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

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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 Extracellualr 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 *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

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also with the grade of malignancy, occurrence of metastases and poor prognosis [9,38-40]. Undigested UL-VWF were observed in patients with disseminated tumors and they result from deficient ADAMTS13 protease activity. Interestingly, the clinical presence and absence of tumor metastases correlated significantly with ADAMTS13 enzymatic activities for <15% and >88%, respectively [41]. Some single nucleotide polymorphisms (SNP) of *ADAMTS13* were associated with a reduced protease activity. In particular, the polymorphisms Val154Ile, Asp187His, Arg421Cys, Tyr603Cys resulted in 15%, 19%, 24% and 28%, respectively [42]. Another study confirmed that another SNP, the P475S mutation, led to a dramatic decreases in VWF-cleaved protease activity [43]. Notably, reduced ADAMTS13 enzymatic activity as well as high plasma VWF/ADAMTS13 ratio may serve as an independent predictive factor for mortality in patients with advanced Non-Small Cell Lung Cancer (NSCLC) [44] .

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 Brinkamnn 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 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.

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