Chinese Medical Journal Pulmonary and Critical Care Medicine 1 (2023) 67–76

Contents lists available at ScienceDirect

Chinese Medical Journal Pulmonary and Critical Care Medicine

journal homepage: www.elsevier.com/locate/pccm

Review Article

TIMP-1 and its potential diagnostic and prognostic value in pulmonary diseases

Sultan Almuntashiri[1,2], Abdullah Alhumaid[1,2], Yin Zhu[1], Yohan Han[1], Saugata Dutta[1], Ohmed Khilji[3], Duo Zhang[1,4,*], Xiaoyun Wang[1,**]

[1] Clinical and Experimental Therapeutics, Department of Clinical and Administrative Pharmacy, College of Pharmacy, University of Georgia and Charlie Norwood VA Medical Center, Augusta, GA 30912, USA

[2] Department of Clinical Pharmacy, College of Pharmacy, University of Hail, Hail 55473, Saudi Arabia

[3] Department of Emergency Medicine, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA

[4] Department of Medicine, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA

ARTICLE INFO

Edited by: Peifang Wei

Keywords:
Metalloproteases
Extracellular matrix
Chronic obstructive pulmonary disease
Idiopathic pulmonary fibrosis
Cystic fibrosis
Lung injury

ABSTRACT

Tissue inhibitors of metalloproteases (TIMPs) have caught the attention of many scientists due to their role in various physiological and pathological processes. TIMP-1, 2, 3, and 4 are known members of the TIMPs family. TIMPs exert their biological effects by, but are not limited to, inhibiting the activity of metalloproteases (MMPs). The balance between MMPs and TIMPs is critical for maintaining homeostasis of the extracellular matrix (ECM), while the imbalance between MMPs and TIMPs can lead to pathological changes, such as cancer. In this review, we summarized the current knowledge of TIMP-1 in several pulmonary diseases namely, acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), pneumonia, asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and pulmonary fibrosis. Considering the potential of TIMP-1 serving as a non-invasive diagnostic and/or prognostic biomarker, we also reviewed the circulating TIMP-1 levels in translational and clinical studies.

Introduction

Since their discovery in the 1980s, tissue inhibitors of metalloproteases (TIMPs) have caught the attention of many scientists due to their role in various physiological and pathological reactions. TIMP-1, 2, 3, and 4 are known members of the TIMPs family, where TIMPs exert their biological effects by, but are not limited to, inhibiting the activity of metalloproteases (MMPs).[1,2] Due to its bodily omnipresence, TIMPs activity varies depending on the affected tissues, where this activity increases in certain tissues and decreases in others in the pathogenesis of certain diseases. TIMPs are involved in inhibiting metastases and angiogenesis in cancer and supporting neuronal regulation and certain cellular functions.[3,4] The balance between MMPs and TIMPs is critical for maintaining homeostasis of the extracellular matrix (ECM), while the imbalance between MMPs and TIMPs can lead to pathological changes, such as cancer.[2,5] Among the four members of the TIMPs family, TIMP-1 uniquely exhibits certain effects through ways other than only binding to different forms of MMPs.[2,6,7] TIMP-1 can be detected in body fluids and most tissues/organs and promotes cell growth.[8,9] These pathways are activated by TIMP-1 via activating p38, mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK).[10] Growth factors, such as transforming growth factor β1 (TGF-β1), fibroblast growth factor (FGF), and epidermal growth factor (EGF), along with phorbol ester and some cytokines such as interleukin-1 (IL-1) and interleukin-6 (IL-6), are known inducers of TIMP-1 expression.[11] Despite the numerous protective and favorable activities of TIMP-1, its dysregulation has been observed in various disease conditions. For instance, a study reported that TIMP-1 is highly expressed in glioblastoma and is associated with poor prognosis.[12] Moreover, the absence of TIMP-1 in immunostaining tests showed a more positive impact on certain types of breast cancer.[13] These facts expand our understanding of TIMPs as it clarifies the role of these enzymes by going against the previous concept of the positive impact of TIMPs in tumor prevention.[14] Therefore, TIMPs exerted preferable effects on some but not all pathological conditions, where their activities should be investigated independently.

TIMP-1 shows a high expression in the lung [Fig. 1A]. The expression of TIMP-1 is markedly altered in pulmonary diseases due to the remodeling or destruction of the ECM. Elevated levels of TIMP-1 than

* Correspondence to: Clinical and Experimental Therapeutics, Department of Clinical and Administrative Pharmacy, College of Pharmacy, University of Georgia and Charlie Norwood VA Medical Center, Augusta, GA 30912, USA.
E-mail address: duozhang@uga.edu (D. Zhang)

** Correspondence to: Department of Medicine, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA.
E-mail address: xiaoyunwang@uga.edu (X. Wang)

https://doi.org/10.1016/j.pccm.2023.05.002
Received 28 September 2022; Available online 19 June 2023
2097-1982/© 2023 The Authors. Published by Elsevier B.V. on behalf of Chinese Medical Association. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

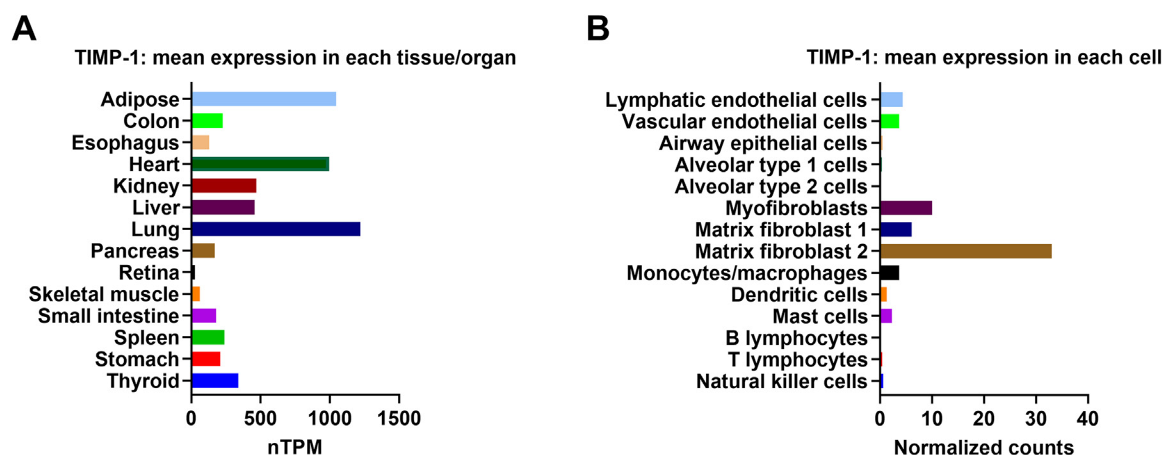

Fig. 1. TIMP-1 expression profiles. (A) RNA-sequencing tissue data generated by the GTEx project are reported as nTPM, corresponding to mean values of the different individual samples from each tissue. TIMP-1 mRNA expression profile was accessed on 02/28/2023. (B) The lung single-cell RNA-sequencing data generated by LungGENS database show the relative TIMP-1 mRNA expression in various human lung cells (accessed on 02/28/2023). GTEx: Genotype-Tissue Expression; LungGENS: Lung Gene Expression iN Single-cell; mRNA: Messenger RNA; nTPM: Normalized protein-coding transcripts per million; RNA: Ribonucleic acid; TIMP: Tissue inhibitors of metalloprotease.

MMPs may contribute to lung fibrosis whereas lower levels of TIMP-1 than MMPs may enhance the degradation of collagen in the interstitial space causing lung injury. In other lung diseases, several factors including etiology, severity, and duration largely alter TIMP-1 regulation within each disease. Thus, emphasizing the significance of this protein in lung diseases might increase the potential of developing novel therapeutic agents or biomarkers. Therefore, we review the roles of TIMP-1 in pulmonary diseases namely, acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), pneumonia, asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and pulmonary fibrosis. Due to the possibility of TIMP-1 serving as a non-invasive blood marker, we focused on circulating TIMP-1 in clinical studies. Additionally, we also reviewed both *in vitro* and *in vivo* studies to provide a better understanding of its function and regulation in lung diseases.

Expression and regulation of TIMP-1

Gene regulation ensures that cells express the necessary genes to proliferate, differentiate, and maintain their proper function by turning gene transcription on and off. Understanding the regulation of TIMP-1 is critical for the investigation of its involvement in various pulmonary diseases. Single-cell RNA sequencing analysis revealed that fibroblasts have the highest transcriptional level among lung cells, while endothelial cells and myeloid cells have moderate TIMP-1 levels [Fig. 1B].[15] The regulation of TIMP-1 by chemicals, inhibitors, cytokines, chemokines, growth factors, etc. has attracted the attention of researchers for decades [Table 1]. Most studies have shown that fibrosis stimulus can trigger TIMP-1 expression in lung fibroblasts, macrophages, and epithelial cells. For instance, TIMP-1 expressions were most prominent in mononuclear inflammatory cells within the regions of tissue damage upon bleomycin stimulation, and TIMP-1 messenger RNA (mRNA) was identified close to areas of inflammatory cell accumulation.[16] Consistently, immunoreactive TIMP-l was expressed in alveolar macrophages in both bleomycin-resistant and bleomycin-prone mice.[17] Following paraquat and hyperoxia exposure, TIMP-1 was also localized in alveolar macrophages in the lungs of fibrosis rats.[18] Similarly, with multi-walled carbon nanotube (MWCNT) model, Mac2+ macrophages were the source of TIMP-1 production, inside the fibrotic foci of the lungs.[19] These studies emphasized that the mononuclear inflammatory cells particularly macrophages as a source of TIMP-1.

Fibroblasts from the other side appeared to be prominent cells for TIMP-1 expression and production. Hsp47+ fibroblasts were the predominant source of TIMP-1 production inside the fibrotic foci of the lungs in response to MWCNT-induced lung fibrosis.[19] Consistently, TIMP-1 mRNA was upregulated in pulmonary fibroblasts derived from fibrosis-sensitive C57BL/6 mice after the stimulation with active TGF-β1 compared to fibroblasts obtained from fibrosis-resistant BALB/c mice.[20] Given that Smad-3 is recognized as a major mediator of TGF-β signaling in progressive fibrosis,[21] one study has assessed *Smad-3* knockout (KO) mice after fibrosis stimulus. These results highlight the importance of fibroblasts as TIMP-1 producers in fibrotic conditions and suggest TIMP-1 as a potential therapy target for pulmonary fibrosis. Other cells including bronchiolar and alveolar epithelial cells were reported to slightly express TIMP-1 in murine fibrotic models.[17,18]

TIMP-1 in ALI/ARDS

ALI and ARDS are life-threatening diseases in critically ill patients.[57-59] The early stage of ALI and ARDS is identified by exudative alveolar flooding due to a disruption in the air-blood barrier and by extensive alveolar collapse due to surfactant abnormalities.[60,61] The heterogeneity in causes of ARDS has resulted in a critical underdiagnosis,[62] and to date, no specific pharmacological therapies have shown an improvement in the severe form of lung injury.[63,64] Thus, one of the obstacles in ARDS is the identification of a promising biomarker that can be targeted later to enhance drug therapy.[65]

MMPs and their tissue inhibitors are thought to participate in leukocyte influx and vascular permeability at sites of lung injury.[66,67] Particularly, TIMP-1, as a critical protein in ECM turnover,[68] has been studied as a biomarker or treatment strategy in lung diseases. Nevertheless, the regulation and function of TIMP-1 in ALI/ARDS are largely unknown.

In a large prospective study of mechanically ventilated patients with acute respiratory failure (ARF), TIMP-1 levels were significantly higher in non-survivors than survivors and were independently associated with 90-day mortality.[69] Among different groups of ARF patients, TIMP-1 levels were significantly higher in ARDS subjects than in the entire cohort.[69] The high levels of TIMP-1 were associated with the severity of partial pressure of oxygen/fraction of inspired oxygen ratio (PaO_2/FiO_2) while improving this ratio was associated with reduced TIMP-1 levels.[69] In a prospective study of critically ill patients admitted to the intensive care unit (ICU), higher plasma of TIMP-1 concentrations and MMP-9/TIMP-1 ratios were significantly associated with ARDS and 30-day mortality risk.[70] Moreover, there was a significant negative correlation between plasma TIMP-1 and MMP-9 levels.[70] Recently, our group measured the plasma TIMP-1 level in ARDS patients enrolled in Albuterol to Treat Acute Lung Injury (ALTA) trial.[71] Higher plasma TIMP-1 lev-

Table 1
TIMP-1 regulation in lung cells.

Cell type	Treatment	Change of TIMP-1
Fibroblasts	TGF-β1[22–27]; CSE[28]; IL-13[27]; IL-33[29]; PI3K inhibitor (LY294002)[24,27]; Oncostatin M[24]; WSE[30]; media of *M. tb* infected monocytes[31]	↑
	IL-1β[32,33]; TNF-α[33]; p38 inhibitor (SB203580)[24,31]; ERK1/2 inhibitor (PD9805)[24]; Rho/Rock signaling inhibitor (Y-27632) and TGF-β/Smad signaling inhibitor (staurosporine)[25]; MEK1 inhibitors (U0126 and PD98059)[26]	↓
	TGF-β1[34]; CSE[35]; HRV[36]	→
Monocytes/macrophages	IL-1β[37,38]; LPS[37–41]; nickel nanoparticles[42]; p38 inhibitor (SB20358)[43]; CSE[37]	↑
	CSE or BCG[44]; p38 inhibitor (SB203580) and MEK inhibitor (PD98059)[45]; *M. tb*[43,46]; ERK inhibitor (PD9805)[43]	→
Epithelial cells	TGF-β1[47]; TNF-α or IL-1β[48]; Xanthohumol[49]; Sinomenine[50]	↑
	TNF-α[51]; *M. tb*[52]; ERK1/2 inhibitor (PD9805)[52]; BSE[53]	↓
	TNF-α[54]; LPS[51]; IL-1β[51,54]; HRV[55]; *M. tb*[56]	→

↑ Upregulation; ↓ Downregulation; → Unchanged; BCG: Bacille Calmette-Guérin; BSE: Biomass smoke extract; CSE: Cigarette smoke extract; ERK: Extracellular signal-regulated kinase; HRV: Human rhinovirus; IL: Interleukin; LPS: Lipopolysaccharide; MEK: Mitogen-activated protein kinase kinase; *M. tb*: Mycobacterium tuberculosis; p38: Mitogen-activated protein kinase 14; PI3K: Phosphoinositide 3-kinase; Rho: Rhodopsin; Rock: Rho associated coiled-coil containing protein; SMAD: Suppressor of Mothers against Decapentaplegic; TGF-β: Transforming growth factor-beta; TIMP: Tissue inhibitors of metalloprotease; TNF-α: Tumor necrosis factor-alpha; WSE: Wood smoke extract.

Table 2
Correlation between circulating TIMP-1 levels and clinical severity of ALI/ARDS.

Conclusions	*P* value	Reference
TIMP-1 levels: non-survivors>survivors	<0.001	69
TIMP-1 was independently associated with 90-day mortality	0.004	
The mortality was significantly higher in patients with TIMP-1 levels exceeding 458.6 ng/mL than in patients with levels below the cutoff	<0.001	
TIMP-1 levels were associated with the severity of hypoxemia	<0.05	
MMP-8/TIMP-1 ratio was not correlated with 90-day mortality	>0.05	
TIMP-1 correlated with MMP-8 ($r = 0.247$), CRP ($r = 0.409$), SOFA score (r = 0.323), and SAPS II score (r = 0.162) at 24 h from ICU admission	<0.001	
TIMP-1 negatively correlated with PaO_2/FiO_2 ratio ($r = -0.260$)	<0.001	
TIMP-1 levels were associated with ARDS	0.01	70
TIMP-1 levels were associated with 30-day mortality	0.02	
MMP-9/TIMP-1 ratios were associated with the increased risk of ARDS	0.02	
MMP-9/TIMP-1 ratios were associated with the increased risk of 30-day mortality	<0.01	
TIMP-1 and MMP-9 show a negative correlation ($r = -0.32$)	<0.01	
TIMP-1 and MMP-3 show a positive correlation ($r = 0.38$)	<0.01	
TIMP-1 levels: ARDS patients>normal subjects	<0.001	71
TIMP-1 levels: No difference between female and male ARDS patients	0.481	
TIMP-1 levels: non-survivors > survivors in female patients	<0.001	
TIMP-1 levels: no difference between non-survivors and survivors in male patients	0.649	
The 90-day mortality was significantly higher in female patients with TIMP-1 levels exceeding 159.7 ng/mL than in patients with levels below the cutoff	<0.001	

ALI: Acute lung injury; ARDS: Acute respiratory distress syndrome; CRP: C-reactive protein; ICU: Intensive Care Unit; MMP: Metalloprotease; PaO_2/FiO_2: Partial pressure of oxygen/fraction of inspired oxygen ratio; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment; TIMP: Tissue inhibitors of metalloprotease.

els were observed in ARDS patients than in normal control subjects.[71] Interestingly, circulating TIMP-1 shows an excellent discriminating ability for the prediction of mortality among female ARDS patients.[71] These findings highly suggest the potential of circulating TIMP-1 as a prognostic biomarker for ALI/ARDS [Table 2]. Nevertheless, more studies are required from the ARDS biomarker discovery to clinical application, especially as a sex-specific biomarker.

The pre-clinical studies on lung injury have focused on various aspects of TIMP-1 by using both wild-type (WT) and *Timp-1* deficient mice. These aspects were mainly TIMP-1 expression, MMP-9/TIMP-1 ratio, weight loss, immune cell infiltrations, and lung hemorrhage. Allen *et al.*[67] have shown that influenza infection caused a substantial induction of TIMP-1 in WT mice. Consistently, TIMP-1 expression and MMP-9/TIMP-1 ratio were significantly higher in the ALI group compared with the control group after lipopolysaccharide (LPS) instillation in mice and rats, respectively.[72,73] In the latter study, the MMP-9/TIMP-1 ratio was positively associated with the lung wet/dry ratio and the pulmonary permeability index.[73] Functionally, *Timp-1* deficient mice showed significantly less body weight loss than WT mice after *Pseudomonas aeruginosa* (*P. aeruginosa*)[74] or influenza infection.[67] In addition, *Timp-1* deficient mice demonstrated fewer immune cell infiltrates and airway inflammation after influenza infection, suggesting that TIMP-1 promotes lung immune response.[67] In line with these findings, the knockdown of TIMP-1 using small interfering RNA (siRNA) leads to a reduced lung inflammatory phenotype during LPS-induced ALI.[75] Collectively, TIMP-1 is induced in response to ALI and promotes immune responses. Loss of *Timp-1* exerts protective effects by reducing lung inflammation.

TIMP-1 in pneumonia

Pneumonia is defined as an infection that occurs in the lung parenchyma and is described by alveoli filling with inflammatory exudates and ultimately leading to pulmonary tissue solidification.[76] In normal physiology, polymorphonuclear neutrophils (PMNs) located in the vascular bed of the lung serve as a powerful host defense barrier.[77] In pneumonia, these cells invade the alveolar compartment by secreting enzymes stored in granules and vesicles like MMPs and TIMP, which play crucial roles including ECM turnover, tissue degradation, and repair mechanisms.[78]

TIMP-1 has been studied in patients with pneumonia including community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP) to evaluate whether the levels of TIMP-1 are related to clinical severity of the disease. TIMP-1 concentration was significantly increased in plasma from CAP patients compared to healthy controls[79] and pa-

Table 3
Circulating TIMP-1 levels and clinical severity of asthma.

Conclusions	*P* value	Reference
Serum TIMP-1 concentrations of asthmatic patients were significantly higher than those of the control subjects	<0.001	86
Lower circulating levels of TIMP-1 in patients with asthma than that in controls, but the differences were not statistically significant	0.27	90
Neither TIMP-1 concentration nor MMP-9/TIMP-1 ratio was related to asthma severity	-	91
Serum TIMP-1 level was higher in asthma patients than in healthy subjects	0.01	87
No difference in the circulating TIMP-1 concentrations between patients with asthma exacerbation or stable asthma	>0.05	92
No difference was seen between asthmatic patients and healthy subjects	>0.05	89

TIMP: Tissue inhibitors of metalloprotease; MMP: Metalloprotease.

tients without lung diseases.[80,81] But no significant difference in the MMP-9/TIMP-1 ratio was seen.[79] Based on these results, TIMP-1 had a more significant change than MMP-9/TIMP-1 in response to pneumonia. Interestingly, a significant decrease in the TIMP-1 and MMP-9/TIMP-1 ratio was noticed after the CAP patients received antibiotic treatment compared with the pre-treatment level.[79,82] Furthermore, both TIMP-1 and MMP-9/TIMP-1 ratios were evaluated in relation to inflammatory cells and pneumonia severity scores. Chiang *et al.*[79] have shown that the plasma MMP-9/TIMP-1 ratio was positively correlated with the number of white blood cells (WBC) and neutrophils. In the same study, the plasma TIMP-1 level was also positively correlated with pneumonia severity scores including Pneumonia Severity Index (PSI), Acute Physiology and Chronic Health Evaluation (APACHE II), and CURB-65 (confusion, uremia, respiratory rate, blood pressure, age ≥65 years) scores.[79] Likewise, Bircan *et al.*[82] have reported that TIMP-1 level was correlated with PSI scores as well as oxygenation indices including PaO_2 and PaO_2/FiO_2 ratio. These results suggest that circulating TIMP-1 levels could reflect the clinical severity of pneumonia.

TIMP-1 in asthma

Asthma is a complex disease triggered by some genetic, epigenetic, and environmental factors. It is characterized by lymphocyte and eosinophil infiltrates leading to chronic inflammation, bronchial fibroblast activation, and airway wall remodeling.[83,84] ECM production and its degradation are involved in this dynamic process. Likewise, MMPs and their specific inhibitors have been reported to play crucial roles in this process.[85] TIMP-1 particularly may contribute to the pathogenesis of exaggerated submucosal ECM accumulation and lack of matrix degradation in asthma.

Overall, circulating TIMP-1 has been studied in clinical cohorts that included asthmatic patients, patients with different stages of asthma, and healthy subjects to assess the variability in TIMP-1 levels between the groups. Although alteration of circulating TIMP-1 levels was seen in patients with asthma, inconsistency in results was reported [Table 3]. For instance, three independent studies have shown that serum TIMP-1 concentrations of asthmatic patients were significantly higher than those of the control subjects.[86-88] However, no difference was seen between asthmatic patients and healthy subjects in another two independent studies.[89,90] Furthermore, the TIMP-1 and MMP-9/TIMP-1 ratios were not related to asthma severity as assessed with forced expiratory volume in one second (FEV_1).[91] Likewise, there were no significant differences in serum TIMP-1, MMP-2/TIMP-1, and MMP-9/TIMP-1 between different groups of asthma.[87,92] These studies suggest that TIMP-1 in circulation may not be largely altered due to asthma prognosis or severity.

The most common preclinical model utilized in asthma studies is ovalbumin (OVA) sensitization.[93] The regulation of TIMP-1 has been studied after OVA challenge, and Lin *et al.*[94] have shown that mice sensitized with OVA had significantly higher concentrations of TIMP-1 in bronchoalveolar lavage (BAL) than the control group. Moreover, Sands *et al.*[95] employed *Timp-1* KO mice in an OVA-induced allergic asthma model (*Timp-1* KO-OVA) to test the hypothesis that the absence of Timp-1 would increase airway hyperactivity, lung inflammation, and remodeling in asthma. They have shown that *Timp-1* KO-OVA mice were deteriorated based on airway activity, methacholine responsiveness, dynamic lung compliance, and lung histological data in comparison to *Timp-1* KO mice receiving PBS. In addition, *Timp-1* KO-OVA mice showed higher cytokines gene expressions than WT-OVA mice, such as interleukin (IL)-5, IL-6, and IL-10.[95] In addition, eosinophil count was significantly higher in *Timp-1* KO-OVA mice than in WT-OVA mice.[95] Their findings suggested that TIMP-1 plays a protective role by modulating inflammatory responses including cytokines expression and eosinophilic inflammation.

In a murine model of toluene diisocyanate (TDI)-induced asthma, TIMP-1 was increased at both mRNA and protein levels in the lung tissues after TDI inhalation in a time-dependent manner.[96] Similarly, the concentration of TIMP-1 in the BAL was increased in the TDI-exposed mice at different time points.[96] In the asthmatic mice, positive TIMP-1 staining was seen on inflammatory cells around bronchioles and significant correlations between the levels of TIMP-1 and the numbers of lymphocytes, neutrophils, and eosinophils were found in the BAL.[96,97]

TIMP-1 in COPD

COPD is an inflammatory lung disease affecting the airways, lung parenchyma, and vasculature. It is characterized by slow progressive airflow limitation leading to dyspnea, chest pain, frequent respiratory infections, exercise limitation, and respiratory failure.[98] There is a widely accepted theory that ECM remodeling is an important causative factor for COPD, which is mediated by exaggerated inflammation and disruption of the proteinase/anti-proteinase balance. Moreover, both MMPs and TIMPs are believed to play crucial roles in the pathogenesis of COPD.[99]

Circulating TIMP-1 levels in COPD have been measured in multiple clinical cohorts [Table 4]. In a few clinical studies, the concentration of TIMP-1 in serum was higher in COPD patients than in controls.[86,100-102] However, Shaker *et al.*[103] have identified that patients with COPD had significantly lower levels of plasma TIMP-1 than smokers and non-smokers control. Similarly, D'Armiento *et al.*[104] have shown that plasma TIMP-1 levels were significantly lower in the emphysema cohort compared to both the control and smoker groups. In the affirmative, current smoking, a major cause of COPD, was associated with reduced TIMP-1 levels in COPD patients.[105] Different factors including disease severity and duration could explain varying results from previous cohort studies.

The molar ratio of MMP-9/TIMP-1 has been generally considered an important parameter in several COPD studies. Gilowska *et al.*[106] found a significant difference in MMP-9/TIMP-1 ratio between control and COPD patients toward higher levels in COPD patients. Moreover, Uysal and Uzun[107] found that the circulating MMP-9/TIMP-1 ratio was higher in patients with emphysema than in patients with other phenotypes of COPD. In contrast, one study has found that the circulating MMP-9/TIMP-1 ratio was significantly lower in COPD than in control subjects.[86] The variability in this ratio from previous reports indicates that TIMP-1 level could not easily exhibit MMPs activities and the relationship between these proteins is quite complicated.

Spirometry parameters including FEV_1 and forced vital capacity (FVC) are well-known clinical parameters to assess lung function de-

Table 4
Circulating TIMP-1 levels and COPD.

Conclusions	*P* value	Reference
TIMP-1 serum levels were higher in COPD patients than in healthy control	<0.0001	86
TIMP-1 serum levels negatively correlated with the FEV_1/FVC	<0.05	
The circulating MMP-9/TIMP-1 ratio was significantly lower in COPD than in control subjects	<0.0001	
TIMP-1 increased in COPD compared with that in healthy subjects	<0.001	100
No significant difference in TIMP-1 levels between survivors and non-survivors	0.839	114
A significant difference in MMP-9/TIMP-1 ratio between survivors and non-survivors	<0.001	
Patients with COPD had significantly lower levels of plasma TIMP-1 than smokers and non-smokers controls	0.02	103
Plasma TIMP-1 levels were significantly lower in the emphysema cohort compared to both non-smoker and smoker groups	<0.0001	104
TIMP-1 in plasma did not correlate with disease parameters and was not predictive of subsequent lung function decline among COPD patients	>0.05	
TIMP-1 did not predict the presence of emphysema in smokers	>0.05	115
Circulating MMP-9/TIMP-1 ratio was higher in patients with emphysema than in patients with other phenotypes of COPD	<0.01	107
FEV_1 was correlated with MMP-9/TIMP-1 ratio in patients with emphysema	<0.001	
No significant differences in the serum TIMP-1 levels between the healthy control group and COPD patients	>0.05	116
Increasing age and overweight were significantly correlated to TIMP-1 in COPD	<0.05	105
Current smoking was associated with reduced TIMP-1 levels in COPD	0.013	
Increasing MMP-9/TIMP-1 ratio was associated with current smoking, overweight, and decreasing FEV_1% predicted	<0.05	
A significant difference in MMP-9/TIMP-1 ratio between non-smoker subjects and COPD patients	0.04	106
No significant difference in MMP-9/TIMP-1 ratio between control smokers and COPD patients	0.9	
TIMP-1 was not correlated with annual changes of % predicted FEV_1	0.961	108
A trend of a higher level of TIMP-1 in current smokers than in COPD patients	0.056	
TIMP-1 concentrations were elevated in COPD than in control subjects	<0.001	101
Serum concentrations of TIMP-1 were higher in non-smoking COPD patients as compared with non-smoking control subjects	0.025	
No significant difference between smokers of COPD and control subjects	>0.05	

COPD: Chronic obstructive pulmonary disease; FEV_1: Forced expiratory volume in one second; FVC: Forced vital capacity; MMP: Metalloprotease; TIMP: Tissue inhibitors of metalloprotease.

cline in COPD and asthma patients. Among COPD patients, TIMP-1 serum levels negatively correlated with the FEV_1/FVC ratio reflecting airway obstruction.[86] Yet, two studies have stated that TIMP-1 measurements in plasma were not predictive of subsequent functional decline as assessed by FEV_1.[104,108] Furthermore, the molar ratio of MMP-9/TIMP-1 has shown a negative correlation with FEV_1% predicted in two studies.[105,107] These studies indicate that circulating TIMP-1 and MMP-9/TIMP-1 may partially reflect lung function decline among COPD patients as assessed with FEV_1 and FVC.

Cigarette smoke (CS) has been widely recognized and utilized as a preclinical model for COPD manifestations.[109] Upon CS exposure, mRNA expression of TIMP-1 was highly increased in mice.[110,111] In the latter study, the increase of TIMP-1 protein level was also seen in CS-exposed murine lungs.[111] These studies indicate the dysregulation of TIMP-1 in response to CS may involve in the pathogenesis of COPD. In addition, the imbalance of TIMP-1 and MMP-9 is believed to be associated with the development of lung emphysema in Klotho mice,[112] which exhibit multiple aging-like phenotypes and pulmonary emphysema.[113] Although evidence links the alteration of TIMP-1 levels and COPD, the role of TIMP-1 in the development of COPD is not well studied using gain-of-function or loss-of-function strategies.

TIMP-1 in cystic fibrosis

Cystic fibrosis (CF) is a lethal genetic disease caused by several mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR dysfunction leads to chloride channel defects resulting in mucus accumulation, endobronchial infection, and exaggerated pulmonary inflammation.[117] One of the hallmarks of CF is the progressive remodeling of tissue and in particular, the accumulation of ECM and the lack of matrix degradation.[118] The identification of the mediators involved in CF pathophysiology may provide prognostic markers with the potential to predict disease prognosis and assess response to treatment. Increasing evidence suggests that dysregulated activities of MMPs and their inhibitors lead to scar formation and subsequent tissue fibrosis in CF.[118]

Both TIMP-1 and its ratios have been assessed in CF patients in relation to pulmonary exacerbations and spirometry parameters including forced expiratory volume and vital capacity. In two independent studies, enhanced TIMP-1 was found in CF patients with pulmonary exacerbations compared to healthy controls and patients without pulmonary exacerbations.[119,120] The MMP-9/TIMP-1 ratio was also increased in patients in comparison to healthy controls.[120] In relation to spirometry parameters, Rath et al.[119] have identified that the serum expression of TIMP-1 was significantly increased in CF adult patients with a declined FEV_1 and vital capacity. Similarly, Devereux *et al.*[121] have shown that the plasma MMP-9/TIMP-1 ratio was negatively correlated with FEV_1. Higher concentrations of plasma TIMP-1 were associated with increased mortality.[121] Altogether, TIMP-1 appears to play a crucial role in CF via controlling ECM homeostasis. The circulating TIMP-1 level may serve as a diagnostic and prognostic blood marker.

TIMP-1 in pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and fibrotic lung disease of unknown etiology characterized by epithelial cell injury, fibroblast proliferation, and excessive accumulation of ECM in the alveolar architecture.[122] This disease leads to decreased lung compliance, impaired gas exchange, and eventually lung failure and death despite therapy, with the median survival time being 2–4 years from diagnosis.[123] Thus, the identification of host genes that participate in the development of IPF may help uncover novel drug targets.

IPF patients have higher circulating TIMP-1 levels than controls.[124-126] Patients with MMP-9 gene polymorphism showed an elevation of TIMP-1 supporting the importance of MMP-9/TIMP-1 ratio.[126] Moreover, TIMP-1 was positively correlated with MMP-9 in another study.[127] Thus, altered TIMP-1 levels in IPF patients influence the MMP-9/TIMP-1 ratio, which involves interstitial lung diseases (ILDs).

Pulmonary fibrosis can be induced experimentally using several chemicals including bleomycin, LPS, silica, asbestosis, MWCNT, paraquat and hyperoxia, and cytokine overexpression.[128] The most reported method in the literature is bleomycin representing the most applied preclinical model of lung fibrosis.[129] TIMP-1 mRNA and protein levels from WT mice were increased after intranasal bleomycin administration in lung tissue and BAL.[16,130] Bleomycin-induced TIMP-1 in BAL has been reported in both fibrosis-resistant (BALB/c) and fibrosis-sensitive (C57BL/6) mice.[17] However, another study showed that a single intratracheal injection of bleomycin upregulated TIMP-1 levels in

the lungs and in the BAL from sensitive C57BL/6 mice but not the bleomycin-resistant BALB/c strain.[131] The dose and route of administration could explain the inconsistent findings that were seen in fibrosis-resistant mice. In paraquat and hyperoxia model, fibrosis rats exhibited a significant increase in TIMP-1 mRNA levels from lung homogenates than control.[18] In MWCNT-induced fibrosis method, TIMP-1 mRNA lung expression and protein levels in BALF and serum were markedly increased compared with baseline level in a time-and dose-dependent manner.[19] These studies emphasize the robust association between TIMP-1 and lung fibrosis.

Although preclinical evidence suggests the altered regulation of TIMP-1 in response to bleomycin administration may play a role in pulmonary fibrosis,[17] there was no difference in lung fibrosis between *Timp-1* deficient mice and control mice after bleomycin treatment.[132,133] In another study, Tang *et al.*[134] repressed TIMP-1 using antisense complementary DNA (cDNA) retroviral vectors. Interestingly, they demonstrated that TIMP-1 knockdown can suppress bleomycin-induced pulmonary fibrosis in the early stages.[134] Thus, more studies are needed to address these controversial findings on the role of TIMP-1 in pulmonary fibrosis.

Summary and conclusions

In this review, we summarized the current knowledge of TIMP-1 in pulmonary diseases, including ALI/ARDS, pneumonia, asthma, COPD, cystic fibrosis, and pulmonary fibrosis. We also reviewed the regulation of TIMP-1 in response to *in vitro* stimulus focusing on various lung cells. These studies mainly focused on fibroblasts, macrophages, and epithelial cells.

TIMP-1 has been reported widely with MMPs denoting the importance of the TIMP-1/MMPs ratios as they may intimately affect each other at tissue and biofluid levels. It is theoretically presumed that TIMP-1 is capable of inhibiting MMPs activities, and this can be simply explained by an inverse relationship. Yet, circulating TIMP-1 was positively correlated with MMP-9 and MMP-3 among IPF and ARDS patients, respectively indicating the complex associations of these proteins.[28,71] Generally, it seems that the TIMP-1 level could not easily exhibit MMPs activities and the relationship between these proteins is quite complicated.

Different pathogens or stimulations like *P. aeruginosa*, influenza, *Mycobacterium tuberculosis* (*M. tb*), LPS or bleomycin were applied to induce or suppress TIMP-1 expression in experimental studies. TIMP-1 responds differently to pathogens, which could be caused by several factors including the intensity of each pathogen, the etiology, the molecular pathways, and infectious versus sterile injury. Nevertheless, in most previous reports, *Timp-1* KO mice showed less injury in response to ALI, such as H1N1 influenza infection[67] and *P. aeruginosa*.[74] These studies not only suggest that the loss of *Timp-1* could protect mice from lung infection and injury, but also indicate the potential role of TIMP-1 as an immune modulator.

Fibroblasts appeared to be the most important cells in driving TIMP-1 dysregulation when compared to other cells based on the findings from the current review. For instance, bleomycin administration elicited an increase in the protein levels of TIMP-1 in the BAL and the transcript levels in lung tissue extracts of mice treated with or without anti-PMN antibodies.[135] This indicates that polymorphonuclear leukocytes including neutrophils were not enough to be targeted in diminishing TIMP-1 secretion. Similarly, epithelial overexpression of TIMP-1 did not alter lung fibrosis in mice.[131] In the study of *Smad-3* KO mice, significantly increased expression of TIMP-1 was seen in WT fibroblasts but not in *Smad-3* deficient fibroblasts after treatment with recombinant TGF-β1 *in vitro*. Consistent findings were also seen after TGF-β1 administration *in vivo* in the same study.[136] In another study, the inhibition of TIMP-1 by its neutralizing antibodies *in vitro* effectively reduced the proliferative effect on fibroblasts.[19] Furthermore, TIMP-1 protein secretion was reduced in MRC5 fibroblast cells in response to *M. tb* infection,[31] but was not affected by macrophage infection with the same pathogen.[43] Likewise, *M. tb* infection did not affect TIMP-1 protein secretion in human bronchial epithelial cells (HBECs).[56] Overall, these findings indicate that fibroblasts are the most important cells in driving TIMP-1 dysregulation. Thus, further investigation of TIMP-1 in fibroblasts may unveil potential treatment strategies for lung fibrosis.

In clinical cohorts, circulating TIMP-1 has been measured mainly as a diagnostic marker providing a possibility to differentiate between different groups of patients and to reflect the disease severity. The potential of TIMP-1 to serve as a prognostic marker after receiving therapies has not received much attention in the literature so far. In pneumonia, a significant decrease in the MMP-9/TIMP-1 ratio was noticed after the CAP patients received antibiotic treatment in comparison with the pre-treatment level in two independent studies.[79,82] Consistently, TIMP-1 levels decreased significantly after glucocorticoid therapy compared with the pre-treatment levels in the IPF patients.[126] Thus, TIMP-1 could be investigated as a non-invasive blood marker to evaluate the effectiveness of drug therapies in pulmonary diseases, particularly lung fibrosis.

Different mechanisms and pathways have been studied in the regulation of TIMP-1. So far, TGF/Smad pathways are confirmed as upstream modulators of TIMP-1 [Fig. 2]. MWCNTs activated the extracellular regulated protein kinases (ERK) pathway in murine fibroblast cells in a TIMP-1-dependent manner.[19] However, inhibition of the ERK pathway using PD980590 had no effect on TIMP-1 secretion on MRC5 cells and normal adult human lung fibroblasts after infection with *M. tb*.[31] Furthermore, inhibition of p38 MAPK pathway using SB203580 reversed infection-induced inhibition of TIMP-1 secretion in the later study.[31] Similarly, p38 MAPK inhibitor (SB203580) increased TIMP-1 secretion in a dose-dependent manner after macrophages were infected with *M. tb*.[43] This effect was also seen in normal human bronchial epithelial cells after infection with *M. tb* and using p38 pathway inhibitor (SB203580).[52] Moreover, the upregulation of TIMP-1 by TGF-β1 has been shown in various types of cells.[22-27,47] A significantly increased expression of TIMP-1 in fibroblasts isolated from WT but not in fibroblasts of *Smad3* KO mice was seen after treatment with recombinant TGF-β1 *in vitro*,[136] indicating that the TGF-β/Smad signaling pathway plays a role in the regulation of TIMP-1.

Several other factors may also affect the expression of TIMP-1, including age, weight, and smoking based on the current studies. In COPD, increasing age and overweight were significantly related to increasing TIMP-1 in plasma.[105] Moreover, current smoking was associated with reduced TIMP-1 levels in the same study.[105] It is well known that TIMP-1 is an adipocyte-secreted protein and can be upregulated by adipokines, which may explain the significant association with overweight patients.[137] Similarly, adipokines are well recognized to increase with age and this could be due to increased adipose tissue mass.[138] However, lower levels of TIMP-1 among smokers can be explained by the association of smoking with lipolysis and body weight loss.[139]

Biological sex has been reported to influence susceptibility to infection, immune response, disease severity, and response to therapy.[140,141] Sex hormones particularly estradiol plays regulatory roles in immune responses as described by the induction of pro-inflammatory cytokines and macrophage activation.[142] Estradiol was also found to upregulate T helper 17 (Th17)-related inflammation and worsen pneumonia in mice.[143] TIMP-1 has a genomic location on the X chromosome.[144,145] Most genes from the inactivated X-chromosome are silenced, while TIMP-1 may escape X-inactivation. Anderson and Brown[146] showed that human TIMP-1 is prone to reactivation and also variable in its inactivation. Under inflammatory conditions, estradiol significantly induced TIMP-1 expression in goat oviductal epithelial cells[147] and also in human aortic endothelial cells.[148] Thus, there is a possibility that TIMP-1 could be largely regulated by estradiol and may be affected more in females. Our recent clinical study of ALI/ARDS showed that circulating TIMP-1 level was a promising predictor of mortality, ventilator-free days, and ICU-free days among females.[71] Nevertheless, no study considered the female sex as a factor that could significantly affect TIMP-1

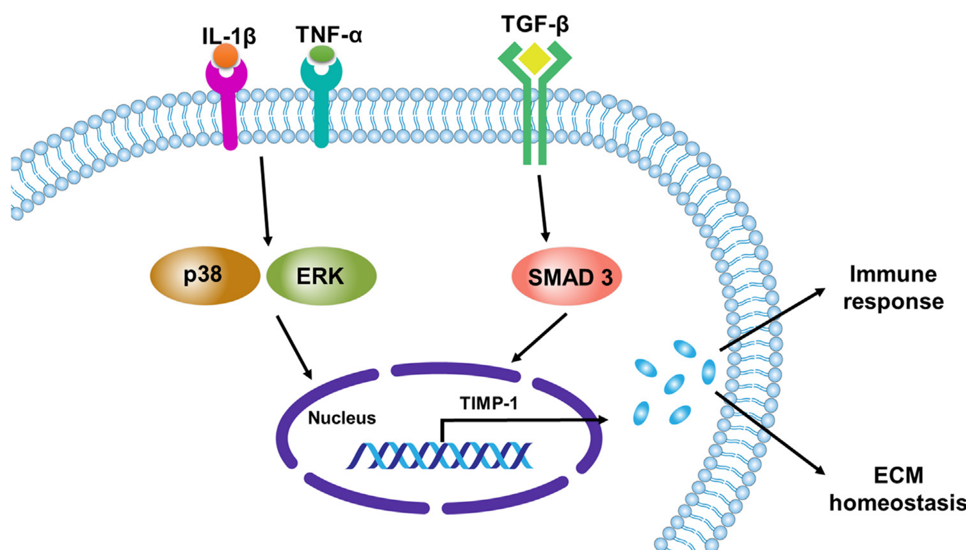

Fig. 2. A schematic representation of TIMP-1 regulation in lung cells. Inflammatory mediators IL-1β, TNF-α, and TGF-β promote TIMP-1 expression via signaling pathways, such as mitogen-activated protein kinases pathways (p38 and ERK) and TGF/SMAD pathway. TIMP-1 may involve in the pathogenesis of pulmonary diseases through the modulation of the immune response and ECM homeostasis. ECM: Extracellular matrix; ERK: Extracellular signal-regulated kinase; IL-1β: Interleukin-1-beta; p38: Mitogen-activated protein kinase 14; SMAD: Suppressor of mothers against decapentaplegic; TGF-β: Transforming growth factor-beta; TIMP: Tissue inhibitors of metalloprotease; TNF-α: Tumor necrosis factor-alpha.

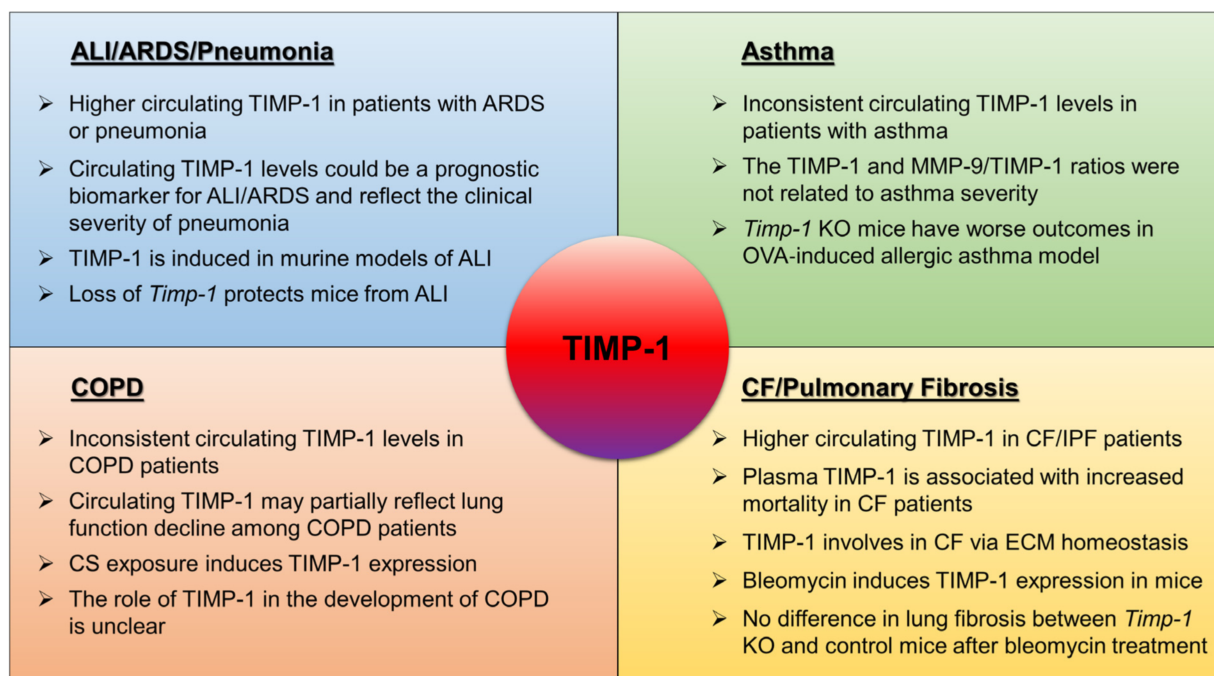

Fig. 3. A diagram illustrating the regulation and function of TIMP-1 in pulmonary diseases. ALI: Acute lung injury; ARDS: Acute respiratory distress syndrome; CF: Cystic fibrosis; COPD: Chronic obstructive pulmonary disease; ECM: Extracellular matrix; IPF: Idiopathic pulmonary fibrosis; KO: Knockout; MMP: Metalloproteases; OVA: Ovalbumin; TIMP: Tissue inhibitors of metalloprotease.

expression under normal and pathological conditions yet. Most previous studies investigating the role of TIMP-1 in lung injury or fibrosis employed male rodents only.[16,19,67,73,74,135,149,150] In addition, some studies only used female rodents[20,75,131] and others did not specify the sex of study subjects.[72,130,136,151] Therefore, the female sex needs to be considered as a key factor that may critically influence TIMP-1 expression under physiological and pathological conditions.

In summary, this review has focused on the dysregulation of TIMP-1 in pulmonary diseases by summarizing the findings in both preclinical and clinical studies [Fig. 3]. It also discussed the heterogeneity and consistency in previous studies. Current findings indicate that TIMP-1 may reflect the pathogenesis of pulmonary diseases, and could serve as a promising targeted therapy for pulmonary fibrosis. In this scenario, neutralizing TIMP-1 using a monoclonal antibody can provide a strategy for inhibiting the abnormally increased TIMP-1 in disease conditions.[152] However, preclinical studies are urgently needed to evaluate the therapeutic efficacy and mechanism before its clinical use in treating pulmonary diseases, especially lung fibrosis.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was supported by National Institutes of Health (NIH) grants NIH/NHLBI R00 HL141685 to D.Z. and NIH/NIAID R03AI169063 to X.W.

Acknowledgment

The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health (NIH), and by National Cancer Institute (NCI), National Human Genome Research Institute (NHGRI), National Heart, Lung, and Blood Institute (NHLBI), National Institute on Drug Abuse (NIDA), National Institute of Mental Health (NIMH), and National Institute of Neurological Disorders and Stroke (NINDS). The data used for the analyses described in Fig. 1A were obtained from the GTEx Portal of The Human Protein Atlas on 02/28/2023.

References

1. Stetler-Stevenson WG, Krutzsch HC, Liotta LA. Tissue inhibitor of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family. *J Biol Chem*. 1989;264:17374–17378. doi:10.1016/S0021-9258(18)71503-2.
2. Justo BL, Jasiulionis MG. Characteristics of TIMP1, CD63, and beta1-integrin and the functional impact of their interaction in cancer. *Int J Mol Sci*. 2021;22:9319. doi:10.3390/ijms22179319.
3. Jackson HW, Defamie V, Waterhouse P, Khokha R. TIMPs: versatile extracellular regulators in cancer. *Nat Rev Cancer*. 2017;17:38–53. doi:10.1038/nrc.2016.115.
4. Remillard TC, Bratslavsky G, Jensen-Taubman S, Stetler-Stevenson WG, Bourboulia D. Molecular mechanisms of tissue inhibitor of metalloproteinase 2 in the tumor microenvironment. *Mol Cell Ther*. 2014;2:17. doi:10.1186/2052-8426-2-17.
5. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochim Biophys Acta*. 2000;1477:267–283. doi:10.1016/s0167-4838(99)00279-4.
6. Triebel S, Bläser J, Gote T, et al. Evidence for the tissue inhibitor of metalloproteinases-1 (TIMP-1) in human polymorphonuclear leukocytes. *Eur J Biochem*. 1995;231:714–719. doi:10.1111/j.1432-1033.1995.0714d.x.
7. Zaoui P, Barro C, Morel F. Differential expression and secretion of gelatinases and tissue inhibitor of metalloproteinase-1 during neutrophil adhesion. *Biochim Biophys Acta*. 1996;1290:101–112. doi:10.1016/0304-4165(96)00008-6.
8. Lizio M, Abugessaisa I, Noguchi S, et al. Update of the FANTOM web resource: expansion to provide additional transcriptome atlases. *Nucleic Acids Res*. 2019;47:D752–D758. doi:10.1093/nar/gky1099.
9. Bertaux B, Hornebeck W, Eisen AZ, Dubertret L. Growth stimulation of human keratinocytes by tissue inhibitor of metalloproteinases. *J Invest Dermatol*. 1991;97:679–685. doi:10.1111/1523-1747.ep12483956.
10. Petitfrère E, Kadri Z, Boudot C, et al. Involvement of the p38 mitogen-activated protein kinase pathway in tissue inhibitor of metalloproteinases-1-induced erythroid differentiation. *FEBS Lett*. 2000;485:117–121. doi:10.1016/s0014-5793(00)02210-9.
11. Lambert E, Dassé E, Haye B, Petitfrère E. TIMPs as multifacial proteins. *Crit Rev Oncol Hematol*. 2004;49:187–198. doi:10.1016/j.critrevonc.2003.09.008.
12. Aaberg-Jessen C, Christensen K, Offenberg H, et al. Low expression of tissue inhibitor of metalloproteinases-1 (TIMP-1) in glioblastoma predicts longer patient survival. *J Neurooncol*. 2009;95:117–128. doi:10.1007/s11060-009-9910-8.
13. Kuvaja P, Talvensaari-Mattila A, Pääkkö P, Turpeenniemi-Hujanen T. The absence of immunoreactivity for tissue inhibitor of metalloproteinase-1 (TIMP-1), but not for TIMP-2, protein is associated with a favorable prognosis in aggressive breast carcinoma. *Oncology*. 2005;68:196–203. doi:10.1159/000086774.
14. Song G, Xu S, Zhang H, et al. TIMP1 is a prognostic marker for the progression and metastasis of colon cancer through FAK-PI3K/AKT and MAPK pathway. *J Exp Clin Cancer Res*. 2016;35:148. doi:10.1186/s13046-016-0427-7.
15. Du Y, Guo M, Whitsett JA, Xu Y. 'LungGENS': a web-based tool for mapping single-cell gene expression in the developing lung. *Thorax*. 2015;70:1092–1094. doi:10.1136/thoraxjnl-2015-207035.
16. Madtes DK, Elston AL, Kaback LA, Clark JG. Selective induction of tissue inhibitor of metalloproteinase-1 in bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2001;24:599–607. doi:10.1165/ajrcmb.24.5.4192.
17. Manoury B, Caulet-Maugendre S, Guénon I, Lagente V, Boichot E. TIMP-1 is a key factor of fibrogenic response to bleomycin in mouse lung. *Int J Immunopathol Pharmacol*. 2006;19:471–487. doi:10.1177/039463200601900303.
18. Ruiz V, Ordóñez RM, Berumen J, et al. Unbalanced collagenases/TIMP-1 expression and epithelial apoptosis in experimental lung fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2003;285:L1026–L1036. doi:10.1152/ajplung.00183.2003.
19. Dong J, Ma Q. TIMP1 promotes multi-walled carbon nanotube-induced lung fibrosis by stimulating fibroblast activation and proliferation. *Nanotoxicology*. 2017;11:41–51. doi:10.1080/17435390.2016.1262919.
20. Kolb M, Bonniaud P, Galt T, et al. Differences in the fibrogenic response after transfer of active transforming growth factor-beta1 gene to lungs of "fibrosis-prone" and "fibrosis-resistant" mouse strains. *Am J Respir Cell Mol Biol*. 2002;27:141–150. doi:10.1165/ajrcmb.27.2.4674.
21. Hu HH, Chen DQ, Wang YN, et al. New insights into TGF-beta/Smad signaling in tissue fibrosis. *Chem Biol Interact*. 2018;292:76–83. doi:10.1016/j.cbi.2018.07.008.
22. Todorova L, Gürcan E, Westergren-Thorsson G, Miller-Larsson A. Budesonide/formoterol effects on metalloproteolytic balance in TGFbeta-activated human lung fibroblasts. *Respir Med*. 2009;103:1755–1763. doi:10.1016/j.rmed.2009.03.018.
23. Wang Z, Chen C, Finger SN, et al. Suberoylanilide hydroxamic acid: a potential epigenetic therapeutic agent for lung fibrosis? *Eur Respir J*. 2009;34:145–155. doi:10.1183/09031936.00084808.
24. Tong L, Smyth D, Kerr C, Catterall J, Richards CD. Mitogen-activated protein kinases Erk1/2 and p38 are required for maximal regulation of TIMP-1 by oncostatin M in murine fibroblasts. *Cell Signal*. 2004;16:1123–1132. doi:10.1016/j.cellsig.2004.03.003.
25. Ji H, Tang H, Lin H, et al. Rho/Rock cross-talks with transforming growth factor-beta/Smad pathway participates in lung fibroblast-myofibroblast differentiation. *Biomed Rep*. 2014;2:787–792. doi:10.3892/br.2014.323.
26. Zhou X, Hu H, Huynh ML, et al. Mechanisms of tissue inhibitor of metalloproteinase 1 augmentation by IL-13 on TGF-beta 1-stimulated primary human fibroblasts. *J Allergy Clin Immunol*. 2007;119:1388–1397. doi:10.1016/j.jaci.2007.02.011.
27. Zhou X, Trudeau JB, Schoonover KJ, et al. Interleukin-13 augments transforming growth factor-beta1-induced tissue inhibitor of metalloproteinase-1 expression in primary human airway fibroblasts. *Am J Physiol Cell Physiol*. 2005;288:C435–C442. doi:10.1152/ajpcell.00035.2004.
28. Guan S, Liu Q, Han F, et al. Ginsenoside Rg1 ameliorates cigarette smoke-induced airway fibrosis by suppressing the TGF-beta1/Smad pathway in vivo and in vitro. *Biomed Res Int*. 2017;2017:6510198. doi:10.1155/2017/6510198.
29. Wu L, Luo Z, Zheng J, et al. IL-33 can promote the process of pulmonary fibrosis by inducing the imbalance between MMP-9 and TIMP-1. *Inflammation*. 2018;41:878–885. doi:10.1007/s10753-018-0742-6.
30. Recillas-Román S, Montaño M, Ruiz V, et al. Wood smoke extract promotes extracellular matrix remodeling in normal human lung fibroblasts. *Int J Toxicol*. 2021;40:506–516. doi:10.1177/10915818211044809.
31. O'Kane CM, Elkington PT, Jones MD, et al. STAT3, p38 MAPK, and NF-kappaB drive unopposed monocyte-dependent fibroblast MMP-1 secretion in tuberculosis. *Am J Respir Cell Mol Biol*. 2010;43:465–474. doi:10.1165/rcmb.2009-0211OC.
32. Kim SH, Baek MS, Yoon DS, et al. Vitamin D inhibits expression and activity of matrix metalloproteinase in human lung fibroblasts (HFL-1) cells. *Tuberc Respir Dis*. 2014;77:73–80. doi:10.4046/trd.2014.77.2.73.
33. Fang Q, Liu X, Al-Mugotir M, et al. Thrombin and TNF-alpha/IL-1beta synergistically induce fibroblast-mediated collagen gel degradation. *Am J Respir Cell Mol Biol*. 2006;35:714–721. doi:10.1165/rcmb.2005-0026OC.
34. Pan R, Zhang Y, Zheng M, Zang B, Jin M. Hydroxysafflor yellow a suppresses MRC-5 cell activation induced by TGF-beta1 by blocking TGF-beta1 binding to TbetaRII. *Front Pharmacol*. 2017;8:264. doi:10.3389/fphar.2017.00264.
35. Kim H, Liu X, Kohyama T, et al. Cigarette smoke stimulates MMP-1 production by human lung fibroblasts through the ERK1/2 pathway. *COPD*. 2004;1:13–23. doi:10.1081/COPD-120030164.
36. Wieczfinska J, Pawliczak R. Thymic stromal lymphopoietin and apocynin alter the expression of airway remodeling factors in human rhinovirus-infected cells. *Immunobiology*. 2017;222:892–899. doi:10.1016/j.imbio.2017.05.010.
37. Russell RE, Culpitt SV, DeMatos C, et al. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol*. 2002;26:602–609. doi:10.1165/ajrcmb.26.5.4685.
38. Lim S, Roche N, Oliver BG, Mattos W, Barnes PJ, Chung KF. Balance of matrix metalloprotease-9 and tissue inhibitor of metalloprotease-1 from alveolar macrophages in cigarette smokers. Regulation by interleukin-10. *Am J Respir Crit Care Med*. 2000;162:1355–1360. doi:10.1164/ajrccm.162.4.9910097.
39. Liang Y, Yang N, Pan G, Jin B, Wang S, Ji W. Elevated IL-33 promotes expression of MMP2 and MMP9 via activating STAT3 in alveolar macrophages during LPS-induced acute lung injury. *Cell Mol Biol Lett*. 2018;23:52. doi:10.1186/s11658-018-0117-x.
40. Jan JS, Yang CH, Wang MH, et al. Hirsutanol a attenuates lipopolysaccharide-mediated matrix metalloproteinase 9 expression and cytokines production and improves endotoxemia-induced acute sickness behavior and acute lung injury. *Mar Drugs*. 2019;17:360. doi:10.3390/md17060360.
41. Wang X, Zhang D, Fucci QA, Dollery CM, Owen CA. Surface-bound matrix metalloproteinase-8 on macrophages: contributions to macrophage pericellular proteolysis and migration through tissue barriers. *Physiol Rep*. 2021;9:e14778. doi:10.14814/phy2.14778.
42. Wan R, Mo Y, Chien S, et al. The role of hypoxia inducible factor-1alpha in the increased MMP-2 and MMP-9 production by human monocytes exposed to nickel nanoparticles. *Nanotoxicology*. 2011;5:568–582. doi:10.3109/17435390.2010.537791.
43. Rand L, Green JA, Saraiva L, Friedland JS, Elkington PT. Matrix metalloproteinase-1 is regulated in tuberculosis by a p38 MAPK-dependent, p-aminosalicylic acid-sensitive signaling cascade. *J Immunol*. 2009;182:5865–5872. doi:10.4049/jimmunol.0801935.
44. Le Y, Cao W, Zhou L, et al. Infection of *Mycobacterium tuberculosis* promotes both M1/M2 polarization and MMP production in cigarette smoke-exposed macrophages. *Front Immunol*. 2020;11:1902. doi:10.3389/fimmu.2020.01902.
45. Lo CY, Huang HY, He JR, et al. Increased matrix metalloproteinase-9 to tissue inhibitor of metalloproteinase-1 ratio in smokers with airway hyperresponsiveness and accelerated lung function decline. *Int J Chron Obstruct Pulmon Dis*. 2018;13:1135–1144. doi:10.2147/COPD.S161257.
46. Elkington PT, Nuttall RK, Boyle JJ, et al. *Mycobacterium tuberculosis*, but not vaccine BCG, specifically upregulates matrix metalloproteinase-1. *Am J Respir Crit Care Med*. 2005;172:1596–1604. doi:10.1164/rccm.200505-753OC.
47. Zhang Q, Guo Y, Dong R, Dai R, Zhou M. Suppressor of cytokine signaling 1-modulated metalloproteinases and tissue inhibitor of metalloproteinase in pulmonary fibrosis. *Mol Med Rep*. 2015;12:3855–3861. doi:10.3892/mmr.2015.3810.

48. Hozumi A, Nishimura Y, Nishiuma T, Kotani Y, Yokoyama M. Induction of MMP-9 in normal human bronchial epithelial cells by TNF-alpha via NF-kappa B-mediated pathway. *Am J Physiol Lung Cell Mol Physiol*. 2001;281:L1444–L1452. doi:10.1152/ajplung.2001.281.6.L1444.
49. Slawinska-Brych A, Mizerska-Kowalska M, Król SK, Stepulak A, Zdzisinska B. Xanthohumol impairs the PMA-driven invasive behaviour of lung cancer cell line A549 and exerts anti-EMT action. *Cells*. 2021;10:1484. doi:10.3390/cells10061484.
50. Shen KH, Hung JH, Liao YC, Tsai ST, Wu MJ, Chen PS. Sinomenine inhibits migration and invasion of human lung cancer cell through downregulating expression of miR-21 and MMPs. *Int J Mol Sci*. 2020;21:3080. doi:10.3390/ijms21093080.
51. Yao PM, Maitre B, Delacourt C, Buhler JM, Harf A, Lafuma C. Divergent regulation of 92-kDa gelatinase and TIMP-1 by HBECs in response to IL-1beta and TNF-alpha. *Am J Physiol*. 1997;273:L866–L874. doi:10.1152/ajplung.1997.273.4.L866.
52. Elkington PT, Emerson JE, Lopez-Pascua LD, et al. *Mycobacterium tuberculosis* up-regulates matrix metalloproteinase-1 secretion from human airway epithelial cells via a p38 MAPK switch. *J Immunol*. 2005;175:5333–5340. doi:10.4049/jimmunol.175.8.5333.
53. Mehra D, Geraghty PM, Hardigan AA, Foronjy R. A comparison of the inflammatory and proteolytic effects of dung biomass and cigarette smoke exposure in the lung. *PLoS One*. 2012;7:e52889. doi:10.1371/journal.pone.0052889.
54. McDougall CM, Blaylock MG, Douglas JG, Brooker RJ, Helms PJ, Walsh GM. Nasal epithelial cells as surrogates for bronchial epithelial cells in airway inflammation studies. *Am J Respir Cell Mol Biol*. 2008;39:560–568. doi:10.1165/rcmb.2007-0325OC.
55. Tacon CE, Wiehler S, Holden NS, Newton R, Proud D, Leigh R. Human rhinovirus infection up-regulates MMP-9 production in airway epithelial cells via NF-{kappa}B. *Am J Respir Cell Mol Biol*. 2010;43:201–209. doi:10.1165/rcmb.2009-0216OC.
56. Brilha S, Chong DLW, Khawaja AA, et al. Integrin alpha2beta1 expression regulates matrix metalloproteinase-1-dependent bronchial epithelial repair in pulmonary tuberculosis. *Front Immunol*. 2018;9:1348. doi:10.3389/fimmu.2018.01348.
57. Ragaller M, Richter T. Acute lung injury and acute respiratory distress syndrome. *J Emerg Trauma Shock*. 2010;3:43–51. doi:10.4103/0974-2700.58663.
58. Lew TW, Kwek TK, Tai D, et al. Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. *JAMA*. 2003;290:374–380. doi:10.1001/jama.290.3.374.
59. Matthay MA, Zimmerman GA. Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. *Am J Respir Cell Mol Biol*. 2005;33:319–327. doi:10.1165/rcmb.F305.
60. Matthay MA, Zemans RL, Zimmerman GA, et al. Acute respiratory distress syndrome. *Nat Rev Dis Primers*. 2019;5:18. doi:10.1038/s41572-019-0069-0.
61. Gonzales JN, Lucas R, Verin AD. The acute respiratory distress syndrome: mechanisms and perspective therapeutic approaches. *Austin J Vasc Med*. 2015;2:1009.
62. Welker C, Huang J, Gil IJN, Ramakrishna H. 2021 acute respiratory distress syndrome update, with coronavirus disease 2019 focus. *J Cardiothorac Vasc Anesth*. 2022;36:1188–1195. doi:10.1053/j.jvca.2021.02.053.
63. Boyle AJ, Mac Sweeney R, McAuley DF. Pharmacological treatments in ARDS; a state-of-the-art update. *BMC Med*. 2013;11:166. doi:10.1186/1741-7015-11-166.
64. Silva PL, Pelosi P, Rocco PRM. Personalized pharmacological therapy for ARDS: a light at the end of the tunnel. *Expert Opin Investig Drugs*. 2020;29:49–61. doi:10.1080/13543784.2020.1699531.
65. García-Laorden MI, Lorente JA, Flores C, Slutsky AS, Villar J. Biomarkers for the acute respiratory distress syndrome: how to make the diagnosis more precise. *Ann Transl Med*. 2017;5:283. doi:10.21037/atm.2017.06.49.
66. Warner RL, Beltran L, Younkin EM, et al. Role of stromelysin 1 and gelatinase B in experimental acute lung injury. *Am J Respir Cell Mol Biol*. 2001;24:537–544. doi:10.1165/ajrcmb.24.5.4160.
67. Allen JR, Ge L, Huang Y, et al. TIMP-1 promotes the immune response in influenza-induced acute lung injury. *Lung*. 2018;196:737–743. doi:10.1007/s00408-018-0154-2.
68. Kubota Y, Nishiwaki K, Ito M, Sugimoto A. The role of tissue inhibitors of metalloproteinases in organ development and regulation of ADAMTS family metalloproteinases in *Caenorhabditis elegans*. *Genetics*. 2019;212:523–535. doi:10.1534/genetics.119.301795.
69. Hästbacka J, Linko R, Tervahartiala T, et al. Serum MMP-8 and TIMP-1 in critically ill patients with acute respiratory failure: TIMP-1 is associated with increased 90-day mortality. *Anesth Analg*. 2014;118:790–798. doi:10.1213/ANE.0000000000000120.
70. Jones TK, Reilly JP, Anderson BJ, et al. Elevated plasma levels of matrix metalloproteinase-3 and tissue-inhibitor of matrix metalloproteinases-1 associate with organ dysfunction and mortality in sepsis. *Shock*. 2022;57:41–47. doi:10.1097/SHK.0000000000001833.
71. Almuntashiri S, Jones TW, Wang X, Sikora A, Zhang D. Plasma TIMP-1 as a sex-specific biomarker for acute lung injury. *Biol Sex Differ*. 2022;13:70. doi:10.1186/s13293-022-00481-9.
72. Guo Y, Ma L, Zhang F, Sun R, Li T. Neutrophil elastase ameliorates matrix metalloproteinase-9 to promote lipopolysaccharide-induced acute lung injury in mice 1. *Acta Cir Bras*. 2016;31:382–388. doi:10.1590/S0102-865020160060000004.
73. Chen G, Ge D, Zhu B, Shi H, Ma Q. Upregulation of matrix metalloproteinase 9 (MMP9)/tissue inhibitor of metalloproteinase 1 (TIMP1) and MMP2/TIMP2 ratios may be involved in lipopolysaccharide-induced acute lung injury. *J Int Med Res*. 2020;48:1–10. doi:10.1177/0300060520919592.
74. Lee MM, Yoon BJ, Osiewicz K, et al. Tissue inhibitor of metalloproteinase 1 regulates resistance to infection. *Infect Immun*. 2005;73:661–665. doi:10.1128/IAI.73.1.661-665.2005.
75. Chernikov IV, Staroseletz YY, Tatarnikova IS, et al. siRNA-mediated *Timp1* silencing inhibited the inflammatory phenotype during acute lung injury. *Int J Mol Sci*. 2023;24:1641. doi:10.3390/ijms24021641.
76. Jain V, Vashisht R, Yilmaz G, Bhardwaj A. *Pneumonia Pathology*. Treasure Island (FL): StatPearls; 2022.
77. Giacalone VD, Margaroli C, Mall MA, Tirouvanziam R. Neutrophil adaptations upon recruitment to the lung: new concepts and implications for homeostasis and disease. *Int J Mol Sci*. 2020;21:851. doi:10.3390/ijms21030851.
78. Chiang TY, Tsao SM, Yeh CB, Yang SF. Matrix metalloproteinases in pneumonia. *Clin Chim Acta*. 2014;433:272–277. doi:10.1016/j.cca.2014.03.031.
79. Chiang TY, Yu YL, Lin CW, Tsao SM, Yang SF, Yeh CB. The circulating level of MMP-9 and its ratio to TIMP-1 as a predictor of severity in patients with community-acquired pneumonia. *Clin Chim Acta*. 2013;424:261–266. doi:10.1016/j.cca.2013.06.013.
80. Herr C, Mang S, Mozafari B, et al. Distinct patterns of blood cytokines beyond a cytokine storm predict mortality in COVID-19. *J Inflamm Res*. 2021;14:4651–4667. doi:10.2147/JIR.S320685.
81. Hartog CM, Wermelt JA, Sommerfeld CO, Eichler W, Dalhoff K, Braun J. Pulmonary matrix metalloproteinase excess in hospital-acquired pneumonia. *Am J Respir Crit Care Med*. 2003;167:593–598. doi:10.1164/rccm.200203-258OC.
82. Bircan HA, Çakir M, Yilmazer Kapulu I, Sütcü R, Kaya S, Öztürk O. Elevated serum matrix metalloproteinase-2 and -9 and their correlations with severity of disease in patients with community-acquired pneumonia. *Turk J Med Sci*. 2015;45:593–599. doi:10.3906/sag-1402-51.
83. Hough KP, Curtiss ML, Blain TJ, et al. Airway remodeling in asthma. *Front Med*. 2020;7:191. doi:10.3389/fmed.2020.00191.
84. Kudo M, Ishigatsubo Y, Aoki I. Pathology of asthma. *Front Microbiol*. 2013;4:263. doi:10.3389/fmicb.2013.00263.
85. Parameswaran K, Willems-Widyastuti A, Alagappan VK, Radford K, Kranenburg AR, Sharma HS. Role of extracellular matrix and its regulators in human airway smooth muscle biology. *Cell Biochem Biophys*. 2006;44:139–146. doi:10.1385/CBB:44:1:139.
86. Higashimoto Y, Yamagata Y, Iwata T, et al. Increased serum concentrations of tissue inhibitor of metalloproteinase-1 in COPD patients. *Eur Respir J*. 2005;25:885–890. doi:10.1183/09031936.05.00092804.
87. Park HS, Kim HA, Jung JW, et al. Metalloproteinase-9 is increased after toluene diisocyanate exposure in the induced sputum from patients with toluene diisocyanate-induced asthma. *Clin Exp Allergy*. 2003;33:113–118. doi:10.1046/j.1365-2222.2003.01563.x.
88. Cao TBT, Quoc QL, Yang EM, et al. Tissue inhibitor of metalloproteinase-1 enhances eosinophilic airway inflammation in severe asthma. *Allergy Asthma Immunol Res*. 2023;15:e25 Forthcoming.
89. Belleguic C, Corbel M, Germain N, et al. Increased release of matrix metalloproteinase-9 in the plasma of acute severe asthmatic patients. *Clin Exp Allergy*. 2002;32:217–223. doi:10.1046/j.1365-2222.2002.01219.x.
90. Kumar M, Bhadoria DP, Dutta K, et al. The alpha(1)AT and TIMP-1 gene polymorphism in the development of asthma. *Comp Funct Genomics*. 2012;2012:968267. doi:10.1155/2012/968267.
91. Ko FW, Diba C, Roth M, et al. A comparison of airway and serum matrix metalloproteinase-9 activity among normal subjects, asthmatic patients, and patients with asthmatic mucus hypersecretion. *Chest*. 2005;127:1919–1927. doi:10.1378/chest.127.6.1919.
92. Oshita Y, Koga T, Kamimura T, Matsuo K, Rikimaru T, Aizawa H. Increased circulating 92 kDa matrix metalloproteinase (MMP-9) activity in exacerbations of asthma. *Thorax*. 2003;58:757–760. doi:10.1136/thorax.58.9.757.
93. Casaro M, Souza VR, Oliveira FA, Ferreira CM. OVA-induced allergic airway inflammation mouse model. *Methods Mol Biol*. 2019;1916:297–301. doi:10.1007/978-1-4939-8994-2_28.
94. Lin SC, Chou HC, Chiang BL, Chen CM. CTGF upregulation correlates with MMP-9 level in airway remodeling in a murine model of asthma. *Arch Med Sci*. 2017;13:670–676. doi:10.5114/aoms.2016.60371.
95. Sands MF, Ohtake PJ, Mahajan SD, et al. Tissue inhibitor of metalloproteinase-1 modulates allergic lung inflammation in murine asthma. *Clin Immunol*. 2009;130:186–198. doi:10.1016/j.clim.2008.08.029.
96. Lee KS, Jin SM, Lee H, Lee YC. Imbalance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in toluene diisocyanate-induced asthma. *Clin Exp Allergy*. 2004;34:276–284. doi:10.1111/j.1365-2222.2004.01849.x.
97. Cho JY, Miller M, McElwain K, et al. Remodeling associated expression of matrix metalloproteinase 9 but not tissue inhibitor of metalloproteinase 1 in airway epithelium: modulation by immunostimulatory DNA. *J Allergy Clin Immunol*. 2006;117:618–625. doi:10.1016/j.jaci.2005.12.1324.
98. Agarwal AK, Raja A, Brown BD. *Chronic Obstructive Pulmonary Disease*. Treasure Island (FL): StatPearls; 2022.
99. Karakioulaki M, Papakonstantinou E, Stolz D. Extracellular matrix remodelling in COPD. *Eur Respir Rev*. 2020;29:190124. doi:10.1183/16000617.0124-2019.
100. Arbaningsih SR, Syarani F, Ganie RA, Lelo A. The levels of vitamin D, metalloproteinase-9 and tissue inhibitor metalloproteinase-1 in COPD patients, healthy smokers and non-smokers of Indonesian citizens. *Open Access Maced J Med Sci*. 2019;7:2123–2126. doi:10.3889/oamjms.2019.612.
101. Navratilova Z, Zatloukal J, Kriegova E, Kolek V, Petrek M. Simultaneous up-regulation of matrix metalloproteinases 1, 2, 3, 7, 8, 9 and tissue inhibitors of metalloproteinases 1, 4 in serum of patients with chronic obstructive pulmonary disease. *Respirology*. 2012;17:1006–1012. doi:10.1111/j.1440-1843.2012.02197.x.
102. Dimic-Janjic S, Hoda MA, Milenkovic B, et al. The usefulness of MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio for diagnosis and assessment of COPD severity. *Eur J Med Res*. 2023;28:127. doi:10.1186/s40001-023-01094-7.

103. Shaker SB, von Wachenfeldt KA, Larsson S, et al. Identification of patients with chronic obstructive pulmonary disease (COPD) by measurement of plasma biomarkers. *Clin Respir J*. 2008;2:17–25. doi:10.1111/j.1752-699X.2007.00032.x.
104. D'Armiento JM, Goldklang MP, Hardigan AA, et al. Increased matrix metalloproteinase (MMPs) levels do not predict disease severity or progression in emphysema. *PLoS One*. 2013;8:e56352. doi:10.1371/journal.pone.0056352.
105. Linder R, Rönmark E, Pourazar J, Behndig A, Blomberg A, Lindberg A. Serum metalloproteinase-9 is related to COPD severity and symptoms - cross-sectional data from a population based cohort-study. *Respir Res*. 2015;16:28. doi:10.1186/s12931-015-0188-4.
106. Gilowska I, Kasper L, Bogacz K, et al. Impact of matrix metalloproteinase 9 on COPD development in polish patients: Genetic polymorphism, protein level, and their relationship with lung function. *Biomed Res Int*. 2018;2018:6417415. doi:10.1155/2018/6417415.
107. Uysal P, Uzun H. Relationship between circulating Serpina3g, matrix metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 and -2 with chronic obstructive pulmonary disease severity. *Biomolecules*. 2019;9:62. doi:10.3390/biom9020062.
108. Higashimoto Y, Iwata T, Okada M, Satoh H, Fukuda K, Tohda Y. Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease. *Respir Med*. 2009;103:1231–1238. doi:10.1016/j.rmed.2009.01.021.
109. Eltom S, Stevenson C, Birrell MA. Cigarette smoke exposure as a model of inflammation associated with COPD. *Curr Protoc Pharmacol*. 2013;5:Unit5.64 Chapter. doi:10.1002/0471141755.ph0564s60.
110. Wright JL, Tai H, Wang R, Wang X, Churg A. Cigarette smoke upregulates pulmonary vascular matrix metalloproteinases via TNF-alpha signaling. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L125–L133. doi:10.1152/ajplung.00539.2005.
111. Xu J, Xu F, Wang R, Seagrave J, Lin Y, March TH. Cigarette smoke-induced hypercapnic emphysema in C3H mice is associated with increases of macrophage metalloelastase and substance P in the lungs. *Exp Lung Res*. 2007;33:197–215. doi:10.1080/01902140701459514.
112. Funada Y, Nishimura Y, Yokoyama M. Imbalance of matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 is associated with pulmonary emphysema in Klotho mice. *Kobe J Med Sci*. 2004;50:59–67.
113. Gao W, Yuan C, Zhang J, et al. Klotho expression is reduced in COPD airway epithelial cells: effects on inflammation and oxidant injury. *Clin Sci*. 2015;129:1011–1023. doi:10.1042/CS20150273.
114. Linder R, Rönmark E, Pourazar J, Behndig AF, Blomberg A, Lindberg A. Proteolytic biomarkers are related to prognosis in COPD- report from a population-based cohort. *Respir Res*. 2018;19:64. doi:10.1186/s12931-018-0772-5.
115. Tsay JJ, Hu Y, Goldberg JD, et al. Value of metalloproteinases in predicting COPD in heavy urban smokers. *Respir Res*. 2020;21:228. doi:10.1186/s12931-020-01496-9.
116. Piesiak P, Brzecka A, Kosacka M, Passowicz-Muszyńska E, Dyła T, Jankowska R. [Concentrations of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinases-1 in serum of patients with chronic obstructive pulmonary disease]. *Pol Merkur Lekarski*. 2011;31:270–273.
117. Morrison CB, Markovetz MR, Ehre C. Mucus, mucins, and cystic fibrosis. *Pediatr Pulmonol*. 2019;54:S84–S96. doi:10.1002/ppul.24530.
118. Gaggar A, Hector A, Bratcher PE, Mall MA, Griese M, Hartl D. The role of matrix metalloproteinases in cystic fibrosis lung disease. *Eur Respir J*. 2011;38:721–727. doi:10.1183/09031936.00173210.
119. Rath T, Zwaschka L, Hage L, et al. Identification of neutrophil activation markers as novel surrogate markers of CF lung disease. *PLoS One*. 2014;9:e115847. doi:10.1371/journal.pone.0115847.
120. Roderfeld M, Rath T, Schulz R, et al. Serum matrix metalloproteinases in adult CF patients: relation to pulmonary exacerbation. *J Cyst Fibros*. 2009;8:338–347. doi:10.1016/j.jcf.2009.06.001.
121. Devereux G, Steele S, Jagelman T, et al. An observational study of matrix metalloproteinase (MMP)-9 in cystic fibrosis. *J Cyst Fibros*. 2014;13:557–563. doi:10.1016/j.jcf.2014.01.010.
122. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. *Lancet*. 2017;389:1941–1952. doi:10.1016/S0140-6736(17)30866-8.
123. Ley B, Collard HR, King Jr TE. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2011;183:431–440. doi:10.1164/rccm.201006-0894CI.
124. Wei LQ, Li ZH, Kang J, Hou XM, Yu RJ. [Changes of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in the bronchoalveolar lavage fluid and the serum of patients with idiopathic pulmonary fibrosis]. *Zhonghua Jie He He Hu Xi Za Zhi*. 2006;29:399–402.
125. Yuan YD, Zhang Y, Sun CH. [Association of genetic polymorphisms of angiotensin converting enzyme and matrix metallo proteinase-1 with idiopathic pulmonary fibrosis]. *Zhonghua Jie He He Hu Xi Za Zhi*. 2013;36:38–45.
126. Zhang HT, Fang SC, Wang CY, et al. MMP-9 1562C>T gene polymorphism and efficacy of glucocorticoid therapy in idiopathic pulmonary fibrosis patients. *Genet Test Mol Biomarkers*. 2015;19:591–597. doi:10.1089/gtmb.2015.0057.
127. Xu L, Bian W, Gu XH, Shen C. Differing expression of cytokines and tumor markers in combined pulmonary fibrosis and emphysema compared to emphysema and pulmonary fibrosis. *COPD*. 2017;14:245–250. doi:10.1080/15412555.2017.1278753.
128. Tashiro J, Rubio GA, Limper AH, et al. Exploring animal models that resemble idiopathic pulmonary fibrosis. *Front Med*. 2017;4:118. doi:10.3389/fmed.2017.00118.
129. Liu T, De Los Santos FG, Phan SH. The bleomycin model of pulmonary fibrosis. *Methods Mol Biol*. 2017;1627:27–42. doi:10.1007/978-1-4939-7113-8_2.
130. Manoury B, Nenan S, Leclerc O, et al. The absence of reactive oxygen species production protects mice against bleomycin-induced pulmonary fibrosis. *Respir Res*. 2005;6:11. doi:10.1186/1465-9921-6-11.
131. Fattman CL, Gambelli F, Hoyle G, Pitt BR, Ortiz LA. Epithelial expression of TIMP-1 does not alter sensitivity to bleomycin-induced lung injury in C57BL/6 mice. *Am J Physiol Lung Cell Mol Physiol*. 2008;294:L572–L581. doi:10.1152/ajplung.00291.2007.
132. Craig VJ, Zhang L, Hagood JS, Owen CA. Matrix metalloproteinases as therapeutic targets for idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2015;53:585–600. doi:10.1165/rcmb.2015-0020TR.
133. Chuliá-Peris L, Carreres-Rey C, Gabasa M, Alcaraz J, Carretero J, Pereda J. Matrix metalloproteinases and their inhibitors in pulmonary fibrosis: EMMPRIN/CD147 comes into play. *Int J Mol Sci*. 2022;23:6894. doi:10.3390/ijms23136894.
134. Tang H, Mao J, Gao L, Liu J, Wu T. Effect of antisense TIMP-1 cDNA on the expression of TIMP-1 and MMP-2 in lung tissue with pulmonary fibrosis induced by bleomycin. *Mol Med Rep*. 2013;7:149–153. doi:10.3892/mmr.2012.1140.
135. Manoury B, Nénan S, Guénon I, Lagente V, Boichot E. Influence of early neutrophil depletion on MMPs/TIMP-1 balance in bleomycin-induced lung fibrosis. *Int Immunopharmacol*. 2007;7:900–911. doi:10.1016/j.intimp.2007.02.009.
136. Bonniaud P, Kolb M, Galt T, et al. Smad3 null mice develop airspace enlargement and are resistant to TGF-beta-mediated pulmonary fibrosis. *J Immunol*. 2004;173:2099–2108. doi:10.4049/jimmunol.173.3.2099.
137. Kralisch S, Klein J, Lossner U, et al. Proinflammatory adipocytokines induce TIMP-1 expression in 3T3-L1 adipocytes. *FEBS Lett*. 2005;579:6417–6422. doi:10.1016/j.febslet.2005.10.033.
138. Mancuso P, Bouchard B. The impact of aging on adipose function and adipokine synthesis. *Front Endocrinol*. 2019;10:137. doi:10.3389/fendo.2019.00137.
139. Zagoriti Z, El Mubarak MA, Farsalinos K, Topouzis S. Effects of exposure to tobacco cigarette, electronic cigarette and heated tobacco product on adipocyte survival and differentiation in vitro. *Toxics*. 2020;8:9. doi:10.3390/toxics8010009.
140. Dias SP, Brouwer MC, van de Beek D. Sex and gender differences in bacterial infections. *Infect Immun*. 2022;90:e0028322. doi:10.1128/iai.00283-22.
141. Giefing-Kröll C, Berger P, Lepperdinger G, Grubeck-Loebenstein B. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell*. 2015;14:309–321. doi:10.1111/acel.12326.
142. Liarte S, Chaves-Pozo E, Abellán E, Meseguer J, Mulero V, García-Ayala A. 17beta-Estradiol regulates gilthead seabream professional phagocyte responses through macrophage activation. *Dev Comp Immunol*. 2011;35:19–27. doi:10.1016/j.dci.2010.07.007.
143. Wang Y, Cela E, Gagnon S, Sweezey NB. Estrogen aggravates inflammation in *Pseudomonas aeruginosa* pneumonia in cystic fibrosis mice. *Respir Res*. 2010;11:166. doi:10.1186/1465-9921-11-166.
144. Woolley DE, Roberts DR, Evanson JM. Inhibition of human collagenase activity by a small molecular weight serum protein. *Biochem Biophys Res Commun*. 1975;66:747–754. doi:10.1016/0006-291x(75)90573-2.
145. Burkhardt J, Petit-Teixeira E, Teixeira VH, et al. Association of the X-chromosomal genes TIMP1 and IL9R with rheumatoid arthritis. *J Rheumatol*. 2009;36:2149–2157. doi:10.3899/jrheum.090059.
146. Anderson CL, Brown CJ. Polymorphic X-chromosome inactivation of the human TIMP1 gene. *Am J Hum Genet*. 1999;65:699–708. doi:10.1086/302556.
147. Peng J, Gao K, Gao T, et al. Expression and regulation of tissue inhibitors of metalloproteinases (TIMP1 and TIMP3) in goat oviduct. *Theriogenology*. 2015;84:1636–1643. doi:10.1016/j.theriogenology.2015.09.003.
148. Nasiri-Ansari N, Spilioti E, Kyrou I, et al. Estrogen receptor subtypes elicit a distinct gene expression profile of endothelial-derived factors implicated in atherosclerotic plaque vulnerability. *Int J Mol Sci*. 2022;23:10960. doi:10.3390/ijms231810960.
149. Kim KH, Burkhart K, Chen P, et al. Tissue inhibitor of metalloproteinase-1 deficiency amplifies acute lung injury in bleomycin-exposed mice. *Am J Respir Cell Mol Biol*. 2005;33:271–279. doi:10.1165/rcmb.2005-0111OC.
150. Zhang X, Zhang W, Chen X, Cai Y. Prevention of bleomycin-induced pulmonary inflammation and fibrosis in mice by bilobalide. *Evid Based Complement Alternat Med*. 2023;2023:1973163. doi:10.1155/2023/1973163.
151. Cabrera S, Selman M, Lonzano-Bolaños A, et al. Gene expression profiles reveal molecular mechanisms involved in the progression and resolution of bleomycin-induced lung fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2013;304:L593–L601. doi:10.1152/ajplung.00320.2012.
152. Parsons CJ, Bradford BU, Pan CQ, et al. Antifibrotic effects of a tissue inhibitor of metalloproteinase-1 antibody on established liver fibrosis in rats. *Hepatology*. 2004;40:1106–1115. doi:10.1002/hep.20425.