BIOLOGICAL ROLE OF COLLAGENASES IN BRONCHIAL ASTHMA

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ABSTRACT

Bronchial asthma is a chronic multifactorial inflammatory disease of the airways characterized by airway inflammation, mucus hyperproduction and thickening of submucosa resulting in reversible bronchoconstriction and airway hyper-responsiveness. Many genetic and environmental risk factors have been associated with asthma development and morbidity. The chronic inflammation causes bronchial remodeling characterized by shedding of airway epithelial cells, increased number of goblet cells, mucous gland hypertrophy, increased fibroblast/myofibroblast number, increased airway smooth muscle mass, degradation of the extracellular matrix and neovascularity.

Matrix metalloproteinases (MMPs) are a family of more than 23 zinc- and calcium-dependent proteinases which mainly degrade extracellular matrix (ECM), but also can hydrolyze other substrates. They are synthesized as pro-enzymez. The key feature of collagenases is their ability to cleave interstitial collagens I, II, and III. They can also digest a number of other ECM and non-ECM molecules.

Several studies have suggested the implication of matrix metalloproteinases in the development of tissue remodeling and fibrosis associated with various inflammatory conditions. Impairment of the balance between MMPs and their endogenous inhibitors TIMPs (protease/antiprotease imbalance) is a key event in the development of pulmonary diseases. Although increased MMP expression in asthmatic patients makes these MMPs candidate diagnostic biomarkers, this does not necessarily mean that inhibiting them will have a beneficial therapeutic effect.

***Key words**: matrix metalloproteinases, collagenases, bronchial asthma, polymorphism, extracellular matrix*

BRONCHIAL ASTHMA

Bronchial asthma is a chronic multifactorial inflammatory disease of the airways characterized by airway inflammation (accumulation of inflammatory cells in the lung and airways), mucus obstruction and thickening of the reticular basement membrane resulting in bronchoconstriction and airway hyper-responsiveness. It is considered to reflect airway remodeling due to shedding of airway epithelial cells, fibroblast proliferation, goblet cell and smooth muscle hyperplasia, increased collagen deposition, neovascularity and degradation of the extracellular matrix (Watson, Benton et al. 2010; Pickholtz, Admon et al. 2011; Vandenbroucke, Dejonckheere et al. 2011; Banerjee and Henderson 2012; Killeen and Skora 2013). As soon as the disease becomes severe, neutrophils also seem to play a significant role in asthma (Gueders, Balbin et al. 2005)

Many genetic and environmental risk factors such as cigarette smoke, viral respiratory infections, psychological stress, beta blocker medications, indoor allergens (especially in children), polymorphisms in over 100 genes have been associated with asthma development and morbidity (Harju, Leinonen et al. 2006; Ober and Hoffjan 2006; Miller and Ho 2008; Watson, Benton et al. 2010)

Asthma is associated with widespread variable recurrent episodes of wheezing, breathlessness and coughing (Kroegel 2009).

The interest about morphological changes that occur in the bronchial tree in patients with bronchial asthma has risen. The chronic inflammation cause bronchial remodeling due to by

shedding of airway epithelial cells, increased number of goblet cells, mucous gland hypertrophy, increased fibroblast/myofibroblast number, increased airway smooth muscle mass, neovascularity and degradation of the extracellular matrix (especially collagen components) (Pickholtz, Admon et al. 2011; Killeen and Skora 2013). During the recovery process the remaining epithelial cells secrete matrix components, composed of collagen I, collagen III and fibronectin. The cells migrate to the site of injury by adhering to this matrix components (Cataldo, Gueders et al. 2004; Gueders, Balbin et al. 2005; Roberts, Magowan et al. 2011)

MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMPs) are a family of more than 23 zinc- and calcium-dependent proteinases which mainly degrade extracellular matrix (ECM) but also can hydrolyze other substrates (Visse and Nagase 2003). They play crucial roles in variety biological and pathological processes, such as wound healing, senescence, cancer, fibrosis and inflammation. MMPs are synthesized as pro-enzymes (Vermeer, Denker et al. 2009). Matrix metalloproteinases can be divided into six groups according to their substrate specificity and structure: 1) Collagenases: MMP-1, MMP-8, MMP-13, and MMP-18; 2) Gelatinases: Gelatinase A (MMP-2) and gelatinase B (MMP-9); 3) Stromelysins: Stromelysin 1 (MMP-3) and stromelysin 2 (MMP-10); 4) Matrilysins: Matrilysin 1 (MMP-7) and matrilysin 2 (MMP-26); 5) Membrane-Type MMPs: There are six membrane-type MMPs (MT-MMPs): MMP-14, MMP-15, MMP- 16, MMP-24, MMP-17 and MMP-25; and 6) other MMPs: MMP-12, MMP-19, MMP-20, MMP-22, MMP-28 (Hu and Beeton 2010).

The pro-enzymes of MMPs require activation in order to achieve their specific function. This process can be performed in several ways: 1) Stepwise activation mechanism – MMPs can be activated by proteinases or chemical agents (oxidized glutathione, reactive oxygen species, SDS). In both cases the active form is obtained after complete removal of the pro-peptide; 2) Intracellular activation – several MMPs have similar furin recognition sequences responsible for the inracellular activation. 3) Cell surface activation - proMMP-2 is activated on the cell surface and is mediated by MT-MMPs. MT4-MMP does not activate proMMP-2. ProMMP-2 forms a tight complex with tissue inhibitor of metalloproteases-2 (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 (Suzuki, Enghild et al. 1990; Nagase, Suzuki et al. 1992; Itoh, Takamura et al. 2001; Visse and Nagase 2003).

Collagenases

MMP-1 (also known as intestinal collagenase, collagenase 1) is synthesized by fibroblasts, chondrocytes, macrophages, keratinocytes, endothelial cells and osteoblasts. MMP-1 is capable of cleaving triple helical fibrillar collagen of type I, II, II and V into fragments, which denature into gelatin and are further degraded by other MMPs, such as gelatinases (Vihinen and Kahari 2002). Particularly, MMP-1 has a preference to type III collagen. Besides, MMP-1 can also cleave other substrates: casein, gelatin, entactin and link proteins.

MMP-8 (also known as neutrophil collagenase and collagenase 2) is accumulated in the specific granules of polymorphonuclear leukocytes (PMNs) as an inactive pro-enzyme. PMNs play an important role in phagocytosis and possess high capacity to connective tissue infiltration. MMP-8 has similar efficiency to MMP-1 in cleaving collage and is produced at increased levels in conditions with prominent inflammation, including COPD and asthma (Segura-Valdez, Pardo et al. 2000; Vernooy, Lindeman et al. 2004). MMP-8 can cleave also proteins such as fibronectin and cartilage aggrecan, as well as peptides such as angiotensin receptor and substance P.

MMP-13, also known as collagenase-3 has broad substrate specificity, and plays an important role in invasion and metastasis. Increased expression of MMP-13 reflects the increased

invasiveness of tumors, squamous cell carcinoma of the upper respiratory tract, larynx, and vulva. Substrates for MMP-13 are collagens (I, II, III, IV, IX, X and XIV); gelatin, aggrecan, fibronectin, osteonectin, MMP-9. Expression of MMP-13 has been detected in a variety of cells, including macrophages, T-lymphocytes and plasma cells (Henry, McMahon et al. 2002; Gueders, Balbin et al. 2005; Rowe, Keena et al. 2011; Wallace, Mercer et al. 2012)

Polymorphisms in genes of MMP-1 and MMP-3

It was found that most of the genes coding for MMPs are highly polymorphic (polymorphisms in the promoter regions of the MMP and TIMP gene). These polymorphisms are functional and it has been demonstrated that they are associated with an alteration of gene expression and/or enzyme activity, which in turn may affect the development and progression of various pathological conditions including Bronchial asthma. Such promoter polymorphisms with allele specific effect on transcriptional activity are MMP1-1607insG (1G> 2G, rs1799750) and MMP3-1171insA (5A> 6A, rs3025058). There are evidence that the 2G allele of -1607 1G> 2G polymorphism in MMP1 leads to increased transcription MMP-1 (37), while the allele on 6A -1171 5A> 6A polymorphism in MMP3 to a significant reduction in gene expression (Henney, Ye et al. 2000; Dimov, Vlaykova et al. 2011).

BRONCHIAL ASTHMA AND COLLAGENASES

Several studies have suggested the implication of matrix metalloproteinases in the development of tissue remodeling and fibrosis associated with various inflammatory conditions. Bronchial epithelial injury and repair are features of chronic respiratory diseases, such as Bronchia asthma. Direct toxic insults, trauma or inflammation cause the loss of airway epithelial cells and degradation of the extracellular matrix (Roberts, Magowan et al. 2011). The pulmonary interstitium is composed of an interwoven network of structural proteins (including collagens and elastin) as well as glycoproteins and proteoglycans (Rowe, Keena et al. 2011). Disruption of the balance between MMPs and their endogenous inhibitors, TIMPs (protease/antiprotease imbalance) is a key event in the development of pulmonary diseases. Many MMPs, including MMP-1, -3, -8 and -9, implicated in mediating type I collagenolytic activity, have been detected by various methods in the sputum or bronchoalveolar lavage (BAL) from asthmatics. Several types of lung cells can release MMPs in response to environmental stimuli (Cataldo, Gueders et al. 2004; Sabeh, Li et al. 2009; Vandenbroucke, Dejonckheere et al. 2011). Pulmonary fibroblasts have been implicated as the key effectors of lung ECM turnover due to the central role that those cells play in synthesis and turnover of type I collagen(Rowe, Keena et al. 2011). Net MMP activity can also be inhibited by TIMPs. For example TIMP1, which is capable to inhibit MMP-1, -3 and -9, is increased in sputum of asthmatic patients (Vandenbroucke, Dejonckheere et al. 2011).

MMP-1 is involved in airway extracellular matrix degradation and is linked to chronic respiratory conditions, such as COPD, chronic asthma, emphysema, lung tuberculosis, and lung carcinoma (Li, Ghio et al. 2009). Activation of MMP-1 has been shown to be of great relevance for airway and lung health and disease. Increased MMP-1 expression by epithelial cells overlying intra-alveolar fibrosis has been observed from Henry et al. in lung samples from patients with lung injury (Henry, McMahon et al. 2002). MMP1-mediated insulin-like growth factor binding protein (IGFBP) proteolysis was shown to induce airway smooth muscle hyperplasia and airway obstruction by modulating the insulin-like growth factor axis (Vandenbroucke, Dejonckheere et al. 2011). Transforming growth factor-β1 (TGF-β1), a mediator reported to be increased in the bronchial tree from asthmatics, have been demonstrated to induce the expression of MMP-1 in different cell types (Cataldo, Gueders et al. 2004).

Previous reports have implicated MMP-8 and MMP-9 in the modulation of neutrophil mediated lung inflammation (Cederqvist, Janer et al. 2006). MMP-8 is expressed in neutrophils and is stored as a pro-MMP-8 within specific granules and released upon neutrophil activation. After release from granules, a significant part of the MMP-8 is associated to the membrane of neutrophils and exerts a pericellular proteolysis (Lamblin, Gosset et al. 1998; Prikk, Maisi et al. 2002; Kong, Li

et al. 2011). Gueders et al. have found that in asthmatics, increased expression of MMP-8 mRNA has been detected in bronchial biopsies and was correlated with the intensity of the disease. Studying the expression after allergen exposure in mice they have found that MMP-8 was significantly increased after allergens.

According to Vandenbroucke et al. MMP8 seems to play a protective role in asthma, since its deficiency promotes allergen-induced airway inflammation, mainly by delaying clearance of recruited neutrophils. MMP8-deficient mice displayed airway hyperresponsiveness and decreased levels of soluble IL-13Ra2, the decoy receptor for the central mediator of asthma IL-13 (Vandenbroucke, Dejonckheere et al. 2011).

MMP-1 is tightly regulated at the level of transcription, post-transcription, and post-translation; it is also known that MMP-1 expression is influenced by genetic variants in the promoter (Wallace, Mercer et al. 2012). In a research Li et al. have shown that the –1607GG MMP-1 allele of MMP1-1607insG polymorphism in the 5′ regulatory region of the human MMP-1 gene exerts a dominant influence on MMP-1 transcription and is more effective than the –1607G allele containing promoter. At least one copy of the –1607GG allele is present in 60% to 80% of humans (Li, Ghio et al. 2009).

In *MMP3* an insertion/deletion of an A nucleotide at position -1171 in promoter region of *MMP3* has been identified. This promoter polymorphism (5A/6A, -1171insA) results in transcriptional activity of the 5A homozygous in approximately double than the 6A homozygous (Ye, Eriksson et al. 1996; Ye 2000).

According to our previous studies there was no difference in distribution of genotypes and alleles of *MMP1* -1607insG (G/GG) polymorphism between patients with Bronchial asthma and controls, whereas 6A allele and 6A/6A genotype of *MMP3* -1171insA (5A>6A) polymorphism were significantly more frequently found in patients than in controls (Dimov, Vlaykova et al. 2011). We suggested that the high productive 5A allele of the promoter polymorphism-1171insA (5A> 6A) in *MMR3* appears to be protective, possibly by increased allele-specific synthesis of MMP-3 results in more effective metabolism of ECM components and delayed collagen deposition in the bronchial walls.

Finally we may conclude that although the increased MMP expression in asthmatic patients makes these matrix proteinases candidate diagnostic biomarkers, this does not necessarily mean that inhibiting them will have a beneficial therapeutic effect.

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