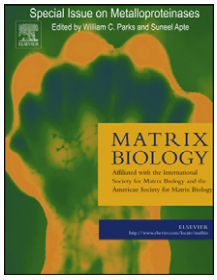
Review




# Moving targets: Emerging roles for MMPs in cancer progression and metastasis

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## Abstract

Matrix metalloproteinases have long been associated with cancer. Clinical trials of small molecule inhibitors for this family of enzymes however, were spectacularly unsuccessful in a variety of tumor types. Here, we discuss some of the newer roles that have been uncovered for MMPs in cancer that would not have been targeted with those initial inhibitors or in the patient populations analyzed. We also consider novel ways of using cancer-associated MMP activity for clinical benefit.



## Introduction

MMPs are traditionally associated with matrix remodeling, in particular with cancer invasion and with angiogenesis. However, the roles for MMPs have expanded to include processing of a variety of different substrates so that all stages of cancer from initiation to outgrowth of clinically-relevant metastases can be associated with various members of this family of proteases. Despite these clear associations, the field of MMP research in cancer is haunted by the resounding failure of small molecule MMP inhibitors that were in multiple phase III trials [1,2]. The reasons for the failures were myriad and have been discussed at length in the literature [2–5]. Two of the most critical issues were: (i) the lack of specificity of the inhibitors used — even those described as 'selective' could still target multiple members of the MMP family as well as potentially other metalloproteinases; and (ii) dose-limiting side-effects that are still poorly understood, but which resulted in questions of whether truly efficacious doses were ever reached. One of the valuable after-effects of the clinical failure has been a concerted effort to truly understand the multiple positive and negative implications of MMP inhibition. This has led to an appreciation of alternative ways of considering MMPs in cancer, and other diseases. Here, we focus on some recently discovered roles for MMPs in cancer that may impact strategies regarding inhibitor design, as well as ways in which MMP activity can potentially be harnessed for diagnostic, prognostic or therapeutic benefit.

## Proteolysis-independent functions of MMPs in Cancer

While MMP catalytic activity is responsible for the processing of ECM and non-matrix molecules, multiple studies now demonstrate roles for MMPs that are independent of proteolysis. This exciting new frontier for MMP research is only beginning to be explored, but evidence from multiple physiological and pathological settings, including cancer, suggest that this is a much more widespread phenomenon than initially considered. One of the first functional examples described was an antimicrobial activity linked to a four amino acid sequence within the c-terminal domain of MMP12 [6]. An anti-viral host defense mechanism also involving MMP12, was delineated recently but this time the MMP acted as a transcription factor [7]. While it is the catalytic domain of MMP12 that is required both for nuclear localization and DNA binding in anti-viral immunity, these activities appear independent of

proteolysis. Similarly, MMP14 was also identified to have DNA binding effects that result in widespread reprogramming of macrophage function [8]. Given the clear intersection between the immune system and cancer, it is quite possible that these new characteristics may have profound implications for cancer progression and metastasis.

MMPs are also significant mediators of cellular differentiation and morphology. Recently, MMP14 was shown to play a role in osteoclast differentiation via a non-catalytic mechanism in which the intracellular domain of the membrane anchored MMP drove myeloid migration and fusion by modulating Rac1 and p130Cas activity [9]. It was already appreciated that MMP14 is a key regulator of skeletal development, and clearly its catalytic activity and ability to process type I collagen and non-matrix molecules such as RANKL make MMP14 an attractive therapeutic target in bone metastatic disease [10–12]. However, this additional non-catalytic role in the generation of osteolytic cells may suggest a new reason for targeting MMP14 in cancers with extensive bone lesions, such as multiple myeloma, and breast and prostate adenocarcinomas. In the developing mammary gland, studies have shown that both MMP3 and MMP14 contribute to the precision of epithelial cell branching via the processing of ECM components [13–15]. However, the non-catalytic hemopexin domain of the enzymes can also impact the process [16,17]. While often considered as important for substrate specificity, the hemopexin domain is a c-terminal MMP domain that mediates protein–protein interactions that may not involve enzymatic processing. Invasion of mammary epithelial cells through a type-1 collagen gel in vitro is critically dependent on interaction of the hemopexin domain of MMP3 with extracellular HSP90β. The use of a catalytically-dead mutant of MMP3 does not affect this process, however inhibiting extracellular HSP-90β with a blocking antibody prevents invasion and mammary branching through an unknown mechanism [17]. Similarly, a non-catalytic domain of MMP14 was demonstrated to play a role in mammary branching via binding to integrin-β1 [16].

In melanoma, the phenotype of cancer cells migrating at the invasive front was found to be predominantly amoeboid. Paradoxically these melanoma cells were found to express more MMPs (in particular MMPs-9, 10 and 13) when compared to melanoma cells displaying a more mesenchymal-like phenotype in vitro [18]. Most surprisingly, MMP9 was found to promote amoeboid migration via non-catalytic mechanisms, by increasing actomyosin contractibility due to activation of ROCK pathways, following MMP9 binding to CD44 on the cancer cell surface. Other studies have identified a non-catalytic role for MMP9 in another process critical to cancer progression: cell survival. In chronic lymphocytic leukemia (CLL), binding of the hemopexin domain of pro-MMP9 to $\alpha_4\beta_1$ integrin and CD44v, activated anti-apoptotic pathways via Lin kinase and Stat3 phosphorylation [19]. Interestingly, the interaction between MMP9, $\alpha_4\beta_1$ integrin and CD44v was only found to occur in malignant B-cells, and not those from healthy individuals.

While these studies demonstrate novel roles for non-catalytic domains of MMPs, they also present a conundrum in regard to therapeutic intervention. Is it possible to inhibit both the catalytic and non-catalytic effects of MMPs or would specific domain targeting be a superior approach? Clearly more work is required in order to truly define the roles for each member of the MMP family, but also for the different domains of each of those family members.

## Utilizing novel MMP activatable probes for cancer imaging

Heightened MMP activity is typical in the tumor microenvironment with several studies correlating the presence of individual MMPs with poor prognoses in regard to overall survival [20]. Given their role in ECM remodeling and regulation of the bioactivity and bioavailability of cytokines and growth factors, this is not surprising, although it should be noted that MMPs can also have protective/tumor suppressor effects depending on the tissue context [5,21]. Despite the limitations of previous clinical trials, specific MMP inhibition remains an active area of translational research for cancer treatment, with several antibody-based inhibitors being explored [22,23]. Indeed, cancer clinical trials with an anti-MMP9 therapeutic antibody were recently initiated by Gilead Biosciences [24]. In addition to being a direct therapeutic target, MMP activity in the tumor-microenvironment is also being exploited to facilitate cancer detection, imaging and the assessment of therapeutic response.

Activity-based probes (ABP), utilize the substrate specificity of MMPs for imaging purposes. Typically, ABP construction involves the labeling of broad spectrum or selective MMP cleavable peptides with fluorophore/quenchers. Cleavage of the substrate by MMPs allows for a readout of enzymatic activity. Incorporation of near infrared fluorophores with wavelengths between 650 and 900 nm, facilitates the imaging of MMP activity in vivo. For example, an MMP7 selective probe was generated using Cy5.5 as an infra-red sensor dye with the addition of a second higher wavelength fluorophore that acted as an internal reference to monitor probe concentration [25]. In vivo, the probe demonstrated a 2.2 fold increase in fluorescence in MMP7 expressing tumors compared to control tumors negative for MMP7. The probe was found to be extremely sensitive, capable of detecting tumors as small as 0.01 $cm^2$ in a mouse model of colon cancer. Similar approaches have been utilized by a number of other