GELATINASES IN COPD AND BRONCHIAL ASTHMA

Tanya Tacheva[1*], Dimo Dimov[2], Tatyana Vlaykova[1]
[1]Dept. of Chemistry and Biochemistry, [2]Dept of Internal Medicine, Medical Faculty, Trakia Univesity , 11 Armeiska str., Stara Zagora, Bulgaria, E-mail: tanya.ta4eva@abv.bg

ABSTRACT

COPD and asthma are two of the most common chronic diseases worldwide, characterized with impaired lung structure and function. Recent studies have demonstrated that matrix metalloproteinases (MMPs - matrixins, hydrolyze components of the extracellular matrix) play a central role in the lung remodeling. MMPs play a central role in many biological processes, such as embryogenesis, normal tissue remodeling, wound healing, and angiogenesis, and in diseases such as rheumatoid arthritis, cancer, and tissue ulceration, COPD and Bronchial asthma.

This review describes members of the matrix metalloproteinases family and discusses substrate specificity, domain structure and function, the activation of proMMPs, the regulation of MMP activity, COPD, asthma (clinical signs and risk factors) and the role of MMPs in both diseases.

Key words: *matrix metalloproteinases, extracellular matrix, chronic obstructive pulmonary disease (COPD), asthma*

Introduction

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

COPD is a heterogeneous chronic condition .The worldwide prevalence has been estimated at 7.6% to 26.1%, with age and smoking history being the most significant risk factors. It also has been estimated that COPD will be the third leading cause of death worldwide by 2020 (Rosendorff 2012). The disease is associated with a number of co-morbidities, which include hypertension, diabetes, coronary artery disease, HF, and CV disease (CVD) in genera (Fabbri, Luppi et al. 2008). Despite the high incidence of COPD awareness among people about the disease is woefully inadequate - 75% of patients do not know they have the disease (www.riokoz-vt.com/hobb.htm 2012).

The Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) describes COPD as a "preventable and treatable disease with some significant extrapulmonary effects that contribute to the severity in individual patients. Its pulmonary component is characterized by airlow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with inflammatory response of the lung to noxious particles and gases" (Lakhdar, Denden et al. 2011).

Clinical signs

COPD includes two main components - chronic bronchitis and pulmonary emphysema. Coughing is usually the first symptom of the disease. Normal airways are small amount of mucus, which serves to capture (adhesion) of bacteria and harmful particles within them through inspired air. Usually this amount is insignificant and not separated as phlegm. When the bronchi are irritated and inflamed, the amount of mucus increases and this leads to sputum. Another typical feature of the disease is shortness of breath, which in this case more often is constant, with periods of strengthening and weakening. Shortness of breath in COPD progresses gradually, limiting physical activity of the patient.

Risk factors

Risk factors for COPD may be endogenous and exogenous. The main exogenous risk factor causing COPD is smoking. Other risk factors include contaminated ambient air, frequent respiratory infections, acute and prolonged exposure to harmful substances in the workplace (dusts and chemicals), socio-economic status (eg, low dietary intake of antioxidant vitamins A, C and E and others), gender, age and others (www.riokoz-vt.com/hobb.htm 2012).

However, the pathogenesis of COPD is not fully resolved and the differing contributions of these mechanisms, along with other factors such as genetic and dietary, are likely to influence the heterogeneity the disease observed between patients (Barnes, Shapiro et al. 2003; MacNee 2005).

BRONCHIAL ASTHMA

Bronchial asthma is a major cause of chronic lung disease worldwide and is the most common chronic childhood disease in Western countries (Watson, Benton et al. 2010). During the past two decades the incidence and severity of asthma have increased in prevalence by more than 80% in all age and ethnic groups and continue to increase particularly in urban areas (Aligne, Auinger et al. 2000). Asthma is a multifactorial disease characterized by airway inflammation, hyperresponsiveness and mucus obstruction and is now considered to reflect airway remodeling due to fibroblast proliferation, goblet cell and smooth muscle hyperplasia, and increased collagen deposition (Watson, Benton et al. 2010).

Clinical signs

The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing particularly at night or in the early morning. These episodes are usually associated with widespread, but variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment" (Kroegel 2009).

Risk factors

Asthma is caused by environmental and genetic factors (Martinez 2007). These factors influence the severity of asthma and the response to medication (Choudhry, Seibold et al. 2007). The interaction is complex and not fully understood (Miller and Ho 2008).

Many environmental risk factors have been associated with asthma development and morbidity. Cigarette smoke (CS) is one of the principal environmental air pollutants and contains various carcinogens and oxidative free radicals (Watson, Benton et al. 2010).

Viral respiratory infections are not only one of the leading triggers of an exacerbation but may increase one's risk of developing asthma especially in young children (Moore 2008).

Respiratory infections such as rhinovirus, *Chlamydia pneumoniae* and *Bordetella pertussis* are correlated with asthma exacerbations (Harju, Leinonen et al. 2006).

Psychological stress and beta blocker medications such as metoprolol may trigger asthma.

Genetic factors:

GSTM1	LTA	STAT6	IL4	HLA-DQB1
IL10	GRPA	NOS1	IL13	TNF
CTLA-4	NOD1	CCL5	CD14	FCER1B
SPINK5	CC16	TBXA2R	ADRB2 (β-2 adrenergic receptor)	IL4R
LTC4S	GSTP1	TGFB1	HLA-DRB1	ADAM33

Over 100 genes have been associated with asthma in at least one genetic association study. However, such studies must be repeated to ensure the findings are not due to chance. Through the end of 2005, 25 genes had been associated with asthma in six or more separate populations (Ober and Hoffjan 2006):

MATRIX METALLOPROTEINASES

Recent studies have demonstrated that matrix metalloproteinases (MMP) play a central role in the lung remodeling in COPD and Bronchial asthma (Brajer, Batura-Gabryel et al. 2008).

Matrix metalloproteases (MMPs) are a large family of zinc- and calcium-dependent enzymes initially characterized by their capacity to degrade components of the ECM. They can also

degrade other substrates. MMP are synthesized as zymogens (pro-enzymes) (Vermeer, Denker et al. 2009).

More than twenty MMPs have been identified and are separated into six groups based on their structure and substrate specificity (Hu and Beeton 2010):

Collagenases: MMP-1, MMP-8, MMP-13, and MMP-18

Gelatinases: Gelatinase A (MMP-2) and gelatinase B (MMP-9)

Stromelysins: Stromelysin 1 (MMP-3) and stromelysin 2 (MMP-10)

Matrilysins: Matrilysin 1 (MMP-7) and matrilysin 2 (MMP-26

Membrane-Type MMPs: There are six membrane-type MMPs (MT-MMPs): MMP-14, MMP-15, MMP- 16, MMP-24, MMP-17 and MMP-25

Other MMPs: MMP-12, MMP-19, MMP-20, MMP-22, MMP-28

Gelatinases

Gelatinases A (MMP-2) and B (MMP-9) digest the denatured collagens, gelatins. These enzymes have three repeats of a type II fibronectin domain inserted in the catalytic domain, which bind to gelatin, collagens, and laminin (Visse and Nagase 2003). MMP-2 digests collagen I, IV, V, VII, X, XI and XIV, gelatin, elastin, fibronectin, laminin, proteoglycan-associated protein, osteonectin. MMP-9 digests collagen IV, V, VII, X, XIV, gelatin, elastin, agregan, proteoglycan-associated protein, fibronectin, osteonectin, plazmynogen.

MMP-2 (gelatinase A) is primarily expressed in mesenchymal cells (mainly fibroblasts) during development and tissue regeneration. It is also synthesized by neutrophils, macrophages and monocytes. MMP-2 is required to activate angiogenesis in tumors, and its level increased in the endothelium of tumor vessels and in the urine of patients with different tumor entities.

Together with the MMP-9, MMP-2 is involved in the degradation of type IV collagen, a major component of basement membranes and gelatin (denatured collagen). MMP-2 may also destroy other types of collagens (V, VI and X), elastin and fibronectin. It is involved in processing of many other molecules that modulate their function in various ways. For example, it cleaves monocyte chemotactic protein-3, which leads to a reduction of inflammation and ensures vasoconstriction.

Matrix metalloproteinase-9 (MMP-9) (also known as gelatinase B) is secreted as a zymogene with molecular mass of 92 kDa. Substrates for the active MMP-9 include denatured collagen type I (gelatin), native collagen types IV, V, VII, X and XI, fibrinogen, vitronectin, IL-1 and entaktin that connects the laminin and collagen type IV. MMP-9 is involved in the processes of inflammation, tissue remodeling and repair, matrix-related growth factors and cytokine processing (www.biochemmmack.ru).

Under normal physiological conditions, the activities of MMPs are precisely regulated at the level of transcription, activation of the precursor zymogens, interaction with specific ECM components, and inhibition by endogenous inhibitors (Lu, Gunja-Smith et al. 2000). MMP activity is regulated at three levels: 1) gene transcription (growth factors, cytokines, and inflammatory components can each modulate *MMP* transcriptional activity); 2) processing (MMPs are synthesized as zymogens that require proteolytic cleavage of the prodomain for enzymatic activity); and 3) secretion of tissue inhibitors of metalloproteases (TIMPs), specific MMP inhibitors that bind in a 1:1 molar ratio and inactivate MMPs (Vermeer, Denker et al. 2009). A loss of activity control may result in diseases such as rheumatoid arthritis, cancer, atherosclerosis, aneurysms, nephritis, tissue ulcers, and fibrosis (Visse and Nagase 2003).

Activation of ProMMPs

Stepwise activation mechanism

MMPs can be activated by proteinases or *in vitro* by chemical agents, such as thiol-modifying agents, oxidized glutathione, SDS, chaotropic agents, and reactive oxygen species, low pH, heat treatment and NO can also lead to activation. The initial proteolytic attack occurs at an exposed loop region between the first and the second helices of the propeptide. The cleavage

specificity of the affected region is dictated by the sequence found in each MMP. Once a part of the propeptide is removed, this probably destabilizes the rest of the propeptide, including the cysteine–zinc interaction, which allows the intermolecular processing by partially activated MMP intermediates or other active MMPs (Suzuki, Enghild et al. 1990; Nagase, Suzuki et al. 1992).

Intracellular activation

Some proMMPs such as proMMP-11 (stromelysin 3) are activated intracellularly by furin. ProMMP-11 possesses a furin recognition sequence, KX(R/K)R, at the C-terminal end of the propeptide (Visse and Nagase 2003).

Cell surface activation of proMMP-2

The main activation of proMMP-2 takes place on the cell surface and is mediated by MT-MMPs. MT4-MMP does not activate proMMP-2. ProMMP-2 forms a tight complex with TIMP-2 through their C-terminal domains, therefore permitting the N-terminal inhibitory domain of TIMP-2 in the complex to bind to MT1-MMP on the cell surface. The cell surface–bound proMMP-2 is then activated by an MT1-MMP that is free of TIMP-2. Alternatively, MT1-MMP inhibited by TIMP-2 can act as a "receptor" of proMMP-2. This MT1-MMP–TIMP-2–proMMP-2 complex is then presented to an adjacent free MT1-MMP for activation. Clustering of MT1-MMP on the cell surface through interactions of the hemopexin domain facilitates the activation process (Itoh, Takamura et al. 2001).

Substrate Specificity of MMPs

In general MMPs cleave a peptide bond before a residue with a hydrophobic side chain, such as Leu, Ile, Met, Phe, or Tyr. The hydrophobic residues fit into the S1 specificity pocket, whose size and shape differ considerably among MMPs. In some cases, noncatalytic domains influence the enzyme activity (Bode, Fernandez-Catalan et al. 1999).

MMPs play a critical role in cell invasion, cartilage degradation, tissue remodeling, wound healing, and embryogenesis. They therefore participate in both normal processes and in the pathogenesis of many diseases, such as rheumatoid arthritis, cancer, or chronic obstructive pulmonary disease (Hu and Beeton 2010).

Polymorphisms in genes of MMPs

There are many promoter polymorphisms in MMP genes that lead to altered gene expression. Promoter polymorphism in the gene for MMP-1 (G-1607GG) introduces a new binding site for ETS-1 transcription factors. Replacing A with G at A82G polymorphism in MMP12 reduces the affinity of the promoter to the transcription factor AP-1, which consequently affects the levels of expression of MMP-1 (low expression). In other studies the role of promoter polymorphisms in lung disease found that variant-1607GG allele in the MMP1 is associated with reduced lung function in smokers. The same study has shown that polymorphisms in genes of MMP-9 and MMP-12 have individual effect of the deterioration of lung function. But the combination of alleles of MMP1-1607GG and MMR12 Asn375 polymorphism showed a statistically significant association with reduced lung function. In contrast, another study found that the promoter polymorphism in the gene for MMP-9 (C-1562) is associated with the development of emphysema in smokers in Japan: variant-allele 1562 was associated with nearly 3 -fold greater risk of developing emphysema. This risk allele has a lower affinity for transcription represor (Henney, Ye et al. 2000).

ROLE OF MMPS IN COPD AND ASTHMA

MMP9 can be synthesized by a variety of cell types including neutrophils, macrophages, and epithelial cells (Vermeer, Denker et al. 2009). Innate immune cells including macrophages and neutrophils are a major source of matrix metalloproteinase-9 (MMP-9) and neutrophil elastase (NE), which are central to these processes. In the normal setting, anti-proteinases such as α1-antitrypsin (α1-AT), secretory leukoprotease inhibitor (SLPI) and tissue inhibitor of metalloproteinases (TIMPs) are in excess and provide an anti-proteinase screen to prevent deleterious effects (Vlahos, Wark et al. 2012). Evidence of ongoing inflammation has been documented via the increased levels of neutrophils and eosinophils in bronchoalveolar lavage

(BAL) fluid, CD4+ and CD8+ cells in bronchial biopsy specimens and eosinophilic cationic protein (ECP) in sputum (Louhelainen, Stark et al. 2010). Activated neutrophils release serine proteinases including NE, Proteinase-3 (PR-3) and MMPs including MMP-9 and MMP-8. As the disease progresses, increased susceptibility to respiratory virus and/or bacteria promote acute exacerbations (AECOPD) that further amplifies inflammation (Vlahos, Wark et al. 2012).

Matrix metalloproteinases (MMPs) have an important role in the breakdown of extracellular matrix (ECM) and they are considered as biomarkers of tissue damage (Louhelainen, Stark et al. 2010).

Neutrophil elastase (NE) is abundant in primary azurophil granules formed during the early stages of myelopoiesis and it is secreted during neutrophil degranulation (Faurschou and Borregaard 2003). Free NE activity may also accumulate in airways from necrotic neutrophils that release their intracellular content. NE activity is elevated in COPD and the degree of NE localized to lung elastic fibers correlates with the degree of emphysema. NE can degrade extracellular matrix components including elastin, collagens I-IV and fibrinogen and the genetic deficiency in α1-AT is associated with early onset of pan lobular emphysema (Vlahos, Wark et al. 2012). Serine proteinases such as NE are considered to be at the apex of the proteinase hierarchy in airways as they have the ability to activate MMPs including MMP- 9 via cleavage of pro-MMP-9 into active-MMP-9 (Ferry, Lonchampt et al. 1997). Furthermore, NE preferentially degrades TIMP1 that is bound to MMP- 9, thereby liberating active MMP-9 (Itoh and Nagase 1995). NE can also activate the inflammatory NF-kB pathway via a Toll-Like Receptor-4 (TLR-4) dependent manner and promote an acute inflammatory response. The promoter of MMP-9 also contains an NF-kB binding site that promotes its expression (Vlahos, Wark et al. 2012).

The levels of neutrophils and eosinophils in bronchoalveolar lavage (BAL) fluid in patients with COPD and asthma are elevated (Louhelainen, Stark et al. 2010).There is also increased MMP9 expression in the airways of asthmatic and COPD patients (Vermeer, Denker et al. 2009).

CONCLUSION

MMPs are important components in many biological and pathological processes because of their ability to degrade ECM components. It has become clear that they play central role in pathogenesis of COPD and bronchial asthma. This puts a new light on the diagnosis, prevention and control of these diseases.

REFERENCES

1. Aligne, C. A., P. Auinger, et al. (2000). "Risk factors for pediatric asthma. Contributions of poverty, race, and urban residence." Am J Respir Crit Care Med **162**(3 Pt 1): 873-7.

2. Barnes, P. J., S. D. Shapiro, et al. (2003). "Chronic obstructive pulmonary disease: molecular and cellular mechanisms." Eur Respir J **22**(4): 672-88.

3. Bode, W., C. Fernandez-Catalan, et al. (1999). "Structural properties of matrix metalloproteinases." Cell Mol Life Sci **55**(4): 639-52.

4. Brajer, B., H. Batura-Gabryel, et al. (2008). "Concentration of matrix metalloproteinase-9 in serum of patients with chronic obstructive pulmonary disease and a degree of airway obstruction and disease progression." J Physiol Pharmacol **6**: 145-52.

5. Choudhry, S., M. A. Seibold, et al. (2007). "Dissecting complex diseases in complex populations: asthma in latino americans." Proc Am Thorac Soc **4**(3): 226-33.

6. Fabbri, L. M., F. Luppi, et al. (2008). "Complex chronic comorbidities of COPD." Eur Respir J **31**(1): 204-12.

7. Faurschou, M. and N. Borregaard (2003). "Neutrophil granules and secretory vesicles in inflammation." Microbes Infect **5**(14): 1317-27.

8. Ferry, G., M. Lonchampt, et al. (1997). "Activation of MMP-9 by neutrophil elastase in an in vivo model of acute lung injury." FEBS Lett **402**(2-3): 111-5.

9. Harju, T. H., M. Leinonen, et al. (2006). "Pathogenic bacteria and viruses in induced sputum or pharyngeal secretions of adults with stable asthma." Thorax **61**(7): 579-84.

10. Henney, A. M., S. Ye, et al. (2000). "Genetic diversity in the matrix metalloproteinase family. Effects on function and disease progression." Ann N Y Acad Sci **902**: 27-37.
11. Hu, X. and C. Beeton (2010). "Detection of functional matrix metalloproteinases by zymography." J Vis Exp **8**(45): 2445.
12. Itoh, Y. and H. Nagase (1995). "Preferential inactivation of tissue inhibitor of metalloproteinases-1 that is bound to the precursor of matrix metalloproteinase 9 (progelatinase B) by human neutrophil elastase." J Biol Chem **270**(28): 16518-21.
13. Itoh, Y., A. Takamura, et al. (2001). "Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion." Embo J **20**(17): 4782-93.
14. Kroegel, C. (2009). "Global Initiative for Asthma (GINA) guidelines: 15 years of application." Expert Rev Clin Immunol **5**(3): 239-49.
15. Lakhdar, R., S. Denden, et al. (2011). "Update in chronic obstructive pulmonary disease: role of antioxidant and metabolizing gene polymorphisms." Exp Lung Res **37**(6): 364-75.
16. Louhelainen, N., H. Stark, et al. (2010). "Elevation of sputum matrix metalloproteinase-9 persists up to 6 months after smoking cessation: a research study." BMC Pulm Med **10**: 13.
17. Lu, L., Z. Gunja-Smith, et al. (2000). "Matrix metalloproteinases and collagen ultrastructure in moderate myocardial ischemia and reperfusion in vivo." Am J Physiol Heart Circ Physiol **279**(2): H601-9.
18. MacNee, W. (2005). "Pathogenesis of chronic obstructive pulmonary disease." Proc Am Thorac Soc **2**(4): 258-66.
19. Martinez, F. D. (2007). "Genes, environments, development and asthma: a reappraisal." Eur Respir J **29**(1): 179-84.
20. Miller, R. L. and S. M. Ho (2008). "Environmental epigenetics and asthma: current concepts and call for studies." Am J Respir Crit Care Med **177**(6): 567-73.
21. Moore, W. C. (2008). "Update in asthma 2007." Am J Respir Crit Care Med **177**(10): 1068-73.
22. Nagase, H., K. Suzuki, et al. (1992). "Activation mechanisms of the precursors of matrix metalloproteinases 1, 2 and 3." Matrix Suppl **1**: 237-44.
23. Ober, C. and S. Hoffjan (2006). "Asthma genetics 2006: the long and winding road to gene discovery." Genes Immun **7**(2): 95-100.
24. Rosendorff, C. (2012). "beta-blockade: benefits beyond blood pressure reduction?" J Clin Hypertens **14**(2): 69-70.
25. Suzuki, K., J. J. Enghild, et al. (1990). "Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin)." Biochemistry **29**(44): 10261-70.
26. Vermeer, P. D., J. Denker, et al. (2009). "MMP9 modulates tight junction integrity and cell viability in human airway epithelia." Am J Physiol Lung Cell Mol Physiol **296**(5): 6.
27. Visse, R. and H. Nagase (2003). "Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry." Circ Res **92**(8): 827-39.
28. Vlahos, R., P. A. Wark, et al. (2012). "Glucocorticosteroids differentially regulate MMP-9 and neutrophil elastase in COPD." PLoS One **7**(3): 7.
29. Watson, A. M., A. S. Benton, et al. (2010). "Cigarette smoke alters tissue inhibitor of metalloproteinase 1 and matrix metalloproteinase 9 levels in the basolateral secretions of human asthmatic bronchial epithelium in vitro." J Investig Med **58**(5): 725-9.
30. www.biochemmmack.ru.
31. www.riokoz-vt.com/hobb.htm (2012).