to have independent validation of the established biomarker results. In this respect, it still remains to be seen whether the 5 prioritized biomarkers from Kubiczkova *et al.*[5] can be confirmed in an independent cohort. The models or gene signatures from biomarker studies easily suffer from being over fit, as typically many more genes are measured than samples. In these circumstances, multiple hypothesis-testing correction measures need to be taken (e.g. checking for the false discovery rate), along with internal cross-validation or preferably external blinded validation. Furthermore, challenging the novel biomarker in a multi-variate analysis along with established diagnostic markers or risk predictors is also crucial in order to establish the added value of the new marker; if not, it's JAM (just another marker)! Only when a biomarker test has withstood the challenge of independent multi-variate validation will it have a chance of actually being used in the clinic and providing benefits for the patients. And that is the ultimate goal of this type of work.

Despite the cautionary notes outlined here, there is a bright future for using circulating RNAs, and in particular microRNAs, because of their tissue specificity and stability, as an embodiment of the long-searched for "Holy Grail" of non-invasive molecular diagnostics.

Jo Vandesompele is a Professor and Senior Lecturer at the Center for Medical Genetics, Ghent University, Belgium, where his main fields of interest are non-coding RNA in cancer, biomarker research, functional genomics, and applied bio-informatics. He is also co-founder and CEO of Biogazelle, Zwijnaarde, Belgium, where he works in the fields of transcriptome analysis, and RNA-based diagnostics and therapeutics. Pieter Mestdagh is a postdoctoral researcher at the Center for Medical Genetics, Ghent University, Belgium. His main fields of interest are non-coding RNA in cancer, biomarker research, and functional and integrative genomics.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

References

1. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA. 2008;105 (30):10513-8.
2. Chim SSC, Shing TKF, Hung ECW, Leung T-Y, Lau T-K, Chiu RWK, et al. Detection and characterization of placental microRNAs in maternal plasma. Clin Chem. 2008;54(3):482-90.
3. Hydbring P, Badalian-Very G. Clinical applications of microRNAs. F1000Research. 2013;2:136.
4. Turchinovich A, Weiz L, Burwinkel B. Extracellular miRNAs: the mystery of their origin and function. Trends Biochem Sci. 2012;37(11):460-5.
5. Kubiczkova L, Kryukov F, Slaby O, Dementyeva E, Jarkovsky J, Nekvindova J,et al. Circulating serum microRNAs as novel diagnostic and prognostic biomarkers for multiple myeloma and monoclonal gammopathy of undetermined significance. Haematologica. 2014;99 (3):511-8.
6. Fan J-B, editor. miRNA expression profiling: from reference genes to global mean normalization. Methods Mol Biol. Totowa, NJ: Humana Press; 2012;822(Chapter 18):261-72.
7. Mestdagh P, Van Vlierberghe P, De Weer A, Muth D, Westermann F, Speleman F, et al. A novel and universal method for microRNA RT-qPCR data normalization. Genome Biol. 2009;10(6):R64.
8. Rekker K, Saare M, Roost AM, Kubo A-L, Zarovni N, Chiesi A, et al. Comparison of serum exosome isolation methods for microRNA profiling. Clin Biochem. 2014;47(1-2):135-8.
9. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids—the mix of hormones and biomarkers. Nature Publishing Group. 2011;8(8):467-77.
10. Kirschner MB, Kao SC, Edelman JJ, Armstrong NJ, Vallely MP, van Zandwijk N, et al. Haemolysis during Sample Preparation Alters microRNA Content of Plasma. PLoS ONE. 2011;6(9):e24145.
11. Page K, Guttery DS, Zahra N, Primrose L, Elshaw SR, Pringle JH, et al. Influence of plasma processing on recovery and analysis of circulating nucleic acids. PLoS ONE. 2012;8(10):e77963-3.

Post-translational arginylation as a novel regulator of platelet function

Markus Bender and Hervé Falet

Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

E-mail: hfalet@rics.bwh.harvard.edu *doi:10.3324/haematol.2013.103119*

In this issue of Haematologica, Lian *et al.* show that megakaryocyte-specific loss of arginyltransferase (ATE1)-mediated post-translational arginylation leads to enhanced clot retraction and *in vivo* thrombus formation in mice, due to enhanced myosin regulatory light chain (RLC) phosphorylation in platelets.[1]

Platelets are small, discoid-shaped cells circulating in the bloodstream. After vascular injury, platelets are recruited to the exposed subendothelial extracellular matrix that triggers platelet activation to seal wound sites by the formation of a hemostatic plug. Consequently, excessive bleeding is prevented under normal circumstances. However, if thrombus formation is uncontrolled, it may lead to vessel occlusion and to life-threatening events, such as myocardial infarction and stroke.[2]

Platelet activation involves a large number of platelet surface receptors, signaling molecules, and cytoskeletal-modifying proteins, as well as their complex interactions. Platelet signaling requires a cascade of intracellular protein post-translational modifications, of which phosphorylation is probably the best studied. Mass spectrometry analysis revealed that more than 270 proteins are phosphorylated in human platelets.[3] The number of studies on how post-translational modifications influence platelet biology is increasing, demonstrating that these modifications constitute an emerging, biologically significant field.

Post-translational arginylation, or tRNA-dependent addition of the amino acid arginine to proteins, was discovered more than 40 years ago. However, it remained a comparatively less-known post-translational modification. Arginylation was once thought to play a singular role in the N-end rule pathway of protein degradation. However,

recent evidence shows that protein arginylation also regulates a wide variety of crucial biological processes, including embryogenesis, cardiovascular development, angiogenesis, and neural crest cell migration, which are just beginning to be understood (reviewed by Saha *et al.*[4]). For example, arginylation of β-actin has been found to regulate lamellipodial formation at the leading edge in fibroblasts, suggesting that similar functions of β-actin in other cell types may also require arginylation. Thus, arginylation is emerging as a regulator protein of function that is reminiscent of phosphorylation.[4,5]

Protein arginylation is mediated by the arginyltransferase ATE1 (for Arginine Transfer Enzyme 1), an enzyme present in all eukaryotic cells.[4] Arginylation requires no additional factors besides the ATE1 enzyme, the charged arginine-tRNA, and the protein substrate. ATE1 can transfer arginine not only to the N-terminus, but also to internal sites in a protein substrate, and is capable of self-arginylation, which is likely involved in its regulation.[6] Every organism, from yeasts to humans, contains the *ATE1* gene, which encodes a single protein in lower eukaryotes, and multiple isoforms in higher species. The mouse *Ate1* gene encodes four ATE1 isoforms, *i.e.* ATE1-1/4, produced by different combinations of four alternatively spliced exons 1 and 2 and exons 8 and 9. ATE1-1/2 have higher activity and more substrate specificity than ATE1-3/4. ATE1-2 is the most ubiquitously expressed ATE1 isoform in different mouse tissues.[7] The essential physiological significance of protein arginylation *in vivo* was shown by the generation of *Ate1*-null mice, which results in embryonic lethality with defects in cardiovascular development and angiogenesis.[8,9]

Lian *et al.* demonstrate that the ubiquitous ATE1-2 is the main isoform expressed in mouse platelets.[1] The total amount of ATE1 protein in platelets is similar to that in fibroblasts. The authors further capitalized on megakaryocyte-specific *Ate1*-null mice to investigate the role of ATE1-mediated protein arginylation in platelet biology, which had been completely unexplored before. *Ate1*$^{fl/fl}$ *Pf4-Cre* mice do not suffer from spontaneous bleeding. Consistently, lack of ATE1 function is dispensable for platelet biogenesis, secretion, aggregation and spreading. In contrast to the situation in fibroblasts, ATE1 is not essential for β-actin assembly in platelets, suggesting that ATE1 might have other targets in platelets. Consequently, Lian *et al.* tested platelets for other candidate proteins as possible targets for arginylation and describe more than 20 proteins that are arginylated in platelets, including myosin essential light chain ELC/MYL6 on Val64 and myosin heavy chain MYH9 on Leu1844.

Myosin is a well-described protein that is involved in mediating contractile forces.[10] Thus, *Ate1*-null platelets were analyzed for the generation of contractile forces, a highly cytoskeletal-dependent process. The authors found that *Ate1*-null platelets exert enhanced contractile forces, as evidenced by accelerated clot retraction, and were able to pinpoint the molecular mechanism by showing that phosphorylation of myosin RLC on Ser19, but not on Thr18 is enhanced in thrombin-stimulated *Ate1*-null platelets. Furthermore, ATE1 and myosin interact in mouse platelets, demonstrating that ATE1 can regulate myosin function. The authors hypothesize that addition of the bulky arginine on ELC/MYL6 Val64 might impair the access of myosin light chain kinase to myosin RLC Ser19 (Figure 1).

It was previously reported that the contractile mechanisms in platelets are critical for maintaining the integrity of a hemostatic plug at wound sites independently of thrombin and fibrin generation and that the tight packing of platelets was reversed after blocking MYH9 activity by using the myosin inhibitor, blebbistatin.[11] In agreement with this, *Ate1*-null platelets with enhanced contractile behavior formed thrombi after $FeCl_3$-induced carotid artery injury and arrested bleeding faster than control platelets.

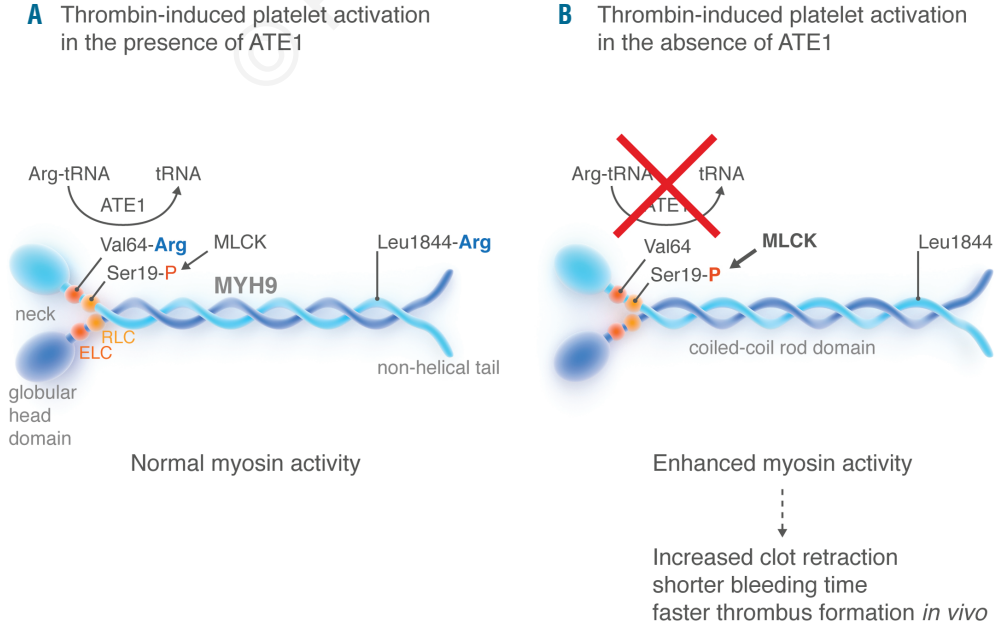

Figure 1. Hypothetical model. (A) The arginyltransferase ATE1 transfers the amino acid arginine (Arg) to myosin essential light chain (ELC) Val64 and myosin heavy chain MYH9 Leu1844. Myosin light chain kinase (MLCK) phosphorylates myosin regulatory light chain (RLC) Ser19 and activates it. (B) Myosin RLC Ser19 phosphorylation is increased in *Ate1*-null platelets, as access of MLCK to its target is influenced by the lack of the bulky arginine addition to myosin ELC Val64. This in turn enhances myosin activity, leading to increased clot retraction, faster thrombus formation and shorter bleeding time in megakaryocyte-specific *Ate1*-null mice.

These data support previous observations that myosin-mediated contractile forces contribute to proper thrombus formation and may have important, clinically related implications for patients with MYH9-related platelet disorders suffering from altered hemostatic function.[12]

In conclusion, Lian *et al*. convincingly revealed a so far unknown role for arginylation, a lesser known post-translational modification, in platelet biology and demonstrate that there is a hierarchical network of post-translational modifications, with myosin ELC/MYL6 arginylation regulating the level of myosin RLC phosphorylation. Whether this is a general regulatory mechanism in cells remains to be determined. For example, the authors identified the arginylation of the cytoskeletal and scaffold protein filamin A in platelets. Filamin A plays a critical role in platelet morphology and signaling, as it cross-links actin filaments, tethers the von Willebrand factor receptor glycoprotein Ib-IX-V complex and integrins to the underlying actin cytoskeleton, and serves as a scaffold for signaling intermediates, e.g. the tyrosine kinase Syk[13] (reviewed by Falet *et al.*[14]). Filamin A arginylation occurs on three different sites, *i.e*. Pro2151, Phe2311 and Tyr2501, among which Pro2151 is particularly important, as it is located near Ser2152, a major phosphorylation site and a possible regulator of integrin binding. It will be interesting to investigate if and how ATE1-mediated arginylation influences the function of the more than 20 arginylated proteins, for example filamin A, in platelets. Thus, we are just at the beginning of understanding the importance of arginylation in platelet biology.

Markus Bender, PhD, is a Postdoctoral Fellow at Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. Hervé Falet, PhD, is Instructor in Medicine at Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. His main field of interest is platelet and megakaryocyte biology.

Acknowledgments: This work was supported by Deutsche Forschungsgemeinschaft (DFG) postdoctoral fellowship BE 5084/1-1 (to MB) and National Institutes of Health (NIH) grant HL059561 (to HF).

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

References

1. Lian L, Suzuki A, Hayes V, Saha S, Han X, Xu T, et al. Loss of ATE1-mediated arginylation leads to impaired platelet myosin phosphorylation, clot retraction, and in vivo thrombosis formation. Haematologica. 2013;99(3):554-60.
2. Nieswandt B, Pleines I, Bender M. Platelet adhesion and activation mechanisms in arterial thrombosis and ischaemic stroke. J Thromb Haemost. 2011;9 (Suppl 1):92-104.
3. Zahedi R, Lewandrowski U, Wiesner J, Wortelkamp S, Moebius J, Schütz C, et al. Phosphoproteome of resting human platelets. J Proteome Res. 2008;7(2):526-34.
4. Saha S, Kashina A. Posttranslational arginylation as a global biological regulator. Dev Biol. 2011;358(1):1-8.
5. Soffer R. Enzymatic modification of proteins. 4. Arginylation of bovine thyroglobulin. J Biol Chem. 1971;246(5):1481-4.
6. Wang J, Han X, Saha S, Xu T, Rai R, Zhang F, et al. Arginyltransferase is an ATP-independent self-regulating enzyme that forms distinct functional complexes in vivo. Chem Biol. 2011;18(1):121-30.
7. Rai R, Kashina A. Identification of mammalian arginyltransferases that modify a specific subset of protein substrates. Proc Natl Acad Sci USA. 2005;102(29):10123-8.
8. Rai R, Wong C, Xu T, Leu N, Dong D, Guo C, et al. Arginyltransferase regulates alpha cardiac actin function, myofibril formation and contractility during heart development. Development. 2008;135(23):3881-9.
9. Kwon Y, Kashina A, Davydov I, Hu R-G, An J, Seo J, et al. An essential role of N-terminal arginylation in cardiovascular development. Science. 2002;297(5578):96-9.
10. Levayer R, Lecuit T. Biomechanical regulation of contractility: spatial control and dynamics. Trends Cell Biol. 2012;22(2):61-81.
11. Ono A, Westein E, Hsiao S, Nesbitt W, Hamilton J, Schoenwaelder S, Jackson S. Identification of a fibrin-independent platelet contractile mechanism regulating primary hemostasis and thrombus growth. Blood. 2008;112(1):90-9.
12. Balduini C, Pecci A, Savoia A. Recent advances in the understanding and management of MYH9-related inherited thrombocytopenias. Br J Haematol. 2011;154(2):161-74.
13. Falet H, Pollitt A, Begonja A, Weber S, Duerschmied D, Wagner D, et al. A novel interaction between FlnA and Syk regulates platelet itam-mediated receptor signaling and function. J Exp Med. 2010;207(9):1967-79.
14. Falet H. New insights into the versatile roles of platelet FlnA. Platelets. 2013;24(1):1-5.

Survey of professional competence in hematology in Europe

Eva Hellström Lindberg,[1] Antonio Almeida,[2] Fredrik Enoksson,[3] Thom Duyvené de Wit,[4] Janet Strivens,[5] Ambjörn Naeve,[3] and Cheng-Hock Toh[6,7]

[1]Karolinska Institute, Department of Medicine, Division of Hematology, Karolinska University Hospital, Stockholm, Sweden; [2]Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisbon, Portugal, [3]KTH Royal Institute of Technology, The Knowledge Management Research Group, School of Education and Communication in Engineering Sciences (ECE), Stockholm, Sweden; [4]EHA Executive Office, The Hague, The Netherlands; [5]Centre for Lifelong Learning, the University of Liverpool, UK; [6]Roald Dahl Haemostasis and Thrombosis Centre, Royal Liverpool University Hospital, UK; [7]Institute of Infection and Global Health, University of Liverpool, UK

E-mail: eva.hellstrom-lindberg@ki.se *doi:10.3324/haematol.2014.104299*

Competence in hematology: a prerequisite for good patient care

The purpose of specialty training in medicine is to supply the population with a sufficient number of adequately educated medical specialists. The majority of hematologic disorders often require immediate attention, investigation and treatment. The diversity of hematologic diagnoses and specialized treatments set high demands on the competence of hematologists and access to specialist care. Moreover, the specialty of hematology is unique in its requirement to blend both clinical and laboratory skill sets. It is, therefore, a concern that the number of hematologic specialists, as well as their training, varies consider-