

COMMENTARY

Matrix metalloproteinase-13 unlucky for the forming thrombus

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Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endoproteases involved in extracellular matrix and non-matrix protein degradation.¹ In the latest issue of *Research and Practice in Thrombosis and Haemostasis*, Howes and colleagues² investigated the role of MMP-13 in platelet aggregation and thrombus formation and identified that MMP-13 could engage important platelet receptors and influence platelet function in vitro. MMP-13 is of great cardiovascular interest as expression of this metalloproteinase is significantly upregulated in a host of atherothrombotic and inflammatory conditions.³

Matrix metalloproteinases are released from most cell types as zymogens and undergo usually pericellular activation to cleave a wide range of extracellular targets in (patho)physiological processes. MMP activity can contribute to pathology related to conditions including atheroma,⁴ arthritis,⁵ and cancer,⁶ with central roles in tissue remodelling, repair, and wound healing.^{1,6} There are at least 23 members of the MMP family, sharing a basic domain structure consisting of pro-, catalytic, and hemopexin domains. The majority of MMPs do not have transmembrane domains, and utilize the hemopexin domain to engage with specific regions of cell membrane proteins in order to localize the MMP to a specific site of activity.

In a series of adhesion assays using washed platelets and recombinant proteins, Howes and colleagues² demonstrate that immobilized pro- or mature MMP-13 is able to mediate static adhesion of platelets, and this adhesion could be disrupted by pretreatment of platelets with anti-GPVI antibodies, or reagents that target the RGD-binding site within integrin α IIb β 3. This suggests that adhesion of platelets to MMP-13 involved both GPVI and α IIb β 3 receptors. Both the catalytic and hemopexin domains of MMP-13 were required for efficient binding, but MMP-13 proteolytic activity was not required

as recombinant catalytically inactive proMMP-13 (bearing an E to A substitution at amino acid position 204, which lies immediately adjacent to the catalytic zinc-binding region of MMP-13) also could mediate platelet adhesion.

Competitive platelet adhesion assays showed MMP-13 could compete with platelet binding to immobilized fibrinogen (IC₅₀ ~150 ng/mL) or collagen-related peptide (CRP)-coated plates (IC₅₀ ~10 ng/mL). A role for α IIb β 3 was supported by data showing platelets isolated from a patient with Glanzmann's thrombasthenia (normal platelet size and presumably normal levels of GPVI, but no detectable α IIb β 3) failed to adhere to MMP-13. Recombinant α IIb β 3 immobilized on plastic in isolation did not bind MMP-13, suggesting that cooperative binding with one or more platelet surface proteins, such as GPVI, was required for efficient binding. Notably, preincubation of washed platelets with 80 nmol/L catalytically inactive proMMP-13 E204A consistently resulted in an inhibition of aggregatory responses to low doses of collagen or CRP. Higher doses of agonist overcame this inhibition. Further, using anticoagulated whole blood, the authors observed significant differences in thrombus size (primarily thrombus height) in flow-based adhesion assays when platelets were pre-treated with MMP-13 before exposure to collagen at arteriolar shear rates. The same effect was not observed if MMP13 was coated on its own or co-immobilized with collagen. It would be of interest to fully assess MMP-13 impact on thrombus formation across a range of (patho)physiological shear rates. This may allow subtle MMP-13-mediated effects to emerge more clearly as the shear rate varies and would help in evaluating to what extent MMP-13 regulates atherothrombotic events and platelet function across a stenotic lesion.⁷

Megakaryocytes and platelets carry mRNA transcripts for up to 10 MMPs and platelets contain several MMPs which are known

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TABLE 1 Platelet-associated MMPs—location of platelet-associated MMPs and their effects on platelet function

MMP	Location in/on platelets			Interacts with	Effect on platelets		References
	Granules	Membrane	Other		Activating ^a	Inhibitory ^b	
MMP-1 Collagenase-1	Yes	Yes		α IIb β 3 PAR1 α 2 β 1	Increase thrombus formation Primes platelets, cleaves PAR1, activates platelet signalling		8,9,12
MMP-2 Gelatinase-A	Yes	Yes	Cytoplasm	GP1b-IX-V α IIb β 3	Increase thrombus formation Cleaves PAR1		10,11
MMP-3 Stromelysin-1	Yes	Yes			No effect	No effect	8
MMP-9 Gelatinase-B	Yes/No	Yes/No	Plasma-derived MMP-9			Decreased activation Reduced Ca ²⁺ mobilisation Increased thrombus area in MMP-9 ^{-/-} mice	16,17
MMP-14 Membrane type 1 (MT1-MMP)		Yes		MMP-2 TIMP-2		Inhibits thrombus growth and stability	8,15
MMP-13 Collagenase-3	?	?	Plasma-derived MMP-13	GPVI and α IIb β 3		Impaired platelet aggregation to low dose collagen, CRP Reduced thrombus formation	2

MMPs, matrix metalloproteinases; TIMP-2, tissue inhibitor of metalloproteinase-2; ?, not determined.

^aPromoting platelet activation (+++).

^bInhibition of platelets (- - -).

to be implicated in hemostasis via platelet function and thrombus formation (Table 1). Both MMP-1 (present on human but not murine platelets), and MMP-2 (present on both), localize to the platelet surface by allosteric engagement with the platelet-specific integrin α IIb β 3 and amplify platelet activation and thrombus formation under arterial shear rates.^{8,9} The MMP-1 and -2 modes of action involve a metalloproteinase-driven proteolysis of protease-activated receptor (PAR) 1, at two sites proximal to, but distinct from, the thrombin cleavage site in PAR1.¹⁰⁻¹² Platelets from MMP-2-deficient mice showed impaired aggregation to low doses of agonists targeting G-protein coupled receptors and reduced adhesion to collagen. Addition of pro-MMP2 to MMP-2-deficient platelets restored platelet function and MMP-2-deficient mice also showed reduced thrombosis in a thromboembolism model.¹¹ MMP-2 has been implicated in human thrombotic conditions, with elevated levels of MMP-2 observed in blood samples from acute coronary syndrome patients.¹³

Platelets also express MMP-14, which has a transmembrane domain and is often found in complex with pro-MMP-2 and an associated inhibitor, tissue inhibitor of metalloproteinase (TIMP)-2.^{8,14,15} Whether MMP-9 is produced by platelets or originates from external

sources and simply binds to platelets remains a matter of some conjecture,^{16,17} and the anti-aggregatory effects of MMP-9 on platelets remain to be elucidated.

A number of the MMPs are noted to have catalytic activity at the platelet surface, and in the case of MMP-2, intracellular cleavage of talin¹⁸ which may contribute to transmission of inside-out signals leading to α IIb β 3 activation. Here,² the mechanism of action of MMP-13 on platelets did not seem to involve direct cleavage of GPVI or α IIb β 3. Whilst MMP-13 could cleave recombinant purified forms of GPVI and α IIb β 3, as well as purified fibrinogen, treatment of platelets with MMP-13 did not result in release of GPVI or α IIb β 3. Intracellular cleavage events mediated by MMP-13 were not investigated. It would be important and interesting to assess whether, like MMP-1 and -2, MMP-13 is able to cleave platelet PAR1, particularly as MMP-13 was already shown to cleave PAR1 on cardiac fibroblasts and cardiomyocytes.¹⁹ Mixing MMP-13 with washed platelets also did not trigger platelet degranulation or α IIb β 3 activation as assessed by flow cytometric measurement of increased P-selectin levels and PAC-1 binding, indicating that the engagement of GPVI and α IIb β 3 by MMP-13 did not mimic ligand-binding and did not trigger inside-out signalling events.

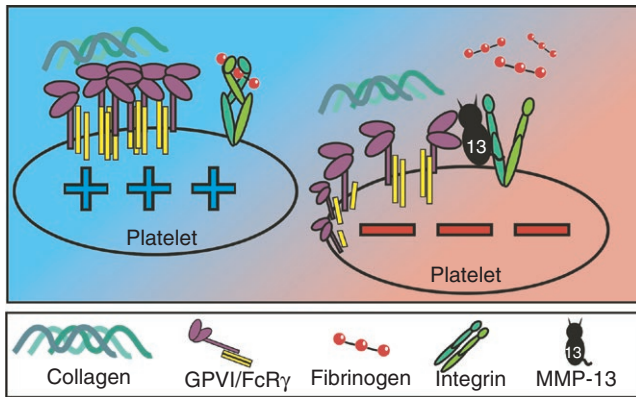


FIGURE 1 Exposure of platelets to collagen triggers the clustering of GPVI and a chain of signalling events culminating in α IIb β 3 becoming activated and binding fibrinogen (+++ = activated platelets with no MMP-13 being present). The addition of MMP-13 to platelets results in MMP-13 engaging with sites within the second immunoglobulin-like domain of GPVI, disrupting receptor clustering, and to a region within α IIb β 3 proximal to the RGD binding pocket. This dual interaction requires both the catalytic and hemopexin domains of MMP-13 and impairs platelet aggregation and thrombus formation (--- = inhibited platelets in presence of MMP-13)

Howes and colleagues included data that indicate that MMP-13 is involved in cooperative binding to both GPVI and α IIb β 3 (Figure 1). Treatment of platelets with MMP-13 disturbed α IIb β 3- and GPVI-mediated adhesion to immobilized fibrinogen and CRP respectively. However MMP-13 did not interfere with GPVI-mediated secretion and higher concentrations of GPVI ligands overcame MMP-13 disruption of GPVI activation in platelet suspensions. This implies that MMP-13 engages with GPVI at a site that is distinct from the ligand binding region. In support of this, the anti-GPVI single chain antibody 1C3 disrupted washed platelet adhesion to MMP-13. 1C3 recognizes an epitope that includes amino acids residing in the second immunoglobulin-like domain of GPVI and in previous studies, treatment of platelets with 1C3 did not alter thrombus size, surface area, or morphology on collagen, but could interfere with GPVI clustering.²⁰ Further, MMP-13 was found to bind strongly to the dimeric form of GPVI over monomeric GPVI, and that there was minimal binding to integrin α 2 β 1, a finding that is somewhat unexpected given that α 2 β 1 binds other MMPs¹⁴ and co-localizes with GPVI dimer clusters.²¹ Taken together, these data suggest that MMP-13 may influence GPVI function by interfering with the lateral movement of GPVI preventing receptor clustering, but not blocking the ligand-binding site of GPVI.

Matrix metalloproteinase-13 has an emerging role in pathobiology of cancer, arthritis, and in cardiovascular disease.³ MMP-13 was originally isolated from breast carcinomas and is upregulated in premetastatic niches, and levels of MMP-13 correlate positively with stroke progression and are implicated as contributing to enhanced plaque vulnerability and rupture.²² Taken together, data presented by Howes and colleagues suggest that MMP-13

could have a regulatory function to limit platelet activation. This process, however, will rely on significant local concentrations of MMP-13 accumulating in the area of plaque rupture, from plasma, or plaque-resident cells as well as a diminution of local inhibitory molecules including α 2-macroglobulin and TIMPs which are present in significant amounts in plasma as well as platelets and other cells, and bound to matrix proteins.¹⁴ Nonetheless, this intriguing study by Howes and colleagues shows MMP-13 can diminish platelet function and thrombus formation, by interfering with GPVI and α IIb β 3 processes. MMP-13-impaired platelet function could impact on atherothrombotic events, and now there may be a role for MMP-13 in modulating the recruitment and activation of platelets in thrombotic pathologies.

RELATIONSHIP DISCLOSURE

S.J.M. and E.E.G. have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

S.J.M. and E.E.G. co-wrote the manuscript.

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