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Pathological remodeling of distal lung matrix in end-stage cystic fibrosis patients

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ABSTRACT

Background: Manifestations of cystic fibrosis, although well-characterized in the proximal airways, are understudied in the distal lung. Characterization of the cystic fibrosis lung 'matrisome' (matrix proteome) has not been previously described, and could help identify biomarkers and inform therapeutic strategies.
Methods: We performed liquid chromatography-mass spectrometry, gene ontology analysis, and multimodal imaging, including histology, immunofluorescence, and electron microscopy for a comprehensive evaluation of distal human lung extracellular matrix (matrix) structure and composition in end-stage cystic fibrosis.
Results: Quantitative proteomic profiling identified sixty-eight (68) matrix constituents with significantly altered expression in end-stage cystic fibrosis. Over 90% of significantly different matrix peptides detected, including structural and basement membrane proteins, were expressed at lower levels in cystic fibrosis. However, the total abundance of matrix in cystic fibrosis lungs was not significantly different from control lungs, suggesting that cystic fibrosis leads to loss of diversity among lung matrix proteins rather than an absolute loss of matrix. Visualization of distal lung matrix via immunofluorescence and electron microscopy revealed pathological remodeling of distal lung tissue architecture and loss of alveolar basement membrane, consistent with significantly altered pathways identified by gene ontology analysis.
Conclusions: Dysregulation of matrix organization and aberrant wound healing pathways are associated with loss of matrix protein diversity and obliteration of distal lung tissue structure in end-stage cystic fibrosis. While many therapeutics aim to functionally restore defective cystic fibrosis transmembrane conductance regulator (CFTR), drugs that target dysregulated matrix pathways may serve as adjunct interventions to support lung recovery.

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Abbreviations

BAL	bronchoalveolar lavage
EMT	epithelial mesenchymal transition
EVG	elastic van Gieson
GO	gene ontology
LC-MS/MS	liquid chromatography mass spectrometry
MMP	matrix metalloproteinase
PCA	principal component analysis
SEM	scanning electron microscopy
TEM	transmission electron microscopy
TIMP	tissue inhibitor of metalloproteinase

1. Introduction

Cystic fibrosis (CF) is characterized by a pathologic cascade of impaired ion transfer, dysregulation of airway surface liquid and mucus, inability to clear infection, chronic inflammation, and ultimately end-stage lung disease necessitating transplantation [1,2]. As disease severity increases, structural remodeling of the airways (i.e., bronchiectasis) presents as a hallmark feature that is routinely observed on chest radiography and evaluated with established scoring systems [3–7]. Though these structural alterations to the airways are well-characterized, changes to the distal lung, including structure and composition of the parenchymal extracellular matrix (matrix), are understudied. Despite the recognized importance of the matrix in lung function as well as its involvement in many chronic respiratory diseases, a comprehensive characterization of the CF lung matrix has not been previously reported.

Lung matrix structure and composition facilitate gas exchange by providing mechanical integrity to withstand dynamic changes in pressure during breathing, elasticity to ventilate the distal alveoli, and biochemical cues to guide cell behavior and respond to injury. Key structural and biological matrix constituents in the lung include collagens and laminins which comprise the basement membrane, and elastin which provides the lungs with elastic recoil enabling ventilation. In healthy patients, coordinated matrix remodeling governs lung tissue repair to restore function of an injured area. In diseased lungs, however, the matrix and its reparative wound-healing functions are compromised in a disease-specific manner. Because matrix structure and composition are intrinsically linked to cell, tissue, and organ-level function, we aimed to identify significantly altered matrix components and dysregulated pathways in CF.

Previous studies identified elevated levels of proteolytic enzymes and matrix breakdown products in the bronchoalveolar lavage (BAL) fluid, sputum, and serum of CF patients, suggesting that matrix alterations mediated by protease/anti-protease imbalance may be a feature of CF [8–11]. However, a comprehensive, quantitative analysis of the CF lung matrisome, which includes constitutive and associated matrix proteins, remains outstanding. Understanding pathologic alterations to the lung matrix in CF may offer prognostic value and could uncover biomarkers of disease severity to inform therapeutic strategies. While gene therapies under development and currently-available cystic fibrosis transmembrane conductance regulator (CFTR) modulators aim to functionally restore CFTR expression, adjunct interventions targeting matrix remodeling could be developed to slow or reverse tissue damage and lung decline.

Here, we report a comprehensive, quantitative analysis of the distal lung matrix proteome (matrisome) in end-stage CF. We hypothesized that dysregulation of matrix pathways and alterations to distal lung matrix structure and composition accompany the well-described hallmarks of CF, including chronic infection, inflammation, structural changes to the airways, and progressive decline in lung function. Changes to the matrix were visualized at multiple scales by histology and electron microscopy. Towards quantitative analysis of lung matrix, we (*i*) extracted matrix from CF and control lung tissues, (*ii*) quantified composition of extracted matrix with liquid chromatography-mass spectrometry (LC-MS/MS), and (*iii*) filtered the resultant LC-MS/MS dataset to identify constituents of the lung matrisome (matrix and matrix-associated proteins) [12–17]. Profiles of collagens, glycoproteins, proteoglycans, secreted factors, matrix-regulating, and matrix-affiliated proteins were quantified, and differentially-regulated pathways were identified through gene ontology (GO) analysis.

Significant loss of expression and structural breakdown of key matrix components, including basement membrane proteins, were observed, and pathways including 'extracellular matrix', 'degradation of the extracellular matrix', and 'structural matrix constituents conferring tensile strength' were significantly downregulated in CF. Correlating with loss of matrix organization and diversity, pathways involved in aberrant wound healing were upregulated in CF. Altogether, our findings suggest that pathological changes to the distal lung matrisome accompany other hallmark indicators of end-stage CF, such as stereotypical remodeling of airway structure, and may serve as therapeutic targets or biomarkers of disease state.

2. Methods

Detailed methods can be found in the **Supplementary Methods**. Briefly, lung tissue was obtained at the time of transplantation from explanted lungs from patients with end-stage CF ($n = 9$) and uninjured regions of donor lungs declined for transplantation to serve as controls ($n = 3$). All CF patient tissue underwent multimodal imaging ($n = 9$), and a subset of patient tissue samples ($n = 8$) underwent LC-MS/MS. For one CF patient, the right middle lobe was unavailable and instead, tissue was obtained from the lingula. CF patient mutations were determined by isolating genomic DNA and sequencing regions of the CFTR gene where common mutations are found (**Supplementary Table 1**). Structure and composition of lung extracellular matrix was analyzed using histology, immunofluorescence, electron microscopy, liquid chromatography mass spectrometry (LC-MS/MS), and gene ontology (GO) analysis.

3. Results

We investigated alterations to the distal lung matrix in end-stage CF, using tissue obtained from explanted lungs of CF patients undergoing lung transplantation. Patient mutations were determined to be ΔF508/ΔF508 ($n = 5$), ΔF508/G542X ($n = 1$), ΔF508/N1303K ($n = 1$), and ΔF508/unknown ($n = 2$) (**Supplementary Table 2**). CF lung tissues were obtained from a random sample of patients with end-stage CF, and none of the samples were excluded from the study. As lung matrix provides structural and biochemical cues to resident cells which together govern development, function, and injury response, we sought to investigate pathologic alterations to matrix structure and composition in end-stage CF, using microscopy and liquid chromatography mass spectrometry (LC-MS/MS) analysis (**Fig. 1**).

3.1. Ultrastructural abnormalities in CF distal lung matrix

The lung matrix is a complex, well-organized, 3D structure that supports lung function by providing a large surface area for gas exchange and allowing elastic recoil during lung ventilation. For a global comparison of matrix structure and tissue morphology in CF compared to control lung, histological staining was performed on lung specimens from all patients to visualize overall tissue histomorphology (H&E), and the distribution of collagen (Masson's trichrome, blue), and elastin (elastic van Gieson, black) (**Fig. 2A,**

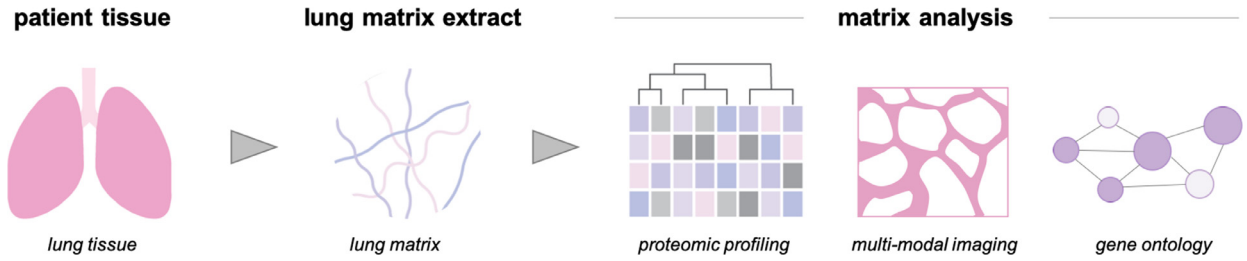

Fig. 1. Experimental overview. Cystic fibrosis and control lung tissues were procured to extract and analyze the distal lung matrix structure and composition through proteomic profiling, multi-modal imaging (e.g., histology, immunostaining, electron microscopy), and gene ontology.

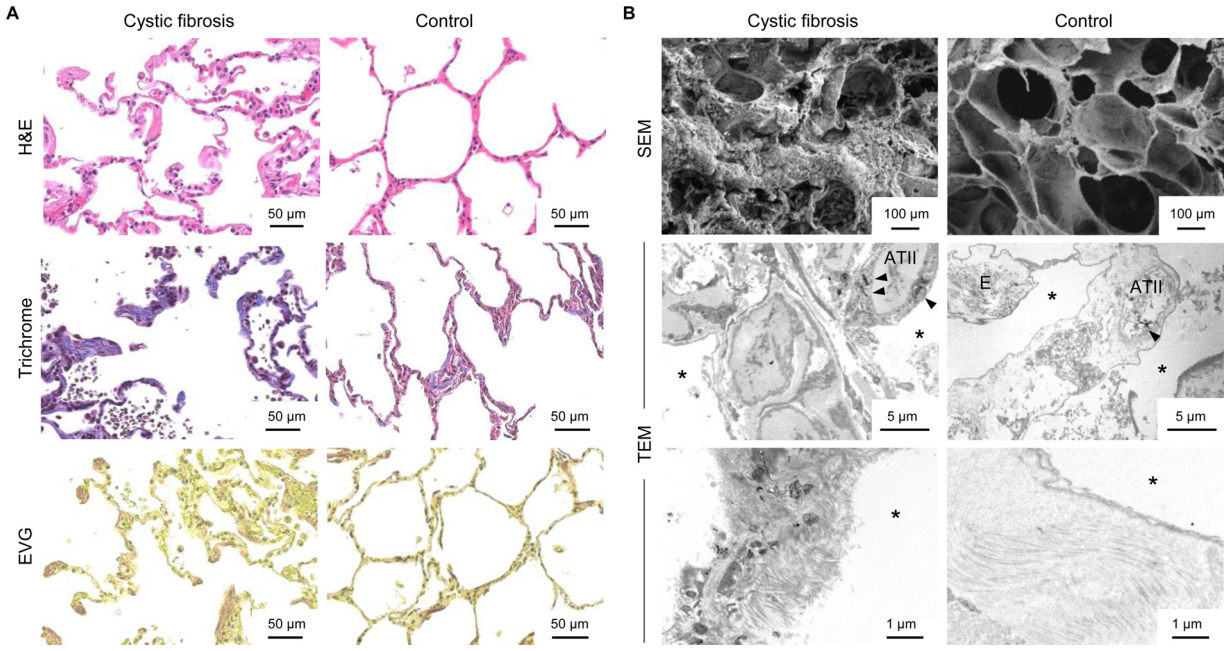

Fig. 2. Structural comparison of cystic fibrosis and control lung tissue. Overall tissue structure and composition of the lung parenchyma were evaluated on (**A**) histology with hematoxylin and eosin (H&E), Masson's trichrome (blue, collagens), and elastic van Gieson (EVG) (black, elastin), and (**B**) scanning electron microscopy (SEM) and transmission electron microscopy (TEM). * = Airspace, *E* = elastic fibers, ATII = alveolar type II pneumocyte, arrows = lamellar bodies.

Supplementary Figs. **1**, **2**). Histological staining of airway sections confirmed stereotypical airway morphology, including elevated levels of glycoproteins and mucins (Alcian blue, blue) in CF (**Supplementary Fig. 3**). Compared to control lung, CF specimens displayed expected septal thickening, fragmented fibers, and proteinaceous fluid-filled airways and alveoli with varying degrees of severity.

Lung parenchymal ultrastructure and alveolar architecture were grossly abnormal in CF with airway and alveolar obstruction and collapse, as evidenced by scanning electron microscopy (SEM) imaging, indicative of parenchymal destruction. Visual assessment of the epithelium, endothelium, and alveolar basement membrane by transmission electron microscopy (TEM) revealed basement membrane disruption and alveolar collapse (Fig. 2**B**).

3.2. Quantitative analysis of CF distal lung matrisome

We performed LC-MS/MS on intact distal lung tissues from CF patients and controls to identify changes to the lung proteome. Gene ontology (GO) analysis of detected peptides revealed that the extracellular space (p = 4.0E-4) and extracellular organelles (p = 1.68E-5) were significantly altered in CF compared to control lung (**Supplementary Figs**. **4–8**). Identification of significant differences in pathways involving the extracellular environment informed deeper analysis of the CF lung matrix. Matrix was then extracted by decellularization of lung tissues, which led to greater than 95% reduction in DNA content and nearly complete removal of DAPI-stained nuclei (**Supplementary Fig. 9**), while maintaining intact matrix. The full list of peptides identified using LC-MS/MS of extracted lung matrix was filtered using the open-access 'Matrisome Project' which categorizes matrix proteins as: core matrisome (i.e., collagens, glycoproteins, proteoglycans), matrix regulators, matrix affiliates, and secreted factors. The number of matrisome proteins and their relative intensities across each of these categories were compared for CF and control lungs (**Supplementary Fig. 10**).

Overall, 243 significantly different proteins were detected in the CF matrix, including 14 collagens, 18 glycoproteins, 4 proteoglycans, 10 matrix regulators, 6 matrix affiliates, 9 secreted factors, and 182 others (i.e., significantly altered but not considered to be matrisome constituents) (Fig. 3**B**). Over 90% of the significantly altered matrix proteins were downregulated in CF. We observed similar changes in the relative intensities of the proteins in the collagens, glycoproteins, and secreted factors categories (**Supplementary Fig. 10**), indicating similar coverage in both groups. However, there was a decreased abundance of proteoglycans in CF compared to control lung (p = 0.016), suggesting loss of proteoglycan activity in CF. To validate the matrix extraction method, LC-MS/MS was performed on whole lung tissues (i.e., tissues that did not undergo matrix extraction), and a decrease in matrix proteins was also detected (**Supplementary Fig. 4**). Using a principal component anal-

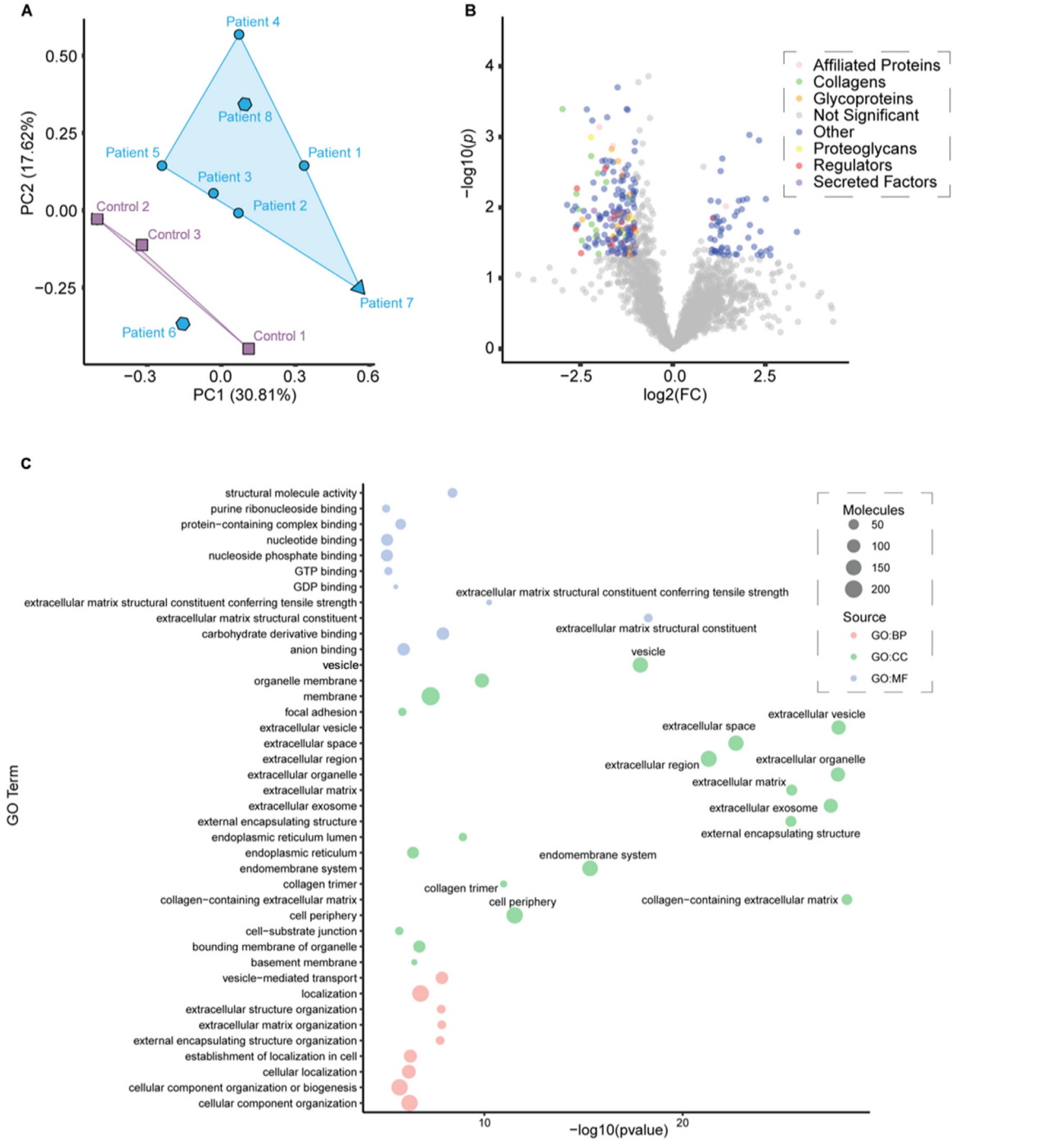

Fig. 3. Global analysis of cystic fibrosis lung matrisome. (A) Principal component analysis (PCA) of CF and control lung matrix. Shape of marker indicates patient mutations: circle, ΔF508/ΔF508; triangle, ΔF508/G542X; hexagon, ΔF508/unknown. (B) Volcano plot of differentially expressed matrisome components. (C) Gene ontology (GO) analysis of pathways significantly altered in CF. BP, biological processes; CC, cellular components; MF, molecular factors.

ysis (PCA) on the extracted matrix LC-MS/MS dataset, we found that 7 out of 8 CF patients clustered together, while control patient samples did not cluster (Fig. 3**A**). The group size was too small to draw definitive conclusions about any mutation-specific alterations to the lung matrix.

GO analysis of the full list of significant peptides identified through LC-MS/MS revealed differences in matrix-related pathways in CF versus control lung matrix. Significantly different GO terms included: extracellular matrix (p = 3.16E-26), degradation of the extracellular matrix (p = 2.73E-7), collagen trimer (p = 1.09E-11), structural matrix constituents conferring tensile strength (p = 5.78E-11), collagen chain trimerization (p = 2.93E-10), and matrix proteoglycans (p = 5.86E-9) (Fig. 3**C**). Additional significant GO terms related to cell-matrix interactions and cell signaling were identified, including anion binding (p = 1.19E-6), focal adhesion (p = 1.39E-6), cell-substrate junction (p = 1.97E-6), extracellular vesicle (p = 1.36E-28), and extracellular exosome (p = 3.38E-28).

After filtering for peptides with $\log_2$FC < -1, GO terms identified included: extracellular matrix structural constituent (p = 6.89E-38), collagen binding (p = 9.02E-7), glycosaminoglycan binding (p = 3.33E-6), extracellular matrix organization (p = 1.00E-

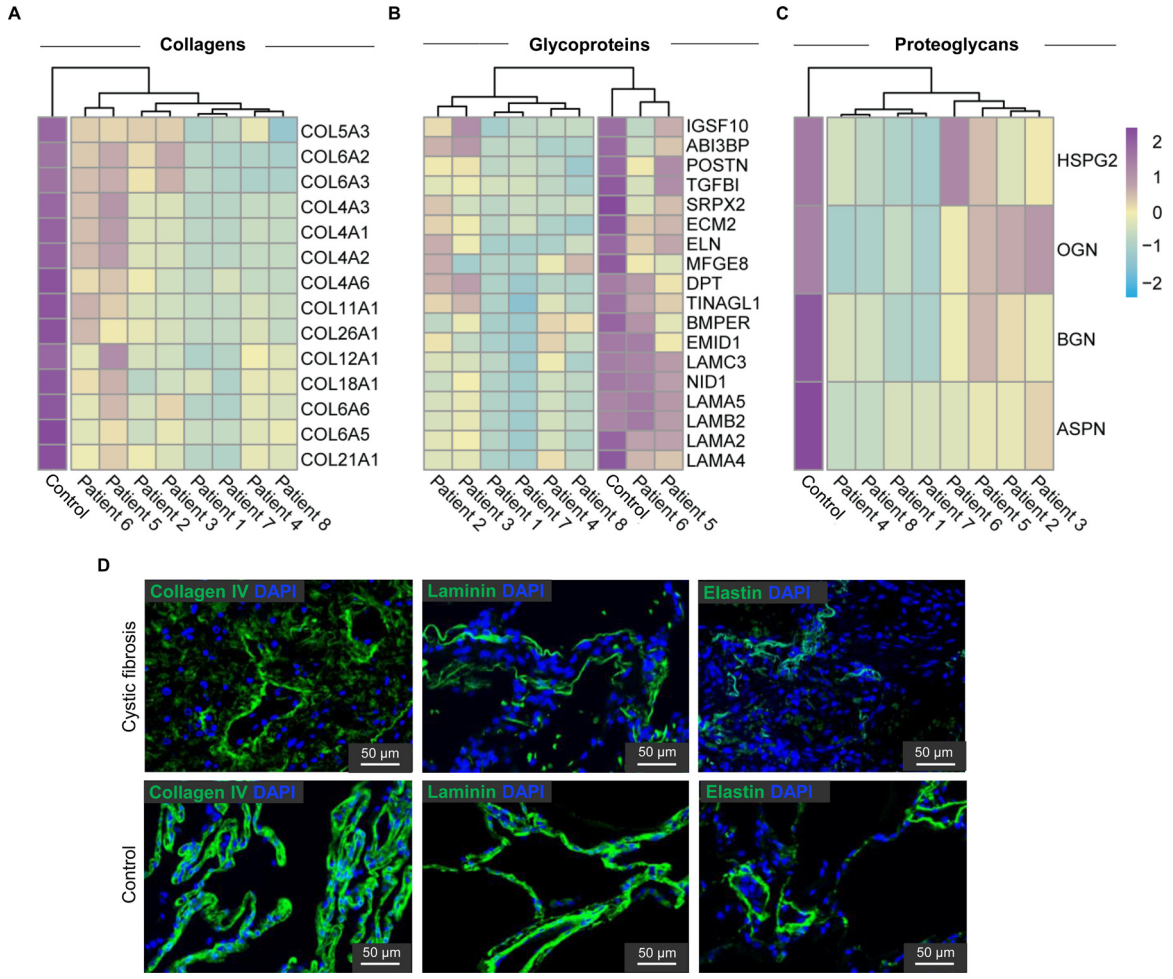

Fig. 4. Characterization of core matrix structure and composition in cystic fibrosis and control lung. Expression of core matrisome components, including (A) collagens, (B) glycoproteins, and (C) proteoglycans quantified using LC-MS/MS. (D) expression of basement membrane constituents and elastin visualized using immunofluorescent staining.

18), and extracellular structure organization (p = 1.12E-18). Filtering for peptides with $\log_2$FC > 1 identified GO terms including: cadherin binding (p = 6.28E-23), unfolded protein binding (p = 3.57E-9), protein-lipid complex (p = 1.78E-12), response to topologically incorrect protein (p = 2.91E-6), response to unfolded protein (p = 2.44E-4), and negative regulation of wound healing (p = 8.16E-3). The complete lists of identified GO pathways can be found in **Supplementary Figs. 11–15** and **Supplementary Tables 3, 4.**

3.3. Degradation of core proteins in distal lung matrix

In total, 36 core matrisome proteins (i.e., collagens, proteoglycans, glycoproteins) of the 143 detected were significantly differentially expressed in CF matrix compared to control lung. The majority of collagens (COL4A1/2/3/6, COL5A3, COL6A2/3/5/6, COL11A1, COL21A1, COL26A1, COL12A1, and COL18A1) were significantly downregulated in CF compared to control tissue (Fig. 4**A**). Other collagens (COL15A1, COL23A1, COL10A1, COL5A1, COL1A2) were upregulated in CF, albeit not significantly (**Supplementary Fig. 16**). Other key basement membrane constituents, including multiple isoforms of laminin (LAMA2, 4, and 5) and nidogen1 (NID1) were downregulated in CF (LAMA2, $\log_2$FC = −1.6, p = 0.025; LAMA4, $\log_2$FC = −1.2, p = 0.012; LAMA5, $\log_2$FC = −1.3, p = 0.047; NID1, $\log_2$FC = −1.3, p = 0.030), indicative of basement membrane dysregulation in CF lung disease and consistent with TEM and SEM findings. Elastin (ELN, $\log_2$FC = −1.6, p = 0.026) which confers lung elasticity was decreased in CF compared to control lung, a trend indicative of possible emphysema (Fig. 4**B**). Notably, perlecan (HSPG2, proteoglycan found in the basement membrane) downregulation accompanied the loss of collagens and laminins (HSPG2, $\log_2$FC = −1.36, p = 0.021). Other collagen-interacting proteoglycans were also downregulated, such as osteoglycin (OGN, $\log_2$FC = −1.21, p = 0.014), biglycan (BGN, $\log_2$FC = −1.08, p = 0.014), and asporin (ASPN, $\log_2$FC = −2.21, p = 0.001) (Fig. 4**C**).

In line with the matrisome quantification and GO analysis indicating collagen trimer and basement membrane as significantly altered pathways, immunofluorescent staining for collagen IV – a major component of the lung basement membrane – was sparse, poorly organized, and partially degraded in the alveolar septa in CF lung compared to control lung where well-organized collagen IV staining was ubiquitous in the basement membrane. Elastin, which enables cyclic movement during breathing, and laminin, which provides structural support and promotes cell adhesion to the basement membrane, appeared fragmented in CF lungs compared to controls (Fig. 4**D**).

3.4. Dysregulation of matrix modifying proteins

Degradation of basement membrane proteins was accompanied by decreased expression of proteins that promote cell adhesion to the basement membrane (C1QTNF5/7, $\log_2$FC = −1.3/−1.4,

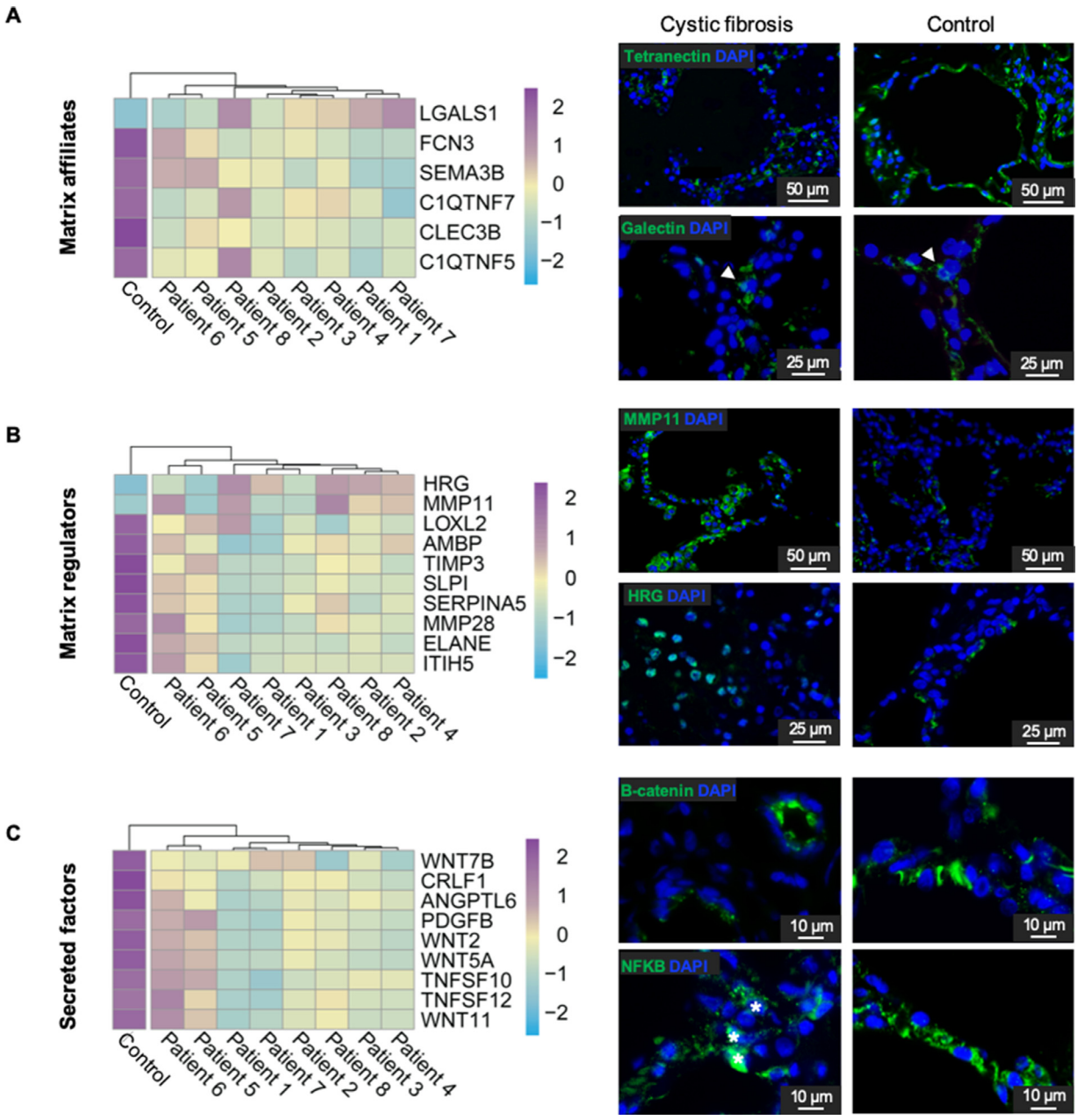

Fig. 5. Analysis of matrix-associated proteins in cystic fibrosis and control lung. (A) matrix affiliates, (B) matrix regulators, and (C) secreted factors CF and control lung matrisome. Representative immunostaining of differentially expressed proteins in each category. Arrows indicate positive cytoplasmic galectin staining. Asterisks indicate positive nuclear NFκB.

$p = 0.017/0.002$; SEMA3B, $\log_2$FC $= -1.6$, $p = 0.046$). Tetranectin (CLEC3B) was found to be downregulated in CF ($\log_2$FC $= -1.62$, $p = 0.001$). However, galectin-1 (LGALS1) was overexpressed in CF, at moderate to high levels ($\log_2$FC $= 1.43$, $p = 0.009$), indicative of infection response and myeloid cell recruitment (Fig. 5**A**). Immunostaining of tetranectin in CF samples showed sparse staining, whereas galectin staining was moderately increased in CF compared to control lung.

Matrix regulators are proteins responsible for crosslinking, degrading, and remodeling the matrix. Consistent with the breakdown of key matrix constituents, we observed significant downregulation of proteins that inhibit matrix degradation, including trypsin inhibitor (ITIH5, $\log_2$FC $= -1.82$, $p = 0.002$), serine protease inhibitor (SERPINA5, $\log_2$FC $= -1.57$, $p = 0.013$), and tissue inhibitor of metalloproteinase 3 (TIMP3, $\log_2$FC $= -2.62$, $p = 0.019$). Similarly, lower levels of LOXL2 ($\log_2$FC $= -1.05$, $p = 0.019$), a collagen and elastin crosslinker, correlates with observed decreases in both collagen and elastin levels in CF lung. Matrix metalloprotease (MMP) expression varied, with upregulation of MMP11 and MMP8 and downregulation of MMP28 (Fig. 5**B**).

Staining for MMP11 was more pronounced in CF compared to control lung. Histidine rich glycoprotein (HRG) which is expressed by platelets and modulates angiogenesis, fibroblast proliferation, complement activation, coagulation, and fibrinolysis, was significantly upregulated (HRG, $\log_2$FC $= 1.08$, $p = 0.014$) and showed increased expression and more nuclear localization in CF compared to control lung. Other alterations in proteins expressed by or associated with platelets were also observed, including upregulation of platelet derived growth factor subunit B (PDGF-B, $\log_2$FC $= -1.7$, $p = 0.028$), fibulin-1 (NS), and type 3 collagen (NS).

Among the secreted factors that were identified, inflammatory markers – including interleukin 16 (IL-16, $\log_2$FC = 3.9, $p = 0.031$) and transforming growth factor beta (TGF-β1, $\log_2$FC = 2.3, $p = 0.024$), were all significantly increased in CF compared to control lung, whereas interleukin 17 (IL-17, $\log_2$FC = −2.2, $p = 0.009$) was downregulated. Several constituents of the WNT pathway were significantly downregulated in CF, and β-catenin and NF-kB demonstrated increased nuclear localization (**Fig. 5C**). Consistent with these findings, WNT ligand biogenesis and trafficking (p = 1.05E-7) was identified as a significantly altered pathway via GO analysis (**Supplementary Table 3**).

4. Discussion

Lung extracellular matrix serves as the major structural component of the lung and provides a large surface area for gas exchange in the form of the alveolar basement membrane. In CF, structural remodeling of the airways and elevated levels of proteolytic enzymes and matrix breakdown products in airway fluid are recognized indicators of worsening disease [4,7–10]. These well-described features suggest changes to the lung matrix may also be present in CF. Despite the recognized importance of the matrix in lung development, injury, and disease, a comprehensive characterization of the CF lung matrix has not been previously reported. This study addresses this gap and provides a comprehensive and quantitative analysis of the distal lung matrix in end-stage CF.

Analysis of CF lung matrix composition and structure revealed pathological remodeling of tissue architecture, destruction of alveolar basement membranes, and significant loss of key constitutive and associated matrix proteins, including collagens, laminins, and elastin. Obliteration of distal lung parenchymal structure and loss of matrix protein diversity were identified as features of end-stage CF. Through LC-MS/MS analysis of end-stage CF samples in comparison to control lung tissue, we determined that the majority of significantly altered matrix proteins (> 90%) were expressed at lower levels in CF compared to control lung. Interestingly, the total matrix protein abundance in CF and control lung tissue was not significantly different, suggesting that CF does not change the total amount of matrix but instead leads to loss of diversity among matrix proteins.

In previous studies, matrix-degrading enzymes including neutrophil elastase, collagenases, serine proteases, and matrix metalloproteinases have been identified in airway secretions, BAL fluid, and sputum of CF patients, with elevated levels correlating with pulmonary exacerbations and respiratory decline [8,18–20]. Pathologically low levels of protease inhibitors including TIMPs, alpha1-antitrypsin, and secretory leukocyte protease inhibitor (SLPI) have also been identified in CF [9,21–25], and matrix breakdown products including collagen and elastin fragments were detected in BAL, serum, and urine of CF patients [11,26,27]. Here we report a pathologically altered lung matrisome in end-stage CF, which includes destruction of key structural matrix and matrix-associated proteins. Taken with the prior evidence in the literature, these findings suggest that matrix breakdown may correlate with functional decline of the lung in CF.

The lung matrix serves as a structural scaffold that provides macro- and micro-scale features to support lung function, including ventilation and oxygenation. For example, elastin confers the lung with appropriate compliance and enables dynamic movement during breathing, and basement membrane proteins provide a well-organized, thin membrane across which gas exchange occurs. Disruption to matrix proteins that enable ventilation and oxygenation has a deleterious effect on lung function. Our findings indicate that matrix constituents involved in lung elasticity (i.e., elastin) and basement membrane integrity (i.e., collagen IV, laminin) are pathologically altered in CF.

Elastin was degraded in end-stage CF, as evidenced by LC-MS/MS and immunofluorescence. We also observed a loss of lysyl oxidase 2 (LOXL2), which functions as an elastin crosslinker to stabilize the matrix. These findings are consistent with features of emphysema, in which protease and antiprotease imbalance and immune cell recruitment lead to irreversible destruction of the lung parenchyma, loss of tissue elasticity, and impairment of proper ventilation [28–31]. Evidence of tissue breakdown, similar to that which occurs in emphysematous lung, was also observed on histology in CF specimens. Similarly, prior work has suggested emphysema as a unifying feature in end-stage CF based on histopathological and radiographic analysis [32].

Basement membrane constituents, including several isoforms of collagen IV and laminin which were visualized with immunofluorescence and quantified with LC-MS/MS, were degraded in end-stage CF, consistent with dysregulation of the basement membrane and alveolar structure observed on SEM and TEM. Disruption to the basement membrane is a feature of many acute and chronic lung pathologies (e.g., emphysema, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS)), contributing to functional impairment of the lung by compromising the integrity of the blood-gas barrier [33–35]. This pattern of destruction may be mediated by an imbalance between proteases and protease inhibitors, and has been shown to correlate with mortality in other respiratory conditions [35–37]. Loss of basement membrane integrity in CF may contribute to cytokine release that triggers paracrine-mediated inflammation, as well as edema and alveolar fluid retention, leading to impaired gas exchange [37,38].

Gene ontology analysis revealed several pathways involving cell-matrix interactions and cellular function, including cell-substrate junction, focal adhesion, extracellular vesicles, cellular component biogenesis and cellular localization, indicating that matrix degradation has downstream effects on cell populations in the lung. Furthermore, significantly altered matrix-associated proteins were identified via LC-MS/MS, including galectin which mediates cell-matrix interactions and proliferation, and tetranectin which binds to plasminogen and may regulate secretion and exocytosis [39,40]. IL-16 and TGF-β were both significantly increased in CF lung tissue and 'response to TGF-β' was an identified GO term, indicative of the inflammatory process that contributes to aberrant wound healing and can signal epithelial-mesenchymal transition (EMT), a common pathway implicated in many lung diseases [41,42]. Proteins involved in EMT, including several WNT proteins, were altered in CF compared to control lungs. Dysregulated EMT has deleterious effects on lung cell phenotype and function by: (*i*) impairing epithelial barrier function and innate defense mechanisms, (*ii*) enabling migration of activated stromal cells throughout the lung matrix, and (*iii*) promoting stromal cell hyperplasia while compromising pseudostratified airway epithelium [41,43].

Interestingly, in the filtered LC-MS/MS dataset for downregulated peptides ($\log_2$FC < −1), GO terms identified included several pathways involved in matrix structure and organization, such as extracellular matrix structural constituent, extracellular matrix organization, and degradation of the extracellular matrix. Additionally, several GO pathways that were identified in the downregulated dataset implicate the basement membrane specifically, including: basement membrane, collagen type IV trimer, and laminin complex. In comparison, when the dataset was filtered for upregulated peptides ($\log_2$FC > 1), the identified GO pathways included: cellular responses to topologically incorrect protein and unfolded protein, and negative regulation of wound healing. These findings suggest that downregulation of matrix organizational pathways may result in destruction of matrix and aberrant cellular response to matrix fragments (i.e., matrikines) present in the extracellular environment.

Several outstanding questions remain towards better understanding of the complex changes in CF matrix. This study only evaluated end-stage CF lung samples obtained at the time of lung transplant. Future studies should assess temporal changes to the matrix in comparison with age-matched controls, and determine specific effects on cell phenotype and function. The number of samples in this study was too small to determine if mutation-specific changes to the matrix occur, and data on clinical history was not available because patient samples were obtained as de-identified surgical waste that was considered non-human subjects research. Future studies with larger patient cohorts could reveal correlations between matrix changes, clinical status, and CFTR mutation(s).

The study was focused on tissue-level changes, therefore alterations to protease levels in BAL or sputum were not analyzed. Longitudinal proteomic analysis of multiple sample types (i.e., tissue, serum, BAL fluid, urine, etc.) from individual patients may enable mechanistic understanding of disease processes involving matrix degradation. While sampling distal lung tissue via biopsy is challenging, identification of serum or BAL markers that correlate with lung matrix degradation could inform patient care, as previous studies have shown that administration of nebulized or intravenous medication can mitigate elevated proteolytic enzyme levels in BAL fluid [18,23,44].

It is unclear if matrix breakdown is intrinsically driven by loss of CFTR expression or if chronic infection is the primary driver of matrix destruction. Comparison of matrix composition in other organs affected by CF (i.e., pancreas, liver) that are not undergoing an infectious process may offer insights into the relative contributions of intrinsic loss of CFTR versus chronic lung infection to matrix breakdown. For example, increased collagen deposition has been reported in liver and pancreas of CF patients [45,46], suggesting that matrix degradation in the lung may be primarily driven by chronic infection and corresponding immune response rather than loss of CFTR. Similarly, elevated protease levels correlate with bronchiectasis and tissue damage in primary ciliary dyskinesia, indicating that persistent respiratory infection triggers matrix breakdown pathways even in the presence of functioning CFTR [47]. Nevertheless, the extent to which CFTR loss contributes to parenchymal obliteration in CF should be further evaluated. Comprehensive matrisome analyses of lung tissues from COPD and idiopathic pulmonary fibrosis (IPF) have been previously reported [12], and when taken with our findings, suggest that changes to the lung matrix are disease-specific. Though these diseases differ in their underlying pathophysiology, cross-disease matrix comparison may unveil common pathways implicated in lung inflammation, matrix remodeling, and tissue repair, which could serve as therapeutic targets.

CFTR modulator drugs such as Trikafta have revolutionized the cycle-of-care for CF patients and led to dramatic improvements in functional lung parameters [48]. The ability of these CFTR-targeting medications to indirectly prevent matrix degradation, recover dysregulated matrix pathways, or repair degraded lung matrix should be evaluated. Drivers of matrix destruction identified in this study could act as therapeutic targets or biomarkers to monitor disease progression. Drugs targeting dysregulated matrix pathways may serve as adjunct interventions to CFTR modulators to support repair of damaged tissue and recovery of lung function in CF patients.

Author contributions

M.R.P., M.A.T., M.B., J.D.O., N.V.D., and G.V.N. designed the study. M.R.P., M.A.T., S.F., J.A.R., M.H., A.E.H., S.R.K., Y.T., R.K.S., and O.P.G. performed experiments. M.R.P., M.A.T., S.F., J.A.R., M.H., A.E.H., S.R.K., R.K.S., B.A.G., J.D.O. analyzed data. C.C.M. performed histopathologic review. M.R.P., M.A.T., S.F., B.A.G., J.D.O., and G.V.N co-wrote manuscript. All authors reviewed and approved the submitted version of the manuscript.

Declaration of Competing Interest

The authors declare that no conflict of interest exists

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2022.04.016.

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