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Role of Matrix Metalloproteinases in Cancer

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Introduction

Matrix metalloproteinases (MMPs) are endopeptidases which depend on zinc for their activity. They belong to the metzincin family of enzymes that code for a highly conserved zinc binding motif. MMPs have a role in degrading all kinds of extracellular matrix proteins and can also act upon a number of bioactive molecules. These are also called matrixins. MMPs cleave cell surface receptors; they release apoptotic ligands like FAS ligand, and also mediate activation or inactivation of chemokine and cytokine [1]. MMPs effect many behavioral patterns of the cell; like its proliferation, differentiation, migration and apoptosis. In addition, they play a major role in angiogenesis and host defence.

The first MMP was discovered when Jerome Gross and Charles Lapiere (1962) found out enzymatic activity during metamorphosis of the tadpole tail. They observed that the collagen triple helix degraded when the tadpole tail placed in collagen matrix metamorphosed [2]. Their suggestion was that the tadpole tissue produced a protease which was diffusible, but as EDTA could inhibit cleavage, it suggested an involvement of MMP.

Released as an inactive proenzyme, MMPs are activated by factors which are regulated by TIMP (tissue inhibitors of matrix metalloproteinases). The endogenous inhibitor family of TIMP is formed by four enzymes. Brew et al., reported in 2010 that pathological conditions may develop due to imbalance in the levels of MMP and TIMP [3]. Various reports show that an increase in expression of MMPs leads to various inflammatory, malignant and degenerative diseases. Thus there is a possibility that inhibitors of MMPs have therapeutic value [4-6].

Structure

The MMPs have three common domains, namely

- The pro-peptide, which is responsible for keeping the enzyme in an inactive form. This domain contains “cysteine switch” - a unique and conserved cysteine residue that interacts with the zinc in the active site. Interaction with the zinc prevents binding and cleavage of the substrate. This domain has to be proteolytically cleaved in order to make the enzyme active. The cleavage is done intracellularly by furin or extracellularly by other MMPs or serine proteinases such as plasmin [7].

- The catalytic domain - the structural signature of which is the zinc binding motif. The Zn2+ ion, bound by three histidine residues, forms the active site. The active site runs horizontally across the molecule as a shallow groove, and binds the substrate.

- The hinge region - A flexible hinge or linker region which connects the catalytic domain to the C-terminal domain. Although this 75 amino acids long region and has no determinable structure, it is very important for the enzyme’s stability.

- The hemopexin-like C-terminal domain - which is so named due to its sequence similarity to a serum protein, haemopexin. The polypeptide chain of this domain organizes into four β sheets. These β sheets or blades arrange themselves symmetrically around a central channel, resulting into a four-bladed β-propeller structure. The flat surface provided by this structure is believed to be involved in interactions between proteins and is a determinant of substrate specificity, for example TIMP, interacts with this site. However, this domain is not present in plants and nematodes.

ADAM (a disintegrin and metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) are the two families of metzincin proteinases that are closely related to the MMPs. Mostly membrane-anchored and pericellular space functioning ADAMs play roles in fertilization, development, and cancer [8]. ADAMs perform their function in a nonproteolytic manner. The generally secreted and soluble ADAMTS enzymes, function during ECM assembly, ovulation, and cancer, and have a protease, a disintegrin, and one or more thrombospondin domains [9].

MMPs are believed to remodel the ECM as they are capable of degrading ECM molecules. Likewise, MMPs may carry out significant functions during embryonic development as ECM remodelling is considered a critical part of tissue growth and morphogenesis. Additionally, MMPs also influence many cellular functions during development and normal physiology, for example:
Figure 1: Modelled structure of the full-length pro MMP. The catalytic domain is shown in standard orientation. The pro-domain and the catalytic domain are taken from the experimental proMMP-3 catalytic domain structure, whereas the linker (blue spheres) and the haemopexin-like domain are taken from and attached as seen in the crystal structure of full-length MMP-1. The catalytic domain from the hinge residue, Pro107, onwards is given as a solid surface colored according to the electrostatic surface potential. In the centre of the active-site cleft running from left to right resides the catalytic zinc (pink half sphere, center). The pro-segment is shown as a yellow ribbon with its ordered segments only, that is with the first (only apparently separated) helix, the second helix, the connecting loop, the third helix and the switch loop (running across the catalytic zinc liganding it via the conserved Cys side chain, shown as a green CPK model), and the rocker arm (green color), which swings to the bottom left after activation cleavage. The haemopexin-like domain consisting of the four blades I to IV and viewed at its exit side is shown as a golden ribbon; the single calcium ion (blue sphere, back) located at the entry side (MMP-1) is shown together with the second calcium (central blue sphere) and the two chlorine ions (black spheres) found in the haemopexin-like domains of gelatinase A and MMP-13.

(1) Allowing cell migration through degradation of ECM molecules;
(2) Altering cellular behavior by changing ECM micro-environment;
(3) Modulating the activity of biologically active molecules by direct cleavage, release from bound stores, or the modulating of the activity of their inhibitors.

Figure 2: Modes of action of the matrix metalloproteinases. (A) MMPs may affect cell migration by changing the cells from an adhesive to non adhesive phenotype and by degrading the ECM. (B) MMPs may alter ECM microenvironment leading to cell proliferation, apoptosis, or morphogenesis. (C) MMPs may modulate the activity of biologically active molecules such as growth factors or growth factor receptors by cleaving them or releasing them from the ECM. (D) MMPs may alter the balance of protease activity by cleaving the enzymes or their inhibitors.

Classification of MMPs

On the basis of the specificity of MMPs for ECM components, they are divided into collagenases, gelatinases, stromelysins and matrilysins. The common names of the MMPs mirror this classification. Out of eight distinct structural classes of MMPs: five are secreted
and three are membrane-type MMPs (MT-MMPs). The MT-MMPs are linked by covalent bonds to the membrane of the cell, the most obvious way of tethering MMP activity to the cell membrane. The other way to localize to the cell surface is by binding to integrins, which is the mode of secreted MMPs or to CD44 or through interactions with cell-surface-associated heparan sulphate proteoglycans, collagen type IV or the extracellular matrix metalloproteinase inducer (EMMPRIN) [7,10–12].

<table>
<thead>
<tr>
<th>MMP designation</th>
<th>Structural class</th>
<th>Common Name(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>Simple hemopexin domain</td>
<td>Collagenase-1, interstitial collagenase, fibroblast collagenase, tissue collagenase</td>
</tr>
<tr>
<td>MMP-2</td>
<td>Gelatin-binding</td>
<td>Gelatinase A, 72-kDa gelatinase, 72-kDa type IV collagenase, neutrophil gelatinase</td>
</tr>
<tr>
<td>MMP-3</td>
<td>Simple hemopexin domain</td>
<td>Stromelysin-1, transin-1, proteoglycanase, procollagenase activating protein</td>
</tr>
<tr>
<td>MMP-7</td>
<td>Minimal domain</td>
<td>Matrilysin, matrin, PUMP1, small uterine metalloproteinase</td>
</tr>
<tr>
<td>MMP-8</td>
<td>Simple hemopexin domain</td>
<td>Collagenase-2, neutrophil collagenase, PMN collagenase, granulocyte collagenase</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Gelatin-binding</td>
<td>Gelatinase B, 92-kDa gelatinase, 92-kDa type IV collagenase</td>
</tr>
<tr>
<td>MMP-10</td>
<td>Simple hemopexin domain</td>
<td>Stromelysin-2, transin-2 MMP-11 Furin-activated and secreted Stromelysin-3</td>
</tr>
<tr>
<td>MMP-12</td>
<td>Simple hemopexin domain</td>
<td>Metalloelastase, macrophage elastase, macrophage metalloelastase</td>
</tr>
<tr>
<td>MMP-13</td>
<td>Simple hemopexin domain</td>
<td>Collagenase-3</td>
</tr>
<tr>
<td>MMP-14</td>
<td>Transmembrane</td>
<td>MT1-MMP, MT-MMP1</td>
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<tr>
<td>MMP-15</td>
<td>Transmembrane</td>
<td>MT2-MMP, MT-MMP2</td>
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<tr>
<td>MMP-16</td>
<td>Transmembrane</td>
<td>MT3-MMP, MT-MMP3</td>
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<td>GPI-linked</td>
<td>MT4-MMP, MT-MMP4</td>
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<td>Simple hemopexin domain</td>
<td>Collagenase-4 (Xenopus; no human homologue known)</td>
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<td>Simple hemopexin domain</td>
<td>RASI-1, MMP-18</td>
</tr>
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<td>MMP-20</td>
<td>Simple hemopexin domain</td>
<td>Enamelysin</td>
</tr>
<tr>
<td>MMP-21</td>
<td>Vitronectin-like insert</td>
<td>Homologue of Xenopus XMMMP</td>
</tr>
<tr>
<td>MMP-22</td>
<td>Simple hemopexin domain</td>
<td>CMMP (chicken; no human homologue known)</td>
</tr>
<tr>
<td>MMP-23</td>
<td>Type II transmembrane</td>
<td>Cysteine array MMP (CA-MMP), femalysin, MIFR,MMP-21/MMP-22</td>
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<td>MMP-24</td>
<td>Transmembrane</td>
<td>MT5-MMP, MT-MMP5</td>
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<td>MMP-27</td>
<td>Simple hemopexin domain</td>
<td>Epilysin</td>
</tr>
<tr>
<td>MMP-28</td>
<td>Furin-activated and secreted</td>
<td>Mcol-A (Mouse)</td>
</tr>
<tr>
<td>No designation</td>
<td>Gelatin-binding</td>
<td>Mcol-B (Mouse)</td>
</tr>
</tbody>
</table>

Table 1: The Matrix Metalloproteinases Family.

*MMP-4, -5 and -6 have been abandoned. †When MMP-19 was cloned it was initially called MMP-18. However, an MMP from *Xenopus* had already received that designation, and therefore this MMP is now known as MMP-19. ‡The cloning of a partial fragment of human MMP-21 has been described, but the sequence has not been submitted to GenBank and the human enzyme has not been characterized. §By similarity with mouse and rat MMP-23. ¶Gururajan and colleagues identified two new MMP genes, which they called MMP21 and MMP22. The nucleotide sequences of the two genes are almost identical, so they are now designated MMP23A and MMP23B. *Sequence submitted to GenBank (access no. AF195192). GPI, glycosylphosphatidylinositol; MMP, metalloproteinase; MT-MMP, membrane type MMP; PMN, polymorphonuclear neutrophil; PUMP, putative metalloproteinase

**Role of Matrix Metalloproteinase**

**MMPs1 (Interstitial collagenase)**

Collagen type I, a major component of bone ECM is degraded by Matrix Metalloproteinase 1 (MMP-1). MMP-1 was significantly down-regulated, while TIMP-1 levels were increased, in a time- and pressure-dependent manner in a smooth muscle cell (SMC) mechanical strain model. Fibroblasts, keratinocytes, endothelial cells, monocytes and macrophages express MMP-1. Additionally, a misexpression screen set up to identify molecules required for motoneuron development also resulted in isolation of *Mmp1*. MMP-1 encoding mRNA was expressed at considerably higher levels in Human OS cells in primary culture than normal human bone cells.

**MMPs2 (Gelatinase-A, 72 kDa gelatinase)**

Whole-mount RNA in *in situ* hybridization characterized the expression pattern of *Mmp2*. Expression of *Mmp2* widely takes place in the embryonic CNS, which is in contrast to *Mmp1*. Expression of MMP-2 and beta-catenin loss have a role in the pathogenesis and progression of ESC. Recently, it has been shown that DNAzyme generated against MMP-2 mRNA reduced the expression of the enzymes *in vitro*, and the size of the C6-glioma *in vivo*, in the animal model. An important role is played by decreased E-cadherin in the development of both ESC and EEC.

**MMPs3 (Stromelysin 1)**

The pattern of IGFBP-3 degradation products produced by MMP-3 is identical in size to that produced by pregnancy serum. The stromelysin subgroup contains stromelysin-1 (MMP-3). A series of apoE/MMP double knockout mice were used in recent studies on atherosclerotic plaque stability to indicate that MMP-3 limits plaque growth and enhances plaque stability, and thus plays a protective role.

**MMPs7 (Matrilysin, PUMP 1)**

A big role in the invasion and metastasis of cancer is played by Matrix metalloproteinase-7 (MMP-7), the matrix-degrading enzyme. Studies have shown that oligonucleotides antisense to MMP-7 inhibit the higher rate of spreading of gastric gland cells infected with *H. pylori* cultures. MMP-7 mRNA was expressed in 53% of primary gastric cancers, but not in normal gastric mucosa, fibroblasts, or mesothelial cells. Induction of MMP-7 takes place during the response of epithelial cell to bacterial infection.
Figure 3: Human MMPs. Schematic representation of the structure of the 24 human matrix metalloproteinases (MMPs), which are classified into four different groups on the basis of domain organization. Archetypal MMPs contain a signal peptide (necessary for secretion), propeptide, a catalytic domain that binds zinc (Zn²⁺) and a hemopexin carboxy (C)-terminal domain. Y, D, and G represent tyrosine, aspartic acid and glycine amino acids that are present in the catalytic domain of all collagenases. Matrilysins contain the minimal domain organization that is required for secretion, latency and catalytic activity. Gelatinases contain fibronectin type II modules that improve collagen and gelatin degradation efficiency. Convertase activatable MMPs contain a basic insert in the propeptide that is targeted by furin-like proteases (convertase cleavage site). MMPs that belong to this group can be secreted enzymes, or membrane-anchored via GPI (glycosylphosphatidylinositol), type I or type II transmembrane (TM) segments. MMP-23A and MMP-23B contain unique cysteine array (CA) and immunoglobulin (Ig)-like domains in their C-terminal region.

MMPs8 (Neutrophil collagenase)

Neutrophil collagenase, a collagen cleaving enzyme, is present in the connective tissue of most mammals. It is also known as (MMP-8) or PMNL collagenase (MNL-CL). It has an exclusive pattern of expression in inflammatory conditions, and is therefore unique among the family of matrix metalloproteinases (MMPs). MMP-8 mRNA and protein were expressed in all the 3 cell types of human atheroma in situ.

MMPs9 (Gelatinase -B, 92 kDa gelatinase)

It is also known as 92 kDa type IV collagenase, 92 kDa gelatinase or gelatinase B (GELB) [13]. MMP-9 releases skit and this enables translocation of BM repopulating cells to a permissive vascular niche, which favours differentiation and reconstitution of the stem/progenitor cell pool.

MMPs10 (Stromelysin 2)

MMP10 gene in humans encodes Stromelysin-2 enzyme, which is also known as matrix metalloproteinase-10 (MMP-10) or transsin-2 [14,15].

MMPs11 (Stromelysin-3)

In humans, MMP11 gene encodes Stromelysin-3 (SL-3) or (MMP-11) [16-19]. Role of Matrix metalloproteinase-11 in neointima formation was tested with the use of a vascular injury model in wild-type (MMP-11+/+) and MMP-11–deficient (MMP-11−/−) mice. Probably, MMP-11 overexpression was associated with the aggressiveness of ovarian carcinoma.

MMPs12 (Macrophage metalloelastase)

Investigation into the role of MMP-12 in the development of COPD in human smokers was undertaken in animal models, and it suggested a predominant role for MMP-9 and MMP-12 in the pathogenesis of pulmonary inflammation.

MMP13 (Collagenase 3)

MMP13 gene in humans encodes Collagenase 3 enzyme [20,21]. Expressed by chondrocytes and synovial cells in human OA and RA, MMP-13 is thought to play a critical role in cartilage destruction. It has been reported that in MMP-13 KO mice, degradation of connective tissue growth factor in wound tissue was transiently prevented. MMP-13 remains the major MMP expressed by chondrocytes to degrade their matrix, when they are stimulated with retinoic acid.
MMP14 (MT1-MMP)

Membrane type 1-matrix metalloprotease (MT1-MMP or MMP-14) is a major activator of pro-MMP-2 and is essential for skeletal development. It is generated in vitro by cleavage of membrane-bound native MT1-MMP with several recombinant MMPs, including both active MT1-MMP and MMP-2.

MMP15 (MT2-MMP)

Ueno et al. found a correlation between positive nodal status and the expression of 15 mRNA. Hypocellular ECs at E10.5 were displayed by mice with targeted snai1 knockdown. This was associated with decreased expression of mesenchyme cell markers and down regulation of the matrix metalloproteinase (mmp) family member, mmp15.

MMP16 (MT3-MMP)

According to a numerical nomenclature for matrix metalloproteinases, this is the new name for MT3-MMP [Membrane-type matrix metalloproteinase-3]. In end-stage osteoarthritis, MT3-MMP expression is elevated in human cartilage. PDGF and fibronectin can upregulate MMP-16 expression by cultured vascular smooth muscle cells under pathologic conditions.

MMPs17 (MT4-MMP)

MMPs-17 (MT4-MMP) is a member of the MT-MMP subfamily. They are anchored to the plasma membrane via a glycosyl-phosphatidyl inositol (GPI) anchor, which confers these enzymes a unique set of regulatory and functional mechanisms that separates them from the rest of the MMP family.

MMP18 (Collagenase 4, xcol4, Xenopus collagenase)

MMP-18 is expressed in the migrating macrophages, and bands corresponding to mRNA for MMP-18 are present in both HTM and CB tissue.

MMP19 (RASI-1, occasionally referred to as stromelysin-4)

MMP-19 was revealed as a novel mediator in laser capture microscope followed by microarray analysis in hyperplastic epithelial cells adjacent to fibrotic regions. Expressed in human epidermis and endothelial cells, it has roles in cellular proliferation, migration, angiogenesis and adhesion. Yu et al., 2012, identified multiple transcript variants encoding distinct isoforms for this gene [22].

MMP20 (Enamelysin)

An MMP-20 mutation which alters the normal splice pattern and results in premature termination of the encoded protein has been associated with amelogenesis imperfecta.

MMP21 (X-MMP)

MMP-21 enhances tumor invasion and metastasis ability in some solid tumors. MMP-21 expression has been investigated in 296 cases of gastric cancer by immunohistochemistry assay.

MMP23A (CA-MMP)

Unlike other MMPs, MMP23a does not possess the signal sequence. This suggests that it may act intracellularly. MMP-23 has a short prodomain and contains a single cysteine residue that can be part of the cysteine-switch mechanism operating for maintaining enzyme latency.

MMP23B

MMP23B degrades various components of the extracellular matrix. Mmp23b was identified as a gene linked to the genomic locus of an enhancer trap transgenic zebrafish line in which GFP expression was restricted to the developing liver.

MMP24 (MT5-MMP)

TIMPs inhibit all MMPs, except MMP -24.

MMP25 (MT6-MMP)

Membrane-type MMPs (MMP -25, also called MT1-, MT2-, MT3-, MT4-, MT5-, and MT6-MMP, respectively) are structurally similar to the other classes of MMPs but are anchored to the exterior of the cell membrane. It is highly expressed in leukocytes and in some cancer tissues.

MMP26 (Matrilysin-2, endometase)

MMP-26 has 998 mRNA nucleotides and no transcript variant. RT-PCR, immunofluorescence analysis and flow cytometry determined the mRNA and protein expression of MMP-26 by. It is the smallest member of the matrix metalloproteinase. The encoded protein degrades type IV collagen, fibronectin, fibrinogen, casein, vitronectin, alpha 1-antitrypsin, alpha 2-macroglobulin, and insulin-like growth factor-binding protein 1, and activates MMP9 by cleavage.

MMP27 (MMP-22, C-MMP)

mRNAs for MMP-27 are generally expressed at a lower level.

MMP28 (Epilysin)

Matrix metalloproteinase-28 (MMP-28, epilysin) is highly expressed in the skin by keratinocytes, the developing and regenerating nervous system and a number of other normal human tissues. MMP-28 expression is associated with cell proliferation during epithelial
repair and is tightly regulated spatially and temporally during wound repair. In primary keratinocytes, expression of MMP-28 is upregulated by treatment with TNF-alpha [23].

**Regulation of MMPs**

MMPs are synthesized as inactive zymogens. They are kept inactive by an interaction between a cysteine-sulphydryl group in the propeptide domain and the zinc ion bound to the catalytic domain. Their activation requires proteolytic removal of the propeptide prodomain. Activation of majority of MMPs occurs outside the cell by other activated MMPs or serine proteinases. MMP-11, MMP-28 and MT-MMPs can however also be activated by furin-like serine proteinases present intracellularly before they reach the cell surface [7]. Activation of MMP-2 at the cell surface occurs by a unique multistep pathway involving MMP-14 (MT1-MMP) and the tissue inhibitor of metalloproteinases 2 (TIMP-2) [24]. During this process, TIMP-2 binds MMP-14 at its amino terminus and pro-MMP-2 at its carboxyl terminus, allowing an adjacent, non-inhibited MMP-14 to cleave the bound pro-MMP-2. MMP-14 does not fully activate MMP-2, as activatedMMP-2 s necessary to remove a residual portion of the MMP-2 propeptide [25]. Alternatively, activation of Pro-MMP-2 might also occur by MMP-15 through a mechanism not requiring TIMP-2.

An abundant plasmaprotein in tissue fluids, α2-macroglobulin acts as the main inhibitor of MMPs by forming a α2-macroglobulin–MMP complex which binds to a ‘scavenger receptor’ and gets irreversibly cleared by endocytosis [26]. The debris of the cell is scavenged by scavenger receptors, which forms a broad class of receptors. They also have other activities, such as adhesion. Similarly, thrombospondin-2 forms a complex with MMP-2, facilitating scavenger-receptor-mediated endocytosis and clearance [27]. Thrombospondin-1 binds to pro-MMP-2 and -9, directly inhibiting their activation [28,29]. TIMPs -1, -2, -3 and -4 remain the best-studied endogenous MMP inhibitors. All of them reversibly inhibit MMPs in a 1:1 stoichiometric fashion [8]. Wang et al., 2000, studied Timp-2-deficient mice and showed that the dominant physiological function of TIMP-2 is activation of MMP-2 [30].

**MMP Substrate**

MMPs make cell migration easier by degrading the structural components of ECM. Cells, on the other hand, have receptors for structural ECM components for example, integrins, therefore cleavage of ECM proteins by MMPs affects cellular signalling and functions [31]. Cleavage of ECM components by MMPs generates fragments with new functions: cleavage of laminin-5 and collagen type IV results in exposure of ‘cryptic sites’ that promote migration [32,33]. A part of protein which is normally hidden within its three dimensional structure constitutes the ‘Cryptic site’. It might get exposed due to conformational changes in the protein. Additionally, cleavage of insulin-like growth-factor-binding protein (IGF-BP) and perlecan releases IGFs and fibroblast growth factors (FGFs), respectively [34-36]. Moreover, MMPs along with the ADAMS participate in the release of cell-membrane-bound precursor forms of many growth factors, including transforming growth factor-α (TGF-α) [37]. However, TGF-β is released by MMP-2 and MMP-9 from an inactive extracellular complex and made available biologically [38].

MMP-2 cleaves the FGF receptor 1, a growth factor receptor [39]. Two members of the epidermal-growth-factor receptor (EGFR) family — HER2/neu (also known as ERBB2) and HER4 (also known as ERBB4) — and the hepatocyte-growth-factor receptor c-MET are substrates for unidentified MMPs or ADAMS [40-42].

MMPs also act on cell-adhesion molecules. For instance, cleavage of E-cadherin and CD44 results in the release of fragments of the extracellular domains and an increased invasive behaviour [43,44]. Cleavage of the α-v integrin subunit precursor by MMP-14 enhances cancer-cell migration. In addition, MMPs cleave other MMPs and proteinase inhibitors such as serpins [7].

Inappropriate expression of MMP activity results in the pathogenic mechanism associated with a wide range of diseases which include;

- Tissue breakdown and remodelling during invasive tumor growth and tumor angiogenesis [45]
- Degradation of myelin-basic protein in neuro-inflammatory diseases [46]
- Increased matrix turnover in restenotic lesions [47]
- Liver fibrosis [48]
- Loss of aortic wall strength in aneurysms [49]
- Tissue degradation in gastric ulceration [50]
- Opening of the blood-brain barrier following brain injury [51]
- Breakdown of connective tissue in periodontal disease [52]
- Acute lung injury and acute respiratory distress syndrome [53] and,
- The destruction of cartilage and bone in rheumatoid and osteoarthritis [54]

However, it is still a subject of interest that which MMPs are involved in which diseases.

**MMPs Inhibition and Anticancer Therapy**

Synthesis of MMPs is blocked by several agents which prevent them from interacting with the molecules that direct their activities to the cell surface or inhibit their enzymatic activity.

**Inhibition of MMP synthesis**

MMP synthesis is inhibited directly by transfecting cells with antisense mRNA or oligonucleotides, or by targeting mRNA with RIBOZYMES. In mouse models, this means was used to downregulate MMP7 or 9 to reduced tumour burden or metastasis [55-57]. Indirect methods to reduce MMP expression are inhibition of the signal-transduction pathways that induce MMP transcription. Several drugs in clinical trials inhibit tyrosine kinase receptor signalling and affect MMP expression levels [58]. Halofuginone, a COCCIDIOSTAT that is used in chickens, is a drug regulating MMP gene expression and experimental cancer-cell metastasis [59]. Coccidiostat is a drug used to treat coccidiosis, an intestinal disease that is caused by a protozoan.
Inhibiting interactions between MMP and other proteins

In order to inhibit interaction of MMP with other proteins, MMP-2 is inhibited from binding to αvβ3 integrin. In clinical practice, this type of strategy could be tested by means of specifically targeting cancer-promoting function, and the compound shows promising results in animal experiments [60].

Exploiting MMP activity

Several cytotoxic agents have been developed that are activated by MMPs. This is useful in treatment of tumours. Cytotoxic agents like recombinant proteins containing ANTHRAX TOXIN fused to an MMP cleavage site, are activated by MMP cleavage at the cell surface and are internalized by the cell, followed by cell death [61].

Blocking of MMP

The MMPs are inhibited by specific endogenous (TIMPs), which comprise a family of four protease inhibitors:

- TIMP-1
- TIMP-2
- TIMP-3 and
- TIMP-4

TIMPs might have MMP-independent cancer-promoting activities [62].

Three categories of synthetic MMP inhibitors are:

- The collagen peptidomimetics, which mimic the cleavage sites of MMP substrates. Examples are Batimastat and Marimastat. While Batimastat is no longer tested for the treatment of human cancer, Marimastat has undergone several Phase III clinical trials.
- The collagen non-peptidomimetics, which are synthesized on the basis of the conformation of the MMP active site. Examples include BAY 12-9566, Prinomastat/AG3340, BMS 275291 and CGS 27023A/MMI270. While treatment with BAY 12-9566 in Phase III trials showed poorer survival than for placebo-treated groups, Prinomastat combined with standard chemotherapy did not show beneficial effects compared with chemotherapy alone. Phase II/III clinical trials with BMS 275291 are being recruited [63].
- The tetracyclines derivatives, which inhibit both the activity and synthesis of MMPs. An example is Col-3 (Metastat) that has entered Phase II trials for Kaposi’s sarcoma and advanced brain tumours [64]. A new class of MMP inhibitors are small peptides which can be selected for high specificity for individual MMPs, and one such peptide that inhibits MMP-2 and -9 enzymatic activity shows promising effects in animal experiments [65].

Bisphosphonates, originally developed for the treatment of disturbances in calcium homeostasis and for the prevention of bone metastasis, also inhibit the enzymatic activity of MMPs [66]. Some unconventional MMP inhibitors like AE-941 (Neovastat), an extract from shark cartilage, inhibits MMPs and is now in Phase III clinical trials for the treatment of metastatic non-small-cell lung cancer [67]. A component in green tea being tested in Phase III trial, acts as an MMP-2 and -9 inhibitor in vitro [68]. Acetylsalicylic acid reduces the risk of colon cancer, by directly inhibits MMP-2 activity [69].

Therapeutic Inhibition of MMPs

Strategies for blocking MMP gene transcription

General approaches to inhibit MMP gene transcription target extracellular factors signal transduction pathways or nuclear factors that activate expression of these genes. Targeting MMP transcripts using ribozymes or antisense constructs downregulate MMP production by cancer cells [55,57,70].

Extracellular factors

IFN-γ inhibits transcription of several MMPs via the transcription factor STAT1 in diverse human cancer cells [71]. Similarly, IFN-β and IFN-α can also be used for this purpose [72-73]. Blocking of signalling by cytokines or growth factors that upregulate MMPs serves as an alternative approach.

Monoclonal antibodies therapeutically reduce TNF-α-induced MMP production in arthritis, and therefore have a potential in cancer too. In a similar strategy for abolishing MMP production in cancer, blocking of IL-1 or epithelial growth factor (EGF) receptors might be useful [74,75]. Retinoids and TGF-β have been reported to downregulate the expression of MMPs and increase TIMP expression, but other studies have reported the opposite [76-79]. Interestingly, blockade of TGF-β with a soluble TGF-β receptor antagonist inhibits tumour metastasis and the production of active MMP-2 and MMP-9 in mouse models of breast carcinomas [80]. Therefore, proposals to use retinoids or TGF-β as therapeutics to block MMP production in cancer must be reconsidered in light of stimulatory effects of these agents on the production of diverse tumour-associated MMPs. As selecting MMP targets for cancer therapy becomes more complex, the need is to define specific proteases that are involved in a particular tumour at each stage.

Signal transduction

MMP production can also be blocked by targeting the signal-transduction pathways that mediate their induction (Figure 4). Halofuginone (an alkaloid from the medicinal plant Dichroa febrifuga) interferes with the TGF-β signalling pathway and inhibits bladder carcinoma metastasis by blocking MMP2 expression [81]. In addition, selective inhibition of p38 MAPK activity with SB203580 abolishes the expression of MMP1, 9 and 13 in transformed keratinocytes and squamous-cell carcinoma cells [82,83]. Inhibitors of other MAPK pathways, including ERK and JNK, also block the production of some MMPs by tumour cells [84]. Malolactomycin D — a potent inhibitor of transcription that is controlled by the RAS responsive element suppresses the expression of several MMPs and the RAS-induced transformed phenotype in NIH3T3 cells, at least in part, by blocking the activation of p38 MAPK [85]. Also, manumycin A — an inhibitor of RAS farnesyltransferase — blocks hyaluronan-mediated MMP-2 secretion in lung carcinoma cells, indicating that RAS...
signalling is required in this process [86]. Since blocking of general oncogenic signalling pathways-RAS–MAPK, can hamper cancer progression by means of many different mechanisms, therefore it will be crucial to show that therapeutic MMP inhibitors have no deleterious effects on signal transduction.

**Figure 4:** Signalling pathways involved in MMP gene transcription, and potential strategies for therapeutic intervention. Compounds that are able to block the transcription of matrix metalloproteinase (MMP) genes at different levels are shown in red boxes. Extracellular factors, such as interferon-γ (IFN-γ) inhibit MMP transcription via the JAK–STAT signalling pathway. Monoclonal antibodies against tumour necrosis factor-α (anti-TNF), soluble forms of the TNF receptor (sTNF-R), natural antagonists of the interleukin (IL)-1 receptor-α (IL-1Rα) or soluble forms of this receptor (sIL-R) can block signalling pathways initiated by extracellular factors such as TNF-α and IL-1, which induce MMPs in cancer cells. Compounds such as manumycin A, SB203580, malolactomycin, SP600125 or PD98059 act at different levels to block the signal transduction pathways that are associated with MMP transcriptional induction in human tumours. Finally, there are several possibilities to target the nuclear factors that are responsible for MMP transcriptional upregulation. Glucocorticoids, terpenoids, curcumin, nobiletin or NSAIDs (nonsteroidal anti-inflammatory drugs) block the activity of transcription factors such as AP1 and NF-xB, which regulate the transcription of several MMP genes. Similarly, restoring the activity of transcription factors such as p53 and TEL, which negatively regulate MMP expression and the activity of which is lost in human tumours, could downregulate these genes. IFN-γ, interferon-γ; IκB, inhibitor of κB; IκB kinase; JAK, JUN-activated kinase; MAPK, mitogen activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen activated protein kinase kinase kinase; NF-κB, nuclear factor of κB; STAT, signal transducer and activator of transcription; TEL, translocation-ETS-leukaemia.

**Nuclear factors**

A third way to block the upregulation of MMPs in human tumours is by targeting the nuclear factors that regulate these genes (Figure 4). Many different extracellular stimuli and signalling pathways that activate MMP expression converge at the AP1 DNA-binding site. Glucocorticoids interact with the AP1 binding site and prevent the upregulation of MMPs [87], but this treatment can affect the expression of many genes and have several side effects. Natural products such as nobiletin—a flavonoid obtained from *Citrus depressa*—have been shown to inhibit AP1 binding activity and suppress both the production of MMP-1 and MMP-9 by human fibrosarcoma cells and the invasive properties of these cells [88]. Similarly, curcuminoids, natural products of the Indian spice turmeric, inhibit MMP9 expression by interfering with AP1-induced transcription [89].

Another factor that can be targeted to prevent MMP transcription is NF-xB in cancer [90,91]. Interestingly, PS-341—a proteasome inhibitor that blocks the degradation of inhibitor of xB (IxB) and thereby maintains NFxB in an inactive status, might be effective in treating multiple myeloma and other cancer types in humans [92]. Synthetic triterpenoids and non-steroidal anti-inflammatory drugs also interfere with the NF-xB pathway [93,94].

Some transcription factors such as p53, PTEN (phosphatase and tensin homologue) and ETS transcription factor (TEL), are involved in negative regulation of MMP expression. Their activity is commonly lost during tumour progression which leads to an increase in the
proteolytic capacity of tumour cells [95,96]. Adenoviral delivery of wild-type p53 into squamous-cell carcinoma cells that carry mutant/forms of p53 inhibits expression of MMPs and invasive properties, independently of the pro-apoptotic effect of p53 on these cells [97].

**Strategies for Blocking proMMP Activation**

Inhibitors of plasmin can prevent cleavage of proMMP, and combined with administration of MMPIs, can profoundly reduce
tissue, highlighting the potential for similar combinatorial treatment of cancer [98]. MT-MMP, an MMP-activating enzyme, is also
overexpressed by different malignant tumours. Together with their general proteolytic behaviour, the MT-MMPs should be considered
primary targets.

Anti-inflammatory cytokines, such as IL-4 and IL-13, have been shown to interfere with the proMMP activation process rather than
with enzyme expression [99]. Natural products such as green tea catechins, have also been reported to block the MT1-MMP-dependent
activation of proMMPs [100]. Similarly, since MMP-3 is a well-characterized activator of proMMPs, inhibitors of this enzyme will also
prevent the activation of other proMMPs.

**Proprotein convertase inhibitors**

A selective furin inhibitor, α1-PDX, has been shown to prevent tumour growth and invasion of human cancer cells [101,102].
Activation of MT1-MMP was prevented by Furin inhibitions, resulting in reduced processing of proMMP-2. Similar results were obtained
using a synthetic furin inhibitor [103]. However, the effect of these inhibitors on the activation of the secreted convertase sensitive MMPs,
such as MMP-11 (stromelysin-3), which is strongly expressed by tumour stroma [104], has not been reported. Convertase inhibitors
drastic MMPs, but in view of the essential roles of convertases in protein processing in many tissues, side effects could limit the dosage that
can be administered, and therefore limit efficacy in humans.

![Figure 5: Strategies for blocking proMMP activation.](image)

**a** | Active MMPs are generated through a multistep proteolytic cascade that involves plasminogen cleavage by urokinase to create
plasmin, which then cleaves proMMPs to create active MMPs. Some of these MMPs go on to cleave other proMMPs. MMP inhibitors
(MMPIs) block MMP generation from proMMPs. Serine proteinase inhibitors (PAIs) of urokinase block plasmin generation, and
plasmin inhibitors block the proMMP conversion to MMPs.

**b** | Blockade of cell-surface and furin-mediated activation of MMPs. ProMMP-2 activation occurs after the formation of a ternary
complex that contains proMMP-2 linked to cell-surface MT1-MMP via a tissue inhibitors of metalloproteinase (TIMP)-2 bridge. The TIMP-2 inhibitory amino (N) domain binds to the active site of MT1-MMP, inhibiting its proteolytic activity. TIMP-2 also binds MMP-2 outside the catalytic domain (C) on the outer rim of the hemopexin carboxy-terminal domain (HxC), at the junction of hemopexin modules III and IV [129]. The crystal structure of the complex proMMP-2/TIMP-2 has shown that the inhibitory N domain of TIMP-2 is not in contact with the HxC domain, and is also distant from the catalytic site of MMP-2 [130]. MT1-MMP has been shown to dimerize in forming the trimeric complex on the cell surface with MMP-2. MT2-MMP does not require TIMP-2 to bind or activate MMP-2 [108], and it is not known whether the other MT-MMPs form a complex with TIMP-2. Compounds that interfere with the activation of proMMP-2 include cathechins, anti-MT1-MMP antibody and MMPIs. MMP-2 HxC domain analogues and TIMP-2 domain analogues disrupt the TIMP-2/MMP interaction, and prevent MMP-2 activation. Endostatin and proteoglycans, which form complexes with MMP-2, inhibit processing and activation by MT1-MMP. Active MT1-MMP is generated through a proteolytic cascade from proMT1-MMP, which are generated by furin-like proprotein convertases. These convertases can be inhibited by inhibitors such as α1-PDX and human immunodeficiency virus (HIV) aspartyl protease inhibitors.

**Targeting MMP-2 Activation**

MMP activation can also be blocked by the use of thrombospondin-1 — an anti-angiogenic factor that inhibits proMMP-2 and proMMP-9 activation [28,29]. Similarly, thrombospondin-2 promotes MMP-2 endocytosis via the low-density lipoprotein-receptor-related protein pathway [27]. Endostatin forms a complex with MMP-2 and inhibits processing and activation by MT1-MMP [105], which might partially explain its antiangiogenic activity. Similarly proteoglycans, such as testican-3 and its splice-variant gene product N-Tes, can suppress proMMP-2 activation that is mediated by MT-MMPs, with the subsequent abrogation of invasive properties of glioma cells [106]. A potent inhibitor of proMMP-2 activation *in vitro* is recombinant hemopexin, indicating the feasibility of targeting this interaction to block MMP-2 activation *in vivo* [24,107,108]. Analogues of the TIMP-2 C-terminal domain might be used to compete for TIMP-2 binding to the MMP-2 hemopexin C-terminal domain, and so might prevent trimeric complex formation that is required for MMP-2 activation. Accordingly, MMPIs have both a direct effect in inhibiting active site MMPs and an indirect effect in blocking TIMP-2 binding and MMP-2 activation by MT1-MMP.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Structure</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marimastat (BB-2516)</td>
<td>Peptido mimetic</td>
<td>Broad spectrum</td>
<td>Survival benefit in a subset of gastric cancer patients Survival benefit in glioblastoma multiforme patients in combination with temozolomide Survival rate similar to gemcitabine in pancreatic cancer No survival benefit in SCL, NSCL and ovarian cancer patients</td>
</tr>
<tr>
<td>Tanomastat (BAY 12-9566)</td>
<td>Non-peptido mimetic</td>
<td>MMP-2, 3, 9</td>
<td>Development halted because treated patients showed poorer survival than controls</td>
</tr>
<tr>
<td>Prinomastat (AG3340)</td>
<td>Non-peptido mimetic</td>
<td>Broad spectrum</td>
<td>No survival benefits in NSCL cancer patients No difference in progression of prostate carcinomas.</td>
</tr>
<tr>
<td>Metastat (COL-3)</td>
<td>Tetracycline derivative</td>
<td>Gelatinases</td>
<td>Multiple mechanisms of action against MMPs Currently recruiting Kaposi’s sarcoma patients</td>
</tr>
<tr>
<td>Neovastat (AE-941)</td>
<td>Shark cartilage extract</td>
<td>Broad spectrum</td>
<td>Multiple mechanisms of action on MMPs Currently recruiting renal-cell carcinoma, multiple myeloma and NSCL cancer patients</td>
</tr>
<tr>
<td>BMS-275291</td>
<td>Non-peptido mimetic</td>
<td>Broad spectrum</td>
<td>Currently recruiting NSCL cancer patients</td>
</tr>
<tr>
<td>MMI270</td>
<td>Non-peptido mimetic</td>
<td>Broad spectrum</td>
<td>Anti-angiogenic and anti-metastatic effects in animal models Phase I studies in patients with advanced malignancies</td>
</tr>
</tbody>
</table>

Table 2: Matrix metalloproteinase inhibitors in clinical development for cancer therapy.

MMPs, matrix metalloproteinases; NSCL cancer, non-small-cell lung cancer; SCL cancer, small-cell lung cancer. Table adapted from Coussens et al., 2002; Hidalgo et al., 2001 [64,109].

**Natural Compounds as MMP-Inhibitors**

Matrix metalloproteinases have been heralded as promising targets for cancer therapy on the basis of their massive up-regulation in malignant tissues and their unique ability to degrade all components of the extracellular matrix. Preclinical studies testing the efficacy of MMP suppression in tumor models were so compelling that synthetic metalloproteinase inhibitors (MMPIs) were rapidly developed and routed into human clinical trials. The results of these trials were, however, disappointing.

**Natural inhibitors of MMPs**

Natural inhibitors of MMPs- TIMPs, were also used to block MMPs activity. Although they have demonstrated efficacy in experimental models, TIMPs may exert MMP-independent promoting effects [3]. To avoid the negative results and toxicity issues raised by the use of synthetic MMPIs, one answer was provided from the field of natural compounds. One compound taken into consideration was extracted from shark cartilage. Oral administration of a standardized extract, neovastat, exerts anti-angiogenic and anti-metastatic activities and inhibits processing and activation by MT1-MMP. Active MT1-MMP is generated through a proteolytic cascade from proMT1-MMP, which are generated by furin-like proprotein convertases. These convertases can be inhibited by inhibitors such as α1-PDX and human immunodeficiency virus (HIV) aspartyl protease inhibitors.

In 2004, Lambert et. al. reported that the matrix metalloproteinase inhibitors (MMPIs) may be derived from natural resources such as herbs, plants, fruits, and other agriculture products [111]. New and potentially beneficial compounds isolated from these sources were shown to exhibit some degree of MMP activity, but they were far less potent and specific than the TIMP family. These natural compounds included long chain fatty acids, epigallocatechin gallate (EGCG) and other polyphenols and flavonoids.

Perhaps the most thoroughly studied class of natural MMP inhibitors are the endogenous tissue inhibitors of metalloproteinases (TIMPs), of which four are currently known, designated as TIMP-1 through -4. It is assumed that the natural ratio of MMPs to TIMPs is tightly regulated, and a disruption in the natural balance between these two families is often associated with the progression of multiple disease states. Each of the four TIMPs forms a complex with the MMPs in a 1:1 stoichiometry, exhibiting high affinity, but varying degrees of selectivity.
MMPIs from marine natural products

**Marine saccharoid MMPIs:** Most of the marine saccharoid MMPIs inhibit MMP by direct down regulation of MMP-9 transcription or via inhibition of activator protein-1 (AP-1) pathway or nuclear factor κB (NF-κB) pathway.

Marine saccharoid MMPIs exhibit high MMPs inhibitory activity either by direct inhibition of the enzyme or by inhibiting the expression of MMPs. These have also shown low toxicity levels. However, due to high molecular weight of these MMPIs, the structure-activity relationship and the mechanism of the activity is hard to be addressed. If these shortcomings are overcome in the future, marine saccharoid MMPIs have a great potential to be used in clinical applications.

**Marine flavonoids and polyphenols MMPIs:** Flavonoid glycosides, isorhamnetin 3-O-b-D-glucosides, and quercetin 3-O-b-D-glucoside were isolated from *Salicornia herbacea* and their inhibitory effects on matrix metalloproteinase-9 and -2 were evaluated in human fibrosarcoma cell line [112].

Flavonoids and polyphenols MMPIs have excellent MMPs inhibitory activities; however they show a high toxicity level. Therefore, the pharmaceutical applications of these MMPIs are limited. Researchers should pay attention to reduce their toxicity levels by altering the structure in a way that it preserves the bioactivity. Then this class of MMPIs will gain a huge potential to be used in clinical applications.

**Marine fatty acid MMPIs:** Long-chain fatty acids can inhibit MMPs. However for different MMPs, the degree of inhibition is different. Oleic acid and elaidic acid can inhibit MMP-2 and MMP-9 with the micromole Kᵢ values, although their inhibitory effects on collagenase-1 (MMP-1) are weak [113]. The fatty acid chain length and its degree of saturation is related to the level of inhibition, as the fatty acids with long carbon chains show stronger inhibition than the short ones, and the non-saturation degree shows a positive correlation to the overall inhibitory capacity of the fatty acid chains. Fatty acids bind to neutrophil elastase, while the parinaric acids are inhibitors of neutrophil elastase. The fatty acids bind to plasmin for example, oleic acid can modulate fibrinolysis. It is well known that the marine fishes are rich in omega-3 long-chain polysaturated fatty acids (ω3 LC-PUFAs), especially eicosapentaenoic (EPA) and docosaheaxenoic acid (DHA). Suzuki et al. found that the inhibition of lung metastasis of a colon cancer cell line by EPA and DHA was associated with a reduced activity of MMP-9. A wide range of biological activities such as cytotoxicity [114], antimicrobial [115], antifouling [116], and enzyme inhibition are shown by acetylenic fatty acids isolated from marine sponges. Sodium 1-(12-hydroxy) octadecanoyl sulfite inhibits MMP-2. Callysponginol sulfate A, extracted from the marine sponge, *Callyspongia truncate*, inhibits recombinant MT1-MMP.

Other marine natural products MMPIs: The compounds extracted from shark cartilage (such as Neovastat®, AE-941, U-995) have been investigated for their potential use as MMPIs. Neovastat® inhibits enzymatic activity of MMP-2 with minor inhibition of MMP-1, -7, -9 and -13. TIMP-like proteins within AE-941 could be responsible for its specific MMP inhibitory property [117]. The Atlantic cod (Gadus morhua) muscle contains a 21-kDa proteinase inhibitor which has properties similar to human TIMP-2. The inhibitor was found to inhibit the gelatin-degrading enzymes present in the gelatin-bound fraction. In addition, it inhibited gelatinolytic activity obtained from a human macrophage cell medium rich in MMP-9 [118].

**Marine plants:** Metabolites from marine plants have outstanding biological activities. A highly effective anticoagulant and antiproliferative agent, sulfated polysaccharide, from brown alga *Ecklonia cava*, exhibited a promising antiproliferative effect on human promyelocytic leukemia (HL-60) and human leukemic monocyte lymphoma (U-937) cells. Fucoidan extracts from sea weed *Cladosiphon novae-zelandiae* reduce the cellular invasiveness in human fibrosarcoma HT1080 cells by suppressing the activity of MMP-2 and MMP-9. Further, it has been reported that these fucoidan extracts suppress the expression and secretion of an angiogenesis factor, vascular endothelial growth factor (VEGF); thereby reporting the inhibitory effects on invasion and angiogenesis of tumor cells [119].

Extracts from *Eisenia bicyclis*, *Ecklonia cava*, and *Ecklonia stolonifera* have strongly reduced MMP-1 expression via inhibiting both NF-kappa B and AP-1 reporter. Free radical scavenging activity of phlorotannin from *Ecklonia species* has been reported. Dieckol from marine brown alga, *E. cava* has been reported to suppress LPS-induced production of nitric oxide (NO), prostaglandin E2 (PGE2), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in murine BV2 microglia; thus establishing dieckol as a potent anti-inflammatory and neuroprotective agent. Kong et al. reported that flavonoid glycosides, isolated from this plant have inhibited the expression of MMP-2 and MMP-9 and elevated the TIMP-1 expression in human fibrosarcoma (HT1080) cells. Moreover, the down regulation of MMP-9 and MMP-2 by these flavonoids is due to the interference with the transcription factor AP-1, there by suggesting that these flavonoid glycosides can be used as potent natural chemopreventive agents for cancer. Phlorotannins from brown algae *E. cava* have been reported to have inhibitory activity on MMP-2 and MMP-9, signifying the role of phlorotannins as potential and safe marine derived MMPIs.

Miscellaneous natural products

Screening has led to the discovery of both synthetic and natural MMP inhibitors. The latter include tetracyclines, such as doxycycline and minocycline, for which it has been found that chemical modification can separate MMP activity from antibiotic activity [120]. Actinonin has been identified as an MMP inhibitor and it is a succinyl hydroxyacid that bears close structural similarity to compounds obtained by substrate based design. Although significant advances have been made in inhibitor design, it is still not clear how to design compounds that specifically inhibit individual MMPs in spite of the available structural data. This remains a major challenge for MMP inhibitor medicinal chemistry [121].

**Other natural compounds**

Fujita et al., 2003 reported that ageladine A, a fluorescent alkaloid isolated from the marine sponge *Agelas nakamura*, inhibits MMP-1, -8, -9, -12, and -13. This compound could also inhibit MMP-2 but N-methylated derivatives did not inhibit MMP-2. The inhibition is not due to Zn⁺⁺ chelation, as ageladine is not capable of chelating to Zn⁺⁺, and a kinetic analysis indicated that the inhibition was not competitive. In addition, bovine aortic endothelial cell migration and vascular formation by murine ES cells were significantly inhibited by this compound [122].

**Future Perspective**

Research into neoepitopes will provide important and novel means of diagnosis, prognostics and increasing treatment efficacy in cancer. However, to fully take advantage of neoepitopes as highly valuable cancer biomarkers, it is very important to understand the
physiological mechanisms and signalling pathways that regulate their generation. Thus, the ultimate goal of new diagnostic tests should be to use highly reliable non-invasive mechanism-based biomarkers. At present, receptors, cell adhesion molecules, growth factors and enzymes, with their related protein substrates (e.g., MMPs and extracellular matrix components), are all hot research areas in the development of cancer drugs and diagnostic assays [123].

In the past year, further evidence establishing the usefulness of β interferons and glatiramer in the treatment of relapsing-remitting multiple sclerosis has been advanced. Interferon-β-1b was also shown to be efficacious in secondary progressive multiple sclerosis. There are more than 20 MMPs identified that share several common features: signal peptides, prodomain, and prodomain, and catalytic domain, with at least eight of these proteins clustered on chromosome 11 (MMPs -1, -3, -7, -8, -10, -12, -13, and -20), probably due to a gene duplication event [124]. Johnson, PR et al., reported in 2001, that although the healthy adult lung is not a major source of MMPs, parenchymal cells such as airway epithelium, fibroblast, and smooth muscle have the capacity to express active MMPs following stimulation by a variety of agents such as infectious pathogens, environmental toxins, growth factors, and cytokines [125]. Lopez-Boado et al. 2000 reported a 25-fold induction of MMP-7 in the lung epithelial cells following infection with Escherichia coli and Pseudomonas aeruginosa, which could explain the up regulation of this enzyme in the airway of cystic fibrosis patients who are commonly infected with bacteria. It also has been shown that proinflammatory cytokines such as interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), upregulate the expression of MMP-9 in human airway epithelial cells following a 1-day treatment [126]. Additionally, inflammatory cells invading the lung during the course of COPD are also a major source of different MMPs. It has been shown that the neutrophils and macrophages, the predominant inflammatory cells in the lungs of COPD patients, have the capacity to release MMPs -2, -3, -7, -9, and -12 [127].

Future of Cell Utilization for Disc Disease

Despite the growing number of research data on cell-based experimental therapy for IVD disease, it is clear that we do not know much about native disc cells and their microenvironment. This lack of information is a major obstacle in building a strategy for the treatment of IVD disease. Investigating the specific differentiation status of native IVD cells and their homeostasis will surely provide more ideas and clues for efficient therapeutic approaches. Although cell-based therapy for IVD disease is still in its infancy, the stage of testing a variety of cells for injection should be toned. To progress to the next step, we should be investigating what exactly IVD cells are, and how they control their homeostasis, along with various studies optimising parameters, such as cell dosage and culture period and the severity of IVD degeneration in the recipient [128].

Moreover, there is the need for attention to the stage and type of cancer that is likely to be evaluated in clinical versus preclinical studies. For example, the selection of advanced pancreatic and lung cancers for clinical trials was based on considerations such as expected survival time and patient availability, both of which affect the time and financial resources required to achieve statistically significant results. Patent issues, competition, and impatience contributed to the decision to proceed at an unprecedented pace in an inappropriate setting, and these factors will undoubtedly continue to influence drug development decisions in the future.

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