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Matrix metalloproteinases and their inhibitors in non-neoplastic otorhinolaryngological disease

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Abstract

Matrix metalloproteinases (MMPs) are a family of zinc and calcium-dependent endopeptidases that play a key role in extracellular matrix (ECM) degradation. MMPs are known to be important in normal remodelling processes. Overexpression and activation of MMPs or an imbalance of active MMPs and tissue inhibitors of metalloproteinases (TIMPs) has been linked with a number of specific disease states associated with the breakdown and remodelling of the extracellular matrix. MMPs and TIMPs play a role in the development and progression of conditions such as acute and chronic otitis media, nasal polyposis and Sjogren's disease of salivary glands. Their role in allergic rhinitis has not been proven although they do appear to have a role in asthma, a condition closely linked to rhinitis. The use of a broad spectrum MMP inhibitor has been shown to alter the outcome of acute otitis media and otitis media with effusion. Therapeutic strategies with anti-MMP molecules are currently being developed and may play a role in modulating the course of non-neoplastic otorhinolaryngological disease in the future.

Key words: Matrix metalloproteinases; Otorhinolaryngological diseases

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc and calcium-dependent endopeptidases that play a key role in extracellular matrix (ECM) degradation.[1] They are capable of breaking down all the constituents of ECM including collagen, elastin, proteoglycans, laminin and fibronectin. They are produced by a range of stromal cells, macrophages and neutrophils.[2]

MMPs are known to be important in normal remodelling processes. Overexpression and activation of MMPs or an imbalance of active MMPs and tissue inhibitors of metalloproteinases (TIMPs), has been linked with a number of specific disease states associated with the breakdown and remodelling of the extracellular matrix, such as rheumatoid arthritis, periodontal disease, tumour invasion and metastasis, and vascular processes such as atherosclerosis, angiogenesis and aneurysms.[3–5]

There is increasing evidence that MMPs do more than degrade extracellular matrix. They also act upon non-matrix proteins, such as cytokines, chemokines, surface receptors and antimicrobial peptides, often potentiating the activity of these proteins.[6]

There is strong evidence that at least some members of the MMP family play a crucial role in the infiltrative growth process of squamous cell carcinomas of the head and neck.[7] It has been demonstrated that activity and expression of MMPs is increased in head and neck squamous cell carcinoma and there is some evidence that elevated levels of some MMPs correlate with more aggressive tumour growth and poor prognosis.[8–10]

In this article, the role of MMPs and their inhibitors in non-neoplastic otorhinolaryngological disease is explored.

Matrix metalloproteinases

There are at least 26 known MMPs, which are grouped according to their substrate specificity.[11] The collagenases include MMP-1, MMP-8 and MMP-13, the stromolysins comprise MMP-3, MMP-10 and MMP-11 while the gelatinases, MMP-2 and MMP-9 are the most widespread. However, there is some crossover between these groups in terms of activity upon the various substrates. For example, MMP-2, which is primarily a gelatinolytic and type IV collagen degrading enzyme is also effective against fibrillar type I collagen.[12]

MMPs are secreted as inactive pro-enzymes. They have three distinct domains: a zinc-containing catalytic domain, an amino-terminal pro-peptide domain containing a cysteine residue that chelates the zinc ion in the catalytic domain and holds the enzyme in a latent pro- form, and a carboxy-terminal

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haemapexin-like domain that contributes to substrate recognition. They require proteolytic cleavage of the N-terminal peptide for activation.[13]

Regulation of synthesis, secretion and activity

MMPs are regulated at multiple levels including gene transcription, activation of the latent enzyme and inactivation by specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Deregulation of MMPs has been implicated in the abnormal matrix degradation leading to tissue damage in tumour invasion and metastasis, rheumatoid arthritis and other inflammatory disorders.

Gene transcription

Most MMPs are expressed by many different cell types. Expression of the MMP gene is controlled at the transcriptional or post-transcriptional level. The expression of MMP genes can be modified by a variety of physiological and pharmacological signals including bacterial endotoxin, hormones, cytokines and growth factors.[14]

In general, MMP expression is up-regulated by interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), transforming growth factor-α (TGF-α), epidermal growth factor (EGF) and platelet derived growth factor (PDGF). On the other hand, expression of MMPs is down-regulated by interferon-α, (IFN-α), transforming growth factor-β (TGF-β), and glucocorticoids.[15] There are differences, however, between the MMPs in terms of their inducibility by cytokines and growth factors.

All MMPs are synthesized as quite large pre-pro-enzymes inside the cell. Most are secreted from the cells as inactive pro-enzymes. These pro-enzymes require activation before they can become fully active.

Pro-enzyme activation

Latency of MMPs is maintained by the link between the cysteine residue in the pro-peptide domain to the zinc atom of the active site. Activation occurs by dissociation of this cysteine-zinc bond, allowing a water molecule to complex with the zinc atom enabling the enzyme to become catalytically active.[16]

In vitro, this activation can occur by using a variety of proteinases such as plasmin trypsin and neutrophil elastase. Chemical dissociation can also be brought about by some organomercury compounds and reagents such as sodium dodecylsulphate.[3]

Many enzymes have been suggested to play a role in MMP activation, for example mast cell proteinases. As well as this, some MMPs are able to initiate activation of other latent MMPs.

Tissue inhibitors of metalloproteinases

Tissue inhibitors of metalloproteinases, or TIMPs, are low-molecular-weight endogenous inhibitors of the protease activity of MMPs.[17] They are locally active and widely distributed. They neutralize MMP activity via interaction of their N-terminal domain with the catalytic site of the MMP. Four mammalian TIMPs have been cloned, purified and characterized. Individual members of the TIMP family may possess selective affinities for different members of the matrix metalloproteinase family. For example, TIMP-2 is 10-fold more effective than TIMP-1 at inhibiting activated MMP-2.[18]

The regulatory role of TIMPs is more intricate than just blocking the active site of activated MMPs. Most MMPs only interact with TIMPs after activation. However, MMP-2 and MMP-9 can be secreted as bimolecular complexes with TIMP-2 and TIMP-1 respectively and hence TIMPs may also influence pro-MMP activation.[11]

Role of MMPs

Extracellular matrix degradation

The main recognized role of MMPs is in the degradation of extracellular matrix components. MMPs can act upon a variety of protein substrates and may indeed act upon different substrates under different circumstances. This activity is important in the inflammatory response to trauma, infection, toxic or autoimmune conditions. Degradation of the basement membrane allows cell migration which is an integral component of inflammation.

It is becoming increasingly clear, however, that MMPs also influence the inflammatory response by their actions on chemokines, cytokines and other receptors.

MMPs and chemokines

Chemokines are a group of chemotactic molecules that specifically attract and recruit populations of immune effector cells to the sites of inflammation. Many studies have demonstrated that specific MMPs control chemokine activity. This may either be a direct interaction between the MMP and the chemokine molecule or indirectly via its effect upon other substrates that influence chemokine activity.[6]

An example of a direct interaction is that of monocyte chemoattractant protein-3 (MCP-3). This has been identified as a physiological substrate of MMP-2. Cleaved MCP-3 binds to the same CC-chemokine receptors as uncleaved MCP-3 but it no longer promotes chemotaxis; instead it acts as a chemokine antagonist that dampens inflammation. This suggests that matrix metalloproteinases are both effectors and regulators of the inflammatory response.[19]

In contrast, MMPs can act indirectly by regulating the formation of chemokine gradients. This is by binding to subtrates that would normally be bound by chemokines. The net result of this type of interaction is often the opposite of the direct effect.

MMPs and cytokines

Cytokines function in mediating inflammation and repair processes. As already mentioned, the production and activation of MMPs can be affected by various cytokines. In addition, MMPs have a role in modulating cytokine activity. The various groups of MMPs including their specific substrates and activity are described below.

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Collagenases

Of the three MMPs (MMPs 1, 8 and 13) known to break down type I collagen, MMP-1 appears to be the most widespread. It is known to be expressed by many different tissues including fibroblasts, keratinocytes, endothelial cells, macrophages and others.[15]

MMP-1

MMP-1 (collagenase 1) is produced by synthesis in response to specific stimuli. It is not stored intracellularly, but is secreted as the pro-enzyme, which then undergoes extracellular activation. In its activated state it occurs both as a 42 kDa and a glycosylated 46 kDa form.[3] It is fairly widespread and degrades collagens I, II, III, VII, VIII, X and gelatin. It also activates pro-MMP-9.

MMP-8

MMP-8 (collagenase 2, neutrophil collagenase) has substrate specificity for native collagens including types I, II and III.[20] MMP-8 was originally thought to be confined to polymorphonuclear leucocytes (neutrophils, PMN), stored in granules and secreted upon activation.[21] Recent studies showed that MMP-8 is also expressed in other cells, such as osteoarthritic chondrocytes,[22] synovial fibroblasts and endothelial cells.[23] MMP-8, like other members of this gene family, is secreted as the precursor form. MMP-8 activity is inhibited by the binding of TIMP-1 or TIMP-2 in a 1:1 molar ratio.

MMP-13

MMP-13 (also known as collagenase-3) has a more restricted pattern of expression within connective tissue, and is usually produced only by cartilage and bone during development, and by chondrocytes in osteoarthritis.[24]

Gelatinases

MMP-2

MMP-2 (Gelatinase A, 72 kDa Gelatinase, Collagenase Type IV-A) is probably the most widely distributed MMP and is expressed by many cell types including fibroblasts, lymphocytes and numerous tumour cell lines.[15] It is also secreted from endothelial cells, neutrophils and macrophages. It specifically cleaves type IV collagen, the major structural component of basement membranes.

The 72 kDa form is inactive and is secreted as a latent pro-enzyme, which may be complexed with tissue inhibitor of metalloproteinase-2 (TIMP-2). Like other members of the collagenase family, this enzyme complex must be converted to a catalytically active form for proteolytic remodelling of extracellular matrix to occur.[25] This occurs by cleavage resulting in a 62 kDa activated enzyme.

MMP-2 acts upon pro-IL-1β, pro-TGF-β2[26] and pro-TNF-α. It also acts upon pro-MMP-1, pro-MMP-9, and inactivates substance P.

MMP-9

MMP-9 (Gelatinase B, 92 kDa Gelatinase) is produced by macrophages and granulocytes as well as T-cells, dendritic cells, epithelial cells, fibroblasts, keratinocytes and osteoblasts. Activation of neutrophils by IL-8 induces the release of MMP-9.[27]

Non-neutrophil pro-MMP-9 is secreted complexed with TIMP-1, which binds via a C-terminal domain.[28] In neutrophils, pro-MMP-9 is secreted as a non-complexed monomer. When pro-MMP-9 is present in excess of TIMP-1, it can form a TIMP-free homodimer but this complexing is prevented when TIMP-1 is in excess.[28]

MMP-9 acts upon pro-IL-1β, pro-TGF-β2[26] and pro-TNF-α. It truncates and potentiates IL-8 and inactivates IL-1β.

Stromolysines, stromolysine-like MMPs and membrane-type MMPs

Stromolysines comprise MMP-3 (stromelysin-1), MMP-10 and MMP-11.[14] They act upon substrates such as fibronectin, laminin, collagens IV, V, IX, X, elastin, pro-MMP-1 and pro-MMP-9. MMP-3 has been studied in cholesteatoma.

Stromolysine-like MMPs consist of MMP-7 (matrilysin), MMP-12 (macrophage metalloelastase) and MMP-26 (matrilysin-2).[14] Membrane-type MMPs include MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25. They are different from the remaining MMPs in that they are membrane-anchored and have a cytoplasmic tail. They activate other MMPs, mainly MMP-2.[29] They can regulate pericellular proteolysis very efficiently by concentrating proteolytic activities in the vicinity of the cell surface. MMPs 7, 10, 11, 12 and membrane-type MMPs have not been investigated with regard to benign ear and nose disease.

MMPs and ear disease

Acute otitis media

Acute otitis media is characterized by bacterial or viral inflammation of the middle-ear mucosa. Both human and bacterial proteases are known to play a role in the pathogenesis of otitis media.

MMPs are produced by *Pseudomonas aeruginosa*, *Staphylococcus aureus* and other bacteria that are implicated in the pathogenesis of acute otitis media.[30] In a study of the inhibitory effect of ilomostat (a broad spectrum MMP-inhibitor), it was shown that ilomostat led to greater protease inhibition in samples of middle-ear otorrhoea when the history was less than one week or when there was isolated *P. aeruginosa* infection. Thus it was felt that early on in the infective process when the host inflammatory response is not so intense, bacterial proteases were more likely to be active than human ones.[31]

Bacterial endotoxin has been demonstrated to cause a rise in MMP-9 but not MMP-2 expression in the host cells.[32] Leucocyte-derived proteases including some MMPs may help to prevent or eradicate bacterial infection but they may also contribute to tissue damage leading to sequelae or persistence.[31]

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Otitis media with effusion

Otitis media with effusion is characterized by persistence of fluid in the middle-ear cleft. Theories abound as to its cause but it is most widely accepted to be a sequela of infection.

In a study of MMPs in middle-ear effusions, it was demonstrated that MMP-2 and MMP-9 activity was higher in thick than in thin effusions.[33] It was also shown that MMP-2 concentration correlated with the chronicity of disease as judged by the number of ventilation tubes inserted previously while MMP-9 concentration did not. The authors surmised that the digestion of basement membrane collagen mediated by MMP-2 might contribute to the damage leading to otitis media with effusion.

Local administration of MMP inhibitors has been shown to improve outcomes in an animal model of otitis media with effusion.[34] In a recently published study by Antonelli *et al.,* significant levels of total MMP activity were found in 52 per cent of middle-ear effusions tested and ilomostat inhibited 64 per cent of MMP activity.[35] Thus, MMPs produced by cells within the middle-ear mucosa as a result of a prolonged inflammatory response, may affect the chronicity and characteristics of middle-ear effusions.

Cholesteatoma

The matrix of cholesteatoma demonstrates proteolytic activity that mediates the destructive processes that characterize this condition. In a study comparing gelatinolytic activity of cholesteatoma, middle-ear granulations and deep meatal skin, there was significantly higher activity at 45–47 kDa corresponding to MMP-1 in the cholesteatoma and middle-ear granulations than in controls (deep meatal skin). All of the cholesteatoma and middle-ear granulations were positive on Western blotting for MMP-1.[36] Zymographic analysis revealed a highly heterogeneous expression of MMP-2 and MMP-9 in cholesteatoma specimens. MMP-9, but not MMP-2, was increased in cholesteatoma when compared to deep meatal skin samples.[37]

By immunocytochemistry of cholesteatoma-cryosections, the expression of MMP-2, MMP-9 , and MMP-3 (stromelysin-1) was strictly confined to the basal and suprabasal cell layer of the cholesteatoma epithelium. The neutrophil collagenase (MMP-8) showed a more disseminated expression in the epithelium and the granulation tissue as well. The tissue inhibitor of metalloproteases, TIMP-1, could be detected only in very limited areas of the granulation tissue in a quite random manner.[38] It was also demonstrated that there was no detectable expression of metalloproteinase activity in normal tympanic membrane or in middle-ear mucosa.

In another study of macrophage migration inhibitory factor[39] (MIF) in cholesteatoma, MMP-3 and MMP-9 expression were found to correlate with MIF expression in infected cholesteatomas, while MMP-2 expression did not. MMP-3 has the potential to activate pro-MMP-9 into MMP-9.[40] It is interesting to note that while the activation of both MMP-3 and MMP-9 is mediated by distinct cytokines and the urokinase/plasminogen/plasmin system, pro-MMP-2 activation is mediated by the membrane-type MMPs (MT-MMPs) and not by cytokines.[41]

It was felt by Wilmoth *et al*. that inflammatory mediators produced by bacteria (i.e. endotoxin) might stimulate a host response that includes expression of cytokines (e.g. TNF-α) and ultimately, MMPs. When they cultured gerbil tympanic membranes with lipopolysaccharide (LPS, the active component of bacterial endotoxin), they found TNF-α and MMPs primarily in the culture media supernatant, not in homogenized tympanic membranes. This led them to surmise that TNF-α and MMPs are secreted into surrounding tissues, such as the extracellular matrix, after their production. Localization of these inflammatory mediators in the extracellular matrix could facilitate destruction of the lamina propria and progression of atelectasis.[42]

MMPs and nasal disease

Nasal polyps

The processes underlying the development of nasal polyposis remain unclear. In most cases, the lamina propria of nasal polyps demonstrates large numbers of eosinophils and lymphocytes. The inflammation leading to nasal polyposis is mediated by neuropeptides, cytokines, and growth factors produced by these cells. In addition, fibroblasts, epithelial, and endothelial cells contribute to the regulation of the inflammatory response in nasal polyps.

It appears that MMPs also contribute towards this process. In a study comparing nasal polyps with normal nasal mucosa, zymography and quantitative image analysis showed that MMP-9 active forms were significantly increased ($p < 0.05$) in nasal polyps compared to control nasal mucosal specimens, while MMP-2 expression was similar in both tissues.[43] Concomitant studies of gelatinase immunoexpression showed that MMP-9 expression was enhanced (4- to 16-fold) in surface epithelium, glands ($p < 0.05$), and submucosal inflammatory cells ($p < 0.05$). In addition, MMP-9 positivity was markedly increased in endothelial cells ($p < 0.01$). These results suggest up-regulation of active MMP-9 in the glands and vessels characteristic of nasal polyps.

MMP-9 up-regulation could be achieved by various molecules, such as plasminogen activators or TNF-α, already detected in nasal polyposis.[44,45] Circulating eosinophils do not express detectable MMP-9 while the eosinophils infiltrating nasal polyps express MMP-9.[46]

It has been surmised that MMP-9 produced by inflammatory cells in nasal polyps, by degrading components of endothelial basement membrane, could increase microvascular permeability, leading to oedema and cell transmigration.[46] MMP-9 could also facilitate epithelial and endothelial cell migration observed during polyp development and growth.

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Allergic rhinitis

Allergic reactions involve both early and late-phase responses. The early phase of allergic rhinitis is based upon interactions of allergen with membrane-bound allergen-specific IgE on the surface of mediator cells, i.e. basophils and mast cells, leading to the release of allergic mediators (both performed and newly synthesized) including histamine, leukotrienes, and eosinophil cationic protein (ECP).[47] Further mediators include prostaglandins, bradykinin and platelet activating factor (PAF). There is good evidence that these agents mediate the immediate allergic response. They cause vasodilatation and increased vascular permeability, stimulation of mucus secretion and stimulation of afferent nerves.

Late-phase responses are characterized by T-lymphocyte activation. When antigen is presented to naïve Th0 cells, they may differentiate into Th1 or Th2 cells. Th1 cells regulate cell mediated defence against tumours, mycobacterial and fungal infections, and Th2 cells are involved in non-phagocytic host responses such as the allergic response. In the presence of IL-4, antigen stimulated T-cells differentiate into Th2 cells. Th2 cells produce a range of cytokines including IL-3, IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. This cytokine profile attracts and activates eosinophils and mast cells as well as inhibits the Th1 response.[48] Th2 cytokines negatively regulate protease balance by limiting the production of MMP-9 while enhancing the production of TIMP-1.[49]

It has been shown that human mast cells produce MMP-9.[50] The levels of MMP-9 in nasal lavage fluid have been shown to rise immediately in response to nasal challenge with allergen extract.[51] However, in another study, there was no significant difference between the expression of MMP-9 RNA in the nasal mucosa in allergic and non-allergic participants who were not subject to allergen challenge.[52] Thus, it may be that MMP-9 has a clear role in the early phase of allergic rhinitis being degranulated along with other mediators from mast cells, while in the late phase there is a more complex interaction between MMP-9, Th2 cytokines, mast cells and eosinophils.

There is more information available regarding the role of MMP-9 in asthma, a condition with many similarities to allergic rhinitis. MMP-9 is the predominant MMP in the airway of asthmatics.[13] In comparison to normal controls, patients with stable asthma have increased MMP-9 levels in sputum, bronchoalveolar lavage fluid and in cultures of alveolar macrophages.[53] Studies performed during asthma exacerbations have also shown enhanced expression of MMP-9 with a return to lower levels after therapy and disease stabilization. After treatment with glucocorticoids, the MMP-9 levels decreased but TIMP-1 levels increased resulting in a net decrease in the MMP-9/TIMP-1 ratio.[54,55]

Further studies are required to improve our understanding of the role that MMPs play in allergic rhinitis.

MMPs and benign salivary gland disease

Sjogren's syndrome

It is thought that cytokines and growth factors effect tissue destruction in autoimmune diseases via the regulation of MMP levels. There is no significant difference in levels of MMP-2 in salivary glands of patients with Sjogren's syndrome and controls.[56] However, MMP-9 levels in saliva of patients with Sjogren's syndrome are higher than in normal controls.[57] The levels and presence of active forms of MMP-9 increase with increasing severity of disease.[58]

It is therefore felt that MMP-9 plays a crucial role in the increased remodelling and/or structural destruction of the basement membrane scaffolding in salivary glands in Sjogren's syndrome. Due to the role of basal lamina as an important molecular sieve and extracellular matrix-cell signal, these pathological changes may contribute to the pathogenesis of the syndrome.

- **Matrix metalloproteinases and their inhibitors are known to play a role in disease by influencing basement membrane breakdown**
- **They are already known to affect the course of malignant disease**
- **MMPs and TIMPs have been demonstrated to influence the course of some non-neoplastic otorhinolaryngological conditions**
- **Further studies are required to delineate their role and to determine whether this knowledge can be used therapeutically**

Conclusions

These data indicate that MMPs and TIMPs play a complex role in the development and progression of conditions such as acute and chronic otitis media, nasal polyposis and Sjogren's disease of the salivary glands. Future studies are required to further explain the part that they play in the pathogenesis of conditions such as allergic rhinitis.

It has also been demonstrated that the use of an MMP inhibitor can alter the outcome of acute otitis media and otitis media with effusion. As therapeutic strategies with anti-MMP molecules are currently being developed,[59] local control of the release and/or activation of MMPs may be another strategy for the treatment of conditions in which disordered activation of MMPs plays a role.

References

1 Morimoto Y, Tsuda T, Nakamura H, Hori H, Yamato H, Nagata N, *et al*. Expression of matrix metalloproteinases, tissue inhibitors of metalloproteinases, and extracellular matrix mRNA following exposure to mineral fibers and cigarette smoke *in vivo*. *Environ Health Perspect* 1997;**105**(suppl 5):1247–51
2 Finlay GA, Russell KJ, McMahon KJ, D'arcy EM, Masterson JB, FitzGerald MX, *et al*. Elevated levels of matrix metalloproteinases in bronchoalveolar lavage fluid of emphysematous patients. *Thorax* 1997;**52**:502–6

https://doi.org/10.1258/0022215054273269 Published online by Cambridge University Press

3 Nagase H, Ogata Y, Suzuki K, Enghild JJ, Salvesen G. Substrate specificities and activation mechanisms of matrix metalloproteinases. *Biochem Soc Trans* 1991;**19**:715–18

4 Stetler-Stevenson M, Mansoor A, Lim M, Fukushima P, Kehrl J, Marti G, *et al*. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in reactive and neoplastic lymphoid cells. *Blood* 1997;**89**:1708–15

5 Vaes G, Delaisse JM, Eeckhout Y. Relative roles of collagenase and lysosomal cysteine-proteinases in bone resorption. *Matrix Suppl* 1992;**1**:383–8

6 Parks WC, Wilson CL, Lopez-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 2004;**4**:617–29

7 Werner JA, Rathcke IO, Mandic R. The role of matrix metalloproteinases in squamous cell carcinomas of the head and neck. *Clin Exp Metastasis* 2002;**19**:275–82

8 Tokumaru Y, Fujii M, Otani Y, Kameyama K, Imanishi Y, Igarashi N, *et al*. Activation of matrix metalloproteinase-2 in head and neck squamous cell carcinoma: studies of clinical samples and *in vitro* cell lines co-cultured with fibroblasts. *Cancer Lett* 2000;**150**:15–21

9 Magary SP, Ryan MW, Tarnuzzer RW, Kornberg L. Expression of matrix metalloproteinases and tissue inhibitor of metalloproteinases in laryngeal and pharyngeal squamous cell carcinoma: a quantitative analysis. *Otolaryngol Head Neck Surg* 2000;**122**:712–16

10 Kawamata H, Uchida D, Hamano H, Kimura-Yanagawa T, Nakashiro KI, Hino S, *et al*. Active-MMP2 in cancer cell nests of oral cancer patients: correlation with lymph node metastasis. *Int J Oncol* 1998;**13**:699–704

11 Corcoran ML, Hewitt RE, Kleiner DE Jr, Stetler-Stevenson WG. MMP-2: expression, activation and inhibition. *Enzyme Protein* 1996;**49**:7–19

12 Aimes RT, Quigley JP. Matrix metalloproteinase-2 is an interstitial collagenase. Inhibitor-free enzyme catalyzes the cleavage of collagen fibrils and soluble native type I collagen generating the specific 3/4- and 1/4-length fragments. *J Biol Chem* 1995;**270**:5872–6

13 Kelly EA, Jarjour NN. Role of matrix metalloproteinases in asthma. *Curr Opin Pulm Med* 2003;**9**:28–33

14 Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001;**17**:463–516

15 Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, *et al*. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;**4**:197–250

16 Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Nat Acad Sci USA* 1990;**87**:5578–82

17 Woessner JF Jr. MMPs and TIMPs – an historical perspective. *Mol Biotechnol* 2002;**22**:33–49

18 Edwards DR, Beaudry PP, Laing TD, Kowal V, Leco KJ, Leco PA, *et al*. The roles of tissue inhibitors of metalloproteinases in tissue remodelling and cell growth. *Int J Obes Relat Metab Disord* 1996;**20**(suppl 3):S9–15

19 McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I, Overall CM. Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* 2000;**289**:1202–6

20 Murphy G, Reynolds JJ, Bretz U, Baggiolini M. Partial purification of collagenase and gelatinase from human polymorphonuclear leucocytes. Analysis of their actions on soluble and insoluble collagens. *Biochem J* 1982;**203**:209–21

21 Hasty KA, Pourmotabbed TF, Goldberg GI, Thompson JP, Spinella DG, Stevens RM, *et al*. Human neutrophil collagenase. A distinct gene product with homology to other matrix metalloproteinases. *J Biol Chem* 1990;**265**:11421–4

22 Shlopov BV, Lie WR, Mainardi CL, Cole AA, Chubinskaya S, Hasty KA. Osteoarthritic lesions: involvement of three different collagenases. *Arthritis Rheum* 1997;**40**:2065–74

23 Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, *et al*. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem* 1997;**272**:31504–9

24 Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res* 2002;**4**:157–64

25 Brown PD, Kleiner DE, Unsworth EJ, Stetler-Stevenson WG. Cellular activation of the 72 kDa type IV procollagenase/TIMP-2 complex. *Kidney Int* 1993;**43**:163–70

26 Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev* 2000;**14**:163–76

27 Pruijt JF, Fibbe WE, Laterveer L, Pieters RA, Lindley IJ, Paemen L, *et al*. Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9). *Proc Nat Acad Sci USA* 1999;**96**:10863–8

28 Goldberg GI, Strongin A, Collier IE, Genrich LT, Marmer BL. Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metalloproteinases prevents dimerization, complex formation with interstitial collagenase, and activation of the proenzyme with stromelysin. *J Biol Chem* 1992;**267**:4583–91

29 Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. *J Biol Chem* 1997;**272**:2446–51

30 Teufel P, Gotz F. Characterization of an extracellular metalloprotease with elastase activity from *Staphylococcus epidermidis*. *J Bacteriol* 1993;**175**:4218–24

31 Avidano MA, Cotter CS, Stringer SP, Schultz GS. Analysis of protease activity in human otitis media. *Otolaryngol Head Neck Surg* 1998;**119**:346–51

32 Albert J, Radomski A, Soop A, Sollevi A, Frostell C, Radomski MW. Differential release of matrix metalloproteinase-9 and nitric oxide following infusion of endotoxin to human volunteers. *Acta Anaesthesiol Scand* 2003;**47**:407–10

33 Jennings CR, Guo L, Collins HM, Birchall JP. Matrix metalloproteinases 2 and 9 in otitis media with effusion. *Clin Otolaryngol* 2001;**26**:491–4

34 Cotter CS, Avidano MA, Stringer SP, Schultz GS. Inhibition of proteases in *Pseudomonas* otitis media in chinchillas. *Otolaryngol Head Neck Surg* 1996;**115**:342–51

35 Antonelli PJ, Schultz GS, Kim KM, Cantwell JS, Sundin DJ, Pemberton PA, *et al.* Alpha 1-antitrypsin and ilomastat inhibit inflammatory proteases present in human middle ear effusions. *Laryngoscope* 2003;**113**:1347–51

36 Banerjee AR, James R, Narula AA, Lee RJ. Matrix metalloproteinase-1 in cholesteatoma, middle ear granulations and deep meatal skin: a comparative analysis. *Clin Otolaryngol* 1998;**23**:515–19

37 Schmidt M, Grunsfelder P, Hoppe F. Up-regulation of matrix metalloprotease-9 in middle ear cholesteatoma – correlations with growth factor expression in vivo? *Eur Arch Otorhinolaryngol* 2001;**258**:472–6

38 Schonermark M, Mester B, Kempf HG, Blaser J, Tschesche H, Lenarz T. Expression of matrix-metalloproteinases and their inhibitors in human cholesteatomas. *Acta Otolaryngol* 1996;**116**:451–6

39 Choufani G, Ghanooni R, Decaestecker C, Delbrouck K, Simon P, Schuring MP, *et al*. Detection of macrophage migration inhibitory factor (MIF) in human cholesteatomas and functional implications of correlations to recurrence status and to expression of matrix metalloproteinases-3/9, retinoic acid receptor-beta, and anti-apoptotic galectin-3. *Laryngoscope* 2001;**111**:1656–62

40 Onodera S, Kaneda K, Mizue Y, Koyama Y, Fujinaga M, Nishihira J. Macrophage migration inhibitory factor up-regulates expression of matrix metalloproteinases in synovial fibroblasts of rheumatoid arthritis. *J Biol Chem* 2000;**275**:444–50

41 Uhm JH, Dooley NP, Villemure JG, Yong VW. Mechanisms of glioma invasion: role of matrix-metalloproteinases. *Can J Neurol Sci* 1997;**24**:3–15
42 Wilmoth JG, Schultz GS, Antonelli PJ. Tympanic membrane metalloproteinase inflammatory response. *Otolaryngol Head Neck Surg* 2003;**129**:647–54
43 Lechapt-Zalcman E, Coste A, d'Ortho MP, Frisdal E, Harf A, Lafuma C, *et al*. Increased expression of matrix metalloproteinase-9 in nasal polyps. *J Pathol* 2001;**193**:233–41
44 Finotto S, Ohno I, Marshall JS, Gauldie J, Denburg JA, Dolovich J, *et al*. TNF-alpha production by eosinophils in upper airways inflammation (nasal polyposis). *J Immunol* 1994;**153**:2278–89
45 Larsen K, de Maat MP, Jespersen J. Plasminogen activators in human nasal polyps and mucosa. *Rhinology* 1997;**35**:175–7
46 Ohno I, Ohtani H, Nitta Y, Suzuki J, Hoshi H, Honma M, *et al.* Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1997;**16**:212–19
47 Bellanti JA, Wallerstedt DB. Allergic rhinitis update: epidemiology and natural history. *Allergy Asthma Proc* 2000;**21**:367–7
48 Durham SR. The inflammatory nature of allergic disease. *Clin Exp Allergy* 1998;**28**(suppl 6):20–4
49 Opdenakker G, Van den Steen PE, Van Damme J. Gelatinase B: a tuner and amplifier of immune functions. *Trends Immunol* 2001;**22**:571–9
50 Kanbe N, Tanaka A, Kanbe M, Itakura A, Kurosawa M, Matsuda H. Human mast cells produce matrix metalloproteinase 9. *Eur J Immunol* 1999;**29**:2645–9
51 van Toorenenbergen AW, Gerth VW, Vermeulen AM. Allergen-induced matrix metalloproteinase-9 in nasal lavage fluid. *Allergy* 1999;**54**:293–4
52 Shaida A, Kenyon G, Devalia J, Davies RJ, MacDonald TT, Pender SL. Matrix metalloproteinases and their inhibitors in the nasal mucosa of patients with perennial allergic rhinitis. *J Allergy Clin Immunol* 2001;**108**:791–6
53 Kelly EA, Busse WW, Jarjour NN. Increased matrix metalloproteinase-9 in the airway after allergen challenge. *Am J Respir Crit Care Med* 2000;**162**:1157–61
54 Tanaka H, Miyazaki N, Oashi K, Tanaka S, Ohmichi M, Abe S. Sputum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio in acute asthma. *J Allergy Clin Immunol* 2000;**105**:900–5
55 Hoshino M, Takahashi M, Takai Y, Sim J. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol* 1999;**104**:356–63
56 Perez P, Goicovich E, Alliende C, Aguilera S, Leyton C, Molina C, *et al*. Differential expression of matrix metalloproteinases in labial salivary glands of patients with primary Sjogren's syndrome. *Arthritis Rheum* 2000;**43**:2807–17
57 Wu AJ, Lafrenie RM, Park C, Apinhasmit W, Chen ZJ, Birkedal-Hansen H, *et al*. Modulation of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) by interferon-gamma in a human salivary gland cell line. *J Cell Physiol* 1997;**171**:117–24
58 Konttinen YT, Halinen S, Hanemaaijer R, Sorsa T, Hietanen J, Ceponis A, *et al*. Matrix metalloproteinase (MMP)-9 type IV collagenase/gelatinase implicated in the pathogenesis of Sjogren's syndrome. *Matrix Biol* 1998;**17**:335–47
59 Brown PD. Matrix metalloproteinase inhibitors in the treatment of cancer. *Med Oncol* 1997;**14**:1–10

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