von Willebrand Factor: More Than a Regulator of Hemostasis and Thrombosis

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Key Words
ADAMTS13 · Angiogenesis · Tumors · von Willebrand factor

Abstract
von Willebrand factor (vWF) was first identified as an adhesive glycoprotein involved in hemostasis by Zimmermann in 1971. Since then, vWF has been shown to play a vital role in platelet adhesion, platelet binding to collagen and factor VIII protection. Recent studies have implicated vWF as a regulator of angiogenesis, smooth muscle cell proliferation, tumor cell metastasis and crosstalk in the immune system. In this review, we will discuss the aspects of vWF structure that facilitate its biological effects and speculate on its newly discovered and hypothesized roles in the pathogenesis of several diseases.

Introduction
The von Willebrand factor (vWF) has two major well-established biological functions: mediating adhesion and aggregation of platelets, and acting as a consort for the essential blood clotting factor: factor VIII [1]. Very recently, research studies have provided evidence to indicate that vWF not only regulates hemostasis and thrombosis, but also the processes of angiogenesis, smooth muscle cell proliferation and tumor cells metastasis. Interestingly, vWF has also been implicated in proper functioning of the immune system [2, 3]. Both the size and conformation of vWF can be modified by genetic, physiological and environmental factors, including its cleavage protease ADAMTS13, the adhesive glycoprotein thrombospondin-1 (TSP-1) and shear stress forces.

vWF was first recognized for its role in the hereditary bleeding disorder known as von Willebrand disease (vWD). Since then, vWF has been associated with the thrombotic thrombocytopenic purpura (TTP) clotting disorder, various vascular disorders, liver diseases and cancers, and has emerged as a useful biomarker of these afflictions. It has been theorized that vWF is in fact a vital endothelial damage indicator and may be a promising diagnostic or prognostic marker of many other diseases. The purpose of this review is to discuss and summarize some of the newly identified and theorized roles of vWF.

The General Aspects of vWF
vWF is produced exclusively by endothelial cells and megakaryocytes, and is synthesized in the precursor pro-vWF form. The precursor is composed of four types of domains that are constructed as repeats in the following order: D1-D2-D′-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK [4]. The D1 and D2 domains comprise the pro-

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peptide and are cleaved during proteolytic processing to generate the mature vWF. The remaining domains in the mature vWF each carry out specific functions (fig. 1).

The pre-pro-vWF is comprised of 2,813 amino acids (aa) which encompass a 22-aa signal peptide, a 741-aa large propeptide and 2,050 aa making up the mature subunit [5]. Proteolytic processing involves cleavage of the signal peptide, which facilitates translocation of vWF into the endoplasmic reticulum. In the endoplasmic reticulum, pre-pro-vWF subunits homodimerize in a 'tail-to-tail' manner through disulfide bonds that form between the C-terminal residues. The vWF dimers are subsequently transported to the Golgi complex, where further modifications take place, such as the proteolytic removal of the large vWF peptide [6], sulfation of N-linked oligosaccharides and O-linked glycosylation [7]. While traveling through the Golgi complex, vWF dimers multimerize through self-association of propeptides on adjacent dimers. Further multimerization and propeptide cleavage occur simultaneously in the post-Golgi compartment. vWF produced by endothelial cells is either secreted constitutively or stored in Weibel-Palade bodies (WPBs) until the cell is stimulated by a secretagogue.

In response to pathological stimuli, such as inflammation, the circulatory concentration of vWF increases rapidly. Secretion of stored vWF from endothelial cells occurs through both a constitutive and a regulated pathway [8]. Megakaryocytes, however, lack the regulatory pathway, and once they are stimulated, vWF is constitutively
secreted. Myriad secretagogues and inhibitors can mediate the secretion effects on both vWF-producing cell types. The secretagogues can be divided into two distinct groups: Ca\(^{2+}\)-raising agonists and cAMP-raising agonists. There also are three well-studied inhibitors of vWF secretion, namely nitric oxide (NO), hydrogen peroxide (H\(_2\)O\(_2\)), and dopamine [9, 10] (table 1). vWF is secreted into plasma in an ultra large form (ULvWF) that consists of several hundred vWF monomers. After secretion, the ULvWF travels in the blood flow until it is broken up by shear stress forces or collisions and molecular interactions with the vessel wall. In a mouse model, circulating vWF was predominantly taken up by the liver (approx. 40–60%), with the remaining being absorbed by the spleen and kidney [11]. van Schooten et al. [12] demonstrated that macrophages and factor VIII, mainly in the liver, mediate the removal of vWF from circulating blood. However, the precise cellular membrane receptors for vWF remain unknown.

### Determination of vWF Size and Conformation

The biological function of vWF depends largely on the size of its multimers [13]. Larger multimers are more likely to bind to platelets and collagen, and to promote platelet adhesion in circulating blood. Thus, the ULvWF multimers are more active than the largest plasma vWF in inducing platelet aggregation [14]. Since many proteolytic proteins target the available ULvWF to promote clotting, it is essential to maintain a fine balance between ULvWF and proteolysis to generate the smaller vWF forms that function in other processes. Therefore, it is very important for researchers to gain a detailed understanding of the mechanisms regulating vWF size and conformation.

### Cleavage by ADAMTS13

The vWF-cleaving protease ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1-motif 13) has been purified from both human plasma and factor VIII/vWF concentrates [15]. ADAMTS13 targets the A2 domain of vWF and specifically cleaves the protein between Tyr1605-Met1606 (fig. 1), thereby reducing the ULvWF and hyperactive vWF multimers to smaller and less active forms which can be more readily unfolded by shear forces in flowing blood.

Studies using DNA molecule recombinant technology have demonstrated that two subsites, D187-R193 and D252-P256, can contribute to the cleavage efficiency and site specificity of ADAMTS13 [16]. Furthermore, Zanardelli et al. [17] identified a binding site for ADAMTS13 in vWF residues 1874–2813, and determined that removal of this fragment can inhibit vWF proteolysis. Still an-

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**Table 1. vWF secretion pathways induced by agonists or inhibitors**

<table>
<thead>
<tr>
<th>Secretagogue</th>
<th>Mediator</th>
<th>Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>Ca(^{2+})</td>
<td>VEGFR2</td>
</tr>
<tr>
<td>NAADP</td>
<td>Ca(^{2+})</td>
<td>H1R</td>
</tr>
<tr>
<td>Histamine</td>
<td>Ca(^{2+})</td>
<td>H1R</td>
</tr>
<tr>
<td>Thrombin</td>
<td>Ca(^{2+})</td>
<td>Thrombin receptor</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Ca(^{2+})</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>LTs, ROIs, calcium ionophore</td>
<td>Ca(^{2+})</td>
<td>UNK</td>
</tr>
<tr>
<td>A23187/ionomycin, PMA, C5a</td>
<td>Ca(^{2+})/camp</td>
<td>PE2R/FA2R</td>
</tr>
<tr>
<td>and C5b-9, superoxide anions</td>
<td>cAMP</td>
<td>V2R</td>
</tr>
<tr>
<td>Purine nucleotides</td>
<td>cAMP</td>
<td>β2-AR</td>
</tr>
<tr>
<td>Vasopressin (DDAVP)</td>
<td>cAMP</td>
<td>UNK</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>cAMP</td>
<td>D2, D3, D4</td>
</tr>
<tr>
<td>5-HT, adenosine, prostacyclin</td>
<td>Not Ca(^{2+}) or cAMP</td>
<td>UNK</td>
</tr>
<tr>
<td>Dopamine</td>
<td>GCy, cGMP, NSF, Ca(^{2+})</td>
<td>UNK</td>
</tr>
<tr>
<td>NO</td>
<td>Ca(^{2+})</td>
<td>UNK</td>
</tr>
<tr>
<td>H(_2)O(_2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

cAMP = Cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; GCy = guanylate cyclase; UNK = unknown; A2R = adenosine A2 receptor; β2-AR = β2-adrenergic receptor; H1R = histamine H1 receptor; P2yR = P2y purinergic receptor; V2R = vasopressin 2 receptor.
other study determined that a vicinal disulfide bond between C1669 and C1670 in the vWF A2 domain inhibits A2 domain proteolytic cleavage [18]. Four leukocyte proteases have been described that can mediate ADAMTS13-targeted A2 domain cleavage. While each of the four have been shown to efficiently process the synthetic vWF substrate FRETsvWF73, as well as multimeric vWF under denaturing conditions and fluid shear stress [19], the precise cleavage sites and underlying mechanism remain to be elucidated. ADAMTS13 mutants deficient in the C-terminal TSP-1 and CUB domains were found to exist in a hyperactive state under flow conditions, indicating that this C-terminal region might act to negatively regulate ADAMTS13 activity [20].

**Disulfide-Bond Reduction by TSP-1**

TSP-1 is a trimeric glycoprotein that acts to further reduce the size of vWF multimers after ADAMTS13 processing. Each TSP-1 subunit is composed of N- and C-terminal globular domains connected by a thin strand. Like vWF, TSP-1 is released from endothelial cells and megakaryocytes and tethers to sites of vascular damage. TSP-1 can then alter multimer size of proximal vWF molecules by splitting the disulfide-bonds that link vWF monomers at its N- and C-terminal ends; in addition, TSP-1 can compete with ADAMTS13 for the same vWF binding site in A2 and A3 domains [21]. Pimanda et al. [22] reported that the vWF-reducing activity of TSP-1 lies in a free thiol located at Cys974 in its C-terminal sequence. Paradoxically, TSP-1 has been shown to protect vWF from degradation by ADAMTS13, both in vitro and in vivo [23]. Moreover, TSP-1-null mice have normal platelet aggregation but impaired Ca2+ ionophore A23187-stimulated endothelial platelet recruitment and reduced thrombus adherence. This phenotype was rescued by injection of human TSP-1 or neutralizing anti-ADAMTS13 antibodies.

**Influence of Fluid Shear Stress**

A shear stress is defined as the force that is applied parallel or tangential to the face of a material or molecule, as opposed to normal stress, which is applied perpendicularly. Siedlecki et al. [24] studied the effects of shear stress on vWF structure by atomic force microscopy, and found that 35 ± 3.5 dyn/cm² of shear stress caused the molecule to change from a globular to a short extended form, and finally to an extended chain conformation. Thus, the function of shear stress on vWF would be expected to correlate with its velocity. Indeed, fluid shear rates above 2,300–6,000/s have been shown to promote the aggregation or self-association of purified vWF [25].

Higher shear stresses not only modulate the large form vWF, but can also unfold the ADAMTS13 cleavage site in the A2 domain. In addition, shear stress has been shown to facilitate ADAMTS13 proteolysis of ULvWF multimers [26], probably by exposing the peptide bond in the A2 domain during the overall conformational change in vWF. Zhang et al. [27] used a technique based on optical tweezers to show how ADAMTS13 cleaves vWF under flow. They demonstrated that every single A2 domain that is present in a vWF molecule requires a force of approximately 11 pN to unfold, and that the A2 domain is not cleaved if the mechanical force is below this unfolding threshold.

**The Biological Effects of vWF**

There are three types of vWFs in the human body: soluble plasma vWF, basement membrane vWF (extracellular matrix vWF) and cellular vWF. Only the larger multimers of vWF are effective in the control of hemostasis. vWF has three main recognized hemostatic functions, which are to mediate interactions of platelet to platelet and platelet to collagen in the vessel wall, and to serve as a carrier of factor VIII in the circulation system. Recent research has suggested that vWF may have other nonhemostatic functions, including in angiogenesis, smooth muscle cell proliferation, tumor cell metastasis and immune cell regulation.

**vWF and Platelet Adhesion**

Under physiological conditions, circulating platelets are recruited from the blood flow to injury sites, where they act to prevent excessive bleeding [28]. vWF is essential in this process, as was demonstrated by platelet agglutination abnormalities in vWD patients who are deficient in vWF. vWD sufferers have a prolonged bleeding time due to impaired platelet plug formation, and transfusion of vWF concentrates can resolve this disorder.

When a vessel membrane is injured, the plasma vWF interacts with circulating platelets through two membrane receptors: known as glycoprotein Ib (GPIb) and the glycoprotein IIb–IIIa (GPIIb–IIIa) complex [29]. GPIb participates mainly in platelet-vessel wall adhesion, while the GPIIb–IIIa complex is involved in both platelet-vessel wall adhesion and crosstalk between platelets. vWF is the only ligand that promotes platelet adhesion by attaching to both of these receptors. However, shear-induced interaction of platelets in the presence of vWF could lead to GPIbα shedding, which can be inhibited through calpain...
cleavage [30]. Mody et al. [31] suggested that the formation of reversible tether bonds between GPIbα and the vWF A1 domain might be formed by hydrodynamic shear flow. Apart from platelet GPIb, Huang et al. [32] discovered that integrin αvβ3 can anchor to vWF strings, as observed by immunofluorescence microscopy of living human umbilical vein endothelial cells (HUVECs).

Interactions between vWF and the Subendothelial Matrix
Endothelium injury exposes the vascular subendothelial matrix to vessel contents. When the subendothelial matrix proteins at the injury site come across the coagulation factors, thrombus formation is initiated. However, this process has to withstand the surrounding high blood flow rates, which can trigger endothelial vWF secretion as the body attempts to ensure that sufficient vWF is available for interactions between platelets [33]. On the other hand, the high shear stress allows conformational changes within vWF, which can result in the exposure of binding sites for platelets or collagen. vWF plays an important role in initiating platelet adhesion onto the extracellular matrix, mainly the collagens. There are two mechanisms that mediate this type of adhesion: a direct mechanism, by which fibrin binds to the GPIIb–IIIa complex directly and an indirect mechanism, by which vWF serves as a bridging agent between fibrin and platelet GPIb.

In static conditions, recombinant soluble vWF with the A3 domain deleted has poor binding ability to collagen fibrils, as compared with a vWF with A1 deleted and intact vWF [34]. The A1 domain of vWF interacts mainly with collagen type VI [35], but can also bind, with less affinity, to collagen types I and III. A3 can also bind to collagens, namely collagen types I and III [36].

Under flow conditions, Bonnefoy et al. [34] found that the A1 domain initiates platelet recruitment to collagen fibers by comparing the binding abilities of intact vWF, an A1-deleted vWF and an A3-deleted vWF. They were also able to confirm that the collagen-vWF antagonist, HI1786A-vWF, binds to collagen only through A1, and not A3.

A Carrier of Factor VIII
Unlike endothelial cell- or megakaryocyte-originated vWF, factor VIII is produced by liver cells as a single chain polypeptide consisting of 2,351 aa. Factor VIII is a cofactor of the intrinsic clotting cascade, and its deficiency manifests as hemophilia A. vWF is the consort for factor VIII [3], effectively extending the half-life of factor VIII. This phenomenon may be mediated by any of the following mechanisms: (1) structural stabilization of factor VIII; (2) inhibition of phospholipid-binding proteins that target factor VIII for proteolytic degradation; (3) inhibition of factor VIII binding to activated factor IX to stimulate the coagulation pathway, and (4) prevention of factor VIII cellular uptake via scavenger cell receptors [3, 12, 37].

Regulation of Angiogenesis
Recent research has revealed that vWF has several previously unrecognized biological functions. A regulatory role for endothelial vWF in angiogenesis has been hypothesized based on the fact that vWF is closely related to other well-known regulators of angiogenesis and angiodysplasia in vWD patients [38, 39]. To test this hypothesis, Starke et al. [40] used short interfering RNA (siRNA) to inhibit vWF expression in HUVECs, and observed stimulated results in angiogenesis, and improved vascular endothelial growth factor (VEGF) receptor-2 (VEGFR-2)-dependent proliferation and migration. VEGFR2 is an important receptor for the key angiogenesis-mediator hormone VEGF-A. That same study also found that vWF-deficient mice had increased angiogenesis and a larger vascular network [41]. Another study used endothelial cells isolated from vWD patients with low levels of plasma activity and antigen to show that the vWD phenotype could be recapitulated. Similarly, blood outgrowth endothelial cell colonies from vWD patients were also observed to have significantly increased tube formation, VEGF-dependent proliferation and basal migration [40]. Collectively, the findings from these studies indicate that endothelial vWF deficiency can promote angiogenesis.

vWF and Smooth Muscle Cell Proliferation
Based on the fact that pigs with vWD also presented with decreased progression of atherosclerosis, Qin et al. [42] speculated that elevated expression of endothelial vWF may have mitogenic effects on smooth muscle cell proliferation. Using an in vitro model system, they found that mouse smooth muscle cell proliferation correlates positively with the concentration of vWF. Further studies in an in vivo system of carotid artery ligation revealed that intimal hyperplasia in carotid arteries was absent in low plasma vWF mice, and that treatment with the synthetic vasopressin desmopressin (DDAVP) moderately rescued plasma vWF levels. These results indicate that expression of vWF is capable of modulating, at least partially, intimal hyperplasia.
vWF and Tumor Cell Metastasis

Platelets are important components of the hemostatic process, which is believed to support metastasis. When the platelet amount is reduced, metastasis is reduced as well. Recent studies have indicated that vWF, in particular, can function as a promoter of tumor cell metastasis [43, 44]. For example, the B16-BL6 melanoma cell transplantable tumor cell line has been demonstrated to adhere to vWF through the membrane-expressed integrin αVβ3. In addition, vWF has been shown to promote adhesion of tumor cells through the Arg-Gly-Asp (RGD) sequence in its C1 domain. Finally, using an experimental pulmonary metastasis model system based on B16-BL6 melanoma cell or Lewis lung carcinoma cell transplantation into wild-type and vWF-deficient mice, vWF deficiency was shown to result in a significantly increased number of metastatic foci, but not increased tumor growth [45].

New Roles for vWF with Immune Cells

Currently, many researchers have begun to investigate potential roles of vWF and the immune response. In particular, a novel role for vWF as a mediator of crosstalk between immune cells has been proposed.

Leukocytes

Leukocyte extravasation is an important inflammatory process that involves chemoattraction to the site of immune response to injury or infection, rolling adhesion as the leukocytes approach the site and begin to merge out of the blood flow, tight adhesion to the vessel wall at the site, and endothelial transmigration through the vessel wall to the site of action. Petri et al. [2] reported that vWF promotes leukocyte extravasation in a platelet- and GPIb-dependent manner, providing evidence that vWF may functionally contribute to inflammation.

Neutrophils

Neutrophil extracellular traps (NETs) are made within the vasculature by activated neutrophils. The NETs, themselves, are composed of DNA fibers, histones and antimicrobial proteins, which are catapatelated out of the neutrophil into the pathogen-containing extracellular space. NETs have been shown to prevent the spread of a bacterial infection not only by physically entrapping the bacteria but by killing them by proteolytic degradation [46]. In the vasculature, NETs also provide a scaffold for platelets and subsequent thrombus formation [47]. Immunocytochemistry has revealed that vWF, fibronectin and fibrinogen are capable of binding to the NETs, thereby promoting platelet binding to NETs.

Macrophages

Macrophages play a key role in rapid immune clearance of infectious agents and tumor cells. A role for vWF in the mechanism of macrophage-based immune clearance was suggested by a study of spleen and liver tissues from vWF-deficient mice treated with recombinant vWF or recombinant factor VIII. Immunohistochemical staining with the anti-mouse macrophage marker F4/80 indicated that vWF was enriched in macrophages using the anti-mouse macrophage marker F4/80 [12]. Of note, when macrophages were reduced in these mice by gadolinium chloride (GdCl3) treatment, vWF clearance was promoted and vWF half-life was nearly doubled. Thus, it was hypothesized that macrophages are involved in the vWF clearance process.

Dendritic Cells

Dendritic cells (DCs) are critical immune modulators as they trigger the activation and proliferation of T cells. By incubating DCs derived from monocytes of healthy blood donors with a panel of bioactive molecules, Dasgupta et al. [3] found that DC-mediated endocytosis of factor VIII can be downregulated by vWF.

Megakaryocytes

Megakaryocytes are the precursors of platelets in mammalian bone marrow. Nurden et al. [48] performed a cohort study of both megakaryocytes and platelets from patients with von Willebrand type 2B disease and concluded that vWF could impair megakaryocytopenia.

The Significance of vWF in Diseases

The concentration of normal plasma vWF ranges from 5 to 15 μg/ml. Defects in vWF secretion, assembly of vWF multimers, or overt proteolytic degradation can result in a low concentration of vWF, which manifests as vWD. On the contrary, impaired vWF degradation of ULvWF is a strong risk factor for TTP. Apart from these two diseases, vWF levels have been regarded as a thrombosis risk. Ongoing research efforts are aimed at gaining a better understanding of how vWF is regulated in disease conditions, particularly vascular-related diseases, and to determine its potential as a biomarker.

vWF and vWD

vWD is the most common inherited human bleeding disorder and results from defects in plasma vWF quantity and/or quality [49]. According to phenotype analysis,
vWD can be classified among six independent types: type 1, types 2A, 2B, 2M and 2N, and type 3 [50]. Type 1 vWD is characterized by a reduction both in the amount and activity of vWF. Type 2 vWD is characterized by abnormal adhesion activity and functional tests will show an abnormality. Type 3 vWD patients present with vWF levels that are nearly or absolutely undetectable. vWD is characterized by excessive mucocutaneous bleeding, which manifests clinically as epistaxis, menorrhagia, easy bruising and nose or oral cavity bleeding. Generally speaking, only a small portion of vWD cases are severe, and most of the vWD patients do not require extensive therapeutic intervention to stop the bleeding. Different therapeutic strategies are needed to treat patients according to the clinical classification of their vWD (table 2).

**vWF and Thrombotic Thrombocytopenic Purpura**

Several clinical forms of TTP are currently recognized, including the acute single-episode TTP, familial TTP and relapsing TTP [51]. Up to half of TTP patients have been shown to have a higher vWF multimer size, as compared to that of healthy, non-TTP individuals. Several researches have reported that the normal vWF-cleaving protease activity is completely absent in patients with TTP [52–54]. Since platelet thrombi formation depends on the amount of platelets adhering to ULvWF, plasma ADAMTS13-targeted cleavage of ULvWF is key to maintaining a balance between coagulation and hemostasis. In TTP, ADAMTS13 fails to control thrombus growth, allowing the thrombus to grow continually and eventually leading to vessel occlusion. Absent or severely reduced quantity or activity of ADAMTS13 always causes patients to fail to timely degrade ULvWF multimers secreted by endothelial cells. The ULvWF multimeric strings remain bound to endothelial cells and cause deposition of platelet-rich thrombi. In addition, fluid shear stresses and the torque formed by ULvWF-platelet adherence may also promote the detachment of ULvWF [55]. Gene mutation of ADAMTS13 and autoimmune inhibitors targeting ADAMTS13 may further promote vWF aggregation and microvascular thrombosis [56]. In fact, ADAMTS13 gene mutations have been show to affect both the function and processing of ADAMTS13, resulting in vWF binding or cleavage inhibition [57].

**vWF in Acquired von Willebrand Syndrome**

Acquired von Willebrand syndrome (AvWS) is a rare hemorrhagic disorder with multiple etiologies, including but not limited to myeloproliferative disorders, lymphoproliferative disorders, immune-mediated diseases, cardiovascular disorders, hypothyroidism, malignancies and altered shear stress [58, 59]. While many mechanisms have been proposed for this disease, such as selective absorption of ULvWF multimers, nonselective absorption of vWF, shear-induced destruction of vWF, and increased proteolysis [59], the true molecular pathogenesis remains unknown. The type 1 AvWS is characterized by hypothyroidism and requires DDAVP therapy [60]. Most AvWS types mimic vWD type 1 or type 2A. Only two of the AvWS cases reported in the worldwide medical literature to date have mimicked vWD type 2B [61, 62]. Still other types of AvWS have been reported, although they appear to be exceedingly rare. As medical research and global communication continue to advance, it is likely that more cases of AvWS will be recognized and reported to the international community.

**vWF as a Potential Clinical Biomarker**

Endothelial cells release a multitude of procoagulation and anticoagulation proteins that regulate the fine balance between hemostasis and thrombosis. The three major hemostatic regulatory molecules are vWF, thrombomodulin and tissue factor pathway inhibitor [63]. Plasma levels of the first two are increased significantly upon endothelial membrane injury, making them promising biomarkers of endothelial dysfunction [64, 65].
Many clinical case-cohort studies have set forth to evaluate the relationship between the onset and extent of endothelial cell-related diseases and vWF plasma levels (summarized in Table 3). Some of the clinical trials and follow-up studies have successfully demonstrated that elevated plasma vWF is a risk factor of coronary heart disease [63], cerebral sinus and venous thrombosis [66], systemic lupus erythematosus [67], ischemia stroke [68], liver cirrhosis [69], atrial fibrillation [70], hypertension [71], sickle cell disease [72] and obesity [73]. On the other hand, several other studies have failed to establish an association between vWF and endothelium-related diseases, including hypertrophic cardiomyopathy [74], Alzheimer’s disease [65] and retinopathy-validated cerebral malaria [75].

While some prospective studies have demonstrated that elevated vWF levels are associated with the severity of vascular disorders, the underlying mechanisms have yet to be determined. It also remains unclear whether vWF levels could act as an accurate marker of severity of endothelial damage. Plasma vWF levels are known to be influenced by many of the established cardiovascular risk factors, such as age, cholesterol and hypertension [76], and any of these may complicate the use of vWF as a diagnostic or prognostic biomarker in clinical practice.

### vWF and Liver Diseases

The liver is one of the primary organs that produce clotting factors, anticoagulant proteins and fibrinolytic factors. Patients with liver dysfunction tend to suffer from bleeding or thrombi disorders that stem from hemostatic disturbances. Recent studies have shown that vWF is involved in the development and progression of liver diseases such as liver cirrhosis, alcoholic hepatitis and liver transplantation-related disorders.

Plasma levels of vWF antigen (vWF:Ag) are significantly higher in patients with liver cirrhosis than in healthy controls [69, 77]. Lisman et al. [77] reported that a substantial elevation of vWF:Ag accompanied aggravation of cirrhosis, according to Child-Pugh classification scores (A: 380%; B: 500%; C: 760%). In addition, vWF:RCo (von Willebrand factor ristocetin cofactor) levels have also been shown to increase in conjunction with vWF:Ag. However, the overall ratio of vWF:RCo/vWF:Ag is lower in cirrhotic patients than in healthy controls, indicating an impairment in the functional capacity of the liver. Interestingly, La Mura et al. [69] reported that a positive linear correlation exists between levels of vWF and the hepatic venous pressure gradient, but also found that the prognostic value of vWF was not associated with the gra-

### Table 3. Recent studies evaluating vWF as a biomarker for vascular disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Plasma/active vWF¹</th>
<th>Potential as a biomarker</th>
<th>p value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
<td>3.04</td>
<td>yes, a risk factor for CHD</td>
<td>0.01</td>
<td>[63]</td>
</tr>
<tr>
<td>Cerebral sinus and venous</td>
<td>1.52</td>
<td>yes, its role as a risk factor may be mediated by factor VIII</td>
<td>0.01</td>
<td>[66]</td>
</tr>
<tr>
<td>thrombosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLE related CVD</td>
<td>1.4</td>
<td>yes, a predictor of first CVD</td>
<td>0.01</td>
<td>[67]</td>
</tr>
<tr>
<td>Ischemia stroke</td>
<td>3.2</td>
<td>yes, associated with the occurrence of acute ischemic stroke</td>
<td>&lt;0.01</td>
<td>[68]</td>
</tr>
<tr>
<td>Cirrhosis and portal hypertension</td>
<td>2.13</td>
<td>yes, correlates with liver function and hepatic venous pressure</td>
<td>&lt;0.001</td>
<td>[69]</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>4.47</td>
<td>yes, may help to identify patients with essential HBP</td>
<td>&lt;0.001</td>
<td>[70]</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.21</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>ULvWF: 1.2–3.6</td>
<td>yes, correlates with the rate of hemolysis</td>
<td>&lt;0.01</td>
<td>[72]</td>
</tr>
<tr>
<td>to normal: 1.06 after gastric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>banding: 0.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>various</td>
<td>no</td>
<td>not given</td>
<td>[74]</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>1.04</td>
<td>no</td>
<td>0.74</td>
<td>[65]</td>
</tr>
<tr>
<td>CM-R</td>
<td>AUROC: 0.58</td>
<td>no, but ppvWF can</td>
<td>0.305</td>
<td>[75]</td>
</tr>
</tbody>
</table>

AUROC = Area under the receiver operating characteristic curve; CHD = coronary heart disease; CM-R = retinopathy-validated cerebral malaria; CVD = cardiovascular disease; HBP = high blood pressure; SLE = systemic lupus erythematosus; ppvWF = pre-pro-vWF.

¹ Fold-change, relative to control.
vWF and Tumors

vWF abnormality has been reported in both hematologic malignancies and nonhematologic malignancies, including chronic lymphocytic leukemia [78], acute lymphoblastic leukemia [79], colorectal carcinoma [80], leiomyosarcoma [81], breast cancer [82] and prostate cancer [83]. In an experimental metastasis mouse model, vWF was observed to inhibit tumor metastasis [45]. In fact, several clinical trials have shown significantly elevated levels of plasma vWF in various cancer patients [84–86]. It is reported that during the process of tumor angiogenesis, platelets produce VEGF [87, 88], which subsequently promotes endothelial proliferation and elevated plasma levels of vWF. One study of tumor angiogenesis determined that vWF staining represented an effective maker of microvessel density [89], which itself may be a prognostic marker for cancer progression or patient survival. Another study of colorectal cancer patients indicated that large numbers of vWF-positive microvessels in the presence of tumor-associated macrophages can be an indicator of poor prognosis [90]. Future studies of the molecular mechanisms underlying tumor angiogenesis and metastasis are expected to provide further insights into the tumor-related roles of vWF.

Concluding Remarks

A substantial amount of data has emerged over the past decades to suggest myriad roles of vWF in a broad range of vascular- and immune-related normal processes and disease, but many questions remain. For example, does vWF’s involvement in angiogenesis and metastasis indicate a consequential (and therapeutically manipulable) correlation with tumorigenesis or tumor progression? Is the association between vWF and cardiovascular disease robust enough for vWF to be used as an accurate and specific biomarker? Until the precise molecular mechanisms of vWF in each disease’s pathogenesis are elucidated, the answers to these questions would be clear. Regardless, the body of vWF data to date has uncovered many new and clinically-promising functions of vWF beyond its original documented action as a mediator of hemostasis.

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References

vWF, Hemostasis and Thrombosis


