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Novel functions for Von Willebrand factor

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Abstract:
For many years, it has been known that VWF interacts with FVIII, collagen and platelets. In addition, the key roles played by VWF in regulating normal hemostasis have been well defined. However, accumulating recent evidence has shown that VWF can interact with a diverse array of other novel ligands. To date, more than 60 different binding partners have been described, with interactions mapped to specific VWF domains in some cases. Although the biological significance of these VWF binding interactions has not been fully elucidated, recent studies have identified some of these novel ligands as regulators of various aspects of VWF biology, including biosynthesis, proteolysis and clearance. Conversely, VWF-binding has been shown to directly impact the functional properties for some of its ligands. In keeping with those observations, exciting new roles for VWF in regulating a series of non-hemostatic biological functions has also emerged. These include inflammation, wound healing, angiogenesis, and bone metabolism. Finally, recent evidence supports the hypothesis that the non-hemostatic functions of VWF directly contribute to pathogenic mechanisms in a variety of diverse diseases including sepsis, malaria, sickle cell disease and liver disease. In this manuscript, we review the accumulating data regarding novel ligand interactions for VWF and critically assess how these interactions may impact cellular biology. In addition, we consider the evidence that non-hemostatic VWF functions may contribute to the pathogenesis of human diseases beyond thrombosis and bleeding.

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Novel functions for von Willebrand factor

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KEY POINTS

1. VWF interacts with a diverse array of binding partners.
2. VWF has important roles in regulating non-hemostatic biological functions including inflammation, wound healing and angiogenesis.
3. The non-hemostatic functions of VWF are of direct translational relevance with respect to normal physiology and disease pathogenesis.

ABSTRACT

For many years, it has been known that VWF interacts with FVIII, collagen and platelets. In addition, the key roles played by VWF in regulating normal hemostasis have been well defined. However, accumulating recent evidence has shown that VWF can interact with a diverse array of other novel ligands. To date, more than 60 different binding partners have been described, with interactions mapped to specific VWF domains in some cases. Although the biological significance of these VWF binding interactions has not been fully elucidated, recent studies have identified some of these novel ligands as regulators of various aspects of VWF biology, including biosynthesis, proteolysis and clearance. Conversely, VWF-binding has been shown to directly impact the functional properties for some of its ligands. In keeping with those observations, exciting new roles for VWF in regulating a series of non-hemostatic biological functions has also emerged. These include inflammation, wound healing, angiogenesis, and bone metabolism. Finally, recent evidence supports the hypothesis that the non-hemostatic functions of VWF directly contribute to pathogenic mechanisms in a variety of diverse diseases including sepsis, malaria, sickle cell disease and liver disease. In this manuscript, we review the accumulating data regarding novel ligand interactions for VWF and critically assess how these interactions may impact cellular biology. In addition, we consider the evidence that non-hemostatic VWF functions may contribute to the pathogenesis of human diseases beyond thrombosis and bleeding.

KEYWORDS

Von Willebrand factor; von Willebrand disease; Ligands; Inflammation; Wound healing; Angiogenesis

Introduction

Von Willebrand factor (VWF) is a large multimeric plasma glycoprotein that plays key roles in normal hemostasis by binding to exposed collagen at sites of vascular injury and recruiting platelets to the site of injury.[1] In addition, VWF also acts a carrier molecule for factor VIII (FVIII). Under normal conditions, approximately 95% of plasma FVIII circulates in high affinity complex (Kd ~ 0.2-0.5 nmol/L) with VWF.[2] Quantitative or qualitative reductions in plasma VWF levels result in von Willebrand disease (VWD), which constitutes the commonest inherited bleeding disorder.[3] Conversely, elevated plasma levels of the VWF-FVIII complex represent a dose-dependent risk factor for thrombosis.[4,5] Recent studies have highlighted that VWF is able to interact with a diverse array of other proteins and reported novel biological functions extending beyond coagulation. In this manuscript, we review these data, together with accumulating evidence that non-hemostatic VWF functions may contribute to the pathogenesis of human diseases beyond thrombosis and bleeding.

Ligands that influence VWF life cycle – including biosynthesis, proteolysis and clearance

For many years, VWF was perceived to bind a limited number of ligands important in regulating its hemostatic efficacy. These included (i) a FVIII binding site in the D'D3 region; (ii) binding sites for collagen in the A1 and A3 domains and (iii) binding sites for platelet receptors GPIbα and $\alpha_{IIb}\beta3$ located in the A1 domain and C4 domain respectively.[6-10] However, accumulating data have shown that additional binding-partners play direct roles in the life cycle of VWF (**Fig. 1**). For example, during post-translational modification within endothelial cells (EC), VWF interacts with a series of glycosyltransferases and glycosidases in the ER and Golgi, as well as chaperone binding proteins (e.g. binding-immunoglobulin protein, BiP) and the protein disulfide isomerase PDIA1 (**Fig. 1A**).[11] Importantly, the VWF propeptide (VWFpp) binds to the N-terminal D'D3 domains and regulates VWF multimerization and trafficking into Weibel Palade Bodies (WPB) (**Fig. 1A**).[12] VWFpp is cleaved from mature VWF by furin and stored within WPB (**Fig. 1A**). Following EC activation,

VWFpp is secreted in equimolar amounts to VWF but has a much shorter plasma half-life (approximately 2-3 hours). Although the VWFpp has no defined extracellular function, some VWFpp dimers associate with the D'D3 domain of mature VWF in a non-covalent manner.[13] Finally, following WPB exocytosis, elongated VWF strings can be tethered on the surface of activated ECs by a number of reported ligands including the integrin $\alpha_v\beta3$, P-selectin and syndecan-1 (SDC-1)-linked heparan sulfate (HS) (**Fig. 1A**).[14-16]

Following secretion from EC, VWF interacts with ADAMTS13 (A Disintegrin And Metalloproteinase with Thrombospondin type-1 repeats) which cleaves at Tyr1605-Met1606 within the A2 domain (**Fig. 1B**).[17] Recent crystal studies have provided insights into the mechanisms through which ADAMTS13 exosites interact with VWF to enable specific A2 domain cleavage in a shear-dependent manner.[17] Several other VWF-binding ligands influence susceptibility to ADAMTS13 proteolysis (**Fig. 1B**). FVIII binding to the D'D3 region and GPIbα binding to the A1 domain significantly enhance VWF A2 domain cleavage by ADAMTS13.[17] Conversely, binding of platelet factor 4 (PF4), human neutrophile peptide (HNP), Complement factor H, hemoglobin and thrombospondin 1 (TSP1) all attenuate VWF proteolysis by ADAMTS13 (**Fig. 1B**).[18-22] Finally, other VWF binding ligands have been implicated in reducing multimers, including plasmin which cleaves in the A1-A2 linker region (**Fig. 1B**).[23]

Recent studies have identified cell surface receptors that bind to plasma VWF and regulate its clearance (**Fig. 1C**). C-type lectin receptors shown to bind VWF glycans include asialo-glycoprotein (ASGPR) expressed predominantly on hepatocytes, macrophage galactose-type lectin (MGL), and C-type Lectin Domain Family 4 Member M (CLEC4M) which is expressed on liver sinusoidal endothelial cells (LSECs) (**Fig. 1C**).[24-26] Scavenger receptors have also been reported to interact with VWF and contribute to its clearance. These include the low-density lipoprotein receptor-related protein-1 (LRP1), scavenger receptor class A member I (SR-A1), Stabilin-2 (STAB2) and Scavenger receptor class A member (SCARA5) (**Fig. 1C**).[27-30] The specific VWF domains involved in modulating interaction with individual clearance receptors have not been fully defined (**Fig. 2**). In addition, in contrast to the ability

of VWF binding partners to modulate susceptibility to ADAMTS13 proteolysis, it remains unclear whether any ligand binding interactions impact VWF clearance in vivo.

Novel ligands for VWF

Recent studies have described a diverse array of novel binding partners for VWF (**Fig. 2**). Some of these ligands have been shown to bind during VWF biosynthesis within ECs. For example, the VWF A1 domain has been shown to mediate binding to angiopoietin-2 (Angpt-2) and osteoprotegerin (OPG).[31-33] Both Angpt-2 and OPG are co-trafficked with VWF into WPB stores. P-selectin and FVIII both bind to the D'D3 domains of VWF and are also recruited into WPBs.[6,34] More recently it has been elucidated that Complement factor H, insulin-like growth factor binding protein 7 (IGFBP7) and interleukin-8 (IL8) can also bind VWF and be recruited into WPBs.[21,35,36] Although Angpt-2 and OPG first bind to VWF during its biosynthesis within EC, these interactions remain intact following VWF secretion into the plasma. Mobayen *et al* recently reported that approximately 70% of plasma Angpt-2 is circulating in high affinity complex with VWF.[33] Importantly with respect to function, Angpt-2 binding to the VWF A1 domain did not alter platelet capture function.[33] Similarly, OPG has been shown to remain attached to VWF strings following WPB exocytosis,[31] and circulate in complex with VWF in normal human plasma.[37] In contrast to Angpt-2, OPG binding to the VWF A1 domain significantly attenuated platelet adhesion to VWF strings tethered on the surface of activated EC.[38]

VWF is heavily glycosylated with 12 N-linked (NLG) and 10 O-linked glycans (OLG) on each monomer.[39] Previous studies have demonstrated that the NLG of VWF interact with galectin-1 (Gal-1) and galectin-3 (Gal-3) (**Fig. 2**).[40] VWF co-localized with galectins in EC and remained attached following WPB exocytosis.[40] Similar to OPG, VWF-platelet string formation was reduced in the presence of galectin binding. Also, thrombus formation in a ferric chloride-induced injury model was significantly more rapid in Gal-1/Gal-3 double-deficient mice.[40]

Besides the ligands that engage with VWF within EC, other ligands encounter VWF for the first time after it has been secreted into the plasma or extravascular vessel wall. An array of binding partners has been reported, including fibronectin, myosin, complement factors, histones, poly P, vimentin, thrombospondin 1 (TSP1) and multiple growth factors (**Fig. 2**). It is important to emphasize that the strength of the biochemical evidence supporting the interaction of VWF with these individual binding partners varies considerably (**Suppl. Table 1**). For some ligands, specific VWF domains have been implicated in binding (**Suppl. Table 1**). Other ligands, notably putative clearance receptors, have been shown to interact with multiple discrete VWF domains (**Fig. 2**). To date, the relative importance of these different VWF binding sites in regulating clearance has not been elucidated. Nevertheless, specific *VWF* missense variants have been shown to promote enhanced clearance in vivo. Some VWF-binding partners have been investigated using both in vivo and in vitro studies (**Suppl. Table 1**). Conversely, other partner interactions have only been assessed using in vitro studies, so it remains unclear whether these ligands will bind VWF in the presence of other abundant plasma glycoproteins such as albumin or immunoglobulin. In addition, binding affinities have been determined for only a minority of putative VWF ligands. Finally, some reported VWF-binding partners have yet to validated by studies performed in other independent laboratories (**Suppl. Table 1**).

Interaction of VWF with structurally diverse binding ligands

Notwithstanding issues that need to be addressed in future studies, it is intriguing that VWF has the potential to interact with such a wide variety of structurally diverse binding partners. This is unusual compared to other coagulation factors but well established for other plasma glycoproteins such as albumin which displays marked ligand heterogeneity.[41] Binding promiscuity may reflect the fact that VWF is a large adhesive molecule present in plasma at higher concentrations than most other coagulation proteins. Each VWF monomer is also highly glycosylated, with N- and O-linked glycans together constituting approximately 20% of the total monomeric mass. Mass spectroscopy studies have shown that many different VWF

glycoforms may be present in a given individual at any specific time. Although the complex N-glycans on VWF have been implicated in limiting some ligand interactions through steric hindrance, these carbohydrates can also mediate binding to ligands that possess carbohydrate-recognition domains.[42]

The unusual array of VWF binding partners is at least in part attributable to the fact that it circulates in normal plasma as a series of heterogeneous multimers that may contain between 40 and 100 monomers. This unusual property facilitates clustering of ligand binding sites in multimeric VWF. The importance of VWF multimer size has been studied for a limited number of ligands but can influence some binding interactions (e.g., GPIbα, collagen and complement C3b). Finally, recent studies have demonstrated that VWF also has the potential to circulate in a range of different allosteric conformational variants determined by variability in intramolecular disulfide bond formation.[43] This serves to further increase the number of VWF epitopes that may be available for potential ligand interactions.

Another intriguing observation is that so many VWF-binding partners appear to target the A1 domain (**Fig. 2**). To date, it remains unclear whether there can be A1 occupancy with different ligand combinations, or how these binding partners may compete for adjacent binding sites within A1. However, it has been shown that some A1 domain ligands (e.g. galectins and OPG) can inhibit interaction with platelet GPIbα. Further studies will be required to define why the A1 domain is so important and to exclude the possibility that some of these reported interactions may be artefactual in nature due to the presence of ristocetin. Nevertheless, the A1 domain does have several important features. First, the domain is flanked by two O-linked glycan clusters which have been shown to influence conformation of the A1 domain and regulate interaction with specific ligands (e.g., GPIbα and MGL). Second, the N- and C-terminal flanking regions of the A1 domain have been shown to form an autoinhibitory module (AIM). This means that A1 can adopt different active or inactive conformational states. Consequently, the precise A1 construct (particularly the N and C-termini limits) utilized in binding studies has the potential to impact interactions.

VWF – novel biological functions beyond hemostasis.

Together with the identification of diverse binding ligands, an array of novel biological functions for VWF beyond hemostasis have also been proposed (**Fig. 3**). The evidence supporting roles for VWF in these processes is undoubtedly stronger for some than others. In addition, interpretation of results from experiments performed in *VWF*$^{-/-}$ mice needs to be considered carefully since these animals also lack WPB storage organelles within their EC. Nonetheless, accumulating data suggest that at least some of these novel VWF functions have direct physiological or pathological significance, notably with respect to inflammation, angiogenesis and wound healing.[44-46]

1. Effects of VWF on inflammation

It is well recognized that EC activation triggers exocytosis of VWF antigen (VWF:Ag) and VWFpp stores from WPB.[1] Moreover, plasma VWF:Ag and VWFpp levels have been used as biomarkers of clinical severity in patients with a variety of inflammatory and septic conditions.[47-49] Rather than merely serving as measure of EC activation and damage, more recent studies have demonstrated that VWF plays direct roles in regulating inflammatory responses. Pendu *et al* demonstrated that VWF binds to polymorphonuclear leukocytes (PMNs).[50] Under shear, this VWF-leucocyte interaction consisted of initial transient rolling mediated by VWF-A1 domain binding to P-selectin glycoprotein ligand-1 (PSGL-1) on leucocytes. This was subsequently followed by more stable adhesion mediated by the VWF-D'D3 and A1A3 domains interacting with β2-integrins on leucocytes (**Fig. 3**).[50] In addition, Slc44a2 on neutrophil surfaces has also been shown to interact with the VWF A1 domain.[51] Functional polymorphisms at the *SLC44A2* locus define expression of human neutrophil antigens (HNA-) 3A and 3B respectively. Interestingly, recent studies have shown that VWF interaction is significantly attenuated for HNA-3b.[51] In vivo studies have confirmed that VWF regulates vascular permeability and PMN extravasation into inflamed tissues (**Fig. 3**). In *VWF*$^{-/-}$ mice, or following VWF inhibition in wild type mice, significantly reduced neutrophil recruitment has been observed in different inflammation models including (i) thioglycollate-

induced peritonitis; (ii) keratinocyte-derived chemokine (KC)-stimulated cremaster muscle; (iii) immune-complex-mediated vasculitis and (iv) irritative contact dermatitis.[52,53]

In addition to binding to PMNs, VWF has also been shown to bind to macrophages but not to undifferentiated monocytes.[54] Recent data have highlighted that VWF interaction with specific macrophage receptors (notably LRP1) initiates significant downstream inflammatory signaling (including activation of MAPKinase p38 and NF-κB) that ultimately triggers macrophages to adopt an M1 pro-inflammatory phenotype (**Fig. 3**).[54] Consequently, VWF binding leads to a significant increase in macrophage glycolysis, together with upregulated secretion of pro-inflammatory cytokines and chemokines.[54] Altogether, these data define a novel biological role for VWF in linking primary hemostasis and innate immunity which may be important at sites of vascular injury.

Beyond its effects on PMNs and macrophages, VWF can influence inflammatory responses in other ways. For example, VWF has also been reported to bind to dendritic cells (**Fig. 3**).[55] This raises the potential for VWF to also impact adaptive immune responses. In addition, VWF has also been implicated in NETosis.[56] In particular, the VWF A1 domain can bind directly to both histones and extracellular DNA (**Fig. 2**).[56] Furthermore, the inclusion of VWF in NETs was associated with significantly enhanced leucocyte recruitment.[51] Finally, VWF has also been reported to influence complement activation (**Fig. 3**). Complement factor H binds to the VWF A1 and A2 domains and has been reported to inhibit VWF cleavage by ADAMTS13.[21,57] In addition, VWF also binds to complement C1q, C3 and C3b respectively, and serves as a cofactor for factor I-mediated cleavage of complement C3b.[58,59]

2. Effects of VWF on angiogenesis

A role for VWF in regulating angiogenesis is supported by various lines of evidence. First, angiodysplasia, particularly involving the GI tract, is a recognized complication in patients with both inherited and acquired VWD (e.g., in Heyde's syndrome with aortic stenosis).[60] For reasons that remain poorly defined, angiodysplasia appears to be more marked in VWD subtypes characterized by loss of HMW-VWF multimers.[60] Second, angiogenesis and

vascular density are significantly increased in *VWF*$^{-/-}$ mice.[61] Third, siRNA inhibition of VWF expression in EC ex vivo is associated with enhanced proliferation, migration velocity and angiogenesis.[61] Fourth, abnormal angiogenesis has also been observed in studies of ECFCs derived from VWD patients with specific *VWF* sequence variants and different types of VWD.[61,62] Overall, these data demonstrate that VWF plays a predominantly inhibitory role in regulating angiogenesis through several distinct mechanisms. These include putative roles for partners Angpt-2 and Gal-3 which are both normally trafficked into WPB stores via interaction with VWF.[63] Recent review manuscripts have considered in detail the mechanisms through which VWF may impact angiogenic regulation.[44,64]

3. Effects of VWF on wound healing

Recent studies suggest a role for VWF in promoting wound healing (**Fig. 3**). Ishihara *et al* observed that healing of dermal skin wounds was significantly delayed in *VWF*$^{-/-}$ mice compared to wild type controls.[46] Furthermore, wounds in the *VWF*$^{-/-}$ mice contained reduced levels of vascular endothelial growth factor (VEGF)-A and fibroblast growth factor (FGF)-2 and exhibited attenuated EC and smooth muscle proliferation.[46] Subsequently, the heparin-binding domain (HBD) in the VWF A1 domain (Tyr1328-Ala1350) was shown to bind to important growth factors, including members of the platelet derived growth factor (PDGF), VEGF, FGF and TGF-β families (**Fig. 2**).[46] Furthermore, VEGF-A2 colocalized with VWF in WPB stores and was co-secreted following EC activation. Finally, co-immunoprecipitation experiments showed that VEGF-A and FGF-2 in normal human plasma both circulate in complex with VWF.[46] In contrast to the concept that VWF is predominantly an inhibitor of angiogenesis, there was impaired local angiogenesis at wound sites in *VWF*$^{-/-}$ mice which may contribute to the impaired wound healing observed.[46] Studies in several other animal models of ischemia have also reported pro- and anti-angiogenic roles for VWF, suggesting that these effects may vary under specific conditions and in different tissues.

4. Additional novel cellular interactions and biological functions for VWF

Although the evidence is less developed, studies have suggested additional biological roles for VWF. Like platelets and macrophages, VWF binding to vascular smooth muscle cells (VSMCs) has also been shown to trigger intracellular signaling (**Fig. 3**).[65,66] These cells regulate vasoconstriction, vasodilatation and thereby blood pressure control. In addition, VSMCs also have an important role in atherosclerosis.[67] Lagrange *et al* recently demonstrated that the LRP4 receptor on the surface of VSMCs binds to the VWF A2 domain.[66] This binding triggers signaling through integrin $\alpha_v\beta_3$, leading to activation of p38-MAPK and Src, followed by downstream phosphorylation of Akt and ERK1/2. Furthermore, this VWF signaling through the LRP4/$\alpha_v\beta_3$ complex led to VSMC proliferation, migration and intimal hyperplasia.[66] Interestingly, previous in vivo studies reported significant differences in atheroma development in *VWF*$^{-/-}$ mice compared to wild type controls. Importantly, macrophage recruitment into the atheromatous plaques was attenuated in the *VWF*$^{-/-}$ mice.[68] Cumulatively, these findings raise the intriguing possibility that VWF may contribute to atheroma progression by influencing both macrophage and VSMC biology.[69]

As already discussed, OPG binds to the VWF A1 domain within ECs, is stored within WPB and circulates in plasma in complex with VWF.[31] OPG also has important intrinsic functions in regulating bone metabolism. In particular, OPG functions as a soluble decoy receptor for RANK ligand (RANKL) and thereby inhibits osteoclastogenesis (**Fig. 3**).[70] Interestingly, interaction of VWF-FVIII complex with OPG has been shown to increase binding of OPG to RANKL, which in turn promoted downregulation of osteoclast differentiation.[70,71] Additional studies will be required to determine whether the effect of VWF in regulating osteoclast biology has a clinically relevant effect on bone metabolism in vivo.

Pathogenic roles for VWF beyond hemostasis

In addition to its established roles in the etiology of both bleeding and thrombotic disorders (including VWD, TTP, DVT, PE, MI, and ischemic stroke) (**Fig. 4**).[4,5,72] additional pathogenic roles for VWF in a range of other non-hemostatic diseases has also been proposed (**Fig. 4 &**

Suppl. Table 2). These putative novel pathogenic roles for VWF are supported in some cases by significant data from in vivo studies involving a variety of different murine models. Nevertheless, the level of evidence regarding the involvement of VWF in these pathologies exhibits considerable variability, with certain disorders still in early stages of investigation.

1. VWF in sickle cell disease and malaria

Elevated plasma VWF levels, pathological ultra-large (UL-) VWF multimers and mild decreases in plasma ADAMTS13 levels have been reported in patients with sickle cell disease (SCD).[48,73] In addition, VWF has been implicated in the pathogenesis of SCD vaso-occlusive crises (VOC) (**Suppl Table 2**).[48,74] In particular, UL-VWF multimers secreted from ECs have been shown to bind and tether sickle erythrocytes under shear conditions. Recent studies in in a murine SCD model demonstrated that *VWF*$^{-/-}$ animals were protected against hemolysis, VOC and organ damage.[74] Moreover, treatment with recombinant ADAMTS13 also significantly reduced hemolysis and organ damage in a humanized SCD murine model.[74] Despite these exciting murine data, the role of the VWF-ADAMTS13 axis in contributing to VOC development in human SCD patients has not yet been defined. For example, there is no clear evidence that ABO blood group (which affects plasma VWF:Ag levels and susceptibility to ADAMTS13 proteolysis) has any effect on SCD severity. In addition, it remains unclear whether the effect of VWF in the murine SCD model is entirely explained via VWF binding to sickle erythrocytes, or whether VWF effects on inflammation may also be important.

Similar to SCD, markedly increased plasma VWF levels and abnormal UL-VWF multimers have also been postulated to contribute to disease pathogenesis in both malaria and acute COVID-19.[47,75] Importantly, platelet decorated VWF strings have been shown to bind to *P. falciparum* infected erythrocytes and thus may contribute to EC cytoadhesion. In particular, the A1 domain of VWF has also been shown to bind to *Plasmodium falciparum* erythrocyte membrane protein 1 (PFEMP-1) on the surface of malaria-infected erythrocytes.[76] In addition, in vivo studies have shown that *VWF*$^{-/-}$ mice are significantly protected in a murine model of

Plasmodium beghei induced experimental cerebral malaria.[47] In contrast to SCD, ABO blood has been shown to significantly regulate malaria severity in human patients.[77]

2. VWF in liver disease

Acute and chronic liver disease are both associated with marked increases in plasma VWF:Ag levels (5-10 fold).[78] In contrast to malaria, SCD or COVID-19, HMWM multimers are typically reduced in patients with liver disease, despite the fact that plasma ADAMTS13 levels are significantly reduced (3-5 fold).[78] This paradox may be due to HMWM consumption in platelet-rich microthrombi within the liver. Alternatively, VWF proteolysis via ADAMTS-13 independent mechanisms may be occurring. Importantly, recent animal studies have demonstrated roles for VWF in contributing to acute and chronic liver pathobiology (**Fig. 4 & Suppl Table 2**).[79,80] Notably, Groeneveld *et al* demonstrated that VWF-platelet aggregates formed in the liver of mice following acetaminophen overdose.[79] In *VWF*$^{-/-}$ mice, these platelet aggregates were cleared more rapidly, which led to accelerated liver repair. Similarly, anti-VWF antibodies also reduced hepatic platelet accumulation in wild type mice, leading to faster liver repair. A direct role for VWF in the pathogenesis of carbon tetrachloride-induced experimental liver fibrosis has also been reported.[80] Once again, *VWF*$^{-/-}$ mice were shown to develop significantly reduced liver fibrosis compared to wild type control animals.[80] Further studies will be needed to elucidate the biological mechanism(s) through which VWF impacts acute and chronic liver disease, and its importance in human patients .

3. VWF and tumor cell biology

Increased plasma VWF levels have been associated with worse outcomes in multiple different types of cancer, including breast, thyroid and gastric malignancies.[45] Increased VWF-FVIII complex levels also likely contribute to cancer-associated thrombosis risk.[45] Interestingly, more recent studies have suggested that VWF may also play roles in regulating specific aspects of tumor cell biology, notably with respect to metastasis, apoptosis and tumor angiogenesis (**Suppl. Table 3**).[45,81] Importantly however, previous in vivo studies

performed in *VWF*$^{-/-}$ mice produced some unexpected results, with significantly enhanced metastasis in the absence of VWF. These findings may relate to the fact that VWF impacts cancer biology differently depending upon the cancer type and stage. The putative mechanisms through which VWF may influence tumor cell biology have recently been reviewed in detail.[82]

Targeting novel functions of VWF as a therapeutic strategy

Recent studies suggest that targeting novel functions of VWF may offer an exciting novel therapeutic approach. For example, a novel A1 domain targeted nanobody significantly reduced leukocyte recruitment and vascular permeability in two different murine models of inflammation (immune complex-complex-mediated vasculitis and irritant contact dermatitis respectively).[53] Collectively, these findings support the concept that anti-VWF targeted therapies may have clinical utilities beyond hemostasis.

It is interesting to consider the potential importance of non-hemostatic roles of VWF in patients with VWD. Angiodysplasia undoubtedly has direct significance with respect to increased risk for GI bleeding, particularly in patients with type 2A VWD and acquired VWD.[60] Although there is limited evidence, it seems likely that non-hemostatic functions of VWF may also be important in the context of heavy menstrual bleeding (HMB). The menstrual cycle is characterized by monthly bleeding, followed by a period of local inflammation and wound repair in the uterus.[83] In turn, this is followed by local angiogenesis which ultimately leads to re-bleeding. Based on our current understanding regarding novel roles for VWF in regulating not only bleeding, but also inflammation, wound repair and angiogenesis, it is evident that VWF has the potential to impact multiple aspects of the menstrual cycle. This '*perfect storm*' may explain the enormous clinical burden associated with HMB, even in women with only mild-to-moderate reductions in plasma VWF levels in the Low VWF range (30-50 IU/dL).[84]

Conclusions

The rapidly expanding list of binding ligands has led to the suggestion that VWF may be considered as a 'shuttle bus' in vivo.[85] Thus, some passengers board the VWF bus at its home terminus (i.e. within EC). Other passengers get onto the VWF bus as it travels along its route (i.e., in the circulation). Passengers may disembark the VWF bus at times and may be replaced by other new passengers. At times, passengers may be involved in competition for seats on the VWF bus. Under specific physiological or pathological conditions, the number of VWF buses may be increased or decreased in number. Furthermore, the VWF bus may be more accessible for specific passenger types under certain conditions (e.g., following A1 domain activation). Finally, the ultimate destination of VWF buses may vary. Under normal condition, most VWF will be cleared from the circulation by macrophages, LSECs and hepatocytes respectively.[86] Alternatively, at times of hemostatic challenge, VWF along with its passengers will instead be recruited to sites of vascular injury. In addition, VWF buses may be directed to additional sites under specific pathological conditions (e.g., to leucocytes at sites of inflammation). Finally, in some circumstances the VWF bus can be hijacked and used by non-human ligands. In particular, different micro-organisms (including *Staphylococcus Aureus, Helicobacter Pylori* and *Schistosoma Mansoni*) have been shown to bind and utilize VWF to facilitate dissemination.[87-89] Clearly, this analogy is a gross simplification but serves to integrate the plethora of novel binding partners for VWF proposed in recent studies. Validating these individual VWF interactions, and elucidating the molecular mechanisms involved, will require significant further research. Nevertheless, disentangling these additional roles of VWF extending beyond its classical pro-hemostatic functions may offer exciting opportunities to develop novel treatment approaches for important unmet clinical needs.

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LEGENDS

Figure 1. Ligands involved in the life cycle of VWF.

(A) Ligands involved in VWF biosynthesis and secretion. VWF trafficking through the endoplasmic reticulum is regulated by interaction with ligands including binding-immunoglobulin protein, BiP). VWF propeptide (VWFpp) plays key roles in regulating VWF multimerization and packing in Weibel Palade bodies (WPB). Furin cleaves VWFpp from mature VWF. VWF strings can be tethered on the surface of activated ECs by ligands including integrin αvβ3, P-selectin and syndecan-1 (SDC-1)-linked heparan sulfate (HS).

(B) Ligands involved in VWF proteolysis. ADAMTS13 and plasmin independently proteolyze VWF. FVIII and GPIbα binding to VWF promote ADAMTS13-mediated proteolysis. Conversely, binding of PF4, Complement factor H, hemoglobin, human neutrophile peptides and TSP1 to VWF all attenuate VWF proteolysis by ADAMTS13.

(C) Ligands involved in regulating VWF clearance. Macrophage receptors implicated in regulating VWF clearance include the low-density lipoprotein receptor-related protein-1 (LRP1), macrophage galactose-type lectin (MGL) and scavenger receptor class A member I (SR-A1). In addition, the asialo-glycoprotein (ASGPR) on hepatocytes, and C-type Lectin Domain Family 4 Member M (CLEC4M) and Stabilin-2 (STAB2) on sinusoidal endothelial cells also contribute to VWF clearance. Finally, Scavenger receptor class A member (SCARA5) on splenic littoral endothelial cells has also been reported to interact with VWF.

Figure 2. A broad spectrum of ligands interacts with VWF through binding sites across all its domains.

VWF has been reported to interact with a wide variety of structurally diverse binding partners. The binding sites for some of these ligands has been localized to specific domains.

FVIII - factor VIII; VWFpp - Von Willebrand factor propeptide; PolyP - Polyphosphate; GPIbα – Glycoprotein Ibα; LRP1 - Low-density lipoprotein receptor-related protein 1; OPG - Osteoprotegerin; β2-GPI - β2 glycoprotein I; CTL-2 - Choline transporter-like protein 2; Slc44a2 - Solute carrier family 44 member 2; PSGL-1 - P-selectin glycoprotein ligand-1;

ADAMTS13 - A Distntegrin and Metalloproteinase with a Thrombospondin Type 1 motif, member 13; PF4 - Platelet factor 4; HNPs - human neutrophil peptides; PDIA1 - Protein Disulfide Isomerase A1; CCN2/CTGF - Connective Tissue Growth Factor; ADAM28 - A Disintegrin and Metalloproteinase 28; SR-A1 - Scavenger receptor class A member 1; TSP1 - Thrombospondin 1; Angpt- Angiopoietin-; IGFBP7 - Insulin-like growth factor-binding protein 7; MGL - Macrophage galactose-type lectin receptor; CLEC4M - C-Type Lectin Domain Family 4 Member M; ASGPR - Asialoglycoprotein Receptor; Siglec-5 - Sialic Acid-Binding Ig-Like Lectin 5; Gal- Galectin-; STAB2 - Stabilin-2; SCARA-5 - Scavenger Receptor Class A Member 5; eNOS - Endothelial Nitric Oxide Synthase; IL-8 - Interleukin-8; HDL - High-density lipoprotein; ApoA-I - Apolipoprotein A-I, LDL - Low-density lipoprotein; SDC-1 - Syndecan-1; HS - heparan sulfate; RANKL - RANK ligand; BiP - binding-immunoglobulin protein; VEGF - Vascular endothelial growth factor; PIGF-2 - Placental growth factor-2; PDGF - Platelet-derived growth factor; FGF - Fibroblast growth factor; TGF - Transforming growth factor; BMP-2 - Bone morphogenetic protein 2; NGF - Nerve growth factor; NT-3 - Neurotrophin-3; CXCL-12γ - C-X-C motif chemokine ligand 12 gamma.

*Growth factor interactions: VEGF-A165, PIGF-2, PDGF-AA/BB/CC/DD, FGF-2/7/18, TGF-β1, BMP-2, NGF-β, NT-3, CXCL-12γ.

Figure 3. VWF interacts with a variety of different cell types to influence both physiological and pathological processes.

The diverse biological roles of VWF are mediated through effects upon endothelial cells, neutrophils, leucocytes, macrophages, dendritic cells, tumor cells, smooth muscle cells and osteoclasts as illustrated.

LRP4 - Low-density lipoprotein receptor-related protein 4; αvβ3 - Alpha-v Beta-3 Integrin; M0 - undifferentiated macrophage; M1 - Pro-inflammatory macrophage; P38 - Protein kinase 38; NF-κB - Nuclear factor kappa B; NETosis - Formation of neutrophil extracellular traps; WPB - Weibel-Palade body; Angpt-2 - Angiopoietin-2; Gal-3 - Galectin-3; VEGF - Vascular Endothelial Growth Factor; VEGFR-2 Vascular Endothelial Growth Factor Receptor 2; Tie-2 -

Tyrosine kinase with immunoglobulin-like and EGF-like domains 2; Angpt-1 - Angiopoietin-1; FGD5 - FYVE, RhoGEF and PH domain-containing protein 5;

Figure 4. VWF may contribute to disease pathobiology for a variety of both hemostatic- and non-hemostatic disorders.

The left side of the figure illustrates hemostatic disorders in which VWF has been implicated in playing a direct pathogenic role. The right side of the figure illustrates a series of non-hemostatic disorders in which putative pathogenic roles for VWF have been proposed. CTEPH Chronic thromboembolic pulmonary hypertension; TTP - thromboembolic thrombocytopenic purpura; VWD - von Willebrand disease; DVT - Deep vein thrombosis.

Figure 1

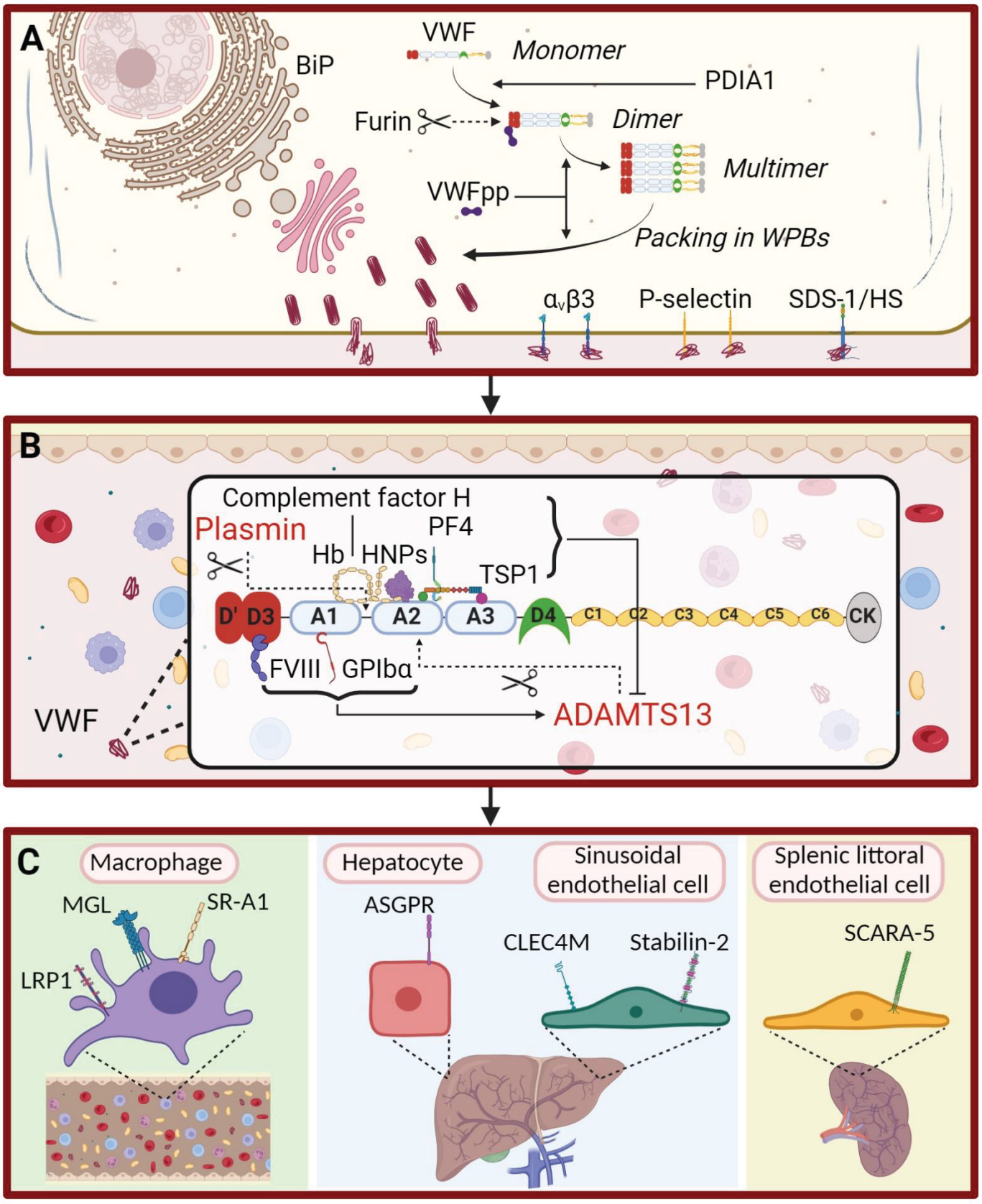


Figure 2

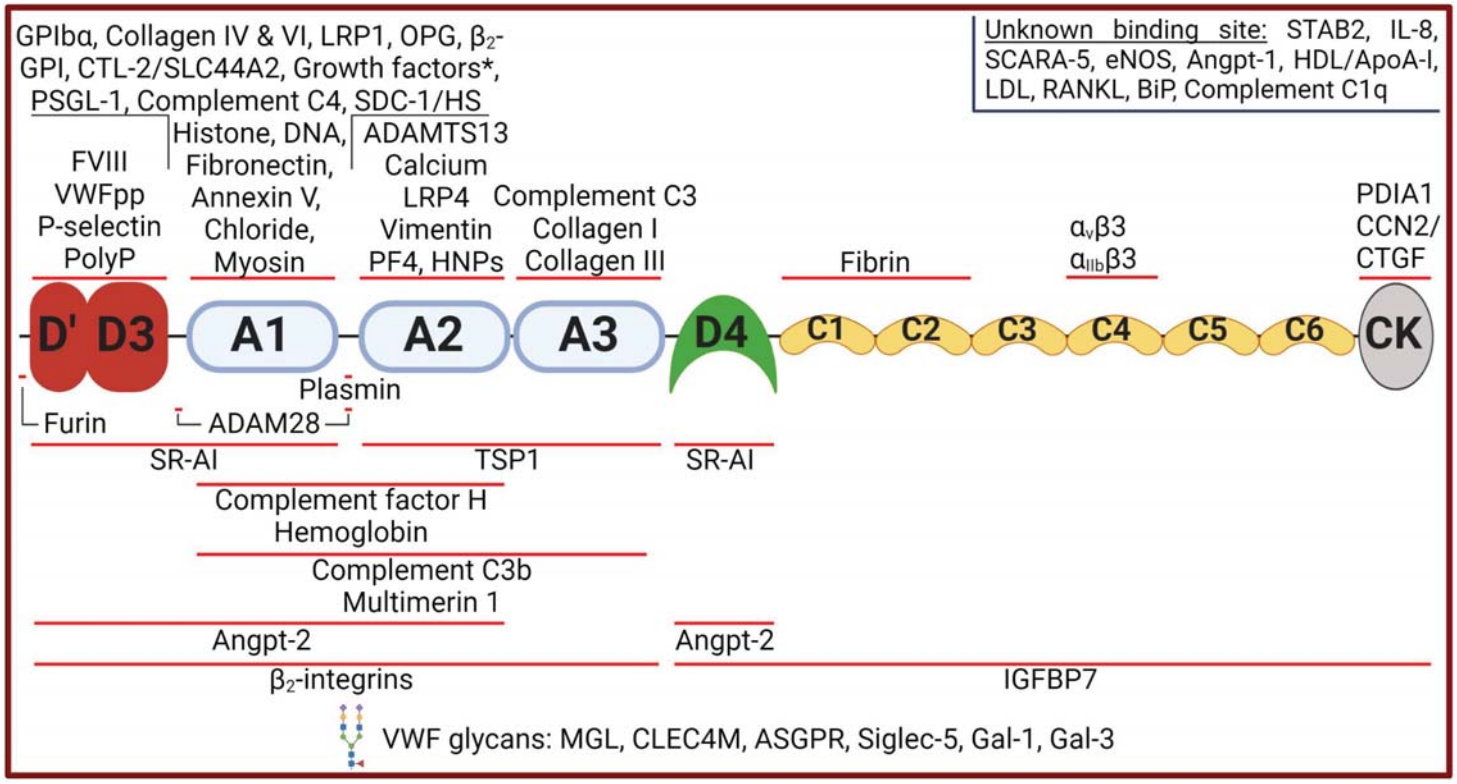


Figure 3

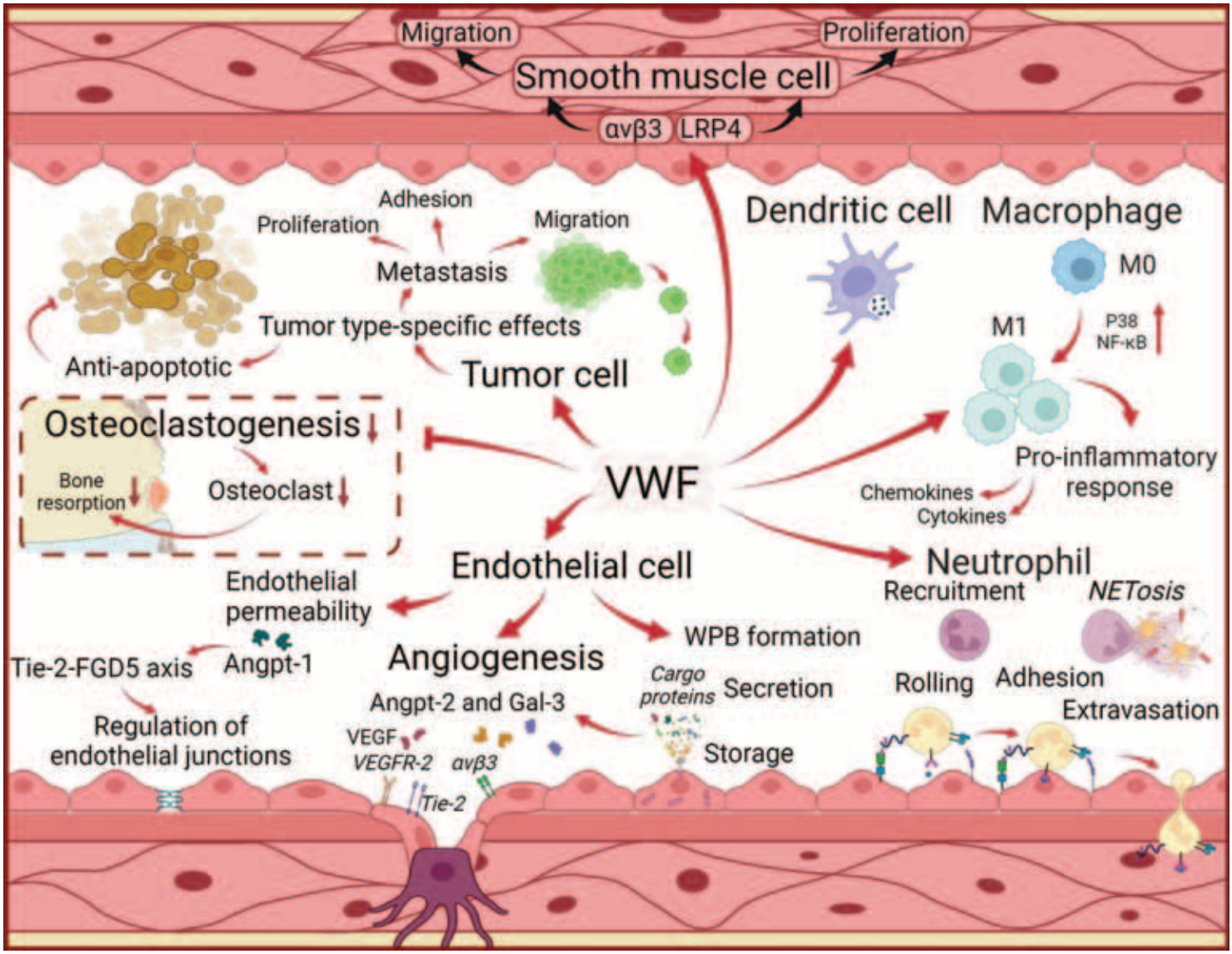
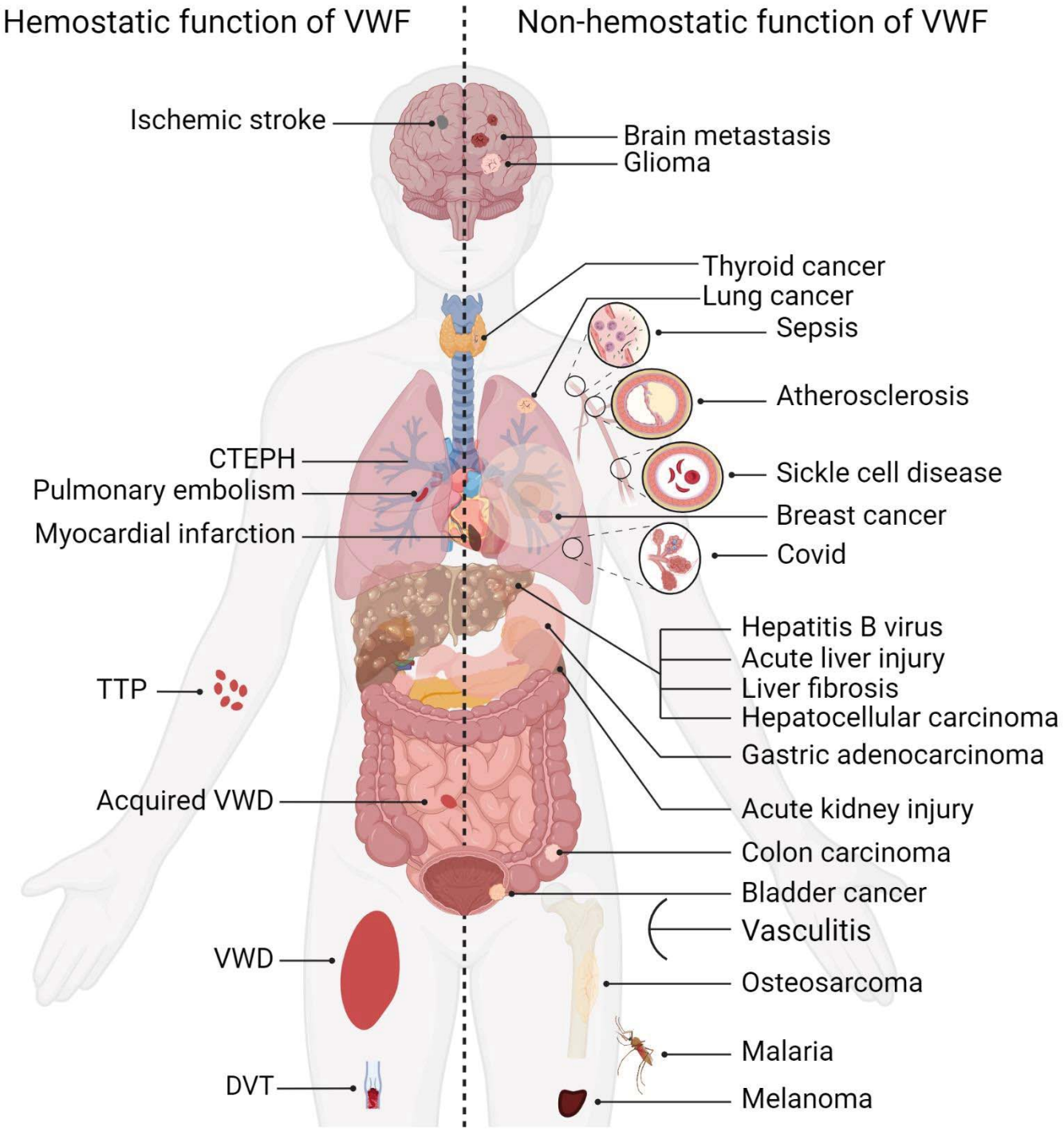
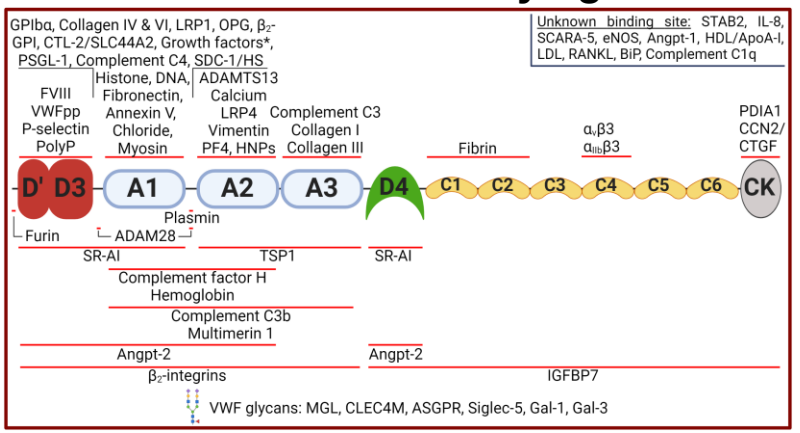


Figure 4

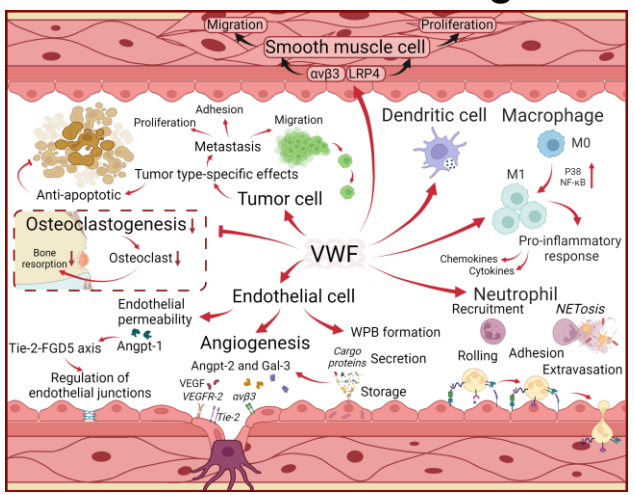


Novel functions for von Willebrand factor

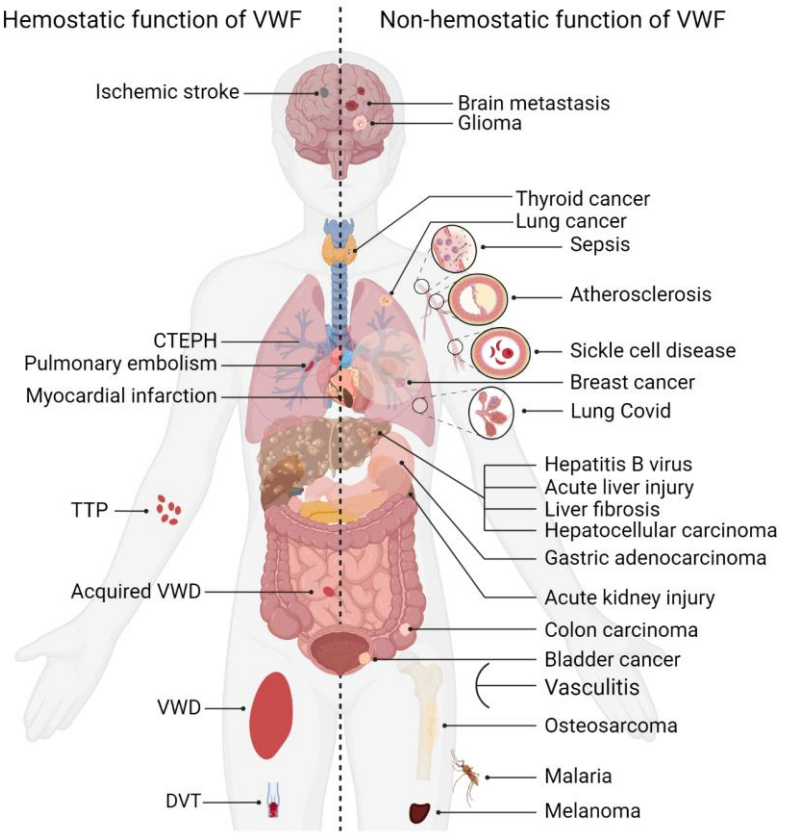
VWF binds over sixty ligands



VWF influences a wide range of cells



VWF is associated with the pathophysiology of numerous disorders



VWF interacts with a diverse array of binding partners through which it regulates important non-hemostatic biological functions in human body

Atiq and O'Donnell. doi: …. PMID: ….