Structure and multiple functions of von Willebrand factor

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Abstract

Since the first description of a patient with von Willebrand disease (VWD) back in 1926, significant advances have been made in understanding the biology of von Willebrand factor (VWF). Under normal conditions, *in vivo* biosynthesis of VWF is restricted to endothelial cells and megakaryocytes only. This biosynthesis involves complex post-translational modifications (including glycosylation and multimerization) which play a key role in enabling the hemostatic functions of VWF. As a result, VWF circulates in normal plasma as a series of heterogeneous multimers that can modulate tethering of platelets and primary hemostasis at sites of vascular injury. In addition, VWF also influences secondary hemostasis by serving as a chaperone molecule and protecting factor VIII from proteolysis and premature clearance. The molecular mechanisms underlying the pro-hemostatic functions of VWF have been comprehensively characterized. These insights serve to underpin the current classification of different VWD subtypes. Interestingly, accumulating evidence over the past decade has identified an array of new ligands that are able to bind to VWF. Consistent with these data, recent studies have further suggested a series of novel and non-hemostatic biological functions for VWF. These include potential roles for VWF in regulating inflammation, wound healing, angiogenesis and tumor cell metastasis. Further research in the coming years will be required to determine the clinical significance of these non-hemostatic roles of VWF. Defining the molecular mechanisms involved may offer exciting opportunities to develop novel anti-VWF targeted treatment approaches for important unmet clinical needs.

Introduction

Von Willebrand factor (VWF) is a complex multimeric plasma glycoprotein that plays critical roles in normal hemostasis.[1,2] First, VWF binds to exposed collagen at sites of vascular injury and then tethers platelets to the site of injury. Second, VWF also serves as a chaperone protein for factor VIII (FVIII).[3,4] Studies over many years have demonstrated that quantitative and/or qualitative reductions in plasma VWF activity result in von Willebrand disease (VWD).[1,5] Conversely, elevated plasma VWF-FVIII complex levels are a risk factor for both venous and arterial thrombosis.[6,7] In this manuscript, we review our current understanding of the biosynthesis and structure of VWF. In addition, we further consider the mechanisms through which this multimeric glycoprotein can play such a critical role in normal hemostasis. Finally, we discuss accumulating recent evidence that VWF may have novel biological functions extending beyond coagulation.

Structure of von Willebrand factor

The *VWF* gene is localized to chromosome 12 and contains 52 exons that span approximately 178 kb with exon size varying between 40 bp and 1.4 kb for exon 28.[8] The primary translation product is a 2,813 amino acid protein. The N-terminal portion of VWF includes a hydrophobic 22-amino acid signal peptide followed by a 741-amino acid propeptide (VWFpp) and the 2,050-amino acid mature VWF protein (Figure 1A). During biosynthesis, the propeptide is removed from the mature VWF protein proteolytically by the enzyme furin (Figure 1A). The VWF protein was historically reported to contain a series of homologous A, B, C and D domains with the propeptide containing two D domains (D1 and D2) and the mature VWF protein comprised of D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK domains. More recently, Zhou and colleagues have updated the VWF domain structure to assign specific nodules, relating these nodules to structure using electron microscopy (Figure 1B).[9]

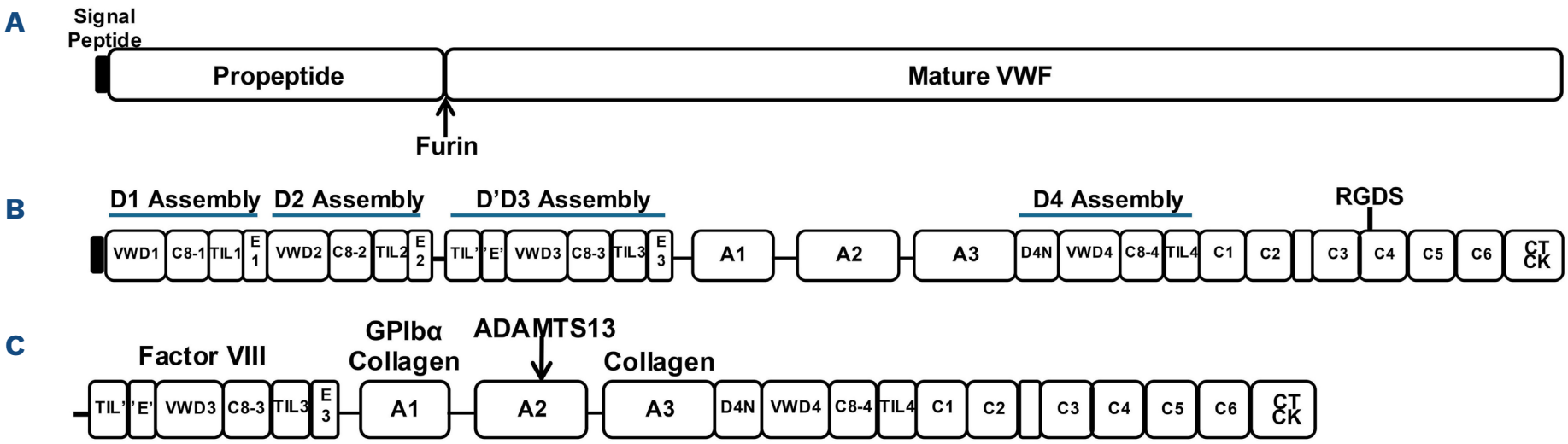

Figure 1. Domain structure of von Willebrand factor. (A) Von Willebrand factor (VWF) is synthesized as pre-pro-VWF containing a 22 amino acid (aa) signal peptide, a 741 aa propeptide (VWFpp) and the 2,050 aa mature VWF protein. (B) VWF domain structure. VWFpp consisting of D1 and D2 domain assemblies. Mature VWF comprises the remainder. (C) Sites within VWF for binding to factor VIII, platelet GPIbα, and collagens. The A2 domain contains the cleavage site of ADAMTS13 (A Disintegrin and Metalloproteinase with ThromboSpondin type-1 repeats 13).

The D domains have been updated to assemblies of small nodules and the B and C domains have been re-annotated as six tandem von Willebrand C (VWC) domains and VWC-like domains. Mature VWF binds to multiple proteins and these binding sites have been mapped to specific VWF domains (Figure 1C).[10]

Biosynthesis of von Willebrand factor

VWF is synthesized exclusively in endothelial cells and megakaryocytes where it undergoes a complex sequence of processing.[11,12] Much of the information regarding VWF synthesis and intracellular processing has been gained through studies of cultured endothelial cells or transfected mammalian cells and fewer studies of megakaryocytes/platelets.[13-20] VWF is synthesized as pre-pro-VWF containing a signal peptide, propeptide and mature VWF protein. In the endoplasmic reticulum (ER), the signal peptide is removed, pro-VWF protein is folded, and disulfide bonds are formed (Figure 2). VWF is rich in cysteine residues with 64 cysteines in VWFpp and 170 cysteines in mature VWF. With 170 cysteines in mature VWF, folding and disulfide formation is a complicated process. Although older reports indicated no free cysteines in the mature VWF protein, more recent data acquired using more sensitive techniques suggest that there may be some unpaired cysteines.[21,22] The mapping of disulfide bonds has been resolved for many of the disulfide bonds; however, many others remain unresolved.[9,23]

In the ER, the pro-VWF proteins form carboxyl-terminal dimers. The large VWFpp is not necessary for dimerization as expression of a propeptide-deleted mature VWF results in a dimeric VWF protein secreted.[17] The pro-VWF molecule is extensively modified in the ER by addition of N-linked glycans (Figure 2). The VWFpp contains four N-linked glycosylation sites and the mature VWF protein contains 13 N-linked sites.[24] Together, the N- and O-linked glycans account for about 20% of the total VWF protein mass. Glycomic analyses have defined approximately 100 distinct N-glycan compositions, including ABO blood group antigens, and a variety of structural features.[24] VWF dimerization, glycosylation and proper protein folding are required for successful exit of VWF from the ER.[25,26] Misfolded proteins are selected in the ER for degradation, and defective processing in the ER may contribute to the VWD phenotype in patients.

Within the Golgi, O-linked glycans are added, glycans are trimmed and galactose and sialic acid are added to form complex carbohydrates (Figure 2). Canis and colleagues identified 18 distinct O-glycan structures including both core 1 and core 2 structures, as well as unusual tetra-sialylated core 1 O-glycans and ABH antigen-containing core 2 glycans.[27] Importantly, the complex N- and O-glycans on VWF have been shown to influence multiple aspects of the glycoprotein's lifecycle including biosynthesis, susceptibility to proteolysis and clearance.[28-30] The large VWFpp is proteolytically removed from mature VWF in the Golgi by the enzyme furin, a Golgi-resident protein. The site of VWFpp cleavage is targeted by the sequence motif Arg-Xxx-Arg/Lys-Arg at the carboxyl-terminal end of VWFpp.[31] The cleaved VWFpp remains non-covalently associated with mature VWF until secretion of both proteins from the cell. In the Golgi, the VWF dimers form very large amino terminal-linked multimers (Figure 2) that can exceed 20 million Da in size.[32] VWF dimerization and multimerization have been shown to be two independent processes.[20] While the ER is the most probable site for disulfide bond formation due to the neutral pH and presence of oxidoreductase enzymes such as protein disulfide isomerase, the acidic Golgi that lacks oxidoreductases is an unlikely environment for disulfide bond formation. In addition, ER-to-Golgi transport vesicles are likely unable to accommodate the very large VWF multimers. VWF has a unique mechanism to

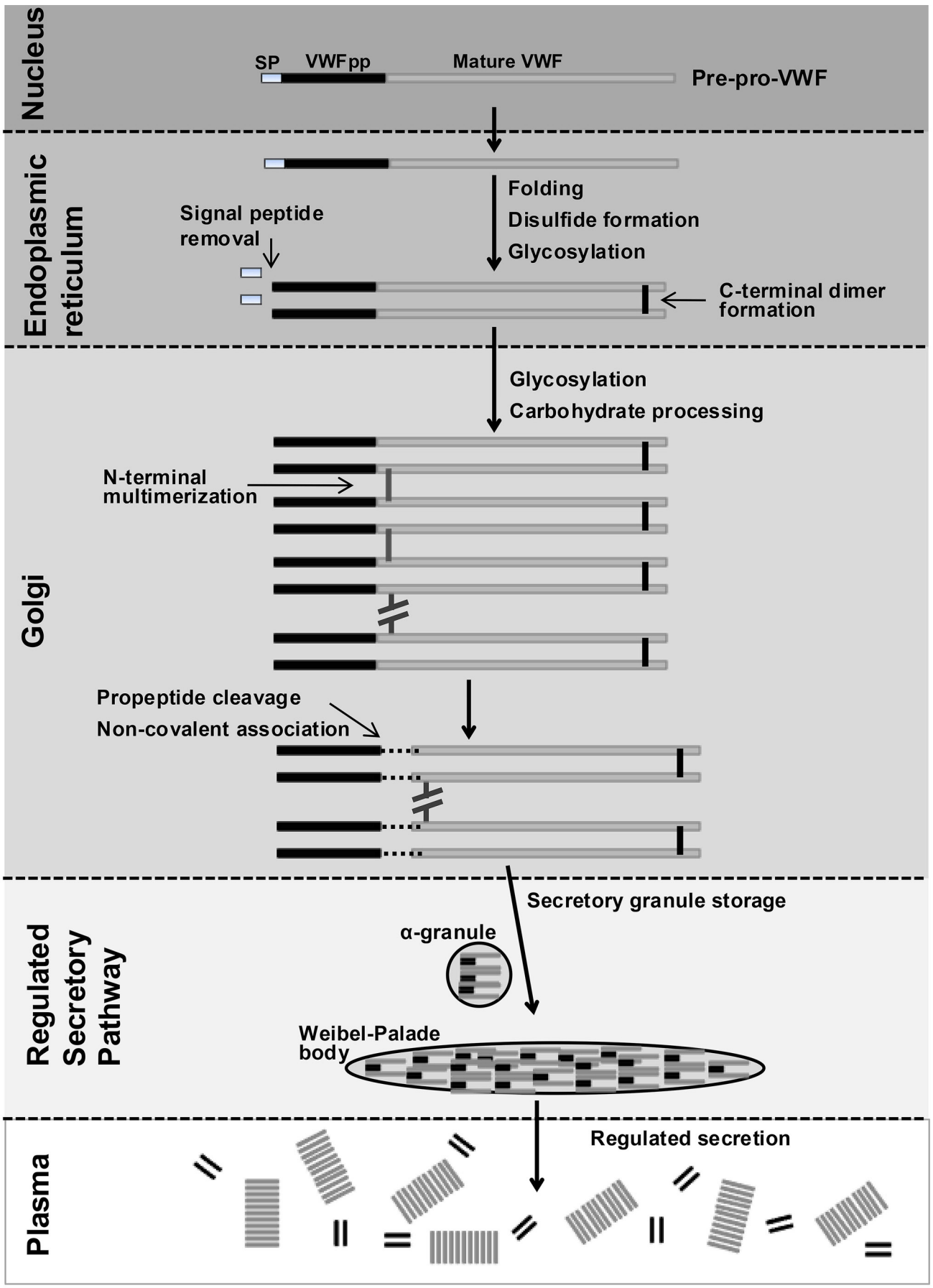

Figure 2. Intracellular processing of von Willebrand factor. In the endoplasmic reticulum, the von Willebrand factor (VWF) signal peptide (SP) is removed, VWF is folded, disulfide-bonded, glycosylated and forms C-terminal dimers. In the Golgi, further glycosylation and processing occurs, dimers are assembled into N-terminal multimers, and VWF-propeptide (VWFpp) is cleaved but remains non-covalently associated. From the Golgi, VWF is trafficked to regulated secretory granules, α-granules in platelets and Weibel-Palade bodies in endothelial cells. Once secreted into plasma, VWF and VWFpp disassociate and circulate independently.

facilitate multimerization in the Golgi by utilizing VWFpp.[21] This role for VWFpp in multimerization of VWF has been intensely studied and reported in numerous studies. Both the D1 and D2 domains in VWFpp contain vicinal cysteine motifs (CXXC sequences) which are similar to those found in disulfide isomerases that participate in multimerization by catalyzing disulfide bond exchange.[18] Thus, the VWFpp functions as an oxidoreductase to facilitate multimerization of VWF in the Golgi.[33] VWFpp remains non-covalently associated with mature VWF multimers when they leave Golgi and are both stored for regulated release (Figure 2) in endothelial cell Weibel-Palade bodies (WPB) or platelet α-granules.[14,17]

Von Willebrand factor secretory pathways

Regulated secretion allows for the swift release of VWF at the site of vascular injury. The WPB is a rod-shaped organelle up to 2 μm in width and up to 4 μm in length.[34] The platelet α-granule appears to be somewhat like a

WPB in terms of morphology.[35] These storage granules contain some of the highest molecular weight VWF multimers, which are the most active for binding to platelets or subendothelial collagen. The mechanisms involved in the trafficking of VWF to regulated storage granules have been extensively studied utilizing cultured endothelial cells and transfected mammalian cells.[17,19,36,37] Several studies have shown that VWFpp is actively involved in the trafficking of VWF to regulated storage.[17,19,36] VWFpp alone traffics to WPB and studies have shown that VWFpp contains the necessary signal (sequence or conformation) for navigating the regulated storage pathway, and co-traffics mature VWF multimers through a non-covalent association.[16,33] VWF multimer formation is not a prerequisite for VWF storage in WPB, although multimerization is accomplished prior to regulated storage.[38,39] Several VWF variants with abnormal multimer structure are stored in WPB and undergo regulated release.[39,40] Although VWFpp plays an active role in VWF multimerization and regulated storage, the regions within VWFpp for each of these processes appear to be independent of one another.[16] In addition, WPB biogenesis appears to be a VWF-driven event. In the absence of VWF, such as in VWF-deficient mice and dogs, endothelial cell WPB are absent.[15,41] After expression of VWF and VWFpp in endothelial cells harvested from VWF-deficient dogs, VWF-containing granules with classic WPB morphology were observed.[15] More recently, tubule assembly such as found in WPB was observed *in vitro* following incubation of recombinant VWFpp and the D'-D3 domain of VWF, with the process being dependent on acidic pH and the presence of calcium.[42] These studies have delineated the requirement for WPB biogenesis to the D1-D3 domains of VWF.

VWF is released from WPB following endothelial cell exposure to various stimuli including epinephrine, thrombin, histamine and desmopressin.[43] These secretagogues trigger endothelial cells to release VWF as well as other WPB proteins such as P-selectin, CD63, and interleukin 8.[38,44,45] In VWD patients, the most common treatment is desmopressin, which results in the release of VWF. VWF-containing WPB are translocated from the cytoplasm to the plasma membrane, followed by fusion and release into the circulation. Once secreted from the cell, VWF and VWFpp circulate independently of one another (Figure 2). VWF and VWFpp are cleared from plasma with half-lives of approximately 12 hours and 2-3 hours, respectively.[46]

Roles of von Willebrand factor in hemostasis

Multimeric VWF plays a critical role in primary hemostasis at sites of vascular injury (Figure 3).[2,47] Following vessel damage, circulating VWF binds to exposed collagen in the subendothelial matrix. Local shear stress causes unwinding of globular inactive VWF multimers into an active elongated conformation.[47] Previous studies have demonstrated that VWF binding to platelets involves two primary sites of interaction. First, the VWF-A1 domain (residues Tyr[1271]-Asp[1459]) binds to the platelet-receptor glycoprotein 1bA (GP1bα). Second, an RGD sequence located in the VWF-C4 domain interacts with the αIIbβIII platelet integrin receptor. Consequently, tethered VWF at sites of injury can promote platelet adhesion and aggregation, ultimately leading to platelet plug formation. In addition, local endothelial cell activation leads to WPB exocytosis of stored high molecular weight multimers of VWF (HMWM-VWF). Under shear conditions and in the absence of effective proteolysis by ADAMTS13 (A Disintegrin and Metalloproteinase with ThromboSpondin type-1 repeats 13), ultra-large VWF strings may be formed and remain tethered to the surface of activated endothelial cells via several ligands including the integrin αvβ3 and P-selectin.[48,49] Although the physiological and pathological significance of these VWF strings remains poorly understood, studies have demonstrated that that they can effectively recruit platelets and other cell types to the endothelial cell surface.[50]

In addition to plasma and endothelial cell-derived VWF, activation of platelets at the site of injury leads to α-granule secretion and consequently high local concentrations of platelet-derived HMWM-VWF.[51,52] This platelet-derived VWF is partially resistant to ADAMTS13 proteolysis since it has significantly altered N-terminal glycosylation compared to endothelial cell-derived VWF.[53] Consequently, platelet-derived VWF also contributes to primary hemostasis under shear conditions.[51,52] Finally, previous studies have shown that endothelial cells also secrete VWF directly into the subendothelial matrix. However, it remains unclear whether this extravascular VWF has any biological role in regulating hemostasis.

Interaction of von Willebrand factor with GP1bα

Under steady state conditions in normal plasma, VWF and platelet GP1bα do not interact. Recent studies by Li and colleagues have identified the mechanism underlying this quiescence.[54-56] They demonstrated that N-terminal (Gln[1238]-His[1268]) and C-terminal (Leu[1460]-Asp[1472]) flanking peptides located either side of the VWF-A1 domain interact to form a so-called autoinhibitory module (AIM).[54,56] This AIM serves to prevent binding of GP1bα to the VWF-A1 domain under static conditions.[56] However, in the presence of shear stress, hydrodynamic forces cause dissociation of the two AIM peptides, leading to VWF-A1 domain activation and thereby enabling an interaction with GP1bα.[55] Thus, conformational changes and VWF multimeric size both play key roles in regulating VWF hemostatic activity.[47,57] In the absence of shear stress, ristocetin can be used as a surrogate to induce VWF interaction with platelet GP1b.[58] Consequently, the VWF ristocetin cofactor activity assay (VWF:RCo) has been used for many years as a gold standard test of VWF function However, recent studies

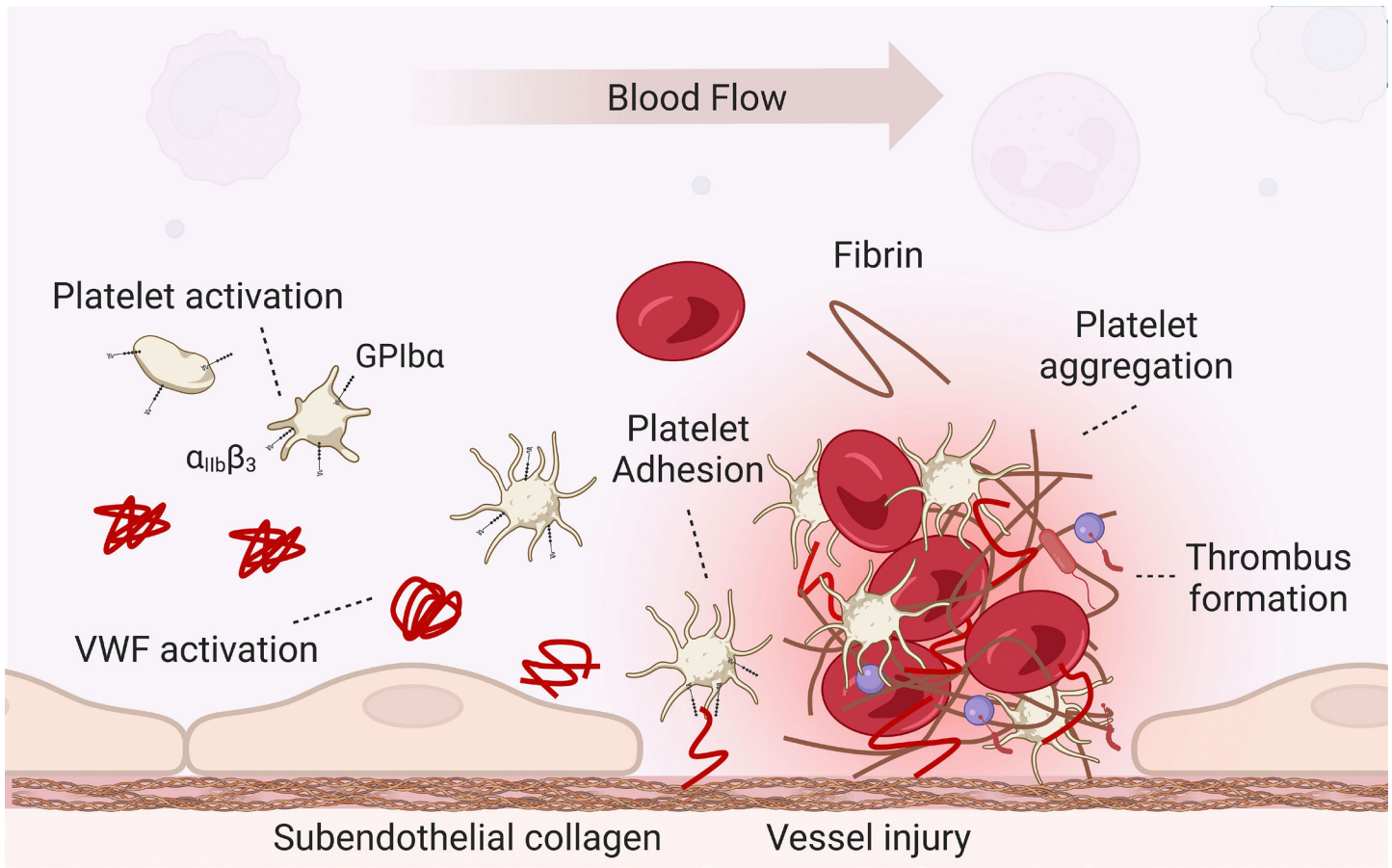

Figure 3. Hemostatic function of von Willebrand factor. Tethered multimeric von Willebrand factor (VWF) at sites of vascular damage is unwound by shear stress such that the binding site for platelet GP1bα within the VWF-A1 domain becomes accessible. This facilitates platelet recruitment, followed by platelet activation and aggregation.

have demonstrated that VWF-A1 domain polymorphisms (notably D1472H) can significantly impair VWF binding to ristocetin.[59] Although D1472H may thus cause significantly reduced VWF:RCo levels, it is not associated with any reduction in VWF functional activity *in vivo*, or a bleeding tendency. These findings are clinically important because D1472H is common in the general population, particularly in African-American individuals.[59]

Interaction of von Willebrand factor with αIIbβIII

Platelet adhesion to VWF triggers platelet activation and intracellular signaling which leads to a conformational change in the αIIbβIII integrin.[29,60] The activated integrin is then able to interact with fibrinogen to enable platelet-platelet interaction and aggregation. The VWF-C4 domain contains an Arg-Gly-Asp-Ser (RGDS) motif (residues 2,507-2,510) that can also interact with αIIbβIII to support fibrinogen-independent platelet aggregation.[61,62] In contrast to the GP1bα interaction, VWF can efficiently bind αIIbβIII under static conditions and VWF multimer size does not appear to affect the interaction.[47]

Interaction of von Willebrand factor with collagen

For VWF to efficiently mediate platelet recruitment at sites of vascular injury, it must bind to exposed subendothelial collagen. Previous studies have demonstrated that two distinct VWF regions participate in binding, depending upon the type of collagen. Thus, the VWF-A3 domain mediates binding to types I and III collagen,[63,64] whereas the VWF-A1 domain interacts with types IV and VI collagen.[65,66] Like GP1bα, HMWM-VWF demonstrates significantly enhanced binding to collagen.[67] However, in striking contrast to GP1bα, VWF can interact with collagen under static conditions, suggesting that the collagen binding sites are constitutively exposed.[47]

Interaction of von Willebrand factor with factor VIII

For more than 40 years it has been recognized that VWF functions as a necessary chaperone protein for FVIII.[3] Studies have demonstrated that VWF binds to FVIII with high affinity (dissociation constant [K_d] of approximately 0.5 nM).[68,69] Under normal conditions, an estimated 95% of FVIII circulates bound to VWF.[3,68,69] Interaction with VWF plays a key role in protecting FVIII from proteolysis and premature clearance.[4] Consequently, in patients with type 3 VWD, absence of VWF binding is associated with a marked reduction in FVIII half-life *in vivo* (from approximately 12 hours to 2 hours). Recent electron microscopy studies have provided important insights into the molecular mechanisms through which VWF interacts with FVIII, demonstrating that the FVIII light chain (a3-A3-C1-C2) interacts with the N-terminal D'D3 domain regions of VWF.[70,71] These studies have highlighted that the FVIII C1-C2 domains interact with the VWF-D3 region, and the FVIII a3-A3 domains bind to the VWF-D' region.[70,71] Consistent with these structural data, expression of a dimeric truncated VWF-D'D3 fragment (spanning residues 764-1,035) has been shown to be sufficient to

normalize plasma FVIII levels in a VWF-deficient murine model.[72] Current evidence suggests that FVIII binding is not influenced by either VWF multimer distribution or shear stress.[47]

Interaction of von Willebrand factor with ADAMTS13

The multimer distribution of VWF is a key determinant of the glycoprotein's functional activity, since HMWM-VWF binds to both collagen and platelet GPIbα with increased affinity compared to low molecular weight multimers.[47] In normal plasma, VWF multimer distribution is tightly regulated by ADAMTS13, which interacts with VWF following secretion from endothelial cells. Recent crystal studies have provided novel insights into the mechanisms through which ADAMTS13 exosites interact with different VWF domains to enable specific cleavage at Tyr^{1605}-Met^{1606} within the VWF-A2 domain.[73] Importantly, VWF proteolysis by ADAMTS13 is shear- and multimer-dependent in nature.[47,73] Several other VWF-binding ligands have been shown to modulate the factor's susceptibility to ADAMTS13 proteolysis.[10] For example, FVIII binding to the D'D3 region and GPIbα binding to the A1 domain both significantly enhance VWF-A2 domain cleavage by ADAMTS13.[73] In contrast, binding of platelet factor 4 and thrombospondin 1 inhibit VWF proteolysis by ADAMTS13.[74,75] ADAMTS13-mediated regulation of VWF multimer distribution is essential for normal hemostasis. Enhanced proteolysis and loss of HMWM-VWF in patients with type 2A VWD results in a significant bleeding phenotype.[1,5] Conversely, inherited or acquired ADAMTS13 deficiency causes accumulation of pathological ultra-large VWF multimers and the formation of platelet-rich thrombi in patients with thrombotic thrombocytopenic purpura.[76] Furthermore, reduced ADAMTS13 levels have been associated with an increased risk of myocardial infarction and may be important in the pathogenesis of other microangiopathies including cerebral malaria and coronavirus disease 2019.[7,77,78]

Other ligand interactions that influence von Willebrand factor activity

Several additional binding ligands have been shown to influence VWF biosynthesis, proteolysis and clearance.[10] For example, during post-translational modification in endothelial cells, VWF interacts with chaperone binding proteins including binding-immunoglobulin protein and protein disulfide isomerase.[79] Importantly, a series of cell surface receptors have been identified that bind to plasma VWF and regulate its clearance *in vivo*. These include C-type lectin receptors such as the asialo-glycoprotein,[80] macrophage galactose-type lectin,[81] and C-type lectin domain family 4 member M.[82] In addition, several scavenger-type receptors also contribute to VWF clearance, including the low-density lipoprotein receptor-related protein (LRP)-1,[83] scavenger receptor class A member I,[84] and stabilin-2.[85]

Non-hemostatic biological roles of von Willebrand factor

Studies over the past decade have shown that an array of more than 50 different binding ligands can interact with VWF.[10] Furthermore, a series of non-hemostatic biological functions for VWF have also been described, including roles in inflammation and angiogenesis and tumor cell metastasis (Figure 4).[10] Emerging evidence suggests that these non-hemostatic functions of VWF may be of physiological and/or pathological significance.

Von Willebrand factor and inflammation

Previous studies have described that plasma VWF antigen (VWF:Ag) and VWFpp levels can be useful as biomarkers of endothelial cell activation and disease severity in patients with a range of inflammatory conditions including various forms of sepsis.[86-88] However, VWF has also been shown to bind directly to immunoregulatory cells and play roles in promoting pro-inflammatory responses (Figure 4). For example, VWF has been shown to bind to polymorphonuclear leukocytes (PMN).[89] Under shear conditions, initial rolling of PMN is mediated by interaction of the VWF-A1 domain with P-selectin glycoprotein ligand-1 on the leukocyte surface. Subsequently, stable adhesion is mediated by binding of the VWF-D'D3 and VWF-A1A3 domains to β2-integrin receptors on leukocytes.[89] In addition to binding to PMN, studies in murine models have demonstrated that VWF also influences vascular permeability and thereby regulates leukocyte extravasation at sites of inflammation.[90] In the absence of VWF, significantly reduced accumulation of *in vivo* PMN accumulation was observed in models of thioglycollate-induced peritonitis, immune-complex-mediated vasculitis and irritative contact dermatitis.[90,91]

Besides PMN, recent data have shown that VWF also binds to macrophages under both static and shear conditions (Figure 4).[92-94] Several different macrophage surface lectin and scavenger receptors can interact with different domains of VWF.[95] These interactions play a key role in regulating the physiological clearance of plasma VWF. Importantly, however, recent studies have highlighted that VWF binding to macrophages can trigger significant pro-inflammatory intracellular signaling pathways, leading to activation of mitogen-activated protein kinase p38 (MAPK p38) and nuclear factor-κB.[94] As a result, VWF binding causes macrophages to develop a pro-inflammatory M1 phenotype with enhanced glycolysis and upregulated pro-inflammatory cytokine secretion.[94] Collectively, these findings suggest that VWF not only initiates primary hemostasis at sites of vascular damage but also activates tissue-resident macrophages in the vicinity to promote local innate immune responses. Additional roles through which VWF can directly impact inflammation have also been proposed. First, VWF has been shown to bind to dendritic cells, and thus may affect adaptive immune responses.[96] Second, roles for VWF in

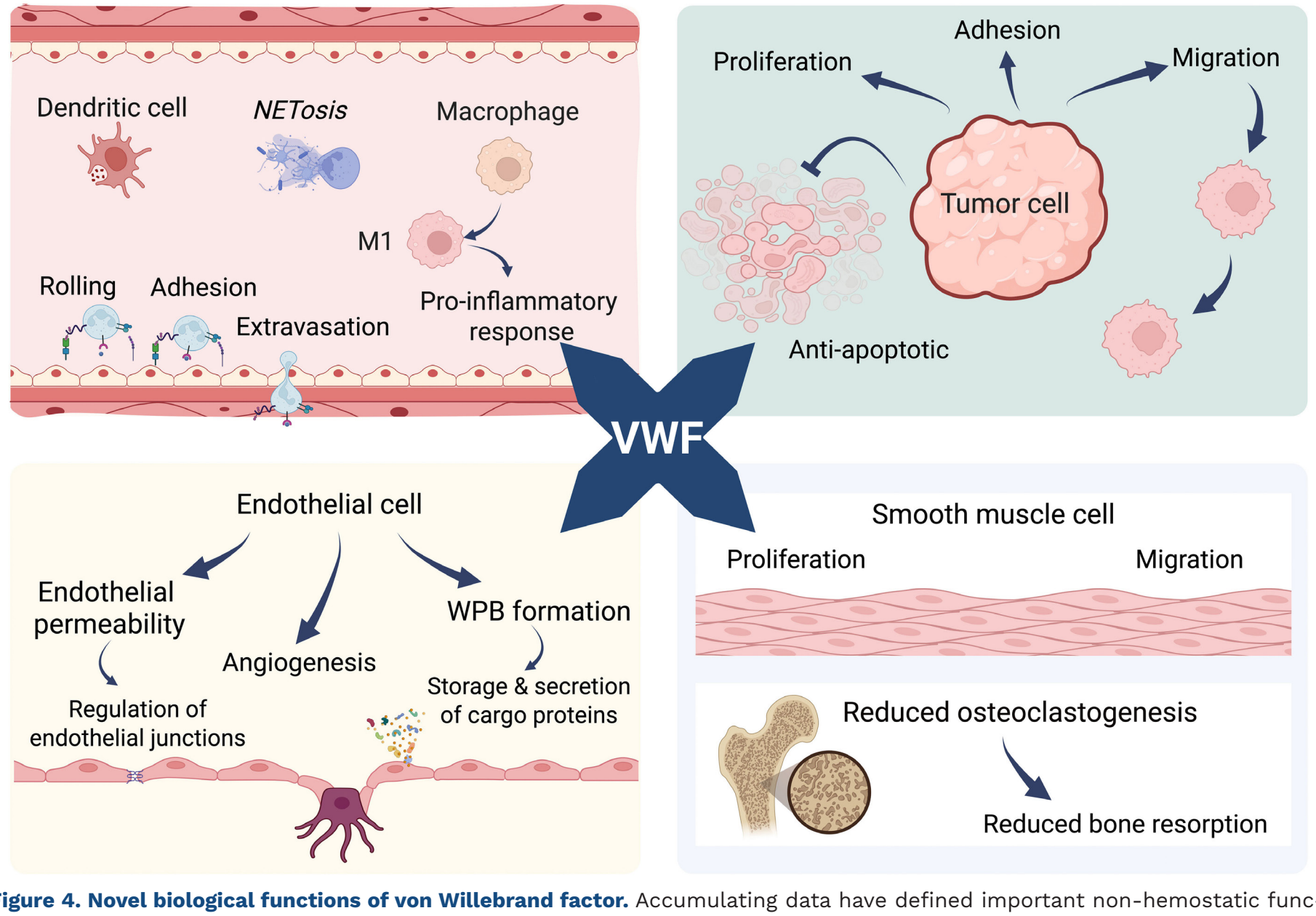

Figure 4. Novel biological functions of von Willebrand factor. Accumulating data have defined important non-hemostatic functions for von Willebrand factor (VWF). With respect to inflammation, VWF can interact with neutrophils to influence their adhesion to endothelial cell surfaces and subsequent extravasation. In addition, VWF can bind to macrophages to trigger pro-inflammatory intracellular signaling. Furthermore, VWF can bind to dendritic cells and influence NETosis. VWF can also: (i) interact with cancer cells to directly influence multiple aspects of tumor biology; (ii) influence angiogenesis through multiple potential pathways; (iii) affect smooth muscle proliferation; and migration and (iv) regulate osteoclast differentiation and bone resorption.

regulating NETosis by binding to histones and extracellular DNA have been described.[97] Finally, VWF has also been reported to bind to various members of the complement family (including C1q, C3, C3b and complement factor H) and to influence complement activation.[98,99]

Von Willebrand factor and angiogenesis

For many years, it has been recognized that gastrointestinal angiodysplasia is common in patients with inherited and acquired VWD, for example in Heyde syndrome associated with aortic stenosis.[100] Interestingly, this angiodysplasia may be more marked in VWD subtypes that are associated with loss of HMWM-VWF.

Consistent with the human data, more recent studies have shown significantly enhanced angiogenesis in VWF-deficient mice.[101] Furthermore, inhibition of VWF expression in endothelial cells using small-interfering RNA led to significantly enhanced angiogenesis *ex vivo*. Cumulatively, these findings suggest that VWF plays a role in regulating angiogenesis.[101,102] Although the biological mechanisms involved have not been fully elucidated, VWF has been shown to bind to both angiopoietin 2 and galectin 3, which are both involved in regulating angiogenesis.[100]

Additional non-hemostatic functions for von Willebrand factor

Beyond inflammation and angiogenesis, additional non-hemostatic functions for VWF have been proposed.[10] For example, recent studies reported a role for VWF in regulating wound healing, with significantly impaired wound healing in VWF-deficient mice.[103] Although further studies will be required to define the underlying mechanisms involved, the VWF-A1 domain has been shown to interact with critical growth factors, including vascular endothelial growth factor-A, fibroblast growth factor-2 and platelet-derived growth factor.[103]

As well as binding to platelets, PMN and macrophages, VWF can also bind to vascular smooth muscle cells (VSMC)

(Figure 4).[104] This interaction is mediated at least in part by VWF interaction with LRP-4 and $\alpha_v\beta_3$ receptors on the VSMC surface. Interestingly, VWF binding has also been shown to initiate intracellular signaling (including activation of MAPK p38) that ultimately leads to VSMC proliferation, enhanced migration and intimal hyperplasia.[104] In addition, a role for VWF in regulating osteoclast differentiation and bone resorption has also been reported.[105] Finally, as in sepsis, elevated plasma VWF levels have been associated with significantly worse outcomes in patients with different types of cancer (e.g., breast, gastric and hematologic malignancies).[106,107] This likely relates in part to a higher risk of cancer-associated thrombosis in patients with markedly increased VWF-FVIII levels. Interestingly, however, recent data have shown that VWF binding to cancer cells can directly influence tumor cell apoptosis, proliferation and metastasis (Figure 4).[107,108]

Conclusions

Since the original publication of Erik von Willebrand back in 1926, we have gained significant insights into the structure of VWF. In particular, we have come to appreciate the unique shear-based regulation of multimeric VWF function and understand how this complex glycoprotein is able to regulate formation of primary hemostasis at sites of vascular injury. However, recent studies have identified an array of other proteins that can bind to VWF and suggested intriguing biological roles for VWF extending well beyond its classical pro-hemostatic function. Further research in the coming years will be required to determine the clinical significance of these novel non-hemostatic roles of VWF. However, defining the underlying molecular mechanisms involved may offer exciting opportunities to develop novel targeted treatment approaches for important unmet clinical needs.

Disclosures

JSO'D has served on speakers' bureau for Baxter, Bayer, Novo Nordisk, Sobi, Boehringer Ingelheim, Leo Pharma, Takeda and Octapharma; has served on the advisory boards of Baxter, Sobi, Bayer, Octapharma, CSL Behring, Daiichi Sankyo, Boehringer Ingelheim, Takeda and Pfizer; and also received research grant funding awards from 3M, Baxter, Bayer, Pfizer, Shire, Takeda and Novo Nordisk. SLH has no conflicts of interest to disclose.

Contributions

SLH and JSO'D designed and wrote the article. Both authors participated in this work, take public responsibility for the content and gave consent to the final version of the article.

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