

receptor VEGFR-2, which has been shown to cooperate with VEGFR-3 in lymphatic endothelial cell migration and proliferation. Whereas VEGF-C is essential in mouse lymphatic vascular development, it remains to be resolved to what extent the lack of VEGFR-2 binding accounts for the dispensable role of VEGF-D in this process.<sup>5,6</sup> Elucidation of the receptor-binding pattern of *Xenopus* VEGF-D and finding whether it explains the functional difference between VEGF-C and VEGF-D in this organism might bring us a step forward in solving this question. A hint to the lymphangiogenic modifier role of VEGF-D is provided by the fact that exogenous VEGF-D protein administration is able to partially rescue the impaired sprouting and migration of lymphatic endothelial cells in VEGF-C-deficient mouse embryos,<sup>6</sup> although loss of endogenous VEGF-D does not aggravate the VEGF-C null phenotype.<sup>7</sup>

Death of VEGFR-3-deficient mice due to blood vascular defects prior to lymphatic vessel development has precluded studies of the role of VEGFR-3 in mouse embryonic lymphangiogenesis.<sup>8</sup> The importance of VEGFR-3 signaling in embryonic lymphatic vascular development, postnatal lymphatic vessel survival, and stimulation of adult lymphangiogenesis has been directly or indirectly demonstrated in various experimental settings in several species. By using genetic down-regulation and chemical inhibition, Ny et al document the critical role of VEGFR-3 in developmental lymphangiogenesis in the *Xenopus* tadpole model and thus the functional conservation of VEGFR-3 in this species.

A profound understanding of the molecular regulation of lymphatic vascular development and the entire range of functions of its

major players and modifiers should enable the development of controlled antilymphangiogenic and prolymphangiogenic therapies. Characterization of the lymphatic system in small model organisms has provided new tools to study lymphangiogenic processes and to complement the methods available in mammals. Ny et al highlight the potential of *Xenopus* tadpoles in lymphatic vascular research by demonstrating the subtle modifier role of VEGF-D in lymphatic vascular development.

*Conflict-of-interest disclosure:* T.K. declares no competing financial interests. K.A. is a minority shareholder of *Lymphatic Ltd.* and the Chairman of the Scientific Advisory Board of *Veogenics Ltd.* ■

## REFERENCES

1. Veikkola T, Jussila L, Makinen T, et al. Signalling via vascular endothelial growth factor receptor-3 is sufficient for lymphangiogenesis in transgenic mice. *EMBO J.* 2001;20:1223-1231.
2. Rissanen TT, Markkanen JE, Gruchala M, et al. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. *Circ Res.* 2003;92:1098-1106.
3. Stacker SA, Caesar C, Baldwin ME, et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat Med.* 2001;7:186-191.
4. Köpfstein L, Veikkola T, Djonov VG, et al. Distinct roles of vascular endothelial growth factor-D in lymphangiogenesis and metastasis. *Am J Pathol.* 2007;170:1348-1361.
5. Baldwin ME, Halford MM, Roufail S, et al. Vascular endothelial growth factor D is dispensable for development of the lymphatic system. *Mol Cell Biol.* 2005;25:2441-2449.
6. Karkkainen MJ, Haiko P, Sainio K, et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. *Nat Immunol.* 2004;5:74-80.
7. Haiko P, Makinen T, Keskitalo S, et al. Deletion of VEGF-C and VEGF-D is not equivalent to VEGFR-3-null in mouse embryos. *Mol Cell Biol.* 2008;28:4843-4850.
8. Dumont DJ, Jussila L, Taipale J, et al. Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. *Science.* 1998;282:946-949.

**A**DAMTS13 cleaves only a single bond,  $\text{A}_{\text{Tyr}^{1605}\text{-Met}^{1606}}$ , buried within the VWF A2 domain. Shear forces unravel the normally tightly packed globular VWF, exposing this cleavage site. How does ADAMTS13 position itself on this flexible and dynamic multimeric glycoprotein, in such a way that it remains in place throughout the shear-induced folding and unfolding that occurs during transit through microcirculatory beds?

Binding is known to take place directly on the A2 domain of VWF spatially adjacent to the cleavage site,<sup>2</sup> but also on a more remote site of the A2 domain.<sup>3,4</sup> There might be additional domain interaction sites on VWF. Complementary binding sites have been identified on ADAMTS13, certainly within its Spacer<sup>5</sup> and Cys-rich regions,<sup>6</sup> but also within the TSR/CUB domains,<sup>7</sup> depending on shear conditions. Binding between each distinct remote site (exosite) ramps up the specificity constant of cleavage. According to Gao et al, collective exosite-driven proteolysis of the  $\text{Tyr}^{1605}\text{-Met}^{1606}$  bond accounts for approximately 2 orders of magnitude increase in cleavage of a small VWF substrate.

Remote exosite interactions are common in the hemostatic process and are exemplified by thrombin,<sup>8</sup> which uses exosites to direct and enhance cleavage of multiple protein and cellular substrates. In order for ADAMTS13 to utilize a similar mechanism of exosite recognition to partner its conformationally-responsive substrate during its conformational dance to shear, this enhanced proteolysis also requires that the protease can retain its binding under changing shear conditions during its transit through vessels of different sizes. Gao et al introduce an important concept of portability of exosites. This can be seen in the figure. The A2 domain model is shown folded as a compact globular molecule with the cleavage site hidden within the structure. When unfolded, it presents several distinct interaction sites near the cleavage site and extending to its C-terminus. Flexibility in spatial relationships (portability) between interacting sites is provided and/or enhanced by a redundant (dispensable) sequence. If there is no fixed distance between exosite and protease cleavage site, then the 2 partner macromolecules can be tightly bound but move together, elongating when shear stress is high in the microcirculation, and contracting in length to globular form in larger vessels when shear stress is low. Such a rhythmic dance depends on mutual and multiple exosite engagements on dynamical structures that nevertheless precisely position the active site

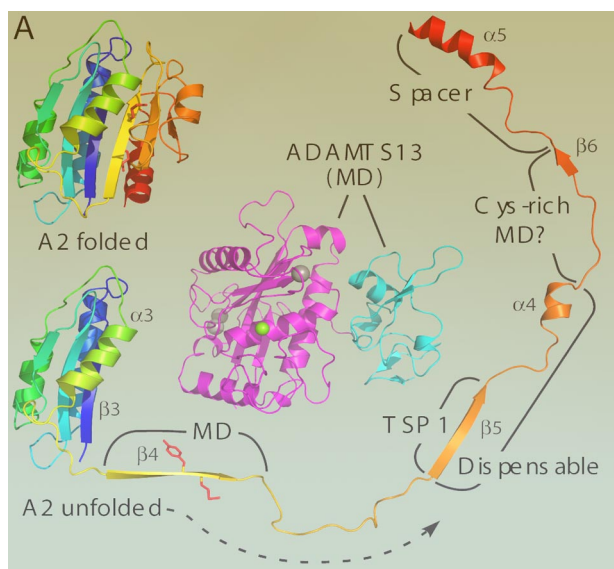
## ● ● ● HEMOSTASIS

Comment on Gao et al, page 1713

# Shear tango: dance of the ADAMTS13/VWF complex

Rens de Groot and David A. Lane IMPERIAL COLLEGE LONDON

ADAMTS13, the metalloprotease that regulates the size of von Willebrand factor (VWF),<sup>1</sup> is critical in preventing microvascular platelet clumping, such as occurs in thrombotic thrombocytopenic purpura. The article in this issue of *Blood* by Gao et al addresses how these two proteins use multiple binding sites to facilitate proteolytic regulation of VWF.



**A** homology model of the ADAMTS13 metalloprotease (magenta) and disintegrin-like domains (cyan) is shown (MD) to provide a sense of scale, with active site Zn<sup>2+</sup> ion (green) and 3 structural Ca<sup>2+</sup> ions (gray) as spheres. The VWF A2 domain is predicted to consist of a 6-stranded  $\beta$ -sheet surrounded by 5  $\alpha$ -helices. Residues Tyr<sup>1605</sup>-Met<sup>1606</sup> (side chains in red) are buried in strand  $\beta$ 4. Exposure of this bond to ADAMTS13 requires substantial unfolding of domain A2; more distal segments that interact with specific domains of ADAMTS13 are labeled. The locations of these ADAMTS13 domains relative to the MD moiety are not known. Deletion of strand  $\beta$ 5 through helix  $\alpha$ 4 (dispensable) has a minimal effect on the rate of substrate cleavage. Molecular graphics prepared with PyMOL (DeLano Scientific, Palo Alto, CA). See the complete figure in the article beginning on page 1713.

within the protease domain. This makes it poised to strike when the cleavage site is revealed by the shear-induced unfolding of the A2 domain.

Ultimately, then, such flexible proximity enables the catalytic center of ADAMTS13 to proteolyse the Tyr<sup>1605</sup>-Met<sup>1606</sup> bond as it becomes exposed and thereby reduces the size and reactivity of the VWF polymer. The unique preference of ADAMTS13 for a single bond contrasts with that of thrombin, the latter being an example of exosite-enhanced (almost) promiscuous proteolysis. Numerous crystal structures have given insight into the mechanisms of

ADAMTS-13 in the A2 domain of von Willebrand factor. *Proc Natl Acad Sci U S A*. 2006;103:18470-18474.

5. Zheng X, Nishio K, Majerus EM, et al. Cleavage of von Willebrand factor requires the spacer domain of the metalloprotease ADAMTS13. *J Biol Chem*. 2003;278:30136-30141.

6. Soejima K, Matsumoto M, Kokame K, et al. ADAMTS-13 cysteine-rich/spacer domains are functionally essential for von Willebrand factor cleavage. *Blood*. 2003;102:3232-3237.

7. Zhang P, Pan W, Rux AH, et al. The cooperative activity between the carboxyl-terminal TSP-1 repeats and the CUB domains of ADAMTS13 is crucial for recognition of von Willebrand factor under flow. *Blood*. 2007;110:1887-1894.

8. Lane DA, Philippou H, Huntington JA. Directing thrombin. *Blood*. 2005;106:2605-2612.

functional specificity of thrombin. What precisely determines the unique cleavage specificity of ADAMTS13 remains to be determined.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

## REFERENCES

1. Tsai HM. Platelet activation and the formation of the platelet plug: deficiency of ADAMTS13 causes thrombotic thrombocytopenic purpura. *Arterioscler Thromb Vasc Biol*. 2003;23:388-396.
2. Zanardelli S, Crawley JT, Chion CK, et al. ADAMTS13 substrate recognition of von Willebrand factor A2 domain. *J Biol Chem*. 2006;281:1555-1563.
3. Gao W, Anderson PJ, Majerus EM, et al. Exosite interactions contribute to tension-induced cleavage of von Willebrand factor by the antithrombotic ADAMTS13 metalloprotease. *Proc Natl Acad Sci U S A*. 2006;103:19099-19104.
4. Wu JJ, Fujikawa K, McMullen BA, et al. Characterization of a core binding site for

also essential for normal hemostasis and mild von Willebrand disease (VWD), due to partial deficiency of VWF, is more common than hemophilia A. However, we all know how bad bleeding can be when factor VIII is absent, so we care whether VWF is able to protect factor VIII. Thus, we also care whether factor VIII stays with VWF, even when it is removed from the blood by a clearance pathway.

VWF is an unusually large protein. It differs from most plasma proteins in that it is synthesized and secreted primarily by endothelial cells rather than hepatocytes. Rolled up like a spool of yarn, the secreted VWF travels in the blood until it unravels under vascular shear stress or after engagement of the vascular wall. The unrolled VWF is a sticky, very long molecule. A single VWF multimer, attached to a vessel wall, can be a good deal longer than the width of an endothelial cell. As blood rushes by, the unrolled VWF thread is soon decorated by platelets that cling tightly enough to resist shear force and the bumping of red cells. Thus, deficiency of VWF leads to bleeding problems caused by failure of platelet deposition. In a pathologic situation, excessive function of VWF is a dramatic feature of thrombotic thrombocytopenic purpura. In that disease, abnormally large VWF multimers in blood causes deposition of platelet-rich thrombi that fragment red blood cells and occlude blood flow to essential organs.

Each of the 20 to 50 subunits that make up a VWF multimer has a high affinity niche for a factor VIII molecule. When bound to VWF, factor VIII is prevented from binding to platelets and participating in the coagulation cascade. Factor VIII is also protected from spontaneous dissociation, from proteolytic degradation, and from clearance by scavenger receptors that line blood vessels. Thus, VWF substantially prolongs the plasma half-life of factor VIII. VWF has a longer plasma half-life than factor VIII and, until now, it hasn't been clear how VWF molecules are removed from the circulation or whether they take bound factor VIII molecules when they go.

In this issue of *Blood*, van Schooten and colleagues identify a cellular clearance pathway for VWF and, apparently, for VWF-bound factor VIII. The surprise is that VWF is cleared via a macrophage pathway rather than via the usual scavenger receptors that are localized primarily on hepatocytes, renal cells, and endothelial cells. It is fitting that VWF, the giant molecule, is cleared by a cellular pathway

## HEMOSTASIS

Comment on van Schooten et al, page 1704

# Where does von Willebrand factor go?

Gary E. Gilbert VA BOSTON HEALTHCARE SYSTEM

VWF is removed from the blood by macrophages rather than scavenger receptors, suggesting a possible strategy for improving treatment of VWD and hemophilia.

In clinical hematology, von Willebrand factor (VWF) is a bit like the actor Jake Gyllenhaal: we care about him because of who clings to his arm rather than for any inherent

quality. VWF is the consort for factor VIII, and many hematologists care more that VWF brings factor VIII to the party than getting to chat with VWF. This is unfair to VWF, of course. VWF is