Review

Inflammation and breast cancer

Metalloproteinases as common effectors of inflammation and extracellular matrix breakdown in breast cancer

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Abstract

Two rapidly evolving fields are converging to impact breast cancer: one has identified novel substrates of metalloproteinases that alter immune cell function, and the other has revealed a role for inflammation in human cancers. Evidence now shows that the mechanisms underlying these two fields interact in the context of breast cancer, providing new opportunities to understand this disease and uncover novel therapeutic strategies. The metalloproteinase class of enzymes is well studied in mammary gland development and physiology, but mostly in the context of extracellular matrix modification. Aberrant metalloproteinase expression has also been implicated in breast cancer progression, where these genes act as tumor modifiers. Here, we review how the metalloproteinase axis impacts mammary physiology and tumorigenesis and is associated with inflammatory cell influx in human breast cancer, and evaluate its potential as a regulator of inflammation in the mammary gland.

Introduction

Breast cancer continues to be one of the leading causes of cancer-related mortality in women in the western world. Much like other cancers, amplifications of oncogenes or deletions of tumor suppressor genes underlie mammary tumorigenesis. In addition, the mammary tissue microenvironment, consisting of structural, biochemical and cellular entities, is known to contribute to tumor cell fate. The extracellular matrix (ECM) acts as a junction through which these components interact [1], with the basement membrane presenting a structural impediment to epithelial cells during mammary remodeling and tumorigenesis [2]. Release and activation of growth factors and cytokines anchored to the ECM or cell surface provide biochemical cues that exert a major influence on tumor cell survival. Further, carcinoma-associated fibroblasts, and inflammatory and immune cells are critical cellular entities that affect the tumorigenic potential of the mammary stroma.

Metalloproteinases are the largest class of proteases in the human genome [3]. Matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs) and tissue inhibitors of metalloproteinases (TIMPs) together comprise an important proteolytic axis. There are 23 MMP [4], 13 catalytically active ADAM [5,6], 19 ADAM with a thrombospondin domain (ADAMTS) [6], and 4 TIMP [7] proteins in humans. As a whole, this axis has long been recognized for its regulatory role in matrix turnover and growth factor bioavailability. Using various model systems, studies have explored the effects of these individual proteases and inhibitors on cancer hallmarks such as cell proliferation, apoptosis, invasion and metastasis (reviewed in [7,8]). A novel dimension to the metalloproteinase axis is its ability to regulate many critical aspects of immunity and inflammation. This is achieved through clipping, shedding, and regulated intramembrane processing (RIPping) of key substrates in the tissue microenvironment, as described in our recent review [9]. These processes now add another mechanistic link between metalloproteinases and the inflammatory contribution to tumorigenesis.

In breast cancer, epidemiological evidence suggests that inflammation is associated with poor prognosis. Here we ask whether the emerging role of metalloproteinases in inflammation extends to breast cancer. We review literature on mammary gland physiology, murine mammary tumor models and clinical breast cancer studies, in each case summarizing

ADAM = a disintegrin and a metalloproteinase; ADAMTS = ADAM with a thrombospondin domain; CSF = colony stimulating factor; ECM = extracellular matrix; EGFR = epidermal growth factor receptor; GM-CSF = granulocyte macrophage colony stimulating factor; HER = human epidermal growth factor receptor; MMP = matrix metalloproteinase; MMTV = mouse mammary tumor virus; RECK = reversion-inducing cysteine-rich protein with Kazal motifs; RIPping = regulated intramembrane processing; TIMP = tissue inhibitor of metalloproteinase; TNF = tumor necrosis factor.

what is known regarding the metalloproteinase axis as well as seeking evidence for its role as a mediator of inflammation. We also comment on the emerging contribution of the metalloproteinase axis to immune cell function, its correlation with lymphocytic infiltrate positivity in breast cancer and its potential to bridge inflammation and ECM breakdown in this disease.

Determinants of mammary gland morphogenesis and involution

Metalloproteinase-mediated ECM remodeling

Altered metalloproteinase activity has a direct impact on mammary gland physiology as controlled remodeling of mammary gland ECM through pericellular proteolysis is important for mammary morphogenesis, cyclical changes during the estrous cycle, and the differentiation necessary for lactation. Although ECM breakdown is required by epithelial cells, stromal cells, including fibroblasts, as well as inflammatory and immune cells are major producers of metalloproteinases [2]. In addition, ADAM proteases operate as sheddases for cell surface substrates and participate in stromal-epithelial cross-talk through paracrine delivery of signals [10]. Finally, TIMPs, as inhibitors of metalloproteinases, are critical regulators of matrix turnover in the mammary gland. The spatial localization of proteins of the metalloproteinase axis may be particularly important for the orchestration of these events.

During mammary morphogenesis in the mouse, MMP3 localizes to elongating ducts [11] and its overexpression results in supernumerary ductal branching [12]. MMP2 and MMP14 deficient mice display diminished ductal elongation, whereas MMP9 deficiency has no effect [13]. ADAM17 plays a role in paracrine communication involving epithelial-specific amphiregulin and stromally restricted epidermal growth factor receptor (EGFR) [14]. Specifically, *Amphiregulin*$^{-/-}$ mammary glands have impaired ductal outgrowth [15], while *Adam17*$^{-/-}$ mammary glands have severe growth and branching retardation that phenocopies EGFR-deficient mammary glands [14]. Manipulation of TIMP levels also leads to alterations in mammary morphogenesis. Reduction of TIMP1 expression through antisense RNA production leads to more extensive branching, increased ductal elongation, and increased proliferative index. Conversely, TIMP1 upregulation leads to inhibition of ductal elongation without affecting bifurcation or lateral budding [16]. TIMP3 deficient mice also show accelerated ductal elongation but normal branching patterns [17]. Orthotopically implanted TIMP-containing pellets result in inhibition by TIMP4, but promotion by TIMP2 of ductal outgrowth [17]. Thus, individual members of the metalloproteinase axis are not necessary for gland development *per se*, but are required for refining the ductal and branching patterns within the mammary gland. This is emphasized by the fact that most phenotypes in genetic models of MMPs and TIMPs are transient; the glands become lactationally competent when brought to parturition.

Reversion of a lactating gland to a virgin-like state during involution requires an extensive remodeling of the ECM along with epithelial cell death. The first and second phases of involution have been designated protease-independent and protease-dependent stages, respectively, based on the expression of MMPs and TIMPs [18]. Mammary gland involution is accelerated upon mammary-specific overexpression of an autoactivating form of MMP3, due to unscheduled apoptosis early in pregnancy [19]. In contrast, excess TIMP1 delivered through an implanted pellet delays gland regression [20]. TIMP3 is produced by epithelia and stroma and its loss leads to accelerated involution that cannot be rescued by re-initiation of suckling [21]. The substrates of MMPs that have been identified during involution include components of the ECM, as well as proteins involved in cell-cell, and cell-ECM adhesion. MMP3 cleaves the ECM protein entactin, which interacts with other ECM proteins and integrins [19]. TIMP3 deficiency leads to fibronectin fragmentation [21] and liberates the DIII fragment of laminin-5 during involution, which activates EGFR [22]. Metalloproteinases fragment E-cadherin, releasing a degradation product that further destabilizes E-cadherin function, compromising epithelial integrity during involution [23,24]. MMPs and TIMPs are also implicated in regulating adipogenesis during the third phase of mammary gland involution. While genetic deletion of MMP3 in the gland does not affect epithelial apoptosis, immature adipocytes have increased differentiation, displaying accelerated adipogenesis; an effect phenocopied by TIMP1 overexpression [25]. These studies highlight the importance of tissue interactions during involution, with the mammary stroma actively contributing towards epithelial cell death.

The immune system

A review by Vorbach and colleagues [26] presented a concept that the mammary gland may have evolved from the innate immune system. This hypothesis suggests that the gland's initial function was the provision of innate immunity, and its nutritional role evolved later. Indeed, failure of passive transfer of immunity from mammary gland secretions to mammalian newborns can contribute to neonatal mortality. Various studies show that immune cells are present in the mammary stroma and are implicated in gland development. In humans, extramedullary hematopoietic cells have been found in the stroma of the infant breast anlage [27]. Leukocytic infiltrates have been documented throughout pubertal and adult breast development [27]. Colony stimulating factor (CSF)-1 mutant mice (*Csf1*$^{op/op}$) that lack macrophages, or mice deficient in eotaxin, a chemokine that recruits eosinophils, have impaired formation of terminal end buds, ductal invasion, and ductal branching [28]. Whole body irradiation with a sublethal dose that depletes the bone marrow leads to impaired epithelial ductal elongation, suggesting a general role for immune cell involvement in murine gland development [28]. Expression of inflammatory mediators and acute phase proteins, along with the presence of neutrophils, plasma cells,

macrophages and eosinophils in involuting glands all point to a role for inflammation [29,30]. The local tissue deconstruction during this process may be facilitated by the activation of innate immune components, with macrophages likely performing a corpse-clearing function [31]. At present, very little is known about the presence or role of components of the adaptive immune system in mammary gland physiology. Further, the role of the metalloproteinase axis in mediating inflammation and immunity during morphogenesis and involution currently remains unexplored.

Determinants of murine mammary tumorigenesis

Metalloproteinases as tumor modifiers

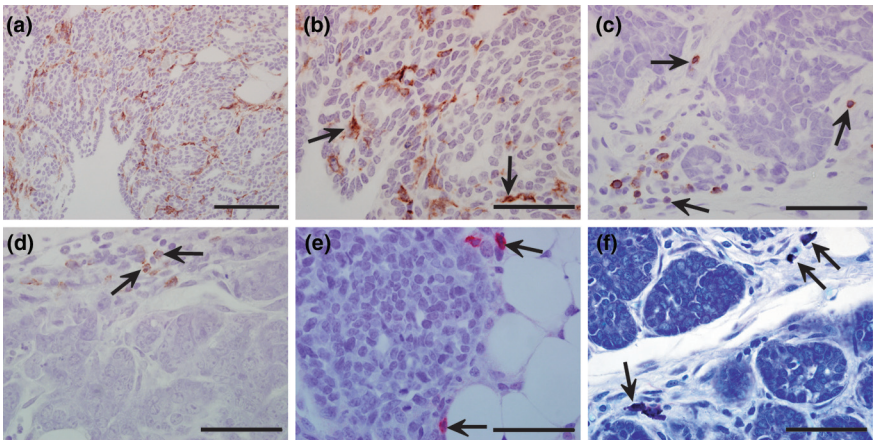
Genetic mouse models are powerful tools for understanding the role of specific genes in breast cancer development. MMP3 overexpression driven by the whey acidic protein promoter or MMP7 overexpression under the mouse mammary tumor virus (MMTV) promoter both lead to mammary tumor formation at low frequency [32,33], while MMP7 deficiency results in 60% reduction of early mammary lesions in a chemical carcinogenesis model [34]. MMTV-ras mice lacking MMP11 have significantly increased survival and a smaller tumor burden compared to wild type but develop significantly more metastatic lesions [35]. Overexpression of the membrane-anchored MMP14 in the mammary epithelium leads to increased lymphocytic infiltrates, periductal fibrosis, ductal hyperplasia with dilated ducts, dysplasia, and adenocarcinoma in multiparous transgenic mice [36]. The effect of TIMP1 on mammary tumorigeneis has been assessed in transgenic mice that either secreted TIMP1 systemically using an albumin promoter, or expressed it in a mammary-specific manner using the MMTV promoter [37]. When subjected to the DMBA model of mammary carcinogenesis or crossed with MMTV-PyMT mice, systemic TIMP1 elevation reduced tumor burden by 70% and 44%, respectively. Metastasis was also inhibited. Interestingly, mammary-specific TIMP1 overexpression was ineffective against mammary tumorigenesis in both models. On the other hand, a recent report showed inhibition of MCF10A (non-transformed, immortalized mammary epithelial cells) apoptosis by recombinant TIMP1 in a metalloproteinase inhibitor-independent capacity [38]. A recent study has revealed that TIMP2 overexpression in the mammary gland increases MMTV-Wnt1-induced mammary tumor latency, with tumors showing lower bromodeoxyuridine and CD31 positivity, and higher TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) positivity compared to wild-type Wnt1 tumors [39]. Thus far, genetic studies that address the role of TIMP3 or TIMP4 in mammary tumorigenesis are lacking, although several *in vitro* and clinical reports suggest that these remaining TIMPs may also be important. For instance, overexpression of engineered mutant TIMP3 protein that mimics the Sorsby's Fundus Dystrophy mutation promotes apoptosis in MCF-7 cells [40], and metastasis of TIMP3 overexpressing MDA-MB-435 breast cancer cells is significantly reduced [41]. Notably, *Timp3* is found silenced by promoter methylation in a panel of human cancer cell lines derived from primary breast cancers and metastases to brain [42-45]. TIMP4 was originally identified in human breast cancer [46] and its overexpression in human breast cancer cells diminishes growth and metastasis in athymic mice [47]. Individual members of the metalloproteinase axis investigated to date are able to function as tumor modifiers in a variety of breast cancer models, with increased MMP or decreased TIMP activity generally associated with tumor promotion. Future investigations exploring non-proteolytic functions of members of this axis, as well as characterizing newer members such as ADAMs and ADAMTSs, will better define their specific contribution to mammary tumorigenesis.

Inflammatory mediators as tumor modifiers

Classically, inflammation is associated with immune surveillance against neoplasms [48], and tumors are known to develop strategies to circumvent immune recognition and clearance. Although mouse models provide an opportunity to directly test the specific role of individual inflammatory and immune cell types and effector molecules such as cytokines in mammary tumourigenesis, there has been very little work addressing this important issue. A few studies using mice point to a protective role of immune cells in tumorigenesis: concurrent lack of the immune mediators granulocyte macrophage CSF (GM-CSF)1 and interferon-γ lead to spontaneous tumor formation in mice, including mammary adenocarcinoma [49]; and the loss of neutrophil collagenase, MMP8, leads to increased susceptibility to skin cancer due to ineffectual neutrophil infiltration, indicating the importance of a timely inflammatory response in protecting against skin carcinogenesis [50]. In contrast, other studies have pointed to a pro-tumorigenic role for inflammatory cells, specifically tumor-associated macrophages [51] and B cells [52]. These cells are postulated to foster tumor growth and metastasis through release of cytokines and matrix remodeling enzymes. Genetic crosses of macrophage-deficient, osteopetrotic mice that are mutant for the macrophage growth factor CSF1 (*Csf1*$^{op/op}$) with MMTV-PyMT mice show reduced progression to malignancy and metastatic disease [53]. Mice that are deficient in cyclooxygenase-2 have decreased levels of prostaglandin E2 and decreased tumor multiplicity [54] when crossed into the breast cancer model expressing the activated form of Neu/human epidermal growth factor receptor (HER)2 (MMTV-NeuNDL - *neu* deletion mutant).

A system to study the importance of a wide variety of immune and inflammatory cells as well as cytokines on mammary tumorigenesis exists in the MMTV-PyMT model. Beyond macrophages, we have observed other inflammatory and immune cell types, namely CD3+ T lymphocytes, B cells, mast cells, and neutrophils in and around the mammary tumors arising in MMTV-PyMT mice (Figure 1). The presence of these cells provides an opportunity to study the effect of

Figure 1



Immune cells in mammary tumours arising from PyMT expression. **(a-e)** Immunostaining for various bone marrow derived cells, and **(f)** toludine blue staining for mast cells in MMTV-PyMT mammary tumors. Macrophages are commonly present within and around tumors (a,b). Arrows indicate macrophages (b), T-cells (c), B-cells (d), neutrophils (e), and mast cells (f). T- and B-cells are often present as groups of mixed lymphocytes at the borders of lesions, and are more frequent than neutrophils unless necrosis is present. Mast cells are the most rare, and are usually solitary or in groups of two to three cells.

specific cell types and effector molecules in mammary tumor progression by crossing this model to mice with the desired gene deficiencies. Although the MMTV-PyMT model has relatively high tumor multiplicity and short latency, histological analyses reveal that this model shares molecular and morphological characteristics with human breast cancer [55] as well as the immune and inflammatory cell populations shown in Figure 1. Additionally, the role of metalloproteinase axis members that are linked to regulation of inflammation can be functionally assessed using this model in combination with mice deficient in the protease or inhibitor of interest.

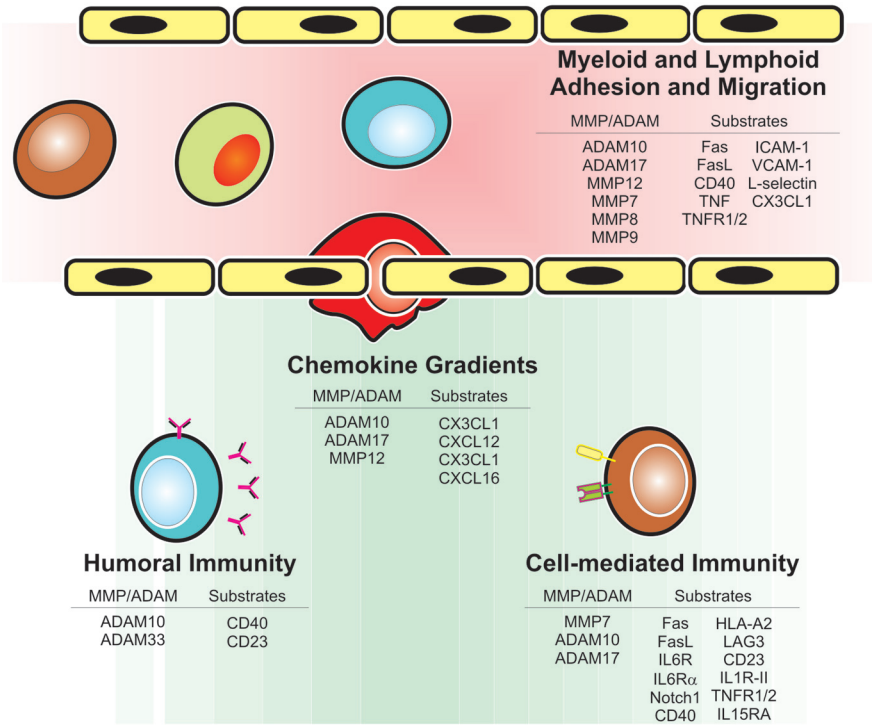
Human breast cancer studies

The metalloproteinase axis in breast cancer progression

Many studies have attempted to correlate MMP, ADAM and TIMP expression profiles with breast cancer progression and common trends have emerged (reviewed in [56,57]). The levels of MMP expression usually correlate with aggressive breast tumors, those of individual TIMPs suggest a more complex association with breast cancer, while data on ADAM and ADAMTS expression in breast cancer are relatively recent [7,57,58]. High levels of MMP9, which degrades type IV collagen in basement membrane, is associated with poor prognosis in breast cancer independent of the cell type expressing this protease [59,60]. Patients with atypical ductal hyperplasia are at increased risk of developing invasive breast cancer. MMP1 protein was detectable in a subgroup of patients with atypical ductal hyperplasia that had a history of cancer [61] and this protease was found in ductal lavage, leading to the suggestion that MMP1 may identify atypical ductal hyperplasia patients that are at risk for developing breast cancer. Studies on TIMP1 expression in breast cancer show both a role for and against it as a positive prognostic factor [58], owing to its diverse effects on cellular proliferation, angiogenesis, and apoptosis, as detailed in a recent review by Cruz-Munoz and Khokha [7]. Lipton and colleagues [62] measured plasma TIMP1 levels via ELISA (enzyme-linked immunosorbent assay) and correlated its elevation with higher serum HER2 levels, increased metastasis, and reduced survival in breast cancer patients. Real time-PCR analysis of breast cancer tissue correlated TIMP3 overexpression with success of adjuvant endocrine therapy [63,64]. Similarly, TIMP3 mRNA levels in breast tumors significantly associated with good prognosis and longer disease-free survival [65]. In contrast, TIMP3 levels were found to be higher in mammographically dense breasts, which are considered to be at a higher risk for developing breast cancer [66]. In another study, higher mRNA expression of the membrane-anchored MMP inhibitor RECK (reversion-inducing cysteine-rich protein with Kazal motifs) in breast tumors was found to be an independent prognostic indicator associated with a longer relapse-free survival time [67].

In a tissue microarray study of primary invasive ductal carcinoma, high individual expression of MMP9, MMP11, TIMP1, and TIMP2 was significantly associated with increased incidence of metastasis at five years post-surgical resection [68]. When the authors accounted for cell type-specific expression (tumor cells, fibroblasts, inflammatory mononuclear cells), additional specific members (MMP1, MMP7, MMP13, MMP14, TIMP3) had significant associations with developing metastastic disease [68]. In a follow-up study [69], the most powerful indicator of distant relapse-free survival in breast cancer patients was a set of MMPs and TIMPs whose expression was specific to tumor-associated mononuclear inflammatory cells. Similarly, separating breast

Figure 2



Involvement of matrix metalloproteinases (MMPs), a disintegrin and a metalloproteinase (ADAMs), and tissue inhibitor of metalloproteinases (TIMPs) in immune function. The substrate repertoire generated through shedding, clipping, and regulated-intramembrane processing (RIPping) provides insight into the role of the metalloproteinase axis in immune cell adhesion and migration, the generation of chemokine gradients, and humoral and cell-mediated immunity [9]. ICAM, intracellular adhesion molecule; IL, interleukin; TNF, tumor necrosis factor; TNFR, TNF receptor; VCAM, vascular cell adhesion molecule.

tumor tissue into different cellular components revealed that TIMP3 was not present in ductal carcinoma *in situ* or normal epithelium, but was significantly overexpressed in myofibroblasts and myoepithelial cells surrounding ductal carcinoma *in situ* [70]. Thus, such profiles were less informative when bulk tumor, fibroblasts, or tumor cells were analyzed, suggesting that monitoring inflammatory cell-specific expression may provide clinically important insights. Future studies must consider the cell- and stage-specific patterns of these proteins to resolve present evidence that is limited and at times contradictory with respect to the association of TIMP expression with breast cancer.

ADAM and ADAMTS metalloproteinases are becoming recognized as important factors in breast cancer. ADAM9, ADAM12, ADAM15, ADAM17, ADAM23, ADAM28, and ADAMTS1 have all been found in breast cancer [5,6]. Levels of ADAM9 correlate positively with HER2 levels [6] and with positive response to Tamoxifen [5]. A possible diagnostic role for the soluble form of ADAM12 has been proposed since urine levels of this metalloproteinase positively correlate with breast cancer progression [5]. ADAM17 is overexpressed in breast tumors and its inhibition leads to decreased cell proliferation *in vitro* or tumor growth in xenograft models [5]. The critical role of ADAM17 in mediating tumor necrosis factor (TNF)-initiated inflammation [71] and/or its role in transactivating EGFR through the cleavage of EGF ligands such as transforming growth factor-α may underlie these effects [10]. Although the biology of ADAMs is less understood than that of MMPs, their ability to shed cell surface molecules qualifies them and their substrates as candidates for breast cancer progression biomarkers.

The metalloproteinase axis potentially links inflammation and breast cancer

The importance of cytokine signaling as a link between inflammation and cancer has been highlighted [72], and the bioavailability of many of these critical molecules is regulated by the metalloproteinase axis. Figure 2 illustrates the metalloproteinases and the potential substrates linked to specific aspects of an inflammatory or immune response, such as the generation of chemokine gradients, immune cell influx, lymphocyte activation and effector functions. Each of these aspects is described in greater detail by Murphy and colleagues [9]. For instance, ADAM17 processes a number of cell surface proteins, including TNF, fractalkine and GM-CSF, all important recruiters and activators of macrophages. Ductal lavage of the breast shows the presence of

macrophages [73] and tumor-associated macrophage density has been correlated with poor prognosis [74]. CSF1, an important growth factor for macrophages, is over-expressed in human breast cancers and its expression correlates with high grade tumors and poor prognosis [51]. Given these clinical observations, an intriguing avenue for investigation is the contribution of metalloproteinases to macrophage function in breast cancer.

Several experimental models have linked TIMP activity to inflammation, although such a function in breast cancer is unexplored. TIMP1 deficiency promotes neutrophil accumulation in an inflammatory model of bleomycin-induced lung injury [75], whereas TIMP2 deficiency has no effect. TIMP3 regulates the bioactivity of the inflammatory cytokine TNF through its physiological inhibition of the TNF sheddase, ADAM17/TNF alpha converting enzyme, which is critical for several physiological systems that depend on TNF [76-79]. Increased numbers of neutrophils are observed in *Timp3*$^{-/-}$ remodeling hearts in an otherwise non-inflammatory model of cardiac pressure overload [77]. *Timp3*$^{-/-}$ mice are also hyper-responsive to endotoxin, which causes systemic release of TNF in a model of innate immunity [78]. Overall, these data point to select candidates of the metalloproteinase axis that can potentially participate in inflammation during breast cancer progression. Specifically, the coordinated action of TIMP3, ADAM17, and TNF in initiating signal transduction pathways essential to innate immune responses that can impact mammary tumourigenesis is currently under investigation in our lab.

In addition to the metalloproteinase-mediated generation of critical inflammation triggers, metalloproteinases are, in turn, utilized by immune cells to further propagate the inflammatory reaction. Of the MMPs, MMP9 is often implicated as an inflammation-related MMP, with reported roles in carcinogenesis models [80,81]. In breast cancer samples, MMP9 in the stroma is found in neutrophils, macrophages, and T lymphocytes [56]. In a mammary tumorigenesis xenograft model, CD4+ T-cells in the periphery as well as within the breast tumor expressed high levels of MMP9 [82]. MMP3 is often present in infiltrating T-lymphocytes when found overexpressed in breast carcinoma [56]. During inflammation, increased TNF has been shown to induce expression of collagenases [83]. Notably, the roles of MMPs, such as the neutrophil collagenase MMP8 primarily produced by inflammatory cells [50], and macrophage elastase MMP12 [84], have yet to be elucidated in mammary tumorigenesis.

Table 1

Metalloproteinase axis mRNA expression in lymphocytic infiltrate-positive breast cancer

mRNA	Differential expression	*P*-value
Timp1	↓	0.043
Timp2	NC	0.565
Timp3	↓	1.4E-7
Timp4	↓	0.004
RECK	↓	1.2E-6
Mmp1	↑	1.4E-5
Mmp2	↓	2.8E-4
Mmp3	NC	0.54
Mmp7	↑	2.2E-5
Mmp8	NC	0.388
Mmp9	↑	0.009
Mmp10	NC	0.47
Mmp11	↓	3.3E-4
Mmp12	↑	3.5E-7
Mmp13	↓	0.02
Mmp14	NC	0.225
Mmp15	NC	0.058
Mmp16	NC	0.416
Mmp17	NC	0.511
Mmp19	↑	0.049
Mmp20	↑	9.4E-4
Mmp23	↓	8.3E-5
Mmp24	NC	0.72
Mmp25	NC	0.794
MmpL1	NC	0.794
Adam8	↑	1.8E-4
Adam9	NC	0.574
Adam10	NC	0.25
Adam12	↓	0.001
Adam15	NC	0.062
Adam17	↑	0.005
Adam19	NC	0.067
Adam23	NC	0.625
Adam28	NC	0.291
Adam33	↓	0.029
Adamts1	NC	0.068
Adamts4	NC	0.16
Adamts5	↓	0.008

Up and down arrows indicate increased and decreased expression, respectively; NC indicates no change. ADAM, a disintegrin and a metalloproteinase; ADAMTS, ADAM with a thrombospondin domain; MMP, matrix metalloproteinase; RECK, reversion-inducing cysteine-rich protein with Kazal motifs; TIMP, tissue inhibitor of metalloproteinase.

The metalloproteinase axis and lymphocytic infiltrate-positive breast cancer

To address the possible role of MMPs, TIMPs, and ADAMs in inflammation in breast cancer, we performed expression profiling of members of these families in the Oncomine database [85], which contains microarray expression data from a variety of human cancers. Of the 31 studies on breast cancer, only the study by van't Veer and colleagues [86] recorded lymphocytic infiltration as one of the many clinical parameters. This study profiled breast tumor mRNA from 117 patients, of which 89 were lymphocytic infiltrate-negative and 28 were lymphocytic infiltrate-positive. Lymphocytic infiltrate positivity correlated with BRCA mutant and estrogen

receptor-negative status in an unsupervised two-dimensional clustering analysis [85]. We found differential expression of specific MMPs, ADAMs, and TIMPs, when the sample set was stratified based on lymphocytic infiltration (Table 1). Of the 22 MMPs examined in their study, several showed differential expression. Specifically, mRNAs of the inflammation associated MMPs, MMP9 and MMP12, were upregulated in lymphocyte infiltrate-positive breast cancers. ADAM8, a reported sheddase for L-selectin, and ADAM17, the sheddase for TNF, were also upregulated, consistent with their suggested pro-inflammatory function. Interestingly, the mRNA expression of the membrane-type MMPs did not correlate with lymphocytic infiltrate status in this study, and ADAMTS expression was also variable. Low expression of TIMP1, TIMP3, TIMP4, and RECK mRNA significantly correlated with lymphocytic infiltrate positivity, while TIMP2 was comparable between groups. While this one study shows intriguing trends, further clinical studies that document lymphocyte involvement are needed to reveal the association between global gene expression patterns, inflammation, and breast cancer.

Conclusion

Although metalloproteinase activity has for some time been linked to breast cancer as well as mammary gland development and physiology, it is only recently that the metalloproteinase axis has been explored in the context of inflammation and immunity. How the inflammation link operates in breast cancer is still an open question. It is apparent that metalloproteinases participate during morphogenesis and involution, but the current knowledge on how they may influence immune cells during these critical windows is completely untested. Similarly, direct evidence for their role in regulating inflammation/immunity in mammary tumorigenesis is lacking. On the other hand, data from clinical breast cancer studies raise intriguing possibilities. By considering MMP, ADAM and TIMP expression in individual cell populations, strong associations have emerged with regard to clinical outcomes in breast cancer patients [68,69]. As discussed above, further analyses of global gene expression profiles may reveal clinically relevant correlations between individual metalloproteinase genes and immune cell involvement in breast cancer. Once the key factors linking inflammation, metalloproteinase activity, and breast cancer have been identified, this knowledge will serve to drive novel therapies and prevention strategies targeting critical components.

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Competing interests

The authors declare that they have no competing interests.

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