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Thesis outline

Emphysema-like phenotype in Ig-Hepta/Gpr116-deficient mice linked to alveolar macrophage activation

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ABBREVIATIONS

7TM	seven transmembrane
AM	alveolar macrophage
BALF	bronchoalveolar lavage fluid
CCL	CC chemokine ligand
CXCL	CXC chemokine ligand
COPD	chronic obstructive pulmonary disease
dpc	days post coitum
ECM	extracellular matrix
GPCR	G protein-coupled receptor
H_2DCFDA	2',7'-dichlorodihydrofluorescein diacetate
Ig	immunoglobulin
IL	interleukin
LPO	lipid hydroperoxide
MCP	monocyte chemotactic protein
MMP	matrix metalloproteinase
NF-κB	nuclear factor-kappa B
ROS	reactive oxygen species
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SP	surfactant protein
TNF	tumor necrosis factor
TIMP	tissue inhibitor of metalloproteinase
WT	wild type

SUMMARY

Ig-Hepta/GPR116 is a member of the Adhesion family of G protein-coupled receptors (GPCRs) with a pair of immunoglobulin (Ig)-like repeats in the long extracellular region and is highly expressed in alveolar type II cells. Previously, it has been shown that mice lacking Ig-Hepta exhibit abnormal lung structure and progressive accumulation of pulmonary surfactant, indicating its essential role in surfactant homeostasis. Ig-Hepta knockout mice (*Ig-Hepta*$^{-/-}$) also exhibit emphysema-like symptoms with enlarged alveoli, accumulation of foamy alveolar macrophages (AMs), and increased expression of matrix metalloproteinase (MMP)-12. These abnormalities are similar to those seen in patients and animal models with emphysema, suggesting its role in emphysema pathogenesis. In this study, I tried to elucidate the underlying mechanism of emphysema pathogenesis in *Ig-Hepta*$^{-/-}$ mice.

Here, an intriguing finding showed that AMs of *Ig-Hepta*$^{-/-}$ mice produce and release inflammatory mediators, lipid hydroperoxides and MMPs. In addition, NF-κB is activated and translocated into the nuclei of the AMs of *Ig-Hepta*$^{-/-}$ mice, mediating the release of MMP-2 and MMP-9. Inhibitors of oxidants and NF-κB decreased the release of MMP-2 and MMP-9 from the AMs. I also found that monocyte chemotactic protein-1 is increased in the embryonic lungs of *Ig-Hepta*$^{-/-}$ mice, in which AMs are not accumulated and activated. These results suggest that Ig-Hepta is involved in the regulation of macrophage immune responses, and its deficiency leads to AM activation though the NF-κB signaling pathway and eventual local inflammation in the lung.

INTRODUCTION

The Adhesion family of G protein-coupled receptors (GPCRs) and Ig-Hepta

The GPCR family comprises the largest class of seven-transmembrane cell-surface receptors that generally transduce extracellular signals into intracellular responses via activating G proteins. There are more than 800 GPCR genes in humans, with a variety of ligands, such as ions, amino acids, peptides, lipids, and nucleotides. GPCRs have diverse cellular and physiological functions, such as neuronal signalling, cell differentiation and growth, metabolism, ion homeostasis and immune response. However, malfunction of GPCRs can cause various human diseases. Therefore, GPCRs constitute the most common target for therapeutic drugs and account for approximately 40% of all newly introduced drug targets. Emerging knowledge of GPCR physiology and pathophysiology will enhance our understanding the molecular mechanisms of their action and will provide insights into new therapeutic strategies [1, 2].

Based on phylogenetics, the GPCR family is divided into five major subfamilies: Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, and Secretin [3]. The Adhesion subfamily is the second largest, but poorly studied, family of GPCRs with more than 33 members in mammals [4]. Most of Adhesion GPCRs possess the N-terminal long extracellular region, which contains multiple domains implicated in cell–cell and cell–matrix interactions, such as the epidermal growth factor (EGF)-like, thrombospondin repeats, leucine-rich repeats, lectin-like, immunoglobulin (Ig)-like, and cadherin repeats [5]. All Adhesion GPCRs contain the GAIN domain and most undergo autoprocessing within the GAIN domain, yielding an N-terminal fragment (NTF) and a C-terminal fragment (CTF) [5]. The NTF and CTF non-covalently associate to form a

heterodimer at the cell surface. Although the autoprocessing is expected to modulate receptor activity and signalling, its biological significance is unknown. Recent studies have reported that Adhesion GPCRs have a variety of biological functions, such as Schwann cell development [6], synaptogenesis [7], oligodendrocyte development [8], and cell-in-cell invasion [9].

Ig-Hepta (also known as Gpr116 or ADGRF5) is a member of Adhesion GPCRs with a pair of Ig-like domains in the N-terminal extracellular region [10]. Like other Adhesion GPCRs, Gpr116 undergoes autoprocessing during its transport to the cell surface, but its significance on the signalling property has not been clarified [11]. Gpr116 is predominantly expressed in the lung and weakly in the kidney [12]. Gpr116 is also expressed in the capillary endothelium in a variety of tissues [12]. Recent functional studies have reported the cellular and physiological function of Gpr116. Tang *et al.* [13] have shown that Gpr116 promotes breast cancer metastasis through Gαq-dependent activation of RhoA and Rac1. Nie *et al.* [14] have shown that Gpr116 contributes to adipogenesis of 3T3-L1 preadipocytes *in vitro* and energy homeostasis *in vivo*. Adipose tissue-specific conditional Gpr116 knockout mice exhibit glucose intolerance and insulin resistance in response to high-fat diet [14]. Most importantly, the studies using Gpr116 knockout (*Ig-Hepta*$^{-/-}$) mice have revealed the critical role of Gpr116 in lung physiology (see below).

Ig-Hepta, a key regulator of pulmonary surfactant homeostasis

The lung is an essential organ of respiration, providing a large surface area for gas exchange between the alveoli and pulmonary capillaries. The alveolar surface is formed by two types of epithelial cells, alveolar type I and type II cells, which cover 95% and 5% of the surface area, respectively. Alveolar type I cells are large, flat

terminally differentiated cells and serve as a thin, gas-permeable membrane. Alveolar type II cells are small, cuboidal cells with progenitor cell activity and multiple functions essential for alveolar homeostasis. Alveolar type II cells proliferate and differentiate into alveolar type I cells during lung development and injury. The major and most extensively studied function of alveolar type II cells is their role in pulmonary surfactant homeostasis [15]. Pulmonary surfactant consists of phospholipids and proteins (surfactant proteins) that forms a surface-active film covering the gas exchange interface of the alveoli. It reduces surface tension in the alveoli, thereby preventing the lungs from collapsing during expiration. Alveolar type II cells are responsible for synthesis/secretion and clearance of pulmonary surfactant. They are also known to play an important role in innate immunity by releasing inflammatory cytokines and chemokines and regulating recruitment, transepithelial migration and activation of immune cells [16, 17]. Alveolar macrophages (AMs) are primary phagocytic cells present within the alveoli and remove inhaled particles and microorganisms and excess pulmonary surfactant [18]. Therefore, alveolar type II cells and AMs are thought to cooperate to regulate surfactant homeostasis and immune defense, but their molecular mechanisms remain to be elucidated.

It is thought that pulmonary surfactant homeostasis is maintained by balancing its release and uptake by alveolar type II cells and the phagocytosis by alveolar macrophages for degradation. However, the question of “how the levels of pulmonary surfactant are monitored” has long been a matter of concern in the field of lung physiology. In 2013, our and two other groups [12, 19, 20] identified Ig-Hepta as a sensor and regulator of surfactant levels through the analyses of *Ig-Hepta*$^{-/-}$ mice. Ig-Hepta is highly expressed in the apical surface (the luminal side) of the alveolar type II cells but not in alveolar macrophages [12, 20]. Targeted disruption of Ig-Hepta

results in progressive accumulation of pulmonary surfactant due to its increased synthesis and reduced catabolism by alveolar type II cells [12, 19]. Moreover, we have shown that Ig-Hepta binds to surfactant protein (SP)-D, a component of pulmonary surfactant, through its extracellular region [12]. Therefore, we hypothesized that Ig-Hepta controls surfactant levels by monitoring the amount of SP-D in the surfactant pool. *Ig-Hepta*$^{-/-}$ mice also exhibit enlargement of the alveoli and accumulation of foamy AMs [12, 19, 20]. These abnormalities are similar to those seen in patients and animal models with emphysema, a lung disease [21, 22]. Therefore, I suspected the possibility that Ig-Hepta is also involved in the pathogenesis of emphysema.

Emphysema

Emphysema is a type of chronic obstructive pulmonary disease (COPD), which is the third most common cause of death in the world [Supplementary Fig. 7]. The main symptoms of emphysema are shortness of breath, coughing and eventually breathlessness due to damage of the alveoli. Emphysema is defined as alveolar airspace enlargement and parenchymal tissue destruction [23]. The major risk factor for emphysema is cigarette smoking, but other environmental (*e.g.*, air pollutants) and genetic (*e.g.*, α1-antitrypsin deficiency) factors also develop this disease [23]. These factors trigger lung inflammation response that leads to an imbalance of protease and anti-protease activity in the lung [23, 24]. A number of studies have been shown that exposure to inhaled cigarette smoke induces chronic lung inflammation, in which inflammatory cells are recruited and release proteases that degrade the extracellular matrix (ECM) of the alveoli. Genetic deficiency in α1-antitrypsin, an endogenous elastase inhibitor, leads to development of emphysema [25]. In a simplistic sense, parenchymal tissue destruction in emphysema is caused by the protease–

antiprotease imbalance. However, the molecular pathogenesis of emphysema is not fully understood because it involves a complicated network of inflammatory cells and mediators, proteases/antiproteases, and reactive oxygen species (ROS) (see below). That is the reason why there is no cause treatment for emphysema.

Alveolar macrophages (AMs) in emphysema

AMs are critical players in emphysema pathogenesis. They are increased in the alveoli of patients with emphysema [26]. There is a positive correlation between AM numbers and the severity of COPD [27-29]. In patients with emphysema and smokers, AMs are activated and release various inflammatory cytokines and chemokines, including tumor necrosis factor (TNF)-α and monocyte chemotactic protein (MCP)-1, which promote local pulmonary inflammation [30-32]. TNF-α is a potent pro-inflammatory cytokine that induces the expression and release of various proinflammatory mediators which lead to inflammatory responses as well as tissue damage and remodelling [33]. MCP-1 is a chemokine which recruits and activates monocytes and AMs into the alveolar and air way epithelium through the activation of its receptor, C-C chemokine receptor 2 (CCR2) on these cells [32, 34]. These recruited cells further promote chronic lung inflammation [32]. AMs also secrete proteases, including matrix metalloproteases (MMPs) (*e.g.*, MMP-2, MMP-9, and MMP-12) and cathepsins K, L, and S, which are capable of degrading ECM [35]. This degradation is widely considered to be the initiator of tissue remodelling in the lungs of emphysema patients [35]. Lung ECM is comprised of collagens, elastin, fibronectin, laminin, and heparin and sulfate proteoglycans, and this complex network of proteins and glycoproteins act as a support for adhesion cells of the alveolar and airway epithelia [36]. MMPs are a family of Ca^{2+}-activated Zn^{2+}-dependent proteases that degrade

these ECM components both in normal physiological condition and in abnormal pathological processes. As mentioned above, a number of studies have pointed out that an imbalance between MMPs and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs), cause emphysematous destruction of the lung parenchyma – the idea is known as the protease–antiprotease hypothesis [36, 37].

Reactive oxygen species (ROS) in emphysema

The lungs are continuously exposed to ROS generated either endogenously as by-products of cellular metabolic reactions, such as mitochondrial respiration and phagocytes, or exogenously from air pollutants and cigarette smoke. ROS are chemically reactive molecules derived from oxygen (O_2). Oxygen readily accepts free electrons produced during normal cellular metabolism, forming superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) as well as hydroxyl radical ($HO^{\bullet -}$) [38]. Because ROS oxidize DNA, RNA, proteins and lipids, cells possess the antioxidant defence system, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, to prevent or alleviate these damages. ROS play important roles in many normal biological processes, including host defense, cell proliferation and apoptosis [38]. However, over-accumulation of ROS causes oxidative stress that disrupts cellular integrity and function and eventually leads to a variety of diseases, such as cancer, asthma, COPD, and retinopathy.

AMs in the lungs of smokers and patients with emphysema are more activated compared with those of nonsmokers. The activated AMs release of excess amounts of ROS that may cause direct lung injury or induce a variety of cellular responses leading to the development of emphysema [39-41]. In particular, ROS induce inflammatory responses in the lungs through the activation of transcription factors,

such as nuclear factor (NF)-κB and activator protein (AP)-1, and other signal transduction pathways, such as mitogen-activated protein (MAP) kinases and phosphoinositide-3-kinase (PI-3K) [42, 43]. A growing body of evidence indicates that ROS induce the expression of MMPs, including MMP-2, MMP-9, and MMP-12, through NF-κB activation in AMs [44, 45].

With the phenotypes shown in *Ig-Hepta*$^{-/-}$ mice, I, therefore, hypothesized that Ig-Hepta may involve in emphysema pathogenesis. This study is aimed to elucidate the underlying mechanism of emphysema pathogenesis in Ig-hepta-deficient mice

MATERIAL AND METHODS

For materials and methods, please see “Donna Maretta Ariestanti, Hikaru Ando, Shigehisa Hirose and Nobuhiro Nakamura “Targeted Disruption of Ig-Hepta/Gpr116 Causes Emphysema-like Symptoms That Are Associated with Alveolar Macrophage Activation” *Journal of Biological Chemistry* (2015) 290, 11032-11040, DOI: 10.1074/jbc.M115.648311”.

RESULTS

In this study, an intriguing finding showed that bronchoalveolar lavage fluid (BALF) obtained from *Ig-Hepta*$^{-/-}$ mice contains high levels of inflammatory mediators, lipid hydroperoxides (LPOs), and MMPs, which are produced by AMs. I examined the levels of ROS in AMs of *Ig-Hepta*$^{-/-}$ and wild type (WT) mice by using H_2DCFDA

staining, a fluorogenic probe for intracellular ROS. Accumulation of ROS was observed in *Ig-Hepta*$^{-/-}$ but not in WT mice. This excessive ROS causes oxidative stress, showed by a marked increase of lipid peroxide levels, an oxidative stress marker, both in BALF and lung tissue of *Ig-Hepta*$^{-/-}$ mice. Oxidative stress is known to be associated with activation of transcriptional pathways such as NF-kB to mediate inflammatory response. Subcellular fractionation and Western blot analysis showed the nuclear localization of the p65 subunit of NF-kB in the AMs of *Ig-Hepta*$^{-/-}$ mice. Consistently, immunofluorescence confocal microscopy also showed nuclear localization of p65 in AMs of *Ig-Hepta*$^{-/-}$ mice. Taken together, these results indicate that NF-kB is activated in AMs of *Ig-Hepta*$^{-/-}$ mice. Next, I examined the levels of MMP-2 and MMP-9 that are known to be implicated in emphysema. Quantitative PCR and Western blot analysis demonstrated that expression of MMP-2 and MMP-9 was significantly increased in BALF and AMs of *Ig-Hepta*$^{-/-}$ mice compared to WT mice. To confirm whether the increased MMPs expression is mediated by oxidative stress-induced NF-κB activation, the AMs of *Ig-Hepta*$^{-/-}$ mice were treated with inhibitors of oxidants and NF-κB. The results showed that the release of MMP-2 and MMP-9 from the AMs was strongly inhibited by treatment with these inhibitors. Through ELISA analysis, I also found that the level of monocyte chemotactic protein-1 (MCP-1) is increased in the embryonic lungs of *Ig- Hepta*$^{-/-}$ mice at 18.5 days postcoitum (dpc), when AMs are not accumulated and activated.

For detail, please see “Donna Maretta Ariestanti, Hikaru Ando, Shigehisa Hirose and Nobuhiro Nakamura “Targeted Disruption of Ig-Hepta/Gpr116 Causes Emphysema-like Symptoms That Are Associated with Alveolar Macrophage Activation” *Journal of Biological Chemistry* (2015) 290, 11032-11040, DOI: 10.1074/jbc.M115.648311”.

DISCUSSION

Ig-Hepta$^{-/-}$ mice exhibit emphysema-like symptoms, in which AMs are activated and release MMPs through ROS-mediated NF-κB activation. The macrophage activation is likely to be mediated by MCP-1 induced by Ig-Hepta deletion, but the underlying mechanism, including Ig-Hepta-mediated signaling, should be elucidated in future studies. My findings suggest that Ig-Hepta is responsible for ensuring homeostasis of the internal environment of the alveoli, such as surfactant homeostasis to prevent macrophage activation and emphysema. Therefore, I propose that *Ig-Hepta*$^{-/-}$ mice are a useful model to gain insights into the pathogenesis and treatment of emphysema.

For detail, please see “Donna Maretta Ariestanti, Hikaru Ando, Shigehisa Hirose and Nobuhiro Nakamura “Targeted Disruption of Ig-Hepta/Gpr116 Causes Emphysema-like Symptoms That Are Associated with Alveolar Macrophage Activation” *Journal of Biological Chemistry* (2015) 290, 11032-11040, DOI:_10.1074/jbc.M115.648311”.

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REFERENCES

1. Millar, R.P. and C.L. Newton, *The year in G protein-coupled receptor research.* Mol Endocrinol, 2010. **24**(1): p. 261-74.
2. Yona, S. and M. Stacey, *Adhesion-GPCRs: structure to function. Preface.* Adv Exp Med Biol, 2010. **706**: p. v-vii.
3. Lagerstrom, M.C. and H.B. Schioth, *Structural diversity of G protein-coupled receptors and significance for drug discovery.* Nat Rev Drug Discov, 2008. **7**(4): p. 339-57.
4. Civelli, O., et al., *G protein-coupled receptor deorphanizations.* Annu Rev Pharmacol Toxicol, 2013. **53**: p. 127-46.
5. Langenhan, T., G. Aust, and J. Hamann, *Sticky signaling--adhesion class G protein-coupled receptors take the stage.* Sci Signal, 2013. **6**(276): p. re3.
6. Petersen, S.C., et al., *The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with laminin-211.* Neuron, 2015. **85**(4): p. 755-69.
7. Sigoillot, S.M., et al., *The Secreted Protein C1QL1 and Its Receptor BAI3 Control the Synaptic Connectivity of Excitatory Inputs Converging on Cerebellar Purkinje Cells.* Cell Rep, 2015.
8. Ackerman, S.D., et al., *The adhesion GPCR Gpr56 regulates oligodendrocyte development via interactions with Galpha12/13 and RhoA.* Nat Commun, 2015. **6**: p. 6122.
9. Purvanov, V., et al., *G-protein-coupled receptor signaling and polarized actin dynamics drive cell-in-cell invasion.* Elife, 2014. **3**.
10. Abe, J., et al., *Ig-hepta, a novel member of the G protein-coupled hepta-helical receptor (GPCR) family that has immunoglobulin-like repeats in a long N-terminal extracellular domain and defines a new subfamily of GPCRs.* J Biol Chem, 1999. **274**(28): p. 19957-64.
11. Abe, J., T. Fukuzawa, and S. Hirose, *Cleavage of Ig-Hepta at a "SEA" module and at a conserved G protein-coupled receptor proteolytic site.* J Biol Chem, 2002. **277**(26): p. 23391-8.
12. Fukuzawa, T., et al., *Lung surfactant levels are regulated by Ig-Hepta/GPR116 by monitoring surfactant protein D.* PLoS One, 2013. **8**(7): p. e69451.

13. Tang, X., et al., *GPR116, an adhesion G-protein-coupled receptor, promotes breast cancer metastasis via the Galphaq-p63RhoGEF-Rho GTPase pathway.* Cancer Res, 2013. **73**(20): p. 6206-18.
14. Nie, T., et al., *Adipose tissue deletion of Gpr116 impairs insulin sensitivity through modulation of adipose function.* FEBS Lett, 2012. **586**(20): p. 3618-25.
15. Perez-Gil, J. and T.E. Weaver, *Pulmonary surfactant pathophysiology: current models and open questions.* Physiology (Bethesda), 2010. **25**(3): p. 132-41.
16. Thorley, A.J., et al., *Differential regulation of cytokine release and leukocyte migration by lipopolysaccharide-stimulated primary human lung alveolar type II epithelial cells and macrophages.* J Immunol, 2007. **178**(1): p. 463-73.
17. Witherden, I.R., et al., *Primary human alveolar type II epithelial cell chemokine release: effects of cigarette smoke and neutrophil elastase.* Am J Respir Cell Mol Biol, 2004. **30**(4): p. 500-9.
18. Schmitz, G. and G. Muller, *Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids.* J Lipid Res, 1991. **32**(10): p. 1539-70.
19. Bridges, J.P., et al., *Orphan G protein-coupled receptor GPR116 regulates pulmonary surfactant pool size.* Am J Respir Cell Mol Biol, 2013. **49**(3): p. 348-57.
20. Yang, M.Y., et al., *Essential regulation of lung surfactant homeostasis by the orphan G protein-coupled receptor GPR116.* Cell Rep, 2013. **3**(5): p. 1457-64.
21. Deacu, M., et al., *Pulmonary alveolar lipoproteinosis associated with emphysematous areas.* Rom J Morphol Embryol, 2012. **53**(1): p. 173-7.
22. Hirama, N., et al., *Increased surfactant protein-D and foamy macrophages in smoking-induced mouse emphysema.* Respirology, 2007. **12**(2): p. 191-201.
23. Taraseviciene-Stewart, L. and N.F. Voelkel, *Molecular pathogenesis of emphysema.* J Clin Invest, 2008. **118**(2): p. 394-402.
24. Barnes, P.J., *Mediators of chronic obstructive pulmonary disease.* Pharmacol Rev, 2004. **56**(4): p. 515-48.
25. DeMeo, D.L., *1-Antitrypsin deficiency {middle dot} 2: Genetic aspects of 1-antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk.* Thorax, 2004. **59**(3): p. 259-264.
26. Finkelstein, R., et al., *Alveolar inflammation and its relation to emphysema in smokers.* Am J Respir Crit Care Med, 1995. **152**(5 Pt 1): p. 1666-72.

27. Pesci, A., et al., *Inflammatory cells and mediators in bronchial lavage of patients with chronic obstructive pulmonary disease.* Eur Respir J, 1998. **12**(2): p. 380-6.
28. Keatings, V.M., et al., *Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma.* Am J Respir Crit Care Med, 1996. **153**(2): p. 530-4.
29. Di Stefano, A., et al., *Severity of airflow limitation is associated with severity of airway inflammation in smokers.* Am J Respir Crit Care Med, 1998. **158**(4): p. 1277-85.
30. Churg, A., et al., *Tumor necrosis factor-alpha drives 70% of cigarette smoke-induced emphysema in the mouse.* Am J Respir Crit Care Med, 2004. **170**(5): p. 492-8.
31. Rahman, I. and I.M. Adcock, *Oxidative stress and redox regulation of lung inflammation in COPD.* Eur Respir J, 2006. **28**(1): p. 219-42.
32. de Boer, W.I., et al., *Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD.* J Pathol, 2000. **190**(5): p. 619-26.
33. Tanni, S.E., et al., *Smoking status and tumor necrosis factor-alpha mediated systemic inflammation in COPD patients.* J Inflamm (Lond), 2010. **7**: p. 29.
34. O'Brien, A.D., et al., *Chemotaxis of alveolar macrophages in response to signals derived from alveolar epithelial cells.* J Lab Clin Med, 1998. **131**(5): p. 417-24.
35. Barnes, P.J., S.D. Shapiro, and R.A. Pauwels, *Chronic obstructive pulmonary disease: molecular and cellular mechanisms.* Eur Respir J, 2003. **22**(4): p. 672-88.
36. Churg, A., S. Zhou, and J.L. Wright, *Series "matrix metalloproteinases in lung health and disease": Matrix metalloproteinases in COPD.* Eur Respir J, 2012. **39**(1): p. 197-209.
37. Janoff, A., *Proteases and lung injury. A state-of-the-art minireview.* Chest, 1983. **83**(5 Suppl): p. 54S-58S.
38. Covarrubias, L., et al., *Function of reactive oxygen species during animal development: passive or active?* Dev Biol, 2008. **320**(1): p. 1-11.
39. Rahman, I. and W. MacNee, *Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary disease.* Thorax, 1996. **51**(4): p. 348-50.

40. Morrison, D., et al., *Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers.* Am J Respir Crit Care Med, 1999. **159**(2): p. 473-9.
41. MacNee, W., *Oxidative stress and lung inflammation in airways disease.* Eur J Pharmacol, 2001. **429**(1-3): p. 195-207.
42. Rahman, I. and W. MacNee, *Role of transcription factors in inflammatory lung diseases.* Thorax, 1998. **53**(7): p. 601-12.
43. Kamata, H., et al., *Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases.* Cell, 2005. **120**(5): p. 649-61.
44. Hsieh, H.L., et al., *Transforming growth factor-beta1 induces matrix metalloproteinase-9 and cell migration in astrocytes: roles of ROS-dependent ERK- and JNK-NF-kappaB pathways.* J Neuroinflammation, 2010. **7**: p. 88.
45. Hemmerlein, B., et al., *The balance between MMP-2/-9 and TIMP-1/-2 is shifted towards MMP in renal cell carcinomas and can be further disturbed by hydrogen peroxide.* Int J Oncol, 2004. **24**(5): p. 1069-76.