Chapter 3

Matrix Metalloproteinases in Asthma-Associated Airway Remodeling – Dr. Jekyll or Mr. Hyde ?

Katarzyna Grzela, Agnieszka Strzelak,
Wioletta Zagórska and Tomasz Grzela

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62440

Abstract

Matrix metalloproteinases (MMPs) are Zn^{2+}-dependent endoproteases, which digest extracellular matrix (ECM) components and various non-ECM molecules. Main physiological role of MMPs concerns regulation of tissue remodeling and regeneration. The production and activity of MMPs are tightly supervised by multistage control mechanisms. These mechanisms include regulation of gene expression, and various post-transcriptional/post-translational modifications. However, without proper control MMPs reveal dual nature, similarly to character from the novella by R.L. Stevenson, "Strange Case of Dr Jekyll and Mr Hyde". They become dangerous molecules, involved in cancer metastasis, or cardiovascular diseases.

Recent studies revealed that MMPs are also engaged in asthma. Despite extensive research, exact role of MMPs in this process remains unclear and there is no agreement among scientists, regarding two opposite concepts. The followers of "destructive hypothesis" postulate detrimental effect of MMPs on mucosa. Accordingly, MMPs-mediated damage stimulates chaotic regeneration, and progressive remodeling. Oppositely, enthusiasts of "protective hypothesis" postulate that MMPs actually do not allow formation of excessive collagen deposits, and thus they protect from tissue fibrosis.

The better understanding of "MMPs — Jekyll or Hyde ?" story may be clinically relevant, especially while considering therapies focused on modulation of MMPs activity. Therefore, this issue requires instant elucidation.

Keywords: airway remodeling, asthma, extracellular matrix, matrix metalloproteinase, destructive hypothesis and protective hypothesis

1. Introduction

Matrix metalloproteinases (MMPs) represent group of 25 endoproteases, which require a presence of zinc ions to reveal their proteolytic activity. According to worldwide accepted nomenclature, MMPs have assigned numbers from 1 to 28. However, till now no respective molecules have been ascribed for numbers 4, 5 and 6, whereas MMP-18 was identified only in *Xenopus* frogs. [1, 2] Apart from regulation of extracellular matrix (ECM) turnover, MMPs are also involved in controlling of numerous non-ECM molecules, including cytokines and growth factors. Thus, MMPs are key molecules in embryo- and organogenesis, angiogenesis and tissue regeneration. However, they are also main destructive factors, responsible for cancer progression, aortic aneurysm rupture or delayed healing of chronic wounds. [3, 4] Recently, their involvement was postulated also in some inflammatory diseases affecting respiratory tract, among them chronic obstructive pulmonary disease and asthma. [5] In this chapter authors will focus especially on possible role of MMPs in asthma and asthma-associated alterations in architecture and function of respiratory tract mucosa, which are better known as airway remodeling.

2. MMPs — portrait of the family

2.1. MMP structure

Based on molecular structure, substrate specificity and mechanism of activation, MMPs are classified into four groups: gelatinases, matrilysins, archetypal MMPs and furin-activated MMPs (Fig. 1.). Formerly, MMPs were divided into six types – collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others. However, nowadays this classification possesses rather historical meaning. The overall structure of MMPs reveals some common features, which are similar in all members of the family. [2, 3] One of these features is the presence of signaling peptide, located on the N-terminus of newly synthesized proteins. This leader sequence is necessary for the insertion of maturating MMP molecule into cistern of endoplasmic reticulum, and then it is removed. Unlike to other family members, in MMP-23 the N-terminal signaling sequence is substituted by a type II transmembrane domain, which allows anchorage of these molecules in cell membrane.

The next part common in MMP structure is approximately 80 amino acid-long prodomain. It contains conserved "cysteine switch" motif, responsible for maintaining the latent form of enzyme by the blockade of its catalytic site. The main constant segment present in all family members is their catalytic domain. This sphere-like domain is composed of 160-170 amino acids. It contains shallow slot with two zinc ions inside, which constitutes an active site of MMP molecule. Exclusively, catalytic domains in both gelatinases, MMP-2 and -9, contain unique fibronectin II-like inserts. Most MMPs (except for MMP-7, -23 and -26) have short hinge segment of approximately 10-30 amino acids, which connects the catalytic domain with hemopexin-like domain. Exceptionally, MMP-9 molecule has the longest hinge region, composed of 64 strongly O-glycosylated amino acids. The C-terminal hemopexin-like domain,

http://dx.doi.org/10.5772/62440

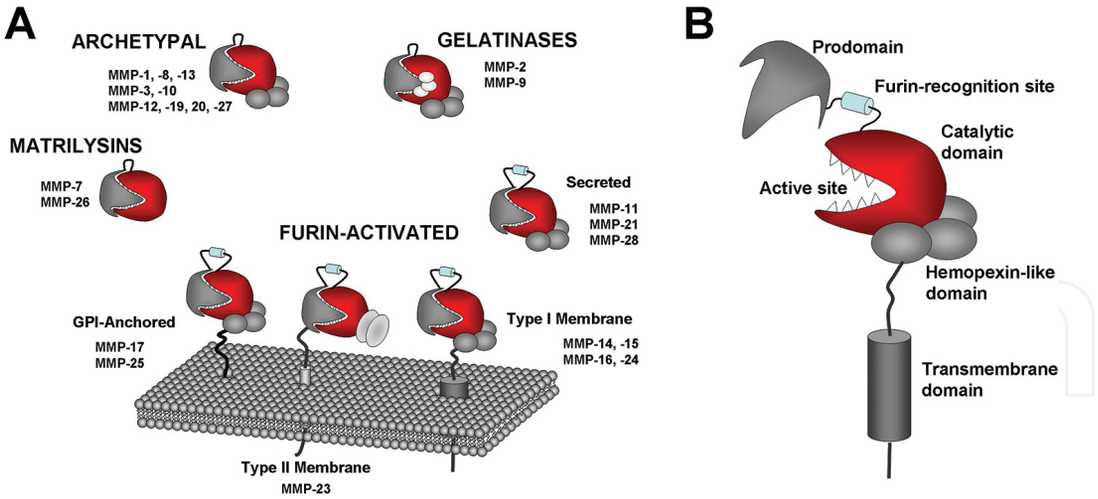

Fig. 1. Schematic representation of MMPs family. (A). Main groups of the family and their structure. (B). An example of schematic structure of MMP. Detailed description in text.

not present in MMP-7, -23 and -26, is composed of approximately 200 amino acids and is considered as docking spot for tissue inhibitors of MMPs (TIMPs). In MMP-23 molecule the hemopexin-like domain was replaced by a cysteine-rich immunoglobulin-like domain.

The representatives of membrane-type (MT) subgroup of MMPs (except of already mentioned MMP-23) have hemopexin-like domain connected to a type I transmembrane domain with a short intracellular tail (MMP-14, -15, -16 and -24, also known as MT1, -2, -3 and -5-MMP, respectively), or a cell membrane-anchoring glycosylphosphatidylinositol (GPI) moiety (MMP-17 and -25, known as MT4- and MT6-MMP) (Fig. 1).

Finally, three of secreted MMPs (MMP-11, -21 and -28), all the membrane-type MMPs and MMP-23 have a specific sequence between their prodomain and the catalytic domain, which is recognized by furin. This subtilisin-like serine proteinase from trans-Golgi apparatus and the endoplasmic reticulum removes the prodomain from the catalytic domain and thus may lead to intracellular activation of MMP molecule. [1–6]

2.2. Substrate specificity

MMPs are able to digest main components of extracellular matrix (ECM), including high molecular weight polymers of native and denatured collagens and elastin, as well as small ECM molecules, like fibronectin, laminin and aggrecan. Moreover, MMPs may process numerous non-ECM molecules, among them various adhesion molecules, dystroglycan, syndecans, growth factors, pro-cytokines, and their receptors. MMPs were shown to activate via proteolysis pro-forms of interleukin (IL)-1β, IL-8, tumor necrosis factor (TNF), Fas ligand, transforming growth factor (TGF)-β, but also other members of MMPs family (Table 1). [1, 3, 7–9] Noteworthy, some of these cytokines, including vascular endothelial growth factor (VEGF) and TGF-β, may be further entrapped in three-dimensional net of extracellular matrix

components or by their binding proteins. Therefore, MMPs may be necessary to reveal biological activity of these factors, through their enzymatic discharge from ECM.

Group	Representatives	Main ECM substrates	Non-ECM substrates
ARCHETYPAL MMPs	**Collagenases** MMP-1, -8- 13	collagens, gelatin, fibronectin, aggrecan…	pro-IL-1β, pro-IL-8, pro-TNF, other MMPs, PAI, IGFBM
	Stromelysins MMP-3, -10	collagens, gelatin, elastin, fibronectin, laminin, aggrecan	pro-IL-1β, other MMPs, IGFBP, MMP/TIMP complex, fibrinogen, plasminogen, antitrombin III
	Others MMP-12, -19, -20, -27	collagen IV, gelatin, elastin, fibronectin, laminin	fibrin, plasminogen, myelin basic protein
MATRILYSINS MMP-7, -26		collagen IV, gelatin, elastin, fibronectin, laminin, integrins…	other MMPs, MMP/TIMP complex, fibrinogen, plasminogen
GELATINASES MMP-2, -9		collagens, gelatin, elastin, fibronectin…	pro-IL-1β, plasminogen, other MMPs
FURIN-ACTIVATED MMPs	**Secreted** MMP-11,-21,-28	collagen IV, gelatin, laminin, fibronectin	casein, IGFBP
	Type 1 transmembrane MMP-14,-15,-16,-24	collagens, gelatin, elastin, laminin, vitronectin	other MMPs
	GPI-anchored MMP-17,-25	UNK	
	Type II transmembrane MMP-23A,-23B	UNK	

Table. 1. Representatives of MMPs family with their main substrates (detailed description in text). ECM – extracellular matrix, Non-ECM- other substrates, PAI – plasminogen activator inhibitor, IGFBP – insulin-like growth factor-binding protein, TIMP – tissue inhibitor of MMPs, UNK – unknown.

2.3. MMP expression

Due to a high proteolytic activity and broad substrate specificity, MMPs are recognized as key molecules, engaged in cell proliferation and migration, tissue growth, remodeling, and regeneration. For this reason their expression and activation has to be maintained under precise multistage control. These controlling mechanisms include regulation of gene expression, post-transcriptional and post-translational modifications, but also several ways of pro-enzyme activation or inhibition of active MMP. [3, 4] Nevertheless, if these mechanisms fail, similarly to the well known character from the famous novella by R.L. Stevenson, "Strange Case of Dr. Jekyll and Mr. Hyde", MMPs may also reveal their dual nature. Without sufficient

http://dx.doi.org/10.5772/62440

supervision, these endoproteases may become highly dangerous effector molecules, engaged in various pathologies. These conditions include cancer metastasis, formation and rupture of aortic aneurysm, delayed healing of chronic wounds and many others. [1, 3, 10, 11] Recent studies have provided evidence that MMPs may also be involved in pathogenesis of asthma, mainly asthma-associated airway remodeling. [5]

Among all MMPs, only MMP-2 and MMP-9 are produced constitutively, whereas the expression of majority of MMP genes requires some trigger, e.g. tissue damage, or inflammatory reaction. It was found that the promoter region of genes encoding for MMPs comprises sequences recognized by two main specific transcription factors, AP-1 and NF-κB. Both transcription factors merge expression of many inflammatory response-engaged molecules, including MMPs with several intracellular signaling pathways, induced by cytokines and growth factors. Indeed, it was proved that MMPs expression may be controlled by variety of growth factors, including TGF-β, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNF, etc.). Moreover, the promoter activity of MMPs may also be supervised by family of Ets transcription factors. Since their conserved binding site is located close to target sequence for AP-1, they may interact each other and thus modulate promoter response to various stimuli. [3, 4, 10, 12–14]

The rigid control of MMP genes expression may also be granted by their epigenetic modification. This mechanism is based on alteration in chromatin conformation, which is mediated by differential acetylation-deacetylation of nucleosomal units, due to activity of an enzyme – histone deacetylase (HDAC). Noteworthy, it has been shown that such regulation may result in various responses of particular MMP genes. *In vitro* stimulation with TNF or IL-1β, with simultaneous suppression of HDAC activity resulted in decreased expression of MMP-1 and MMP-9, but increased production of MMP-3. [14, 15] Finally, the expression of MMPs may also be modified on the post-transcriptional level, by the influence on stability or degradation of their transcripts. Recently, it has been proven that the expression of several MMPs may be negatively regulated by the small molecules of non-coding RNA, known as microRNAs (miRs), in mechanism of RNA interference. It has been demonstrated that miR-9, miR-24 and miR-133a may bind to the 3'-UTR of mRNA for MMP-14 (MT1-MMP) and they directly block its translation. On the other hand, down-regulation of miR-199a-5p in murine model induced MMP-1, possibly via Ets-1 derepression. [16–19]

2.4. MMP activity

All members of MMPs family are expressed as inactive pro-enzymes. This condition is assured by previously mentioned "cysteine switch", a specific interaction between zinc cations from the active site of the catalytic domain, and a cysteine thiol group from the prodomain. The renouncement of inhibitory influence of the prodomain on the catalytic domain is critical for activation of pro-enzyme and may take place in two concurrent ways (Fig. 2). [3, 14, 20]

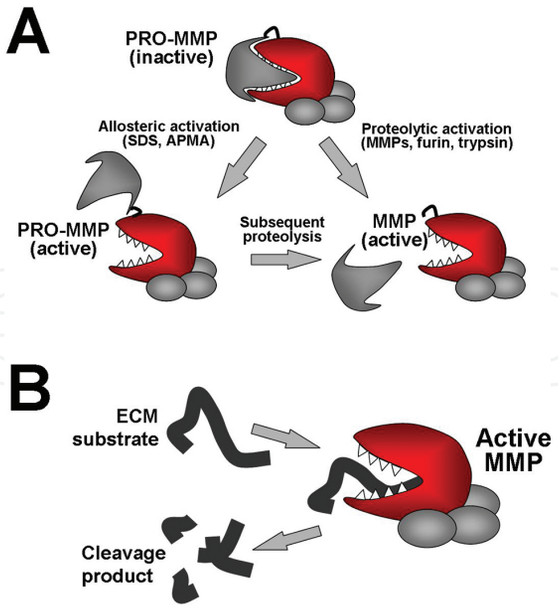

Fig. 2. MMPs activation. (A) Two pathways of MMPs activation. (B) Schematic representation of substrate cleavage. Detailed description in text.

2.4.1. Activation

The first pathway of MMPs activation is based on direct cleavage of their prodomain. It may be carried by several extracellular proteolytic enzymes, including other MMPs, as well as cysteine, serine and aspartate proteases. This pathway also involves already mentioned intracellular processing and activation by furin. Due to removal of prodomain, molecular weight of pro-enzyme activated in this pathway is significantly reduced, as compared to its initial size. Thus, in zymograms of substrate-specific zymography with SDS-polyacrylamide gel electrophoresis (PAGE) the activated MMP appears as the lower band, below that, corresponding to latent form of enzyme. [21–23]

The second pathway depends on interaction of cysteine thiol groups from prodomain with various compounds, including free radical, disulfides, some detergents with sodium dodecyl sulphate (SDS), alkylating agents, heavy metal ions and organomercurials, with 4-aminophenylmercuric acetate (APMA). This interaction may induce allosteric conversion in MMP structure, which leads to an exposure of the active site in the catalytic domain. Therefore, although the prodomain still remains attached to the entire molecule, such MMP may reveal its proteolytic activity. On the other hand, that MMP, despite being activated, has the same molecular weight, as its inactive pro-form. That explains, why such full length-MMP may be visualized in zymograms on the same level as latent pro-MMP. Noteworthy, the prodomain may be further removed by auto-cleavage, that results in decrease of MMP molecular size and, similarly to the first pathway, an appearance of the lower band in zymograms. [3]

Results of recent studies suggest that *in vitro* proteolysis requires only a substrate and respective MMP, whereas *in vivo* systems usually involve some additional component. These accessory factors may include membrane-, or ECM-associated peptides and glycosaminogly-

http://dx.doi.org/10.5772/62440

cans, which may determine specificity, as possibly, catalytic rate of MMPs. Accordingly, such accessory molecules may work as a kind of adapters, which bind a substrate and MMP and thus enable their close interaction with an effective concentration. [21–23]

2.4.2. Inhibition

As was already noticed, the precise control of MMPs expression and activity is essential for homeostasis of the entire body. Therefore, to counterbalance the mentioned stimulators and activators of MMPs, some agents revealing inhibitory properties are also required. Apart from best known family of specific tissue inhibitors of metalloproteinases (TIMPs), there are also less specific endogenous inhibitors, among them α2-macroglobulin, family of serine proteinase inhibitors (serpins), thrombospondin-1 (TSP-1), tissue factor-pathway inhibitor (TFPI)-2, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), etc. [3, 10, 21, 24]

Members of TIMPs family (numbered from 1 to 4) are the best identified specific endogenous inhibitors of MMPs. They are expressed and released by various cell populations, including macrophages, platelets, smooth muscle cells, etc. The mechanism of their action depends on reversible chelating of Zn^{2+} cations from active center of MMP's catalytic domain and, thus, abolishes its proteolytic properties. Since MMP – TIMP interaction occurs in a stoichiometric ratio 1:1, the MMP/TIMP ratio seems to better reflect presumable biological impact of both agents, instead of absolute amount of each of both proteins. Moreover, it is noteworthy that studies concerning *in vivo* interactions between MMPs and TIMPs are also interfered by the highly effective serum antiprotease – α2-macroglobulin. [3, 6, 14]

Although all TIMPs may interact with various MMPs, they differ in their specificity, e.g. TIMP-1 preferentially binds to membrane type-MMPs, whereas TIMP-2 is considered as important regulator of MMP-2 activity. Interestingly, the latter regulation actually involves TIMP-2-dependent activation of MMP-2. In this unique mechanism TIMP-2 works as bridging molecule between hemopexin domain of MMP-2 and MT1-MMP (MMP-14), which mediates cleavage of prodomain in "immobilized" MMP-2.

Apart from mentioned above endogenous MMPs inhibitors, there is also an increasing number of exogenous compounds, which reveal direct and/or indirect modulatory properties towards the activity of MMPs. [3, 4] Since they have potential clinical relevance in a treatment of asthma and asthma-associated remodeling, they will be further described in next paragraph (see 2.7).

2.5. Methods of MMP measurement

Increasing interest in the role of MMPs in asthma and, especially, asthma-associated remodeling encouraged scientists to develop more specific and sensitive methods to detect MMPs in analyzed samples. However, main obstacle in MMPs research is that most commonly used methods, i.e. enzyme-linked immunosorbent assay (ELISA) and zymography, do not allow simultaneous assessment of amount and activity of MMPs. [3]

2.5.1. ELISA

Standard ELISA is a routine laboratory technique, which allows a quantitative detection of minute amounts of MMPs in solution (picograms per ml) using specific antibodies, usually conjugated with peroxidase-based detection system. Noteworthy, standard method provides data concerning specific protein concentration, without any information regarding actual activity of MMPs. Nevertheelss, such activity could be roughly estimated using specially designed ELISA sets, which enable differentiation between truncated forms of activated MMPs and prodomain-containing latent MMPs. However, as mentioned above, allosteric activation not necessarily leads to prodomain removal. Therefore, data provided by ELISA alone are not fully conclusive, and should be verified by some activity assay. [3]

2.5.2. Zymography

The substrate-specific zymography is the most commonly used method to evaluate MMPs activity in tested samples. This assay is based on initial separation of samples using electrophoresis in modified polyacrylamide gel, followed by its incubation in reaction buffer and subsequent staining. The key component of such modified gel is substrate, specific for enzyme being analyzed (e.g. collagen for MMP-1 and -13, gelatin for MMP-2 and -9, casein for MMP-1, -3, -7, -10, -12, -13), which is homogenously distributed in whole gel volume. Since polyacrylamide gel contains sodium dodecyl sulphate (SDS), the speed of migration in electrophoresis is determined by molecular weight of separated proteins, resulting in shifted local condensation of full length pro-enzyme and truncated forms of MMPs. During incubation in calcium- and zinc-rich reaction buffer, MMP molecules become reactivated and digest own specific substrate only in place of their condensation. After wash in the staining solution, e.g. Coomassie Brilliant Blue, the entire gel becomes stained, with except of unstained area corresponding to digested substrate. Noteworthy, when compared to respective molecular weight standard, the localization of unstained area enables better identification of analyzed MMP, whereas the size of digested / unstained bands well correlates with amounts of detected enzyme. This amounts may be further determined by comparison to reference sample, e.g. known amounts of recombinant MMP. [3, 25]

Although substrate-specific zymography is sensitive (picograms per sample), and relatively cheap method, it has some weak points. The first issue is long and time-consuming protocol. The next, more important concern, is uncontrolled allosteric activation of MMP mediated by SDS. Since it may strongly affect results of assessment, in current research standard zymography is often replaced by other, faster and more reliable activity assays.

2.5.3. Fluorescent activity assay

The unintended interaction with SDS may be avoided, when instead of polyacrylamide gel, MMPs activity is assessed in SDS-free reaction mixture. The measurement of substrate proteolysis in solution implements technology of fluorescence resonance energy transfer (FRET) using substrate (e.g. casein or gelatin) tagged with fluorochrome and quencher. Until labeled substrate stays untouched, the entire energy from fluorochrome is absorbed by

http://dx.doi.org/10.5772/62440

quencher, with no fluorescence detectable. When the substrate is cleaved, the fluorochrome-quencher interaction becomes disrupted, that is associated with increased emission of fluorescence under UV light. Since the increase of fluorescence is proportional to enzyme activity, with known quantities of MMP as reference, and with fluorescence reader, this method allows very fast (within few minutes) measurement of proteolytic activity revealed by small amounts (nanograms per ml) of MMP in tested samples. [3, 26]

Noteworthy, in contrast to standard zymography, the fluorescence assay enables studies on proteolytic activity of MMPs, and analysis of modulation of this activity by various agents, e.g. natural and synthetic inhibitors. However, the method is very sensitive to reaction conditions, which may vary depending on protocol of sample preparation. The key factors are concentration of non-ionic detergents, and presence of exogenous protease inhibitors (frequently used to prevent proteolysis in biological material) or metal ion chelators (e.g. EDTA). On the other hand, the tissue sample preparation itself may lead to artificial activation of MMPs or release of their natural inhibitors. [3]

The main disadvantage of the basic variant of mentioned fluorescent method is its non-specificity. Therefore, when analyzing biological samples, to determine, which MMP contributes to the degradation of labeled substrate, it is necessary to use a panel of MMP-specific antibodies, to inhibit proteolytic activity of selected enzyme. Although a such approach enables precise identification of all contributors of observed proteolytic activity, it also significantly increases the cost of that analysis.

2.5.4. Immunozymography assay

Recently, a modification of mentioned above fluorescent method was introduced into market. The method combines specificity of standard ELISA and functionality of fluorescent activity assay. In a first step the sample is applied onto test plate, coated by antibody specific for MMP of interest. Then MMP molecules, which are captured by antibody, convert a latent detection reagent into its active form. The activated detection reagent catalyzes enzymatic conversion of colorless substrate into color product. Since the amount of product directly correlates with number of active MMPs, the use of standard calibration curve allows precise measurement of active MMP molecules concentration in tested samples. Furthermore, when using organomercurials (e.g. APMA) to activate pro-MMPs in tested material, it also allows an assessment of latent form of MMPs. Thus, the assay incorporates advantages of standard zymography (the assessment of MMP activity and discrimination between pro- and active forms of these enzymes), specificity of ELISA and exceptional sensitivity, reaching 0.1 pg/ml. Therefore, it may be the best choice for research concerning MMPs activity in samples, where the minute amounts of MMPs are expected, e.g. condensates of exhaled air. [27]

2.5.5. In situ zymography

The distribution of MMPs in tissue specimens may be studied using immunohistochemistry. However, to assess the local activity of these enzymes, directly on the place of their production, the *in situ* zymography may be used. Similarly to mentioned above fluorescent activity assay,

this method also utilizes FRET technology. The tested specimen is incubated with substrate labeled with fluorochrome-quencher complex and then analyzed using fluorescent microscope, or confocal laser scanning microscope. Similarly to fluorescent activity assay, *in situ* zymography does not identify particular MMPs, unless used with specific neutralizing antibodies. Furthermore, it does not provide information concerning quantities of active MMP. Nevertheless, it is still valuable supplement to other methods in MMPs research. [3]

2.5.6. Reverse zymography

As previously mentioned, various factors which affect MMPs activity, may be analyzed using fluorescent activity assay, western blot or respective ELISA. However, to detect natural tissue inhibitors of MMP (TIMPs) some functional assay, better known as reverse zymography, has been developed. The method is based on specific interaction between TIMPs from analyzed sample and MMP of interest. Similarly to standard zymography, samples are separated in polyacrylamide gel, which is supplemented with homogenously distributed substrate (e.g. gelatin), but also selected MMP. After electrophoresis the gel is incubated in reaction buffer. Since both, MMP and substrate, are present in the entire gel volume, MMP cleaves the whole substrate, except of places corresponding to the TIMPs condensation after electrophoresis. In these places TIMPs protect substrate from digestion, therefore, after Coomassie staining they appear as blue bands, whereas the remaining gel volume stays unstained. [3]

2.6. MMPs in patients with asthma

2.6.1. Mr. Hyde…?

Extensive studies, focused on asthma and asthma-associated airway remodeling, have revealed clear involvement of MMPs in pathogenesis of that disease. [5] However, the exact role of MMPs in this process remains vague. The postulated link between metalloproteinases and asthma was based mainly on observations concerning increased amounts and/or activities of various MMPs in samples collected in patients with asthma. The samples were obtained using various methods of collection and/or various material, among them serum or plasma, mucosal biopsies, induced sputum, broncho-alveolar lavage (BAL) fluid and, most recently, exhaled breath condensates (EBC). [27–31] Majority of studies concerned MMP-9, however, other MMPs, including MMP-1, -2, -3, or -12 were also studied. It is noteworthy that substrate specificity of mentioned MMPs entirely enables their self-sufficient work with full repertoire of ECM components. Collagen IV, and laminin, two main components of basement membranes, are cleaved by MMP-9 and MMP-12. Native molecules of collagen I, main fibrillar ECM component of mucosal connective tissue, are initially digested by MMP-1, whereas their further degradation may be continued by all mentioned MMPs (MMP-2, -3, -9, and -12). Elastin molecules are degraded mainly by MMP-12, but also MMP-2 and -9. [3] Although nominal values of MMPs (especially MMP-9) concentrations differed between various studies, in vast majority of mentioned reports similar regularity was observed. MMPs levels and /or activity in individuals with asthma were several fold higher than in control subjects. [28, 29, 32] The number of MMPs-positive cells in sputum or BAL inversely correlated with values of forced

http://dx.doi.org/10.5772/62440

expiratory volume in 1 second (FEV1), whereas MMPs amounts in sputum, BAL and EBC positively correlated with severity of disease. They were significantly higher in severe asthma or in asthma exacerbation, and lower in mild asthma or in remission. [31, 33] Also bronchial smooth myocytes/myofibroblasts (BSM) from mucosal biopsies of patients with fatal asthma produced increased levels of MMP-9 and -12, whereas BSM from non-asthmatics expressed only small quantities of MMP-2,-3 and -9. [34]

These observations could support concept of "destructive hypothesis", which emphasizes detrimental effect of metalloproteinases on disease progression. In this scenario, similarly to Mr. Hyde from previously mentioned novella by R.L. Stevenson, MMPs reveal their dark nature. The overexpression and hyperactivation of these enzymes may result in progressive damage of epithelium, basement membrane and subepithelial connective tissue. These events may endorse local inflammatory reaction, and thus further increase the damage zone. [5, 35] On the other hand, they may induce excessive and poorly controlled tissue repair, with increased deposition of ECM components, proliferation and hypertrophy of myofibroblasts, as well as goblet cells hyperplasia with mucus hypersecretion. These changes result in structural and functional changes in bronchial tree mucosa, which is known as airway remodeling (Fig. 3). [5, 34] In fact, in animal model of asthma it was found that an increase in MMP-9 activity in the airway mucosa was associated with epithelial damage, alteration of subepithelial basement membrane, but also increased levels of TGF-β and subepithelial collagen deposition. [36, 37] In patients with asthma Vignola and coauthors have observed positive correlation between sputum levels of MMP-9 and the intensity of functional and structural abnormalities, which may be easily visualized using air flow measurement and high resolution computed tomography, respectively. [38, 39]

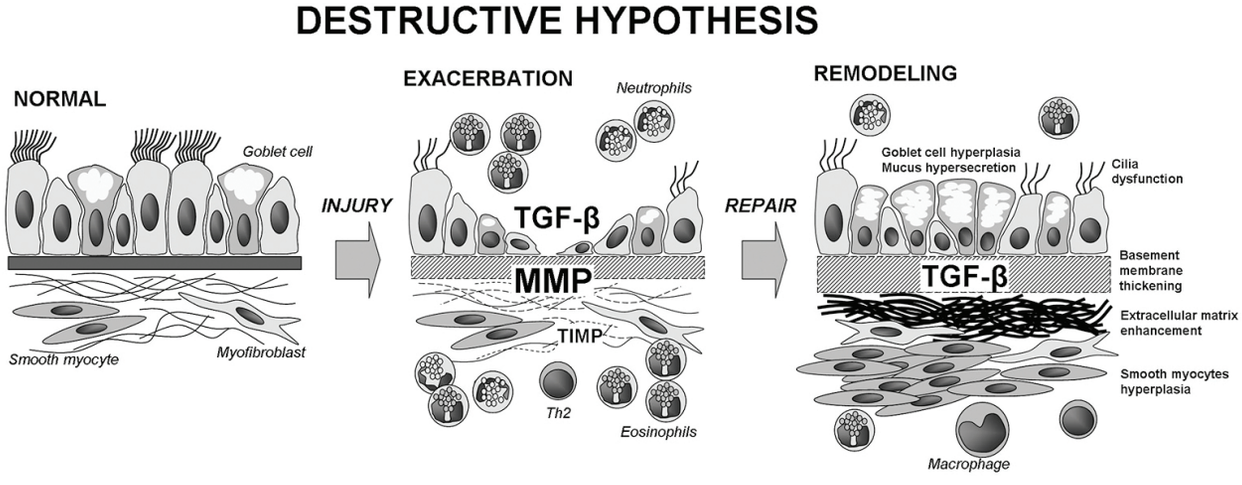

Fig. 3. Schematic representation of the "destructive hypothesis". Detailed description in text.

Interestingly, some authors did not confirm direct correlation of MMPs levels with symptoms severity, especially when using serum or plasma samples for the analysis. [30] The last finding may suggest that a main source of MMPs overproduction in asthma is located in airway system, with limited systemic influence. This assumption may be supported by association

between MMP-9 level measured in breath condensates and predominant population of inflammatory cells in induced sputum or BAL. Barbaro and coauthors have shown that patients with neutrophilic airway inflammation revealed MMP-9 concentrations significantly higher than individuals with severe eosinophilic asthma. [40] Thus, one could conclude that, essentially, neutrophils and, to a lesser extent, eosinophils would be main sources of MMPs in mild and severe asthma. Nevertheless, there is strong evidence that functional and structural changes in bronchial wall are also contributed by other producers of MMPs – epithelial cells, bronchial smooth myocytes, fibroblasts and mast cells. [5, 27] In fact, recent studies have shown that epithelium- and myocytes-derived metalloproteinases may be involved in pathogenesis of asthma and asthma-associated remodeling much earlier, even before clinical manifestation of first symptoms. That hypothesis emerged as an ancillary result of embryological studies, focused on development of bronchial tree. It has been proposed that pouches of epithelium, submerged in mesenchyma, work together during organogenesis as an functional entity, which was named the epithelial-mesenchymal trophic unit (EMTU). [41] Apart from large variety of cytokines and growth factors, produced by EMTU during embryogenesis, the important role in regulation of bronchial growth play metalloproteinases. Their list is still expanding and includes several soluble MMPs, mainly MMP-3, -9 and -12 [34, 42], as well as membrane-type 1 MMP (MT1-MMP/MMP-14). [43] The latter is involved in regulation of cell proliferation, migration and differentiation, since all these events are in some extent associated with pericellular proteolytic activity of this membrane-bound MMP.

Recently, another membrane-bound metallopeptidase, member of distinct class of disintegrin and metalloproteinases (ADAM), denoted as ADAM33, has been added to this list. [44] Similarly to MT-MMPs, function of these metalloproteinases relies on degradation of ECM components located in the close proximity to the cell, that enables further growth and branching of respiratory tree. However, this involvement also comprises processing of cytokines and their receptors. Therefore, although they remain under strict control, including methylation-dependent epigenetic regulation of promoter activity [45], even small abnormality in that system may be responsible for aberrant function of EMTU. This may lead to enhanced response to some stimuli, e.g. oxidative stress or viral infection. [46] Such triggers may result in reactivation of EMTU in adulthood, excessive stimulation of epithelial cells and bronchial myofibroblasts. They start again to express large quantities of metalloproteinases and cytokines, among them TGF-β. Both mentioned enzymes, ADAM33 and MT1-MMP/MMP-14, are supposed to trigger TGF-β-dependent stimulation of BSM and subepithelial fibroblasts, which, in response, start with excessive production and deposition of ECM components. Hereby EMTU reactivation may be associated with an increased risk of airway malfunction. Interestingly, in some individuals the initial ultrastructural changes in basement membrane, a characteristic feature of asthma-associated remodeling, were observed long before the onset of clinical symptoms of disease. [47, 48] Accordingly, one could expect, that EMTU hypothesis should be supported by some genetic background. Indeed, analysis of genome-wide association has indicated the possible role of nucleotide polymorphisms of metalloproteinases in increased susceptibility to asthma. [49] Among them, a significant association with early onset of bronchial hyperresponsiveness and asthma was noted in case of several polymorphic variants of ADAM33. [50] However, due to ethnic variability, the

http://dx.doi.org/10.5772/62440

significance level of this association differs between analyzed populations. Noteworthy, among analyzed polymorphisms of ADAM33, only T1, V4, T+1 and F+1 variants were found to correlate with asthma in the general population [51], whereas other, including ST+7 or haplotype H4, were characteristic solely for certain populations. [52, 53]

Furthermore, several groups have suggested correlation between increased risk of asthma development and occurrence of some nucleotide polymorphisms in genes encoding for "classic" matrix metalloproteinases, mainly MMP-9. The postulated associations concerned single nucleotide polymorphisms (SNPs) of MMP-9 gene, located in promoter region (-1562C/T), the substrate binding site in catalytic domain (279Q/R) and TIMP docking region of hemopexin domain (574P/R and 668R/Q). [5, 54] However, majority of mentioned studies were conducted in rather small groups, with various ethnic origins. Therefore, results of these studies, although relevant, should be considered with some caution. Till now, based on studies involving group of 4,000 children, only allele R of 279 SNP was confirmed as being associated with significant increase of asthma risk. [54] On the other hand, some SNPs in MMP genes may also be associated with eventual benefit for a patient. Recently, the mutant T allele of MMP-2 promoter (-1306C/T) SNP, supposed to decrease MMP-2 production, has been described as conferring significant protection against asthma in North Indian population. [55]

2.6.2. … or Dr. Jekyll ?

As already mentioned, research involving asthma patients, animal models and *in vitro* studies provide strong support for "destructive hypothesis". [56] MMPs are abundantly produced and activated during acute and chronic asthma, and their level negatively correlates with lung function. However, those observations should be interpreted with some caution, especially since recent studies yielded some contradicting data. [57, 58] Based on these data an opposite, or "protective hypothesis" has been formulated. According to this concept, MMPs are responsible for cleavage of excessive amounts of ECM components, which are secreted in response to inflammation-mediated damage of mucosa. [59] Therefore, MMPs are supposed to protect mucosa from uncontrolled fibrosis, whereas their natural inhibitors – TIMPs (especially TIMP-1), since they prevent cleavage of abnormal ECM deposits, would, paradoxically, appear as key detrimental molecules in that system (Fig. 4). In fact, increased TIMP-1 concentrations in BAL were related to persistent wheezing in preschool children. [31] However, an attribution of altered ECM turnover solely to MMPs activity or TIMPs concentration seems to be unfounded simplification. Presumably, enhanced accumulation of matrix components results rather from imbalance between proteolytic activity of MMPs and anti-proteolytic properties of TIMPs. Consequently, instead of absolute levels of MMPs or TIMPs, their relative amounts, expressed as respective MMP/TIMP ratios, may be more relevant for course of disease. Indeed, in several studies decreased MMP-9/TIMP-1 ratio was observed in sputum, BAL and mucosal biopsies of adults and children with asthma. [60, 61] Moreover, it was associated with the airflow aggravation and reduced airway lumen, observed in computed tomography of asthmatic patients. [62] The low MMP-9/TIMP-1 ratio was also reported in asthmatic smokers with persisted airflow obstruction and thickening of bronchial mucosa. [63]

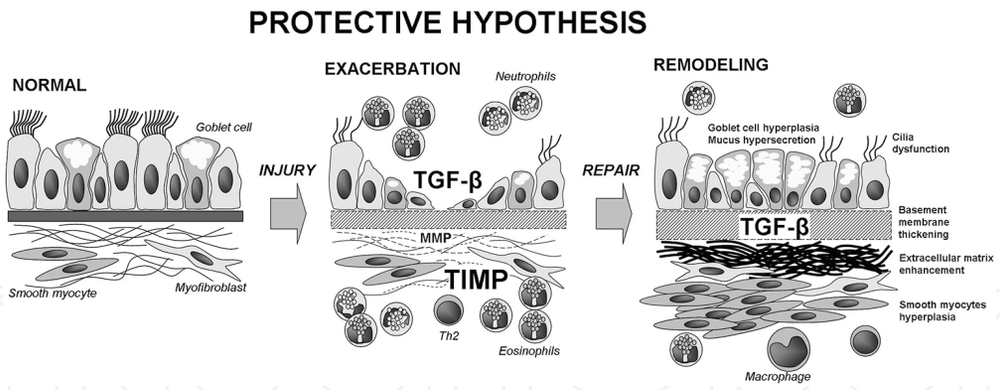

Fig. 4. Schematic representation of the "protective hypothesis". Detailed description in text.

When considering protective role of MMPs in asthma and asthma-associated remodeling, their main function concerns normalization of ECM turnover. However, one has to mention MMP-mediated regulation of cell-to-cell and cell-to-ECM interactions, as well as their involvement in cytokines/growth factors network. [56] As previously described, MMPs have been shown to process some cytokines, including TGF-β and VEGF, but also cleave several cell surface receptors, among them fibroblast growth factor (FGF) receptor 1 (FGFR1), CD44, or alpha subunits of receptors for IL-2, -5 and -13. [56, 64–67] Possibly, MMPs could also reveal their protective effect on asthma progression by interference with trafficking of immune cells and/or shedding key receptors engaged in Th2 signaling, thus attenuating allergic inflammation. This hypothesis was confirmed in murine models with MMP-deficient animals. Indeed, in mice lacking MMP-2, -8, -9 or -19 an allergen challenge resulted in increased allergic inflammation and airway hyperresponsiveness with augmented release of Th2 cytokines – IL-4, -5 and -13. [65, 68–74] Interestingly, MMPs deficiency was also associated with delayed clearance of immune cells from the airway. [68, 69, 71] This finding could be explained by involvement of MMPs in conditioning of leukocytes. [71] The possible mechanism of that phenomenon may exploit MMP-9-mediated cleavage of IL-2Rα subunit on the surface of T lymphocytes, which results in down-regulation of their proliferative capacity and subsequent apoptosis. [75] Apart from mentioned Th2 cytokines, MMPs may modulate inflammatory and immune response via processing of CC and CXC chemokines. Metalloproteinases have been shown to cleave macrophage inflammatory protein (MIP)-2 and monocyte chemoattractant proteins (MCPs) – MCP-2, -3, and -4. [76, 77]

The data mentioned above imply, that allergic inflammation and airway remodeling seem to be intricately related to MMPs activity, since MMPs may represent key mediators, or rather modulators, involved in vigorous crosstalk between airway constituent cells, invading inflammatory cells, and the extracellular matrix. [78] From that point of view the idea concerning protective role of MMPs in asthma and asthma-associated remodeling seems to be convincing. However, the issue becomes more complicated, when analyzing involvement of MMPs in processing of ECM components and their direct input in airway destruction and remodeling. Noteworthy, both concepts, "destructive" and "protective", may be supported by some clinical data. In fact, there is still reasonable doubt, whether MMPs reveal some

http://dx.doi.org/10.5772/62440

similarity to Dr. Jekyll, or they are recognized rather unfriendly, like Mr. Hyde. However, in addition to some philosophical background, this issue has also an outstanding practical meaning, especially in context of possible pharmacological interventions in asthma-associated remodeling, which may be addressed to modulate MMPs activity. Obviously, when favoring "protective" role of MMPs, they would require some support to increase MMP/TIMP ratio. In contrast, if considering the "destructive hypothesis" as more likely, an opposite action should be undertaken. According to that concept, actually the inhibition of MMPs should provide some benefit for patient. Therefore, univocal clarification of that issue is of great clinical relevance.

2.7. MMP modulation — pharmacological interventions

Apart from previously mentioned (see chapter 2.4.2) endogenous or "physiological" inhibitors, several exogenous MMP modulators are also available. Noteworthy, in addition to few agents originally designed as MMP inhibitors (e.g. batimastat or marimatsat), nowadays in clinical practice are used many drugs, originally not intended to modulate MMPs activity. [3, 4, 10] The list of these agents is still expanding and includes tetracyclines, inhibitors of angiotensin converting enzyme (ACE), inhibitors of cholesterol synthesis (better known as statins), corticosteroids, etc. Some of them display direct inhibitory influence on MMPs activity (tetracyclines, ACE inhibitors), whereas in others mechanism of their action is indirect and more complex. Moreover, modulation of MMPs expression has been related to the use of clarithromycin, imatinib, inhibitors of Rho-kinase, antagonists of VEGF receptors, as well as inhibitors of some signaling pathways, including NF-κB, MAPK, and others (Fig. 5). [79–81]

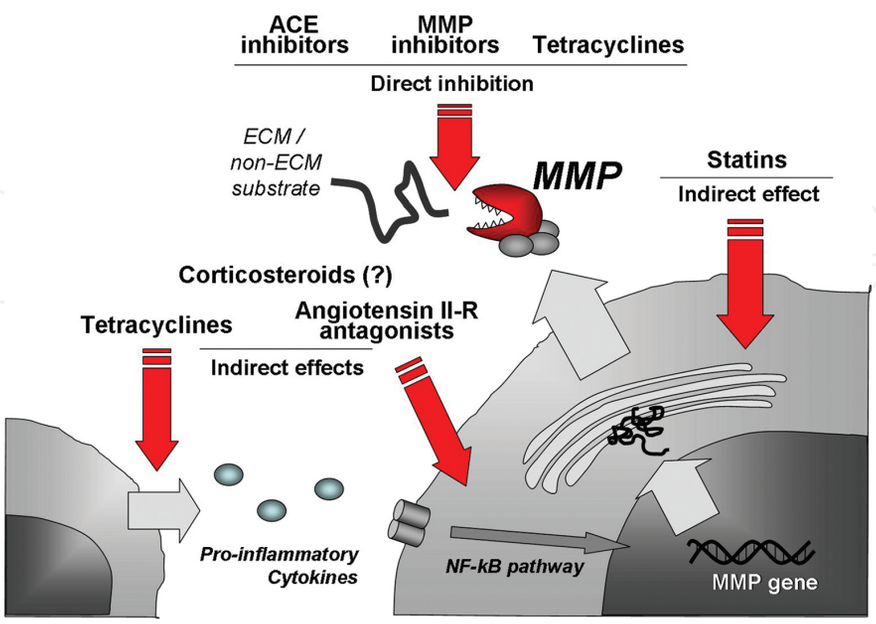

Fig. 5. Schematic representation of main strategies for MMPs modulation. Detailed description in text.

2.7.1. MMP inhibitors

The first group of broad-spectrum MMP inhibitors (batimastat, marimastat, ilomastat, etc.) is based on hydroxamate derivatives. These small zinc ion chelators were originally developed as anti-cancer drugs, and were expected to protect from cancer metastasis and tumor-related angiogenesis. [82] In murine asthma model a treatment with these compounds (marimastat, neovastat, GM6001, and others) was associated with reduced development of allergic inflammation, whereas in patients with atopic asthma these agents decreased bronchial hyperresponsiveness after allergen challenge. [68, 83, 84] However, due to low specificity, nearly all first generation synthetic MMP inhibitors may reveal inhibitory activity against various zinc-containing metalloproteins, including numerous non-MMP enzymes and transcription factors. Therefore, due to reported severe adverse effects, including so-called musculo-skeletal syndrome, mentioned compounds are currently withdrawn from the clinical practice. Regrettably, also novel MMP inhibitors, designed to specifically target particular MMPs, although encouraging in animal studies, did not ensure better safety and, most importantly, satisfactory clinical efficacy in humans. [85–90] The possible explanation of unexpectedly low clinical effectiveness of specific MMP inhibitors concerned compensatory induction of other MMPs after specific down-regulation of the target one.

Noteworthy, some other strategies of MMPs inhibition include use of monoclonal antibodies or anti-sense technologies. [3, 91] However, relatively high cost due to sophisticated technological process, and parenteral route for administration are enumerated as main limitations for development and broad use of these solutions.

2.7.2. Tetracyclines

Tetracyclines are natural antibiotics discovered in *Streptomyces*, which, apart from well defined anti-microbial properties, may also reveal some other, non-antibiotic activities. Tetracyclines may stabilize ECM turnover, presumably by direct inhibition of catalytic site of MMPs, but also indirectly, by suppression of inflammatory cascade and modulation of MMPs expression. [92] Indeed, anti-MMP properties of semi-synthetic tetracycline — doxycycline have been reported in various clinical conditions, including adult periodontitis, abdominal aortic aneurysm, atherosclerosis, autoimmune diseases and in cancer research. [93–96] Thus, doxycycline has received a Food and Drug Administration approval as potent MMP inhibitor, nevertheless its effect on asthma and asthma-associated remodeling still requires extensive studies. Data available from animal studies are promising in terms of attenuated airway hyperresponsiveness after allergen challenge and decreased airway inflammation. [97–99] Moreover, it was observed that long-term administration of doxycycline together with standard therapy was associated with significant improvement in lung function parameters and possible reversal of remodeling in patients with chronic asthma. [100]

2.7.3. Statins

The inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, better know as statins, are potent inhibitors of cholesterol biosynthesis. Due to their mechanism of

http://dx.doi.org/10.5772/62440

action statins are currently used as standard constituent of primary and secondary prevention of atherosclerosis and arterial insufficiency. In addition to decreasing serum cholesterol levels, statins are know to exert various pleiotropic, cholesterol-independent effects. [3, 101] The latter are mediated by inhibition of isoprenoids, which modulate the function of intracellular signaling molecules. Thus, statins, among them lovastatin, cerivastatin, simvastatin, rosuvastatin, or pitavastatin, may reveal some anti-inflammatory activities, including inhibition of MMPs. Therefore, they have been extensively studied mainly in cancer and cardiovascular diseases. [102, 103] Recently, in a randomized controlled study atorvastatin has been shown to significantly reduce sputum concentrations of acute inflammatory mediators, including MMP-8 and -9, in smoking asthmatic patients. [104] Thus, statins, especially when combined with standard therapy, may offer some protection from exacerbation and, possibly, airway remodeling. Noteworthy, simvastatin was found to modulate TGF-β-induced mesenchymal-epithelial transition of alveolar epithelial cells *in vitro*. Interestingly, simvastatin sufficiently suppressed TGF-β to induce expression of connective tissue growth factor (CTGF) and MMP-2 and -9, but it failed to reverse TGF-β-induced morphological changes in epithelial cells. [105] Therefore, although that observation could be a rationale behind the use of statins in prevention of subepithelial fibrosis and airway remodeling, this issue still requires further research.

2.7.4. Renin-Angiotensin System modulators

Another group of potential MMP modulators comprises several compounds designed to control the function of the renin-angiotensin system (RAS). These agents, although originally designed to manage arterial hypertension through the inhibition of angiotensin-converting enzyme, appeared to be effective also against MMPs. The mechanism of their action is based on dose-dependent, direct blockage of active site in catalytic domain of the enzyme. [3] Interestingly, also antagonists of angiotensin II receptor were found to modulate MMPs expression, possibly by inhibition of NF-κB pathway. [106] Since early clinical experience with modulators of RAS may suggest their great potential in novel therapy of respiratory diseases, these compounds are recently in focus of interest of several groups. [5, 107, 108]

2.7.5. Inhaled corticosteroids

Inhaled corticosteroids (ICS) are currently used as a standard treatment to control asthma symptoms. However, it has to be determined, whether they can block or even reverse epithelial–mesenchymal transition and subepithelial fibrosis in the respiratory tract of asthmatic patients. Noteworthy, decreased amounts of MMP-9 in reticular lamina of basement membrane have been recently shown to contribute to the beneficial effect of ICSs on epithelial–mesenchymal transition in chronic obstructive pulmonary disease. [109] Unexpectedly, data from clinical studies in asthmatic patients are limited and disappointing. [27, 110] In particular, no significant decrease in levels and/or activity of MMP-9 was observed after prolonged therapy with ICS in patients with asthma. [27, 111] This finding may suggest that inhaled corticosteroids alone could not be as effective in preventing asthma-associated airway remodeling, as postulated previously. [27, 110]

In contrast to ICS alone, the improved control of asthma severity, possibly due to better modulation of MMPs system, may be achieved by introducing a combination therapy, which comprises ICS and long acting β-agonists (LABA). Although both, *in vitro* and *in vivo* studies, confirmed superiority of combination therapy over ICS or LABA alone in this regard, they did not determine exactly, how this combination may affect MMP levels. [112–114] Possibly, the augmented effect of ICS-LABA combination can be, at least partially, explained by LABA ability to modulate NF-κB signaling pathway. Thus, inhibition of NF-κB will result in decreased expression of MMP-9 gene, as has been recently shown for ultra-LABA – indacaterol. [115] Indeed, combination of ICS with LABA as both, maintenance and reliever therapy, significantly reduced MMP-9 levels in induced sputum of asthmatic patients. [116, 117] Remarkably, MMP-9 levels, observed in patients with asthma before and after combined treatment with ICS and LABA, seem to reflect the intensity of airways remodeling, as they revealed good correlation with bronchial wall thickening, visualized using high-resolution computed tomography. [116]

2.7.6. Leukotriene-receptor antagonists

Leukotriene-receptor antagonists (LTRA) may be considered as an alternative to ICS as "first-line asthma-controller therapy" or as "add-on therapy" in patients already receiving ICS. Montelukast, most commonly used LTRA, was found to decrease the expression of MMP-9 in activated eosinophils *in vitro*. [118] In children with asthma a treatment with LTRA resulted in clinical improvement –reduction of symptoms and increase of peak expiratory flow, which were associated with significant decrease of MMP-9 levels in plasma. [119] In experimental asthma model in mice LTRA treatment was shown to reverse airway remodeling and decreased airway hyperresponsiveness after allergen challenge. Again, mentioned improvement correlated with decrease of MMP-2 and -9 levels in BAL fluid. [120]

3. Conclusions

Despite extensive studies focused on role of MMPs in asthma and asthma-associated remodeling, our knowledge regarding this issue is still far from a satisfactory level. Since there is no agreement among scientists regarding superiority of "destructive" or "protective" concept, the clarification of "Dr. Jekyll or Mr. Hyde ?" issue seems to have outstanding clinical relevance, especially when considering possible pharmacological interventions. Regrettably, the interpretation of results concerning exact place of MMPs in asthma pathogenesis may be impeded by different methodology and various populations analyzed in these studies. Such differences may certainly affect result of MMPs assessment across the studies. [57, 121] On the other hand, these discrepancies can be ascribed to real differences in MMPs amount and/or activity, depending on sample type and disease severity. Furthermore, local expression and activity of individual MMPs may vary in different airway compartments, thus adding complexity to the network of allergic inflammatory response. [61, 78, 122] Accordingly, the distribution of MMP-2, MMP-9 and MMP-12 in bronchial wall was shown to differ between

large and small airways. Moreover, it varied between healthy controls and patients with asthma, and further changed depending on severity of disease. [61, 78]

Noteworthy, mentioned compartmental differences may be easily averaged for the entire bronchial tree, especially when using site-unspecific samples, like induced sputum, BAL, breath condensate and, obviously, serum or plasma. On the other hand, due to such averaging, small local changes, although clinically relevant, may be disregarded. Therefore, further research should focus on more precise assessment of distribution and activity of MMPs, the balance between MMPs and their natural inhibitors, as well as association of those findings with alterations in architecture and function of respiratory tract mucosa.

Author details

Katarzyna Grzela, Agnieszka Strzelak, Wioletta Zagórska and Tomasz Grzela*

*Address all correspondence to: tomekgrzela@gmail.com

The Medical University of Warsaw, Department of Paediatrics, Pneumonology and Allergology (KG, AS, WZ), and Department of Histology and Embryology (TG), Poland

References

[1] Amălinei C, Căruntu ID, Bălan RA. Biology of metalloproteinases. Rom J Morphol Embryol. 2007;48:323–334.

[2] Fanjul-Fernández M, Folgueras AR, Cabrera S, López-Otín C. Matrix metalloproteinases: evolution, gene regulation and functional analysis in mouse model. Biochim Biophys Acta. 2010;1803:3–19. DOI: 10.1016/j.bbamcr.2009.07.004.

[3] Grzela T, Bikowska B, Litwiniuk M. Matrix metalloproteinases in aortic aneurysm—executors or executioners? In: Grundmann RT, editors. Etiology, pathogenesis and pathophysiology of aortic aneurysms and aneurysm rupture. Rijeka: Intech Publ; 2011:25–54. DOI: 10.5772/17861.

[4] Krejner A, Litwinuk M, Grzela T. Matrix metalloproteinases in the wound microenvironment: therapeutic perspectives. Chronic Wound Care Management and Research 2016 (accepted for publication)

[5] Grzela K, Litwiniuk M, Zagorska W, Grzela T. Airway remodeling in chronic obstructive pulmonary disease and asthma: the role of matrix metalloproteinase-9. Arch Immunol Ther Exp 2016;64:47–55. DOI: 10.1007/s00005-015-0345-y.

[6] Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res. 2006;69:562–573. DOI: 10.1016/j.cardiores.2005.12.002.

[7] Endo K, Takino T, Miyamori H, et al. Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. J Biol Chem. 2003;278:40764–40770. DOI: 10.1074/jbc.M306736200.

[8] Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol. 2004;16:558–564. DOI:10.1016/j.ceb.2004.07.010.

[9] Yamada H, Saito F, Fukuta-Ohi H, et al. Processing of beta-dystroglycan by matrix metalloproteinase disrupts the link between the extracellular matrix and cell membrane via the dystroglycan complex. Hum Mol Genet. 2001;10:1563–159. DOI: 10.1093/hmg/10.15.1563.

[10] Hadler-Olsen E, Fadnes B, Sylte I, Uhlin-Hansen L, Winberg JO. Regulation of matrix metalloproteinase activity in health and disease. FEBS J. 2011;278:28–45. DOI: 10.1111/j.1742-4658.2010.07920.x.

[11] Ravanti L, Kahari VM. Matrix metalloproteinases in wound repair. Int J Mol Med. 2000;6:391–407. DOI: 10.3892/ijmm.6.4.391

[12] Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. J Cell Physiol. 2007;211:19–26. DOI: 10.1002/jcp.20948.

[13] Kapila S, Xie Y, Wang W. Induction of MMP-1 (collagenase-1) by relaxin in fibrocartilaginous cells requires both the AP-1 and PEA-3 promoter sites. Orthod Craniofac Res. 2009;12:178–186. DOI: 10.1111/j.1601-6343.2009.01451.x.

[14] Clark IM, Swingler TE, Sampieri CL, Edwards DR. The regulation of matrix metalloproteinases and their inhibitors. Int J Biochem Cell Biol. 2008;40:1362–1378. DOI: 10.1016/j.biocel.2007.12.006.

[15] Clark IM, Swingler TE, Young DA. Acetylation in the regulation of metalloproteinase and tissue inhibitor of metalloproteinases gene expression. Front Biosci. 2007;12:528–535. DOI: 10.2741/2079.

[16] Di Gregoli K, Jenkins N, Salter R, White S, Newby AC, Johnson JL. MicroRNA-24 regulates macrophage behavior and retards atherosclerosis. Arterioscler Thromb Vasc Biol. 2014;34:1990–2000. DOI: 10.1161/ATVBAHA.114.304088.

[17] Zhang H, Qi M, Li S, et al. microRNA-9 targets matrix metalloproteinase-14 to inhibit invasion, metastasis, and angiogenesis of neuroblastoma cells. Mol Cancer Ther. 2012;11:1454–1466. DOI: 10.1158/1535-7163.MCT-12-0001.

[18] Xu M, Wang YZ. miR-133a suppresses cell proliferation, migration and invasion in human lung cancer by targeting MMP-14. Oncol Rep. 2013;30:1398–1404. DOI: 10.3892/or.2013.2548.

[19] Chan YC, Roy S, Huang Y, Khanna S, Sen CK. The microRNA miR-199a-5p down-regulation switches on wound angiogenesis by derepressing the v-ets erythroblastosis

virus E26 oncogene homolog 1-matrix metalloproteinase-1 pathway. J Biol Chem. 2012;287:41032–41043. DOI: 10.1074/jbc.M112.413294.

[20] Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem. 2003;253:269–285.

[21] Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. Amino Acids. 2011;41:271–290. DOI: 10.1007/s00726-010-0689-x.

[22] Pei D, Weiss SJ. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature. 1995;375:244–247. DOI: 10.1038/375244a0.

[23] Ra HJ, Parks WC. Control of matrix metalloproteinase catalytic activity. Matrix Biol. 2007;26:587–596. DOI: 10.1016/j.matbio.2007.07.001.

[24] Litwiniuk M, Bikowska B, Niderla-Bielinska J, et al. Potential role of metalloproteinase inhibitors from radiation-sterilized amnion dressings in the healing of venous leg ulcers. Mol Med Rep. 2012;6:723–728. DOI: 10.3892/mmr.2012.983.

[25] Grzela T, Brawura-Biskupski-Samaha R, Jelenska MM, Szmidt J. Low molecular weight heparin treatment decreases MMP-9 plasma activity in patients with abdominal aortic aneurysm. Eur J Vasc Endovasc Surg. 2008;35:159–161.

[26] Grzela T, Niderla-Bielinska J, Litwiniuk M, White R. The direct inhibition of MMP-2 and MMP-9 by an enzyme alginogel: a possible mechanism of healing support for venous leg ulcers. J Wound Care. 2014;23:278-285. DOI: 10.12968/jowc.2014.23.5.278.

[27] Grzela K, Zagorska W, Krejner A, et al. Prolonged treatment with inhaled corticosteroids dose not normalize high activity of matrix metallopreoteinase-9 in exhaled breath condensates of children with asthma. Arch Immunol Ther Exp. 2015;63:231–237. DOI: 10.1007/s00005-015-0328-z.

[28] Suzuki R, Kato T, Miyazaki Y, et al. Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in sputum from patients with bronchial asthma. J Asthma. 2001;38:477–484.

[29] 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;31:217–223. DOI: 10.1046/j.1365-2222.2002.01219.x.

[30] 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.

[31] Erlewyn-Lajeunesse M, Hunt L, Pohunek P, et al. Bronchoalveolar lavage MMP-9 and TIMP-1 in preschool wheezers and their relationship to persistent wheeze. Pediatr Res 2008;64:194–199. DOI: 10.1203/PDR.0b013e318175dd2d.

[32] Lee Y, Lee B, Rhee Y, Song C. The involvement of matrix metalloproteinase-9 in airway inflammation of patients with acute asthma. Clin Exp Allergy. 2001;31:1623–1630. DOI: 10.1046/j.1365-2222.2001.01211.x.

[33] Karakoc G, Yukselen A, Yilmaz M, Altintas D, Kendirli G. Exhaled breath condensate MMP-9 level and its relationships with asthma severity and interleukin-4/10 levels in children. Ann Allergy Asthma Immunol. 2012;108:300–304. DOI: 10.1016/j.anai.2012.02.019.

[34] Bara I, Ozier A, Tunon de Lara JM, Marthan R, Berger P. Pathophysiology of bronchial smooth muscle remodeling in asthma. Eur Respir J. 2010;36:1174–1184. DOI: 10.1183/09031936.00019810.

[35] Grzela K, Zagorska W, Jankowska-Steifer E, Grzela T. Chronic inflammation in the respiratory tract and ciliary dyskinesia. Centr Eur J Immunol. 2013;38:122–128. DOI: 10.5114/ceji.2013.34369.

[36] Royce SG, Shen M, Patel KP, Huuskes BM, Ricardo SD, Samuel CS. Mesenchymal stem cells and serelaxin synergistically abrogate established airway fibrosis in an experimental model of chronic allergic airways disease. Stem Cell Res. 2015;15:495–505. DOI: 10.1016/j.scr.2015.09.007.

[37] Wenzel SE, Balzar S, Cundall M, Chu HW. Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. J Allergy Clin Immunol. 2003;111:1345–1352. DOI: 10.1067/mai.2003.1464.

[38] Vignola AM, Riccobono L, Mirabella A, et al. Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airflow obstruction in asthma and chronic bronchitis. Am J Respir Crit Care Med. 1998;158:1945–1950. DOI: 10.1164/ajrccm.158.6.9803014.

[39] Vignola AM, Paganin F, Capieu L, et al. Airway remodelling assessed by sputum and high-resolution computed tomography in asthma and COPD. Eur Respir J. 2004;24:910–917. DOI: 10.1183/09031936.04.00032603.

[40] Barbaro M, Spanevello A, Palladino G, Salerno F, Lacedonia D, Carpagnano G. Exhaled matrix metalloproteinase-9 (MMP-9) in different biological phenotypes of asthma. Eur J Int Med. 2014;25:92–96. DOI: 10.1016/j.ejim.2013.08.705.

[41] Holgate S. A brief history of asthma and its mechanisms to modern concepts of disease pathogenesis. Allergy Asthma Immunol Res. 2010;2:165–171. DOI: 10.4168/aair.2010.2.3.165.

[42] Lavigne MC, Thakker P, Gunn J, et al. Human bronchial epithelial cells express and secrete MMP-12. Biochem Biophys Res Commun. 2004;324:534–546. DOI: 10.1016/j.bbrc.2004.09.080.

[43] Araya J, Cambier S, Morris A, Finkbeiner W, Nishimura SL. Integrin-mediated transforming growth factor- activation regulates homeostasis of the pulmonary

epithelial-mesenchymal trophic unit. Am J Pathol. 2006;169:405–415. DOI: 10.2353/ajpath.2006.060049.

[44] Davies DE. The Role of the epithelium in airway remodeling in asthma. Proc Am Thorac Soc. 2009;6:678–682. DOI: 10.1513/pats.200907-067DP.

[45] Yang Y, Haitchi HM, Cakebread J, et al. Epigenetic mechanisms silence a disintegrin and metalloprotease 33 expression in bronchial epithelial cells. J Allergy Clin Immunol. 2008;121:1393-1399,1399.e1-14. DOI: 10.1016/j.jaci.2008.02.031.

[46] 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-κB. Am J Respir Cell Mol Biol. 2010;43:201–209. DOI: 10.1165/rcmb.2009-0216OC.

[47] Barbato A, Turato G, Baraldo S, et al. Airway inflammation in childhood asthma. Am J Respir Crit Care Med. 2003;168:798–803. DOI: 10.1164/rccm.200305-650OC.

[48] Saglani S, Payne DN, Zhu J, et al. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med. 2007;176:858–864. DOI: 10.1164/rccm.200702-212OC.

[49] Madore AM, Laprise C. Immunological and genetic aspects of asthma and allergy. J Asthma Allergy. 2010;3:107–121. DOI: 10.2147/JAA.S8970.

[50] Holgate ST, Yang Y, Haitchi HM, et al. The genetics of asthma: ADAM33 as an example of a susceptibility gene. Proc Am Thorac Soc. 2006;3:440–443. DOI: 10.1513/pats.200603-026AW.

[51] Liang S, Wei X, Gong C, Wei J, Chen Z, Deng J. A disintegrin and metalloprotease 33 (ADAM33) gene polymorphisms and the risk of asthma: a meta-analysis. Hum Immunol. 2013;74:648–657. doi: 10.1016/j.humimm.2013.01.025.

[52] Werner M, Herbon N, Gohlke H, et al. Asthma is associated with single-nucleotide polymorphisms in ADAM33. Clin Exp Allergy. 2004;34:26–31. DOI: 10.1111/j.1365-2222.2004.01846.x.

[53] Schedel M, Depner M, Schoen C, et al. The role of polymorphisms in ADAM33, a disintegrin and metalloprotease 33, in childhood asthma and lung function in two German populations. Respir Res. 2006;7:91. DOI: 10.1186/1465-9921-7-91.

[54] Pinto LA, Depner M, Klopp N, et al. MMP-9 gene variants increase the risk for non-atopic asthma in children. Respir Res. 2010;11:23. DOI: 10.1186/1465-9921-11-23.

[55] Birbian N, Singh J, Jindal SK. Highly protective association of MMP-2 -1306C/T promoter polymorphism with wsthma in a North Indian population: a pilot study. Allergy Asthma Immunol Res. 2014;6:234–241. DOI: 10.4168/aair.2014.6.3.234.

[56] Gueders MM, Foidart JM, Noel A, Cataldo DD. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and

other lung diseases. Eur J Pharmacol. 2006;533:133–144. DOI: 10.1016/j.ejphar.2005.12.082.

[57] Bissonnette ÉY, Madore AM, Chakir J, et al. Fibroblast growth factor-2 is a sputum remodeling biomarker of severe asthma. J Asthma. 2014;51:119–126. DOI: 10.3109/02770903.2013.860164.

[58] McDougall CM, Helms PJ, Walsh GM. Airway epithelial cytokine responses in childhood wheeze are independent of atopic status. Respir Med. 2015;109:689–700. DOI: 10.1016/j.rmed.2015.04.001.

[59] Roberts ME, Magowan L, Hall IP, Johnson SR. Discoidin domain receptor 1 regulates bronchial epithelial repair and matrix metalloproteinase production. Eur Respir J. 2011;37:1482–1493. DOI: 10.1183/09031936.00039710.

[60] Doherty GM, Kamath SV, de Courcey F, et al. Children with stable asthma have reduced airway matrix metalloproteinase-9 and matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio. Clin Exp Allergy. 2005;35:1168–1174. DOI: 10.1111/j.1365-2222.2005.02326.x.

[61] Weitoft M, Andersson C, Andersson-Sjöland A, et al.Controlled and uncontrolled asthma display distinct alveolar tissue matrix compositions. Respir Res. 2014;15:67. DOI: 10.1186/1465-9921-15-67.

[62] Matsumoto H, Niimi A, Takemura M, et al. Relationship of airway wall thickening to an imbalance between matrix metalloproteinase-9 and its inhibitor in asthma. Thorax. 2005;60:277–281. DOI: 10.1136/thx.2004.028936.

[63] Chaudhuri R, McSharry C, Brady J, et al. Low sputum MMP-9/TIMP ratio is associated with airway narrowing in smokers with asthma. Eur Respir J. 2014;44:895–904. DOI: 10.1183/09031936.00047014.

[64] Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. Am J Respir Cell Mol Biol. 2003;28:12–24. DOI: 10.1165/rcmb.2002-0166TR.

[65] Chen W, Tabata Y, Gibson AM, et al. Matrix metalloproteinase 8 contributes to solubilization of IL-13 receptor alpha2 in vivo. J Allergy Clin Immunol. 2008;122:625–632. DOI: 10.1016/j.jaci.2008.06.022.

[66] Cook-Mills JM. Hydrogen peroxide activation of endothelial cell-associated MMPs during VCAM-1-dependent leukocyte migration. Cell Mol Biol (Noisy-le-grand). 2006;52:8-16.

[67] Matsumura M, Inoue H, Matsumoto T, et al. Endogenous metalloprotease solubilizes IL-13 receptor α2 in airway epithelial cells. Biochem Biophys Res Commun. 2007;360:464–469. DOI: 10.1016/j.bbrc.2007.06.076.

[68] Corry DB, Rishi K, Kanellis J, et al. Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. Nat Immunol. 2002;3:347–353. DOI: 10.1038/ni773.

[69] McMillan SJ, Kearley J, Campbell JD, et al. Matrix metalloproteinase-9 deficiency results in enhanced allergen-induced airway inflammation. J Immunol 2004;172:2586–2594. DOI: 10.4049/jimmunol.172.4.2586.

[70] Owen CA, Hu Z, Lopez-Otin C, Shapiro SD. Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. J Immunol. 2004;172:7791–7803. DOI: 10.4049/jimmunol.172.12.7791.

[71] Gueders MM, Balbin M, Rocks N, et al. Matrix metalloproteinase-8 deficiency promotes granulocytic allergen-induced airway inflammation. J Immunol. 2005;175:2589–2597. DOI: 10.4049/jimmunol.175.4.2589.

[72] Page K, Ledford JR, Zhou P, Wills-Karp M. A TLR2 agonist in German cockroach frass activates MMP-9 release and is protective against allergic inflammation in mice. J Immunol. 2009;183:3400–3408. DOI: 10.4049/jimmunol.0900838.

[73] Gueders MM, Hirst SJ, Quesada-Calvo F, et al. Matrix metalloproteinase-19 deficiency promotes tenascin-C accumulation and allergen-induced airway inflammation. Am J Respir Cell Mol Biol. 2010;43:286–295. DOI: 10.1165/rcmb.2008-0426OC.

[74] Mehra D, Sternberg DI, Jia Y, et al. Altered lymphocyte trafficking and diminished airway reactivity in transgenic mice expressing human MMP-9 in a mouse model of asthma. Am J Physiol Lung Cell Mol Physiol. 2010;298:L189–96. DOI: 10.1152/ajplung.00042.2009.

[75] Sheu BC, Hsu SM, Ho HN et al. A novel role of metalloproteinase in cancer-mediated immunosuppression. Cancer Res. 2001;61:237–242.

[76] Yoon HK, Cho HY, Kleeberger SR. Protective role of matrix metalloproteinase-9 in ozone-induced airway inflammation. Environ Health Perspect. 2007;115:1557–1563. DOI: 10.1289/ehp.10289.

[77] McQuibban GA, Gong JH, Wong JP, et al. Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo. Blood. 2002;100:1160–1167.

[78] Araujo BB, Dolhnikoff M, Silva LF, et al. Extracellular matrix components and regulators in the airway smooth muscle in asthma. Eur Respir J. 2008;32:61–69. DOI: 10.1183/09031936.00147807.

[79] Simpson JL, Powell H, Boyle MJ, et al. Clarithromycin targets neutrophilic airway inflammation in refractory asthma. Am J Respir Crit Care Med. 2008;177:148–155. DOI: 10.1164/rccm.200707-1134OC.

[80] Righetti RF, Pigati PA, Possa SS, et al. Effects of Rho-kinase inhibition in lung tissue with chronic inflammation. Respir Physiol Neurobiol. 2014;192:134–146. DOI: 10.1016/j.resp.2013.12.012.

[81] Azizi G, Haidari MR, Khorramizadeh M, et al. Effects of imatinib mesylate in mouse models of multiple sclerosis and in vitro determinants. Iran J Allergy Asthma Immunol. 2014;13:198–206.

[82] Shono T, Motoyama M, Tatsumi K, et al. A new synthetic matrix metalloproteinase inhibitor modulates both angiogenesis and urokinase type plasminogen activator activity. Angiogenesis. 1998;2:319–329.

[83] Bruce C, Thomas PS. The effect of marimastat, a metalloprotease inhibitor, on allergen-induced asthmatic hyper-reactivity. Toxicol Appl Pharmacol. 2005;205:126–132. DOI: 10.1016/j.taap.2004.10.005.

[84] Lee SY, Paik SY, Chung SM. Neovastat (AE-941) inhibits the airway inflammation and hyperresponsiveness in a murine model of asthma. J Microbiol. 2005;43:11–16.

[85] Jung YW, Zindl CL, Lai JF, Weaver CT, Chaplin DD. MMP induced by Gr-1+ cells are crucial for recruitment of Th cells into the airways. Eur J Immunol. 2009;39:2281–2292. DOI: 10.1002/eji.200838985.

[86] Mukhopadhyay S, Sypek J, Tavendale R, et al. Matrix metalloproteinase-12 is a therapeutic target for asthma in children and young adults. J Allergy Clin Immunol. 2010;126:70–76. DOI: 10.1016/j.jaci.2010.03.027.

[87] Li W, Li J, Wu Y, et al. Identification of an orally efficacious matrix metalloprotease 12 inhibitor for potential treatment of asthma. J Med Chem. 2009;52:5408–5419. DOI: 10.1021/jm900809r.

[88] Yu Y, Chiba Y, Sakai H, Misawa M. Effect of a matrix metalloproteinase-12 inhibitor, S-1, on allergic airway disease phenotypes in mice. Inflamm Res. 2010;59:419–428. DOI: 10.1007/s00011-009-0153-0.

[89] Lagente V, Le Quement C, Boichot E. Macrophage metalloelastase (MMP-12) as a target for inflammatory respiratory diseases. Expert Opin Ther Targets. 2009;13:287–295. DOI: 10.1517/14728220902751632.

[90] Dahl R, Titlestad I, Lindqvist A, et al. Effects of an oral MMP-9 and -12 inhibitor, AZD1236, on biomarkers in moderate/severe COPD: a randomised controlled trial. Pulm Pharmacol Ther. 2012;25:169–177. DOI: 10.1016/j.pupt.2011.12.011.

[91] Vandenbroucke RE, Dejonckheere E, Libert C. A therapeutic role for matrix metalloproteinase inhibitors in lung diseases? Eur Respir J. 2011;38:1200–1214. DOI: 10.1183/09031936.00027411.

[92] Hu J, Van den Steen PE, Sang QX, Opdenakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat Rev Drug Discov. 2007;6:480–498. DOI: 10.1038/nrd2308

[93] Cerisano G, Buonamici P, Valenti R, et al. Effects of a timely therapy with doxycycline on the left ventricular remodeling according to the pre-procedural TIMI flow grade in patients with ST-elevation acute myocardial infarction. Basic Res Cardiol. 2014;109:412. DOI: 10.1007/s00395-014-0412-2.

[94] Fiotti N, Altamura N, Moretti M, et al. Short term effects of doxycycline on matrix metalloproteinases 2 and 9. Cardiovasc Drugs Ther. 2009;23:153–159. DOI: 10.1007/s10557-008-6150-7.

[95] Prins HJ, Daniels JM, Lindeman JH, et al. Effects of doxycycline on local and systemic inflammation in stable COPD patients, a randomized clinical trial. Respir Med. 2016;110:46–52. DOI: 10.1016/j.rmed.2015.10.009.

[96] Nukarinen E, Tervahartiala T, Valkonen M, et al. Targeting matrix metalloproteinases with intravenous doxycycline in severe sepsis — A randomised placebo-controlled pilot trial. Pharmacol Res. 2015;99:44–51. DOI: 10.1016/j.phrs.2015.05.005.

[97] Lee KS, Jin SM, Kim SS, at al. Doxycycline reduces airway inflammation and hyperresponsiveness in a murine model of toluene diisocyanate-induced asthma. J Allergy Clin Immunol. 2004;113:902–909. DOI: 10.1016/j.jaci.2004.03.008.

[98] Gueders MM, Bertholet P, Perin F et al. A novel formulation of inhaled doxycycline reduces allergen-induced inflammation, hyperresponsiveness and remodeling by matrix metalloproteinases and cytokines modulation in a mouse model of asthma. Biochem Pharmacol. 2008;75:514–526. DOI: 10.1016/j.bcp.2007.09.012.

[99] Avincsal MO, Ozbal S, Ikiz AO, Pekcetin C, Güneri EA. Effects of topical intranasal doxycycline treatment in the rat allergic rhinitis model. Clin Exp Otorhinolaryngol. 2014;7:106–111. DOI: 10.3342/ceo.2014.7.2.106.

[100] Bhattacharyya P, Paul R, Bhattacharjee P, et al. Long-term use of doxycycline can improve chronic asthma and possibly remodeling: the result of a pilot observation. J Asthma Allergy. 2012;5:33–37. DOI: 10.2147/JAA.S31402.

[101] Feleszko W, Młynarczuk I, Olszewska D, et al. Lovastatin potentiates antitumor activity of doxorubicin in murine melanoma via an apoptosis-dependent mechanism. Int J Cancer. 2002;100:111–118. DOI: 10.1002/ijc.10440.

[102] Yoshimura K, Nagasawa A, Kudo J, et al. Inhibitory effect of statins on inflammation-related pathways in human abdominal aortic aneurysm tissue. Int J Mol Sci. 2015;16:11213–11228. DOI: 10.3390/ijms160511213.

[103] Shen YY, Yuan Y, Du YY, Pan YY. Molecular mechanism underlying the anticancer effect of simvastatin on MDA-MB-231 human breast cancer cells. Mol Med Rep. 2015;12:623–630. DOI: 10.3892/mmr.2015.3411.

[104] Thomson NC, Charron CE, Chaudhuri R, Spears M, Ito K, McSharry C. Atorvastatin in combination with inhaled beclometasone modulates inflammatory sputum mediators in smokers with asthma. Pulm Pharmacol Ther. 2015;31:1–8. DOI: 10.1016/j.pupt.2015.01.001.

[105] Yang T, Chen M, Sun T. Simvastatin attenuates TGF-β1-induced epithelial-mesenchymal transition in human alveolar epithelial cells. Cell Physiol Biochem. 2013;31:863–874. DOI: 10.1159/000350104.

[106] Fujiwara Y, Shiraya S, Miyake T, et al. Inhibition of experimental abdominal aortic aneurysm in a rat model by the angiotensin receptor blocker valsartan. Int J Mol Med. 2008;22:703–708. DOI: 10.3892/ijmm_00000075.

[107] Kaparianos A, Argyropoulou E. Local renin-angiotensin II systems, angiotensin-converting enzyme and its homologue ACE2: their potential role in the pathogenesis of chronic obstructive pulmonary diseases, pulmonary hypertension and acute respiratory distress syndrome. Curr Med Chem. 2011;18:3506–3515. DOI: 10.2174/092986711796642562.

[108] Shrikrishna D, Astin R, Kemp PR, Hopkinson NS. Renin-angiotensin system blockade: a novel therapeutic approach in chronic obstructive pulmonary disease. Clin Sci. 2012;123:487–498. DOI: 10.1042/CS20120081.

[109] Sohal SS, Soltani A, Reid D, et al. A randomized controlled trial of inhaled corticosteroids (ICS) on markers of epithelial-mesenchymal transition (EMT) in large airway samples in COPD: an exploratory proof of concept study. Int J Chron Obstruct Pulmon Dis. 2014;9:533–542. DOI: 10.2147/COPD.S63911.

[110] Obase Y, Rytilä P, Metso T, et al. Effects of inhaled corticosteroids on metalloproteinase-8 and tissue inhibitor of metalloproteinase-1 in the airways of asthmatic children. Int Arch Allergy Immunol. 2010;151:247–254. DOI: 10.1159/000242362.

[111] Mattos W, Lim S, Russell R, Jatakanon A, Chung KF, Barnes PJ. Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. Chest. 2002;122:1543–1552. DOI: 10.1378/chest.122.5.1543.

[112] 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.

[113] Todorova L, Bjermer L, Westergren-Thorsson G, Miller-Larsson A. TGFβ-induced matrix production by bronchial fibroblasts in asthma: budesonide and formoterol effects. Respir Med. 2011;105:1296–1307. DOI: 10.1016/j.rmed.2011.03.020.

[114] Perng DW, Su KC, Chou KT, et al. Long-acting β2 agonists and corticosteroids restore the reduction of histone deacetylase activity and inhibit H2O2-induced mediator release from alveolar macrophages. Pulm Pharmacol Ther. 2012;25:312–318. DOI: 10.1016/j.pupt.2012.04.001.

[115] Lee SU, Ahn KS, Sung MH, et al. Indacaterol inhibits tumor cell invasiveness and MMP-9 expression by suppressing IKK/NF-κB activation. Mol Cells. 2014;37:585-591. DOI: 10.14348/molcells.2014.0076.

[116] Wang K, Liu CT, Wu YH, et al. Effects of formoterol-budesonide on airway remodeling in patients with moderate asthma. Acta Pharmacol Sin. 2011;32:126–132. DOI: 10.1038/aps.2010.170.

[117] Lin CH, Hsu JY, Hsiao YH, et al. Budesonide/formoterol maintenance and reliever therapy in asthma control: acute, dose-related effects and real-life effectiveness. Respirology. 2015;20:264–272. DOI: 10.1111/resp.12425.

[118] Langlois A, Ferland C, Tremblay GM, Laviolette M. Montelukast regulates eosinophil protease activity through a leukotriene-independent mechanism. J Allergy Clin Immunol. 2006;118:113–119. DOI: 10.1016/j.jaci.2006.03.010.

[119] Chuang SS, Hung CH, Hua YM et al. Suppression of plasma matrix metalloproteinase-9 following montelukast treatment in childhood asthma. Pediatr Int. 2007;49:918–922. DOI: 10.1111/j.1442-200X.2007.02497.x.

[120] Hsu CH, Hu CM, Lu KH, et al. Effect of selective cysteinyl leukotriene receptor antagonists on airway inflammation and matrix metalloproteinase expression in a mouse asthma model. Pediatr Neonatol. 2012;53:235–244. DOI: 10.1016/j.pedneo.2012.06.004.

[121] Todorova L, Bjermer L, Miller-Larsson A, Westergren-Thorsson G. Relationship between matrix production by bronchial fibroblasts and lung function and AHR in asthma. Respir Med. 2010;104:1799–1808. DOI: 10.1016/j.rmed.2010.06.015.

[122] Katainen E, Kostamo K, Virkkula P, et al. Local and systemic proteolytic responses in chronic rhinosinusitis with nasal polyposis and asthma. Int Forum Allergy Rhinol. 2015;5:294–302. DOI: 10.1002/alr.21486.

IntechOpen