Review

Missing the target: matrix metalloproteinase antitargets in inflammation and cancer

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Matrix metalloproteinases (MMPs) are reputed to cause the inflammatory tissue destruction characterizing chronic inflammatory diseases and to degrade basement membrane collagen, thereby facilitating cancer cell metastasis. However, following the disappointing MMP drug cancer trials, recent studies using mouse models of disease coupled with high-throughput methods for substrate discovery have revealed surprising and unexpected new biological roles of MMPs in inflammatory diseases and cancer *in vivo*. Thus, MMPs modify signaling pathways and regulate the activity of whole families of cytokines of the immune response by precise proteolytic processing. By cleaving and inactivating cytokine-binding proteins and protease inhibitors, cytokine activities are unmasked and activities of diverse proteases are increased in an interconnected protease web. With new substrates come new roles, and 10 of 24 murine MMPs have antitumorigenic and anti-inflammatory roles making them drug antitargets; that is, their beneficial actions should not be inhibited. Here, we examine whether the discovery that MMPs are drug antitargets for one disease might pave the way for their use for other indications or whether this is a serious threat to the development of MMP inhibitors.

MMPs as inflammation and cancer drug targets

MMPs were identified as cancer drug targets (see Glossary) more than 30 years ago. At this time, MMP1, MMP2, and MMP3 were the only MMPs known and the therapeutic strategy was focused on inhibiting the degradation of the extracellular matrix (ECM) proteins that facilitate metastasis and angiogenesis [1,2]. The first anticancer MMP inhibitors were peptidomimetics that were not orally available (e.g., Batimastat, BB94; Ilomastat, GM-6001) and these were followed by improved second-generation inhibitors (Marimastat, BB2516; Prinomastat [AG3340], BAY12-9566) that entered cancer clinical trials [2,3]. These MMP inhibitors were tested alone and in combination with standard chemotherapeutic drugs in patients with various advanced cancers (pancreas, brain, lung, prostate, and gastrointestinal tract), but drug development was terminated after Phase III trials showed a lack of efficacy or musculoskeletal pain in some patients [2,4].

As their name suggests, the 23 members of the human MMP family (24 in the mouse) were initially thought to just degrade ECM proteins. However, their contribution to pathology extends well beyond remodeling the ECM. MMPs are involved in various inflammatory, infectious, and repair processes [5–7]. They precisely cleave most if not all chemokines, a family of 54 chemotactic proteins (in humans) involved in inflammatory and immune cell recruitment. By influencing chemokine activity, MMPs regulate innate immunity [8], are involved in the process of killing bacteria [9,10], and are implicated in a plethora of acute and chronic inflammatory diseases by cleavage of diverse substrates (reviewed in [11]). The importance of MMPs in inflammation is highlighted by the approval of the first MMP inhibitor for clinical use, Periostat® (low-dose doxycycline), for the treatment of the inflammatory gum disease (chronic periodontitis) [12]. However, the treatment efficacy is considered marginal.

MMPs as drug antitargets

Human studies ultimately are the most relevant test of the efficacy of blocking a drug target. From the failure of broad-spectrum MMP inhibitors in cancer clinical trials, we have learned that: (i) in tumors, MMPs are produced by both the cancer cells and the surrounding stromal cells, and infiltrate inflammatory and immune cells; (ii) the role of MMPs differs according to the stage of cancer; and (iii) some MMPs are antitargets. Drug antitargets are molecules with essential normal roles in cell and tissue function and perform beneficial functions that should not be

Glossary

Anti-target: a molecule or protein with essential or host protective roles in the normal state of a cell or tissue function. Inhibition of its activity results in clinically unwanted side effects and initiation or worsening of disease.
Degradomics: all system-wide genomics, proteomics, and systems biology techniques that study the structural and functional roles of the proteases, inactive homologs, and protease inhibitors that are present in an organism.
Target: a molecule or protein that unambiguously contributes to a disease. Inhibition of a validated drug target by a drug reverses the disease course or at the very least holds it static or slows disease progression; at best, drug targeting restores the normal state of a cell or tissue function.

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inhibited. Down-modulation of the activity of an antitarget leads to unacceptable side effects or, worse, initiates disease or worsens the course of disease. In patients, this may manifest as shorter disease onset times, increased disease burden, or poorer outcomes [2]. To date, 10 of the 24 MMPs have been demonstrated to have beneficial roles in various pathologies *in vivo* (Table 1). However, does this mean that these beneficial activities preclude drug development? A molecule possessing antitarget activities poses interesting challenges to consider in drug development – risk to benefit ratio, ethical, and legal – that are discussed throughout this paper.

MMP8 was the first MMP that was clearly demonstrated to have antitarget properties. In a chemical carcinogenesis model, *Mmp8*$^{-/-}$ mice were more susceptible to skin tumors than wild type animals and transplantation of bone marrow, transferring MMP8-rich neutrophils into the *Mmp8*$^{-/-}$ animals, restored the natural protection against tumorigenesis [13]. The protective role of MMP8 against cancer was also demonstrated in breast [14] and tongue cancers [15]. In several murine arthritis models, a lack of *Mmp8* caused exacerbated inflammation and increased bone erosion and accumulation of neutrophil infiltrates [16] due to a decreased rate of apoptosis [17]. In an acute lung injury model, *Mmp8*$^{-/-}$ mice had increased numbers of lung neutrophils and macrophages compared with wild type animals due to persistence of the chemokine MIP-1α in the absence of MMP8 cleavage [18]. Similarly, in an allergen-induced inflammation model, an increased number of neutrophils and eosinophils was observed in the bronchoalveolar lavage fluid of *Mmp8*$^{-/-}$ mice compared with wild type animals [19], the mechanism for which was not explored but might also relate to the role of MMP8 in apoptosis [17]. Nonetheless, tissue and model context is important, because MMP8 also generates a feed-forward mechanism in neutrophil recruitment *in vitro* and *in vivo* whereby it potently activates the neutrophil chemoattractant mCXCL5 (LIX) in mice and CXCL8 (IL8) and CXCL5 (ENA-78) in humans in acute inflammation [20]. Overall, MMP8 from neutrophils plays a crucial role in dampening inflammation in various pathologies.

MMP8 is not the only MMP that is crucial for the resolution of inflammation and in inflammatory and immune cell trafficking. In an allergic asthma model, MMP2 facilitated the egression of inflammatory cells into the airway lumen. In the absence of MMP2, upregulation of T_H2 cytokines occurred, thus affecting the chemotactic activity of the inflammatory cells, and this resulted in massive accumulation of immune cells in the lung parenchyma [21]. During lung inflammation, both MMP2 and MMP9 disrupt transepithelial chemokine gradients (CCL7, CCL11, and CCL17) that contribute to this protective effect [1]. Indeed, MMP2 is the archetypical MMP for regulating chemokine activity [6], with MMP2 cleaving the N terminus of all monocyte chemoattractant proteins, leading to inactivation and converting agonists to antagonists (CCL2, CCL4, CCL7, CCL8, CCL11, CCL13, CCL15, and CXCL12) [6,7,22] or activating potent macrophage chemoattractants (CCL16 and CCL23) [7,22]. Neutrophil-specific MMP25 (MT6-MMP or leukolysin) also processes many chemokines, including activation of CXCL5, a chemoattractant involved in the recruitment of neutrophils to the site of inflammation [23].

Degradomics, the system-wide study of the complete set of proteases that are present in a tissue or an organism and their substrates and inhibitors, was recently used to discover another unexpected role for MMPs in an analysis of skin inflammation using the N-terminomics approach terminal amino isotope labeling of substrates (TAILS) [24]. Serum and acute-phase proteins were greatly diminished in inflammation in *Mmp2*$^{-/-}$ mice due to less exudation in inflammation compared with wild type mice. The authors reported that complement 1 (C1) inhibitor is cleaved and inactivated in wild type mice by MMP2. When this is reduced by MMP2 gene ablation, the relevant cascades controlled by C1 inhibitor are dampened, including control of vascular permeability by bradykinin generation and complement activation. Thus, in wild type mice in which MMP cleavage occurs, there is less functional (intact) inhibitor and so plasma kallikrein can act on kininogen to release more of the vasoactive bradykinin peptide. With increased vascular permeability the amount of acute-phase and other serum proteins rise in the inflamed tissue. Conversely, in the *Mmp2*$^{-/-}$ mouse there is less of an increase in vascular permeability and hence less exudation of serum and acute-phase proteins. The effect of loss of MMP2 cleavage on C1 inhibitor was to also reduce complement activation in knockout animals; this was validated by proteomic quantification of multiple complement protein cleavages *in vivo* using multiplex isobaric tags for relative and absolute quantitation (iTRAQ)–TAILS analyses. Thereby, these recent studies revealed the crucial importance of MMP2 as a key control point for multiple inflammatory pathways *in vivo* and illustrated the power of high-throughput proteomic strategies to identify new substrates, to formulate hypotheses based on the roles of the substrate, and to test these leading to new, clinically relevant insights.

Other diverse anti-inflammatory roles for MMPs have been revealed by degradomic analyses. MMP25 generates 'eat-me' signals by cleaving vimentin outside the cell on the exterior of the neutrophil cell membrane, which in turn stimulates phagocytosis and chemotaxis of activated monocytes to the targeted neutrophil [23]. Thus, MMP25-cleaved vimentin on the surface of apoptotic neutrophils is a signal for their removal by activated macrophages. Unexpectedly, many other multitasking intracellular proteins moonlighting outside the cell were also found to be MMP substrates [25,26] and further developments in this nascent field are expected to reveal many novel roles for MMPs. Most of the important roles of MMPs in biology are enhanced during inflammation; all MMP knockout mice, with the exception of MMP14 (MT1-MMP), which is lethal at 6 weeks and so has yet to be studied in depth in inflammation models, display minimal phenotypic abnormalities. However, once inflammation is triggered, whether by a chemical, an autoimmune response, or a pathogen, the difference between wild type and MMP knockout mice becomes more pronounced and the roles of MMPs in the resolution of inflammation become apparent.