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MMP generated Matrikines

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Abstract

Matrikines originate from the fragmentation of extracellular matrix proteins and regulate cellular activities by interacting with specific receptors. Matrikines are implicated in inflammation, immune responses, organ development, wound repair, angiogenesis, atherosclerosis, tumor progression and metastasis due to their ability to alter cellular migration, chemotaxis, and mitogenesis. Matrix metalloproteinases (MMPs) degrade extracellular matrix components under normal circumstances and in disease processes. Of the 20 MMPs identified, MMP-1, MMP-2, MMP-8, MMP-9, and MMP-12 have been implicated in regulating the matrikines val-gly-val-ala-pro-gly (elastin peptide) and proline-glycine-proline (PGP). Elastin peptide fragments are generated from elastolytic enzymes and have implications in atherosclerosis, neovascularization, chronic obstructive pulmonary disease, skin disease, as well as tumor invasion and spread. PGP is produced through a multistep pathway that liberates the tripeptide fragment from extracellular collagen. PGP is best described for its role in neutrophil chemotaxis and is implicated in the pathogenesis of corneal ulcers and in chronic lung conditions. In chronic cigarette smoke related lung disease, the PGP pathway can become a self-propagating cycle of inflammation through cigarette-smoke mediated inhibition of leukotriene A4 hydrolase, the enzyme responsible for degrading PGP and halting acute inflammation. This review highlights the roles of MMPs in generating these important matrikines.

Keywords

Matrix metalloproteinase; elastin; proline-glycine-proline; copd

Background

Matrikines are peptides originating from the fragmentation of extracellular matrix proteins and regulate cellular activities by interacting with specific receptors¹. Matrikines are

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involved in the process of extracellular matrix renewal and cellular proliferation. Currently, intact proteins including elastin¹, extracellular matrix collagen², osteopontin³, tenascin, laminin, decorin, thrombospondin, integrin, versican⁴, type XIX collagen⁵, biotinylated peptide⁶, mactinin⁷, tetrastatin⁸, and lumican⁹ have been identified as the sources of matrikines.

Matrikines have been implicated in inflammation, immune responses, organ development⁹, wound repair¹⁰, angiogenesis, atherosclerosis, tumor progression and metastasis due to their ability to alter cellular migration, chemotaxis, and mitogenesis^{8; 11; 12}. Inhibition of matrikine signaling has been implicated in halting tumor growth⁵. Matrikine activity is dictated via differing regulatory mechanisms.

Matrix-metalloproteinases (MMPs) are a family of zinc-dependent metalloendopeptidases that can degrade or cleave many components of the extracellular matrix, as well as a wide range of other extracellular proteins, during both normal and disease processes. MMPs are produced as proenzymes or zymogens and their action is typically in concert with other proteases¹³. MMP-1, MMP-2, MMP-8, MMP-9, and MMP-12 have been implicated in regulating the matrikines Val-Gly-Val-Ala-Pro-Gly (VGVAPG; elastin peptide) and PGP and will be discussed. The biology of the parent proteins of these matrixins will be highlighted in a separate dedicated issue of *Matrix Biology*.

Elastin peptides as matrikines

Elastin is present in elastic fibers in the extracellular matrix and contributes to the elastic properties of skin, lungs, and larger blood vessels. Elastin is a polymer of tropoelastin, which is characterized by domains rich in Lys and Ala that function in generating desmosine-isodesmosine cross-linking and hydrophobic domains rich in Gly, Val, and Pro and occur in repeats of three to six-mers¹⁴. Tropoelastin is coded by the ELN gene¹⁵. ELN expression, and subsequent elastin production, is down regulated in the first years of life¹⁶, highlighting the need for elastic connective tissues to rely on the persistence of elastin. Elastolytic enzymes, including aspartic proteases, cysteine proteases, serine proteases, and metalloproteinases, degrade elastin and play significant roles in disease processes¹⁷. Val-Gly-Val-Ala-Pro-Gly (VGVAPG) is an elastin peptide fragment that is a repeating peptide sequence from the c-terminal portion of the tropoelastin molecule. This sequence is chemotactic for both monocytes and fibroblasts^{18; 19} and can be detected by HPLC²⁰.

The pathobiologic importance of elastin fragments is especially significant in the organ systems with an abundance of elastin. For instance, overproduction of elastin in vascular walls contributes to the development of atherosclerosis. Exogenous administration of elastin peptides stimulates vascular smooth muscle cell proliferation while simultaneously inhibiting elastin expression²¹, cause neovascularization, increased vascular wall thickness, and wall diameter²², and decrease vascular tone in a dose-dependent manner²³. Some groups have reported that elastin binding protein (EBP67) binds VGVAPG resulting in smooth muscle migration through elastin fiber membranes, leading to vascular intimal thickening and development of vascular disease^{24; 25}. These highlight the role of elastin peptides in vascular disease. In the lungs, elastin peptides are generated at diseased sites and specifically

attract monocytes, the precursors to macrophages²⁶ while impairing neutrophil reactivity²⁷. The chemotactic sites on elastin fragments contains lysyl-derived cross-links that recruit inflammatory cells to the lungs, resulting in elastase-induced emphysema²⁸. In melanocyte precursors, VGVAPG stimulates melanogenesis and dendrite formation²⁹. Finally, elastin fragments have pathologic roles in tumor migration, invasion, adhesion, and angiogenesis. VGVAPG has implications for lung cancer tumor progression through several mechanisms including the high affinity binding of the peptide to distinct surface receptors³⁰, altered receptor affinity and chemotactic responsiveness through a membrane-bound protein kinase C dependent process³¹, and post-transcriptional regulation of MMP-2 and uPA³². In fibrosarcoma, VGVAPG stimulates heat-shock protein 90, pro-MMP-2, and urokinase plasminogen activator secretion and cell-invasive capacity³³. In melanoma, VGVAPG increases tumor cell migration and expression of MMP-2 and MMP-3³⁴, NF-kappaB activation, and IL-1beta upregulation³⁵ resulting in increased metastatic and invasive potential.

Elastin peptides are generation by MMPs

The elastin fragments XGXXPG (where X is a generic hydrophobic residue) lead to expression and activation of MMP-1 and MMP-3 in human fibroblasts, suggesting the consensus sequence found in VGVAPG is important for binding to the elastin binding protein (EBP) receptor^{36; 37}. This MMP activation in turn worsens local connective tissue damage. In melanoma, VGVAPG increases the expression of MMP-2 and MMP-3, which in turn further degrade elastin³⁴. The invasive potential occurs predominantly through the galectin-3 receptor. Similarly, MMP-2, MMP-3, and MMP-9 expression are increased in smooth muscle cells in the intimal layer of the aorta³⁸. This increased expression occurs at sites where spiral collagen and degenerated elastin are abundant³⁹. In vascular tissue, these occur at sites of hemodynamic stress, leading to vascular wall vulnerability which has implications for development of aortic dissection and remodeling. Administration of doxycycline, an MMP inhibitor, results in preservation of aortic elastin accompanied by a reduction in MMP-9⁴⁰.

In mice, MMP-12 action on elastin results in the generation of GXXPG and XGXPG peptide fragments. Cleavage sites include Leu and Ile, amino acids with large aliphatic side chains in the P₁' position. These sites are readily accessible to MMP-12⁴¹. Antibodies to the GXXPG and XGXPG sequences abolish chemotactic activity to monocytes and prevent elastase-induced emphysema^{28; 42}. Macrophages accumulate in the lungs of mice chronically exposed to cigarette smoke, and this accumulation is ameliorated in *Mmp 12*^{-/-} mice⁴³.

Recently, investigators have directly examined patients with emphysema for the presence of bioactive elastin fragments via mass spectrometry, identifying 4 distinct elastin-derived fragments in chronic obstructive pulmonary disease (COPD) subjects compared to non-lung disease control⁴⁴, suggesting these elastin-derived peptides are operative in COPD. In addition, there is accumulating data that patients with COPD generate autoantibodies directed at portions of elastin suggesting a potential autoimmune component directed at specific epitopes of elastin^{45; 46}.

PGP as a matrikine

The neutrophil chemotactic activity of the collagen-derived tri-peptide PGP and its acetylated form (AcPGP) were originally described in models of direct alkaline hydrolysis of corneal proteins leading to corneal ulcers⁴⁷. The biologic activities of many similar synthetic peptides, including possible antagonist peptides were investigated. PGP alone was found to be sufficient to elicit bioactivity and the synthetic peptide gly-pro-hyp was able to inhibit PGP-mediated chemotaxis. Chemotaxis stimulated by PGP/AcPGP is dose dependent⁴⁸ and occurs through CXCR2 interaction^{2; 49; 50}. Neutrophil chemotaxis is more pronounced for AcPGP compared with PGP, and the acetylated form present in the lung compartment of smokers may be due in part to direct chemical acetylation by components found in cigarette smoke⁵¹.

Using NMR conformational analysis, the dominant solution conformation for each cis- and trans- isomer of PGP was described, aiding in peptide and non-peptide inhibitors for the chemoattractant^{52; 53}. Complementary peptides to PGP including arginine-threonine-arginine (RTR), an RTR-dimer, an RTR-tetramer, RTR-glycine-glycine, and alanine-serine-alanine (ASA) were tested on PGP-mediated neutrophil activation⁵⁴. Of these, the RTR-tetramer was found to be the most powerful antagonist of AcPGP induced neutrophilic chemotaxis. This inhibition was not observed for leukotriene B4 (LTB4) mediated chemotaxis, highlighting the specificity of RTR for PGP. PGP-mediated corneal ulceration was reduced by RTR^{55; 56}. RTR impedes PGP and IL-8 induced chemotaxis and ameliorates the development of an AcPGP mediated emphysema-like phenotype in mice⁴⁸.

The multistep pathway that generates PGP from extracellular collagen was reported in a cystic fibrosis model⁵⁷, chronic lung transplant rejection⁵⁸, and in models of chronic cigarette smoke exposure⁵⁹ and COPD⁶⁰. Through a stepwise process, collagen is degraded by MMP8, MMP9, and prolyl endopeptidase (PE)^{59; 61; 62}. Cigarette smoke induced increases in MMP-8, MMP-9, PE, and PGP accompany neutrophil influx and improve following smoking cessation⁵⁹. Sputum from patients with cystic fibrosis is able to generate PGP from collagen *ex vivo*, and this cascade is disrupted by inhibitors of MMP8, MMP9, and PE. Leukotriene A4 hydrolase (LTA4H), the pro-inflammatory enzyme responsible for the generation of LTB4 also possesses aminopeptidase activity that serves to degrade PGP⁶³. In the setting of acute inflammation, this serves to stop the PGP-mediated neutrophil chemotaxis. However, cigarette smoke selectively inactivates LTA4H's aminopeptidase function, leading to accumulation of PGP and neutrophils. This ultimately results in the development of COPD^{60; 63}. Once COPD is established, aminopeptidase activity remains selectively inactive through the effects of acrolein. This pathway is depicted in Figure 1.

MMPs in PGP generation

MMP-8 and MMP-9 play several roles in inflammation, including extracellular matrix degradation. Neutrophils contribute to the production of both MMPs. In a lipopolysaccharide (LPS) model of corneal inflammation, MMP-8 and MMP-9 expression is increased⁶⁴. Both MMP-8 and MMP-9 activity are absent in CXCR2^{-/-} mice⁶⁴, which have impaired neutrophil recruitment. In response to LPS, MMP8^{-/-} mice had diminished

neutrophil migration and decreased PGP production. In contrast, MMP-9^{-/-} mice had preserved LPS-induced neutrophil accumulation without alterations to PGP production, highlighting the contribution of MMP-8 to PGP generation. These findings were recapitulated in a murine model of chronic cigarette smoke⁶². In an ex vivo PGP generation assay, inhibitors to MMP-1, MMP-9, and PE prevent PGP release from neutrophils⁶⁵. MMP-9 blockade was the strongest inhibitor of PGP generation. Further, AcPGP induces time-dependent MMP-9 release from activated neutrophil tertiary granules. Activation of the ERK1/2 MAPK pathway is necessary for this MMP-9 release in response to AcPGP. This in turn mediates intracellular ERK1/2 phosphorylation, suggesting a feed-forward pathway to augment AcPGP production⁶⁶. This model is represented in Figure 2. In a murine model of cigarette smoke exposure, chronic smoke exposure increases MMP-8 and MMP-9 expression and activity⁵⁹. These are highly correlated with AcPGP ($r^2=0.82$ for MMP-8 and $r^2=0.96$ for MMP-9, $p<0.001$ for both). MMP-8, -9, and AcPGP levels fall following cigarette smoke cessation, demonstrating the importance of these proteinases in the formation of PGP.

Other bioactive ECM fragments generated by MMPs

Although much of the current literature on ECM fragments has focused on collagen and elastin, other components of ECM have been shown to be fragmented by MMPs leading to bioactive peptides. Laminins are glycoprotein found in the basement membrane important in maintaining tissue architecture and may have important roles in regulating cellular differentiation, survival, and adhesion. A number of laminins have been shown to be cleaved by MMPs, leading to bioactive ECM products. A previous manuscript highlighted that liberation of a specific cryptic epitope of laminin $\alpha 5$ (presumably by proteolytic digestion) releases a 16 amino acid peptide capable of recruiting PMNs and macrophages, along with inducing MMP-9 release from immune cells⁶⁷. More recently, MMP-2 has been shown to cleave laminin-111, liberating a 60kD fragment capable of inducing epithelial-mesenchymal transition in embryonic stem cells⁶⁸. These results strongly suggest that proteolytic cleavage of laminins may impart critical paracrine regulation of structural cells and immune cells. Additionally, matrikines generated from type IV collagen, including tumstatin, have effects on angiogenesis. Tumstatin is a cleavage fragment of the $\alpha 3$ chain of type IV collagen, is associated with normal tissue growth and regulation of angiogenesis. In mice deficient of either the $\alpha 3$ chain of type IV collagen or MMP-9, the enzyme responsible for generating tumstatin, angiogenesis is enhanced and accelerated tumor growth occurs⁶⁹. Other MMP-9 generated ECM fragments have similar angiogenesis inhibiting properties and have implications in cardiac disease⁷⁰ and are therapeutics in cancer treatment^{71; 72}.

Summary and Conclusion

Recent data provide strong evidence that rather than being mere processors of matrix proteins, MMPs are major regulators of normal and pathologic processes. Two important cleavage products – VGVAPG and PGP can both promote well-defined processes such as angiogenesis and chronic lung disease. Targeting the MMPs central to these pathways may result in more selective and efficient therapeutic targets for disease and represent an exciting area of investigation.

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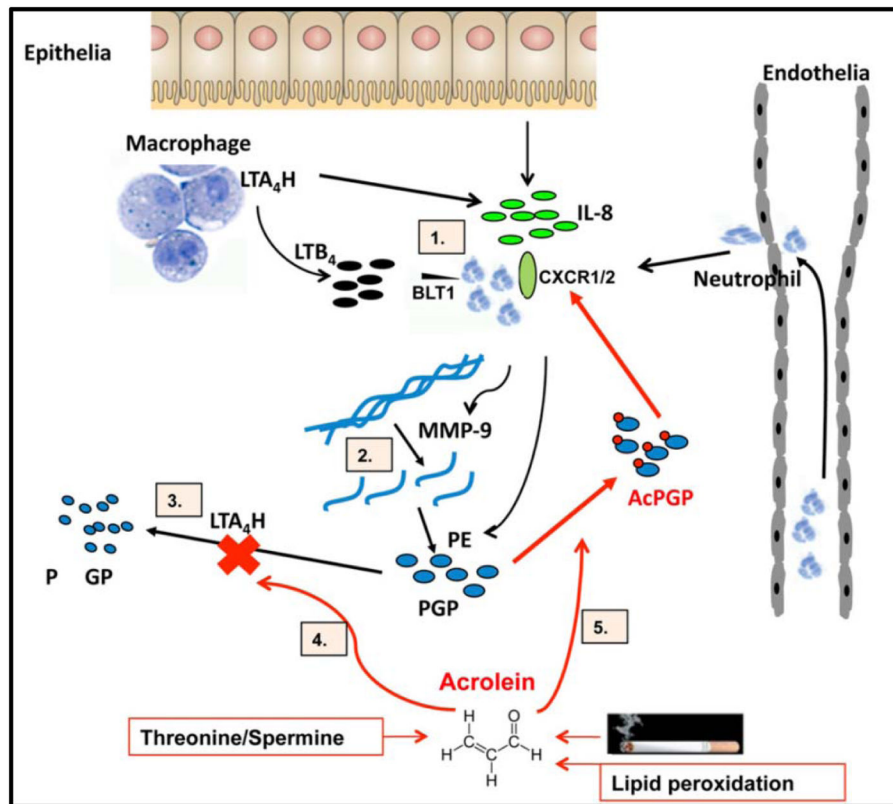


Figure 1. Central role of MMP-derived PGP in smoking-induced pulmonary inflammation
 (1) In response to infection or injury, resident cells within the lung release chemoattractants that promote neutrophil recruitment from the vasculature into the tissue. Epithelial cells and alveolar macrophages, for example, release IL-8 that binds to CXCR1/2 on the neutrophil surface and promote recruitment. The intracellular activity of LTA₄H within leukocytes can generate the lipid mediator LTB₄ that promotes neutrophil recruitment by binding to LTB₄ receptor (BLT1). (2) Neutrophils release an array of proteases within the lung tissue—the coordinated action of matrix metalloproteinases (MMPs; especially MMP-1, -8, and -9) and prolyl endopeptidase (PE) released from the neutrophil targets extracellular matrix collagen, releasing the neutrophil chemoattractant, proline-glycine-proline (PGP). PGP binds CXCR1/2 on the neutrophil and sustains neutrophil recruitment. (3) To terminate PGP-directed neutrophilic inflammation, LTA₄H is released into an extracellular environment to degrade the peptide. (4) Acrolein, derived from cigarette smoke or physiologically during inflammation (lipid peroxidation, metabolism of threonine or spermine), can inhibit LTA₄H-mediated degradation of PGP, allowing the peptide to accumulate and maintain neutrophilic inflammation. (5) Acrolein (and other components of cigarette smoke) can also chemically acetylate PGP on its N terminus, completely protecting the peptide from LTA₄H-mediated degradation, and thus facilitating neutrophil recruitment. AcPGP = acetylated PGP; PE = prolyl endopeptidase. Reproduced with permission⁷³.

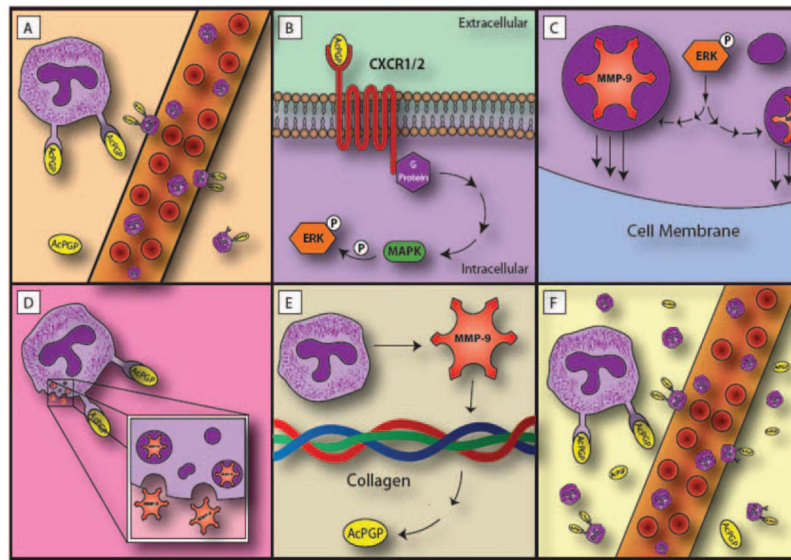


Figure 2. A model of persistent matrikine production and neutrophilic inflammation
 During chronic PMN inflammation, collagen is hydrolyzed and releases AcPGP causing ongoing neutrophilic influx (A). In addition to causing neutrophilic influx, AcPGP ligation of CXCR1 and CXCR2 leads to intracellular ERK phosphorylation and activation (B) and degranulation of MMP-9 from tertiary granules of neutrophils (C and D). This MMP-9 acts on exposed collagen leading to AcPGP generation (E) and a feed-forward inflammatory response on neutrophils (F). Reproduced with permission ⁶⁶.