $P < 0.05$) at 30 days. Up-regulation of hypoxia-inducible genes *VEGFA*, *FLT1*, *VEGFC*, *HMOX1*, and *TIE2* was significant in donor airways relative to native airways (all $P < 0.05$). *VEGFA*, *KDR*, and *HMOX1* were associated with prolonged respiratory failure, prolonged hospitalization, extensive airway necrosis, and CAS ($P < 0.05$).

Conclusions: These findings implicate donor bronchial hypoxia as a driving factor for post-transplantation airway complications. Strategies to improve airway oxygenation, such as bronchial artery re-anastomosis and hyperbaric oxygen therapy merit clinical investigation.

Keywords: lung transplantation; bronchial diseases; oximetry; cell hypoxia; angiogenic proteins

Large airway complications such as central airway stenosis (CAS) are common after lung transplantation and are associated with increased morbidity and mortality (1–5). The pathophysiology is unclear, but post-transplantation bronchial oxygen saturations (Sto_2) and perfusion are subnormal (6). Although the native lung has a dual blood supply from the pulmonary and bronchial arteries, the bronchial circulation is routinely sacrificed at transplantation. As a result, the bronchial mucosa becomes dependent on oxygen-poor, low-flow, pulmonary arterial collaterals. Although studies in dogs suggest that bronchial collaterals can form as early as 2 weeks post-transplantation (7, 8), a human study has found compromised donor bronchial blood flow for up to 1 year post-transplantation (6). The extent to which this physiological defect in large airway oxygenation is clinically significant is uncertain (6, 9), but small airway studies suggest that loss of the airway microvasculature after lung transplantation may contribute to rejection and fibrosis (10–15), which suggests that inadequate

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Correspondence and requests for reprints should be addressed to Scott L. Shofer, M.D., Ph.D., Duke University Medical Center, Box 3683, Durham, NC 27710. E-mail: scott.shofer@duke.edu

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At a Glance Commentary

Scientific Knowledge on the Subject: Bronchial circulation is not routinely re-anastomosed during lung transplantation, which leaves donor airways hypoxemic, although the clinical significance of this is unknown.

What This Study Adds to the Field: Donor airways displayed a greater epithelial hypoxic response and significant up-regulation of hypoxia-responsive genes at 30 days post-transplantation. Hypoxic gene expression was most pronounced in patients with prolonged respiratory failure, airway necrosis, and central airway stenosis, implicating poor bronchial oxygenation as a significant factor in post-transplantation airway complications.

oxygenation may play a role in large airway complications as well.

We therefore sought to extend and define the significance of large airway hypoxia by measuring endobronchial oximetry and bronchial epithelial gene expression of hypoxia-inducible genes in both native and donor lung bronchial tissues of patients who underwent lung transplantation. We found that endobronchial oximetry values were significantly reduced in donor tissue compared with native tissue, and that hypoxia-inducible gene expression was significantly up-regulated in donor tissues relative to native tissues. Moreover, patients with the most up-regulated gene expression profiles displayed significantly more post-transplantation airway complications. Some of the results of these studies have been previously reported in the form of an abstract (16).

Methods

Study Enrollment

Fifteen patients underwent single orthotopic lung transplantation (SOLT) (n = 9) or bilateral orthotopic lung transplantation (BOLT) (n = 6) at Duke University Medical Center (Durham, North Carolina) as described (2, 17). SOLT laterality was determined by technical considerations, history of thoracic surgery, size of the chest cavity, involvement of the underlying lung disease, and ventilation/perfusion imaging as appropriate. Subjects were enrolled between January 2013 and September 2014. All subjects provided written informed consent for participation in the study before transplantation. The study was approved by the Duke University Institutional Review Board (IRB #Pro00038323).

Bronchoscopy

Informed consent was obtained before each procedure. One subject consented to endobronchial oximetry but refused endobronchial biopsy. Vital signs were monitored before, during, and after the procedure. Supplemental oxygen was provided as needed to maintain pulse oximetry >90%. Lidocaine (1%) was administered topically for local anesthesia, and anxiolytics/analgesics (e.g., midazolam and fentanyl) were administered intravenously as needed to achieve moderate sedation.

Endobronchial oximetry was performed in 11 patients (SOLT = 8, BOLT = 3) immediately after transplantation (time 0), and at 3 days and 30 days after transplantation using a T-stat white light tissue oximeter (Spectros Corp., Portola Valley, California) (6, 18). Measurements were taken at the main carina and at the upper lobe bronchus, first subcarina, and lower lobe bronchus of each lung.

Endobronchial biopsies were performed (n = 14 patients) at the 30-day bronchoscopy only. Samples were collected from both donor and native bronchial tissue: in the SOLT patients (n = 8), endobronchial biopsies were taken from the donor and native lung first subcarina; in the BOLT patients (n = 6), biopsies were taken from both donor first subcarinae and the native main carina. In patients with airway necrosis, biopsies were taken from nearby viable tissue. The endobronchial biopsy specimens were labeled numerically for blinding and placed immediately into RNA*later* RNA stabilization reagent (Qiagen, Venlo, the Netherlands) and stored at −80°C.

Real-Time Polymerase Chain Reaction

Total RNA was extracted from the endobronchial biopsy specimens using the RNeasy Midi Kit (Qiagen). RNA purity was confirmed on a 1.2% agarose gel, and the RNA was reverse transcribed into cDNA using the ImProm-II reverse transcription system (Promega, Fitchburg, Wisconsin). Quantitative real-time polymerase chain reaction for a set of hypoxia-responsive genes known to be involved in vasculogenesis was performed on an ABI StepOnePlus using gene expression assays (Applied Biosystems, Waltham, Massachusetts). Gene expression assay primers were used to amplify *HMOX1*, *VEGFA*, *VEGFC*, *FLT1* (*VEGFR1*), *KDR* (*VEGFR2*), *GLUT1* (*SLC2A1*), *ANGPT1*, *TIE2* (*TEK*), and *TGFB1*. 18S rRNA was used as an endogenous control. Quantification of gene expression was determined by the comparative threshold cycle and relative quantification method. Each sample was assayed in triplicate. All mRNA work was performed in a blinded fashion.

Clinical Outcomes

Patients were monitored for 6 months after transplantation for several clinical outcomes, including primary graft dysfunction (PGD) within 72 hours of transplant (19), prolonged respiratory failure (defined as need for re-intubation or tracheostomy after transplantation), time to hospital discharge, development of acute cellular rejection (20), development of airway necrosis ("limited" necrosis was defined as an extension of the necrotic airway plaques to within 2 cm of the anastomosis, and "extensive" necrosis was defined as plaque extension >2 cm distal to the anastomosis and/or involvement of the lower lobe) (21), development of CAS (defined as the inability of a 6.2-mm bronchoscope to traverse the central airway), and change in spirometry between 30 days and 6 months post-transplantation.

Statistical Analysis

Grouped data were normally distributed and expressed as mean ± SEM, except where specified. Differences between groups were analyzed using the unpaired Student's *t* test, Wilcoxon signed-rank test, or the Mann-Whitney *U* test based on normality testing (Prism 6, GraphPad Software, Inc., San Diego, California or Microsoft Excel, Redmond, Washington). All *P* values are two-tailed, and $P < 0.05$ was accepted as significant. Using 80% power to detect significant differences, 13 patients per group were needed to detect a 5% (absolute) difference in StO_2, and 10 per group were needed to detect a two-fold change in gene expression between native and donor tissues.

Table 1. Subject Characteristics

Subject ID	Age	Sex	Lung Disease	Donor Lung	Donor Age	Cold Ischemic Time (*min*)	Warm Ischemic Time (*min*)
001	68	M	IPF	Right	39	202	48
002	69	M	IPF	Left	39	260	48
003	72	F	UIP/CTD	Right	34	692	44
004	61	M	IPF	Left	52	252	51
005	72	M	IPF	Right	26	377	56
006	72	M	IPF	Right	39	160	74
007	65	M	IPF	Right	50	n/a	n/a
008	68	M	IPF	Right	31	294	47
009	66	M	IPF	Right	19	253	56
010	74	F	IPF	Bilateral	67	250	32
011	63	M	IPF/COPD	Bilateral	61	441	37
012	39	F	CF	Bilateral	44	339	44
013	52	F	Sarcoid	Bilateral	39	370	38
014	60	M	IPF/CTEPH	Bilateral	63	333	47
015	57	M	NSIP	Bilateral	63	140	39

Definition of abbreviations: CF = cystic fibrosis; COPD = chronic obstructive pulmonary disease; CTD = connective tissue disease; CTEPH = chronic thromboembolic pulmonary hypertension; ID = identification number; IPF = idiopathic pulmonary fibrosis; n/a = data not available; NSIP = nonspecific interstitial pneumonia; Sarcoid = sarcoidosis; UIP = usual interstitial pneumonia.

For comparisons that were underpowered, trends were noted for $P \leqslant 0.1$.

Results

Patient characteristics are shown in Table 1. Patient age (mean ± SD) was 63.9 ± 9.2 years. There were 11 men (73%) and 4 women (27%). The most common indication for transplantation was interstitial lung disease (n = 14) due to idiopathic pulmonary fibrosis (IPF) (n = 9), connective tissue disease–associated usual interstitial pneumonia (n = 1), nonspecific interstitial pneumonia (n = 1), sarcoidosis (n = 1), IPF and chronic obstructive pulmonary disease (n = 1), or IPF and chronic thromboembolic pulmonary hypertension (n = 1). One patient had cystic fibrosis. Six patients (40%) underwent BOLT and nine patients (60%) underwent SOLT. Donor age (mean ± SD) was 39 ± 13.5 years, and cold and warm ischemic

Table 2. Subject Outcomes

Subject ID	Prolonged Respiratory Failure	Primary Graft Dysfunction Score	Days to Discharge	Days to Rejection*	Rejection Grade	Airway Necrosis	Stenosis Present	% Change in FVC †	% Change in FEV_1 †
001	No	0	42	72	A2Bx	Limited	Yes	29	−8
002	No	0	11	29	A2B0	None	No	16	23
003	No	0	16	29	A1B0	Limited	No	24	20
004	Reintub, trach	2	26	No rejection	n/a	Extensive	Yes	40	61
005	No	2	16	24	A1B0	Limited	No	−21	−28
006	No	0	11	33	A1B0‡	Limited	No	15	17
007	No	1	11	27	A1B0	Limited	No	35	34
008	No	1	6	94	A1B0	Extensive	No	33	25
009	No	0	7	No rejection	n/a	Limited	No	8	7
010	Trach	3	88§	No rejection	n/a	Limited	No	Not avail	Not avail
011	No	0	12	30	A1B0	Limited	No	−2	−28
012	Trach	3	117	No rejection	n/a	Extensive	Yes	30	24
013	No	0	18	99	A1B0	Limited	No	44	56
014	Reintub	3	72	No rejection	n/a	Extensive	No	22	2
015	Reintub	1	27	92	A2B0	Extensive	No	3	−12

Definition of abbreviations: ID = identification number; n/a = not applicable; Not avail = data not available; reintub = reintubation; trach = tracheostomy.
*Up to 6 months post-transplantation.
†Change between approximately 1 month post-transplantation and 6 months post-transplantation.
‡Ischemia-reperfusion injury also present.
§Patient died on hospital day 88.

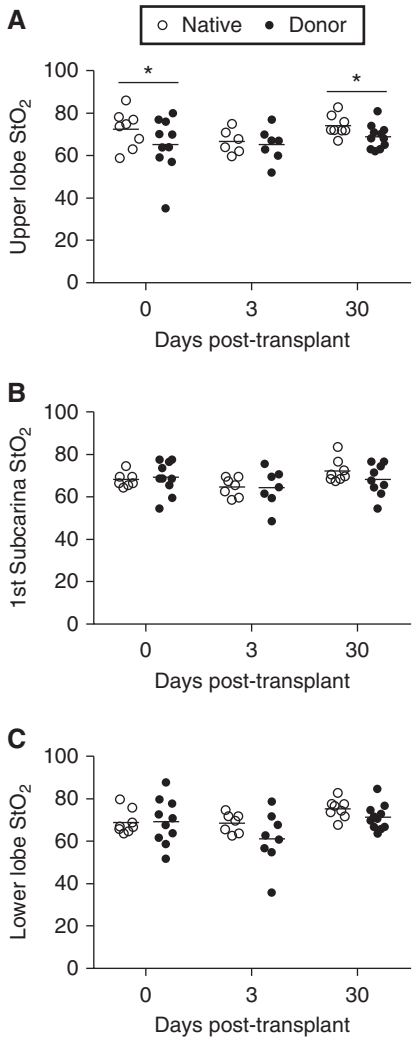

Figure 1. Absolute tissue oxygen saturations (StO_2) of native (*open circles*) and donor (*solid circles*) (*A*) upper lobe, (*B*) first subcarina, and (*C*) lower lobe bronchi in individual patients at 0, 3, and 30 days after single or bilateral lung transplantation. N = 6–11 patients per group per time point. *Bars* represent means. *$P < 0.05$ calculated by the unpaired Student's *t* test. There was also a trend for reduced absolute StO_2 levels in donor lower lobe bronchi at 30 days (71.5 ± 1.8%, n = 11 vs. 75.6 ± 1.6%, n = 8; $P = 0.065$).

times were 311.6 ± 138.3 and 47.2 ± 10.4 minutes, respectively.

Patient outcomes are shown in Table 2. Eight patients developed PGD within the first 72 hours of transplantation, and five developed prolonged respiratory failure requiring reintubation and/or tracheostomy placement. Median time to hospital discharge after transplantation was 16 days (interquartile range, 23.5 d). Median time to the first episode of acute cellular rejection was 31.5 days (interquartile range, 58 d), and ranged in severity from minimal (A1) to mild (A2). One patient had no airway necrosis, nine developed limited necrosis, and five had extensive airway necrosis involving the lower lobe (21). Two patients with extensive necrosis and one with limited necrosis developed CAS. The percent change in FVC and FEV_1 was 19.9 ± 4.8% and 13.8 ± 7.2%, respectively; four patients displayed no improvement (<12% increase) or worsening of both FVC and FEV_1.

To investigate signs of hypoxia in donor bronchi, we serially measured endobronchial tissue StO_2 in the bronchial epithelium of the upper lobe, subcarina, and lower lobe in donor lungs (SOLT and BOLT groups), corresponding native lung sites (SOLT group only), and in native main carina (SOLT and BOLT groups). Bilateral lobar/subcarina StO_2 measurements in BOLT patients were averaged. In a few patients, reliable oximetry measurements could not be obtained at one or more sites because of blood or necrotic plaques in the airways. Compared with native upper lobe bronchi, absolute StO_2 levels of the donor upper lobe bronchi were significantly reduced immediately after transplantation (65.2 ± 4.1%, n = 10 vs. 72.5 ± 3.1%, n = 8; $P < 0.05$) and at 30 days post-transplantation (68.8 ± 1.7%, n = 11 vs. 74.1 ± 1.8%, n = 8; $P < 0.05$). We noticed a trend for reduced absolute StO_2 levels in donor lower lobe bronchi at 30 days (71.5 ± 1.8%, n = 11 vs. 75.6 ± 1.6%, n = 8; $P = 0.065$) (Figures 1A–1C). The StO_2 changes over time for individual patients are shown in Figure E1 in the online supplement. Among the subset of SOLT patients with paired donor and native bronchial StO_2 measurements, the "contralateral StO_2 difference" (donor minus native) was less than zero in the upper lobes at 0 and 30 days post-transplantation (−9.8 ± 3.3 and −6.1 ± 2.2, respectively; n = 8 at each time point; $P < 0.05$) and displayed a trend in the lower lobes at 30 days post-transplantation (−2.9 ± 1.3, n = 8; $P = 0.069$) (Figure 2A). StO_2 levels at the native main carina (above the anastomosis) were also compared with those of the donor bronchi (below the anastomosis) in all patients (SOLT and BOLT groups). The "anastomosis StO_2 difference" (donor minus main carina) was less than zero in the donor upper lobe (−8.8 ± 4.3 points, n = 10; $P < 0.05$) and the donor subcarina (−4.4 ± 1.4, n = 7; $P < 0.05$) immediately after transplantation, and in the donor upper lobe and subcarinal mucosa at 30 days after transplantation (−3.9 ± 1.5, n = 10, and −4.8 ± 2.1, n = 8, respectively; $P < 0.05$) (Figure 2B).

Because endobronchial oximetry values were moderately lower in donor mucosa than in native mucosa, we selected a set of hypoxia-inducible genes associated with vasculogenesis for measurement in endobronchial biopsy specimens taken at 30 days after transplantation. Due to the limited RNA sample, not every transcript could be measured for every patient. Absolute mRNA levels for *KDR* (*VEGFR2*), *ANGPT1*, *GLUT1*, and *TGFB1* were not statistically different between donor and native tissues, but expression of *VEGFA*, *FLT1* (*VEGFR1*), *VEGFC*, *TIE2* (*TEK*), and *HMOX1* mRNA was significantly increased in donor tissues relative to native tissues (all $P < 0.05$) (Figure 3 and Figure E2). The calculated ratios of donor to native gene expression in paired samples revealed a trend for increased *TIE2* (*TEK*) (n = 5) and *VEGFC* (n = 5) expression (both $P = 0.062$), and significant elevations in *VEGFA* (n = 14), *FLT1* (*VEGFR1*) (n = 11), and *HMOX1* (n = 11) expression (all $P < 0.05$) (Figure 4).

To investigate the relationships between gene expression and patient outcomes, we compared the ratios of donor/native mRNA expression to predefined clinical parameters. We did not find associations between mRNA levels and older donor age (>55 yr), longer cold ischemic time (>300 min), acute rejection, or changes in spirometry after transplantation (data not shown), but we did find significant associations between mRNA expression and the development of post-transplantation airway complications. Specifically, patients with prolonged postoperative respiratory failure (i.e., requiring re-intubation or tracheostomy) displayed significantly higher donor/native gene product ratios for *VEGFA* (4.0 ± 0.8, n = 5 vs. 1.5 ± 0.3, n = 9) and *KDR* (*VEGFR2*) (4.1 ± 1.3, n = 4 vs. 0.6 ± 0.1, n = 5) than did patients who were successfully extubated after transplantation (both $P < 0.05$). In addition, patients with PGD scores of 2 or 3 (19) showed a trend for higher *KDR* expression compared with patients scoring 0 or 1 (3.5 ± 2.9, n = 3 vs.

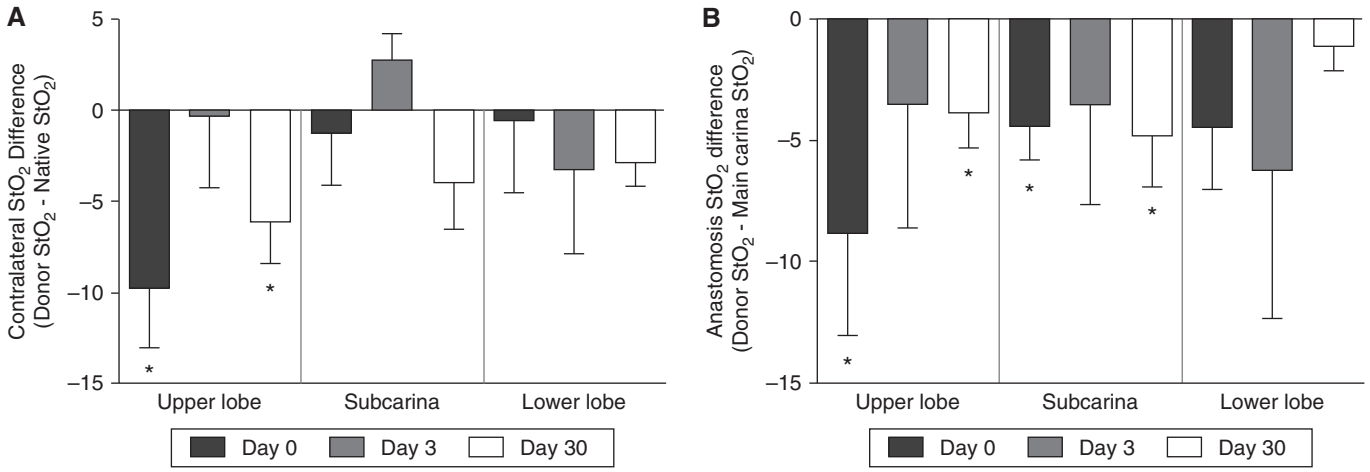

Figure 2. Differential endobronchial tissue oxygen saturation (Sto_2). (*A*) Sto_2 levels were measured in single orthotopic lung transplantation (SOLT) patients only. Paired measurements (SOLT donor lung vs. native contralateral lung) were collected from donor and native upper lobes, subcarinae, and lower lobes at 0 (*black bars*), 3 (*gray bars*), and 30 (*white bars*) days after single lung transplantation. The contralateral Sto_2 difference (donor – native) was calculated for upper lobe, subcarina, and lower lobe sites and analyzed for statistical significance (*$P < 0.05$) by the Wilcoxon signed-rank test against a hypothetical value of 0. The contralateral Sto_2 difference was significantly less than 0 in the upper lobes at 0 and 30 days, and there was a trend in the lower lobes at 30 days (-2.9 ± 1.3, n = 8; $P = 0.069$). N = 5–8 patients per group per time point. *Bars* represent mean ± SEM. (*B*) Sto_2 levels were measured in both SOLT and bilateral orthotopic lung transplantation (BOLT) patients. Paired measurements were collected in each patient at the native main carina (above the anastomosis) and the donor upper lobe, subcarina, and lower lobe tissues (below the anastomosis) at 0 (*black bars*), 3 (*gray bars*), and 30 (*white bars*) days after SOLT or BOLT. The anastomosis Sto_2 difference (donor – main carina) was calculated for upper lobe, subcarina, and lower lobe tissues and analyzed by the Wilcoxon signed-rank test against a hypothetical value of 0. Differences were significantly less than 0 in the upper lobes and subcarinae at 0 and 30 days post-transplantation (*$P < 0.05$). N = 7–11 patients per group. *Bars* represent mean ± SEM.

1.5 ± 2.1, n = 6; $P = 0.053$). Furthermore, patients with prolonged hospital stays (defined as ⩾15 d) displayed significantly higher *HMOX1* expression (11.5 ± 4.2, n = 5 vs. 1.9 ± 0.4, n = 6; $P < 0.05$) and trends for higher *VEGFA* and *KDR* (*VEGFR2*) expression (both $P \leqslant 0.1$) than patients discharged within 15 days of transplantation. We also found significantly higher *VEGFA* (3.7 ± 1.0, n = 5 vs. 1.7 ± 0.3, n = 9) and *KDR* (*VEGFR2*) (4.1 ± 1.3, n = 4 vs. 0.7 ± 0.1, n = 5) mRNA expression in adjacent viable mucosa in patients with extensive airway necrosis (both $P < 0.05$) and higher *VEGFA* expression in patients who developed CAS (4.8 ± 1.2, n = 2 vs. 2.0 ± 0.4, n = 12; $P < 0.05$) (Figure 5).

Discussion

In evaluating the airway hypoxic response in patients undergoing lung transplantation, we detected lower oxygen saturations in donor bronchial mucosa from the time of transplantation that persisted for at least 30 days post-transplantation. In conjunction, hypoxia-responsive genes (e.g., *VEGFA*) were significantly up-regulated in donor airways, which was associated with substantially greater post-transplantation morbidity, including prolonged respiratory failure, prolonged hospital stay, more severe airway necrosis, and the development of CAS.

As the only solid organ transplanted without surgical anastomosis of the arterial circulation, the lung's conducting airways would be expected to be at risk for complications. The bronchial arterial circulation is critical for normal mucociliary clearance, airway lining fluid composition, and bronchial temperature and nourishment (22–24). Its disruption at the time of transplantation has been postulated to increase the risk of airway complications, such as necrosis, infection, and bronchiolitis obliterans syndrome (6, 10–15, 24–26). Although several studies have presented circumstantial evidence of airway microvascular insufficiency linked to airway fibrosis (10–12), the first direct measurements of airway oxygenation after lung transplantation by Dhillon and colleagues demonstrated significantly reduced Sto_2 levels in large airway mucosa of the donors (6). Murine studies using orthotopic tracheal transplantation have also supported these observations (10, 13–15). Our study was the first to serially measure Sto_2 in patients, which in conjunction with the published murine studies (13, 14), indicated that although donor airway oxygenation might recover slightly over the first month after transplantation, donor airways remain significantly hypoxic relative to native airways. The upper lobes in particular were hypoxic, which might be due to their intrinsically higher ventilation/perfusion ratio. Our endobronchial Sto_2 measurements were slightly higher than those obtained by Dhillon and colleagues (∼67% vs. ∼60%) (6) for unknown reasons. There might be institutional differences in surgical anastomoses or donor selection, differences in supplemental oxygen administration during bronchoscopies, and/or differences in timing of the measurements (0–30 days after lung transplantation in our study vs. 1–12 months in the Dhillon and colleagues study). The magnitude of the differences between donor and native airway Sto_2 was similar between the two studies (<10% absolute difference), suggesting precision in our oximetry data. We also did not know whether this modest reduction in donor endobronchial Sto_2 was large enough to cause clinically significant changes in the airway mucosa (9). However, our data indicated that even this small reduction in Sto_2 was enough to activate hypoxic gene expression in donor bronchial epithelium,

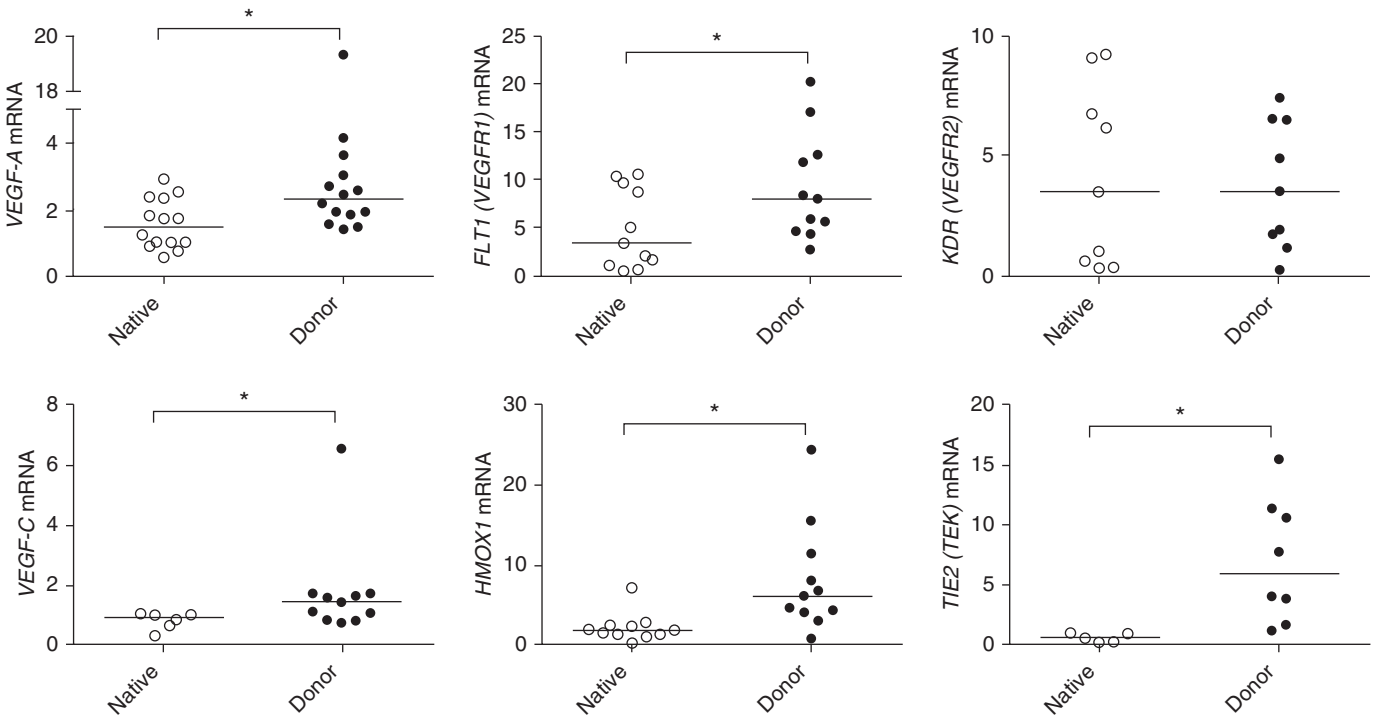

Figure 3. Real-time polymerase chain reaction of hypoxia-inducible genes in native (*open circles*) and donor (*solid circles*) endobronchial tissues of individual subjects. N = 5–14 per group. *Bars* represent median. *$P < 0.05$ is calculated by the Wilcoxon signed-rank test (*VEGF-A*, *FLT1*, *KDR*, and *HMOX1*), or by the Mann-Whitney *U* test if transcript data were missing (*TIE2* and *VEGF-C*). Because of limited RNA sample, not every transcript could be measured for every patient. See Figure E2 for additional genes tested.

which was a biomarker for clinically significant airway complications. Clearly, these associations require further prospective validation; however, the oxygen levels of the donor airways were measured by T-stat oximeters, which measure bronchial mucosal capillary hemoglobin saturation, and might have been disproportionately influenced by higher oxygen diffusion rates from the airways during supplemental oxygen administration. In addition, although not tested in this study, any bronchial mucosa oxygen deficit might be exaggerated by cardiopulmonary stresses, such as anemia, hypoperfusion, infection, or even exercise after lung transplantation.

The cell's main transcriptional responses to hypoxia are generated by activation of hypoxia-inducible factor-1α (HIF-1α), a constitutive and constitutively degraded transcription factor that is stabilized by low cytosolic oxygen tensions. After stabilization, the protein heterodimerizes and translocates to the nucleus to activate transcription of hypoxia-responsive genes (27, 28). HIF-1α activation is critical to the early airway hypoxia response, and its overexpression after orthotopic tracheal transplantation in mice can restore airway microvasculature and prevent chronic rejection and airway fibrosis (15, 29–31). We did not measure HIF-1α protein levels directly due to its instability and the limited quantities of the endobronchial biopsy specimens, but instead measured the mRNA expression of HIF-1α–dependent genes, such as *VEGFA*. *VEGFA* is critical for vascular endothelial cell proliferation, differentiation, mobilization, and survival (32, 33), and its expression together with the expression of *FLT1* receptor (*VEGFR1*) are increased in donor bronchial tissues relative to native tissues. In contrast to *FLT1*, *KDR* expression was not induced by hypoxia, but rather displayed heterogeneity, perhaps representing two populations (high and low). These findings are consistent with known hypoxic regulation of *FLT1* but not *KDR* (34), and suggest *VEGFA* and *FLT1* expression may be useful markers for large airway hypoxia.

In contrast to the cytoprotective effects of *VEGF*, it is also known that *VEGFA* over-expression is associated with the development of bronchiolitis obliterans syndrome (35–37) and PGD (38, 39). In this study, higher *VEGFA* expression was found in patients who developed prolonged respiratory failure, severe airway necrosis, and CAS, and displayed a trend in patients with prolonged hospitalization after transplantation. A similar pattern was observed for *KDR*, which in addition to effecting pro-survival VEGFA signaling also mediates VEGFA-induced vascular permeability (32, 33). In addition, *KDR* expression trended higher in patients with

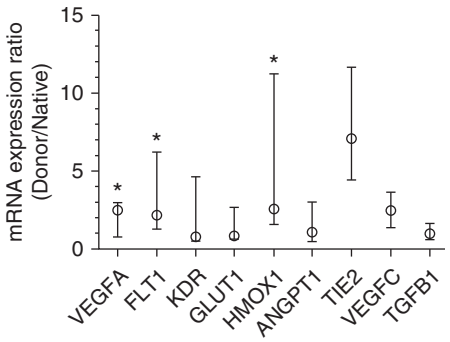

Figure 4. Median ratios of donor to native gene expression for paired samples. N = 5–14 per group. *Bars* represent interquartile range. *$P <$ 0.05 is calculated by the Wilcoxon signed-rank test against a hypothetical value of 1. There was also a trend for increased *TIE2* (*TEK*) and *VEGFC* expression (both $P = 0.062$).

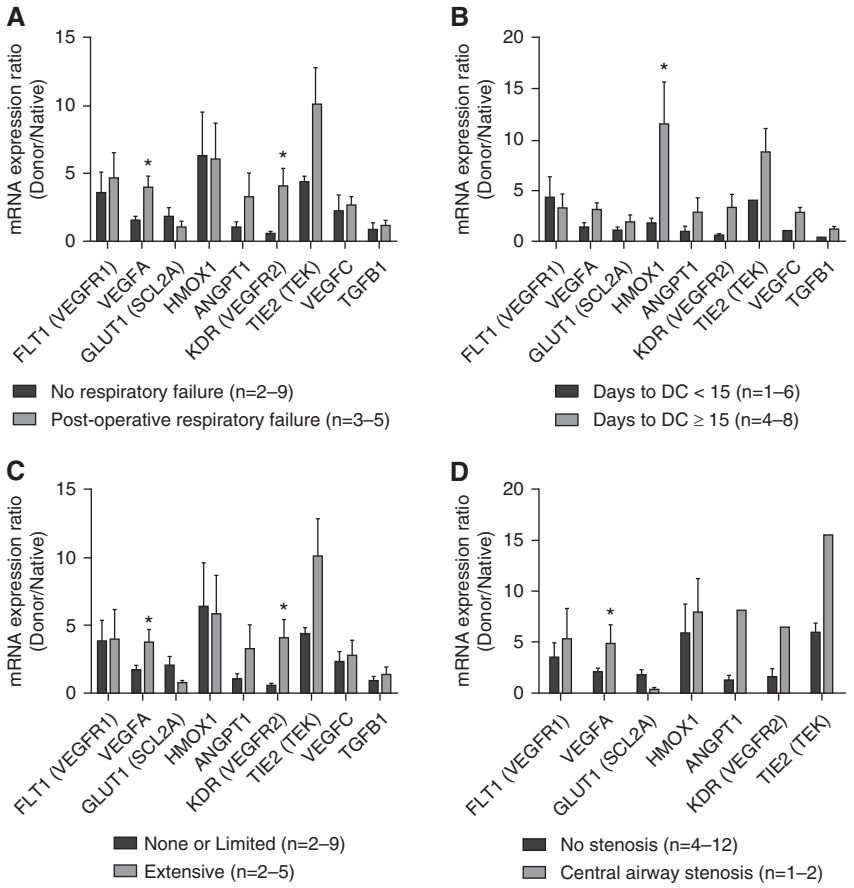

Figure 5. Comparison of donor/native gene expression ratios and clinical outcomes. Gene expression ratios in patients who developed prolonged respiratory failure (*gray bars*, n = 3–5) or did not (*black bars*, n = 2–9) (*A*), with time to hospital discharge (DC) ⩾15 days (*gray bars*, 4–8) or <15 days (*black bars*, 1–6) (*B*), with extensive airway necrosis (*gray bars*, n = 2–5) or no/limited airway necrosis (*black bars*, n = 2–9) (*C*), and with central airway stenosis (*gray bars*, n = 1–2) or no stenosis (*black bars*, n = 4–12) (*D*). *VEGFC* and *TGFB1* polymerase chain reactions were not performed in the patients that developed central airway stenosis owing to lack of remaining cDNA sample. *Bars* represent mean ± SEM. Patients with prolonged hospital stays after transplantation also displayed trends for higher *VEGFA* (3.1 ± 0.7, n = 8, vs. 1.5 ± 0.4, n = 6, $P = 0.076$) and *KDR* (*VEGFR2*) expression (3.4 ± 1.3, n = 5, vs. 0.7 ± 0.08, n = 4, $P = 0.10$). n represents number of transcripts measured per patient and ranges from 1 to 12 per group. Because of limited RNA sample, not every transcript could be measured for every patient. *$P < 0.05$ is calculated by the unpaired Student's *t* test.

more severe PGD (PGD score ⩾2). Overall, these findings support earlier observations of the opposing effects of VEGFA in promoting both tissue angiogenesis and tissue edema, and highlight *KDR* expression as a potentially useful marker for large airway complications after lung transplantation.

Tissue hypoxia also activates *HMOX-1* gene expression both early and late (40) via HIF-1α binding (41), but this gene is also activated by inflammation and pro-oxidants (42). We found significantly higher *HMOX-1* expression in donor bronchial tissue relative to native bronchial tissue, and patients with the highest levels had more severe large airway necrosis and significantly prolonged hospitalizations post-transplantation. Thus, endobronchial *HMOX-1* expression might serve as a biomarker for persistent ischemic, inflammatory, and/or oxidative stresses after transplantation, similar to previous observations in patients with bronchiolitis obliterans and acute rejection (43). However, *HMOX-1* is crucial for coordinating cellular repair programs, such as the counter-inflammatory response and mitochondrial biogenesis (42), and is also associated with good allograft survival (44). Therefore, the increased endobronchial *HMOX-1* expression observed here is also consistent with a cytoprotective response and impending tissue repair. This distinction will require confirmation in additional studies.

The following limitations may temper the interpretation of our findings. First, that the association of the expression of hypoxia-responsive genes with airway complications after lung transplantation does not address the causes of these transcriptional changes, which could be due to other factors (e.g., respiratory failure). However, the differences between native and donor tissues implicate local processes (e.g., cellular hypoxia) that predominate over systemic ones (e.g., respiratory failure). A second issue is that comparing gene expression between native and donor tissues represents the expression profiles of two different individuals (donor and recipient) and may therefore reflect genetic variability between donor and recipient rather than tissue response to the extracellular environment. Nevertheless, our findings in this hypothesis-driven study were consistent enough to implicate hypoxia at the very least as a contributing factor to the development of airway complications and invites further prospective investigation.

In summary, patients undergoing lung transplantation display low donor airway Sto_2 at multiple sites in the lungs accompanied by selected hypoxia-responsive gene expression over the first month post-transplantation. Elevated levels of *VEGFA* and *KDR* mRNA were associated with increased post-transplantation respiratory complications, including prolonged respiratory failure, extensive airway necrosis, and CAS. Although these associations do not demonstrate cause and effect, the consistency of the findings does suggest bronchial hypoxia as a clinically significant factor in airway complications. Strategies to preserve the bronchial circulation during lung transplantation, such as bronchial artery revascularization, may reduce airway complications (45, 46), but these strategies require validation. Alternatively, intermittent oxygenation of ischemic bronchial tissues with postoperative hyperbaric oxygen therapy may improve anastomotic healing and reduce CAS (47, 48), and is currently undergoing investigation at our institution (NCT02363959). ■

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