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The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis

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Abstract

Metalloproteases are important in many aspects of biology, ranging from cell proliferation, differentiation and remodeling of the extracellular matrix (ECM) to vascularization and cell migration. These events occur several times during organogenesis in both normal development and during tumor progression. Mechanisms of metalloprotease action underlying these events include the proteolytic cleavage of growth factors so that they can become available to cells not in direct physical contact, degradation of the ECM so that founder cells can move across tissues into nearby stroma, and regulated receptor cleavage to terminate migratory signaling. Most of these processes require a delicate balance between the functions of matrix metalloproteases (MMPs) or metalloprotease-disintegrins (ADAMs) and natural tissue inhibitors of metalloproteases (TIMPs). In this review, we discuss recent progress in identifying an essential role for metalloproteases in axon outgrowth, as an example of a focal invasive event. We also discuss the evolving concept of how MMPs might regulate stem cell fate during tumor development.

Proteolytic machinery was initially thought to be devoted primarily to the recognition and elimination of unassembled or misfolded proteins. However, recent progress in this area has uncovered additional roles for regulated proteolysis in a variety of important biological processes. In particular, the developmental significance of modulation of the extracellular microenvironment by metalloproteases has become apparent¹.

Most, if not all cancers, acquire the same set of ectopic functions during their development: enhancement of growth signalling during hyperplasia and dysplasia, tissue invasion, enriched angiogenesis and metastasis². Each of these steps represents an evasion of normal regulation in cells and tissues. Breaching in all steps is unlikely to happen by accident, and thus this multi-layered protection is virtually fail-safe and might explain why cancer is a rare occurrence in an average lifetime. The routes by which cells become malignant vary, as does as the particular order in which ectopic functions are acquired.

Metalloproteases are the extrinsic regulators of each of these functions during not only malignant transformation but also normal development (Fig. 1)³. There are two closely related metalloprotease families: matrix metalloproteases (MMPs) and metalloprotease-disintegrins (ADAMs). MMPs are a family of over 20 enzymes that are characterized by their ability to degrade the extracellular matrix (ECM) and their dependence upon Zn²⁺ binding for proteolytic activity. ADAMs are transmembrane proteins that contain disintegrin and metalloprotease domains, indicative of cell adhesion and protease activities. Extensive work on the mechanisms of tumor development has identified MMPs and ADAMs as potential key players in the events that underlie tumor dissemination. Studies have so far provided compelling evidence that MMP

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activity can induce or enhance tumor growth or survival, invasion, angiogenesis and metastasis, whereas ADAM function is more focused, regulating growth signaling and tumor cell adhesion. Tissue inhibitors of metalloproteases (TIMPs) are a family of secreted proteins that selectively, but reversibly, inhibit metalloproteases in a 1:1 stoichiometric manner⁴. Below, we describe each function of metalloproteases and focus on the changing view of each capacity. Finally, we introduce an emerging role for metalloproteases in axon outgrowth, with an emphasis on the similarity of signaling regulation by metalloproteases – from growth control and angiogenesis to axon extension.

Role of metalloproteases in cell proliferation and the release of growth regulators

In elucidating the deregulation of tumor cell growth, inter-cellular signaling between the diverse cell types within a tumor mass is as important as intracellular signaling acquired from growth autonomy. Successful tumor cells are those that induce the release of growth-stimulating signals from neighboring cells. In this review, the release of extracellular domains of proteins from the cell surface by a mechanism involving metalloprotease-directed proteolysis is referred to as ectodomain shedding (Fig. 2). In addition to ectodomain shedding, release from matrix binding also serves as a major mechanism for making growth regulators bioavailable to cells not in direct physical contact (Fig. 2). Both MMPs and ADAMs participate in cell-surface proteolysis, leading to the release of a growing list of cell-surface growth regulators (Fig. 2).

Because MMPs degrade proteins in the ECM, their primary function has been presumed to be remodeling of the ECM. However, MMPs also act on the non-matrix substrates, including cell-surface and matrix-bound growth regulators, releasing them from stores. Coordinated regulation of MMPs and TIMPs therefore governs the cleavage and the release of many important growth factors and cell-surface receptors. For example, transforming growth factor β (TGF- β) signaling is important for both osteoblastic differentiation from osteoprogenitor cells to terminally differentiated osteocytes and for osteoclastic bone resorption. *In vitro* studies show that MMP-9 and MMP-2 can proteolytically cleave latent TGF- β , providing a novel and potentially important mechanism for TGF- β activation⁵. Indeed, phenotypic analysis of mutant mice clearly indicates a genetic interaction between MMPs and the TGF- β signaling cascade. The phenotypes of MMP-2 deficiency in a human hereditary osteolysis syndrome are similar to those of knockout mice of Smad3 or membrane-type 1 MMP, or a mouse mutant expressing a dominant-negative TGF- β receptor⁶. In another study, MMP-2 was shown to influence cell-surface receptor-mediated signaling by releasing the active ectodomain of fibroblast growth factor receptor 1 (FGFR-1)⁷. By contrast, the function of TIMPs is opposite to that of MMPs. For example, TIMP-3 or a broad-spectrum synthetic MMP inhibitor prevents tumor necrosis factor α (TNF- α) shedding in cell-culture models⁴.

More than 40 cell-surface proteins are known to undergo ectodomain shedding by proteolytic cleavage. Many examples of growth regulators have been documented thus far, such as TGF- α , TNF- α , Delta, FGF-R, stem cell factor (SCF), DCC (deleted in colon cancer) and ephrin⁸. Although some MMPs can act as 'sheddas', most of the known shedding events are mediated by ADAMs. Nevertheless, ADAM 17, ADAM 10/Kuzbanian and ADAM 9/MDC9 are the only ADAMs with documented ectodomain-shedding function. As ADAMs are themselves transmembrane proteases, the best-known substrates are other cell-surface proteins. However, a recent study showed that, rather than being a sheddase, ADAM 13 degrades the ECM, and its protease activity plays a crucial role in migration of neural crest cells⁹. One should not adopt the mistaken impression that MMPs and ADAMs are the only proteases capable of cell-surface proteolysis. Many other enzymes do this as well. For example, heparanase expressed by

platelets and tumor and inflammatory cells was shown to release an active heparan sulfate-bound form of FGF-2 from the ECM¹⁰.

Platelet-derived growth factors (PDGFs) are members of the PDGF/vascular endothelial growth factor (PDGF/VEGF) family of growth factors that are important for growth, survival and function in several types of connective tissue cells. After two decades of searching, PDGF-A and PDGF-B were thought to be the only ligands for PDGF receptors. Recently, however, data mining has resulted in the discovery of two additional members of the PDGF family, PDGF-C and PDGF-D (Ref. 11). PDGF-D, like PDGF-C, is activated upon limited proteolysis by an unknown protease, becoming a specific agonistic ligand for a subtype of PDGF receptor^{11,12}.

Role of metalloproteases in invasion

Throughout cancer progression, the microecology of the local host tissue is a consistently active participant in the evolving tumor (Fig. 2). Invasion occurs at the tumor–host interface, where tumor and stromal cells exchange enzymes and cytokines that modulate the local ECM and stimulate cell migration. Similar mechanisms are shared by physiological and tumorigenic invasion. In either case, the rate-limiting step is the breakdown of connective tissue barriers comprising collagens, laminins, fibronectin, vitronectin and heparan sulfate proteoglycans, which requires the action of MMPs. The difference between them is that physiological invasion is regulated, whereas tumorigenic invasion appears to be perpetual. Correlating with this observation, MMPs are invariably upregulated in the stromal compartment of invasive epithelial cancers, suggesting that deregulation of the ratio of MMP-to-TIMP activities underlies the discrepancy (Fig. 1). Examples of physiological invasion include wound repair, vasculogenesis and axon outgrowth (to be detailed later), in which cells respond to signals to migrate and penetrate the basement membrane in a controlled manner. In tumorigenic invasion, most MMPs are made by stromal cells of the host, but their expression is regulated by tumor cells through chemokines, cytokines and extracellular matrix metalloprotease inducer (EMMPRIN) (Fig. 2). EMMPRIN is enriched on the surface of tumor cells and stimulates the production of MMPs by adjacent stromal cells, which in turn activates tumor-cell invasion¹³. By contrast, during the invasive events, TIMPs are expressed primarily by the tumor cells¹⁴ and could serve as a regulatory mechanism for fine-tuning the activity of stromal MMPs, so that tumor cells can have an active role in determining where and when they invade.

Role of metalloproteases in angiogenesis

The process of angiogenesis is governed by an integrated signaling circuitry, and its modulation is dependent upon soluble angiogenic factors, cytokines and insoluble ECM components that surround the participating vessels (Fig. 2). Angiogenesis is necessary for persistent tumor growth because the sprouting capillaries are conduits for gas exchange and nutrient supply¹⁵. Without vascular growth, the tumor mass is restricted to within a tissue-diffusion distance of approximately 0.2 mm. Tumor vessels are recruited by sprouting or intussusception from pre-existing vessels, in which interstitial tissue columns are inserted into the lumen of pre-existing vessels and partition the vessel lumen. MMPs and TIMPs are essential regulators during various phases of the angiogenic process – from deposition and breakdown of the basement membrane of vascular structures, which depends on the effects of TIMP/MMP on the matrix substrates, to endothelial cell proliferation and migration, which depends on the mobilization of latent growth factors and receptor shedding.

Part of the influence of MMPs and TIMPs on angiogenesis is mediated by their effects on pro-angiogenic molecules (e.g. members of the VEGF and angiopoietin family) and anti-angiogenic molecules (e.g. angiostatin). A recent study has demonstrated that the switch from vascular quiescence to angiogenesis involves MMP-9/gelatinase B, which is upregulated in

angiogenic islets and tumors, releasing VEGF-A from an extracellular reservoir¹⁶. By contrast, another study has revealed that mice null for integrin $\alpha 1$, a molecule that is normally an inhibitor of MMP synthesis, display increased plasma levels of angiostatin because of the action of MMP-7 and MMP-9 on plasminogen, resulting in reduced tumor vascularization¹⁷. Taken together, during angiogenesis, MMPs can have both a pro-angiogenic role, by releasing matrix-bound pro-angiogenic factors, and also an anti-angiogenic role, by cleaving the ECM components into anti-angiogenic factors (Fig. 2). The balance between two roles in development dictates the outcome of the angiogenic switch by MMPs. It is also important to note that the ECM fragments cleaved by MMPs can also be pro-angiogenic molecules; for example, the trimeric NC1 domain of collagen XVIII induces endothelial cell migration in angiogenesis¹⁸.

Several angiogenesis inhibitors seem to be involved primarily in tumor angiogenesis. For example, TIMP-1 and TIMP-2 display biphasic phenotypic effects as a result of overexpression in tumor models¹⁹. Despite promotion of the *in vitro* growth rate of cells overexpressing TIMPs, *in vivo* tumor growth is delayed because of the suppression of tumor-induced host angiogenesis^{20,21}. It is not surprising that TIMPs also exert growth-promoting activity, as TIMPs were first identified for their erythroid-potentiating activity. However, this mitogenic activity is independent of the metalloprotease-inhibiting anti-angiogenic activity as mutations can selectively abolish the activity of one but not the other, suggesting that these two activities play different developmental roles. Expression of thrombospondin 2 (TSP-2), an endogenous angiogenesis inhibitor, is upregulated in the mesenchymal stroma of wild-type mice challenged with carcinogens²². The upregulation of TSP-2, which occurs throughout the multistep carcinogenesis process, provides a novel host anti-tumor defence strategy. So how might TSP-2 inhibit tumor angiogenesis? TSP-2-deficient fibroblasts show enhanced MMP-2 activity *in vitro*, suggesting modulation of MMP activity as a mechanism by which TSP-2 exerts its effect²³.

Although VEGF-A is well established as being an inducer of angiogenesis, the function of other VEGF isoforms is less well characterized. VEGF-C is particularly intriguing because it regulates lymphangiogenesis and thus might contribute to metastasis. In addition, its activity is regulated by proteolysis²⁴. Proteolytic cleavage of VEGF-C dramatically increases its binding to VEGFR-3 and enhances its efficacy for lymphangiogenesis, and also makes it acquire binding for VEGFR-2 and promotes its angiogenic potential. The enzymes that modify VEGF-C and the biological relevance of this pathway remain to be determined. The role of a second group of angiogenic factors in development, the FGFs, remains elusive, even though they are required for maintaining tumor angiogenesis²⁵. As in the case for VEGF, MMPs might regulate the bio-availability of FGFs.

Role of metalloproteases in metastasis

Because of their ECM-degrading activity, and the correlation between high levels of their activity and increased tumor metastasis, MMPs were initially thought to facilitate tumor cell metastasis by destroying the basement membrane and other components of the ECM. The ECM blocks tumor metastasis not only in the sense of being a physical barrier but also because it forms a self-protective, apoptosis-resistant microenvironment. This model is supported by studies of epithelial cells, under conditions of basement-membrane attachment when they acquire polarity and form organized tissue structures. A $\beta 4$ integrin mutation that leads to a nonpolarized ECM deposition renders normal and malignant mammary epithelial cells permissive to apoptosis (V.M. Weaver and M.J. Bissell, pers. commun.). However, there is increasing evidence to suggest a supportive role for ECM components in metastasis. A recent study based on the whole-genomic analysis of metastasis reveals that enhanced expression of several genes normally involved in ECM assembly correlates with progression to a metastatic

phenotype²⁶. Most noticeably, fibronectin is expressed at higher levels in all metastatic states tested. As other ECM proteins are also prominent in the assays, fibronectin is not likely to be the only ECM molecule that promotes migration. Although these studies are correlative, they further strengthen the observation made previously that competition between a peptide containing the cell-adhesive region of fibronectin and the wild-type fibronectin results in inhibition of metastasis²⁷. Furthermore, recent studies have shown that MMPs expose cryptic domains within ECM molecules (e.g. laminin-5) that can promote migration and metastasis²⁸. Taken together, these findings support a model in which tumor cells must interact with certain matrix molecules to metastasize and settle at a new site. Other non-ECM molecules have also been implicated in metastasis. Chemokines might determine the directional migration of tumor cells into specific organs²⁹. Although chemokines could provide the driving force for metastasis, MMPs are required to facilitate migration towards the chemokine source, perhaps by regulating chemokine bioavailability.

Insights into cancer from the role of metalloproteases in axon outgrowth

Like epithelial cells, neuronal cells are polarized and must navigate through an ECM-rich environment during development under the influence of attractive and repellent cues. Axon outgrowth can thus act as a paradigm for tissue invasion in tumorigenesis, as described below. It has long been suspected that proteolytic activity on neuronal growth cones controls their migratory activity³⁰. This possibility is further strengthened by the observation that *Drosophila* mutants with a mutation in *kuzbanian* (ADAM 10 in human) display an axon-stalling phenotype. It is believed that the main function of proteases is to create penetrable paths for axon extension and to modulate the activities of receptors and ligands through proteolytic processing. Both roles have been clearly demonstrated in recent studies. Plasminogen activators and MMPs are expressed by neurons and released by growth cones, implicating the growing tip of axons in the proteolysis of ECM substrata. MMP-9 is required for outgrowth by oligodendrocytes, presumably through its activity in remodeling the pericellular environment so that oligodendrocytes can advance processes from the soma³¹. In another study, neurite outgrowth by dorsal root ganglionic neurons cultured on normal nerve sections was increased significantly by first treating the nerve sections with MMP-2, which lifts the inhibitory activity of chondroitin sulfate proteoglycans and unmasks the neurite-promoting activity of associated laminin³². In parallel, two groups have reported that metalloproteases might play a role in the termination of axon extension signaling by modulating either the ligand or the receptor. The triggering of ephrin cleavage by Kuzbanian allows contact-mediated axon detachment by ephrin³³. It is possible that timing withdrawal allows growth cones to navigate to their final targets in a dynamic manner, which usually takes several cycles of retractions and advances. This might explain the axon-stalling phenotype in the *kuzbanian* mutants. In another report, inhibition of ectodomain shedding of DCC by a metalloprotease inhibitor (MPI) potentiates the axon outgrowth-promoting effects of netrin, a cognate ligand of DCC (Ref. 34). Interestingly, ephrins and DCC are also implicated in tumor progression. It has been suggested that ephrins are involved in human cancers through autocrine and/or juxtacrine activation³⁵. The association of DCC with the caspase-activating complex links DCC to tumor suppression³⁶. Because ephrins and DCC play a role in tumorigenesis, this raises the possibility of a crucial role for their sheddases in cancer development.

Concluding remarks

The substantial contribution of the extracellular microenvironment is as important to cell behavior as intracellular events in processes as diverse as neuronal development and neoplastic progression³⁷. This is demonstrated by the recent discoveries that inflammatory cells stimulate malignant conversion of keratinocytes via paracrine factors and that MMPs play a crucial role³⁸.

A large body of correlative data suggests that MMPs facilitate neoplastic progression in cancer. Most of these data have come from cell-culture and experimental transplant models showing that inhibition of MMP activity by over-expression of TIMPs or administration of synthetic MPIs can lead to either reduced tumor burden or diminished invasive or metastatic capacity. Study of specific MMP-null experimental mice similarly susceptible to *de novo* carcinogenesis supports these observations and further reveals novel *in vivo* roles for MMPs aside from matrix degradation, such as activation of angiogenesis by release of matrix-sequestered angiogenic growth factors and regulation of genomic instability, cell proliferation, apoptosis and inflammation⁴. A new paradigm has emerged, suggesting that MMPs function in many aspects of tumor evolution, in addition to mere degradation and/or remodeling of ECM molecules, and that MPIs in cancer trials may be more efficacious if given at an earlier stage and in combination with other therapeutics. If MMPs promote cancer development, then their inhibitors should prevent it. However, this has not always proven to be the case³⁹. Surprisingly, TIMP1 overexpression had no effect on tumor development in one line of *Apc^{min}* mice and augmented intestinal adenoma formation in another, whereas an MPI slowed adenoma formation⁴⁰. A recent analysis of the role of MMP-9 in squamous carcinogenesis indicates that, although MMP-9 mutation blunts premalignant progression and limits malignant conversion into carcinomas, the cancers that do emerge are skewed towards less-differentiated higher grades³⁸. Other studies of experimental metastasis models indicate that some tumors are promoted by MPIs^{14,41}, but importantly at the same time the specificity of the inhibitors for specific MMPs might influence the outcome⁴¹. Recent data from clinical trials testing the efficacy of MPIs in late-stage human patients show a similar tendency³⁹. The molecular mechanisms responsible for these failures in clinical settings are beginning to be elucidated, but might arise from the trial designs in which MPIs are administered with cytotoxic agents, but do no better than the cytotoxic agent alone. Whether MPIs work as well as cytotoxic agents remains to be determined. Significantly, results from animal studies suggest that MPI efficacy might be highest when administered during early neoplastic progression before frank tumor invasion.

Genetic dissection of signaling cascades important for metalloprotease-mediated developmental processes using *Caenorhabditis elegans* or *Drosophila* as model systems will undoubtedly help define the physiological targets and fundamental mechanisms of regulation by metalloproteases. The powerful genetics, short generation time and the completion of genome sequencing in these model organisms facilitate these analyses. Approximately 40% of *C.elegans* genes have apparent homologs in humans, and almost all protein domains found in humans are present in *C. elegans*⁴². So far, at least four MMPs, one TIMP, four ADAMs, and two ADAM-like ADAM-TS homologs have been identified in *C. elegans* by sequence homology [the *C. elegans* Sequencing Consortium (1998)] and by genetic experiments. Many developmental processes in *C. elegans* appear analogous to tumor invasion, vasculogenesis and tumor migration and thus could provide genetic model systems to investigate the essential role for metalloproteases in these processes⁴³.

Mammals differ from insects and nematodes in that during evolution they have acquired the ability to repair damaged tissues (Fig. 1). *Drosophila* and *C. elegans* have fixed cell lineages that could explain the lack of differentiation potential in cells after the embryonic stage. By contrast, even in postembryonic mammals, cells that are primitive and totipotent continue to exist⁴⁴. Because such mammalian stem cells are multipotent, they can choose a differentiation fate based on the demands placed on them for self-renewal or repair. As a result, mammals consistently set aside a cache of 'pro'-repair molecules, such as growth factors, angiogenic factors and metalloproteases, so that they are poised for executing a repair command at any time. Because mammalian cells can be flexible in their fate decision and commit themselves to respond to growth and differentiation cues, they are also tumor prone. In mammalian development, most MMPs are used several times. Their dynamic expression and activation

patterns are dependent on the specific cell-type and developmental stage. Similarly, in the malignant (deregulated repairing) status, different tumor cell types have unique MMP expression profiles. Any given MMP might act positively in promoting the evolution of certain tumor phases while acting negatively by inhibiting other steps in tumor progression. Without a mechanism regulating MMP functions selectively, spatially and temporally, as well as both positively and negatively, cancer therapy based on metalloprotease inhibition alone might not succeed.

Stem cells are pluripotent cells within adult tissue. Because only cells capable of regeneration can be transformed, one of the evolving concepts of cancer development is that stem cells are the targets of tumorigenesis by providing a reservoir of cell types required for multistep carcinogenesis. Resting stem cells are physically insulated from the microenvironmental influences that signal proliferation or differentiation^{44,45}. This raises the question of whether the mechanisms that arrest their differentiation potential are the same mechanisms used for reprogramming mutant cells in tumor development. Because metalloproteases have a pivotal role in modifying the cellular microenvironment during development and tumor progression, these enzymes might be responsible for orchestrating fate decision by stem cells in normal and disease states.

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References

1. Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. *Cell* 1997;91:439–442. [PubMed: 9390552]
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
3. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000;14:2123–2133. [PubMed: 10970876]
4. Sternlicht MD, Werb Z. How metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001;17:463–516. [PubMed: 11687497]
5. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- β and promotes tumor invasion and angiogenesis. *Genes Dev* 2000;14:163–176. [PubMed: 10652271]
6. Vu TH. Don't mess with the matrix. *Nat Genet* 2001;28:202–203. [PubMed: 11431682]
7. Levi E, et al. Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1. *Proc Natl Acad Sci U S A* 1996;93:7069–7074.
8. Primakoff P, Myles DG. The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet* 2000;16:83–87. [PubMed: 10652535]
9. Alfandari D, et al. *Xenopus* ADAM 13 is a metalloprotease required for cranial neural crest-cell migration. *Curr Biol* 2001;11:918–930. [PubMed: 11448768]
10. Vlodavsky I, Friedmann Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. *J Clin Invest* 2001;108:341–347. [PubMed: 11489924]
11. LaRochelle WJ, et al. PDGF-D, a new protease-activated growth factor. *Nat Cell Biol* 2001;3:517–521. [PubMed: 11331882]
12. Bergsten E, et al. PDGF-D is a specific, protease-activated ligand for the PDGF β -receptor. *Nat Cell Biol* 2001;3:512–516. [PubMed: 11331881]
13. Sun J, Hemler ME. Regulation of MMP-1 and MMP-2 production through CD147/extracellular matrix metalloproteinase inducer interactions. *Cancer Res* 2001;61:2276–2281. [PubMed: 11280798]

14. Soloway PD, et al. 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* 1996;13:2307–2314. [PubMed: 8957071]
15. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249–257. [PubMed: 11001068]
16. Bergers G, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol* 2000;2:737–744. [PubMed: 11025665]
17. Pozzi A, et al. Elevated matrix metalloprotease and angiostatin levels in integrin alpha 1 knockout mice cause reduced tumor vascularization. *Proc Natl Acad Sci U SA* 2000;97:2202–2207.
18. Kuo CJ, et al. Oligomerization-dependent regulation of motility and morphogenesis by the collagen XVIII NC1/endostatin domain. *J Cell Biol* 2001;152:1233–1246. [PubMed: 11257123]
19. Yan L, Moses MA. Commentary—A case of tumor betrayal – Biphasic effects of TIMP-1 on Burkitt's lymphoma. *Am J Pathol* 2001;158:1185–1190. [PubMed: 11290534]
20. Guedez L, et al. Tissue inhibitor of metalloproteinases (TIMP)-1 alters the tumorigenicity of Burkitt's lymphoma via divergent effects on tumor growth and angiogenesis. *Am J Pathol* 2001;158:1207–1215. [PubMed: 11290537]
21. Hajitou A, et al. Down-regulation of vascular endothelial growth factor by tissue inhibitor of metalloproteinase-2: effect on *in vivo* mammary tumor growth and angiogenesis. *Cancer Res* 2001;15:3450–3457. [PubMed: 11309307]
22. Hawighorst T, et al. Thrombospondin-2 plays a protective role in multistep carcinogenesis: a novel host anti-tumor defense mechanism. *EMBO J* 2001;20:2631–2640. [PubMed: 11387198]
23. Yang Z, et al. Matricellular proteins as modulators of cell-matrix interactions: adhesive defect in thrombospondin 2-null fibroblasts is a consequence of increased levels of matrix metalloproteinase-2. *Mol Biol Cell* 2000;11:3353–3364. [PubMed: 11029041]
24. Joukov V, et al. Proteolytic processing regulates receptor specificity and activity of VEGF-C. *EMBO J* 1997;16:3898–3911. [PubMed: 9233800]
25. Compagni A, et al. Fibroblast growth factors are required for efficient tumor angiogenesis. *Cancer Res* 2000;60:7163–7169. [PubMed: 11156426]
26. Clark EA, et al. Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* 2000;406:532–535. [PubMed: 10952316]
27. Humphries MJ, et al. A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. *Science* 1986;233:467–469. [PubMed: 3726541]
28. Koshikawa N, et al. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. *J Cell Biol* 2000;148:615–624. [PubMed: 10662785]
29. Muller A, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001;410:50–56. [PubMed: 11242036]
30. Krystosek A, Seeds NW. Plasminogen activator release at the neuronal growth cone. *Science* 1981;213:1532–1534. [PubMed: 7197054]
31. Oh LY, et al. Matrix metalloproteinase-9/Gelatinase B is required for process outgrowth by oligodendrocytes. *J Neurosci* 1999;19:8464–8475. [PubMed: 10493747]
32. Zuo J, et al. Neuronal matrix metalloproteinase-2 degrades and inactivates a neurite-inhibiting chondroitin sulfate proteoglycan. *J Neurosci* 1998;18:5203–5211. [PubMed: 9651203]
33. Hattori M, et al. Regulated cleavage of a contact-mediated axon repellent. *Science* 2000;289:1360–1365. [PubMed: 10958785]
34. Galko MJ, Tessier-Lavigne M. Function of an axonal chemoattractant modulated by metalloprotease activity. *Science* 2000;289:1365–1367. [PubMed: 10958786]
35. Dodelet VC, Pasquale EB. Eph receptors and ephrin ligands: embryogenesis to tumorigenesis. *Oncogene* 2000;19:5614–5619. [PubMed: 11114742]
36. Forcet C, et al. The dependence receptor DCC (deleted in colorectal cancer) defines an alternative mechanism for caspase activation. *Proc Natl Acad Sci U SA* 2001;98:3416–3421.
37. Radisky D, et al. Tumors are unique organs defined by abnormal signaling and context. *Semin Cancer Biol* 2001;11:87–95. [PubMed: 11322828]
38. oussens LM, Werb Z. Inflammatory cells and cancer: think different! *J Exp Med* 2001;193:23–26.

39. Sternlicht MD, Bergers G. Matrix metalloproteinases as emerging targets in anticancer therapy: status and prospects. *Emerg Therapeut Targets* 2000;4:609–633.
40. Heppner-Goss KJ, et al. Differing effects of endogenous and synthetic inhibitors of metalloproteinases on intestinal tumorigenesis. *Int J Cancer* 1998;78:629–635. [PubMed: 9808534]
41. Kruger A, et al. Hydroxamate-type matrix metalloproteinase inhibitor batimastat promotes liver metastasis. *Cancer Res* 2001;61:1272–1275. [PubMed: 11245418]
42. Sternberg PW. Working in the post-genomic *C. elegans* world. *Cell* 2001;105:173–176. [PubMed: 11336667]
43. Chang C, Sternberg PW. *C. elegans* vulval development as a model system to study the cancer biology of EGFR signaling. *Cancer Metastasis Rev* 1999;18:203–213. [PubMed: 10728984]
44. Watt FM, Hogan BLM. Out of Eden: stem cells and their niches. *Science* 2000;287:1427–1430. [PubMed: 10688781]
45. Arnold I, Watt FM. c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. *Curr Biol* 2001;11:558–568. [PubMed: 11369200]

Figure 1. The switches between states of development and physiology and between states of physiology and pathology by controlling the ratio of MMP/ADAM-to-TIMP activities
P indicates the matrix metalloprotease (MMP)/metalloprotease-disintegrin (ADAM) and I indicates the tissue inhibitors of metalloprotease (TIMP). Homeostasis and repair are symbolic of two chemical reactions with the activity of TIMP dictating the shift of the equilibrium. When the function of MMPs is balanced by that of TIMPs, the cells are in a state of physiological quiescence. There is a correlation between upregulation of MMP activity and the progression of cells from normalcy through a series of premalignant steps into invasive cancers. It is unclear whether the malignant cells can be redirected into the program of a normal state. Cells in red represent invasive capacity acquired during developmental and pathological states, and the cell in green represents quiescence.

Figure 2. Crosstalk between stromal and malignant epithelial cells

Most of the proteases complexed at the invasive front are contributed by the stromal cells (fibroblasts, inflammatory cells and endothelial cells). The invading carcinoma cells use these enzymes to disrupt basement membranes, invade nearby tissues and metastasize to different organs. The production of matrix metalloproteases (MMPs) by stroma is regulated by tumor cells through the expression of chemokines, cytokines and extracellular matrix metalloprotease inducer (EMMPRIN). The influence of tissue inhibitors of metalloprotease (TIMP) on the invasive behavior of tumorigenic cells is cell-autonomous, depending only on the TIMP genotype of the tumor and not that of the host. Arrows indicate the exchange of chemokines or cytokines or EMMPRIN and enzymes between the participating cells, modifying the adjacent extracellular matrix (ECM) and releasing cell-surface and matrix-bound molecules by proteolytic cleavage.

Membrane-type (MT) MMPs and metalloprotease-disintegrins (ADAMs) are transmembrane proteases, the best-known substrates of which are other transmembrane proteins (e.g. transmembrane ligands). Metalloprotease-mediated ligand release from the surface of the carcinoma cell regulates autocrine signaling. MMPs and ADAM-like proteases with

thrombospondin domains (ADAM-TS) are secreted proteases, the best-known substrates of which are extracellular matrix proteins.