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An odyssey from breast to bone: Multi-step control of mammary metastases and osteolysis by matrix metalloproteinases

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Abstract

Development of metastases distant to the primary site of solid tumors marks late stages of tumor progression. Almost all malignant mammary tumors are carcinomas arising from the breast epithelium, but the morphological and molecular alterations in the mammary stroma surrounding the premalignant and the growing tumor contribute to its conversion into neoplastic tissue. Two parameters are critical for initiation of the metastatic process and access of tumor cells to the circulation. These are the ability of tumor cells to invade the basement membrane and the stroma, and the neovascularization of breast tumor tissue. A major site for development of distant metastases is the skeleton. After colonizing the bone, tumor cells promote a cascade of events leading to recruitment of osteoclasts and subsequent osteolytic bone destruction. A ubiquitous theme of neoplastic progression of breast tumors is the overproduction of matrix metalloproteinases. In this review, we summarize the recent insights into the functional consequences of matrix metalloproteinase expression and activation during malignant conversion in the breast, and after bone colonization. The current literature supports the hypothesis that matrix metalloproteinases play a key role in the metastatic expansion of most, if not all, mammary tumors and in the ensuing bone loss.

Keywords

Breast cancer; migration; invasion; transdifferentiation; bone resorption; stromelysin-1; collagenase

Breast cancer is often fatal, and in the United States it is the most frequently occurring female cancer (1). Investigations on the molecular mechanisms giving rise to breast cancer have pinpointed a large number of molecules that show altered expression concomitant with progression of the disease. Studies in transgenic mice have identified genes that can cause or suppress breast cancer (2,3). While these investigations suggest that breast cancer is a multicausal disease and can arise through different molecular defects, a general observation in breast tumors is the alteration in expression of matrix metalloproteinases (MMPs). Three of the sixteen currently known MMPs, collagenase-3 (MMP-13), stromelysin-3 (MMP-11) and membrane-type 4 MMP (MT4-MMP, MMP-17) were initially cloned from breast tumor cDNA libraries (4–6). While named originally for their capability to degrade extracellular matrix (ECM), it is now apparent that MMPs have a number of other proteolytic targets that include cell surface molecules and growth factors (7). Since the discovery of expression of collagenolytic activity by breast tumor tissues (8,9), the overexpression and tissue compartmentalization of MMP proteins and mRNAs have been well documented. Commonly,

the excess MMPs are produced by stromal fibroblasts, but are sequestered by carcinoma cells (10). Only in a few instances, such as in the case of collagenase-3, gelatinase B (MMP-9), MT1-MMP (MMP-14) and MT2-MMP (MMP-15), were carcinoma cells, in addition to stromal cells, identified as the source of MMP synthesis (11–13). The notable exception is matrilysin (MMP-7), which is exclusively expressed by carcinoma cells (14). Probably the best correlation between high expression of a particular MMP, breast tumor progression and poor prognosis exists for stromelysin-3 (15,16), but abundant overexpression in breast tumors has also been noted for stromelysin-1 (MMP-3) (17), gelatinases A (MMP-2) and B (18), MT1-MMP (19) and MT2-MMP (6,13). In spite of the existing ample literature on MMP expression, only recently has there been significant insight towards the understanding of MMP function during the multiple stages of malignant progression.

As a first observable step in tumor formation, mammary epithelial cells escape the local and systemic growth control and begin an unscheduled proliferation (see Fig. 1 for major events associated with neoplastic conversion of breast tissue). According to the commonly accepted view, tumor cells will then induce alterations in the mesenchymal tissue compartment of the mammary gland giving rise to what is referred to as the stromal reaction (20).

Neovascularization of tumor tissue, which is a limiting step for tumor growth and metastasis, is only one of the many features of a reactive stroma. Tumor cells, stromal fibroblasts and myofibroblasts produce a plethora of growth factors, ECM constituents and other molecules that define the tumor microenvironment and convey the reciprocal communication between the two compartments (20,21). Whereas the epithelial cells of the early tumor grow within the confinement of a continuous basement membrane, basement membrane structure and also some of its constituents are generally lost in high grade tumors (20–22). Once basement membrane structures and thus acini are lost, tumors commonly become invasive, i.e. clusters of tumor cells infiltrate the stroma and, in more advanced tumors, enter the blood. However, cases have been reported where microscopic loss of basement membrane did not appear to precede the metastatic phenotype (22), possibly because small groups of invasive cells cannot always be detected by histological analysis. Nevertheless, the acquisition of invasive propensity by tumor cells appears to be closely linked to loss of integrity of basement membrane and is a prerequisite for carcinoma cells to reach the newly formed vasculature, to infiltrate the blood, and to extravasate into other tissues in order to be truly malignant.

The bone is the most or second most favored site for mammary metastasis formation (23–25). Although it is not known why breast tumor cells have a strong predilection to colonize the skeleton, the abundance of local cytokines and growth factors in bone may play a role (26, 27). Following exit of carcinoma cells from the circulation into the bone marrow sinus, the metastasis-associated bone remodeling process is initiated with the migration of surplus osteoclasts to the bone surface (see Fig. 2 for schematic summary). Osteoclasts are the major catabolic cells of the bone, whose biological task is to remove calcified matrix (28). In normal adult bone, bone remodeling is a process where bone resorption and bone formation are in equilibrium ensuring homeostasis. The major cell type responsible for deposition of new bone is the osteoblast (29). Osteoclast recruitment to the bone surface involves development of progenitor cells of the monocyte lineage into preosteoclast, and subsequent fusion of preosteoclasts to multinucleated giant cells, the mature osteoclasts. Proteolytic degradation of the unmineralized matrix that demarcates the endosteal bone surface, the osteoid, is prerequisite for access of osteoclasts to the calcified matrix. Breast tumor cells can promote bone resorption by secreting factors that target osteoclasts either directly or indirectly through mediation of osteoblasts. Among these molecules are macrophage colony-stimulating factor, parathyroid hormone-related peptide, hepatocyte growth factor and prostaglandins (30–33).

Processes of tissue remodeling, cell proliferation, survival, migration, and invasion are influenced by proteolytic enzymes of the MMP family. Evidence is now accumulating that the

multiple steps of breast tumor development and metastasis-associated osteolysis are also under control of MMPs and, consequently, influenced by the physiological inhibitors of MMPs, the tissue inhibitors of MMPs (TIMPs).

The Classical Function of MMPs: Regulation of Cell Migration and Invasion

By virtue of their MMP-degrading capacity, MMPs have been suggested to play a role in tumor cell invasion (34). Since then, it has been demonstrated repeatedly that overexpression of MMPs (10) increases, and inhibition of MMP activity decreases invasion of a variety of different tumor cell types. For mammary cells, it was shown that transfection of TIMP-4 into the human breast cancer cell line MDA-MB-435 impaired invasion in a transwell assay (35). In two mouse mammary carcinoma cell lines, SCg6 and TCL1, inhibition of stromelysin-1 expression by use of antisense oligodeoxynucleotides essentially abolished invasion of a reconstituted basement membrane and cut it by half in stromal collagen (36). Furthermore, when a stromelysin-1 cDNA was transfected into noninvasive and nonmalignant mouse mammary cells, cell migration was increased and invasion was induced (36,37). A similar observation was made also for gelatinase A, which promoted cell migration of human mammary epithelial cells by cleavage of laminin-5 (38). Thus, gelatinase A fragmentation of laminin-5 may expose cryptic epitopes that could interact with specific cell surface receptors to promote cell locomotion. Similar principles are likely to operate also in other migration/invasion associated processes. MMPs may also be directly involved in intravasation and extravasation, as is suggested by experiments where transfection of MDA-231 cells with TIMP-2 inhibited their transendothelial migration (39).

The Unorthodox Function of MMPs: Regulation of Tumor Initiation, Growth and Transdifferentiation

Although control of tumor cell migration and invasion is their most apparent function, MMPs are also involved in events that occur prior to acquisition of the invasive properties of mammary carcinoma cells. In mice where the stromelysin-3 gene was knocked out, tumor incidence and size were reduced after carcinogen treatment (40). Furthermore, after injection into immunocompromized mice, stromelysin-3 expressing fibroblasts from wild type mice promoted tumor growth of MCF7 cells much better than fibroblasts from knock-out mice. In an earlier study, MCF7 cells transfected with a stromelysin-3 antisense construct showed a reduced tumor incidence compared to MCF7 cells transfected with a stromelysin-3 sense cDNA (41). Likewise, in addition to decreasing the number of metastases, the synthetic MMP inhibitor batimastat reduced the rate of tumor formation in mice injected with MDA-MB-435 cells (42). In the same cell line, tumor growth and metastasis were also inhibited by overexpression of TIMP-4 (35). These results indicate that MMPs are not only important mediators of tumor cell invasion, but also play pivotal roles in regulating tumor initiation and growth, if expressed inappropriately.

In the models described above, cells that were already tumorigenic, or agents that promote tumor formation were used to study MMP function. Overexpression of stromelysin-1 in transgenic mice, however, was found to be sufficient to generate preneoplastic and malignant mammary gland lesions (43). The mammary phenotype that was observed prior to tumor formation gives insight into the complexity of MMP function: The mammary stroma of stromelysin-1 transgenic mice displayed morphological and molecular alterations reminiscent of the stromal reaction in breast cancer (44). There was an increase in the deposition of collagen around mammary ducts and alveoli, and an increase in the number of blood vessels. Furthermore, expression of tenascin-C, an ECM glycoprotein whose synthesis correlates with poor prognosis in breast cancer (45,46), and MMPs including stromelysin-3, matrilysin, gelatinase A and endogenous stromelysin-1 were augmented. This increase in MMP expression

may directly contribute to the early increase in vascularization, because MMPs, including gelatinase A, promote angiogenesis (47,48). These data suggest that MMPs modify the stromal phenotype, and that the stromal microenvironment in the breast favors tumor initiation and growth (20). The latter notion is supported also by data which show that fibroblasts from disease-free tissues of breast cancer patients, as well as from their cancer-free relatives, showed abnormal behavior and expression of a migration-stimulating factor (49), although these data have not been replicated by other laboratories.

The histopathology of stromelysin-1 transgenic mice argues in favor of an early stromal involvement for tumor formation. If the epithelial cells are already deficient in formation of an endogenous basement membrane, however, stromelysin-1 appears to be sufficient to induce premalignant and malignant changes in mammary cell lines in culture in the absence of stromal cells (Fig. 3) (37,50). Inducible expression of stromelysin-1 in a nonmalignant and functionally normal mouse mammary epithelial cell line, SCp2, resulted in loss of adhesive cell-cell contacts and transdifferentiation into invasive cells that had phenotypic properties of mammary carcinoma cells, including the ability to grow anchorage-independently (50). As in the case of stromelysin-1 overproducing mice, exogenous expression of stromelysin-1 in culture resulted in upregulation of endogenous MMP activity, suggesting a positive feedback regulation of MMP expression in mammary cells. We showed in the same study that stromelysin-1 induced keratinocyte growth factor expression. Since keratinocyte growth factor transgenic mice develop tumors (51), it is possible that increased expression of keratinocyte growth factor in response to a stromelysin-1 transgene may contribute to the formation of stromelysin-1-induced lesions. A trigger for the altered keratinocyte growth factor expression may be the stromelysin-1-stimulated cleavage of E-cadherin and the subsequent internalization of E-cadherin and catenins (50). This may directly lead to loss of cell-cell contacts, and may trigger β -catenin signaling which in turn could modulate expression of genes that favor tumor growth and invasion, such as keratinocyte growth factor (52,53).

An experimental antithesis to the evidence that MMP activity favors initiation and growth of mammary tumors is found in another study where stromelysin-1 transgenic mice were generated using a different promoter (54). Here, transgenic mice did not develop spontaneous tumors, and indeed, chemically-induced tumor formation was reduced in stromelysin-1-overexpressing mice. The differences in results between the two transgenic lines (43,54) may be due to differences in the genetic background of the mouse strains used, differences in the extent and profile of stromelysin-1 expression caused by the different promoters chosen in the two studies, and the tumor induction protocol used. In this context, it must be stressed also that prior to tumor initiation, MMPs fulfill multiple physiological functions that include the control of branching morphogenesis, tissue-specific gene expression, growth and apoptosis (55,56). Thus, the outcome of MMP activity will depend on the tissue context and the microenvironment, which determine the fate of mammary cells exposed to MMPs (57).

The Osteolytic Function of MMPs: Regulation of Destructive Bone Turnover

The presence of mammary carcinoma cells in bone upsets the balance of bone resorption and bone formation resulting in net bone loss (27). Thus, the major pathology of bone metastasis is bone destruction. Bone degradation promotes both growth of metastases and migration of tumor cells to the bone surface, resulting in a vicious cycle of tumor cell expansion and osteolysis (58,59). There is compelling evidence that bone degradation in breast cancer is brought about by osteoclasts (27), although one study showed that breast tumor cells in culture are able to destroy devitalized bone (60).

Whether production of MMPs by breast cancer cells at the sites of bone metastases contributes to bone resorption has not been examined. However, MMPs are required for osteoclastic bone

dissolution. In an early investigation, it was observed that a poorly defined inhibitor of collagenolytic activity reduces release of mineral from fetal bones in culture (61). Since then, a number of synthetic MMP inhibitors, and recombinant TIMP-1 and TIMP-2, were shown to inhibit bone resorption in a variety of different bone culture systems (62–68).

Although a key role for MMPs in mediating bone resorption is uncontested, it is not clear at which stage(s) of osteoclast differentiation and activation MMPs affect bone degradation. It is also still a subject of debate whether or not MMPs contribute to the removal of ECM constituents from mineralized matrix after establishment of a resorption compartment by the osteoclast. In only one out of four studies, bone resorption of osteoclasts seeded on osteoid-free bone slices could be reduced by MMP inhibitors (67,69–71). However, under more physiological conditions in cultures of intact calvarial bone, electron microscopic studies showed that degradation of ECM in the resorption zone was dramatically reduced by MMP inhibitors (72,73). Resorption compartments constitute an acidic microenvironment in which MMPs, including collagenases and gelatinases, are less proteolytically active than at neutral pH (74–76). It was therefore suggested that in the sequence of events that result in bone matrix dissolution in the resorption compartment, a less acidic environment, found during later stages of matrix dissolution, may favor collagen removal by MMPs, whereas a more acidic microenvironment found in the young resorption zone, may favor proteoglycan degradation by cysteine proteinases (73).

MMPs may also regulate the motile and invasive behavior of osteoclasts which is directed towards the bone surface. In bone organ cultures, migration of osteoclast precursors to the bone marrow cavity could be blunted by different MMP inhibitors (77). This may be due to inhibition of both MMPs that are produced by osteoclasts, such as gelatinase B and MT1-MMP (78), and MMPs produced by bone cells and encountered by the osteoclasts, such as collagenases, gelatinase A, or stromelysin-1 (79). In this system, MMP inhibitors may reduce the release of ECM fragments from the matrix that are chemoattractants for osteoclasts (80), or may affect the concentration of osteoclast-stimulating cytokines that are sequestered by ECM (81). A direct involvement of MMPs in motility of osteoclasts is suggested by experiments where collagen invasion of osteoclastic cell protrusions was retarded by synthetic MMP inhibitors and TIMP-2 (71).

Evidence is accumulating that the degradation of osteoid by MMPs also plays a major role in controlling access of osteoclasts to the calcified matrix. At the bone surface, dissolution of osteoid is achieved by degradation of type I collagen (82), the major ECM component in bone. Pretreatment of cell-free endocranial bone surfaces with collagenase enhanced osteoclastic bone resorption (83), and antibodies against collagenase inhibited bone degradation (64). Thus, degradation of collagen by collagenases, collagenase-3 in rodents and collagenases-1 and -3 in humans (82), promotes bone dissolution by osteoclasts. The major cells responsible for collagenase production in bone are cells of the osteoblast lineage, although localization of both collagenase protein and mRNA in osteoclasts also has been reported (82,84). Therefore, the removal of osteoid by osteoblast-produced collagenase is likely to facilitate osteoclastic activity (83). A consequence of collagenase action may involve the exposure of the osteoclast plasma membrane to ECM glycoproteins, such as fibronectin, vitronectin, osteopontin and bone sialoprotein, for which osteoclasts have a higher affinity through their $\alpha\text{v}\beta 3$ integrins than to native collagens to which they adhere using $\alpha 2\beta 1$ integrins (85). This strong adhesion may promote establishment of the resorption compartment, an hypothesis that is supported by data where fibronectin and bone sialoprotein were shown to increase both osteoclast adhesion to bone surfaces and bone resorption (86). Interestingly, mammary tumor cells express major bone proteins such as bone sialoprotein and osteopontin which could directly promote osteoclastic functions (87,88). Bone resorption by osteoclasts is also augmented by collagenase-cleaved collagen when coated onto mineralized matrix (64). The observation that

mammary carcinoma cells produce factors that stimulate collagenase synthesis by osteoblasts (89) may therefore be an additional mode by which these cells exert control over the osteolytic process. Furthermore, breast tumor cells respond to collagen and collagen fragments with chemotactic migration (90). Thus, collagenase appears to couple bone destruction to recruitment of mammary carcinoma cells to the site of bone resorption.

Conclusions

The critical involvement of MMPs in breast tumor initiation, growth, and formation of osteolytic metastases is becoming well established. However, the precise molecular mechanism by which MMPs function is far from understood. Because MMPs have such a variety of different substrates within the ECM network, their exact target molecules will be difficult to identify *in vivo*. Furthermore, while it may be straightforward to understand how MMPs are utilized at the invasive front of cells (91), the molecular basis for the long term effects of MMP action presents a conundrum. Nevertheless, almost all available literature supports the conclusion that MMPs favor tumor initiation, growth and transdifferentiation, as well as tumor cell migration, invasion and osteoclast-mediated osteolysis after establishment of bone metastases (Table 1). Thus, MMPs may prove excellent targets for breast cancer therapy, and indeed, hydroxamic acid-based MMP inhibitors are currently being tested in clinical trials (92,93). However, the fact that MMPs are also involved in many other physiological processes involved in tissue maintenance and repair in the adult may limit the therapeutic use of orally or systemically administered MMP inhibitors. Furthermore, it must be appreciated that MMP inhibitors may not completely revert or obliterate, but only retard tumorigenesis-associated phenomena. Thus, combination therapy using MMP inhibitors in conjunction with other anticancer drugs may be indicated.

Bisphosphonates are common therapeutics used to reduce the burden of bone metastases in breast cancer. They have a high affinity for bone matrix and target the osteoclast and other bone cells (94). They have pleiotropic effects, one of which is the inhibition of protein tyrosine phosphatases and another is the ability to reduce MMP activity both in cell-free systems and *in vivo* (95,96). In an *in vivo* model to study development of osteolytic bone metastases using the human breast cancer cell line MDA-231, cells were transfected with TIMP-2 and injected into the heart ventricle of mice from where they colonized the skeleton (39). Bone metastases were then monitored in animals that had or had not received subcutaneous injections of ibandronate, a potent bisphosphonate. Osteolytic lesions induced by MDA-231 cells not transfected with TIMP-2 could be significantly reduced by treatment of animals with ibandronate, and a marked reduction of bone destruction was observed also in the absence of ibandronate when TIMP-2 transfected cells were used instead of wild type cells. However, a significant reduction in tumor burden and complete inhibition of osteolysis could be obtained only when ibandronate was used on animals inoculated with cells transfected with TIMP-2.

In this short review, we have outlined major discoveries that have contributed to elucidating the effects of MMPs on breast cancer progression and tumor cell-induced osteolysis. However, some of the data reviewed have to be interpreted with caution because of the heterogeneity of cells within a tumor, the differences between the rodent and human mammary gland that pertain to both the parenchyma and the stroma (20), and the apparent differences between the human pathological condition and the model systems employed by scientists at the bench. In spite of these cautionary notes, it appears that MMPs are able to control the malignant process at many levels and during various stages of tumor progression. Thus, it is hoped that the future development of more specific MMP inhibitors that can be administered to patients easily, in conjunction with a better understanding of the physiological mechanisms of MMP function, will facilitate the therapy of breast cancer and other malignancies.

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References

1. Wingo PA, Tong T, Bolden S. Cancer statistics, 1995. *CA Cancer J Clin* 1995;45:8–30. [PubMed: 7528632]
2. Dankort DL, Muller WJ. Transgenic models of breast cancer metastasis. *Cancer Treat Res* 1996;83:71–88. [PubMed: 8826642]
3. Webster MA, Muller WJ. Mammary tumorigenesis and metastasis in transgenic mice. *Semin Cancer Biol* 1994;5:69–76. [PubMed: 8186390]
4. Freije JM, Diez-Itza I, Balbin M, Sanchez LM, Blasco R, Tolivia J, et al. Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. *J Biol Chem* 1994;269:16766–73. [PubMed: 8207000]
5. Puente XS, Pendas AM, Llano E, Velasco G, Lopez-Otin C. Molecular cloning of a novel membrane-type matrix metalloproteinase from a human breast carcinoma. *Cancer Res* 1996;56:944–9. [PubMed: 8640782]
6. Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Limacher JM, et al. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature* 1990;348:699–704. [PubMed: 1701851]
7. Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. *Cell* 1997;91:439–42. [PubMed: 9390552]
8. Feller WF, Stewart SE, Kantor J. Primary tissue culture explants of human breast cancer. *J Natl Cancer Inst* 1972;48:1117–20. [PubMed: 4336875]
9. Langer E, Keiditsch E, Strauch L, Hannig K. Die Kollagenaseaktivität im carcinoma solidum simplex mammae. *Verh Dtsch Ges Pathol* 1968;52:438–41. [PubMed: 4182276]
10. MacDougall JR, Matrisian LM. Contributions of tumor and stromal matrix metalloproteinases to tumor progression, invasion and metastasis. *Cancer Metastasis Rev* 1995;14:351–62. [PubMed: 8821095]
11. Heppner KJ, Matrisian LM, Jensen RA, Rodgers WH. Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. *Am J Pathol* 1996;149:273–82. [PubMed: 8686751]
12. Soini Y, Hurskainen T, Hoyhtya M, Oikarinen A, Autio-Harminen H. 72 KD and 92 KD type IV collagenase, type IV collagen, and laminin mRNAs in breast cancer: a study by in situ hybridization. *J Histochem Cytochem* 1994;42:945–51. [PubMed: 8014478]
13. Ueno H, Nakamura H, Inoue M, Imai K, Noguchi M, Sato H, et al. Expression and tissue localization of membrane-types 1, 2, and 3 matrix metalloproteinases in human invasive breast carcinomas. *Cancer Res* 1997;57:2055–60. [PubMed: 9158005]
14. Wilson CL, Matrisian LM. Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions. *Int J Biochem Cell Biol* 1996;28:123–36. [PubMed: 8729000]
15. Chenard MP, O'Siorain L, Shering S, Rouyer N, Lutz Y, Wolf C, et al. High levels of stromelysin-3 correlate with poor prognosis in patients with breast carcinoma. *Int J Cancer* 1996;69:448–51. [PubMed: 8980245]
16. Rouyer N, Wolf C, Chenard MP, Rio MC, Chambon P, Bellocq JP, et al. Stromelysin-3 gene expression in human cancer: an overview. *Invasion Metastasis* 1994;14:269–75. [PubMed: 7657519]
17. Matrisian LM, Bowden GT. Stromelysin/transin and tumor progression. *Semin Cancer Biol* 1990;1:107–15. [PubMed: 2103488]
18. Tryggvason K, Hoyhtya M, Pyke C. Type IV collagenases in invasive tumors. *Breast Cancer Res Treat* 1993;24:209–18. [PubMed: 8435476]
19. Polette M, Nawrocki B, Gilles C, Sato H, Seiki M, Tournier JM, et al. MT-MMP expression and localisation in human lung and breast cancers. *Virchows Arch* 1996;428:29–35. [PubMed: 8646366]

20. Ronnov-Jessen L, Petersen OW, Bissell MJ. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. *Physiol Rev* 1996;76:69–125. [PubMed: 8592733]
21. Lochter A, Bissell MJ. Involvement of extracellular matrix constituents in breast cancer. *Semin Cancer Biol* 1995;6:165–73. [PubMed: 7495985]
22. Flug M, Kopf-Maier P. The basement membrane and its involvement in carcinoma cell invasion. *Acta Anat (Basel)* 1995;152:69–84. [PubMed: 7660759]
23. Body JJ. Clinical trials in metastatic breast cancer to bone: past – present – future. *Can J Oncol* 1995;5 (Suppl 1):16–27. [PubMed: 8853520]
24. Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. *Br J Cancer* 1987;55:61–6. [PubMed: 3814476]
25. Walther, HE. Krebsmetastasen. Bens Schwabe Verlag; Switzerland: 1948.
26. Hauschka PV, Mavrakos AE, Iafrati MD, Doleman SE, Klagsbrun M. Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-Sepharose. *J Biol Chem* 1986;261:12665–74. [PubMed: 3745206]
27. Mundy GR. Mechanisms of bone metastasis. *Cancer* 1997;80:1546–56. [PubMed: 9362421]
28. Suda T, Nakamura I, Jimi E, Takahashi N. Regulation of osteoclast function. *J Bone Miner Res* 1997;12:869–79. [PubMed: 9169344]
29. Aubin JE, Liu F, Malaval L, Gupta AK. Osteoblast and chondroblast differentiation. *Bone* 1995;17:77S–83S. [PubMed: 8579903]
30. Fuller K, Owens J, Chambers TJ. The effect of hepatocyte growth factor on the behaviour of osteoclasts. *Biochem Biophys Res Commun* 1995;212:334–40. [PubMed: 7626045]
31. Scholl SM, Crocker P, Tang R, Pouillart P, Pollard JW. Is colony-stimulating factor-1 a key mediator of breast cancer invasion and metastasis? *Mol Carcinog* 1993;7:207–11. [PubMed: 8352880]
32. Subbaramaiah K, Telang N, Ramonetti JT, Araki R, DeVito B, Weksler BB, et al. Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. *Cancer Res* 1996;56:4424–9. [PubMed: 8813136]
33. Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, et al. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. *J Clin Invest* 1996;98:1544–9. [PubMed: 8833902]
34. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 1980;284:67–8. [PubMed: 6243750]
35. Wang M, Liu YE, Greene J, Sheng S, Fuchs A, Rosen EM, et al. Inhibition of tumor growth and metastasis of human breast cancer cells transfected with tissue inhibitor of metalloproteinase 4. *Oncogene* 1997;14:2767–74. [PubMed: 9190892]
36. Lochter A, Srebrow A, Simpson CJ, Terracio N, Werb Z, Bissell MJ. Misregulation of stromelysin-1 expression in mouse mammary tumor cells accompanies acquisition of stromelysin-1-dependent invasive properties. *J Biol Chem* 1997;272:5007–15. [PubMed: 9030563]
37. Lochter A, Sternlicht MD, Werb Z, Bissell MJ. The significance of matrix metalloproteinases during early stages of tumor progression. *Ann NY Acad Sci* 1998;857:101–18.
38. Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. Induction of cell migration by matrix metalloproteinase-2 cleavage of laminin-5. *Science* 1997;277:225–8. [PubMed: 9211848]
39. Yoneda T, Sasaki A, Dunstan C, Williams PJ, Bauss F, De Clerck YA, et al. Inhibition of osteolytic bone metastasis of breast cancer by combined treatment with the bisphosphonate ibandronate and tissue inhibitor of the matrix metalloproteinase-2. *J Clin Invest* 1997;99:2509–17. [PubMed: 9153295]
40. Masson R, Lefebvre O, No, Fahime ME, Chenard MP, Wendling C, et al. In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. *J Cell Biol* 1998;140:1535–41. [PubMed: 9508784]
41. Noel AC, Lefebvre O, Maquoi E, VanHoorde L, Chenard MP, Mareel M, et al. Stromelysin-3 expression promotes tumor take in nude mice. *J Clin Invest* 1996;97:1924–30. [PubMed: 8621777]

42. Sledge GWJ, Qulali M, Goulet R, Bone EA, Fife R. Effect of matrix metalloproteinase inhibitor batimastat on breast cancer regrowth and metastasis in athymic mice. *J Natl Cancer Inst* 1995;87:1546–50. [PubMed: 7563189]
43. Simpson CJ, Bissell MJ, Werb Z. Mammary gland tumor formation in transgenic mice overexpressing stromelysin-1. *Semin Cancer Biol* 1995;6:159–63. [PubMed: 7495984]
44. Thomasset N, Lochter A, Simpson CJ, Lund LR, Williams PJ, Behrendtsen O, et al. Expression of autoactivated stromelysin-1 in mammary glands of transgenic mice leads to a reactive stroma during early development. *Am J Pathol* 1998;153:457–67. [PubMed: 9708806]
45. Yoshida T, Ishihara A, Hirokawa Y, Kusakabe M, Sakakura T. Tenascin in breast cancer development – is epithelial tenascin a marker for poor prognosis? *Cancer Lett* 1995;90:65–73. [PubMed: 7536626]
46. Mackie EJ, Chiquet-Ehrismann R, Pearson CA, Inaguma Y, Taya K, Kawarada Y, et al. Tenascin is a stromal marker for epithelial malignancy in the mammary gland. *Proc Natl Acad Sci USA* 1987;84:4621–5. [PubMed: 2440026]
47. Brooks PC, Silletti S, von Schalscha TL, Friedlander M, Cheresh DA. Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. *Cell* 1998;92:391–400. [PubMed: 9476898]
48. Moses MA. The regulation of neovascularization of matrix metalloproteinases and their inhibitors. *Stem Cells* 1997;15:180–9. [PubMed: 9170209]
49. Schor SL, Schor AM, Howell A, Crowther D. Hypothesis: persistent expression of fetal phenotypic characteristics by fibroblasts is associated with an increased susceptibility to neoplastic disease. *Exp Cell Biol* 1987;55:11–7. [PubMed: 3569635]
50. Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ. 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* 1997;139:1861–72. [PubMed: 9412478]
51. Kitsberg DI, Leder P. Keratinocyte growth factor induces mammary and prostatic hyperplasia and mammary adenocarcinoma in transgenic mice. *Oncogene* 1996;13:2507–15. [PubMed: 9000125]
52. Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, et al. Functional interaction of β -catenin with the transcription factor LEF-1. *Nature* 1996;382:638–42. [PubMed: 8757136]
53. Takeichi M. Cadherins in cancer: implications for invasion and metastasis. *Curr Opin Cell Biol* 1993;5:806–11. [PubMed: 8240824]
54. Witty JP, Lempka T, Coffey RJJ, Matrisian LM. Decreased tumor formation in 7,12-dimethylbenzanthracene-treated stromelysin-1 transgenic mice is associated with alterations in mammary epithelial cell apoptosis. *Cancer Res* 1995;55:1401–6. [PubMed: 7882342]
55. Lochter A, Bissell MJ. Mammary gland biology and the wisdom of extracellular matrix. In: Wilde, CJ.; Peaker, M.; Taylor, E., editors. *Biological signaling and the mammary gland*. Ayr. UK: 1997. p. 77-92.
56. Bissell MJ. The central role of basement membrane in functional differentiation apoptosis and cancer. In: Tilly, JF.; Strauss, JF., III; Tenniswood, H., editors. *Cell Death in Reproductive Physiology*. Sereno Symposia; USA: 1997. p. 125-40.
57. Hagios C, Lochter A, Bissell MJ. Tissue structure: the ultimate regulator of epithelial function? *Phil Transactions Roy Soc Lond*. 1998 in press.
58. Manishen WJ, Sivananthan K, Orr FW. Resorbing bone stimulates tumor cell growth. A role for the host microenvironment in bone metastasis. *Am J Pathol* 1986;123:39–45. [PubMed: 3457536]
59. Giunciuglio D, Cai T, Filanti C, Manduca P, Albini A. Effect of osteoblast supernatants on cancer cell migration and invasion. *Cancer Lett* 1995;97:69–74. [PubMed: 7585480]
60. Eilon G, Mundy GR. Direct resorption of bone by human breast cancer cells in vitro. *Nature* 1978;276:726–8. [PubMed: 732878]
61. Horton JE, Wezeman FH, Kuettner KE. Inhibition of bone resorption in vitro by a cartilage-derived anticollagenase factor. *Science* 1978;199:1342–5. [PubMed: 204011]
62. Hill PA, Reynolds JJ, Meikle MC. Inhibition of stimulated bone resorption in vitro by TIMP-1 and TIMP-2. *Biochim Biophys Acta* 1993;1177:71–4. [PubMed: 8485170]

63. Shimizu H, Sakamoto M, Sakamoto S. Bone resorption by isolated osteoclasts in living versus devitalized bone: differences in mode and extent and the effects of human recombinant tissue inhibitor of metalloproteinases. *J Bone Miner Res* 1990;5:411–8. [PubMed: 2343781]
64. Holliday LS, Welgus HG, Fliszar CJ, Veith GM, Jeffrey JJ, Gluck SL. Initiation of osteoclast bone resorption by interstitial collagenase. *J Biol Chem* 1997;272:22053–8. [PubMed: 9268345]
65. Witty JP, Foster SA, Stricklin GP, Matrisian LM, Stern PH. Parathyroid hormone-induced resorption in fetal rat limb bones is associated with production of the metalloproteinases collagenase and gelatinase B. *J Bone Miner Res* 1996;11:72–8. [PubMed: 8770699]
66. Delaisse JM, Eeckhout Y, Sear C, Galloway A, McCullagh K, Vaes G. A new synthetic inhibitor of mammalian tissue collagenase inhibits bone resorption in culture. *Biochem Biophys Res Commun* 1985;133:483–90. [PubMed: 3002346]
67. Hill PA, Murphy G, Docherty AJ, Hembry RM, Millican TA, Reynolds JJ, et al. The effects of selective inhibitors of matrix metalloproteinases (MMPs) on bone resorption and the identification of MMPs and TIMP-1 in isolated osteoclasts. *J Cell Sci* 1994;107:3055–64. [PubMed: 7699005]
68. Hill PA, Docherty AJ, Bottomley KM, O'Connell JP, Morphy JR, Reynolds JJ, et al. Inhibition of bone resorption in vitro by selective inhibitors of gelatinase and collagenase. *Biochem J* 1995;308:167–75. [PubMed: 7755562]
69. Delaisse JM, Boyde A, Maconnachie E, Ali NN, Sear CH, Eeckhout Y, et al. The effects of inhibitors of cysteine-proteinases and collagenase on the resorptive activity of isolated osteoclasts. *Bone* 1987;8:305–13. [PubMed: 2827713]
70. Fuller K, Chambers TJ. Localisation of mRNA for collagenase in osteocytic, bone surface and chondrocytic cells but not osteoclasts. *J Cell Sci* 1995;108:2221–30. [PubMed: 7673342]
71. Sato T, Foged NT, Delaisse JM. The migration of purified osteoclasts through collagen is inhibited by matrix metalloproteinase inhibitors. *J Bone Miner Res* 1998;13:59–66. [PubMed: 9443791]
72. Everts V, Delaisse JM, Korper W, Niehof A, Vaes G, Beertsen W. Degradation of collagen in the bone-resorbing compartment underlying the osteoclast involves both cysteine-proteinases and matrix metalloproteinases. *J Cell Physiol* 1992;150:221–31. [PubMed: 1734028]
73. Everts, V.; Delaisse, JM.; Korper, W.; Beertsen, W. Involvement of matrix metalloproteinases and cysteine proteinases in degradation of bone matrix. In: Davidovitch, Z., editor. *The biological mechanism of tooth eruption, resorption and replacement by implants*. 1994. p. 85-97.
74. Eeckhout Y. Possible role and mechanism of action of dissolved calcium in the degradation of bone collagen by lysosomal cathepsins and collagenase. *Biochem J* 1990;272:529–32. [PubMed: 2176466]
75. Okada Y, Naka K, Kawamura K, Matsumoto T, Nakanishi I, Fujimoto N, et al. Localization of matrix metalloproteinase 9 (92-kilodalton gelatinase/type IV collagenase=gelatinase B) in osteoclasts: implications for bone resorption. *Lab Invest* 1995;72:311–22. [PubMed: 7898050]
76. Okada Y, Morodomi T, Engchild JJ, Suzuki K, Yasui A, Nakanishi I, et al. Matrix metalloproteinase 2 from human rheumatoid synovial fibroblasts. Purification and activation of the precursor and enzymic properties. *Eur J Biochem* 1990;194:721–30. [PubMed: 2269296]
77. Blavier L, Delaisse JM. Matrix metalloproteinases are obligatory for the migration of preosteoclasts to the developing marrow cavity of primitive long bones. *J Cell Sci* 1995;108:3649–59. [PubMed: 8719871]
78. Reponen P, Sahlberg C, Munaut C, Thesleff I, Tryggvason K. High expression of 92-kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. *J Cell Biol* 1994;124:1091–102. [PubMed: 8132709]
79. Meikle MC, Bord S, Hembry RM, Compston J, Croucher PI, Reynolds JJ. Human osteoblasts in culture synthesize collagenase and other matrix metalloproteinases in response to osteotropic hormones and cytokines. *J Cell Sci* 1992;103:1093–9. [PubMed: 1336777]
80. Malone JD, Teitelbaum SL, Griffin GL, Senior RM, Kahn AJ. Recruitment of osteoclast precursors by purified bone matrix constituents. *J Cell Biol* 1982;92:227–30. [PubMed: 6976967]
81. Ruoslahti E, Yamaguchi Y. Proteoglycans as modulators of growth factor activities. *Cell* 1991;64:867–9. [PubMed: 2001586]
82. Partridge NC, Walling HW, Bloch SR, Omura TH, Chan PT, Pearman AT, et al. The regulation and regulatory role of collagenase in bone. *Crit Rev Eukaryot Gene Expr* 1996;6:15–27. [PubMed: 8882305]

83. Chambers TJ, Fuller K. Bone cells predispose bone surfaces to resorption by exposure of mineral to osteoclastic contact. *J Cell Sci* 1985;76:155–65. [PubMed: 4066784]
84. Delaisse JM, Eeckhout Y, Neff L, Francois-Gillet C, Henriot P, Su Y, et al. (Pro)collagenase (matrix metalloproteinase-1) is present in rodent osteoclasts and in the underlying bone-resorbing compartment. *J Cell Sci* 1993;106:1071–82. [PubMed: 8126092]
85. Rodan SB, Rodan GA. Integrin function in osteoclasts. *J Endocrinol* 1997;154(Suppl):S47–S56. [PubMed: 9379137]
86. Raynal C, Delmas PD, Chenu C. Bone sialoprotein stimulates in vitro bone resorption. *Endocrinology* 1996;137:2347–54. [PubMed: 8641185]
87. Bellahcene A, Merville MP, Castronovo V. Expression of bone sialoprotein, a bone matrix protein, in human breast cancer. *Cancer Res* 1994;54:2823–6. [PubMed: 8187059]
88. Oates AJ, Barraclough R, Rudland PS. The identification of osteopontin as a metastasis-related gene product in a rodent mammary tumour model. *Oncogene* 1996;13:97–104. [PubMed: 8700559]
89. Ohishi K, Fujita N, Morinaga Y, Tsuruo T. H-31 human breast cancer cells stimulate type I collagenase production in osteoblast-like cells and induce bone resorption. *Clin Exp Metastasis* 1995;13:287–95. [PubMed: 7606891]
90. Mundy GR, DeMartino S, Rowe DW. Collagen and collagen-derived fragments are chemotactic for tumor cells. *J Clin Invest* 1981;68:1102–5. [PubMed: 7287902]
91. Chen WT. Proteases associated with invadopodia, and their role in degradation of extracellular matrix. *Enzyme Protein* 1996;49:59–71. [PubMed: 8796997]
92. Denis LJ, Verweij J. Matrix metalloproteinase inhibitors: present achievements and future prospects. *Invest New Drugs* 1997;15:175–85. [PubMed: 9387040]
93. Rasmussen HS, McCann PP. Matrix metalloproteinase inhibition as a novel anticancer strategy: a review with special focus on batimastat and marimastat. *Pharmacol Ther* 1997;75:69–75. [PubMed: 9364582]
94. Lipton A. Bisphosphonates and breast carcinoma. *Cancer* 1997;80:1668–73. [PubMed: 9362434]
95. Teronen O, Konttinen YT, Lindqvist C, Salo T, Ingman T, Lauhio A, et al. Inhibition of matrix metalloproteinase-1 by dichloromethylene bisphosphonate (clodronate). *Calcif Tissue Int* 1997;61:59–61. [PubMed: 9192515]
96. Stearns ME, Wang M. Effects of alendronate and taxol on PC-3 ML cell bone metastases in SCID mice. *Invasion Metastasis* 1996;16:116–31. [PubMed: 9186547]

Fig. 1. Schematic representation of major events associated with neoplastic progression in mammary tissue. Epithelial cells are depicted in red, stromal cells in blue. See text for details.

Fig. 2. Schematic representation of major events associated with mammary carcinoma cell-induced osteolysis. Carcinoma cells are depicted in red, cells of the osteoclast lineage in blue and cells of the osteoblast lineage in green. See text for details.

Fig. 3.

Transdifferentiation of mammary epithelial cells in response to stromelysin-1. Functionally normal mouse mammary epithelial cells (SCp2 cell line) were cultured for 6 days without (w/o) or with 1 $\mu\text{g}/\text{ml}$ of activated recombinant stromelysin-1 (rh SL-1). Cells were then immunostained for epithelial cytokeratins (green) and mesenchymal vimentin (red). Note the scattered morphology of cells exposed to stromelysin-1 and the concomitant induction of vimentin intermediate filaments.

TABLE 1
Influence of MMPs on tumor progression and osteolysis

Event	References
Tumor initiation	40, 43
Tumor growth	35, 40, 42
Neovascularization	44
Epithelial-to-mesenchymal conversion	50
Tumor cell migration/invasion	35_38
Intravasation/extravasation	39
Osteoclast migration/invasion	70, 76
Bone resorption	61_67, 72