

NEW FUNCTIONS FOR THE MATRIX METALLOPROTEINASES IN CANCER PROGRESSION

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Matrix metalloproteinases (MMPs) have long been associated with cancer-cell invasion and metastasis. This provided the rationale for clinical trials of MMP inhibitors, unfortunately with disappointing results. We now know, however, that the MMPs have functions other than promotion of invasion, have substrates other than components of the extracellular matrix, and that they function before invasion in the development of cancer. With this knowledge in hand, can we rethink the use of MMP inhibitors in the clinic?

EXTRACELLULAR MATRIX
All secreted molecules that are immobilized outside cells, see BOX 1.

CLINICAL TRIALS
Phase I: the first testing of a new drug in humans. A small group of people is tested to determine drug safety, safe dosage range and side effects. Phase II: the drug is tested on a larger group of patients to see if it is effective and to further evaluate its safety. Phase III: conducted on a large group of patients to confirm the effectiveness, monitor side effects and compare the drug to commonly used treatments.

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Basic cancer research has mainly focused on mutations in cancer cells that result in either gain-of-function in oncogenes or loss-of-function in tumour-suppressor genes¹. However, the **EXTRACELLULAR MATRIX** (ECM) of tumours and the non-cancerous, stromal cells within tumours also have an important impact on tumour progression². Matrix metalloproteinases (MMPs) can regulate the tumour microenvironment, and their expression and activation is increased in almost all human cancers compared with normal tissue.

MMPs are proteolytic enzymes and their basic mechanism of action — degradation of proteins — regulates various cell behaviours with relevance for cancer biology. These include cancer-cell growth, differentiation, apoptosis, migration and invasion, and the regulation of tumour angiogenesis and immune surveillance.

Pharmaceutical inhibitors of MMPs have been developed, but the results from **CLINICAL TRIALS** with these drugs have proved disappointing. However, these first clinical trials were designed on the basis that MMPs were important for the late steps of tumour progression — that is, invasion and metastasis. With the current understanding of the diverse role of MMPs — particularly their relevance for the early steps in cancer progression, such as angiogenesis — these disappointing results seem less surprising. To better target MMPs for cancer treatment, an appreciation of their many functions — often acting in opposing directions in cancer progression — is needed.

The MMP family

The MMPs are endopeptidases that — between the > 21 human MMPs and the homologues from other species — can cleave virtually any component of the ECM (**ONLINE TABLE 1**). Historically, the MMPs were divided into collagenases, gelatinases, stromelysins and matrilysins on the basis of their specificity for ECM components, and the common names of the MMPs reflect this classification (**TABLE 1**). However, as the list of MMP substrates has grown, a sequential numbering system for the MMPs has been adapted, and the MMPs are now grouped according to their structure. There are eight distinct structural classes of MMPs: five are secreted and three are membrane-type MMPs (MT-MMPs) (**FIG. 1**).

Many of the extracellular signalling events that regulate cell behaviour occur at or near the cell membrane and are regulated by pericellular proteolysis³. The MT-MMPs are covalently linked to the cell membrane, which is the most obvious way of tethering MMP activity to the cell membrane. The secreted MMPs can, however, also localize to the cell surface by binding to integrins⁴ or to **CD44** (**REFS 5,6**), or through interactions with cell-surface-associated heparan sulphate proteoglycans, **collagen type IV** or the extracellular matrix metalloproteinase inducer (**EMMPRIN**) (reviewed in **REF. 7**; **ONLINE TABLE 1**).

Regulation of MMP activity

The MMPs are synthesized as inactive **ZYMOGENS** (pro-MMPs). They are kept inactive by an interaction

Summary

- The matrix metalloproteinases (MMPs) comprise a family of zinc-dependent endopeptidases that consist of more than 21 human MMPs and numerous homologues from other species. They can be divided into eight structural classes, three of which are membrane bound.
- The MMPs are synthesized as inactive zymogens and activated by proteinase cleavage. Their activity is regulated by endogenous inhibitors, including α 2-macroglobulin; tissue inhibitors of metalloproteinases (TIMPs); small molecules with TIMP-like domains; and the membrane-bound inhibitor RECK (reversion-inducing cysteine-rich protein with kazal motifs).
- Direct evidence for a role of MMPs in tumour progression comes from xenograft experiments using cancer cells with decreased and increased expression levels of MMPs or TIMPs, and from carcinogenesis experiments with mice that either lack a specific Mmp or Timp-1 or have organ-specific Mmp or Timp-1 overexpression.
- MMPs are upregulated in almost every type of human cancer, and their expression is often associated with poor survival. Whereas some of the MMPs (for example, MMP-7) are expressed by the cancer cells, other MMPs (for example, MMP-2 and MMP-9) are synthesized by the tumour stromal cells, including fibroblasts, myofibroblasts, inflammatory cells and endothelial cells.
- MMPs can promote cancer progression by increasing cancer-cell growth, migration, invasion, metastasis and angiogenesis. MMPs exert these effects by cleaving a diverse group of substrates, which include not only structural components of the extracellular matrix, but also growth-factor-binding proteins, growth-factor precursors, receptor tyrosine kinases, cell-adhesion molecules and other proteinases.
- Several synthetic MMP inhibitors are undergoing Phase III clinical trials. Although a few encouraging results have been reported, some trials were prematurely terminated due to either lack of benefits or major adverse effects.
- The clinical trials have so far focused on patients with advanced-stage disease. Based on animal experiments, we would expect, however, that clinical efficacy might be improved either by using MMP inhibitors in the treatment of early disease (in combination with conventional therapy), or as preoperative and postoperative treatment to prevent surgical-induced micrometastatic spread and recurrence of the disease.

ZYMOGEN

An enzyme that is secreted by cells as an inactive precursor. Activation of the enzymes occurs as one or more peptide bonds in the zymogen are cleaved.

INTRACELLULAR FURIN-LIKE SERINE PROTEINASES

A family of intracellular proteinases, including furin, that are localized in the *trans*-Golgi network, where they have an important role in the intracellular processing of secreted proteins.

SCAVENGER RECEPTORS

A broad class of receptors that 'scavenge' cellular debris: the ligands are endocytosed and subsequently degraded. Scavenger receptors also have other activities, such as adhesion.

between a cysteine-sulphydryl group in the propeptide domain and the zinc ion bound to the catalytic domain: activation requires proteolytic removal of the propeptide prodomain (reviewed in REF 7). Most of the MMPs are activated outside the cell by other activated MMPs or serine proteinases. However, MMP-11, MMP-28 and the MT-MMPs can also be activated by INTRACELLULAR FURIN-LIKE SERINE PROTEINASES before they reach the cell surface (reviewed in REF 7).

MMP-2 is activated at the cell surface through a unique multistep pathway that involves MMP-14 (MT1-MMP) and the tissue inhibitor of metalloproteinases 2 (TIMP-2) (REF 8): TIMP-2 binds MMP-14 at its amino terminus and pro-MMP-2 at its carboxyl terminus, which allows an adjacent, non-inhibited MMP-14 to cleave the bound pro-MMP-2. MMP-14 does not fully activate MMP-2 and another, already activated, MMP-2 is required to remove a residual portion of the MMP-2 propeptide⁹. Pro-MMP-2 might also be activated by MMP-15 by means of a mechanism that does not require TIMP-2 (REF 10).

MMP activity is tightly controlled by endogenous inhibitors. The main inhibitor of MMPs in tissue fluids is α 2-macroglobulin, an abundant plasma protein¹¹. α 2-Macroglobulin binds to MMPs and the

α 2-macroglobulin-MMP complex then binds to a SCAVENGER RECEPTOR and is irreversibly cleared by endocytosis. In a similar way to α 2-macroglobulin, thrombospondin-2 forms a complex with MMP-2 and facilitates scavenger-receptor-mediated endocytosis and clearance¹². By contrast, thrombospondin-1 binds to pro-MMP-2 and -9 and directly inhibits their activation^{13,14}. Curiously, thrombospondin-1 has also been reported to increase MMP-2 and -9 activation¹⁵. The best-studied endogenous MMP inhibitors are TIMPs -1, -2, -3 and -4, which reversibly inhibit MMPs in a 1:1 stoichiometric fashion (reviewed in REF 16). They differ in tissue-specific expression and ability to inhibit various MMPs (reviewed in REF 16; ONLINE TABLE 2). Studies with Timp-2-deficient mice indicate that the dominant physiological function of TIMP-2 is activation of MMP-2 (REF 17). MMP inhibitors that contain subdomains with structural similarity to the TIMPs also exist, and these include the carboxy-terminal fragment of the procollagen C-terminal proteinase enhancer protein¹⁸ and the NC1 domain of collagen type IV¹⁹. Finally, RECK (reversion-inducing cysteine-rich protein with kazal motifs) is the only known membrane-bound MMP inhibitor²⁰.

MMP substrates

Historically, MMPs were thought to predominantly degrade structural components of the ECM (BOX 1), thereby facilitating cell migration. But because cells have receptors for structural ECM components (for example, integrins), cleavage of ECM proteins by MMPs also affects cellular signalling and functions²¹. Cleavage of ECM components by MMPs can also generate fragments with new functions: cleavage of laminin-5 and collagen type IV results in exposure of CRYPTIC SITES that promote migration^{22,23}. Moreover, cleavage of insulin-like growth-factor-binding protein (IGF-BP) and perlecan releases IGFs and fibroblast growth factors (FGFs), respectively²⁴⁻²⁶.

In addition to cleaving structural ECM components, MMPs and the related proteinases, the ADAMS (a disintegrin and metalloproteinases), participate in the release of cell-membrane-bound precursor forms of many growth factors, including transforming growth factor- α (TGF- α)²⁷. Bioavailability of TGF- β is regulated differently: it is released by MMP-2 and MMP-9 from an inactive extracellular complex²⁸.

Growth-factor receptors are also MMP substrates. The FGF receptor 1 is cleaved by MMP-2 (REF 29), whereas 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 ADAMS³⁰⁻³². In all cases, extracellular domains of the receptors are released, and these might function as decoy receptors for the respective ligands.

Cell-adhesion molecules are also MMP substrates. Cleavage of E-cadherin and CD44 results in the release of fragments of the extracellular domains and in increased invasive behaviour^{33,34}, and cleavage of

Table 1 | The matrix metalloproteinase family

MMP designation*	Structural class	Common name(s)
MMP-1	Simple hemopexin domain	Collagenase-1, interstitial collagenase, fibroblast collagenase, tissue collagenase
MMP-2	Gelatin-binding	Gelatinase A, 72-kDa gelatinase, 72-kDa type IV collagenase, neutrophil gelatinase
MMP-3	Simple hemopexin domain	Stromelysin-1, transin-1, proteoglycanase, procollagenase-activating protein
MMP-7	Minimal domain	Matrilysin, matrin, PUMP1, small uterine metalloproteinase
MMP-8	Simple hemopexin domain	Collagenase-2, neutrophil collagenase, PMN collagenase, granulocyte collagenase
MMP-9	Gelatin-binding	Gelatinase B, 92-kDa gelatinase, 92-kDa type IV collagenase
MMP-10	Simple hemopexin domain	Stromelysin-2, transin-2
MMP-11	Furin-activated and secreted	Stromelysin-3
MMP-12	Simple hemopexin domain	Metalloelastase, macrophage elastase, macrophage metalloelastase
MMP-13	Simple hemopexin domain	Collagenase-3
MMP-14	Transmembrane	MT1-MMP, MT-MMP1
MMP-15	Transmembrane	MT2-MMP, MT-MMP2
MMP-16	Transmembrane	MT3-MMP, MT-MMP3
MMP-17	GPI-linked	MT4-MMP, MT-MMP4
MMP-18	Simple hemopexin domain	Collagenase-4 (<i>Xenopus</i> ; no human homologue known)
MMP-19	Simple hemopexin domain	RASI-1, MMP-18 [†]
MMP-20	Simple hemopexin domain	Enamelysin
MMP-21 [‡]	Vitronectin-like insert	Homologue of <i>Xenopus</i> XMMP
MMP-22	Simple hemopexin domain	CMMP (chicken; no human homologue known)
MMP-23	Type II transmembrane	Cysteine array MMP (CA-MMP), femalysin, MIFR, MMP-21/MMP-22
MMP-24	Transmembrane	MT5-MMP, MT-MMP5
MMP-25	GPI-linked	MT6-MMP, MT-MMP6, leukolysin
MMP-26	Minimal domain	Endometase, matrilysin-2
MMP-27 [#]	Simple hemopexin domain	
MMP-28	Furin-activated and secreted	Epilysin
No designation	Simple hemopexin domain	Mcol-A (Mouse)
No designation	Simple hemopexin domain	Mcol-B (Mouse)
No designation	Gelatin-binding	75-kDa gelatinase (chicken)

*MMP-4, -5 and -6 have been abandoned. [†]When MMP-19 was cloned it was initially called MMP-18 (REF. 147). 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 described¹⁴⁸, 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 (REFS 149,150). [¶]Gururajan and colleagues identified two new MMP genes, which they called *MMP21* and *MMP22*. The nucleotide sequences of the two genes are almost identical⁴⁸, so they are now designated *MMP23A* and *MMP23B*. [#]Sequence submitted to GenBank (access no. AF195192). GPI, glycosylphosphatidylinositol; MMP, matrix metalloproteinase; MT-MMP, membrane type MMP; PMN, polymorphonuclear neutrophil; PUMP, putative metalloproteinase.

CRYPTIC SITE

Part of a protein that is normally hidden within the three-dimensional structure. The cryptic site might be exposed following conformational changes in the protein — for example, as a result of proteolytic cleavage.

ADAM

(A disintegrin and metalloproteinase). A family of transmembrane proteinases with metalloproteinase, disintegrin (integrin-binding), cysteine-rich and epidermal growth factor (EGF)-like domains. ADAMs participate in cell-surface proteolysis and cleave substrates such as tumour necrosis factor- α and Notch.

the α v integrin subunit precursor by MMP-14 enhances cancer-cell migration³⁵. Finally, the MMPs cleave and activate their own zymogen forms and, in addition, cleave other MMPs and proteinase inhibitors such as **serpins** (reviewed in REF. 7).

MMPs in experimental cancer models

The evidence for MMPs as active contributors to cancer progression comes from animal studies. In transplantation assays, relatively benign cancer cells acquire malignant properties when MMP expression is upregulated. Conversely, highly malignant cells become less aggressive when MMP expression or activity is reduced (reviewed in REF. 36).

Stromal cells also secrete MMPs, which affects tumour aggressiveness: in contrast to wild-type fibroblasts, *MMP11* null fibroblasts do not support the *in vivo* growth of **breast cancer** cells³⁷. Furthermore, after intravenous injection, cancer cells are less capable of colonizing the lungs of *Mmp2*- or -9-deficient mice than the lungs of wild-type mice^{38,39}.

In mice, tissue-specific overexpression of *Mmp1* or *Mmp7* leads to hyperproliferative disease and increased cancer susceptibility (reviewed in REF. 7; TABLE 2). In addition, expression of *Mmp3* or *14* in the mammary gland results in spontaneous breast cancers^{40,41} (FIG. 2a). Conversely, mice that lack *Mmp2*, *7*, *9* or *11*, or overproduce *Timp1*, develop fewer cancers than wild-type

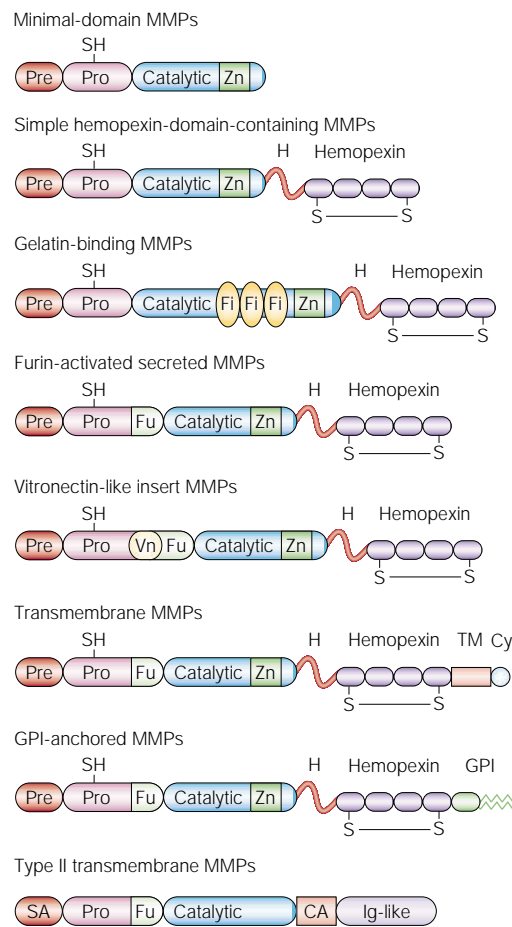


Figure 1 | The protein structure of the MMPs. Matrix metalloproteinases (MMPs) can be divided into eight distinct structural groups, five of which are secreted and three of which are membrane-type MMPs (MT-MMPs). Secreted MMPs: The minimal-domain MMPs contain an amino-terminal signal sequence (Pre) that directs them to the endoplasmic reticulum, a propeptide (Pro) with a zinc-interacting thiol (SH) group that maintains them as inactive zymogens and a catalytic domain with a zinc-binding site (Zn). In addition to the domains that are found in the minimal domain MMPs, the simple hemopexin-domain-containing MMPs have a hemopexin-like domain — that is connected to the catalytic domain by a hinge (H) — which mediates interactions with tissue inhibitors of metalloproteinases, cell-surface molecules and proteolytic substrates. The first and the last of the four repeats in the hemopexin-like domain are linked by a disulphide bond (S–S). The gelatin-binding MMPs contain inserts that resemble collagen-binding type II repeats of fibronectin (Fi). The furin-activated secreted MMPs contain a recognition motif for intracellular furin-like serine proteinases (Fu) between their propeptide and catalytic domains that allows intracellular activation by these proteinases. This motif is also found in the vitronectin-like insert (Vn) MMPs and the membrane-type MMPs (MT-MMPs). MT-MMPs: MT-MMPs include transmembrane MMPs that have a carboxy-terminal, single-span transmembrane domain (TM) and a very short cytoplasmic domain (Cy), and the glycosylphosphatidylinositol (GPI)-anchored MMPs. MMP-23 represents a third type of membrane-linked MMP. It has an N-terminal signal anchor (SA) that targets it to the cell membrane, and so is a type II transmembrane MMP. MMP-23 is also characterized by its unique cysteine array (CA) and immunoglobulin (Ig)-like domains. Adapted from REF. 7.

mice (reviewed in REF. 7; TABLE 2; FIG. 2). Notably, the development of squamous-cell carcinomas in the *Mmp9*-null mice is restored by transplanting *Mmp9*-expressing bone marrow cells, showing the importance of the inflammatory cells in carcinogenesis⁴² (FIG. 2b).

MMPs and TIMPs in human cancer

The expression and activity of MMPs are increased in almost every type of human cancer, and this correlates with advanced tumour stage, increased invasion and metastasis, and shortened survival (ONLINE TABLE 3). So, the clinical data strongly support a role for MMPs in the progression of human cancer.

Most studies show a negative association between MMPs and prognosis. However, there are a few cases in which increased expression of specific MMPs reflects a favourable prognosis. In **colon cancer**, for example, **MMP-12** expression by the carcinoma cells is associated with increased survival⁴³, and MMP-9 expression by infiltrating macrophages is associated with reduced metastases⁴⁴. Whether MMP activity is required for generating antitumour molecules such as pro-apoptotic factors, or for recruitment of cytotoxic T-cells, or whether MMP activity in these cases is irrelevant and the MMPs are just markers of differentiation of the cancer cells or of an immune reaction, remains to be determined.

Several clinical studies find, however, that high levels of TIMP-1 and -2 also correlate with a poor prognosis (ONLINE TABLE 3). This might reflect the fact that the balance between expression of MMPs and TIMPs, although still favouring the MMPs, is at a higher overall level during the increased matrix remodelling that occurs in tumour progression. High TIMP levels would therefore be associated with tumour progression and a worse prognosis, but would not cause it.

It does, however, seem that in some experimental settings the TIMPs might favour cancer progression: TIMP-1 and -2 can inhibit tumour-cell apoptosis, TIMP-2 and -3 can promote tumour-cell growth, and TIMP-1 can promote tumour angiogenesis (ONLINE TABLE 2). It is not clear whether these functions are results of proteinase inhibition, as the TIMPs also seem to have proteinase-independent activities that include upregulation of the anti-apoptotic protein **BCL-X_L**⁴⁵ and growth-factor-like effects⁴⁶. Furthermore, TIMPs can upregulate vascular endothelial growth factor (**VEGF**) secretion and thereby tumour angiogenesis⁴⁷. Significantly, animal experiments show that TIMP-2, despite its name, is an important activator of MMP-2 (REF. 17).

Regulation of MMPs in tumours

Unlike classical oncogenes, MMPs are not upregulated by gene amplification or activating mutations. The only two reported genetic alterations in cancer cells are translocation of the **MMP23** genes in **neuroblastoma**⁴⁸ and amplification of the **MMP24** gene⁴⁹. So, the increased MMP expression in tumours is probably due to transcriptional changes rather than genetic alterations. This might be the result of activation of oncogenes or

Box 1 | The extracellular matrix

The extracellular matrix (ECM) supports adhesion of cells and transmits signals through cell-surface adhesion receptors. The ECM contains collagens, non-collagenous glycoproteins and proteoglycans. Alternative ECM constituents — such as **tenascin**, **fibronectin** and variant isoforms of laminin — are found in tumours and might stimulate cancer progression². The basement membrane (BM) is a specialized ECM that separates the epithelial cells from the underlying stroma, thereby providing the first barrier against invasion of carcinomas.

Collagens

Collagen molecules have three α -chains that form a triple helix¹⁴¹. Fibrillar collagens (types I, II, III, V and XI) form fibrils and influence cellular functions through interactions with integrins. Basement membrane (BM) collagens (type IV collagens) are network-forming collagens of the BM. Cells interact with collagen type IV molecules through integrins, laminin and heparan-sulphate proteoglycans. Proteolytic fragments of these collagens (for example, tumstatin) can inhibit tumour angiogenesis¹⁴². The 'multiple-triple-helix domains with interruptions' collagens — the multiplexins (type XV and XVIII) — are mainly found in the BM of internal organs. A proteolytic fragment of the $\alpha 1$ chain of collagen type XVIII, known as endostatin, is an inhibitor of angiogenesis⁹¹. There are several other groups of collagens, including the transmembrane collagens that are a part of focal adhesion sites. Gelatin is denatured collagen, the product of collagenase-digested collagens.

Glycoproteins

Laminins are heterotrimeric glycoproteins that are composed of α , β and γ chains. They are primarily located in BMs, where they form networks with collagen IV and **nidogen**. Laminins affect cellular functions by binding to integrins and non-integrin receptors¹⁴³. Fibronectins are dimeric glycoproteins that are present in the ECM and in the blood. They form fibrils and affect cell morphology, adhesion, migration and differentiation by binding to integrins^{144,145}.

Proteoglycans

Proteoglycans have post-translational modifications of glycosaminoglycan chains such as heparan, **keratan** and chondroitin sulphate. Perlecan is the most common heparan-sulphate proteoglycan of the BM and is also found in tumour stroma. Other important proteoglycans are **decorin**, which 'decorates' collagen fibrils, and **aggrecan**, which is found as large 'aggregates' in cartilaginous tissues. **Versican** is the main chondroitin-sulphate proteoglycan of non-cartilaginous tissues, and **syndecans**, glypicans and CD44 are cell-surface proteoglycans¹⁴⁶. Hyaluronan is also a glycosaminoglycan, but not a proteoglycan, as it is not covalently attached to any protein.

loss of tumour suppressors; for example, **MMP7** is upregulated through combined activation of the transcription factors PEA3, **c-JUN**, **β -catenin** and **LEF-1** (REF. 50), all downstream of classical oncogenes, and the transcription of **MMP1** and **MMP13** is repressed by the tumour suppressor **p53** (REFS 51,52).

In human tumours, cancer cells are not the only source of MMPs. Whereas some MMPs are synthesized by cancer cells (for example, **MMP7**), many other MMPs (including **MMP2** and **9**) are predominantly made by stromal cells (ONLINE 3; FIG. 3). Cancer cells might stimulate tumour stromal cells to synthesize MMPs in a paracrine manner through secretion of interleukins, interferons, emmprin and growth factors (reviewed in REF. 7). MMPs that are secreted by stromal cells can still be recruited to the cancer-cell membrane. Indeed, **MMP2** mRNA is expressed by stromal cells of human breast tumours, whereas MMP-2 protein is found on both stromal and cancer-cell membranes⁵³.

Despite the general lack of genetic alterations in the MMP genes of cancer cells, polymorphisms in MMP promoters exist that affect gene transcription and influence cancer susceptibility. A single nucleotide polymorphism in the **MMP1** promoter contains either one or two guanines (G). The presence of two Gs creates a functional **ETS**-binding site that is adjacent to an AP-1 site and enhances transcription, so MMP-1 protein expression is higher in tumours from patients who carry

the 2G allele than in 1G homozygotes⁵⁴. The frequency of the 2G polymorphism is higher in cancer patients than in the healthy population⁵⁴. In addition, 2G homozygotes are more likely to develop invasive tumours^{55,56}. Another polymorphism that is linked with cancer is in the promoter of the **MMP3** gene, which contains either five or six adenosines (A). The frequency of homozygotes for the 6A allele, which has half the transcriptional activity of the 5A allele⁵⁷, is lower in cancer patients than in the control population⁵⁸. The differences in allele transcription caused by polymorphisms in the **MMP** promoters are subtle compared with the overexpression that arises from the amplification of oncogenes. Nevertheless, the increased level of MMPs over a lifetime might foster increased susceptibility to tumorigenesis, resembling the observations in transgenic mice that overexpress MMPs.

MMPs and the hallmarks of cancer

Six fundamental alterations in cell physiology underlie cancer progression: self-support in growth signals; insensitivity to growth-inhibitory signals; escape from apoptosis; infinite replication; sustained angiogenesis; and tissue invasion and metastasis¹. Originally, MMPs were considered to be important almost exclusively in invasion and metastasis; however, recent studies document that MMPs are involved in several steps of cancer development (FIG. 4).

Table 2 | Matrix metalloproteinases promote carcinogenesis in genetically modified mice

Genotype	Carcinogenic stimulus	Phenotype	Reference
<i>Haptoglobin-Mmp1</i>	None	Hyperkeratosis, acanthosis	151
	DMBA + PMA	↑ Skin carcinogenesis	151
	DMBA + chrysarobin	↑ Skin papilloma formation	152
<i>WAP-Mmp3</i>	None	Mammary hyperplasia and cancer	40
<i>MMTV-Mmp3</i>	None	Mammary hyperplasia and cancer	153
	DMBA	↓ Mammary hyperplasia and cancer*	64
<i>MMTV-Mmp7</i>	None	Mammary hyperplasia	154
	<i>MMTV-HER2/neu</i>	↑ Mammary carcinogenesis	154
<i>MMTV-Mmp14</i>	None	Mammary hyperplasia and cancer	41
<i>Mmp2^{-/-}</i>	<i>RIP-TAg</i>	↓ Pancreatic carcinogenesis	59
	Injected cells	↓ Angiogenesis and tumour growth	38
<i>Mmp7^{-/-}</i>	<i>Apc^{Min}</i>	↓ Intestinal adenoma formation	155
<i>Mmp9^{-/-}</i>	<i>K14-HPV16</i>	↓ Skin carcinogenesis	42
	<i>RIP-TAg</i>	↓ Pancreatic carcinogenesis	59
	Injected cells	↓ Experimental metastasis	39
<i>Mmp11^{-/-}</i>	DMBA	↓ Mammary carcinogenesis	37
	Injected cells	↓ Tumour-cell survival and growth	69
<i>H2-Timp1</i>	<i>Crp-TAg</i>	↓ Liver carcinogenesis	156
	Injected cells	↓ Tumour growth and spontaneous metastasis	107
<i>WAP-Timp1</i>	<i>WAP-Mmp3</i>	↓ Mammary neoplasia	40
<i>Albumin-Timp1</i>	DMBA + PMA	↓ Mammary carcinogenesis	157
<i>LFABP-Timp1</i>	<i>Apc^{Min}</i>	↑ Intestinal adenoma formation [†]	158
<i>MT-Timp1</i>	Injected cells	↓ Experimental brain metastasis	108
<i>H2-as-Timp1</i>	<i>Crp-TAg</i>	↑ Liver carcinogenesis	156
	Injected cells	↑ Tumor growth and infiltrating metastases	107

*The decreased tumour incidence in the *Mmp3*-overexpressing mice might be the result of increased apoptosis in the *Mmp3*-overexpressing mammary glands⁶⁴. [†]Increased numbers of adenomas were observed in the transgenic line with the lowest expression, out of two tested. In four out of six tumours assayed, the expression of *Timp1* was lost¹⁵⁸. *Apc^{Min}*, adenomatous polyposis coli gene with multiple intestinal neoplasia mutation; as, antisense; *Crp*, C-reactive protein gene promoter; DMBA, dimethylbenz[*a*]anthracene; *H2*, major histocompatibility complex class I promoter; *HPV16*, human papillomavirus 16 early region; *K14*, keratin-14 promoter; *LFABP*, liver fatty-acid-binding-protein promoter; *MMTV*, mouse mammary tumour virus promoter; *MT*, metallothionein-1 promoter; PMA, phorbol myristate acetate; *RIP*, rat insulin II promoter; *TAg*, SV40 T antigens; *WAP*, whey-acidic-protein promoter. Adapted from REF. 7.

MMPs regulate growth. Cancer-cell proliferation is decreased in tumours from *Mmp9*-deficient mice compared with wild-type mice^{42,59} (FIG. 2b), indicating that MMPs generate growth-promoting signals. There are three known ways in which MMPs can promote cancer-cell proliferation (FIG. 4a). First, MMPs or ADAMs release the cell-membrane-bound precursors of some growth factors — for example, TGF- α ²⁷. Second, peptide growth factors that are sequestered by ECM proteins become bioavailable once these proteins are degraded by MMPs. For example, IGFs are released when IGF-BPs are cleaved by MMPs^{24,25} (FIG. 4a). Third, through their effects on the ECM composition, the MMPs might indirectly regulate proliferative signals through integrins⁶⁰. MMPs might also negatively regulate cancer-cell growth, by means of activation of TGF- β ⁶¹ or generation of pro-apoptotic molecules such as FAS ligand (FASL) or TNF- α .

MMPs regulate apoptosis. Evasion of apoptosis permits survival in the presence of genetic instability; low levels of oxygen and nutrients; attacks from the immune system; anticancer treatment; and detachment from the ECM — a necessary step in metastasis⁶². MMPs have both apoptotic and anti-apoptotic actions (FIG. 4b).

MMP-3, -7, -9 and -11 regulate apoptosis. MMP-3 induces apoptosis when overexpressed in mammary epithelial cells^{63,64}, possibly by degrading laminin⁶⁵. MMP-7 releases membrane-bound FASL, a transmembrane stimulator of the death receptor FAS^{66,67}. Released FASL induces apoptosis of neighbouring cells⁶⁶, or decreases cancer-cell apoptosis⁶⁷, depending on the system. In addition, MMP-7 inhibits apoptosis by cleaving pro-heparin-binding epidermal growth factor (pro-HB-EGF) to generate mature HB-EGF, which promotes cell survival by stimulating the ERBB4 receptor tyrosine kinase⁶. MMP-11 also inhibits cancer-cell apoptosis: overexpression of *MMP11* decreases spontaneous apoptosis in tumour XENOGRAFTS⁶⁸. Conversely, cancer cells injected into *Mmp11*-null mice have a higher rate of spontaneous apoptosis than in wild-type hosts⁶⁹. MMP-11 might inhibit apoptosis by releasing IGFs²⁴, which can act as survival factors⁷⁰. Although MMP-9 (REF. 59) and -11 (REF. 69) decrease cancer-cell apoptosis, they increase apoptosis during development^{71,72}.

MMPs or ADAMs are also part of the apoptotic process: they cleave VE-cadherin⁷³, PECAM-1 (REF. 74) and E-cadherin⁷⁵ during apoptosis of endothelial or epithelial cells. Shedding of these adhesion molecules might contribute to the typical rounding up of apoptotic cells.

XENOGRAFT
Transplantation of tissue or cells from one species to another. In cancer research, most xenografts are human cancer-cell lines or human tumours that have been transplanted to immune-deficient rodents.

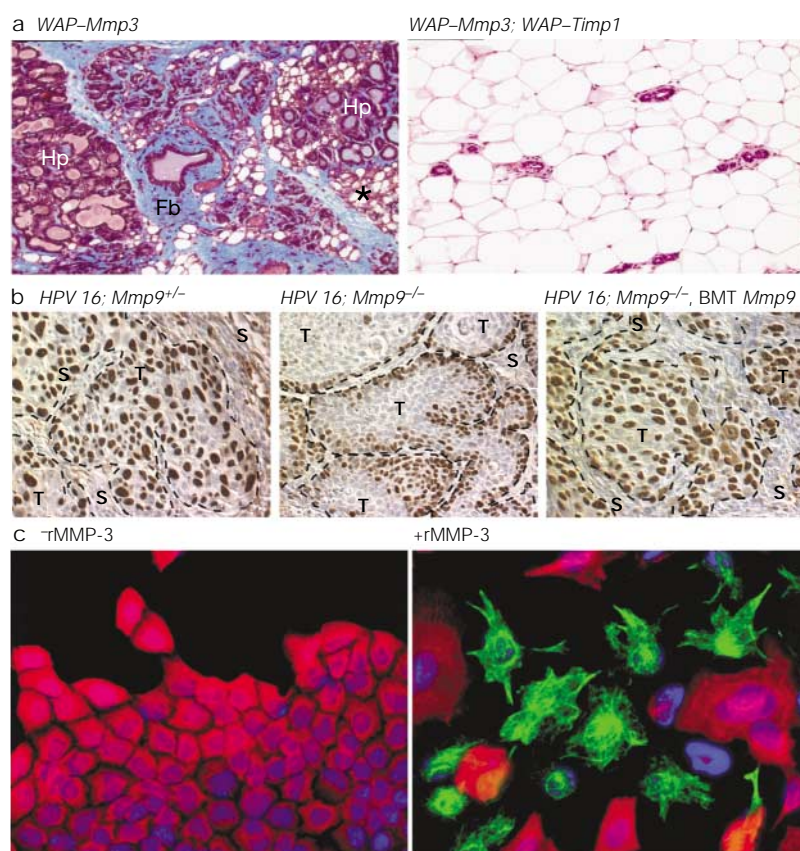


Figure 2 | Properties of MMPs in models of cancer. a | Overexpression of active *Mmp3* under the whey-acidic-protein promoter (WAP) in the mammary glands of transgenic mice results in hyperplastic lesions (Hp) with reactive intervening fibrosis (Fb; stained blue) and multilocular adipocytes (asterisk). This contrasts the normal appearance of glands that co-overexpress *Mmp3* and tissue inhibitor of metalloproteinase (*Timp1*) under WAP promoters, showing that the effect is dependent on the proteinase activity of *Mmp3*. Adapted with permission from REF. 40. © (1999) Elsevier Science. **b** | *Mmp-9* that originates from infiltrating reactive cells is essential for the phenotype of squamous-cell carcinomas that evolve in mice expressing the human papillomavirus 16 (HPV16) early region under the keratin-14 promoter. For instance, the proliferation of malignant cells — detected by immunohistochemistry for proliferating cell nuclear antigen (PCNA) — is restored by bone-marrow transplantation (BMT) with *Mmp-9*-positive cells to *Mmp-9*-deficient mice. Islets of tumour cells (T) are interspersed with stroma (S). Adapted with permission from REF. 42. © (2000) Elsevier Science. **c** | Incubation of normal mammary epithelial cells with active recombinant matrix metalloproteinase 3 (rMMP-3) results in a scattered phenotype and a transition from the cyokeratin-positive epithelial phenotype to a vimentin-positive mesenchymal phenotype — epithelial-to-mesenchymal-transition. Cyokeratins (red) and vimentin (green) are detected by immunofluorescence; nuclei are stained blue with DAPI. Adapted with permission from REF. 40. © (1999) Elsevier Science. Images in parts **a** and **c** courtesy of Mark Sternlicht, University of California at San Francisco; image in part **b** courtesy of Lisa Coussens, University of California at San Francisco.

K14-HPV16 MOUSE MODEL OF SQUAMOUS-CELL CARCINOMA
A transgenic mouse strain that expresses the human papillomavirus type 16 (HPV16) early-region genes, including the E6/E7 oncogenes, under the control of the human keratin-14 promoter (K14) in basal keratinocytes. Invasive squamous carcinomas of the epidermis develop through characteristic stages.

MMPs regulate angiogenesis. The angiogenic response — vessels sprouting into tumour tissue from existing neighbouring vessels — is essential for tumour growth⁷⁶. Both endogenous and synthetic MMP inhibitors reduce tumour angiogenesis in animal experiments^{14,20,77–79}, indicating that MMPs are important positive regulators. MMPs might simply act by degrading the ECM, which would allow endothelial cells to invade the tumour stroma. Indeed, cleavage of collagen type I is required for endothelial-cell invasion of the ECM and for vessel formation⁸⁰.

MMP-2, -9 and -14 directly regulate angiogenesis, and MMP-19 might also be important as it is expressed in blood vessels⁸¹. Downregulation of *MMP2* expression in cancer cells decreases angiogenesis in a chicken chorioallantoic membrane model⁸². Furthermore, tumour angiogenesis and growth is reduced in *Mmp2*-deficient mice compared with wild-type mice³⁸. Cleavage of collagen type IV by MMP-2 exposes a cryptic, $\alpha v\beta 3$ integrin binding site within the collagen. Blockage of this new site with an antibody decreases migration of endothelial cells and *in vitro* angiogenesis, and reduces tumour growth in animal experiments²³. *Mmp-9* has been shown to be important for angiogenesis in two transgenic models of tumour progression — the K14-HPV16 SKIN CANCER MODEL⁴² and the RIP1-TAG2 INSULINOMA MODEL⁵⁹. Surprisingly, *Mmp-2* is not required for angiogenesis in the RIP1-TAG2 model⁵⁹. *Mmp-9* acts by increasing the bioavailability of the pro-angiogenic factor VEGF⁵⁹, although it is not known exactly how. *Mmp-14* is also thought to promote tumour angiogenesis: antibodies directed against the catalytic domain of *Mmp-14* block endothelial-cell migration, invasion and capillary-tube formation *in vitro*⁸³. *Mmp-14* can degrade the fibrin matrix that surrounds newly formed vessels⁸⁴, thereby potentially allowing the endothelial cells to invade further into the tumour tissue, and *Mmp14*- and *Mmp9*-null mice have impaired angiogenesis during development^{72,85}, supporting a role for these MMPs in angiogenesis.

MMPs also produce fragments that are angiogenesis inhibitors (FIG. 4c). Cleavage of PLASMINOGEN by MMP-2, -3, -7, -9 and -12 generates ANGIOSTATIN^{86–88}, and MMP-3, -9, -12, -13 and -20 might be involved in the generation of ENDOSTATIN, a C-terminal fragment of the basement-membrane collagen type XVIII⁸⁹. Both angiostatin and endostatin reduce endothelial-cell proliferation^{90,91} and, in addition, endostatin might inhibit endothelial-cell invasion by acting as an inhibitor of MMP-14 and MMP-2 (REF. 92). MMP-12 might also inhibit tumour angiogenesis by cleavage and shedding of cell-surface-bound UROKINASE-TYPE PLASMINOGEN-ACTIVATOR RECEPTOR, which is required for endothelial-cell invasion into fibrin⁹³.

MMPs in invasion and metastasis. During metastasis, cancer cells must cross several ECM barriers. First, they cross the epithelial basement membrane and invade the surrounding stroma, and then they enter blood vessels or lymphatics, extravasate and establish new proliferating colonies.

Experimental evidence for the role of MMPs in metastasis relies on *in vitro* invasion assays and *in vivo* xenograft METASTASIS ASSAYS. Overexpression of *TIMPs* inhibits, and *MMP2*, *3*, *13* and *14* promotes, invasion of cell lines through either collagen type I, optic nerve explants or MATRIGEL^{94–98}. In experimental metastasis assays, the number of colonies formed in the lungs of mice is reduced by downregulation of *MMP9* in cancer cells⁹⁹, and is also reduced in the *Mmp2*- and *Mmp9*-null mice as compared with wild-type mice^{38,39}.

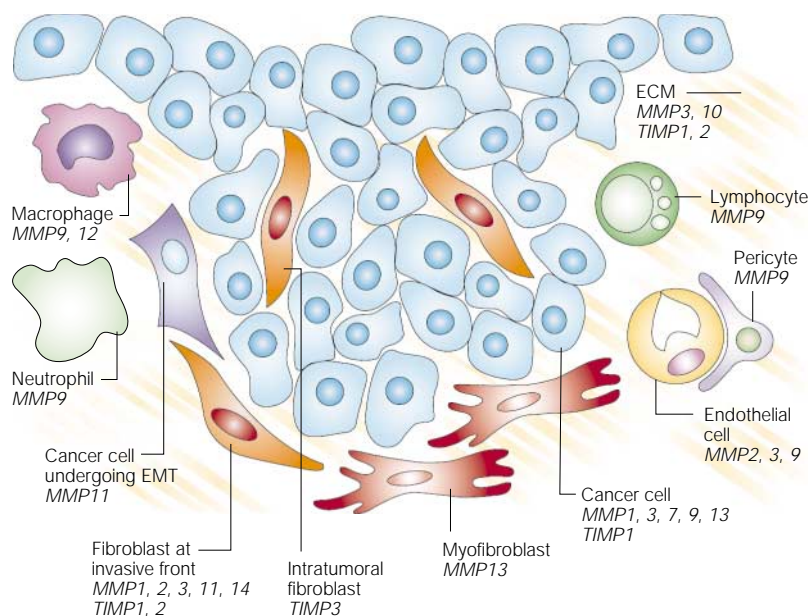


Figure 3 | Expression of MMPs and TIMPs in breast tumours. In addition to cancer cells, tumours of the breast consist of stromal cells, which include fibroblasts, myofibroblasts, endothelial cells, pericytes, macrophages, neutrophils and lymphocytes. Different matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are synthesized by stromal cells, cancer cells and cancer cells undergoing the epithelial-to-mesenchymal transition (EMT). Data are compiled from ONLINE TABLE 2.

RIP-TAG MOUSE MODEL OF ISLET-CELL CARCINOMA
A transgenic mouse strain that expresses the simian virus T antigen (TAg) under the rat insulin II promoter (RIP) in the pancreatic islet β -cells. Carcinomas develop in the pancreatic islets cells and progress through characteristic stages.

PLASMINOGEN
The precursor of plasmin, a trypsin-like serine proteinase that cleaves fibrin in blood clots.

ANGIOSTATIN
A proteolytic fragment of plasminogen and an inhibitor of angiogenesis.

ENDOSTATIN
A proteolytic fragment of the non-collagenous domain of collagen type XVIII and an inhibitor of angiogenesis.

UROKINASE-TYPE PLASMINOGEN-ACTIVATOR RECEPTOR
(uPAR). A cell-surface receptor for urokinase-type plasminogen activator (uPA), a serine proteinase that catalyses the formation of plasmin from plasminogen.

One of the first steps in invasion is migration. Cleavage of laminin-5 by MMP-2 and MMP-14 reveals a cryptic site that triggers cell motility^{22,100} (FIG. 4d). This cleaved form is found in experimental tumours²², and MMP-14 is co-localized with laminin-5 in human cancer¹⁰⁰. During migration, cancer cells must detach from both neighbouring cells and the surrounding matrix. CD44, the main receptor for HYALURONAN, is cleaved by MMP-14 and the extracellular domain is released³⁴. When the cleavage site is mutated, cell migration is inhibited³⁴. In addition to binding to the ECM, CD44 also binds MMP-9, thereby localizing the enzyme to the cell surface. This localization is required for MMP-9 to promote tumour invasion and angiogenesis⁵. Interestingly, disruption of the formation of CD44–MMP-9 complexes at the cell surface — by overexpression of the extracellular domain of CD44 — inhibits tumour invasiveness *in vivo*⁵. Cleavage of CD44 by MMP-14 probably also results in downregulation of MMP-9 cell-surface-associated activity. Together, these results indicate that migration is regulated by cycles of MMP activity or localized MMP activity, rather than by continuously high activity. In support of this hypothesis, there is no linear relationship between *MMP2* expression following transfection and the ability of cancer-cell lines to invade in a matrigel assay: cells expressing intermediate levels of *MMP2* are the most invasive⁹⁷.

E-cadherin is a cell-adhesion molecule that is involved in cell–cell adhesion; deregulation of its normal function is associated with cancer progression¹⁰¹. E-cadherin is cleaved by MMP-3 or -7 (REF. 33). The released fragment of E-cadherin promotes tumour-cell invasion

in a paracrine manner *in vitro*, possibly by binding to and interfering with the function of other full-length E-cadherin molecules³³. Cleavage of E-cadherin also triggers the epithelial-to-mesenchymal transition^{40,95} (FIGS 2c and 4e) — a change in cellular phenotype that is associated with aggressive malignant behaviour¹⁰¹.

During invasion, the localization of MMPs to specialized surface protrusions — INVADOPODIA — on the cell membrane is necessary for their ability to promote invasion¹⁰². MMP-2, -9 and -14 are known to localize to invadopodia, albeit by different mechanisms. MMP-14 is recruited to invadopodia by means of its transmembrane and cytoplasmic domains¹⁰². MMP-9 is probably recruited to invadopodia by binding to CD44, as the proteins co-localize to invadopodia during tumour-cell migration, and antibodies against CD44 inhibit both invadopodia formation and tumour-cell migration¹⁰³. It is not known how MMP-2 is localized to invadopodia, but it might be recruited by either binding to $\alpha v \beta 3$ integrin⁴ or by binding to MMP-14.

MMPs also participate in the late events in the metastatic process, when the cancer cells must enter, survive and exit the blood vessels or lymphatics. MMP-9 is required for INTRAVASATION in an intravasation model¹⁰⁴. Overexpression of *MMP14* increases the number of cancer cells that survive intravenous injection in an experimental metastasis assay¹⁰⁵. By contrast, MMP activity might not be important for EXTRAVASATION, as *TIMP1*-overexpressing cancer cells exit the vasculature equally well as control cells¹⁰⁶. However, they yield fewer and smaller metastases due to diminished cancer-cell growth after they leave the bloodstream¹⁰⁶. So, the final step in the establishment of secondary tumours — proliferation at the secondary site — probably also involves MMP activity.

There might be tissue-specific differences in the proteinases that promote metastasis. Spontaneous and experimental metastasis to the liver is reduced in *Timp1*-overexpressing mice, and is increased in mice that express antisense *Timp1* (REF. 107). Similarly, ectopic overexpression of *Timp1* in the brain of transgenic mice reduces experimental metastasis to the brain¹⁰⁸. However, experimental metastasis to the lungs is not increased in the *Timp1*-null mice¹⁰⁹.

MMPs and the immune responses to cancer. Inflammatory reactions are an important component of human neoplasia (reviewed in REF. 110). The immune system is capable of recognizing and attacking cancer cells, but cancer cells have developed many ways to escape immune surveillance. MMPs are involved in the escape mechanisms (FIG. 4f).

Tumour-specific cytotoxic T lymphocytes, natural killer cells, neutrophils and macrophages are among the inflammatory cells that infiltrate tumours. The proliferation of T lymphocytes is regulated by cytokine signalling by means of the interleukin-2 receptor- α (IL-2R α). MMPs, including MMP-9, can cleave IL-2R α and thereby suppress the proliferation of the T lymphocytes¹¹¹. MMPs also activate TGF- β ²⁸,

METASTASIS ASSAYS

In spontaneous metastasis assays, the tumour cells are inoculated either subcutaneously or orthotopically in animals, and spontaneous metastases from this primary site to distant locations are monitored. In experimental metastasis assays, tumour cells are injected into the bloodstream (for example, intravenously for lung metastasis, into the left heart ventricle for bone metastases and into the portal vein for liver metastases), thereby circumventing the first steps in the metastatic process.

MATRIGEL

The extracellular matrix secreted by the Engelbrecht–Holm–Swarm mouse sarcoma cell line. It contains laminin, collagen IV, nidogen/entactin and proteoglycans, and so resembles the basement membrane.

HYALURONAN

A high molecular weight glycosaminoglycan. It is widely distributed both in the extracellular matrix and at the cell surface.

INVADOPODIA

Specialized membrane protrusions (also known as an invasive pseudopodia) where active extracellular matrix degradation takes place.

INTRAVASATION

Passage from tissue into blood or lymph vessel.

EXTRAVASATION

Passage from blood or lymph vessel into tissue.

CHEMOKINES

A family of chemotactic proteins that are divided into C, CC, CXC and CX3C chemokines, depending on the number and spacing of conserved cysteine residues in the amino-terminal part of the protein. Chemokines are involved in inflammatory-cell recruitment and act through G-protein-coupled receptors.

RIBOZYMES

RNA molecules that function like enzymes and exert a catalytic activity. Ribozymes can be designed to cleave specific mRNAs and thereby inhibit protein synthesis.

an important inhibitor of the T-lymphocyte response against tumours¹¹². A cleavage product of α 1-proteinase-inhibitor, generated by MMP-11 (REF. 113), decreases the sensitivity of tumour cells to natural killer cells. In addition, an increased number of neutrophils and macrophages infiltrate tumours in *Mmp11*-null mice compared with wild-type mice, indicating that Mmp-11 inhibits a chemoattractant for these cells⁶⁹.

Several CHEMOKINES are known targets of MMPs, resulting in increased or reduced infiltration and migration of leukocytes. MMP-9 cleaves the neutrophil chemoattractant **CXCL8** (also known as interleukin-8; IL-8) and its murine homologue, thereby increasing its activity tenfold, whereas it inactivates the **CXCL7** precursor (also known as connective-tissue-activating peptide-III; CTAP-III), **CXCL4** (also known as platelet factor-4; PF4) and **CXCL1** (also known as growth-related oncogene- α ; GRO α) (reviewed in REF. 114). MMP-2 cleaves **CCL7** (also known as monocyte chemoattractant protein-3; MCP-3), and the cleaved fragment not only is inactivated but also becomes an antagonist to the receptors¹¹⁵. Finally, **CXCL12** (also known as stromal-cell-derived factor 1; SDF1) is cleaved and inactivated by MMP-1, -3, -9, -13 and -14 (REF. 116). CXCL12 is a ligand for the CXC chemokine receptor 4 (**CXCR4**) on leukocytes. Intriguingly, breast cancer cells also express CXCR4, and inhibition of the binding of CXCL12 to CXCR4 by blocking antibodies greatly reduces metastasis to lung and lymph nodes *in vivo*¹¹⁷. Therefore, cleavage of CXCL12 by MMPs might inhibit metastasis.

Although the immune system reacts against the cancer cells and probably delays tumour progression, chronic inflammation is also associated with cancers of the **skin**, breast, **prostate** gland, **ovary**, **gastric** mucosa, large bowel, **liver** and urinary **bladder** (reviewed in REF. 110). In animal models, mast cells, neutrophils and macrophages are contributors to the progression of cancer (reviewed in REF. 110). Inflammatory cells synthesize several MMPs, including -9, -12 and -14 (ONLINE TABLE 3), so they might stimulate cancer progression by releasing MMPs (FIG. 4f). Indeed, mice that are prone to develop skin cancer have reduced tumour incidence when they are *Mmp9* null, but carcinogenesis is restored by bone-marrow transplantation of *Mmp9*-expressing cells⁴² (FIG. 2b).

MMP inhibition in anticancer therapy

Several agents have been developed that block the synthesis of MMPs, 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, downregulation of *Mmp7* or *9* by these means results in reduced tumour burden or metastasis^{99,118,119}. Whether these techniques can be translated into clinical practice is, however, an

open question. MMP expression can also be reduced indirectly, by inhibition of the signal-transduction pathways that induce MMP transcription. Several drugs that are in clinical trials at present and that inhibit tyrosine kinase receptor signalling¹²⁰, are also likely to affect MMP expression levels. Other drugs that regulate MMP gene expression and experimental cancer-cell metastasis include halofuginone, a COCCIDIOSTAT that is used in chickens¹²¹.

Inhibiting interactions between MMP and other proteins. MMPs can hypothetically be inhibited by blocking their interactions with other proteins. This has been achieved with a compound that inhibits MMP-2 binding to α v β 3 integrin (REF. 122). Although this type of strategy has not been tested in clinical practice, it could be a means of specifically targeting cancer-promoting functions of MMPs without impeding their potential tumour-defying functions, and the compound shows promising results in animal experiments¹²².

Exploiting MMP activity. To take advantage of the increased MMP activity in tumours, cytotoxic agents that are activated by MMPs have been developed. These include recombinant proteins containing ANTHRAX TOXIN fused to an MMP cleavage site. These recombinant toxin proteins are activated by MMP cleavage at the cell surface and are internalized by the cell, leading to cell death¹²³. Tumour stromal cells secrete many of the MMPs, but whether these agents are sufficiently selective for the cancer cells *in vivo* has not yet been tested in animals.

Blocking MMP activity. A straightforward way of inhibiting MMPs is by blocking enzymatic activity. The natural inhibitors TIMP-2 and -4 show efficacy in experimental models^{124,125}, but TIMPs might have MMP-independent cancer-promoting activities, and systemically administered TIMPs can also promote cancer⁴⁵. Instead, three categories of synthetic MMP inhibitors have been developed: the collagen peptidomimetics, the collagen non-peptidomimetics and the tetracycline derivatives. A new class of MMP inhibitors that have yet to reach clinical testing is small peptides. These 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 experiments¹²⁶.

The peptidomimetic MMP inhibitors mimic the cleavage sites of MMP substrates and include Batimastat and Marimastat. Batimastat cannot be administered orally and is no longer tested for the treatment of human cancer. Marimastat has undergone several Phase III clinical trials (reviewed in REF. 159). A trial for advanced **pancreatic cancer** — intended to detect differences in survival between patients treated with various doses of Marimastat and conventional chemotherapy — failed to detect increased survival for the Marimastat-treated groups. However, the highest dose of Marimastat was as effective as the conventional therapy¹²⁷. In another trial — for advanced gastric cancer — patients who were treated with Marimastat did not have increased survival

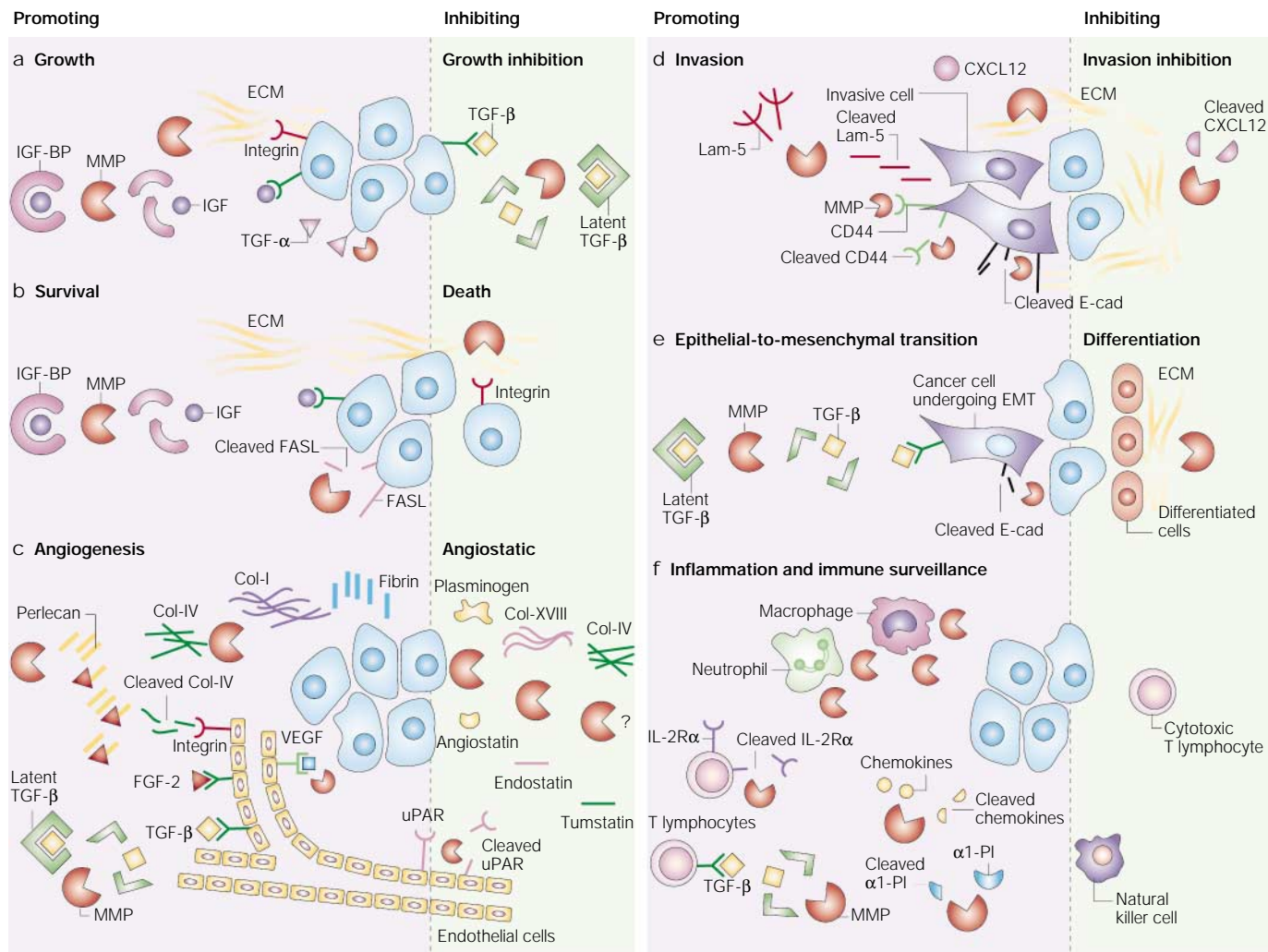


Figure 4 | Functions of MMPs in cancer progression. The matrix metalloproteinases (MMPs) have both cancer-promoting and cancer-inhibiting functions, and pathways with opposing effects on cancer progression are sometimes initiated by cleavage of the same substrate. **a** | MMPs promote growth of cancer cells by cleaving insulin-growth-factor-binding protein (IGF-BP), thereby liberating IGF; by shedding transmembrane precursors of growth factors including transforming growth factor- α (TGF- α); and by regulating the extracellular matrix (ECM), which promotes growth indirectly through interactions between ECM molecules and integrins. MMPs can inhibit cancer-cell growth by liberating transforming growth factor- β (TGF- β) from the latent TGF- β complex. **b** | MMPs promote survival of cancer cells by liberating IGF and by cleavage of FAS ligand (FASL), a ligand for the death receptor FAS. MMPs also promote apoptosis, probably indirectly by changing the ECM composition, which influences integrin signalling. **c** | MMPs promote angiogenesis by increasing the bioavailability of the pro-angiogenic growth factors vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), and TGF- β . These factors stimulate proliferation and migration of endothelial cells. FGF-2 is liberated by cleavage of the ECM protein perlecan, whereas the mechanism that is responsible for increased VEGF bioavailability is unknown. In addition, MMPs promote invasion of endothelial cells by cleaving structural components of the ECM, such as collagen types I (Col-I) and IV (Col-IV) and fibrin. Cleaved Col-IV acts pro-angiogenically by binding to $\alpha v \beta 3$ integrin. MMPs act anti-angiogenically through cleavage of plasminogen and Col-XVIII, resulting in generation of the anti-angiogenic factors angiostatin and endostatin. MMPs might also participate in the generation of tumstatin, a fragment of Col-IV. Cleavage of urokinase-type plasminogen activator receptor (uPAR) on the cell surface of endothelial cells might inhibit angiogenesis, as uPAR is required for endothelial cell invasion *in vitro*. **d** | The MMPs regulate invasion by degrading structural ECM components. Specifically, the MMPs promote invasion and migration by cleaving laminin 5 (Lam-5). MMPs also promote invasion by cleavage of the adhesion molecules CD44 and E-cadherin (E-cad). The released part of E-cad might then bind and inhibit the function of other uncleaved E-cad molecules. In addition, docking of MMP-9 to CD44 is required for cancer-cell invasion. MMPs might inhibit metastasis by cleavage of CXCL12, a chemokine of the CXC family that promotes breast cancer metastasis. **e** | MMPs promote the epithelial-to-mesenchymal transition (EMT) — a transition that is associated with malignant behaviour — by cleaving the cell-adhesion molecule E-cad and by liberating TGF- β . MMPs also promote differentiation. For instance, tumours evolving in *Mmp9*-null mice are less differentiated than tumours evolving in wild-type mice. The mechanism is unknown, but might involve changing the ECM composition and influencing integrin signalling. **f** | Reactive inflammatory cells provide some of the key MMPs involved in cancer progression, but MMPs also inhibit immune reactions against the cancer cells. MMPs cleave the interleukin-2 receptor- α (IL-2R α) on T lymphocytes, thereby inhibiting the proliferation of the T lymphocytes; they liberate TGF- β , an important suppressor of T-cell reactions against cancer cells; they cleave $\alpha 1$ -proteinase inhibitor ($\alpha 1$ -PI), resulting in decreased cancer-cell sensitivity to natural killer cells; and they cleave various members of the CC and CXC chemokine families, with the result that these factors no longer attract leukocytes.

COCCIDIOSTAT
A drug used to treat coccidiosis, an intestinal disease that is caused by a protozoan.

ANTHRAX TOXIN
Anthrax toxin is a three-part toxin that is secreted by the bacterium *Bacillus anthracis* and consists of protective antigen (PA), oedema factor (EF) and lethal factor (LF). PA is cleaved by furin-like proteases at the cell surface, and cleaved PAs form a complex with EF and LF. The complex is endocytosed, and LF and EF are translocated to the cytosol where they act cytotoxically. The requirement for proteolysis of PA has been exploited by replacing the furin-like cleavage site with a sequence recognized by MMPs, in a recombinant anthrax toxin derivative.

compared with placebo-treated patients when the study reached its predefined cut-off point. Nevertheless, in the subgroups of patients without metastases at the time of diagnosis, or those that had received previous chemotherapy, survival was significantly higher than for placebo-treated patients (see the [British Biotech Press Release web site](#)). In addition, as the follow-up time has increased, a significant improved survival in the Marimastat-treated population as a whole, compared with the placebo-treated patients, has been observed (see the [British Biotech Press Release web site](#)). These results are encouraging, as pancreatic and gastric cancer are diseases with very poor survival and limited therapeutic options.

The non-peptidomimetic MMP inhibitors are synthesized on the basis of the conformation of the MMP active site. This group includes BAY 12-9566, Prinomastat/AG3340, BMS 275291 and CGS 27023A/MMI270. The Phase I trial with BAY 12-9566 showed relatively mild adverse effects, and in some cases, disease was stabilized¹²⁸. However, all studies with BAY 12-9566 were terminated when preliminary results from Phase III trials on advanced pancreatic or **small-cell lung cancers** showed significantly poorer survival for groups treated with the drug than for placebo-treated groups (REF. 129, and see the [Bayer Press Release web site](#)). Phase III trials with Prinomastat for advanced prostate and lung cancer were also stopped prematurely when Prinomastat combined with standard chemotherapy did not show beneficial effects compared with chemotherapy alone (see the [Agouron Pfizer Press Release web site](#)). Phase II studies with earlier-stage cancer are, however, continuing (see the [Agouron Pfizer Press Release web site](#)). Finally, patients are now being recruited for Phase II/III clinical trials with BMS 275291 (see the [ClinicalTrials web site](#)).

The tetracycline derivatives inhibit both the activity and synthesis of MMPs. Tetracycline analogues without antibiotic activity have been developed for the treatment of cancer (by removal of a dimethylamino group)¹³⁰. Col-3 (Metastat) is one such analogue that has entered Phase II trials for **Kaposi's sarcoma** and advanced **brain tumours** (see the [ClinicalTrials web site](#)).

Also, an old group of drugs might possess unrecognized activity against the MMPs. The bisphosphonates were originally developed for the treatment of disturbances in calcium homeostasis and for the prevention and palliation of bone metastasis. Their mechanism of action has not been completely elucidated, but they inhibit the enzymatic activity of MMPs¹³¹.

Finally, unconventional MMP inhibitors are also being tested in clinical trials: AE-941 (Neovastat) — an extract from shark cartilage — inhibits MMPs¹³², shows anti-angiogenic effects in animal experiments and is now in Phase III clinical trials for the treatment of metastatic **non-small-cell lung cancer**¹³³. Also, a component in green tea, which is being tested, at present, in a Phase III trial (see the [ClinicalTrials web site](#)), acts as an MMP-2 and -9 inhibitor *in vitro*¹³⁴. Finally, acetylsalicylic acid, which reduces the risk of colon cancer¹³⁵, not only decreases inflammation,

thereby possibly affecting cancer development, but also directly inhibits MMP-2 activity and decreases cancer-cell invasion *in vitro*¹³⁶.

Improving the efficacy of MMP inhibitors. After several clinical trials with MMP inhibitors have ended with limited effects, this therapeutic approach is considered less promising than it was a couple of years ago. However, on the basis of what we now know, these trials might not have been ideally designed.

MMP inhibitors have been compared with cytotoxic drugs and tested in patients with late-stage tumours. However, MMP inhibitors are cytostatic and, furthermore, MMPs have an important role in tumour angiogenesis^{42,59}. So, using MMP inhibitors to treat late-stage tumours with established blood supplies would not be predicted to result in tumour shrinkage. Animal experiments show that MMPs should be targeted early in cancer progression: in cells with inducible expression of MMP-3, tumours form even if the cells are induced to express MMP-3 only during the first two weeks after inoculation⁴⁰. Furthermore, Batimastat reduces end-point tumour burden in an animal model of progressive pancreatic carcinoma if given at the early hyperplastic stage, but effects are limited if treatment begins at later stages¹³⁷.

Instead of using MMP inhibitors to treat inoperable tumours, they might be useful as preventive drugs. For instance, patients with a genetic disposition to cancer development, or patients in which the primary tumour is successfully removed and metastasis not detected, could benefit from treatment with MMP inhibitors. In support of such an approach, mice have 100% survival and no metastases when pretreated before removal of a primary tumour and subsequently continuously treated with Batimastat, whereas all control animals die from massive metastases¹³⁸.

Most trials use the maximum tolerated dose of MMP inhibitors. However, at the highest tolerated doses, the inhibitors might lose selectivity for specific MMPs. Some functions of MMPs — for instance, the generation of angiotensin by MMP-12 (REF. 88) — might help defy cancer. Therefore, it might be important to better target specific MMPs or specific functions of MMPs. The dosage used in some trials could, however, also have been too low as the side effects of MMP inhibitors — most commonly musculoskeletal pain — sometimes necessitate reduced dosing or drug holidays.

Inhibition of MMP for cancer treatment has not lived up to the expectations. Nevertheless, this therapeutic principle should not be discarded before the results of clinical trials with early-stage cancer are known. And finally, it is worth remembering that MMP inhibitors did, in fact, work as well as conventional therapy in some trials (REF. 127, and see [British Biotech Press release web site](#)).

Perspectives

The MMPs are a large and diverse group of enzymes, and the difference between the various MMPs and their functions in cancer is only just emerging. So, one of the

ADAMTS
(A disintegrin and metalloproteinase with thrombospondin type 1 motifs). A family of soluble proteinases, closely related to the ADAMs, that contains domains with high homology to thrombospondin.

important future prospects is to address the differences between the MMPs — why are there so many: is it redundancy or is it because they have different substrates, regulation or tissue expression? Should inhibitors that act only on specific MMPs be developed for the treatment of cancer?

The MMPs are related to the ADAM and the ADAMTS (a disintegrin and metalloproteinase with thrombospondin type 1 motif) families — two other large proteinase families that have ECM proteins and growth factors as substrates, regulate angiogenesis and are inhibited by the TIMPs (reviewed in REFS 139,140; ONLINE TABLE 2). So, although the relevance of these proteinases for cancer progression is unclear at present, it might be anticipated that they have equally as important and diverse roles as the MMPs.

During the past couple of years, it has become clear that MMPs do more than degrade structural ECM proteins to promote invasion and metastasis (FIG. 4).

So, do these many — and opposing — functions make the MMPs tumour promoting or inhibiting; are MMPs 'good' or 'bad'? Most of the literature supports the notion that the sum of MMP action promotes cancer progression, and the actions of the MMPs that negatively regulate cancer progression have received relatively little appreciation. These actions might, however, be part of the reason why the results with the MMP inhibitors have not been more encouraging. It will be important to better characterize the tumour-defying functions of the MMPs, so that these might be exploited — or at least not inhibited — when MMPs are therapeutically targeted.

The basic action of MMPs — cleavage of proteins — has proven sufficiently sophisticated to orchestrate various functions. More substrates and functions of MMPs might still be uncovered. These could include actions that can be used more rationally and effectively in the treatment of cancer.

- Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
- Bissell, M. J. & Radisky, D. Putting tumours in context. *Nature Rev. Cancer* **1**, 46–54 (2001).
- Werb, Z. ECM and cell surface proteolysis: regulating cellular ecology. *Cell* **91**, 439–442 (1997).
- Brooks, P. C. *et al.* Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin α v β 3. *Cell* **85**, 683–693 (1996).
Localization of active MMP-2, secreted by stromal cells, to the cell surface of invasive cells by binding to α v β 3 integrin is necessary for invasive behaviour. Similar results relating to the importance of invasion, angiogenesis and TGF- β processing of tethering MMP-9 to the cell surface by binding to CD44 were reported in references 5 and 28.
- Yu, Q. & Stamenkovic, I. Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev.* **13**, 35–48 (1999).
- Yu, W.-H., Woessner, J. F. Jr, McNeish, J. D. & Stamenkovic, I. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Dev.* **16**, 307–323 (2002).
- Sternlicht, M. D. & Werb, Z. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol.* **17**, 463–516 (2001).
- Strongin, A. Y. *et al.* Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. *J. Biol. Chem.* **270**, 5331–5338 (1995).
- Deryugina, E. I. *et al.* MT1-MMP initiates activation of pro-MMP-2 and integrin α v β 3 promotes maturation of MMP-2 in breast carcinoma cells. *Exp. Cell Res.* **263**, 209–223 (2001).
- Morrison, C. J. *et al.* Cellular activation of MMP-2 (Gelatinase A) by MT2-MMP occurs via a TIMP-2-independent pathway. *J. Biol. Chem.* **276**, 47402–47410 (2001).
- Sottrup-Jensen, L. & Birkedal-Hansen, H. Human fibroblast collagenase- α -macroglobulin interactions. Localization of cleavage sites in the bait regions of five mammalian α -macroglobulins. *J. Biol. Chem.* **264**, 393–401 (1989).
- Yang, Z., Strickland, D. K. & Bornstein, P. Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2. *J. Biol. Chem.* **276**, 8403–8408 (2001).
- Bein, K. & Simons, M. Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. *J. Biol. Chem.* **275**, 32167–32173 (2000).
- Rodriguez-Manzanique, J. C. *et al.* Thrombospondin-1 suppresses spontaneous tumor growth and inhibits activation of matrix metalloproteinase-9 and mobilization of vascular endothelial growth factor. *Proc. Natl Acad. Sci. USA* **98**, 12485–12490 (2001).
- Taraboletti, G. *et al.* The heparin binding 25 kDa fragment of thrombospondin-1 promotes angiogenesis and modulates gelatinase and TIMP-2 production in endothelial cells. *FASEB J.* **14**, 1674–1676 (2000).
- Edwards, D. R. in *Matrix Metalloproteinase Inhibitors in Cancer Therapy* (eds Clendeninn, N. J. & Appelt, K.) 67–84 (Humana Press, Totowa, New Jersey, 2001).
- Wang, Z., Juttermann, R. & Soloway, P. D. TIMP-2 is required for efficient activation of proMMP-2 *in vivo*. *J. Biol. Chem.* **275**, 26411–26415 (2000).
- Mott, J. D. *et al.* Post-translational proteolytic processing of procollagen C-terminal proteinase enhancer releases a metalloproteinase inhibitor. *J. Biol. Chem.* **275**, 1384–1390 (2000).
- Netzer, K. O., Suzuki, K., Itoh, Y., Hudson, B. G. & Khalifah, R. G. Comparative analysis of the noncollagenous NC1 domain of type IV collagen: identification of structural features important for assembly, function, and pathogenesis. *Protein Sci.* **7**, 1340–1351 (1998).
- Oh, J. *et al.* The membrane-anchored MMP inhibitor RECK is a key regulator of extracellular matrix integrity and angiogenesis. *Cell* **107**, 789–800 (2001).
Reports that deficiency of an MMP inhibitor, RECK, is embryonic lethal, due to a defect in vascular development. Also shows that the blood vessels in tumours that overexpress RECK have a reduced branching phenotype.
- Streuli, C. Extracellular matrix remodelling and cellular differentiation. *Curr. Opin. Cell Biol.* **11**, 634–640 (1999).
- Giannelli, G., Falk-Marzillier, J., Schiraldi, O., Stetler-Stevenson, W. G. & Quaranta, V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. *Science* **277**, 225–228 (1997).
Shows, together with reference 100, that cleavage of laminin-5 by MMPs triggers the migration of cells by unmasking a cryptic site that might act as a ligand for an unidentified cellular receptor.
- Xu, J. *et al.* Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth *in vivo*. *J. Cell Biol.* **154**, 1069–1080 (2001).
Shows that a cryptic site within collagen type IV that is exposed following proteinase cleavage promotes angiogenesis and tumour growth, probably by interacting with α v β 3 integrin. This cryptic site is detected in blood vessels from tumours but not normal tissue.
- Manes, S. *et al.* Identification of insulin-like growth factor-binding protein-1 as a potential physiological substrate for human stromelysin-3. *J. Biol. Chem.* **272**, 25706–25712 (1997).
- Manes, S. *et al.* The matrix metalloproteinase-9 regulates the insulin-like growth factor-triggered autocrine response in DU-145 carcinoma cells. *J. Biol. Chem.* **274**, 6935–6945 (1999).
- Whitelock, J. M., Murdoch, A. D., Iozzo, R. V. & Underwood, P. A. The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases. *J. Biol. Chem.* **271**, 10079–10086 (1996).
- Peschon, J. J. *et al.* An essential role for ectodomain shedding in mammalian development. *Science* **282**, 1281–1284 (1998).
- Yu, Q. & Stamenkovic, I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- β and promotes tumor invasion and angiogenesis. *Genes Dev.* **14**, 163–176 (2000).
- Levi, E. *et al.* Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1. *Proc. Natl Acad. Sci. USA* **93**, 7069–7074 (1996).
- Codony-Servat, J., Albanell, J., Lopez-Talavera, J. C., Arribas, J. & Baselga, J. Cleavage of the HER2 ectodomain is a pervanadate-activable process that is inhibited by the tissue inhibitor of metalloproteinases-1 in breast cancer cells. *Cancer Res.* **59**, 1196–1201 (1999).
- Vecchi, M., Rudolph-Owen, L. A., Brown, C. L., Dempsey, P. J. & Carpenter, G. Tyrosine phosphorylation and proteolysis. Pervanadate-induced, metalloproteinase-dependent cleavage of the ErbB-4 receptor and amphiregulin. *J. Biol. Chem.* **273**, 20589–20595 (1998).
- Nath, D., Williamson, N. J., Jarvis, R. & Murphy, G. Shedding of c-Met is regulated by crosstalk between a G-protein coupled receptor and the EGF receptor and is mediated by a TIMP-3 sensitive metalloproteinase. *J. Cell Sci.* **114**, 1213–1220 (2001).
- Noe, V. *et al.* Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. *J. Cell Sci.* **114**, 111–118 (2001).
- Kajita, M. *et al.* Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J. Cell Biol.* **153**, 893–904 (2001).
Reports that CD44 is shed by MMP-14 and that mutation of the cleavage site inhibits migration.
- Deryugina, E. I., Ratnikov, B. I., Postnova, T. I., Rozanov, D. V. & Strongin, A. Y. Processing of integrin α v subunit by MT1-MMP stimulates migration of breast carcinoma cells on vitronectin and enhances tyrosine phosphorylation of FAK. *J. Biol. Chem.* 2001 Nov 27; [epub ahead of print].
- Coussens, L. M. & Werb, Z. Matrix metalloproteinases and the development of cancer. *Chem. Biol.* **3**, 895–904 (1996).
- Masson, R. *et al.* *In vivo* evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. *J. Cell Biol.* **140**, 1535–1541 (1998).
- Itoh, T. *et al.* Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. *Cancer Res.* **58**, 1048–1051 (1998).
- Itoh, T. *et al.* Experimental metastasis is suppressed in MMP-9-deficient mice. *Clin. Exp. Metastasis* **17**, 177–181 (1999).
- Sternlicht, M. D. *et al.* The stromal proteinase MMP-3/stromelysin-1 promotes mammary carcinogenesis. *Cell* **98**, 137–146 (1999).
Shows, together with reference 95, that active MMP-3 changes the phenotype of mammary epithelial cells to

- mesenchymal-like cells, and that the E-cadherin- β -catenin complex is involved in this regulation. Expression of an MMP, even when expressed only in the early stages of cancer development, is also sufficient to initiate tumour formation.**
41. Ha, H. Y. *et al.* Overexpression of membrane-type matrix metalloproteinase-1 gene induces mammary gland abnormalities and adenocarcinoma in transgenic mice. *Cancer Res* **61**, 984–990 (2001).
 42. Coussens, L. M., Tinkle, C. L., Hanahan, D. & Werb, Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* **103**, 481–490 (2000). **In a transgenic mouse model of skin cancer, the reduced carcinogenesis in mice that are deficient for MMP9 can be completely restored by bone-marrow transplantation of MMP9-expressing cells. Tumours that arise in the absence of MMP9 are of a more advanced stage than those that arise in wild-type mice.**
 43. Yang, W. *et al.* Human macrophage metalloelastase gene expression in colorectal carcinoma and its clinicopathologic significance. *Cancer* **91**, 1277–1283 (2001).
 44. Takeha, S. *et al.* Stromal expression of MMP-9 and urokinase receptor is inversely associated with liver metastasis and with infiltrating growth in human colorectal cancer: a novel approach from immune/inflammatory aspect. *Jpn. J. Cancer Res.* **88**, 72–81 (1997).
 45. Jiang, Y. *et al.* Stimulation of mammary tumorigenesis by systemic tissue inhibitor of matrix metalloproteinase 4 gene delivery. *Cancer Res.* **61**, 2365–2370 (2001).
 46. Hayakawa, T., Yamashita, K., Ohuchi, E. & Shinagawa, A. Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2). *J. Cell Sci.* **107**, 2373–2379 (1994).
 47. Yoshiji, H. *et al.* Mammary carcinoma cells over-expressing tissue inhibitor of metalloproteinases-1 show enhanced vascular endothelial growth factor expression. *Int. J. Cancer* **75**, 81–87 (1998).
 48. Gururajan, R. *et al.* Duplication of a genomic region containing the Cdc2L1-2 and MMP21-22 genes on human chromosome 1p36.3 and their linkage to D1Z2. *Genome Res.* **8**, 929–939 (1998).
 49. Llano, E. *et al.* Identification and characterization of human MT5-MMP, a new membrane-bound activator of progelatinase A overexpressed in brain tumors. *Cancer Res.* **59**, 2570–2576 (1999).
 50. Crawford, H. C. *et al.* The PEA3 subfamily of Ets transcription factors synergizes with β -catenin-LEF-1 to activate matrix metalloproteinase transcription in intestinal tumors. *Mol. Cell Biol.* **21**, 1370–1383 (2001).
 51. Sun, Y. *et al.* p53 down-regulates human matrix metalloproteinase-1 (collagenase-1) gene expression. *J. Biol. Chem.* **274**, 11535–11540 (1999).
 52. Sun, Y. *et al.* Wild type and mutant p53 differentially regulate the gene expression of human collagenase-3 (hMMP-13). *J. Biol. Chem.* **275**, 11327–11332 (2000).
 53. Polette, M. *et al.* Gelatinase A expression and localization in human breast cancers. An *in situ* hybridization study and immunohistochemical detection using confocal microscopy. *Virchows Arch.* **424**, 641–645 (1994).
 54. Kanamori, Y. *et al.* Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. *Cancer Res.* **59**, 4225–4227 (1999).
 55. Ye, S. *et al.* Invasiveness of cutaneous malignant melanoma is influenced by matrix metalloproteinase 1 gene polymorphism. *Cancer Res.* **61**, 1296–1298 (2001).
 56. Zhu, Y., Spitz, M. R., Lei, L., Mills, G. B. & Wu, X. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. *Cancer Res.* **61**, 7825–7829 (2001).
 57. Ye, S. *et al.* Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J. Biol. Chem.* **271**, 13055–13060 (1996).
 58. Biondi, M. L. *et al.* MMP1 and MMP3 polymorphisms in promoter regions and cancer. *Clin. Chem.* **46**, 2023–2024 (2000).
 59. Bergers, G. *et al.* Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nature Cell Biol.* **2**, 737–744 (2000). **MMP-9 controls tumour angiogenesis by increasing the bioavailability of VEGF, and is expressed by stromal cells but not by cancer cells. MMP-2 also influences tumour growth but not angiogenesis. Invasion is not affected by MMP-2 or MMP-9.**
 60. Agrez, M., Chen, A., Cone, R. I., Pytela, R. & Sheppard, D. The α 6 β 1 integrin promotes proliferation of colon carcinoma cells through a unique region of the β 6 cytoplasmic domain. *J. Cell Biol.* **127**, 547–556 (1994).
 61. Derynck, R., Akhurst, R. J. & Balmain, A. TGF- β signaling in tumor suppression and cancer progression. *Nature Genet.* **29**, 117–129 (2001).
 62. Reed, J. C. Mechanisms of apoptosis avoidance in cancer. *Curr. Opin. Oncol.* **11**, 68–75 (1999).
 63. Alexander, C. M., Howard, E. W., Bissell, M. J. & Werb, Z. Rescue of mammary epithelial cell apoptosis and entactin degradation by a tissue inhibitor of metalloproteinases-1 transgene. *J. Cell Biol.* **135**, 1669–1677 (1996).
 64. Witty, J. P., Lempka, T., Coffey, R. J. Jr & Matrisian, L. M. Decreased tumor formation in 7,12-dimethylbenzanthracene-treated stromelysin-1 transgenic mice is associated with alterations in mammary epithelial cell apoptosis. *Cancer Res.* **55**, 1401–1406 (1995).
 65. Simpson, C. J. *et al.* Targeted expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression. *J. Cell Biol.* **125**, 681–693 (1994).
 66. Powell, W. C., Fingleton, B., Wilson, C. L., Boothby, M. & Matrisian, L. M. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr. Biol.* **9**, 1441–1447 (1999).
 67. Mitsiades, N., Yu, W.-H., Poulaki, V., Tsokos, M. & Stamenkovic, I. Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res.* **61**, 577–581 (2001).
 68. Wu, E. *et al.* Stromelysin-3 suppresses tumor cell apoptosis in a murine model. *J. Cell Biochem.* **82**, 549–555 (2001).
 69. Boulay, A. *et al.* High cancer cell death in syngeneic tumors developed in host mice deficient for the stromelysin-3 matrix metalloproteinase. *Cancer Res.* **61**, 2189–2193 (2001).
 70. Baserga, R. The contradictions of the insulin-like growth factor 1 receptor. *Oncogene* **19**, 5574–5581 (2000).
 71. Ishizuwa-Oka, A. *et al.* Requirement for matrix metalloproteinase stromelysin-3 in cell migration and apoptosis during tissue remodeling in *Xenopus laevis*. *J. Cell Biol.* **150**, 1177–1188 (2000).
 72. Vu, T. H. *et al.* MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* **93**, 411–422 (1998).
 73. Herren, B., Levkau, B., Raines, E. W. & Ross, R. Cleavage of β -catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases. *Mol. Biol. Cell* **9**, 1589–1601 (1998).
 74. Ilan, N., Mohsenin, A., Cheung, L. & Madri, J. A. PECAM-1 shedding during apoptosis generates a membrane-anchored truncated molecule with unique signaling characteristics. *FASEB J.* **15**, 362–372 (2001).
 75. Steinhilber, U. *et al.* Cleavage and shedding of E-cadherin after induction of apoptosis. *J. Biol. Chem.* **276**, 4972–4980 (2001).
 76. Hanahan, D. & Folkman, J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* **86**, 353–364 (1996).
 77. Martin, D. C. *et al.* Transgenic TIMP-1 inhibits simian virus 40 T antigen-induced hepatocarcinogenesis by impairment of hepatocellular proliferation and tumor angiogenesis. *Lab. Invest.* **79**, 225–234 (1999).
 78. Li, H. *et al.* AdTIMP-2 inhibits tumor growth, angiogenesis, and metastasis, and prolongs survival in mice. *Hum. Gene Ther.* **12**, 515–526 (2001).
 79. Gatto, C. *et al.* BAY 12-9566, a novel inhibitor of matrix metalloproteinases with antiangiogenic activity. *Clin. Cancer Res.* **5**, 3603–3607 (1999).
 80. Seandel, M., Noack-Kunmann, K., Zhu, D., Aimes, R. T. & Quigley, J. P. Growth factor-induced angiogenesis *in vivo* requires specific cleavage of fibrillar type I collagen. *Blood* **97**, 2323–2332 (2001).
 81. Kolb, C., Mauch, S., Peter, H. H., Krawinkel, U. & Sedlacek, R. The matrix metalloproteinase RASI-1 is expressed in synovial blood vessels of a rheumatoid arthritis patient. *Immunol. Lett.* **57**, 83–88 (1997).
 82. Fang, J. *et al.* Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. *Proc. Natl Acad. Sci. USA* **97**, 3884–3889 (2000).
 83. Galvez, B. G., Matias-Roman, S., Albar, J. P., Sanchez-Madrid, F. & Arroyo, A. G. Membrane type 1-matrix metalloproteinase is activated during migration of human endothelial cells and modulates endothelial motility and matrix remodeling. *J. Biol. Chem.* **276**, 37491–37500 (2001).
 84. Hiraoka, N., Allen, E., Apel, I. J., Gyetko, M. R. & Weiss, S. J. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. *Cell* **95**, 365–377 (1998).
 85. Zhou, Z. *et al.* Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. *Proc. Natl Acad. Sci. USA* **97**, 4052–4057 (2000).
 86. Dong, Z., Kumar, R., Yang, X. & Fidler, I. J. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* **88**, 801–810 (1997). **The first paper to show that MMP cleavage can produce anti-angiogenic fragments of ECM components.**
 87. Cornelius, L. A. *et al.* Matrix metalloproteinases generate angiostatin: effects on neovascularization. *J. Immunol.* **161**, 6845–6852 (1998).
 88. Gorris-Rivas, M. J. *et al.* Mouse macrophage metalloelastase gene transfer into a murine melanoma suppresses primary tumor growth by halting angiogenesis. *Clin. Cancer Res.* **6**, 1647–1654 (2000).
 89. Ferreras, M., Felbor, U., Lenhard, T., Olsen, B. R. & Delaisse, J. Generation and degradation of human endostatin proteins by various proteinases. *FEBS Lett.* **486**, 247–251 (2000).
 90. O'Reilly, M. S. *et al.* Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* **79**, 315–328 (1994).
 91. O'Reilly, M. S. *et al.* Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* **88**, 277–285 (1997).
 92. Kim, Y. M. *et al.* Endostatin inhibits endothelial and tumor cellular invasion by blocking the activation and catalytic activity of matrix metalloproteinase. *Cancer Res.* **60**, 5410–5413 (2000).
 93. Koolwijk, P. *et al.* Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices. *Blood* **97**, 3123–3131 (2001).
 94. Ahonen, M., Baker, A. H. & Kahari, V. M. Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells. *Cancer Res.* **58**, 2310–2315 (1998).
 95. Lochter, A. *et al.* Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *J. Cell Biol.* **139**, 1861–1872 (1997).
 96. Belien, A. T., Paganetti, P. A. & Schwab, M. E. Membrane-type 1 matrix metalloproteinase (MT1-MMP) enables invasive migration of glioma cells in central nervous system white matter. *J. Cell Biol.* **144**, 373–384 (1999).
 97. Deryugina, E. I., Luo, G. X., Reisfeld, R. A., Bourdon, M. A. & Strongin, A. Tumor cell invasion through matrigel is regulated by activated matrix metalloproteinase-2. *Anticancer Res.* **17**, 3201–3210 (1997).
 98. Ala-Aho, R., Johansson, N., Baker, A. H. & Kahari, V. M. Expression of collagenase-3 (MMP-13) enhances invasion of human fibrosarcoma HT-1080 cells. *Int. J. Cancer* **97**, 283–289 (2002).
 99. Hua, J. & Muschel, R. J. Inhibition of matrix metalloproteinase 9 expression by a ribozyme blocks metastasis in a rat sarcoma model system. *Cancer Res.* **56**, 5279–5284 (1996).
 100. Koshikawa, N., Giannelli, G., Cirulli, V., Miyazaki, K. & Quaranta, V. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. *J. Cell Biol.* **148**, 615–624 (2000).
 101. Birchmeier, C., Birchmeier, W. & Brand-Saberi, B. Epithelial-mesenchymal transitions in cancer progression. *Acta Anat. (Basel)* **156**, 217–226 (1996).
 102. Nakahara, H. *et al.* Transmembrane/cytoplasmic domain-mediated membrane type 1-matrix metalloprotease docking to invadopodia is required for cell invasion. *Proc. Natl Acad. Sci. USA* **94**, 7959–7964 (1997).
 103. Bourguignon, L. Y. *et al.* CD44v(3,8-10) is involved in cytoskeleton-mediated tumor cell migration and matrix metalloproteinase (MMP-9) association in metastatic breast cancer cells. *J. Cell Physiol.* **176**, 206–215 (1998).
 104. Kim, J., Yu, W., Kovalski, K. & Ossowski, L. Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay. *Cell* **94**, 353–362 (1998).
 105. Tsunozuka, Y. *et al.* Expression of membrane-type matrix metalloproteinase 1 (MT1-MMP) in tumor cells enhances pulmonary metastasis in an experimental metastasis assay. *Cancer Res.* **56**, 5678–5683 (1996).
 106. Koop, S. *et al.* Overexpression of metalloproteinase inhibitor in B16F10 cells does not affect extravasation but reduces tumor growth. *Cancer Res.* **54**, 4791–4797 (1994).
 107. Krüger, A., Fata, J. E. & Khokha, R. Altered tumor growth and metastasis of a T-cell lymphoma in Timp-1 transgenic mice. *Blood* **90**, 1993–2000 (1997).

108. Krüger, A. *et al.* Host TIMP-1 overexpression confers resistance to experimental brain metastasis of a fibrosarcoma cell line. *Oncogene* **16**, 2419–2423 (1998).
109. Soloway, P. D., Alexander, C. M., Werb, Z. & Jaenisch, R. Targeted mutagenesis of Timp-1 reveals that lung tumor invasion is influenced by Timp-1 genotype of the tumor but not by that of the host. *Oncogene* **13**, 2307–2314 (1996).
110. Coussens, L. M. & Werb, Z. Inflammatory cells and cancer: think different! *J. Exp. Med.* **193**, F23–F26 (2001).
111. Sheu, B.-C. *et al.* A novel role of metalloproteinase in cancer-mediated immunosuppression. *Cancer Res.* **61**, 237–242 (2001).
- Shows that MMPs can suppress the proliferation of activated T lymphocytes by shedding the interleukin-2 receptor- α from their surface.**
112. Gorelik, L. & Flavell, R. A. Immune-mediated eradication of tumors through the blockade of transforming growth factor- β signaling in T cells. *Nature Med.* **7**, 1118–1122 (2001).
113. Kataoka, H. *et al.* Enhanced tumor growth and invasiveness *in vivo* by a carboxyl-terminal fragment of α 1-proteinase inhibitor generated by matrix metalloproteinases: a possible modulatory role in natural killer cytotoxicity. *Am. J. Pathol.* **154**, 457–468 (1999).
114. Opendakker, G., Van den Steen, P. E. & Van Damme, J. Gelatinase B: a tuner and amplifier of immune functions. *Trends Immunol.* **22**, 571–579 (2001).
115. McQuibban, G. A. *et al.* Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* **289**, 1202–1206 (2000).
- MMPs regulate the inflammatory response by cleaving CCL7 — a chemokine that is involved in leukocyte recruitment — thereby converting it to an antagonist of its receptors.**
116. McQuibban, G. A. *et al.* Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. *J. Biol. Chem.* **276**, 43503–43508 (2001).
117. Müller, A. *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**, 50–56 (2001).
118. Yonemura, Y. *et al.* Inhibition of peritoneal dissemination in human gastric cancer by MMP-7-specific antisense oligonucleotide. *J. Exp. Clin. Cancer Res.* **20**, 205–212 (2001).
119. Kondraganti, S. *et al.* Selective suppression of matrix metalloproteinase-9 in human glioblastoma cells by antisense gene transfer impairs glioblastoma cell invasion. *Cancer Res.* **60**, 6851–6855 (2000).
120. Noonberg, S. B. & Benz, C. C. Tyrosine kinase inhibitors targeted to the epidermal growth factor receptor subfamily: role as anticancer agents. *Drugs* **59**, 753–767 (2000).
121. Elkin, M. *et al.* Inhibition of matrix metalloproteinase-2 expression and bladder carcinoma metastasis by halofuginone. *Clin. Cancer Res.* **5**, 1982–1988 (1999).
122. Silletti, S., Kessler, T., Goldberg, J., Boger, D. L. & Cheresch, D. A. Disruption of matrix metalloproteinase 2 binding to integrin α v β 3 by an organic molecule inhibits angiogenesis and tumor growth *in vivo*. *Proc. Natl Acad. Sci. USA* **98**, 119–124 (2001).
- Characterizes a compound that inhibits tumour growth by specifically disrupting the protein–protein interaction between MMP-2 and α v β 3 integrin, without any effects on MMP-2 activity in general.**
123. Liu, S., Netzel-Amett, S., Birkedal-Hansen, H. & Leppla, S. H. Tumor cell-selective cytotoxicity of matrix metalloproteinase-activated anthrax toxin. *Cancer Res.* **60**, 6061–6067 (2000).
124. Celiker, M. Y. *et al.* Inhibition of Wilms' tumor growth by intramuscular administration of tissue inhibitor of metalloproteinases-4 plasmid DNA. *Oncogene* **20**, 4337–4343 (2001).
125. Brand, K. *et al.* Treatment of colorectal liver metastases by adenoviral transfer of tissue inhibitor of metalloproteinases-2 into the liver tissue. *Cancer Res.* **60**, 5723–5730 (2000).
126. Koivunen, E. *et al.* Tumor targeting with a selective gelatinase inhibitor. *Nature Biotechnol.* **17**, 768–774 (1999).
- Characterizes a peptide inhibitor that specifically inhibits the gelatin-binding MMPs, but not other MMPs.**
127. Bramhall, S. R., Rosemurgy, A., Brown, P. D., Bowry, C. & Buckels, J. A. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. *J. Clin. Oncol.* **19**, 3447–3455 (2001).
128. Hirte, H. *et al.* A phase I dose escalation study of the matrix metalloproteinase inhibitor BAY 12-9566 administered orally in patients with advanced solid tumours. *Ann. Oncol.* **11**, 1579–1584 (2000).
129. Moore, M. J. *et al.* A comparison between gemcitabine (GEM) and the matrix metalloproteinase (MMP) inhibitor BAY12-9566 (9566) in patients (PTS) with advanced pancreatic cancer. ASCO Online [online] (cited 05 Feb 2002). <http://www.asco.org/prof/me/html/00abstracts/gic/m_930.htm> (2000).
130. Hidalgo, M. & Eckhardt, S. G. Development of matrix metalloproteinase inhibitors in cancer therapy. *J. Natl Cancer Inst.* **93**, 178–193 (2001).
131. Boissier, S. *et al.* Bisphosphonates inhibit breast and prostate carcinoma cell invasion, an early event in the formation of bone metastases. *Cancer Res.* **60**, 2949–2954 (2000).
132. Gingras, D. *et al.* Matrix proteinase inhibition by AE-941, a multifunctional antiangiogenic compound. *Anticancer Res.* **21**, 145–155 (2001).
133. Falardeau, P., Champagne, P., Poyet, P., Hariton, C. & Dupont, E. Neovastat, a naturally occurring multifunctional antiangiogenic drug, in phase III clinical trials. *Semin. Oncol.* **28**, 620–625 (2001).
134. Garbisa, S. *et al.* Tumor invasion: molecular shears blunted by green tea. *Nature Med.* **5**, 1216 (1999).
135. Thun, M. J., Nambodiri, M. M. & Heath, C. W. Jr. Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.* **325**, 1593–1596 (1991).
136. Jiang, M. C., Liao, C. F. & Lee, P. H. Aspirin inhibits matrix metalloproteinase-2 activity, increases E-cadherin production, and inhibits *in vitro* invasion of tumor cells. *Biochem. Biophys. Res. Commun.* **282**, 671–677 (2001).
137. Bergers, G., Javaherian, K., Lo, K. M., Folkman, J. & Hanahan, D. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science* **284**, 808–812 (1999).
138. Eccles, S. A. *et al.* Control of lymphatic and hematogenous metastasis of a rat mammary carcinoma by the matrix metalloproteinase inhibitor batimastat (BB-94). *Cancer Res.* **56**, 2815–2822 (1996).
139. Kheradmand, F. & Werb, Z. Shedding light on sheddases: role in growth and development. *Bioessays* **24**, 8–12 (2002).
140. Tang, B. L. ADAMTS: a novel family of extracellular matrix proteases. *Int. J. Biochem. Cell Biol.* **33**, 33–44 (2001).
141. Olsen, B. R. & Ninomiya, Y. in *Guidebook to the Extracellular Matrix, Anchor and Adhesion Proteins* (eds Kreis, T. & Vale, R.) 380–408 (Oxford Univ. Press, Oxford, UK, 1999).
142. Maeshima, Y. *et al.* Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. *Science* **295**, 140–143 (2002).
143. Sasaki, T. & Timpl, R. in *Guidebook to the Extracellular Matrix, Anchor and Adhesion Proteins* (eds Kreis, T. & Vale, R.) 434–443 (Oxford Univ. Press, Oxford, UK, 1999).
144. Hynes, R. in *Guidebook to the Extracellular Matrix, Anchor and Adhesion Proteins* (eds Kreis, T. & Vale, R.) 422–425 (Oxford Univ. Press, Oxford, UK, 1999).
145. Gustafsson, E. & Fassler, R. Insights into extracellular matrix functions from mutant mouse models. *Exp. Cell Res.* **261**, 52–68 (2000).
146. Lander, A. D. in *Guidebook to the Extracellular Matrix, Anchor and Adhesion Proteins* (eds Kreis, T. & Vale, R.) 351–356 (Oxford Univ. Press, Oxford, UK, 1999).
147. Cossins, J., Dudgeon, T. J., Catlin, G., Gearing, A. J. & Clements, J. M. Identification of MMP-18, a putative novel human matrix metalloproteinase. *Biochem. Biophys. Res. Commun.* **228**, 494–498 (1996).
148. Marchenko, G. N. *et al.* Characterization of matrix metalloproteinase-26, a novel metalloproteinase widely expressed in cancer cells of epithelial origin. *Biochem. J.* **356**, 705–718 (2001).
149. Pei, D., Kang, T. & Orl, H. Cysteine array matrix metalloproteinase (CA-MMP)/MMP-23 is a type II transmembrane matrix metalloproteinase regulated by a single cleavage for both secretion and activation. *J. Biol. Chem.* **275**, 33988–33997 (2000).
150. Ohnishi, J. *et al.* Cloning and characterization of a rat ortholog of MMP-23 (matrix metalloproteinase-23), a unique type of membrane-anchored matrix metalloproteinase and conditioned switching of its expression during the ovarian follicular development. *Mol. Endocrinol.* **15**, 747–764 (2001).
151. D'Armiento, J. *et al.* Collagenase expression in transgenic mouse skin causes hyperkeratosis and acanthosis and increases susceptibility to tumorigenesis. *Mol. Cell Biol.* **15**, 5732–5739 (1995).
152. Colandrea, T. D., D'Armiento, J., Kesari, K. V. & Chada, K. K. Collagenase induction promotes mouse tumorigenesis by two independent pathways. *Mol. Carcinog.* **29**, 8–16 (2000).
153. Matrisian, L. M. Cancer biology: extracellular proteinases in malignancy. *Curr. Biol.* **9**, R776–R778 (1999).
154. Rudolph-Owen, L. A., Chan, R., Muller, W. J. & Matrisian, L. M. The matrix metalloproteinase matrilysin influences early-stage mammary tumorigenesis. *Cancer Res.* **58**, 5500–5506 (1998).
155. Wilson, C. L., Heppner, K. J., Labosky, P. A., Hogan, B. L. & Matrisian, L. M. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. *Proc. Natl Acad. Sci. USA* **94**, 1402–1407 (1997).
156. Martin, D. C., Rütter, U., Sanchez-Sweetman, O. H., Orr, F. W. & Khokha, R. Inhibition of SV40 T antigen-induced hepatocellular carcinoma in TIMP-1 transgenic mice. *Oncogene* **13**, 569–576 (1996).
157. Buck, T. B., Yoshiji, H., Harris, S. R., Bunce, O. R. & Thorgeirsson, U. P. The effects of sustained elevated levels of circulating tissue inhibitor of metalloproteinases-1 on the development of breast cancer in mice. *Ann. NY Acad. Sci.* **878**, 732–735 (1999).
158. Goss, K. J., Brown, P. D. & Matrisian, L. M. Differing effects of endogenous and synthetic inhibitors of metalloproteinases on intestinal tumorigenesis. *Int. J. Cancer* **78**, 629–635 (1998).
159. Coussens, L. M., Fingleton, B. & Matrisian, L. M. Status of matrix metalloproteinase inhibitors as anti-cancer therapeutics. *Science* (in the press).

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Online links

DATABASES

The following terms in this article are linked online to: **CancerNet:** <http://www.cancer.gov/search/> brain tumours | breast cancers | colon cancer | gastric cancer | Kaposi's sarcoma | liver cancer | non-small-cell lung cancer | ovarian cancer | pancreatic cancer | prostate cancer | skin cancer | small-cell lung cancers | bladder cancer **LocusLink:** <http://www.ncbi.nlm.nih.gov/LocusLink/> aggrecan | BCL-X_i | CCL7 | CD44 | c-JUN | c-MET | collagen type IV | CXCL1 | CXCL12 | CXCL4 | CXCL7 | CXCL8 | CXCR4 | decorin | E-cadherin | EGFR | emmprin | endostatin | ETS | FAS | FASL | FGF receptor 1 | FGFs | fibronectin | HB-EGF | HER2 | HER4 | IL-2R α | α v integrin | α v β 3 integrin | keratan | laminin | laminin-5 | LEF-1 | MMP-1 | Mmp-2 | MMP-2 | MMP3 | Mmp-7 | MMP-7 | Mmp-9 | MMP-9 | Mmp-11 | MMP-11 | MMP-12 | MMP-13 | Mmp-14 | MMP-14 | MMP-15 | MMP-20 | MMP23 | MMP24 | MMP-28 | nidogen | p53 | PECAM-1 | perlecan | RECK | serpins | syndecans | tenascin | TGF- α | TGF- β | thrombospondin-1 | thrombospondin-2 | Timp-1 | TIMP-1 | Timp-2 | TIMP-2 | TIMP-3 | TIMP-4 | VE-cadherin | VEGF | versican | vitronectin **OMIM:** <http://www.ncbi.nlm.nih.gov/Omim/> neuroblastoma

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