Platelets and matrix metalloproteinases

Peter Seizer; Andreas E. May
Medizinische Klinik III, Eberhard Karls Universität Tübingen, Germany

Summary
Matrix metalloproteinases (MMPs) and their inhibitors essentially contribute to a variety of pathophysiologies by modulating cell migration, tissue degradation and inflammation. Platelet-associated MMP activity appears to play a major role in these processes. First, platelets can concentrate leukocyte-derived MMP activity to sites of vascular injury by leukocyte recruitment. Second, platelets stimulate MMP production in e.g. leukocytes, endothelial cells, or tumour cells by direct receptor interaction or/and by paracrine pathways. Third, platelets synthesise and secrete a variety of MMPs including MMP-1, MMP-2, MMP-3, and MMP-14 (MT1-MMP), and potentially MMP-9 as well as the tissue inhibitors of metalloproteinase (TIMPs). This review focuses on platelet-derived and platelet-induced MMPs and their inhibitors.

Keywords
Platelets, MMP, EMMPRIN

Introduction

Structure
Matrix metalloproteinases (MMP) are zinc-dependent endopeptidases, which share a common domain structure comprising a pro-peptide domain, a catalytic domain, a hinge region, and a haemopexin-like C-terminal domain (1). Together with the ADAM family (A Disintegrin And Metalloproteinase), they belong to the family of metalloproteinases (2). MMPs can be classified by their respective substrates and their cellular localisation as collagenases (e.g. MMP-1, MMP-8, MMP-13, MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-1), and membrane-type MMPs (MT-MMP) (1–3). In order to allow a fine-tuned balance of matrix degradation MMPs are synthesised and secreted as inactive pro-enzymes. For activation the pro-peptide domain needs to be cleaved from MMPs. The haemopexin-like C-terminal domain determines the specificity of MMPs for their substrates and is linked to the catalytic domain via the hinge region (3). Furthermore, this domain is thought to represent the interaction site for TIMPs (tissue inhibitor of metalloproteinases) (3, 4). The TIMPs comprise a family of four protease inhibitors (TIMP-1 to TIMP-4), which can bind and inactivate (pro)-MMPs (5).

History
In 1962, the matrix metalloproteinases have been initially described during tadpole tail metamorphosis (6). In the following years several MMPs have been discovered in humans. They have been characterised as key players in processes associated with a turnover of extracellular matrix, e.g. embryogenesis, tumour growth and metastasis, or wound healing (7). Consistently, MMP activity enables various cell types to proliferate, migrate and differentiate. Accordingly, invasive cells such as monocytes or tumour cells have been described to focus protease activity at their leading edge (8, 9). MMPs are required for cancer invasion (10, 11) or an effective host defense (12–14). Nevertheless, MMPs are crucially involved in a variety of physiological and pathophysiological processes besides matrix degradation, e.g. cleavage of cell surface receptors, release of soluble ligands (such as the CD40 ligand) and modulation of cytokine functions (15–17). Therefore MMPs are considered as central mediators of inflammation.

Platelets can stimulate and focus MMP activity to specific target sites by various mechanisms: First, platelets can attract other inflammatory cells to any site of vascular injury and, thereby, concentrate proteolytic activity of those cells. Second, by forming cellular conjugates with e.g. leukocytes, endothelial cells, or tumour cells, platelets stimulate MMP production in these cells by direct receptor interaction or/and by paracrine pathways (18–23). Third, a growing body of evidence shows direct platelet expression of several MMPs including MMP-1, MMP-2, MMP-3, and MMP-14 (MT1-MMP) and/or on their own cell surface (24–26).

Expression and function of MMPs in platelets
It has been generally assumed, that mRNA expression patterns in megakaryocytes and platelets reflect one another. Interestingly,
Cecchetti et al. recently found that megakaryocytes differentially sort mRNAs for MMPs and their inhibitors into platelets. While mRNA and protein expression coincided in many cases with one another, differences were found as well (27).

**MMP-1**

Since 1974 platelets have been associated with collagenase activity (28). In fact, resting platelets constitutively express significant amounts of MMP-1 (type I collagenase, primarily pro-MMP-1) which is elevated (and activated) upon thrombin stimulation (25). MMP-1 is much more abundant (~10–20 fold) than MMP-2 and dramatically exceeds MMP-3 and MMP-9, if those are indeed present in platelets (27). In addition to its collagenase activity, endogenous and exogenous MMP-1 can regulate outside-in signalling events in platelets leading to phosphorylation of intracellular proteins, which distributes β3-integrins to areas of cellular contact and primes platelets for aggregation (25). Trivedi et al. have shown that platelet exposure to collagen converts surface bound pro-MMP-1 to active MMP-1, which activates protease-activated receptor-1 (PAR-1) by cleaving the receptor at a cryptic ligand site, and promotes platelet aggregation through PAR-1 (29). Moreover, blockade of the MMP-1-PAR-1 interaction inhibits thrombus formation on collagen surfaces under arterial flow conditions *in vitro* as well as in guinea pigs in a model of carotid denudation by FeCl$_3$ (29). These data lead to a novel hypothesis of a collagen-initiated pathway of thrombogenesis that is mediated by an autocrine action of platelet MMP-1 and PAR-1 (Figure 1A). Together, this study links matrix-dependent MMP activation with platelet G protein signalling and it has identified the MMP-1-PAR-1 axis as a potential target for the prevention or treatment of arterial thrombosis (for detailed review on MMPs and PAR-1 activation see Austin et al. [30]).

Consistent with these *in vitro* data patients with high MMP-1 promoter activity haplotypes showed a significantly increased risk for myocardial infarction, whereas patients with low MMP-1 promoter activity haplotypes were at decreased risk (31). In addition, MMP-1 has been found elevated in sera of the culprit coronary artery in comparison to peripheral blood of patients with acute myocardial infarction (32), raising the question of whether the MMP-1 is cause or consequence in this arterial thrombus formation (29).

**MMP-2, MMP-3 and MMP-9**

MMP-2 (gelatinase A) is the most abundant MMP and is constitutively expressed in cells of mesenchymal origin (33). MMP-2 as protein is present in platelets, released upon platelet stimulation with e.g. thrombin or collagen or upon aggregation (24, 34). Notably, platelets do not contain mRNA for MMP-2, whereas megakaryocytes contain relevant amounts of it (27). It has been reported that MMP-2 is randomly distributed within resting platelets and is not associated with platelet granules as detected by immunogold electron microscopy (34). Upon platelet activation MMP-2 was translocated from the cytosol into the extracellular space (34).

The exogenous addition of low concentrations (0.01–1.0 ng/ml) of active MMP-2 (but not pro-MMP-2) enhanced the aggregation of prestimulated (but not resting) platelets, while higher concen-

![Figure 1: Simplified model of MMP and EMMPRIN activities on platelets.](image-url)
Platelets: basic mechanisms and translational implications

Platelets also express MT1-MMP (MMP-14) protein, while there are divergent reports on the existence of mRNA for MT1-MMP in platelets (27). On the cell surface MT1-MMP (MMP-14) has been studied in various cell types and appears to form a so-called trimeric complex with pro-MMP-2 and TIMP-2 (26, 47, 48). Flow cytometric analysis showed an upregulation of both MT1-MMP, MMP-2, and TIMP-2 expressions at the activated platelet surface. While exogenous active (but not latent) MMP-2 enhances platelet aggregation triggered by collagen (see above), MMP inhibition by recombinant TIMP2 or the synthetic BB94 inhibited collagen-induced platelet aggregation in a concentration-dependent manner indicating the ability of MMP inhibitors to modulate the aggregating response (26).

**Tissue inhibitors of matrix metalloproteinases (TIMP-1, -2, -3, -4)**

Four endogenous tissue inhibitors of matrix metalloproteinases (TIMP-1, -2, -3, -4) have been described controlling MMP activity in nucleate cells (5). In both megakaryocytes and platelets, mRNA for TIMP-1, TIMP-2 and TIMP-3 have been identified together with the respective protein (27). TIMP-2 interacts with the catalytic site of MT1-MMP and the C-terminal domain of MMP-2 forming a trimeric complex in response to platelet stimulation that controls the cleavage of pro-MMP-2 (26). The role of MT1-MMP for MMP-2 activation has been described in detail on endothelium, where it seems to be relevant for angiogenesis (47). Interestingly, low concentrations of TIMP-2 contribute to MMP-2 activation, while higher concentrations inactivate MMP-2. These concentration-dependent divergent activities of TIMP-2 may allow a fine-tuned balancing of MMP-2 activity during the dy-
namic process of platelet activation. These considerations fit to the observation, that TIMP-2 mRNA translation is controlled by signal dependent events (27). The presence of TIMP-4 in platelets is still under discussion (27, 49).

Role of platelets and MMPs in cardiovascular disease

Various cardiovascular diseases such as atherosclerosis, myocardial infarction, ischaemia and reperfusion injury, inflammatory cardiomyopathy and abdominal aortic aneurysms have been associated with enhanced or disbalanced activity of MMPs, especially MMP-2 and MMP-9 (12, 50–54). In vitro and in vivo data provide evidence that the main source leading to disease progression in atherosclerosis or aortic aneurysms are macrophages and smooth muscle cells (39, 55–57). However in cardiovascular diseases MMPs are not only the drivers for disease progression, but also exert a pivotal role in healing and regenerative mechanisms (53, 58).

EMMPRIN (CD147, Basigin) and MMPs in platelet-leukocyte interactions

The so-called Extracellular Matrix Metalloproteinase Inducer (EMMPRIN, Basigin, CD147) is an immunoglobulin-like receptor which has been recently identified on platelets (Figure 1C) (59). Stored in the alpha granules EMMPRIN becomes upregulated on the platelet surface upon platelet stimulation with e.g. thrombin or ADP. Platelet interaction with monocytes induces EMMPRIN-dependent monocyte proteolytic MMP-9 and inflammatory cytokines such as interleukin (IL)-6 and tumour necrosis factor (TNF)–alpha (59). If homotypic EMMPRIN-EMMPRIN mediates this activation pathway still awaits clarification. In patients with acute coronary syndromes EMMPRIN expression on platelets is slightly enhanced in patients with coronary artery disease (60). Currently, several extracellular ligands of EMMPRIN are known including EMMPRIN (homotypic binding), platelet GPIIbα (61, 62), as well as soluble factors like cyclophilin A, cyclophilin B or S100A9 (63–65). Besides the observation that binding of soluble recombinant Fc-EMMPRIN to platelets induces platelet degranulation assessed by enhanced P-selectin expression, EMMPRIN-mediated outside-in signalling has not been investigated yet (59).

Platelet-leukocyte interaction is thought to represent an important trigger for both physical leukocyte recruitment to the site of vascular inflammation and for leukocyte activation (Figure 2) (19, 21). For example, platelets can induce proteolytic MMP-activity. Upon contact with collagen monocytes release MMP-9 which can be effectively amplified in the presence of platelets (20). Inhibition of intercellular contacts mediated by β1-integrins or PSGL-1 dramatically reduced synthesis of MMP-9 (20). In addition, in monocyte-platelet aggregates the secretion of inflammatory cytokines (MCP-1, IL-8) and MMP-9 can be inhibited by the anti-platelet agent dipyridamole via reduction of monocytic inflammatory gene expression (66). However eicosanoid production by cyclooxygenase-2 in monocyte/platelet interaction not only involves adhesion molecules but also cytokine signalling by IL-1β (67). Moreover as described above EMMPRIN is crucially involved in inducing the synthesis and release of MMP-9 and MT1-MMP as well as inflammatory cytokines IL-6, IL-8 and TNF-α in a NFκB-dependent manner in monocytes (59). In turn, neutrophils, which contain no relevant amounts of MMP-2, can also stimulate platelets to release MMP-2, which stimulates platelet- neutrophil aggregation (68). In contrast, neutrophil supernatant alone has no effect on platelet MMP-release supporting the concept that physical neutrophil-platelet interaction is required. This direct interaction of neutrophils and platelets seems to be mediated by neutrophilic PSGL-1 and platelet P-selectin binding (68). Treatment of neutrophil/platelet co-cultures with αIbβ3 antagonists reduced MMP-2 release by platelets as well suggesting fibrinogen-bridging to neutrophilic Mac-1. Notably, in this study platelet-binding to neutrophils did not induce MMP-9 secretion by neutrophils. In neutrophils MMP-9 release seems to critically involve the engagement of L-selectin and Mac-1 (69), which cannot be provided by platelets alone, since they do not express the respective receptors. In a mouse model of angiotensin II-induced abdominal aortic aneurysm formation, the inhibition of the P2Y12 (ADP receptor) mediated activation pathway by clopidogrel significantly attenuated the progression of aneurysm formation, which was accompanied by a reduced MMP-2 expression within the vascular wall (70). Moreover, platelets and their interaction with leukocytes are essential mediators of MMP-2 and MMP-9 in myocardial infarction leading to myocardial remodelling and ventricular rupture (71). Remarkably, using a mouse model of permanent occlusion of the left anterior descending artery platelet inhibition with thienopyridines appeared to be equally effective in preventing ventricular rupture as the complete gene disruption of MMP-2 and MMP-9. Platelets may enter the vascular wall or the infarcted myocardium (and thus contribute to MMP-release/induction) by various potential mechanisms: i) As part of platelet-leukocyte-aggregates; ii) in microvascular bleedings; iii) by active platelet migration (71, 72). Nevertheless, the exact contribution of platelet-derived MMPs to cardiovascular pathologies still awaits clarification.

Platelet-derived inducers of MMP activity

Platelets release a variety of inflammatory chemokines and cytokines (2), which can induce MMPs in various cell types. For example, platelet-derived CD40L induces MT-1-MMP, MMP-1, MMP-2 and MMP-9 on endothelial cells (18). Notably, statins treatment can reduce endothelial-derived MMP-synthesis, which may accounts in part for their pleiotropic effects (73). Moreover, platelet-derived CD40L plays a role in atherosclerosis as well as other inflammatory diseases such as Behcet disease or Crohns disease (74, 75). As described above, CD40L is shed from the platelet surfaces by MMPs (15), which critically depends on MMP-2 and its interaction with αIbβ3 (37).
Platelet-derived MMPs in tumour growth and metastasis

Platelets have also been shown to facilitate metastasis by interacting with tumour cells. MMPs are critically involved in tumour growth and metastasis (10, 11). Platelets are crucially involved by using various pathways: i) Tumour cells “use” adhesion receptors on adherent platelets as “docking stations” for their recruitment and settlement in target organs; ii) tumour cells can induce platelet aggregation, and thereby, support their own settlement in the microvasculature; iii) platelets can induce MMP release in tumour cells (36, 76–79). The role of TIMPs is multifunctional and appears to be in part paradoxical. Depending on the respective environment they can both inhibit or stimulate activities on tumour growth. TIMP-4 for example enhances cell proliferation in mammmary tumour cells and reduces cell proliferation in Wilms tumour cells (4). On the one hand TIMP-1 stimulates metastasis formation and on the other hand TIMP-1 reduces tumour angiogenesis in the liver (4). The exact role of platelet-derived TIMPs remains rather unclear and has to be elucidated in future (36, 80).

Interestingly, it was shown that tumour cells have the ability to induce platelet aggregation in vitro (81). One publication described the ability of tumour cells to induce platelet aggregation in vitro and showed that platelets can stimulate tumour cells to secrete MMPs and thereby facilitate metastasis (82).

The potential of tumour cells to induce platelet aggregation correlates with their ability to metastasise (83). Besides classical platelet-activators such as thromboxane or ADP, the release of MMP-2 from platelets and tumour cells promotes platelet-tumour cell aggregation, while treatment with broad spectrum MMP-inhibitors or TIMP-4 dramatically reduces platelet-tumour cell aggregate formation. For example addition of low and medium concentrations of TIMP-4 to fibrosarcoma cells reduced aggregate formation, whereas high concentration had no effect (49). In specific, active MMP-2 and MT1-MMP (via activation of pro-MMP-2 into active MMP-2) is crucially involved in cancer cell induced platelet aggregation (78, 84).

In addition, platelets can induce MMP-9 in various tumour cell lines in vitro, e.g. breast adenocarcinoma, pancreatic adenocarcinoma, colon adenocarcinoma and fibrosarcoma cells (85–88). Besides this interaction platelet derived microvesicles induce MT1-MMP and MMP-9 in lung carcinoma cell lines (22). However, the exact mechanism by which platelets induce MMPs in tumour cells remains to be elucidated in detail.

Pharmacological therapy with exogenous MMP inhibitors

The therapeutic application of exogenous MMP inhibitors represents an attractive approach for treatment of postischaemic myocardial remodelling or cancer growth and metastasis. However, to date all studies using more or less-specific MMP-inhibitors have failed due to major side effects and/or minor therapeutic benefit. For example PG-116800, an oral MMP inhibitor with high affinity for MMP-2, −3, −8, −9, −13, and −14 and low affinity for MMP-1 and −7, failed to improve cardiac function in patients with myocardial infarction. Furthermore, the patients suffered from musculoskeletal side effects and enhanced occurrence of severe arrhythmias by trend (89). Currently, the only MMP-inhibitor, which has been approved in patients, is the established antibiotic doxycycline, which additionally acts as an MMP-inhibitor besides its microbiological activity (90, 91). For example, doxycycline has been shown to improve lung function in patients with chronic obstructive pulmonary disease (91).

Specific inhibition of platelet-derived MMPs such as MMP-2 may represent a promising therapeutic approach. Nevertheless, to some extent MMP activity is beneficial and is required for a balanced matrix turnover and an effective and successful healing process (53, 58). Thus, successful therapeutic inhibition of MMPs is not only a question of specificity, but may also depend on the exact timing and dosing of the treatment. To date, a platelet-specific anti-MMP therapy does not exist yet.

Conclusion

Platelets play a predominant role in various pathophysologies including atherosclerosis and cancer. The regulation of the MMP activity – either directly through secretion of the proteases and their inhibitors or indirectly through influencing the behaviour of other cells – represents a central mechanism of platelet activities. Any imbalance in this fine-tuned multi-loop feedback system may have direct pathophysiological consequences. Nevertheless, the organisation of the platelet proteome and secretome regarding platelet-derived MMPs and TIMPs appears to be even more complex than previously thought and awaits further intensive investigations.

Acknowledgement

The study was supported by the Deutsche Forschungsgemeinschaft (KFO-274: “Platelets-Molecular Mechanisms and Translational Implications” and the SFB TR19: “Inflammatory Cardio-myopathy”).

Conflicts of interest

None declared.

References

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