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Presence of Insulin-Like Growth Factor Binding Proteins Correlates With Tumor-Promoting Effects of Matrix Metalloproteinase 9 in Breast Cancer [1,2]

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Abstract

The stroma of breast cancer can promote the disease's progression, but whether its composition and functions are shared among different subtypes is poorly explored. We compared stromal components of a luminal [mouse mammary tumor virus (MMTV)–Neu] and a triple-negative/basal-like [C3(1)–Simian virus 40 large T antigen (Tag)] genetically engineered breast cancer mouse model. The types of cytokines and their expression levels were very different in the two models, as was the extent of innate immune cell infiltration; however, both models showed infiltration of innate immune cells that expressed matrix metalloproteinase 9 (MMP9), an extracellular protease linked to the progression of many types of cancer. By intercrossing with *Mmp9* null mice, we found that the absence of MMP9 delayed tumor onset in the C3(1)-Tag model but had no effect on tumor onset in the MMTV-Neu model. We discovered that protein levels of insulin-like growth factor binding protein-1 (IGFBP-1), an MMP9 substrate, were increased in C3(1)-Tag;*Mmp9*$^{-/-}$ compared to C3(1)-Tag;*Mmp9*$^{+/+}$ tumors. In contrast, IGFBP-1 protein expression was low in MMTV-Neu tumors regardless of *Mmp9* status. IGFBP-1 binds and antagonizes IGFs, preventing them from activating their receptors to promote cell proliferation and survival. Tumors from C3(1)-Tag;*Mmp9*$^{-/-}$ mice had reduced IGF-1 receptor phosphorylation, consistent with slower tumor onset. Finally, gene expression analysis of human breast tumors showed that high expression of *IGFBP* mRNA was strongly correlated with good prognosis but not when *MMP9* mRNA was also highly expressed. In conclusion, MMP9 has different effects on breast cancer progression depending on whether IGFBPs are expressed.

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Introduction

Breast cancer is classified on the basis of gene expression: About 20% of human breast cancers overexpress human epidermal growth factor receptor 2 (HER2/ErbB2) and these cancers have a worse prognosis than the more common estrogen and progesterone (hormone) receptor–positive subtypes [1]. Cases negative for hormone receptors and ErbB2 (triple negative subtypes) have the worst prognosis and fewest treatment options [2]. Besides the classification based on

Abbreviations: αSMA, α smooth muscle actin; C3(1)-Tag, transgene mouse expressing Simian virus 40 large T antigen under a C3(1) promoter; HER2, human epidermal growth factor receptor 2; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IL, interleukin; MMP, matrix metalloproteinase; MMTV, mouse mammary tumor virus; NF-κB, nuclear factor κB; PyMT, polyoma middle T antigen

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[2] This article refers to supplementary materials, which are designated by Figures S1 to S6 and are available online at www.neoplasia.com.

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receptor expression status, breast cancer is also commonly classified into luminal and basal-like subtypes, based on global gene expression profiles [3]. Luminal breast cancers are often estrogen receptor– and/or HER2/ErbB2-positive, while the majority of basal-like breast cancers are triple negative [4].

In the mouse mammary tumor virus long terminal repeat driven-Neu (MMTV-Neu) model [5], the rodent homologue of HER2/ErbB2 is overexpressed. The MMTV-Neu model is a model of human luminal breast cancer as defined by expression profiling [6]. In another breast cancer model, expression of the Simian virus (SV) 40 large T antigen (C3(1)-Tag) [7] in the mammary epithelium induces estrogen receptor–, progesterone receptor–, and HER2-negative tumors [8], with expression profiles resembling human basal-like breast cancers [6]. In the MMTV-Neu model, the activation of the Ras/phosphoinositide 3-kinase pathway drives tumor progression, while in the C3(1)-Tag model, it is the inactivation of p53 and Rb that is the driving force.

Breast tumors contain stromal cells, such as immune, mesenchymal, and vascular cells, which communicate with cancer cells. Genetically engineered mouse models of cancer enable studies of how driving oncogenic changes influence tumor progression in the context of a stromal response [9–11]. The communication between cancer and stromal cells is mediated by, e.g., growth factors, cytokines, proteases, and extracellular matrix proteins [12–14]. These extracellular factors, together with the stromal cells, constitute the tumor microenvironment. Experimental studies have shown that the components of the tumor microenvironment possess functions that are vital for tumor growth, including support of angiogenesis (reviewed in [12]). However, it is still unclear whether specific stromal components have the same effects on tumor progression in different subtypes of cancer arising in the same organ.

Many approaches have been undertaken to block the influence of the microenvironment on cancer progression, including the pharmacological inhibition of matrix metalloproteinases (MMPs) [15–17]. MMPs belong to a large family of proteases involved in the degradation and modulation of extracellular proteins. The enzymatic activities of MMPs are important in the tumor microenvironment because proteolysis can regulate a range of different processes, such as angiogenesis and growth factor bioavailability [18].

Despite strong experimental proof that MMPs promote tumor initiation and progression, clinical trials using MMP inhibitors have failed (reviewed in [15,19]). In hindsight, the trials were not optimally designed: For example, they enrolled patients with late-stage cancer even though preclinical experiments showed that MMPs should be blocked in early-stage cancer [20]. Furthermore, no effort was made to ensure that MMPs were overexpressed in the cancer of the treated patients [19]. The design of the clinical trials also ignored the fact that MMPs can have different effects on tumor progression depending on the substrates they act on [21].

MMP9, one of the most studied MMPs in cancer, is mainly expressed by tumor-infiltrating myeloid cells [20,22–25]. Deletion of *Mmp9*, the gene encoding for MMP9, delays tumor onset or slows tumor progression in many genetically engineered mouse models of cancer [20,22–25]. Interestingly, in these models, the p53 and Rb tumor suppressors were inactivated, e.g., by interaction with the SV40 large T antigen or human papilloma virus early region oncogenes [20,22,23]. In contrast, the genetic deletion of *Mmp9* in a model of mammary carcinoma driven by expression of tyrosine kinases [MMTV–polyoma middle T antigen (PyMT)] had no effect on tumor onset or primary tumor growth [24]. It is unclear whether this difference in the effects of MMP9 on tumor progression between the models was due to the different oncogenic events that drive the cancers or because the tumors originated from different tissues.

In this study, we compared expression levels of different stromal factors between the luminal MMTV-Neu and the basal-like C3(1)-Tag murine breast cancer models. Interestingly, we found that MMP9 was expressed by myeloid cells in both models, yet it only influenced tumor onset in the basal-like C3(1)-Tag model. We discovered that the protein levels of the MMP9 substrate insulin-like growth factor binding protein-1 (IGFBP-1) were increased in the absence of MMP9 only in the C3(1)-Tag model, the model that depended on MMP9 for tumor progression. Furthermore, in data sets of human breast cancer samples, high mRNA expression of *IGFBPs* correlated with a good prognosis, except when these tumors also expressed high levels of *MMP9* mRNA. Collectively, our findings show that MMP9 and IGFBPs have different subtype-dependent effects on breast cancer and that a nuanced understanding of tumor biology is necessary to successfully target these stromal factors.

Materials and Methods

Mice

MMTV-Neu [5], C3(1)-Tag [7], and $Mmp9^{-/-}$ [26] mice have all been described previously. All three mice strains were used on the FVB/n background. MMTV-Neu and C3(1)-Tag mice were each crossed with $Mmp9^{-/-}$ mice, and the offspring were further intercrossed to generate MMTV-Neu;$Mmp9^{+/+}$, MMTV-Neu;$Mmp9^{+/-}$, MMTV-Neu;$Mmp9^{-/-}$, C3(1)-Tag;$Mmp9^{+/+}$, C3(1)-Tag;$Mmp9^{+/-}$, and C3(1)-Tag;$Mmp9^{-/-}$ mice. Only mice hemizygous for the MMTV-Neu or C3(1)-Tag transgenes were used to compare tumor onset. All animal experiments were conducted in accordance with procedures approved by the Institutional Animal Care and Use Committee at the University of California, San Francisco.

Tumor Growth

Tumor growth was monitored in all 10 mammary glands by weekly palpations in MMTV-Neu;$Mmp9^{+/+}$ (n = 47), MMTV-Neu;$Mmp9^{+/-}$ (n = 48), MMTV-Neu;$Mmp9^{-/-}$ (n = 39), C(3)1-Tag;$Mmp\text{-}9^{+/+}$ (n = 18), C(3)1-Tag;$Mmp9^{+/-}$ (n = 25), and C(3)1-Tag;$Mmp9^{-/-}$ (n = 13) mice. The length and width of all palpable tumors were measured by caliper, and the volume was calculated using the formula: volume = width^2 × length/2.

Histology and Immunostaining

Dissected mammary carcinomas and lungs were fixed in 4% paraformaldehyde, processed in alcohol, embedded in paraffin, and cut into 5-μm-thick sections. Sections were stained with hematoxylin and eosin, Mayer's hematoxylin with Masson's trichrome, or Picrosirius Red using standard protocols. Lung metastatic burden was examined in tissue sections from lungs of C3(1)-Tag;$Mmp9^{+/+}$ (n = 21), C3(1)-Tag;$Mmp9^{+/-}$ (n = 19), and C3(1)-Tag;$Mmp9^{-/-}$ (n = 14) mice, which were sacrificed at Institutional Animal Care and Use Committee–approved end stage (i.e., when the largest tumor reached 2 cm in diameter or a tumor had ulcerated). The percentage of the lung area occupied by metastasis, the number of metastatic foci, and the average size of each metastatic lesion were quantified on cross sections of lungs stained with hematoxylin and eosin using ImageScope software (Aperio Technologies, Vista, CA). Fibrillar