Matrix metalloproteinase-13 unlucky for the forming thrombus

Samantha J. Montague PhD | Elizabeth E. Gardiner PhD

ACRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia

This is a commentary on Howes et al [2018]: https://doi.org/10.1002/rth2.12088

[Article updated on June 8, 2018 after first online publication on May 1, 2018: At the authors' request, the sentence "Treatment of platelets with MMP-13 disturbed RGD- sequence binding to αIIbβ3 but not collagen or CRP binding to GPVI" was updated to "Treatment of platelets with MMP-13 disturbed αIIbβ3- 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 overcome MMP-13 disruption of GPVI activation in platelet suspensions."]

Correspondence
Elizabeth E. Gardiner, ACRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia.
Email: elizabeth.gardiner@anu.edu.au

Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endopeptidases involved in extracellular matrix and non-matrix protein degradation. In the latest issue of Research and Practice in Thrombosis and Haemostasis, Howes and colleagues 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.

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, arthritis, and cancer, with central roles in tissue remodelling, repair, and wound healing. 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 colleagues 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 αIIbβ3. This suggests that adhesion of platelets to MMP-13 involved both GPVI and αIIbβ3 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 αIIbβ3 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 αIIbβ3) failed to adhere to MMP-13. Recombinant αIIbβ3 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 pretreated 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.

Megakaryocytes and platelets carry mRNA transcripts for up to 10 MMPs and platelets contain several MMPs which are known...
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 αIβ3 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 αIβ3 activation. Here,2 the mechanism of action of MMP-13 on platelets did not seem to involve direct cleavage of GPVI or αIβ3. Whilst MMP-13 could cleave recombinant purified forms of GPVI and αIβ3, as well as purified fibrinogen, treatment of platelets with MMP-13 did not result in release of GPVI or αIβ3. 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 αIβ3 activation as assessed by flow cytometric measurement of increased P-selectin levels and PAC-1 binding, indicating that the engagement of GPVI and αIβ3 by MMP-13 did not mimic ligand-binding and did not trigger inside-out signalling events.

### 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 |
|------------|--------------------------|-------------------------|--------------------------------------------------------|------------|
| MMP-1      | Granules Yes, Membrane Yes, Other | αIβ3, PAR1, α2β1 | Increase thrombus formation Primes platelets, cleaves PAR1, activates platelet signalling | 8,9,12     |
| MMP-2      | Yes, Cytoplasm | GPIb-IX-V αIβ3 | Increase thrombus formation Cleaves PAR1 | 10,11      |
| MMP-3      | No effect | No effect | Decreased activation Reduced Ca2+ mobilisation Increased thrombus area in MMP-9−/− mice | 16,17      |
| MMP-4      | MMP-2 TIMP-2 | MMP-2 TIMP-2 | Inhibits thrombus growth and stability | 8,15       |
| MMP-13     | GPVI and αIβ3 | GPVI and αIβ3 | 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 (– – –).
Matrix metalloproteinase-13 has an emerging role in pathobiology of cancer, arthritis, and in cardiovascular disease. 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. 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 α2-macroglobulin and TIMPs which are present in significant amounts in plasma as well as platelets and other cells, and bound to matrix proteins. Nonetheless, this intriguing study by Howes and colleagues shows MMP-13 can diminish platelet function and thrombus formation, by interfering with GPVI and αllβ3 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.

**REFERENCES**

1. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res. 2006;69:562–73.
2. Howes JM, Pugh N, Hamaia S, et al. MMP-13 binds to platelet receptors αIIbβ3 and GPVI and impairs aggregation and thrombus formation. Res Pract Thromb Haemost. 2018;2:370–9.
3. Amar S, Smith L, Fields GB. Matrix metalloproteinase collagenolysis in health and disease. Biochim Biophys Acta. 2017;1864:1940–51.
4. Johnson JL. Metalloproteinases in atherosclerosis. Eur J Pharmacol. 2017;816:93–106.
5. Rose BJ, Kooyman DL. A tale of two joints: the role of matrix metalloproteinases in cartilage biology. Dis Markers. 2016;2016:4895050.
6. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014;15:786–801.
7. Yong ASC, Pennings GJ, Chang M, et al. Intracoronary shear-related upregulation of platelet P-selectin and platelet-monocyte aggregation despite the use of aspirin and clopidogrel. Blood. 2011;117:11–20.
8. Mastenbroek TG, Feijge MA, Kremers RM, et al. Platelet-associated matrix metalloproteinases regulate thrombus formation and exert local collagenolytic activity. Arterioscler Thromb Vasc Biol. 2015;35:2554–61.
9. Galt SW, Lindemann S, Allen L, et al. Outside-in signals delivered by matrix metalloproteinase-1 regulate platelet function. Circ Res. 2002;90:1093–9.
10. Sebastiano M, Momi S, Falcinelli E, Bury L, Hoyaerts MF, Gresele P. A novel mechanism regulating human platelet activation by MMP-2-mediated PAR1 biased signaling. Blood. 2017;129:883–95.
11. Momi S, Falcinelli E, Giannini S, et al. Loss of matrix metalloproteinase 2 in platelets reduces arterial thrombosis in vivo. J Exp Med. 2009;206:2365–79.

12. Trivedi V, Boire A, Tchernychev B, et al. Platelet matrix metalloprotease-1 mediates thrombogenesis by activating PAR1 at a cryptic ligand site. Cell. 2009;137:332–43.

13. Gresele P, Falcinelli E, Loffredo F, et al. Platelets release matrix metalloproteinase-2 in the coronary circulation of patients with acute coronary syndromes: possible role in sustained platelet activation. Eur Heart J. 2011;32:316–25.

14. Gresele P, Falcinelli E, Sebastiano M, Momi S. Matrix metalloproteinases and platelet function. Prog Mol Biol Transl Sci. 2017;147:133–65.

15. Kazes I, Elalamy I, Sraer JD, Hatmi M, Nguyen G. Platelet release of trimolecular complex components MT1-MMP/TIMP2/MMP2: involvement in MMP2 activation and platelet aggregation. Blood. 2000;96:3064–9.

16. Falcinelli E, Bury L, Tolley N, et al. MMP-9 in platelets: maybe, maybe not. Blood. 2011;118:6471–3.

17. Mannello F, Medda V. Differential expression of MMP-2 and MMP-9 activity in megakaryocytes and platelets. Blood. 2011;118:6470–1.

18. Soslau G, Mason C, Lynch S, et al. Intracellular matrix metalloproteinase-2 (MMP-2) regulates human platelet activation via hydrolysis of talin. Thromb Haemost. 2014;111:140–53.

19. Jaffre F, Friedman AE, Hu Z, Mackman N, Blaxall BC. b-adrenergic receptor stimulation transactivates protease-activated receptor 1 via matrix metalloproteinase 13 in cardiac cells. Circulation. 2012;125:2993–3003.

20. O’Connor MN, Smethurst PA, Davies LW, et al. Selective blockade of glycoprotein VI clustering on collagen helices. J Biol Chem. 2006;281:33505–10.

21. Poulter NS, Pollitt AY, Owen DM, et al. Clustering of glycoprotein VI (GPVI) dimers upon adhesion to collagen as a mechanism to regulate GPVI signaling in platelets. J Thromb Haemost. 2017;15:549–64.

22. Sukhova GK, Schonbeck U, Rabkin E, et al. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. Circulation. 1999;99:2503–9.