Genomic Differences Distinguish the Myofibroblast Phenotype of Distal Lung Fibroblasts from Airway Fibroblasts

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Primary human distal lung/parenchymal fibroblasts (DLFs) exhibit a different phenotype from airway fibroblasts (AFs), including the expression of high levels of α -smooth muscle actin (α -SMA). The scope of the differences between these anatomically differentiated fibroblasts, or the mechanisms driving them, has remained unknown. To determine whether the different characteristics of regional fibroblasts are predicted by distinct genomic differences in AFs versus DLFs, matched human fibroblast pairs were isolated from proximal and distal lung tissue and evaluated. Microarray analysis was performed on 12 matched fibroblast pairs (four normal and eight asthmatic samples) and validated by quantitative real-time PCR. The potential functional implications of these differences were analyzed using computational approaches. Four hundred seventy-four transcripts were up-regulated in AFs, and 611 were up-regulated in DLFs via microarray analysis. No differences in normal and asthmatic fibroblasts were evident, and the data were combined for subsequent analyses. Gene ontology and network analyses suggested distinct patterns of pathway activation between AFs and DLFs. The up-regulation of extracellular matrix-associated molecules in AFs was observed, whereas genes associated with actin binding and cytoskeletal organization were up-regulated in DLFs. The up-regulation of activated/total SMAD3 and c-Jun N-terminal kinase in DLFs may partly explain these myofibroblast-like characteristics in DLFs. Thus, marked genomic differences exist between these two populations of regional lung fibroblasts. These striking differences may help identify potential mechanisms by which AFs and DLFs differ in their responses to injury, regeneration, and remodeling in the lung.

Keywords: human lung fibroblasts; α -smooth muscle actin; microarray; SMAD; JNK; MAPK8

We previously observed regional phenotypic differences in human lung fibroblasts derived from airway or distal lung/parenchymal tissue (1). Distal lung fibroblasts (DLFs) proliferate faster and are more myofibroblast-like, expressing higher levels of α -smooth muscle actin (α -SMA) than do airway fibroblasts (AFs). In contrast, AFs synthesize more collagen and eotaxin-1 than DLFs (1). A recent study confirmed these findings, and expanded on

CLINICAL RELEVANCE

To the best of our knowledge, this is the first comprehensive study of differences in global gene expression between human fibroblasts from the airways compared with those from the parenchyma. The identification of these two inherently different human lung fibroblast phenotypes has profound implications for a mechanistic understanding of the maintenance of normal lung structure and function, and of fibrotic diseases.

them by demonstrating functional differences in contractile forces of DLFs compared with AFs (2).

Fibroblasts are intriguing cell types, consisting of a number of markedly different phenotypes, dependent on location of origin (3, 4). Recent studies suggest that fibroblasts from different anatomic locations of the human body should be considered different cell types, because large-scale differences in basilar gene expression profiles were observed among these cells (5, 6). In particular, 337 genes were variably expressed among 47 human fibroblast lines throughout the body (6). These differences in gene expression allowed for the classification of fibroblasts according to their anatomic sites of origin, based on anterior-posterior, proximal-distal, and dermal-nondermal divisions. These findings suggest that fibroblasts carry positional signals critical for tissue formation and morphogenesis during development, guiding cell migration and determining differentiation during tissue injury and repair (7–9). However, little is known regarding such differences between proximal and distal human lung fibroblasts.

Myofibroblast differentiation is thought to be highly dependent on TGF- β (3, 4, 10). After engagement of the TGF- β receptor complex, SMAD and the mitogen-activated protein kinase (MAPK) superfamily are activated to promote the synthesis of α -SMA (11, 12). Whether alterations in these TGF- β -related pathways contribute to the phenotypic differences between distal lung and airway fibroblasts has yet to be determined.

Based on these studies and the known importance of TGF- β in myofibroblast differentiation (1, 2), we hypothesized that AFs and DLFs would be genomically distinct cells, and that specific differences in TGF- β 1-associated SMAD and c-Jun N-terminal kinase (JNK) pathways would define and contribute to these phenotypic characteristics. To evaluate this hypothesis, primary human lung fibroblasts were bronchoscopically obtained from paired proximal airway and distal lungs and were cultured at early passages. Global gene expression profiling and validation were also performed. These data support the concept that heterogeneous populations of lung fibroblasts are critical to lung morphogenesis, and to the development and progression

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of chronic fibrotic lung diseases such as asthma and interstitial fibrosis.

MATERIALS AND METHODS

Subjects and Lung Tissue Biopsies

In total, 18 subjects underwent bronchoscopy with endobronchial (airway) and transbronchial (distal) biopsies, including 7 with mild/moderate asthma and 11 with severe asthma. Paired samples were also obtained from four normal lungs that had been rejected for lung transplantation. Twelve of the 22 paired samples were used for microarray analysis (four normal and eight asthmatic samples; for further details, see Table E1 in the online supplement). Samples from the remaining 10 subjects were used for validation mRNA and protein experiments. The sample size varied in each experiment, based on cell availability.

AF and DLF Culture

Endobronchial and transbronchial biopsy lung tissue was processed as previously described (1, 13). Briefly, biopsy samples were cultured until fibroblasts grew out from the tissue. Fibroblasts were passed to the next passage during the proliferate state, and only third-passage or fourth-passage primary AFs or DLFs were plated onto 24-well or 6-well plates and grown to near confluence, using Dulbecco's minimum essential medium with 10% FBS. To compare AFs and DLFs, parallel experiments were performed on paired fibroblasts (AFs compared with DLFs from the same subject). After 24-hour serum starvation, cells were harvested at various time points, depending on the experiment.

Microarray Hybridization and Data Analysis

Microarray experiments were performed as previously described (14–16). Briefly, third-passage matched pairs of AFs and DLFs were harvested for RNA, and processed for microarrays. The array data were processed as previously described, and are available at the Gene Expression Omnibus database (accession number GSE27335) of the National Center for Biotechnology Information (NCBI) (14, 17). The processed microarray data were log₂-transformed, and normalized using CyclicLoess (14). The log₂-transformed, normalized data were used in downstream computational analyses. Additional details are provided in the online supplement.

Determining Differentially Expressed Genes

Paired Wilcoxon rank sum tests were used to determine differentially expressed genes between AFs and DLFs. Differentially expressed genes between samples from normal and asthmatic subjects in either AF or DLF cells were determined according to unpaired Wilcoxon rank sum tests. The procedure of Benjamini and Hochberg was used to control the false-discovery rate (FDR) (18). Additional details are provided in the online supplement.

Gene Ontology Statistical Analysis

To identify functional groups of genes enriched among genes differentially expressed between AFs and DLFs, a gene ontology (GO) analysis was performed, using the Gostat program (18). Additional details are provided in the online supplement.

Network Detection and Functional Network Analyses

To identify regulatory networks in AFs or DLFs, genes up-regulated in AFs and DLFs were analyzed using Ingenuity

Pathways Analysis (IPA) software (<http://www.ingenuity.com>; Ingenuity Systems, Redwood City, CA), as described (17). To identify molecular functions, physiological malfunctions, and canonical pathways significantly associated with a network, a functional network analysis was performed, using IPA as described previously (17). Additional details are provided in the online supplement.

Quantitative Real-Time PCR

We performed quantitative RT-PCR on selected and significantly differentially expressed genes, as previously described (14, 19–21). Full details are available in the online supplement.

Western Blot Analysis

Samples were processed for Western blot analysis as previously described, and details are provided in the online supplement (21).

Statistical Analysis for Validation and Functional Studies

For quantitative RT-PCR and Western densitometry, data from DLFs were reported as the ratio of DLFs/AFs, because of varying baselines from subject to subject. Data normally distributed were presented as means $\pm$ SEM. If data were not normally distributed, they were log-transformed for analysis and then reconverted back to the original scale for presentation. Specific group comparisons were performed with a paired *t* test, and *P* < 0.05 was considered statistically significant.

RESULTS

Confirmation of Phenotypic Differences

The differential expression of α -SMA at the basal level from three matched fibroblast pairs was confirmed by Western blot analysis, and further analyzed by densitometry (3.20 ± 0.86 -fold higher in DLFs compared with AFs; *P* < 0.05) (1, 2).

Differences in Terms of Disease Presence and Absence

We expanded on these phenotypic differences by describing the microarray data for AFs and DLFs. Comparisons were performed between four normal and eight asthmatic fibroblasts from both the airway and distal lung compartments for disease-related differences. No genes were differentially expressed between normal and asthmatic fibroblasts from either lung region (FDR-adjusted *P* < 0.05), suggesting that the described heterogeneity is regional rather than disease-associated. If less stringent statistical criteria for the Wilcoxon tests had been applied, differentially expressed genes between normal and asthmatic subjects may have been detected. Our data, however, show that according to the same stringent and standard criteria, many differentially expressed genes were detected between AFs and DLFs, strongly suggesting that the overwhelming difference between AFs and DLFs is attributable to regional as opposed to disease-specific differences. This lack of difference between normal and asthmatic samples allowed us to combine the data for subsequent comparisons across lung compartments.

Differential Gene Expression across Lung Compartments

The combined asthmatic and normal fibroblast microarrays (*n* = 12) were then compared across the proximal (AF) and distal (DLF) compartments. Although the majority of transcripts were expressed at similar levels, 1,085 genes were differentially expressed between AFs and DLFs (FDR-adjusted *P* < 0.05; fold change $\geq$ 1.2; Figure 1 and Table E2A). Of these 1,085

Figure 1. Differences in global gene expression between primary airway fibroblasts (AFs) and distal lung fibroblasts (DLFs). Profiles of genes differentially expressed between AFs and DLFs. Every row represents a gene, and every column represents each individual subject. Genes with higher expression values are shown in yellow; those with lower expression values are shown in purple. MFAP5, microfibrillar-associated protein 5; TLR4, Toll-like receptor 4; CCRL1, Chemokine (C-C motif) receptor-like 1; WNT2, Wingless-type mouse mammary tumor virus (MMTV) integration site family 2; SMAD3, Drosophila mothers against decapentaplegic homolog 3; MAPK8, mitogen-activated protein kinase 8; MYH9, Non-muscle myosin heavy chain 9; DES, desmin; MYH11, smooth muscle myosin heavy chain 11.

genes, 474 were up-regulated in AFs (relative to DLFs), and 611 were up-regulated in DLFs (relative to AFs). One hundred twenty-five of these 474 genes were overexpressed by at least 2-fold in AFs compared with DLFs, including transcripts for proteins associated with chemokine attractants and extracellular matrix (ECM) metabolism. On the other hand, 209 of these 611 genes were overexpressed at least 2-fold in DLFs compared with AFs, including transcripts for proteins associated with cytoskeletal maintenance, cell motility, and cell contractility (Table E2B).

Confirmation of Candidate Genes by Quantitative RT-PCR

Genes were selected to be confirmed on a hypothesis-driven basis and according to their level of expression. To validate the observed differences, quantitative RT-PCR was performed to measure the expression levels of selected genes in the same RNA samples used for microarray analysis ($n = 12$), and an additional 2–4 pairs not included in the microarrays were also evaluated. Nine differentially expressed genes were validated and differentially expressed in AFs compared with DLFs. The up-regulated genes selected for validation among the AFs included Toll-like receptor (TLR)–4, microfibrillar-associated protein 5 (MFAP5), and chemokine (C–C motif) receptor-like 1 (CCRL1). Similarly, wingless-type mouse mammary tumor virus (MMTV) integration site family–2, nonmuscle myosin heavy chain–9, smooth muscle myosin heavy chain–11, desmin,

SMAD3, and MAPK8 (JNK1) were significantly up-regulated in DLFs (Figure 2).

Because the expression level of α -SMA protein distinguished DLFs from AFs, we also checked α -SMA mRNA levels by both microarray and quantitative RT-PCR. Based on the standard criteria (FDR-adjusted $P < 0.05$, and fold change ≥ 1.2), no difference was evident for the expression of α -SMA, as measured by microarray, between AFs and DLFs. This result was confirmed by quantitative RT-PCR. This result is interesting in that α -SMA is thought to be posttranscriptionally or translationally regulated.

AFs and DLFs Express Different Enriched Functional Groups

GO characterizes and identifies functional categories of differentially expressed genes and their products from microarray data, according to biological processes, molecular functions, and cellular components, facilitating the interpretation of data from high-throughput genomic technologies (22). Using GO functional analysis, we found marked differences in the functional groups of genes enriched in AFs compared with DLFs. Genes involved in ECM turnover, ECM organization, and immune-system processes were significantly overrepresented in AFs (Figure 3A), whereas genes involved in cadmium ion binding, microtubule-based processes, cytoskeletal organization, actin binding, enzyme-linked receptor protein signaling pathways, and system development (including lung and respiratory tube development) were overrepresented in DLFs (Figure 3B). Moreover, more functional groups were represented among up-regulated genes in DLFs than in AFs. These results were consistent with our previous observations that AFs produced greater secretions of ECM, whereas DLFs produced more α -SMA (1).

Gene Regulatory Network in AFs and DLFs

IPA promotes an understanding of biological interactions at multiple levels by integrating data from a variety of experimental platforms and by providing insights into molecular and chemical interactions, cellular phenotypes, and disease processes of a system. Using IPA, 489 genes were identified in the AF network (Figure 4A and Table E3A), whereas 474 genes were identified in the DLF network (Figure 4B and Table E3B). Numerous network differences were evident between AFs and DLFs. Two important TGF- β 1–signaling molecules (SMAD3 and MAPK8) were identified in the DLF network, supporting the data from the microarray analysis (Figure 4B). The AF network contained more genes associated with the assembly of ECM and inflammatory responses, whereas the DLF network was enhanced in genes involved with hyperproliferation, the proliferation of smooth muscle cells and the cell cycle, organismic injury/abnormalities and survival, the development and function of the skeletal and muscular systems, cellular morphology and development, and energy production (Table E4; FDR-adjusted $P < 0.05$). Moreover, the AF networks were associated with the canonical pathways of altered T-cell and B-cell signaling in rheumatoid arthritis and with PI3K signaling in B lymphocytes, whereas genes enhanced in the DLF (but not in the AF) network were associated with actin cytoskeleton signaling ($P = 0.02$; Figure E1A), cardiac β -adrenergic signaling (which leads to myofibrillogenesis; $P = 0.04$; Figure E1B), and cell division control protein 42 (cdc42) signaling (which leads to cell migration; $P = 0.005$; Figure E1C), consistent with their myofibroblast-like characteristics.

Disease and functional-relevance analyses showed that SMAD3 and MAPK8 were associated with many of the molecular functions and physiological disorders linked with the

Figure 2. Verification of microarray data was performed by quantitative RT-PCR in AFs and DLFs. (A) Toll-like receptor 4 (TLR-4). (B) Microfibrillar-associated protein 5 (MFAP5). (C) Chemokine (C-C motif) receptor-like 1 (CCRL1). (D) Wingless-type MMTV integration site family 2 (WNT2). (E) Nonmuscle myosin heavy chain 9 (MYH9). (F) Smooth muscle myosin heavy chain 11 (MYH11). (G) Desmin (DES). (H) SMAD3; (I) Mitogen-activated protein kinase 8 (MAPK8). *y* axis, level of candidate gene expression (relative to glyceraldehyde 3-phosphate dehydrogenase [GAPDH]). Open bars, AFs; solid bars, DLFs. * $P < 0.05$ and ** $P < 0.01$, DLFs versus AFs ($n = 14-16$).

network in DLFs but not in AFs, including cellular growth and proliferation, movement, cancer, cardiovascular disease, and skeletal and muscular disorders (Table E4).

Validation of SMAD3 and MAPK8 Protein in AFs and DLFs

SMAD3 mRNA was highly expressed in DLF microarrays, as verified by quantitative RT-PCR, and was suggested to be functionally and centrally involved in DLF networks. Western blot analysis further confirmed that the levels of both phospho-SMAD3 and total SMAD3 proteins were significantly higher in DLFs than in AFs, suggesting a basally more active SMAD3 signaling that contributes to the differentiation of DLFs (Figures 5A and 5C). The level of phospho-SMAD2 was not significantly higher in DLFs than in AFs, although the level of total SMAD2 was higher in DLFs (Figures 5A and 5B), supporting a key role for active SMAD3 in differentiating DLFs from AFs.

Similar to SMAD3, MAPK8 (JNK1) mRNA was also highly expressed in DLFs, as verified by quantitative RT-PCR, and was suggested to be functionally involved in the DLF networks. In contrast to SMAD3, total MAPK8 protein did not differentiate AFs from DLFs, whereas the phosphorylation of MAPK8 was higher in all five DLFs compared with AFs (Figures 5A and 5D), suggesting that basal MAPK8 activation in DLFs could also contribute to the differentiation of DLFs.

DISCUSSION

Observations in previous studies indicate that DLFs are morphologically and functionally distinct from AFs (1, 2). The present study expands on these earlier observations by identifying global differences in gene expression, and by evaluating their relationship to regulatory networks and their potential functional importance. In total, 1,085 genes were differentially regulated between AFs and DLFs, confirming their genomically distinct nature. Genes up-regulated in AFs were significantly

enriched with functional groups involved in ECM and ECM organization, whereas genes up-regulated in DLFs were significantly enriched with functional groups participating in actin binding and cytoskeletal organization. All these GO categories are consistent with the myofibroblast-like characteristics of DLFs, which may normally play an important role in parenchymal changes with respiration. However, this function is only one of many that DLF may play in the lung parenchyma, and unfortunately, gene expression studies are unable to define these specific roles. Moreover, specific molecular functions and pathobiologic disorders associated with the gene expression profile in DLFs were also strongly associated with SMAD3 and MAPK8. The up-regulation of SMAD3 and MAPK8 expression and activation in DLFs was subsequently confirmed by quantitative RT-PCR and protein analysis. The TGF- β canonical signaling molecules, SMAD3 and MAPK8, were significantly expressed and activated in DLFs. Thus, genomic and phenotypic analyses, along with activation pathways, all support the critical importance of TGF- β signaling pathways, SMAD3, and MAPK8 in differentiating DLFs from AFs.

Our work is related to two previous microarray studies that, rather than characterizing fibroblasts from the same anatomic location (lung) as described here, compared gene expression from skin fibroblasts of various anatomic sites (including the foot, arm, and scalp) to lung, liver, prostate, and aortic fibroblasts (5, 6). The differential expression of genes involved in the synthesis of ECM, cell migration, and growth/differentiation, including TGF- β signaling, distinguished fibroblasts from different anatomic sites. These findings suggest that fibroblasts encode positional signals to guide cells to differentiate or migrate. Thus, it is tempting to postulate that despite their close anatomic proximity, genomic differences between AFs and DLFs encode distinct positional signals that facilitate their differential roles in the lungs. AFs may be more involved in the organization of ECM and immune responses, whereas DLFs may be more involved in lung regeneration and repair. TGF- β 1

Figure 3. Potential functional differences between primary AFs and DLFs. Functional groups of genes were enriched significantly among genes up-regulated in AFs (A) or DLFs (B). Within parentheses below each gene ontology (GO) functional group name, (n_i , p_i %) is used to represent the number (n_i) of up-regulated genes in AFs or DLFs in GO group i , and the percentage (p_i %) of up-regulated genes in AFs or DLFs assigned to GO group i (i.e., p_i % = n_i / N_i , where N_i is the total number of genes in GO group i). GO groups in this diagram satisfied: (1) false-discovery rate-adjusted $P < 0.05$, and (2) $n_i \geq 5$.

signaling, through differences in the expression and activation of SMAD2/3 and MAPK8 (JNK1), appears to be central to this differentiation.

Although considerable interest has arisen regarding the differences in fibroblast phenotypes from various diseases, including interstitial pulmonary fibrosis (23–25), pulmonary hypertension (26), scleroderma (27, 28), systemic sclerosis (29), and asthma (30), as well as the migration of fibrocytes into the lung (31), little discussion has centered on the possibility that different fibroblast phenotypes normally exist in the lung. To our knowledge, the finding of two genomically different human lung fibroblast phenotypes (i.e., AFs, innately more fibroblast-like, and DLFs, more “myofibroblast-like”) in structurally different lung regions is unique, with implications for fibrotic lung disease. Moreover, at least in asthma, the differences in gene expression have more to do with location than disease state in human lung fibroblasts. A clear precedent for “normal” phenotypic differences exists in the liver, where four different liver fibroblast–myofibroblast phenotypes were described (32, 33). These different fibroblasts in liver are thought to contribute differentially to the fibrogenic process in hepatic disease (34). Because these different fibroblast phenotypes have not been clearly delineated in the lung, studies that purport to show a different “diseased” fibroblast phenotype may in actuality show a fibroblast–myofibroblast infiltration from a different region of the lung. In fact, despite the global differences in gene expression profiles according to lung region, no disease-specific differences were evident in gene expression between asthmatic

Figure 4. Regulatory networks identified in primary AFs and DLFs. These plots are graphical representations of molecular relationships between nodes (i.e., genes and gene products) in the networks identified in AFs (A) and DLFs (B). An edge (line) represents the biological relationship between two nodes. The intensity of a node color indicates the magnitude of up-regulation of a gene in AFs (shown in green, relative to DLFs), or up-regulation of a gene in DLFs (shown in red, relative to AFs). Hub nodes in the network are also depicted.

and normal control fibroblast regions. This evaluation is the most extensive to date of differences in gene expression profiles between asthmatic and normal lungs, and supports the concept that in asthma, only small (or no) basal differences in gene expression are present compared with normal control samples. Finally, studies that use purchased “lung fibroblasts” should also be interpreted with caution, because these likely represent either a mix of fibroblast phenotypes, or a predominance of parenchymal fibroblasts. Further, regenerative therapies for lung disease should also consider these differences in fibroblasts, replacing diseased areas of the lung with appropriately “matched” fibroblasts.

The DLF network is significantly enriched with genes linked to myofibroblast-like characteristics, suggesting that the distal lung may be preprogrammed to repair injury more rapidly than the airways, and may contain the more important fibroblast cell type for fibrotic processes. This may also partly explain the relative differences in levels of fibrosis between traditional parenchymal disease (e.g., idiopathic pulmonary fibrosis [IPF]) and airway disease (e.g., asthma).

TGF- β 1 is a myogenic factor that induces the differentiation of fibroblasts to myofibroblasts (3). However, the relative importance of TGF- β -related pathways to regional lung fibroblast phenotypic differences was not previously reported. The literature supports SMAD3 as a key canonical signaling molecule for the effects of TGF- β 1 in the differentiation of fibroblasts (35–38). Network analysis predicted that SMAD3 plays an important role in the expression of α -SMA, differentiating DLFs from AFs. In addition to significant differences in the expression of

Figure 5. Expression and activation of SMAD2/3 and MAPK8 in primary AFs and DLFs. (A) A representative Western blot of total and phosphorylated (p) SMAD2/3 and MAPK8 in AFs and DLFs ($n = 5$). (B and C) Fold-changes of p-SMAD2 and total SMAD2 (B) and p-SMAD3 and total SMAD3 (C) are presented as means $\pm$ SEM in DLFs relative to AFs. $*P < 0.05$ (DLFs versus AFs, $n = 5$). (D) Fold-changes of p-MAPK8 and total MAPK8 are presented as means $\pm$ SEM in DLFs relative to AFs. $*P < 0.05$ (DLFs versus AFs, $n = 5$).

SMAD3 in gene arrays, total and phospho-SMAD3 proteins were also significantly increased in DLFs, supporting a critical role of SMAD3 in driving their myofibroblast-like characteristics. Interestingly, our network analysis suggested distinct roles for SMAD2 and SMAD3 in TGF- β 1 signaling. Although SMAD2 was involved in both AF and DLF networks, only SMAD3 was involved in DLF networks. Finally, the overexpression and activation of SMAD3, but not of SMAD2, increased the expression of α -SMA and cytoskeletal organization, perhaps explaining differences in the impact of TGF- β on the differentiation of myofibroblasts in DLFs, compared with collagen and ECM in AFs (39). The molecular mechanisms driving the active SMAD3 pathway in DLFs require further study.

In addition to the canonical SMAD pathways, microarray and quantitative RT-PCR data support a role for MAPK8 (JNK1) in the distal lung myofibroblast phenotype. Interestingly, regional differences in total MAPK8 protein were not evident. However, the phosphorylation of MAPK8 in Western blot analyses was greater in DLFs than in AFs. Numerous factors, including the lower sensitivity of Western blots and of translational and other protein-directed processes, could dampen the differences observed in MAPK8 at the mRNA level. However, the concurrent enhanced expression and activation of threonine and tyrosine kinase could promote the activation of MAPK8 (40). In particular, cdc42 signaling (enhanced in the DLF network) can cause the activation of JNK through a mixed-lineage kinase-3 pathway (Figure E1C). Activated JNK could then also phosphorylate SMADs (in particular, SMAD3), and further contribute to the differentiation of myofibroblasts through TGF- β 1-activated pathways (35, 41). Interestingly, the elevated activation of JNK was a feature of fibrotic lung fibroblasts isolated from patients with pulmonary fibrosis associated with systemic sclerosis. Whether this activation is attributable to a disease-specific effect, as previously thought, or to the overgrowth of a DLF phenotype in patients with systemic sclerosis, remains unclear (42).

In conclusion, to the best of our knowledge, this is the first study to compare global gene expression profiles between fibroblasts from distinctly different lung regions (airway and

parenchyma). As microarray and network analyses suggest, and as follow-up studies confirm, DLFs exhibit a higher basal activation of SMAD3 and MAPK8, which promotes the more myofibroblast-like phenotype present in distal lung and parenchymal tissue. The differences in AFs and DLFs suggest that they will respond differently to injury, activate alternative regenerative pathways, and control different lung activities, thus confirming profound differences between these cell types. Further studies are needed to determine the genomic reasons for the increased SMAD3 and MAPK8 signaling and the mechanisms governing the myofibroblast-like phenotype in DLFs, as well as the structural, immunological, and even disease-related implications of these phenotypic differences. The existence of these two distinct lung phenotypes should be considered in all future studies of injury, regeneration, and repair in the human lung.

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