Von Willebrand factor, ADAMTS13 and Neutrophil Extracellular Traps: allies in cancer-associated thrombosis and tumor progression?

Marcello Monti*

Department of Clinical Medicine and Surgery, “Federico II” University, Naples, Italy

Abstract

Cancer and thrombotic events are strongly associated and von Willebrand factor (VWF) plays a key role in these biological processes. Degradation by ADAMTS13 of ultralarge VWF multimers (UL-VWF) enriched in VWF is crucial to avoid their accumulation in blood resulting in thrombosis. Neutrophil Extracellular Traps (NETs) represent a relatively new mechanism involved in cancer-associated thrombosis and metastases. Since levels of plasma VWF are significantly elevated in cancer patients and NETs DNA positively correlates with VWF and negatively with levels of ADAMTS13, here is highlighted how VWF, ADAMTS 13 and NETs can be correlated in cancer-associated thrombosis and tumor progression.

Abbreviations: NETs: Neutrophil Extracellular Traps; VWF: von Willebrand factor; UL-VWF: Ultralarge VWF multimers, VTE: venous thromboembolism

Introduction

A 4- to 7-fold increased risk of venous thromboembolism (VTE) has been reported for cancer patients as compared to general population and VTE is the second most prevalent cause of death in cancer [1-3]. Patients with metastatic disease have a higher risk of VTE than those with localized tumors [4-6] and platelets play a key role in cancer development and they can interact with metastasizing cancer cells becoming deadly allies [7]. Tumor cell-induced platelet aggregation (TCIPA) is a multistep process in which tumor cells can activate and aggregate platelets leading in the blood stream to the initiation of thrombus formation as well as metastatic cascade [8]. In vitro model system, platelet rich plasma (PRP) of cancer patients resulted in enhanced aggregation by 127% compared to healthy control PRP [9]. From a molecular point of view, it’s an old notion that cancer cells have also been widely reported to secrete platelet agonists such as ADP and thromboxane A2 to induce two different pathways for platelet aggregation [10,11] and conversely platelet receptor P2Y12 was found to influence mechanisms leading to cancer progression as well, mainly by regulating tumor cell/platelet interaction and angiogenesis [12].

Besides, platelets can protect metastatic tumor cells from immune surveillance and help them to attach at the endothelium upon their arrest at metastatic sites [13]. Platelets and endothelial cells with their adhesion properties may facilitate metastasis by augmenting tumor cell extravasation which is favoured by irreversible and complete platelet aggregation achieved after ATP secretion by platelets granules [14]. In this scenario, Wahrenbrock M, et al. demonstrated in vivo that tumor metastases were reduced in mice lacking the vascular adhesion molecules P- and L-selectin [15] and vascular endothelial growth factor (VEGF) released by α-granules of activated platelet was able to promote vasculogenesis in the circulation of patients with cancer [16,17].

Von Willebrand factor (VWF) and ADAMTS13 in thrombosis and cancer

Endothelial cells, megakaryocytes and platelets synthesize von Willebrand factor (VWF), the largest multimeric glycoprotein in human blood involved in regulating hemostasis [18-20]. Circulating VWF connect at the site of vascular injury in the subendothelial matrix by binding the platelet GP Ib-IX-V complex, promoting platelet accumulation (i.e. adhesion, activation and aggregation) in the classical first wave of hemostasis or primary hemostasis [21-23].

After being synthesized, pro-VWF monomers dimerize through C-terminal disulfide bonds and a variable number of dimers then multimerize through N-terminal disulfide bonds [24,25]. Newly synthesized VWF multimers are either constitutively released or stored in the Weibel–Palade bodies of endothelial cells and in the α-granules of megakaryocytes and platelets before to be constitutively secreted in ultralarge VWF multimers (UL-VWF), which are enriched in VWF and hyperreactive in their ability to bind platelets [22,26,27]. UL-VWF are typically cleaved between tyrosine 842 and methionine 843 amino acid residues in the A2 domain of VWF in smaller fragments of 176 and 140-kDa by VWF-cleaved protease also identified as ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif 13), a Zn2+/Ca2+-dependent metalloprotease [28-31] essential for the physiological vascular homeostasis obtained upon VWF proteolysis [32]. The detection of VWF plasma level is mainly a reliable marker of thrombosis and thromboembolism [33,34]. Moreover, levels of plasma VWF are significantly elevated in cancer patients [35,36] and associated not only with cancer-associated thrombosis [37], but

*Correspondence to: Marcello Monti, Department of Clinical Medicine and Surgery, “Federico II” University, Naples, Italy, E-mail: marcello.monti@unina.it

Key words: NETs, VWF, ADAMTS13, cancer, thrombosis

Received: April 19, 2020; Accepted: April 27, 2020; Published: April 30, 2020
Neutrophil Extracellular Traps (NETs) in thrombus formation and in cancer dissemination

Neutrophil Extracellular Traps (NETs) formation in a process termed NETosis is emerged as a relevant process involved in thrombosis and cancer progression. Discovered in 2004 by Brinkmann and colleagues [45], these traps composed by decondensed chromatin and extracellular DNA represent an alternative to phagocytosis, by which neutrophils are able to kill different microorganisms such as virus, bacteria, and fungi through the action of myeloperoxidase, neutrophil elastase/histones and calprotectin, respectively [46,47].

Interestingly, a crucial role for NETs was demonstrated in promoting thrombosis [48] since NETs can provide a scaffold for platelet and red blood cell adhesion and aggregation thus enhancing coagulation [49,50]. All the major constituents of NETs that is extracellular DNA, histones and protease all have procoagulant properties. Nucleic acids activate coagulation through RNA binding to factor XII and XI in the intrinsic pathway as well as histones increase thrombin generation in a platelet-dependent manner promoting coagulation [51-53].

Several plasma protein important for platelet adhesion and thrombus propagation such as fibronectin and VWF may bind to NETs [51]. In these respect, it was demonstrated that both these proteins are key components of NETs not derived from plasma. In a murine model of deep vein thrombosis (DVT), it was observed that citrullinated histone H3 (citH3) by Peptidyl arginine deiminase 4 (PAD4) in NETs colocalized with VWF, suggesting that this VWF and NETs create a complex favoring thrombus growth and stabilization [49].

Furthermore, the UL-VWF adhered to the endothelium can bind and immobilize the DNA released by NETs acting as a linker between leukocyte adhesion to the endothelium and supporting leukocyte extravasation and inflammation [54,55].

Monti, et al. discovered fibronectin as a endogenous component of NETs [56]. This finding opened an interesting setting in various biological processes in which NETs are involved since the fibronectin in the web-like structure of NETs provides specific binding sites for several integrins expressed on the plasma membrane of different cell types, such as platelets, endothelial cells and cancer cells [56]. In this regard, NETs have a key role in cancer progression [57] and the presence of fibronectin in the NETs structure may explain the adhesion of cancer cells of different origin by the expression of RGD-binding integrins (specially α5β1 and αvβ3) mediating the entrapment onto NETs allowing the first step of the metastatic cascade [58].

In preclinical models of lung and colon cancer, it was demonstrated that NETs functionally regulated disease progression and that blocking NETosis through multiple strategies significantly inhibited spontaneous metastasis to the lung and liver [59]. Moreover, in a murine model of infection using cecal ligation and puncture, NETs deposition drove the entrapment of circulating lung carcinoma cells associated with increased formation of hepatic micrometastases at 48 hours after tumor cell injection. These effects were abrogated by NETs inhibition with DNase or a neutrophil elastase inhibitor [60]. Neutrophils from mice with chronic myelogenous leukemia were prone to generate extracellular DNA traps and extracellular chromatin released through NETs formation was demonstrated to be the source for cancer-associated thrombosis [61].

Since the regulation in size of UL-VWF multimers by ADAMTS13 is a relevant mechanism to control invasion of PMNs and higher perivascular leukocyte infiltration was observed in ADAMTS13−/− mice [62], it would be interesting to study the correlation between ADAMTS-13 genetic polymorphisms and its reduced enzymatic activity. This should imply accumulation of UL-VWF multimers and also an improved leukocyte infiltration more prone to NETosis. In turn these events could drive NETs-dependent cancer-associated thrombosis and tumor progression.

Conclusion

In the tumor microenvironment, a clear correlation between NETs and VWF is established and the optimal strategy would be a therapy able to target NETs and inhibit its dual role as fuel of the the metastatic dissemination and as trigger of thrombus formation after entrapping cancer cells in the blood vessels lumen.

Competing Interests

No conflicts of interest exist.

References

1. Fernandes CJ, Morinaga LTK, Alves JL Jr, Castro MA, Calderano D, et al. (2019) Cancer-associated thrombosis: the when, how and why. Eur Respir Rev 28. [Crossref]
2. Noble S, Pasi J (2010) Epidemiology and Pathophysiology of Cancer-Associated Thrombosis. Br J Cancer 1: S2-S9.
3. Sheth RA, Niekamp A, Quencer KB, Shamoun F, Knuttineng MG, et al. (2017) Thrombosis in Cancer Patients: Etiology, Incidence, and Management. Cardiovasc Diagn Ther 7: S178-S185.
4. Hisada Y, Geddings JE, Ay C, Mackman N, et al. (2015) Venous thrombosis and cancer: from mouse models to clinical trials. J Thromb Haemosst 13: 1372-1382. [Crossref]
5. Razak NBA, Jones G, Bhandari M, Berndt MC, Metharom P (2018) Cancer-Associated Thrombosis: An Overview of Mechanisms, Risk Factors, and Treatment. Cancers (Basel) 10: 380.
6. Riedl JM, Posch F, Bexan A, Seckadiz J, Smolle MA, et al. (2017) Patterns of venous thromboembolism risk in patients with localized colorectal cancer undergoing adjuvant chemotherapy or active surveillance: an observational cohort study. BMC Cancer 17: 415. [Crossref]
7. Erpenbeck L, Schön MP (2010) Deadly Allies: The Fatal Interplay Between Platelets and Metastasizing Cancer Cells. Blood 115: 3427-36.
8. Bastida E, Almiron L, Ordinas A (1987) Tumor-cell-induced Platelet Aggregation Is a Glycoprotein-Dependent and Lipoygenase-Associated Process. Int J Cancer 39: 760-3.
9. Olekszowicz L, Bhagwati N, DeLeon-Fernandez M (1999) Deficient activity of von Willebrand's factor-cleaving protease in patients with disseminated malignacies. Cancer Res 59: 2244-2250. [Crossref]
10. Canez A, Dupuy E, Bellucci S, Calvo F, Bryckaert MC, et al. (1986) Human Platelet-Tumor Cell Interactions Vary With the Tumor Cell Lines. Invasion Metastasis 6: 321-334.
11. Honn KV, Steiner BW, Moin K, Onoda JM, Taylor JD, et al. (1987) The Role of Platelet Cyclooxygenase and Lipoprotein Pathways in Tumor Cell-Induced Platelet Aggregation. Biochem Biophys Res Commun 145: 384-389.

12. Best MG, Wesseling P (2018) Tumor-Educated Platelets as a Noninvasive Biomarker Source for Cancer Detection and Progression Monitoring. Cancer Res 78: 3407-3412. [Crossref]

13. Nieswandt B, Hafner M, Echtenacher B, Männel DN (1999) Lysis of Tumor Cells by Natural Killer Cells in Mice Is Impeded by Platelets. Cancer Res 59: 1295-300.

14. Schumacher D, Strilie B, Sivaraj KK, Wetschrecke N, Oeffmann S (2013) Platelet-derived Nucleotides Promote Tumor-Cell Transendothelial Migration and Metastasis via P2Y2 Receptor. Cancer Cell 24: 130-137.

15. Wahrenbrock M, Borsig L, Varki M, Varki A (2003) Selectin-mucin Interactions. Blood 101: 987-995. [Crossref]

16. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, et al. (2002) ADAMTS-13 Rapidly Cleaves Newly Secreted Ultralarge Von Willebrand Factor Multimers on the Endothelial Surface Under Flowing Conditions. Blood 100: 4033-4039.

17. Yamashita K, Yagi H, Hayakawa M, Abe T, Hayata Y, et al. (2016) Rapid Restoration of Thrombus Formation and High-Molecular-Weight Von Willebrand Factor Multimers in Patients With Severe Aortic Stenosis After Valve Replacement. J Atheroscler Thromb 23: 1150-1158.

18. Sadler JE (2013) Von Willebrand Factor in Its Native Environment. Blood 121: 2583-2584.

19. Yang AJ, Wang M, Wang Y, Cai W, Li Q, et al. (2018) Cancer Cell-Derived Von Willebrand Factor Predicts Venous Thromboembolism in Patients With Cancer. J Thromb Haemost 16: 264-270. [Crossref]

20. Ammollo CT, Semeraro F, Xu J, Esmon NL, Esmon CT (2011). Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. Blood 118: 1952-1961.

21. Grassle S, Huck V, Pappelbaum KI, Gorzelanny C, Aponte-Santamaria C, et al. (2014) Von Willebrand factor directly interacts with DNA from neutrophil extracellular traps. Arterioscler Thromb Vasc Biol 34: 1382-1389.

22. Monti M, Iommelli F, De Rosa V, Carriero MV, Miceli R, et al. (2017) Integrin-dependent Cell Adhesion to Neutrophil Extracellular Traps Through Engagement of Fibronectin in Neutrophil-Like Cells. PLoS One 12: e0171362.
57. Cools-Lartigue J, Spicer J, Najmeh S, Ferri L (2014) Neutrophil Extracellular Traps in Cancer Progression. *Cell Mol Life Sci* 71: 4179-4194.

58. Monti M, De Rosa V, Iommelli F, Carriero MV, Terlizzi C, et al. (2018) Neutrophil extracellular traps as an adhesion substrate for different tumor cells expressing RGD-binding integrins. *Int J Mol Sci* 19: 2350.

59. Rayes RF, Mouhanna JG, Nicolau I, Bourdeau F, Giannias B, et al. (2019) Primary Tumors Induce Neutrophil Extracellular Traps With Targetable Metastasis Promoting Effects. *JCI Insight* 5: e128008.

60. Cools-Lartigue J, Spicer J, McDonald B, Gowing S, Chow S, et al. (2013) Neutrophil Extracellular Traps Sequester Circulating Tumor Cells and Promote Metastasis. *J Clin Invest* 123: 3446-3458.

61. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, et al. (2012) Cancers Predispose Neutrophils to Release Extracellular DNA Traps That Contribute to Cancer-Associated Thrombosis. *Proc Natl Acad Sci U S A* 109: 13076-13081.

62. Alffen A, Prüfer S, Ehener K, Reuter S, Lopez PA, et al. (2017) ADAMTS-13 Regulates Neutrophil Recruitment in a Mouse Model of Invasive Pulmonary Aspergillosis. *Sci Rep* 7: 7184.