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Transcript Signatures of Lymphocytic Bronchitis in Lung Allograft Biopsy Specimens

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Abstract

Background—Rejection and obliterative bronchiolitis are barriers to sustained graft function in recipients of transplanted lungs. Early detection is hindered by inadequate tests and an incomplete understanding of the molecular events preceding or accompanying graft deterioration.

Methods—Hypothesizing that genes involved in immune responses and tissue remodeling produce biomarkers of rejection, we measured the expression of 192 selected genes in 72 sets of biopsy specimens from human lung allografts. Gene transcripts were quantified using a 2-step, multiplex, real-time polymerase chain reaction approach in endobronchial and transbronchial biopsy specimens from transplant recipients without acute infections undergoing routine surveillance bronchoscopy.

Results—Comparisons of histopathology in parallel biopsy specimens identified 6 genes correlating with rejection as manifested by lymphocytic bronchitis, a suspected harbinger of obliterative bronchiolitis. For example, β 2-defensin and collagenase transcripts in inflamed bronchi increased 37-fold and 163-fold, respectively. By contrast, these transcripts did not correlate with acute rejection in transbronchial specimens. Further, no correspondence was noted between histopathologic bronchitis and parenchymal rejection when endobronchial and transbronchial samples were obtained from the same patient.

Conclusions—Our highly sensitive method permits quantitation of many gene transcripts simultaneously in small, bronchoscopically acquired biopsy specimens of allografts. Transcript signatures obtained by this approach suggest that airway and alveolar responses to rejection differ and that endobronchial biopsy specimens assess lymphocytic bronchitis and chronic rejection but are not proxies for transbronchial biopsy specimens. Further, they reveal changes in airway expression of the specific genes involved in host defense and remodeling and suggest that the measurement of transcripts correlating with lymphocytic bronchitis may be diagnostic adjuncts to histopathology.

Lung transplantation is an option for patients with end-stage lung disease. Advances in organ preservation, surgery, and perioperative management have improved outcome.^{1–3} However, rejection continues to contribute to morbidity and mortality. The greatest barrier to sustained allograft performance is obliterative bronchiolitis (OB), which is poorly understood but may be related to rejection-associated airway inflammation.^{4–6}

Allograft recipients are at risk of acute rejection, with characteristic perivascular infiltration of lymphocytes in lung parenchyma. The present standard for the diagnosis of acute rejection is transbronchial biopsy (TBB) histopathology.^{4,7,8} Surveillance TBB can detect asymptomatic rejection but can cause bleeding and pneumothorax. The endobronchial biopsy (EBB) is potentially a safer means of sampling and can complement diagnosis by TBB⁹; however, there is no evidence that EBB can replace TBB for monitoring rejection.

Results obtained with tests less invasive than biopsies, including spirometry,¹⁰ high-resolution tomography,^{11,12} exhaled nitric oxide,¹³ and blood and bronchoalveolar lavage cell phenotyping,¹⁴ are encouraging but have not yet produced compelling evidence that they can substitute for histologic analysis. Several mediators, cytokines, and chemokines are implicated in rejection.^{15,16} Recently, microarray profiling of lavage cells identified a number of genes that correlate with acute rejection.¹⁷

Chronic rejection is graft dysfunction characterized histologically by OB and physiologically by airflow limitation, often termed *bronchiolitis obliterans syndrome* (BOS).¹⁸ Early predictive markers are lacking.¹⁵ Its connection with alloimmunity is based partly on a correlation with the severity of prior acute rejection.^{6,19,20} Curiously, experimental OB can progress even if transplanted organs are removed from an alloimmune environment;²¹ thus, persistent rejection may not be essential for progression. Airway virus infection, ischemia–perfusion injury, human leukocyte antigen mismatching, and lymphocyte–epithelial interactions also are implicated.^{4,19,20,22–24} Unlike acute rejection, which reverses with increased immunosuppression, chronic OB/BOS responds poorly.

Although histopathology is now the main means of diagnosing acute rejection, safer and more sensitive means of detecting rejection and pre-clinical OB are needed to test the hypothesis that earlier detection and intervention will forestall irreversible impairment.¹⁵ The molecular basis of acute rejection and OB also needs to be better understood. Among the potential benefits of such an understanding is the identification of surrogate markers of rejection. Lung transplantation studies have been limited by the inability to measure potential markers in small samples. Seeking transcriptional signatures of rejection in human allograft biopsy specimens for diagnostic purposes, we used multiplex, real-time polymerase chain reaction (PCR) amplification of cDNA from EBB and TBB specimens to profile the expression of selected genes with postulated roles in rejection and remodeling.

Methods

Study Design, Biopsy Specimens, and Grading

The subjects in this prospective study were consecutively enrolled lung transplant recipients undergoing surveillance bronchoscopy according to the following schedule: every 2 weeks for the first 2 months after transplantation, then monthly for 6 months, quarterly for the next year, and every 6 months thereafter. EBB and TBB were performed at the same sitting. Pre-bronchoscopic monitoring on the same day included pulmonary function testing and computed tomography scans. Six to 10 TBB specimens were sought from allograft lower lobes. Five to 6 EBB specimens were obtained from the sub-segmental carinae.

Two TBB and 2 EBB specimens were frozen immediately in liquid nitrogen, then stored at -80°C . The remaining samples were fixed, sectioned, stained, and examined by light microscopy. Rejection was classified by a pulmonary pathologist (KDJ) according to standard criteria,²⁵ with lymphocytic bronchitis graded B0 (none) to B4 (highest) and acute parenchymal rejection graded A0 to A4. Pulmonary function results were used to give participants a BOS grade (0, 0-p, 1, 2, or 3)¹⁸ based on forced expiratory volume in the first

second and maximal mid-expiratory flow rate as a percentage of the baseline (mean of 2 best values obtained post-transplantation at least 3 weeks apart).

RNA Extraction, Primer Design, and PCR

Frozen EBB or TBB specimens from a given bronchoscopy were pooled and powdered. Total RNA was isolated and incubated with DNase to remove residual genomic DNA. Repurified aliquots were applied to Nano LabChips (Agilent Technologies, Palo Alto, CA) to determine RNA quality and quantity.

We selected 192 genes for analysis. The first set of primers was used in multiplex reverse transcriptase-PCR amplification using a previously described strategy.^{26,27} A second set of primers, corresponding to the sequence lying within amplicons generated with the initial primers, was used in a real-time quantitation of individual transcripts in the biopsy specimens in conjunction with gene-specific, oligonucleotide probes labeled with reporter 6-carboxy-fluorescein and quencher BHQ1 at the 5'- and 3'-termini, respectively. EBB and TBB cDNAs were generated by reverse transcription.

To test for genomic DNA contamination, control samples were incubated without reverse transcriptase. The multiplex, hot-start PCR step was optimized²⁶ and carried out for 20 cycles, allowing measurements over a broad range of abundance without loss of proportionality. Each pre-amplification involved 192 gene-specific primer sets. Individual transcript levels were determined by real-time PCR in 384-well plates (2 biopsy specimens per plate). Amplifications were performed for 40 cycles at 95°C for 15 seconds and 60°C for 1 minute using an ABI Prism 7900HT cycler (Applied Biosystems, Foster, CA).

Data Transformation and Analysis

Transcript cycle threshold (Ct) values were transformed to copy number and normalized to housekeeping transcript expression.²⁶ The most stably expressed housekeeping gene was determined using geNorm software.²⁸ The linear correlation between transcript levels in paired EBB and TBB specimens was assessed via Pearson's coefficient. Differences in transcript levels in EBB and TBB specimens were assessed via Student's *t*-test. Differences between groups stratified by pathologic grade were assessed by one-way analysis of variance and Bonferroni's multiple comparison test. Sensitivity and specificity were calculated by using the histopathologic grade as the gold standard.

Results

Patient Characteristics

We recruited 22 patients prospectively. Of these, 10 had 1 bronchoscopy during the recruitment interval and 12 patients had 2 or more. Forty bronchoscopies were performed, for 32 of which paired EBB and TBB specimens were obtained at the same sitting. In 7 participants, an EBB specimen was obtained without TBB. In 1 instance, a TBB specimen was obtained without EBB. Patient characteristics are summarized in Table 1. All participants received maintenance immunosuppressive medications, including prednisone, cyclosporine or tacrolimus, and mycophenolate; 2 also received sirolimus.

At the time of biopsy, most participants manifested subtle obstruction on spirometry compared with baseline and were classified BOS 0-p. Of those with greater obstruction, 2 were graded BOS 1, 1 was graded BOS 2, and 2 were graded BOS 3. None had a histopathologic diagnosis of OB, which is uncommonly established by a TBB specimen.¹⁸ Of all the patients, only 1 had a lower BOS grade at the time of biopsy than at an earlier time after transplantation, consistent with prior observations that BOS seldom improves. Of participants sampled more

than once, only 1 increased BOS grade between biopsies. None of these patients undergoing surveillance testing were diagnosed with acute lung infections based on symptom assessment and chest imaging on the day of bronchoscopy. Routine bacterial and viral cultures of lavage fluid were negative, except for a few instances largely attributable to colonization upon correlation with radiologic and pathologic data.

Histopathology

Most subjects lacked airway inflammation and acute parenchymal rejection and thus received grades of B0 and A0 on histopathologic examination of the EBB and TBB specimens. With regard to lymphocytic airway inflammation, 8 patients ranked B1 (minimal), 7 ranked B2 (mild), and 3 ranked B3 (moderate). At the B3 level, bronchial tissue contains dense bands of sub-mucosal mononuclear cells infiltrating the mucosa, with epithelial necrosis or apoptosis. For those with acute parenchymal rejection, 5 ranked A1 (minimal), 2 ranked A2 (mild), and 1 ranked A3 (moderate). At the A3 level, venules and arterioles are cuffed by dense lymphocytic infiltrates with extension into the adjacent interstitium. No participants ranked grade 4 (severe) in either category.

Thus, as expected of surveillance samples, these biopsy samples are weighted toward lower grades of rejection. Few subjects exhibited simultaneous airway inflammation and acute parenchymal rejection (Table 2). Of 27 pairs of histologically gradable samples in which the EBB specimens contained airway mucosa and the TBB specimens contained alveoli, just 3 achieved grades greater than 0 (in each case A1:B1) in both types of biopsy samples. These specimens included 9 EBB graded B1–B3 paired with TBB graded A0, and 5 TBB graded A1–A3 paired with EBB graded B0. Thus, lymphocytic bronchitis and parenchymal rejection appear to be mostly independent by histopathologic criteria.

Transcript Measurements

Intact mRNA suitable for analysis was obtained from all samples. In the recruited population, pilot studies suggested the importance of freezing biopsy samples immediately after acquisition and of manually crushing frozen samples in small-volume polypropylene tubes to minimize tissue loss and mRNA degradation. Mean yield of total RNA was 1.7 and 1.4 μg per pooled EBB and TBB samples, respectively. For all samples, the difference in Ct obtained from the products remaining after incubation with or without reverse transcriptase was between 13 and 27, indicating negligible (1.2×10^{-3} to $7.5 \times 10^{-7}\%$ or less) contamination of pre-amplified cDNA with genomic DNA.

Our procedure quantified a large number of gene transcripts simultaneously, with levels varying over 6 to 7 orders of magnitude, demonstrating extensive dynamic range (see Figures 1 and 2.) Of 5 candidate housekeeping gene transcripts, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was 1 of the 2 most stably expressed in the EBB and TBB specimens and was used for normalization in this study. For each gene product in a given sample, real time Ct was converted to a GAPDH-normalized relative copy number to allow comparisons between biopsy samples.

Among the biopsy samples, relative transcript levels were nearly invariant for some genes. These transcripts achieved copy numbers of greater than 300 but did not vary with pathologic grade or biopsy site and thus are not markers of rejection. In this group, the most abundantly expressed are serine peptidase inhibitor SPINT1 (an epithelial inhibitor of matrix-degrading matriptase), histamine H2 receptor, protease-activated receptor 2, platelet-derived growth factor- α , and MUC1 mucin. Seventy-three of 192 genes were expressed at low levels, with copy numbers consistently less than 300 and were excluded from analysis. Expression of other

genes, including some of the most informative, was variable. Examples are shown in Figures 1 and 2.

Correlation of Transcript Levels With Rejection

Levels of selected transcripts stratified by pathologic grade are shown in Figure 1. In selecting genes for analysis, we hypothesized that increased expression of collagen, matrix metalloproteinases (MMPs) and their inhibitors, and other extracellular matrix components accompanies airway remodeling and precedes the development of clinical OB. We found that levels of several of this type of transcript correlated strongly with increasing grades of airway inflammation, including interstitial collagenase/MMP1 ($p < 0.001$), MMP2 ($p < 0.05$), collagen type I α chain ($p < 0.05$) and fibronectin ($p < 0.01$).

In contrast, these transcripts are unchanged or tend to decline with acute parenchymal rejection (Figure 1). Thus, they are not linked to lymphocytic inflammation per se. While levels of some MMP transcripts rise, those of their inactivators, tissue inhibitors of metalloproteases (TIMPs) 1 and 2, remain steady. Additionally, transcripts encoding β -defensin-2 (DEFB-2), an anti-microbial inflammation-induced product of respiratory epithelium,²⁹ are 37-fold higher in grade B3-inflamed bronchi than in B0 (un-inflamed) bronchi, and transcripts encoding lung chemokine SCYA18³⁰ also rise significantly. Neither transcript increases in parenchymal rejection (Figure 2).

Sensitivity and specificity of EBB transcript measurements in detecting lymphocytic bronchitis vary by gene, pathologic grade, and cutoff point employed. For example, the sensitivity of a MMP1 copy number greater than 10^4 in detecting a pathologic grade of B1 or greater is 67%, with specificity also of 67%. For fibronectin, with a $10^{3.75}$ cutoff, sensitivity and specificity for the same type of comparison is 61% and 68%, respectively. Sensitivity and specificity of other transcripts correlated with pathologic grade in EBB specimens are similar.

Transcript levels and BOS grade were not significantly correlated, as expected, given that most subjects belonged to one BOS category (0-p).

Comparison of Transcript Levels in EBB and TBB Specimens

Of transcripts expressed at higher levels in TBB vs EBB specimens (Table 3), the most differentially expressed in TBB specimens are intercellular adhesion molecule (ICAM)-1 and arachidonate 5-lipoxygenase (ALOX5), both of which were 12-fold higher in TBB specimens ($p < 0.001$ in each case). Transcripts differentially overexpressed in EBB specimens include mucins 4 (MUC4) and MUC5AC ($p < 0.001$ for each), as well as tryptase- ϵ , MMP11, and connective tissue growth factor (CTGF) (see Table 3 and Figure 2).

Regarding the 32 bronchoscopies in which EBB and TBB specimens were obtained from the same patient during the same procedure, transcript levels for 71 of the genes are significantly correlated in paired samples (Figure 2 and Table 4). Several patterns emerge. For example, although MUC1 levels do not differ between TBB and EBB specimens overall, they are highly correlated ($r = 0.816$, $p < 0.001$) in EBB/TBB pairs. The positive correlation is just as striking for CTGF ($r = 0.829$, $p < 0.001$), even though mean transcript levels are significantly higher in EBB than in TBB specimens, with individual levels varying over a range of more than 200-fold (Figure 2).

Despite correlating well in paired biopsy specimens, neither transcript correlates with pathologic grade. For these genes, correlation in a given patient appears to have a basis other than acute rejection pathology. Additional genes vary widely and are neither differentially transcribed nor significantly correlated in EBB/TBB pairs—and yet some of these, such as MMP1, correlate strongly with pathology (Figure 1). Other genes, such as TIMP2, are

differentially expressed in one type of biopsy and, though not linearly correlated in plots of paired samples, exhibit a narrow range of expression (Figure 2). This spectrum of observations is a reminder that the utility of a given transcript in the diagnosis or prediction of rejection can be independent of correlation in paired biopsies.

Discussion

Gene expression profiling is rapidly becoming an essential tool for research and drug discovery and may soon play a central role in clinical diagnostics. This study profiles a large cohort of genes in human lung allografts by applying a high-sensitivity PCR approach to bronchoscopically acquired biopsy specimens. We identify several genes whose transcripts correlate strongly with histopathologic airway rejection/lymphocytic bronchitis. The data also identify genes overexpressed in EBB or TBB specimens, and others correlated between EBB and TBB samples from a given patient. Our work applies multiplex, real-time PCR to bronchoscopic biopsy specimens from lung transplant recipients. This approach has potential advantages of sensitivity and wide dynamic range, and therefore, can assess high-abundance and low-abundance transcripts in the same assay; reproducibility, as validated by the sub-set of genes, subject to little variation; and speed as PCR steps can be completed in 1 day.

The success in harvesting mRNA suitable for analysis is also notable. Once the extraction technique was refined, no sample was insufficient for analysis because of degradation or small size. This implies that we have not stretched the sensitivity limits of this assay. The most recent applications of this technique suggest that it can profile similar numbers of genes in a few cells obtained from tissue sections by laser capture microscopy (unpublished data).

Although the expression of about 33% of the transcripts was consistently low, this relates to low relative abundance rather than small sample size. In our samples, enough mRNA remained to assay more than 1,000 additional unique transcripts. These archived transcripts, indexed for pathologic grade in parallel EBB and TBB specimens, are a resource for future profiling experiments. The current study identifies a sub-set of genes of potential diagnostic utility. Screening of further cohorts of genes may identify additional informative transcripts. The ultimate goal is to winnow the list of candidate genes to a maximally informative sub-set. Our experience with these experiments leads us to predict that a panel of transcripts, rather than the product of a single gene, will be more useful.

Adaptation of this technique to a 384-well format raises the possibility that such an assay can be used in high-throughput fashion in a clinical lab setting with samples from many patients. One potential drawback is the high initial cost of TaqMan-type probes and primers for each gene included in the analysis; although once synthesized, these probe/primer sets can be used for many assays. Another limitation, compared with microarray approaches, is the number of genes that can be analyzed simultaneously (practically, around 200). However, this is offset by much higher sensitivity and dynamic range compared with current, hybridization-based microarrays, which require larger amounts (1–10 µg) of starting RNA and therefore are not applied easily to small biopsy samples.

Although our results identify 6 transcripts that correlate strongly with pathologic grade in EBB specimens, they also reveal variability in transcript levels relative to histopathology. This is reflected in the modest sensitivity and specificity of individual transcript levels compared with the gold standard of histopathologic grading of biopsy specimens obtained in parallel at the same sitting.

Even if levels of these particular transcripts perfectly reflected the degree of lymphocytic bronchitis in a given sample, there would, however, remain limits to sensitivity and specificity in relation to the histopathology of parallel biopsy specimens because of patchy distribution

of disease. We know that patchiness exists from the variation in pathologic grade in separate biopsy samples obtained from the same patient during the same bronchoscopy. This is also implied by the low yield of bronchoscopic biopsy specimens for diagnosing OB, which can produce severe functional deficits despite overt involvement of a small fraction of airways.¹⁸ Additionally, OB may show scarring without inflammation in its inactive phase.

Thus, sensitivity and specificity likely would improve if we had a better gold standard, though presently no suitable alternative exists.³¹ A more practical strategy for improving sensitivity and specificity of transcriptional profiling is to increase the number of samples—perhaps pooling them to obtain a broader representation of a potentially patchy process.

A comparison of transcription profiles of EBB and TBB specimens in the same patient, as shown in Figure 2 and Table 3, identifies transcripts strongly overexpressed in one type of biopsy specimen compared with the other. This suggests the possibility of deriving a sub-set of genes characteristic of airway mucosa and alveolar parenchyma, respectively. Such a sub-set would be useful in estimating airway and alveolar contributions in potentially mixed samples, as in TBB, in which a sample can range from mostly airway to mostly lung parenchyma.

Of the genes surveyed, the most consistently ($p < 0.001$) overexpressed in EBB relative to TBB specimens are mucin genes MUC4 and MUC5AC (but not MUC1). Interestingly, neither MUC4 nor MUC5AC correlates with rejection, suggesting that they are markers of airways but not necessarily of inflammation or disease. Expression of these mucin genes in EBB specimens is so disproportionate that in the few paired biopsy samples in which EBB and TBB expression is approximately equal (see Figure 2) it is likely that the TBB contained mainly airway rather than alveolar material.

Among the larger set of genes overexpressed in TBB relative to EBB specimens, the most extreme examples are ALOX5 and ICAM1. Because neither of these genes correlates with pathologic grade and both are expressed 12-fold higher in TBB than EBB samples, they may reflect alveolar content of a given sample independent of pathology. Other genes overexpressed in TBB specimens are MMP9 and TIMP2 (Figure 2). In the case of MMP9, disproportionate expression in TBB samples may be due to alveolar macrophages, which are a source of MMP9 and also of ALOX5.³² On the other hand, the most likely sources of differential alveolar expression of ICAM1 are vascular endothelium and type I epithelium.³³

A comparison of paired EBB and TBB samples also reveals several transcripts with very high linear correlations in the same patient (Figure 2 and Table 4). Interestingly, none of the transcripts that correlate well ($r > 0.8$, $p < 0.001$) in EBB/TBB pairs correlate with pathologic grade. CTGF is a prominent example featuring a wide range of expression, the basis of which is intriguing because of the correlation of CTGF with lung fibrosis³⁴ and, potentially, with OB. The variation in CTGF transcription could be inherited or acquired and be due to differences unrelated to the accumulation of mononuclear cells in bronchial and alveolar tissues.

In this regard, the lack of correspondence between high-grade bronchial inflammation and acute alveolar rejection in the same patient and bronchoscopy (Table 2) is also interesting. This suggests that the processes are at least partly independent and may reflect a different pathogenesis. It is also consistent with the recent appreciation of sub-types of acute allograft rejection featuring differences in immune activation and cellular proliferation indistinguishable by light microscopy,³⁵ recognition of a chronic onset form of BOS not strongly linked to acute rejection,³⁶ and involvement of innate as well as adaptive immune mechanisms in rejection.³⁷

These data not only shed light on the feasibility and diagnostic implications of measuring multiple transcripts in bronchoscopic biopsies but also provide clues about the mechanism. The finding that type I collagen, MMP, and fibronectin transcripts increase with increasing grades of lymphocytic bronchitis is especially meaningful because these gene products are associated with tissue remodeling and fibrosis, as in OB. It is perhaps significant that while airway MMP transcripts rise, those encoding natural MMP-inhibiting TIMPs do not. This may produce a protease–anti-protease imbalance in the airway wall.

On the other hand, TIMP levels are high in absolute terms—higher than many housekeeping genes. If mRNA levels mirror TIMP and MMP levels in these samples, there may be an excess of TIMP even after induction of MMP expression. Nonetheless, some MMPs can be activated even while bound to TIMP³⁸ so that the TIMP/MMP ratio may be less important than the amount of MMP produced and the presence of an activating mechanism.

The nature and importance of protease/anti-protease imbalance in the evolution of remodeling and fibrosis in the airways of transplanted lungs is not well understood and is likely to be complex. Although the inhibition of some MMPs may favor the accumulation of collagen and other matrix proteins, it is also likely that active proteases are required for fibrosis to develop. This is because when one cell type replaces another (such as fibroblasts for epithelium) in remodeling tissues, or when new cells migrate into areas previously occupied by extracellular matrix, existing matrix proteins must be broken down. Thus, the action of MMPs and other proteases likely are essential for the evolution of fibrotic airway lesions, but the process may be altered by imbalances or dysregulation at critical phases.

Another transcript that increases greatly with higher grades of bronchitis is DEFB2. Defensins are a family of cationic peptides with broad-spectrum anti-microbial activity. DEFB2 is expressed in epithelia of many organs, including airway, where it is found in surface epithelia and serous cells of sub-mucosa glands.²⁹ Moreover, β -defensins may be chemotactic for dendritic and T cells.³⁹ Levels of SCYA18, a CC-class lymphocyte-selective chemokine preferentially expressed in lung,³⁰ also increase in high-grade bronchitis, although transcript levels are lower overall than those of other positively correlated genes. Because of their chemotactic effects, DEFB2 and SCYA18 induced in the allograft airway could augment rejection responses.

In summary, this work demonstrates a method for measuring large cohorts of gene transcripts in small, bronchoscopically acquired EBB and TBB specimens. The results provide a view of molecular events that accompany allograft inflammation and identify specific bronchitis-associated transcripts, which may be useful in diagnosing rejection events in the airways. These findings help explain mechanisms of acute rejection and OB, the major obstacles to long-term survival in lung transplant recipients.

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References

1. Stewart KC, Patterson GA. Current trends in lung transplantation. *Am J Transplant* 2001;1:204–10. [PubMed: 12102253]
2. DeMeo DL, Ginns LC. Lung transplantation at the turn of the century. *Annu Rev Med* 2001;52:185–201. [PubMed: 11160774]
3. Reichart B, Gulbins H, Meiser BM, Kur F, Briegel J, Reichenspurner H. Improved results after heart–lung transplantation: a 17-year experience. *Transplantation* 2003;75:127–32. [PubMed: 12544884]

4. Reichenspurner H, Girgis RE, Robbins RC, et al. Obliterative bronchiolitis after lung and heart-lung transplantation. *Ann Thorac Surg* 1995;60:1845–53. [PubMed: 8787504]
5. Husain AN, Siddiqui MT, Holmes EW, et al. Analysis of risk factors for the development of bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 1999;159:829–33. [PubMed: 10051258]
6. Sharples LD, McNeil K, Stewart S, Wallwork J. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. *J Heart Lung Transplant* 2002;21:271–81. [PubMed: 11834356]
7. Weill D, McGiffin DC, Zorn GL Jr, et al. The utility of open lung biopsy following lung transplantation. *J Heart Lung Transplant* 2000;19:852–7. [PubMed: 11008074]
8. Hopkins PM, Aboyoun CL, Chhajed PN, et al. Prospective analysis of 1,235 transbronchial lung biopsies in lung transplant recipients. *J Heart Lung Transplant* 2002;21:1062–7. [PubMed: 12398870]
9. Ward C, Snell GI, Orsida B, Zheng L, Williams TJ, Walters EH. Airway versus transbronchial biopsy and BAL in lung transplant recipients: different but complementary. *Eur Respir J* 1997;10:2876–80. [PubMed: 9493677]
10. Morlion B, Knoop C, Paiva M, Estenne M. Internet-based home monitoring of pulmonary function after lung transplantation. *Am J Respir Crit Care Med* 2002;165(5):694–7. [PubMed: 11874816]
11. Bankier AA, Van Muylem A, Knoop C, Estenne M, Gevenois PA. Bronchiolitis obliterans syndrome in heart-lung transplant recipients: diagnosis with expiratory CT. *Radiology* 2001;218:533–9. [PubMed: 11161175]
12. Gotway MB, Dawn SK, Sellami D, et al. Acute rejection following lung transplantation: limitations in accuracy of thin-section CT for diagnosis. *Radiology* 2001;221:207–12. [PubMed: 11568342]
13. Silkoff PE, Caramori M, Tremblay L, et al. Exhaled nitric oxide in human lung transplantation. A noninvasive marker of acute rejection. *Am J Respir Crit Care Med* 1998;157:1822–8. [PubMed: 9620912]
14. Whitford HM, Orsida B, Pais M, et al. Bronchoalveolar lavage (BAL) neutrophilia in lung transplant recipients (LTR): infection and bronchiolitis obliterans syndrome (BOS), the chicken or the egg? *J Heart Lung Transplant* 2001;20:260.
15. Hertz MI, Lande J, Gimino V, King R. Gene expression patterns in lung rejection. *Transplant Proc* 2001;33:3566–7. [PubMed: 11750517]
16. Zeevi A, Pavlakis M, Spichty K, et al. Prediction of rejection in lung transplantation. *Transplant Proc* 2001;33:291–2. [PubMed: 11266823]
17. Gimino VJ, Lande JD, Berryman TR, King RA, Hertz MI. Gene expression profiling of bronchoalveolar lavage cells in acute lung rejection. *Am J Respir Crit Care Med* 2003;168:1237–42. [PubMed: 12958056]
18. Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. *J Heart Lung Transplant* 2002;21:297–310. [PubMed: 11897517]
19. Girgis RE, Tu I, Berry GJ, et al. Risk factors for the development of obliterative bronchiolitis after lung transplantation. *J Heart Lung Transplant* 1996;15:1200–8. [PubMed: 8981205]
20. Kroshus TJ, Kshetry VR, Savik K, John R, Hertz MI, Bolman RM 3rd. Risk factors for the development of bronchiolitis obliterans syndrome after lung transplantation. *J Thorac Cardiovasc Surg* 1997;114:195–202. [PubMed: 9270635]
21. King MB, Pedtke AC, Levrey-Hadden HL, Hertz MI. Obliterative airway disease progresses in heterotopic airway allografts without persistent alloimmune stimulus. *Transplantation* 2002;74:557–62. [PubMed: 12352919]
22. Billings JL, Hertz MI, Savik K, Wendt CH. Respiratory viruses and chronic rejection in lung transplant recipients. *J Heart Lung Transplant* 2002;21:559–66. [PubMed: 11983546]
23. Fiser SM, Tribble CG, Long SM, et al. Ischemia-reperfusion injury after lung transplantation increases risk of late bronchiolitis obliterans syndrome. *Ann Thorac Surg* 2002;73:1041–7. [PubMed: 11996238]
24. Borger P, Kauffman HF, Scholma J, Timmerman JA, Koeter GH. Human allogeneic CD2+ lymphocytes activate airway-derived epithelial cells to produce interleukin-6 and interleukin-8. Possible role for the epithelium in chronic allograft rejection. *J Heart Lung Transplant* 2002;21:567–75. [PubMed: 11983547]

25. Yousem SA, Berry GJ, Cagle PT, et al. Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. *J Heart Lung Transplant* 1996;15:1–15. [PubMed: 8820078]
26. Dolganov GM, Woodruff PG, Novikov AA, et al. A novel method of gene transcript profiling in airway biopsy homogenates reveals increased expression of a Na⁺-K⁺-Cl-cotransporter (NKCC1) in asthmatic subjects. *Genome Res* 2001;11:1473–83. [PubMed: 11544191]
27. Ordóñez CL, Khashayar R, Wong HH, et al. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001;163:517–23. [PubMed: 11179133]
28. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;3:1–12.
29. Singh PK, Jia HP, Wiles K, et al. Production of beta-defensins by human airway epithelia. *Proc Natl Acad Sci U S A* 1998;95(25):14961–6. [PubMed: 9843998]
30. Guan P, Burghes AH, Cunningham A, et al. Genomic organization and biological characterization of the novel human CC chemokine DC-CK-1/PARC/MIP-4/SCYA18. *Genomics* 1999;56:296–302. [PubMed: 10087196]
31. Jeffery P, Holgate S, Wenzel S. Methods for the assessment of endobronchial biopsies in clinical research. *Am J Respir Crit Care Med* 2003;168(Pt 2):S1–S17. [PubMed: 14555461]
32. Drazen JM, Austen KF. Leukotrienes and airway responses. *Am Rev Respir Dis* 1987;136:985–98. [PubMed: 2821857]
33. Kang BH, Manderschied BD, Huang YC, Crapo JD, Chang LY. Contrasting response of lung parenchymal cells to instilled TNF alpha and IFN gamma: the inducibility of specific cell ICAM-1 in vivo. *Am J Respir Cell Mol Biol* 1996;15:540–50. [PubMed: 8879188]
34. Bonniaud P, Margetts PJ, Kolb M, et al. Adenoviral gene transfer of connective tissue growth factor in the lung induces transient fibrosis. *Am J Respir Crit Care Med* 2003;168:770–8. [PubMed: 12816739]
35. Sarwal M, Chua MS, Kambham N, et al. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. *N Engl J Med* 2003;349:125–38. [PubMed: 12853585]
36. Jackson CH, Sharples LD, McNeil K, Stewart S, Wallwork J. Acute and chronic onset of bronchiolitis obliterans syndrome (BOS): are they different entities? *J Heart Lung Transplant* 2002;21:658–66. [PubMed: 12057699]
37. Palmer SM, Burch LH, Davis RD, et al. The role of innate immunity in acute allograft rejection after lung transplantation. *Am J Respir Crit Care Med* 2003;168:628–32. [PubMed: 12773319]
38. Frank BT, Rossall JC, Caughey GH, Fang KC. Mast cell tissue inhibitor of metalloproteinase-1 is cleaved and inactivated extracellularly by alpha-chymase. *J Immunol* 2001;166:2783–92. [PubMed: 11160345]
39. Yang D, Biragyn A, Kwak LW, Oppenheim JJ. Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol* 2002;23:291–6. [PubMed: 12072367]

Figure 1.

Correlation of gene transcript levels with histopathology in endobronchial biopsy (EBB) and transbronchial biopsy (TBB) specimens. Results for selected pairs of genes are shown in each panel. Transcript levels were determined by multiplex, real-time polymerase chain reaction (PCR). For all panels the y-axis unit is the $\log_{10}$ value of the relative copy number, which is the transcript level of the gene of interest normalized to that of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the sample. All data are plotted on the same scale to facilitate comparison. Each data point is the mean $\pm$ standard error of the relative copy number of the transcript in patients with a given histopathologic grade as determined in parallel biopsy samples obtained from the same patient at the same sitting. For EBB specimens, the scale ranges from B0 (no lymphocytic airway inflammation) to B4 (severe inflammation with epithelial dissociation, ulceration and necrosis). B3 is the most severe grade in these subjects. For TBB, the scale ranges from A0 (normal parenchyma) to A4 (severe acute rejection with diffuse perivascular, interstitial and air space infiltrates of mononuclear cells). The highest grade in these biopsies is A3 (1 patient), which is grouped with A2 (2 patients). The genes profiled here are collagenase (matrix metalloproteinase-1, MMP1), MMP2, tissue inhibitor of metalloproteinases (TIMP) 1 and 2, β 2-defensin (DEFB2), lung chemokine SCYA18, fibronectin (FN1) and collagen α chain (COL1A1). Results of statistical tests applied between a given data point and the value at B0 or A0 are indicated (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$).

Figure 2.

Transcript profiles in paired endobronchial biopsy (EBB) and transbronchial biopsy (TBB) specimens. The position of each data point is determined by transcript level (copy number relative to level of glyceraldehyde 3-phosphate dehydrogenase [GAPDH] transcript) of a given gene product in EBB and TBB specimens obtained from the same patient at the same sitting. In each graph, the diagonal line marks where data points will fall if expressed at identical levels in both specimens of a given EBB/TBB specimen pair. Data points representing genes expressed preferentially in EBB or TBB specimens fall above and below the line, respectively. Examples are grouped according to relationships between gene products. The upper 6 panels display results involving selected matrix metalloproteinases (MMPs, MMP1, MMP2, MMP9, MMP11) and tissue inhibitor of metalloproteinases (TIMPs). Note the large differences in expression patterns between products, with MMP1 exhibiting a wide range of expression compared with TIMPs, which are near-constantly expressed at higher levels than any MMP. MMP9 is an example of a gene that is strongly overexpressed in TBB relative to EBB specimens, whereas the opposite is the case for MMP11. Among mucin genes depicted in the third row, MUC4 and MUC5AC are strongly overexpressed in EBB specimens. However, this is not true of all mucin genes, for MUC1 transcripts, while varying about 100-fold, are expressed at near-equivalent levels in any given EBB/TBB pair and a strongly correlated ($r = 0.816$, $p < 0.001$). Other strongly correlated genes are shown in the bottom row, including PRSS22 (ϵ -tryptase), MIF (macrophage migration inhibitory factor) and CTGF (connective tissue growth factor), with correlation coefficients of 0.862, 0.800, and 0.829, respectively (each with $p < 0.001$). These examples underscore the diversity of the expression of transcripts in terms of magnitude, range, and biopsy site.

Table 1

Subject Characteristics

Age (years, at time of transplant)	48 ± 11 (mean ± SD)
Gender	14 men 8 women
Ethnicity	16 Caucasian 3 African-American 2 Asian/Pacific Islander 1 Hispanic
Pre-transplant lung disease	6 emphysema 4 lymphangioleiomyomatosis 4 primary pulmonary hypertension 3 idiopathic pulmonary fibrosis 3 cystic fibrosis 1 sarcoidosis
Transplant type	1 silicosis 13 bilateral 8 left 1 right
Months since transplant *	27 ± 25 (mean ± SD)

* Until first biopsy if biopsied more than once. SD, standard deviation.

Pathological Grade of Lymphocytic Bronchitis and Alveolar Acute Rejection in Paired Samples, Each Obtained from the Same Patient at the Same Bronchoscopy

	Pathologic Grade: Bronchi				Total
	B0	B1	B2	B3	
Pathologic Grade: Alveoli					
A0	12	2	4	3	21
A1	2	3	0	0	5
A2	2	0	0	0	2
A3	1	0	0	0	1
Total	17	5	4	3	

Table 3
Differential Transcription in Endo bronchial Biopsy (EBB) vs. Transbronchial Biopsy (TBB) Specimens

Gene	Accession #	Transbronchial Transcript level*	Endobronchial Transcript level*	p [†]
TBB > EBB				
ANGPT1	NM_001146	8.79 ± 1.36	1.52 ± 0.20	<0.0001
ANGPT2	NM_001147	3.64 ± 0.87	1.70 ± 0.39	0.010
CSF1	NM_000757	0.0218 ± 0.0056	0.00491 ± 0.00114	0.002
EDN1	NM_001955	1.88 ± 0.48	0.177 ± 0.029	0.001
HGF	M73239	0.661 ± 0.137	0.135 ± 0.025	<0.0001
IGF1	NM_000618	2.70 ± 0.73	0.623 ± 0.130	0.004
IL16	NM_004513	1.29 ± 0.30	0.331 ± 0.062	0.001
IL18	NM_001562	2.25 ± 0.59	1.37 ± 0.27	0.041
NGFB	NM_002506	1.22 ± 0.38	0.276 ± 0.056	0.016
VEGF	NM_003376	44.6 ± 9.2	8.66 ± 1.19	<0.0001
VEGFC	NM_005429	3.26 ± 0.60	1.15 ± 0.19	0.001
CCRL2	NM_003965	0.583 ± 0.156	0.0357 ± 0.0079	0.001
SCYA18	NM_002988	0.785 ± 0.254	0.0304 ± 0.0159	0.005
SCYA5	NM_002985	12.9 ± 3.3	3.01 ± 0.80	0.001
SCYA3	NM_002983	0.177 ± 0.055	0.0881 ± 0.0316	0.003
PDGFB	NM_002608	9.22 ± 1.74	1.92 ± 0.38	<0.0001
TNFA	NM_000594	0.119 ± 0.033	0.0210 ± 0.0071	0.001
TNFSF10	NM_003810	8.46 ± 1.79	2.93 ± 0.58	0.002
IL13RA1	NM_001560	1.87 ± 0.49	0.521 ± 0.067	0.009
CCR2	NM_000648	2.71 ± 0.64	0.857 ± 0.217	0.003
CXCR4	NM_003467	27.7 ± 7.5	7.86 ± 1.49	0.009
CYSLT1	NM_006639	2.19 ± 0.46	1.18 ± 0.19	0.007
GPR9	NM_001504	2.56 ± 0.57	1.40 ± 0.68	0.006
NRP1	NM_003873	47.7 ± 6.6	14.2 ± 1.5	<0.0001
NRP2	NM_003872	0.0145 ± 0.0045	0.00587 ± 0.00119	0.030
CD14	NM_000591	10.3 ± 1.5	1.86 ± 0.41	<0.0001
CD23A	NM_002002	0.0187 ± 0.0055	0.00204 ± 0.00062	0.004
CD40L	NM_000074	0.223 ± 0.060	0.0779 ± 0.0253	0.008
CD3E	NM_000733	0.00771 ± 0.00257	0.0024 ± 0.0009	0.012
CD44	NM_000610	44.2 ± 5.8	11.9 ± 1.5	<0.0001
ICAM1	NM_000201	75.3 ± 9.1	6.20 ± 1.35	<0.0001
ITGA4	NM_000885	0.00255 ± 0.00088	0.000460 ± 0.000190	0.01
ITGB1	NM_002211	236 ± 26	113 ± 19	<0.0001
PECAM1	NM_000442	194 ± 28	33.1 ± 3.9	<0.0001
SELL	NM_000655	2.17 ± 0.95	0.234 ± 0.062	0.048
ADAM17	NM_003183	1.29 ± 0.41	0.327 ± 0.056	0.014
CTSC	NM_001814	0.0477 ± 0.0140	0.0231 ± 0.0050	0.026
CTSG	NM_001911	0.0314 ± 0.0082	0.00463 ± 0.00168	0.003
CTSL	NM_001912	61.5 ± 11.1	16.9 ± 2.6	<0.0001
CTSS	NM_004079	9.32 ± 1.59	3.06 ± 0.48	<0.0001
PLAT	NM_000931	3.21 ± 0.53	1.54 ± 0.24	0.002
PLAU	NM_002658	12.9 ± 2.8	5.36 ± 1.73	0.015
PRSS8	NM_002773	110 ± 20	40.7 ± 5.2	0.001
TPSB1	NM_003294	13.4 ± 2.6	4.40 ± 0.82	0.001
MMP9	NM_004994	0.800 ± 0.227	0.169 ± 0.069	0.009
TIMP1	NM_003254	65.7 ± 8.9	41.9 ± 6.8	0.006
TIMP2	NM_003255	68.8 ± 8.2	21.7 ± 2.9	<0.0001
FN1	X02761	1.02 ± 0.36	0.190 ± 0.108	0.031
ALOX5	NM_000698	56.1 ± 9.3	4.64 ± 0.86	<0.0001
GNLY	NM_006433	0.0893 ± 0.0265	0.00711 ± 0.00219	0.002
GZMB	NM_004131	1.37 ± 0.23	0.401 ± 0.276	<0.0001
BAX	NM_004324	14.7 ± 2.8	5.36 ± 0.78	<0.0001
DTR	NM_001945	9.86 ± 1.85	4.51 ± 0.79	0.003
EFNB1	NM_004429	0.669 ± 0.146	0.180 ± 0.027	0.001
EBB > TBB				
EGR1	NM_001964	0.00121 ± 0.00069	0.00279 ± 0.00074	0.046
CTGF	NM_001901	1.25 ± 0.33	3.13 ± 0.87	0.005
TGFB3	NM_003239	1.14 ± 0.16	1.66 ± 0.22	0.026
MUC4	NM_018406	2.04 ± 0.56	8.42 ± 1.51	<0.0001
MUC5AC	AF043909	4.62 ± 1.70	21.4 ± 4.2	<0.0001
MMP11	NM_005940	0.0513 ± 0.0144	0.216 ± 0.078	0.047
PRSS22	NM_022119	0.322 ± 0.122	0.490 ± 0.079	0.017

* To obtain the value shown, relative copy number (Mean ± standard error) was multiplied by 10⁻⁵.

† Mean endobronchial vs transbronchial transcript levels.

Table 4
Correlation of Transcripts in Paired Endobronchial Biopsy and Transbronchial Biopsy Specimens

Gene	GenBank Accession #	Transbronchial transcript level*	Endobronchial transcript level*	Correl. Coeff.	p
Cytokines, Chemokines, Receptors					
ANGPT1	NM_001146	8.79 ± 1.36	1.52 ± 0.20	0.483	0.005
ANGPT2	NM_001147	3.64 ± 0.87	1.70 ± 0.39	0.598	<0.0001
CSF1	NM_000757	0.0218 ± 0.0056	0.00491 ± 0.00114	0.668	<0.0001
CTGF	NM_001901	1.25 ± 0.33	3.13 ± 0.87	0.829	<0.0001
EDN1	NM_001955	1.88 ± 0.48	0.177 ± 0.029	0.403	0.022
HGF	M73239	0.661 ± 0.137	0.135 ± 0.025	0.643	<0.0001
IFNG	NM_000619	0.0155 ± 0.0073	0.00447 ± 0.00297	0.610	<0.0001
IGF1	NM_000618	2.70 ± 0.73	0.623 ± 0.130	0.611	<0.0001
IK	NM_006083	0.503 ± 0.107	0.649 ± 0.121	0.769	<0.0001
IL16	NM_004513	1.29 ± 0.30	0.331 ± 0.062	0.708	<0.0001
IL18	NM_001562	2.25 ± 0.59	1.37 ± 0.27	0.790	<0.0001
LIF	NM_002309	0.828 ± 0.232	0.982 ± 0.148	0.401	0.023
MIF	NM_002415	0.772 ± 0.138	0.608 ± 0.103	0.800	<0.0001
VEGFC	NM_005429	3.26 ± 0.60	1.15 ± 0.19	0.405	0.022
CCR2	NM_003965	0.583 ± 0.156	0.0357 ± 0.0079	0.733	<0.0001
SCYA5	NM_002985	12.9 ± 3.3	3.01 ± 0.80	0.639	<0.0001
SCYA3	NM_002983	0.177 ± 0.055	0.0881 ± 0.0316	0.942	<0.0001
PDGFA	NM_002607	4.70 ± 0.71	3.57 ± 0.35	0.558	0.001
PDGFB	NM_002608	9.22 ± 1.74	1.92 ± 0.38	0.404	0.022
TGFA	NM_003236	0.144 ± 0.046	0.130 ± 0.022	0.771	<0.0001
TGFB3	NM_003239	1.14 ± 0.16	1.66 ± 0.22	0.352	0.048
TNFA	NM_000594	0.119 ± 0.033	0.0210 ± 0.0071	0.756	<0.0001
TNFSF10	NM_003810	8.46 ± 1.79	2.93 ± 0.58	0.437	0.012
CCR2	NM_000648	2.71 ± 0.64	0.857 ± 0.217	0.436	0.013
CYSLT1	NM_001838	11.5 ± 2.47	7.01 ± 1.65	0.467	0.007
FLT1	NM_002019	0.0111 ± 0.0065	1.18 ± 0.19	0.722	<0.0001
GPR9	NM_001504	2.56 ± 0.57	0.00133 ± 0.00028	0.359	0.044
IFNGR1	NM_000416	0.00889 ± 0.00424	1.40 ± 0.68	0.818	<0.0001
NRP1	NM_003873	47.7 ± 6.6	0.00135 ± 0.00028	0.714	<0.0001
NRP2	NM_003872	0.0145 ± 0.0045	14.2 ± 1.5	0.471	0.006
CD proteins			0.00587 ± 0.00119	0.691	<0.0001
CD14	NM_000591	10.3 ± 1.5	1.86 ± 0.41	0.466	0.007
CD40L	NM_000074	0.223 ± 0.060	0.0779 ± 0.0253	0.540	0.001
CD3E	NM_000733	0.00771 ± 0.00257	0.0024 ± 0.0009	0.724	<0.0001
CD44	NM_000610	44.2 ± 5.8	11.9 ± 1.5	0.687	<0.0001
Mucins					
MUC1	NM_002456	2.06 ± 0.43	2.35 ± 0.49	0.816	<0.0001
MUC4	NM_018406	2.04 ± 0.56	8.42 ± 1.51	0.638	<0.0001
MUC5AC	AF043909	4.62 ± 1.70	21.4 ± 4.2	0.394	0.026
Defensins					
DEFA1	NM_004084	183 ± 92	12.0 ± 4.6	0.762	<0.0001
DEFA3	NM_005217	259 ± 125	18.8 ± 7.3	0.741	<0.0001
Adhesion Molecules					
ICAM1	NM_000201	75.3 ± 9.1	6.20 ± 1.35	0.429	0.014
ITGA4	NM_000885	0.00255 ± 0.00088	0.00046 ± 0.00019	0.719	<0.0001
ITGB1	NM_002211	236 ± 26	113 ± 19	0.358	0.044
Proteases, Inhibitors, Receptors					
ADAM17	NM_003183	1.29 ± 0.41	0.327 ± 0.056	0.723	<0.0001
CTSC	NM_001814	0.0477 ± 0.0140	0.0231 ± 0.0050	0.788	<0.0001
CTSK	NM_000396	1.12 ± 0.24	1.20 ± 0.45	0.376	0.034
CTSL	NM_001912	61.5 ± 11.1	16.9 ± 2.6	0.513	0.003

Gene	GenBank Accession #	Transbronchial transcript level*	Endobronchial transcript level*	Correl. Coeff.	p
CTSS	NM_004079	9.32 ± 1.59	3.06 ± 0.48	0.708	<0.0001
ELA2	NM_001972	0.177 ± 0.087	0.0136 ± 0.0019	0.369	0.038
PRSS8	NM_002773	110 ± 20	40.7 ± 5.2	0.586	<0.0001
PRSS22	NM_022119	0.322 ± 0.122	0.490 ± 0.079	0.862	<0.0001
TMPSR53	NM_024022	6.57 ± 1.53	6.38 ± 1.13	0.559	0.001
TPSB1	NM_003294	13.4 ± 2.6	4.40 ± 0.82	0.427	0.015
MMP2	NM_004530	0.650 ± 0.116	0.441 ± 0.089	0.446	0.010
TIMP1	NM_003254	65.7 ± 8.9	41.9 ± 6.8	0.505	0.003
SPINT1	NM_003710	20.1 ± 2.70	18.9 ± 2.4	0.759	<0.0001
F2RL1	NM_005242	7.68 ± 1.92	6.56 ± 1.07	0.360	0.043
Transcription Factors					
LEF1	AF288571	0.183 ± 0.059	0.142 ± 0.017	0.750	<0.0001
STAT6	NM_003153	0.00588 ± 0.00339	0.00100 ± 0.00025	0.717	<0.0001
Others					
ADORA2B	NM_000676	2.16 ± 0.46	1.45 ± 0.19	0.464	0.007
ALOX5	NM_000698	56.1 ± 9.3	4.64 ± 0.86	0.474	0.006
BAX	NM_004324	14.7 ± 2.8	5.36 ± 0.78	0.741	<0.0001
CFTR	NM_000492	0.00762 ± 0.00292	0.00276 ± 0.00061	0.506	0.003
CHRM2	NM_000739	0.867 ± 0.474	0.0128 ± 0.0036	0.431	0.014
DTR	NM_001945	9.86 ± 1.85	4.51 ± 0.79	0.415	0.018
EFNB1	NM_004429	0.669 ± 0.146	0.180 ± 0.027	0.563	0.001
EGRI	NM_001964	0.00121 ± 0.00069	0.00279 ± 0.00074	0.450	0.010
GNLY	NM_006433	0.0893 ± 0.0265	0.00711 ± 0.00219	0.745	<0.0001
GSN	NM_000177	0.0174 ± 0.0074	0.00715 ± 0.00154	0.630	<0.0001
GZMB	NM_004131	1.37 ± 0.23	0.401 ± 0.276	0.621	<0.0001
NOS2A	NM_000625	0.332 ± 0.156	0.493 ± 0.105	0.383	0.030

* To obtain the value shown, relative copy number (mean ± SE standard error) was multiplied by 10^{-5} .