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Angiodysplasia in von Willebrand disease: Understanding the clinical and basic science

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Abstract

Severe and intractable gastrointestinal (GI) bleeding caused by angiodysplasia is a debilitating problem for up to 20% of patients with von Willebrand disease (VWD). Currently, the lack of an optimal treatment for this recurrent problem presents an ongoing challenge for many physicians in their management of affected patients. Over the past few years, studies have pointed to a regulatory role for the hemostatic protein, von Willebrand factor (VWF), in angiogenesis, providing a novel target for the modulation of vessel development. The current article will review the clinical implications and molecular pathology of angiodysplasia in VWD.

Keywords

Angiodysplasia; gastrointestinal bleeding; Heyde's syndrome; von Willebrand disease; von Willebrand factor

VON WILLEBRAND FACTOR

von Willebrand factor (VWF) is a multimeric plasma glycoprotein that is required for normal hemostasis. It is primarily synthesized in endothelial cells (ECs) from where it is either constitutively secreted or stored as ultra large molecular weight multimers (ULMWM; comprising VWF moieties of 10,000 kD or more) alongside other bioactive molecules in Weibel Palade-bodies (WPBs).[1] VWF is also produced in megakaryocytes; however, circulating VWF is almost exclusively derived from ECs.[2,3] To render the multimers less thrombogenic, a disintegrin-like and metalloprotease with thrombospondin type 1 motifs 13 (ADAMTS13) cleaves VWF at the A2 domain, thereby reducing VWF multimer size.[4]

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In the circulation, VWF functions as a chaperone of coagulation factor VIII (FVIII) and forms adhesive strings that mediate platelet adhesion and aggregation to sites of vascular damage.[5] In addition to playing an integral role in hemostasis, recent research has revealed that VWF functions in several other important biological processes such as angiogenesis, cell proliferation, and inflammation.[6,7] Individuals with abnormal or absent VWF are commonly afflicted with excessive bleeding tendencies due to von Willebrand disease (VWD) or acquired von Willebrand syndrome (AVWS), and may be prone to abnormalities in vessel formation, or angiogenesis.

VON WILLEBRAND DISEASE

von Willebrand disease (VWD) was first described in 1926 by Dr. Erik von Willebrand, when he cared for a family with a severe mucocutaneous bleeding problem.[8] The index case, a 14 year old female, bled to death during her fourth menstrual period. In the decades since Dr. von Willebrand noted this “pseudohemophilia”, developments in hemostasis and molecular genetics have paved the way for significant progress in our understanding of the disease and allowed for the development of effective treatments. It is now recognized that VWD is an autosomally inherited bleeding disorder, affecting approximately 1% of the population, with a 0.1% symptomatic prevalence.[9,10] The disease arises from defective or dysfunctional VWF and, consequently, patients present with excessive mucocutaneous bleeding and, in some severe cases, spontaneous bleeding into joints and soft tissues.[11]

The International Society on Thrombosis and Hemostasis (ISTH) currently classifies VWD by either quantitative (types 1 and 3) or qualitative (type 2) defects in VWF.[3] Type 1 VWD is generally inherited in an autosomal dominant manner and represents 65 – 80% of VWD cases.[12] Type 1 VWD is most often caused by a heterozygous missense mutation in the *VWF* gene that results in a mild to moderate reduction of VWF.[13] Type 2 VWD constitutes 20–35% of patients and reflects abnormalities in platelet, collagen or FVIII binding.[14] There are four variants of type 2 VWD – 2A (platelet dependent function loss associated with loss of high molecular weight multimers (HMWM) of VWF), 2B (increased binding of VWF to GPIbα), 2M (decreased binding of VWF to GPIbα and/or collagen, but not associated with loss of HMW VWF), and 2N (decreased binding of VWF to FVIII).[12] Lastly, type 3 VWD is inherited in an autosomal recessive fashion and is the least common (0.5 – 4 per million in developed countries) and most severe form of the disease.[12] Affected individuals are generally homozygous or compound heterozygous for null alleles and have undetectable levels of VWF and substantially reduced levels of FVIII (undetectable to ~5%)[13,18]; consequently, these patients have a more severe clinical phenotype than the other VWD subtypes.

VWD and Angiodysplasia

Vascular malformations have been documented in VWD patients since the 1960s when Dr. Armand Quick first reported the presence of dilated blood vessels or telangectasias in the noses of several patients.[17] We now acknowledge that patients with VWD have an increased frequency of angiodysplasia and vessel abnormalities have also been identified in their gastrointestinal (GI) tract, nail bed, prostate, and skin.[18–21] Moreover, when compared with

other bleeding disorders, there is a 99% positive predictive value for VWD when there is a high degree of capillary torquation, dilation, and extravasation of blood.[19]

This association between angiodysplasia and GI bleeding in VWD patients was first reported in 1976 by Ramsey *et al.,* when they described "gastrointestinal vascular dysplasia as a cause of persistently recurring melena in two VWD patients".[22] Though decades have elapsed since this observation, an effective treatment for GI bleeding still evades researchers, and angiodysplasia remains a serious complication for many VWD patients. In fact, recurrent GI bleeding as a result of angiodysplasia is one of the most challenging problems faced by some patients.[9,17,23–25]

In the GI tract, the presentation of angiodysplasia most commonly occurs in the cecum and ascending colon, but has also been noted throughout the small intestine and stomach.[26,27] Patients often present with multiple lesions in the GI tract that are characterized by a fragile vascular network.[28] Disruption of this delicate vascular architecture can result in severe intractable bleeding, often leading to anemia and requiring hospitalization for transfusions of packed red blood cells.[29] Consequently, many VWD patients experience a decrease in quality of life.[29] While GI bleeding is also present in the general population, it is uncommon (prevalence, 0.83%) and the lesions are usually small with a low risk of bleeding.[30]

The diagnosis of angiodysplasia can be difficult and often requires invasive techniques such as GI endoscopy. Consequently, the exact prevalence of angiodysplasia in VWD is not known. In a retrospective study of VWD patients with occult or angiodysplastic bleeding in the VWD Prophylaxis Network (PN), angiodysplasia was only confirmed as the cause of GI bleeding in one third of patients.[31] Although multiple diagnostic methods were used, the cause of bleeding remained unexplained in most patients, highlighting the struggle to determine bleeding etiology.[31]

The incidence of angiodysplasia varies across VWD subtypes in patients where angiodysplasia is confirmed as the main source of bleeding. In a landmark survey of 4503 VWD patients, Fressinaud and Meyer identified that angiodysplasia was almost exclusively present in patients lacking HMWM of VWF. [9] Specifically, angiodysplasia was reported in 2% of type 2 VWD patients and 4.5% of type 3 VWD patients.[9]

ACQUIRED VON WILLEBRAND SYNDROME

AVWS is a rare bleeding disorder due to non-inherited structural and/or functional changes in VWF resulting in hemostatic defects. The condition was first described in 1968 in a case study of a patient with lupus erythematosus.[32] In the last few decades, researchers have discovered that AVWS is often caused by lymphoproliferative disorders, cardiovascular defects such as aortic stenosis, or myeloproliferative disorders among other risk factors.[33] These conditions can influence VWF levels and/or function by decreasing synthesis, inhibiting VWF, increasing clearance due to binding of paraproteins, and pathologically increasing fluid shear stress resulting in increased proteolysis of VWF by ADAMTS13.[34–45] In many patients, treating the underlying condition, if possible, often restores the normal VWF function and results in improvement or resolution of AVWS.

Heyde's Syndrome

Epistaxis and GI bleeding resulting from angiodysplastic lesions have been reported in individuals with AVWS caused by aortic stenosis and left ventricular assist devices (LVAD). Increased bleeding tendencies in these patients point to a direct role for VWF in the development of angiodysplasia.[23,46–50]

The association between GI bleeding and aortic stenosis was first described in 1958 by E.C. Heyde in a letter to the *New England Journal of Medicine*. Heyde recounted that at least 10 patients with calcific aortic stenosis had massive gastrointestinal bleeding for which he could discover no cause.[51] This triad of conditions, including aortic stenosis, angiodysplasia, and GI bleeding is now eponymously referred to as Heyde's syndrome (Figure 1).

In the early 1990s, Warkentin *et al.*, suggested that the link between aortic stenosis and GI bleeding was a deficiency of the largest VWF multimers.[52] In patients with aortic stenosis, elevated shear stress modulates VWF from a globular protein to an elongated one in which the A2 domain is unfolded.[53] This unfolding exposes cryptic exosites to which ADAMTS13 binds and the VWF multimers are more rapidly cleaved.[53] Consequently, patients exhibit a loss of HMWM and develop AVWS. *In vitro* studies showed that this is often accompanied by abnormalities in platelet adhesion and aggregation.[54] Having an aortic valve replacement normalizes the deficiency of VWF HMWM and results in an improvement in AVWS.[49,55] King *et al.*, reported the cessation of GI bleeding in 93% of patients with aortic stenosis following an aortic-valve replacement.[56]

TREATING ANGIODYSPLASTIC GI BLEEDING

The diagnosis and treatment of recurring GI bleeding in VWD is challenging and associated with significant morbidity.[9,23,31] Patients frequently experience intractable, bleeding episodes resulting in recurrent hospital admissions and exposure to multiple therapies of limited efficacy. Bleeding episodes can also develop and increase in severity with age.[47]

Management of GI bleeding can be done via endoscopy or pharmacological methods, but the difficulty in accessing and identifying the angiodysplastic lesions hinders the development and deliverance of effective treatments.[31] While acute management of GI bleeding has been successful with VWF replacement therapy, prophylaxis has been less effective for preventing recurrent GI bleeding.[23,57] These clinical observations were supported by findings from the VWD PN study, in which prophylaxis was more efficacious at reducing joint bleeding and menorrhagia than GI bleeding.[58] This may be because the recovery of normal VWF levels is insufficient to reduce GI bleeding and normalization of VWF in cellular compartments such as ECs, platelets, and subendothelial matrix is also required.[59] Furthermore, current VWF replacement treatments are plasma-derived, and may be less effective in preventing recurrent bleeding episodes due to their relative lack of HMWM.[26] This loss of HMWM results from proteolytic degradation by ADAMTS-13 during the manufacturing process.[60] Given the known association between the development of angiodysplasia and a lack of VWF HMWM, the use of a recombinant VWF replacement product with HMWM and/or even ULMWM could be valuable for the prevention or treatment of recurrent bleeding episodes.[60,61]

Since VWF replacement therapy is not adequate for preventing recurrent GI bleeding, physicians have attempted alternate treatments. In cases where the angiodysplastic lesions can be identified through endoscopy, embolization of the main vessel in the area and surgical resection can be used to manage bleeding.[25,62] This is limited however in cases with multiple, diffuse lesions throughout the GI tract.[18] Thalidomide is an inhibitor of neo-angiogenesis, an effect mediated by suppression of vascular endothelial growth factor (VEGF). The use of this agent has been somewhat successful in treating angiodysplasia with or without VWD, but it has been associated with a high incidence of side effects.[63–65] Atorvastatin is another drug with anti-angiogenic properties. When administered at high doses (up to 80mg daily), it can also be successful in reducing GI bleeding.[63]

The clinical difficulties in treating patients with GI bleeding and the continued need for an ideal treatment emphasizes the importance of further research on the regulation of angiogenesis. Elucidating the association between angiodysplasia and abnormalities in VWF on a cellular and molecular level may point to novel therapeutic targets.

CELLULAR AND MOLECULAR BASIS OF ANGIOGENESIS

Angiogenesis is the formation of new, functional blood vessels from the existing vasculature. This is an important process in many physiological events such as embryogenesis, wound-healing, and the menstrual cycle. Deviations of this highly regulated process can give rise to a number of pathological conditions such as proliferative retinopathies, rheumatoid arthritis, and tumourigenesis.[66] The dysregulation of angiogenesis is central to the development of angiodysplasia.[67]

Normally, expansion of the nascent vascular bed arises under hypoxic conditions that require additional vessel growth to satisfy the oxygen requirements of the surrounding tissue. Hypoxia induces signaling through hypoxia-inducible transcription factors (HIFs) that upregulate many angiogenic factors.[68] Chiefly, HIF-1α induces VEGF.[69] VEGF-A is the best characterized member of the VEGF family of molecules. The binding of VEGF-A to its receptor vascular endothelial growth factor receptor-2 (VEGFR-2) initiates an intracellular signaling cascade prompting EC proliferation and migration toward the hypoxic environment.[70]

Stabilization and maturation of the newly formed vessel requires a fine balance of the interactions between pro-angiogenic and anti-angiogenic molecules.[71] Structural integrity is controlled primarily by Angiopoiein-1 (Ang-1) and Angiopoietin-2 (Ang-2) through signaling of the Tie-2 receptor on ECs.[72] Ang-1 is produced in perivascular cells and promotes blood vessel maturity by recruiting pericytes and decreasing vascular leakage.[73,74] Ang-2 is synthesized by ECs and stored in WPBs alongside VWF (Figure 2) and is an antagonistic ligand of Tie-2.[75] Ang-2 competitively inhibits the binding of Ang-1 at the Tie-2 receptor to promote vessel destabilization and growth by acting synergistically with VEGF.[76]

Interactions between the extracellular environment (ECM) and ECs are vital for preserving the stability of the newly formed sprout. These interactions are facilitated by cell surface

receptors of the integrin family. One member of this family that plays a central role in angiogenesis is integrin αvβ3. This heterodimeric, transmembrane protein is expressed on the surface of ECs and functions as both a pro- and anti-angiogenic molecule through VEGFR-2 signaling depending on the extracellular environment and ligand bound.[76,77] Integrin αvβ3 is the best-characterized endothelial receptor for VWF, and is significantly upregulated during wound healing as it can facilitate ligand binding to VEGFR-2.[78,79] Further research is still required on this protein as its role appears quite complex and variable depending on the ligand bound.[80]

Over the years, many molecules have been implicated in the process of angiogenesis, and the list continues to grow with the addition of many chemokines, hormones, neuropeptides, and cytokines among others.[81] At every phase of angiogenesis, these molecules work in harmony to prevent pathologic vessel growth.[81,82] Angiodysplasia can result from an aberration in this delicate balance between pro- and anti-angiogenic molecules and the association of VWF with angiogenic mediators like Ang-2 has pointed to a regulatory mechanism for this hemostatic protein in angiogenesis.

VWF is a negative regulator of angiogenesis

For many years, we have known that VWF defects can play a role in the formation of angiodysplastic lesions. However, the cellular and molecular pathway through which VWF regulates angiogenesis remained relatively unknown until 2011, when Starke and colleagues published an innovative paper pointing to a novel role for VWF as a negative regulator of angiogenesis.[28] In this paper, inhibition of VWF expression in human umbilical vein endothelial cells (HUVECs) using short interfering ribonucleic acid (siRNA) caused an increase in VEGF-dependent cell proliferation, migration, and tubule formation.[28] The mechanism of VWF regulation was suggested to be through two pathways with intracellular and extracellular components converging to modulate VEGF signaling (Figure 3).

In the extracellular pathway, VWF influences angiogenesis through interactions with integrin αvβ3. VWF is proposed to inhibit VEGF-2 induced proliferation of endothelial cells when bound to integrin αvβ3. In HUVECs lacking VWF, an internalization of β3 from the surface of cells has been observed. The addition of extracellular VWF only partially rescued the phenotype, suggesting the presence of an intracellular pathway involving VWF. In this intracellular pathway, defective WPB formation in the absence of VWF results in improper storage of Ang-2. Thus, Ang-2 is free to escape the ECs and act on Tie-2 receptors to promote vessel destabilization. In fact, increased Ang-2 release was detected from HUVECs lacking VWF and other studies have found that Ang-2 levels are elevated in the plasma of type 3 VWD patients.[28,83] These two pathways are interconnected with previous studies showing that Ang-2 also contributes to the degradation and internalization of αvβ3.[84] Furthermore, both pathways are known to influence VEGF signaling,[85,86] which is enhanced when they are disturbed in the absence of VWF.[28] Starke *et al.,* also showed increased vascularization in the ears of VWF-deficient mice, further validating the role of VWF in angiogenesis.[28]

In its many interactions with an ever-growing list of molecules, VWF has come to be known as much more than a hemostatic protein.[6] The discovery by Starke and colleagues that VWF

is a negative regulator of angiogenesis is an important contribution to the existing body of knowledge, as it provides an explanation for the vascular malformations typical of angiodysplasia found in patients with absent or abnormal VWF.

CELLULAR MODELS OF VWD

The anatomic location of endothelial cells within the vascular bed presents a considerable drawback for the routine examination of angiodysplasia and studies of VWD. As such, physicians often use clinical proxies such as malformations in the nail bed capillaries to identify vascular pathologies.[19] From a research perspective, this limitation in accessing patients' ECs has also hindered our understanding of the molecular pathology of VWD, and studies have mainly been done using non-EC based heterologous cell systems.[87–94]

Human embryonic kidney (HEK) cells can be transfected with recombinant VWF and were preferred models over other cell types (such as COS cells) since they could form pseudo-WPBs capable of exocytosis.[89] However, these cells are not native VWF-producing cells, and important factors such as post-translational modifications are potentially altered.[92] Furthermore, co-transfections of different mutations may not be a precise reproduction of heterozygous states *in vivo*.

For many years, HUVECs have also been used to extensively study VWF mutations.[95–99] However, it is quite difficult to generate many HUVECs with specific VWF mutations and so the search for a more readily accessible source of ECs continued until 2000 when Dr. Robert Hebbel's group identified endothelial cells that could be isolated from peripheral blood. These cells, known as blood outgrowth endothelial cells (BOECs), are derived from endothelial progenitor cells (EPCs)[100] and have significantly advanced the study of many physiological mechanisms including angiogenesis. BOECs grow with a cobblestone morphology typical of mature endothelial cells and express endothelial specific proteins in culture. Importantly, they are representative of the donor's native vascular endothelium and maintain their differentiated phenotype through multiple passages.[28,94,101,102] Of relevance to VWD studies, BOECs contain and store VWF in WPBs allowing for the examination of cellular properties of VWF such as storage and exocytosis which cannot be achieved using standard VWF functional assays. BOECs are also ideal for studies of angiogenesis, since they can be cultured to form tubules in a three-dimensional matrix and can be grown under shear stress.

VWD STUDIES IN BOECS

To date, four major studies on the properties of BOECs from VWD patients have been published by Dr. Jeroen Eikenboom's group in the Netherlands and Dr. Anna Randi's group in England.[28,101–103] These studies provide great insight into the role of VWF in the pathology of VWD and angiogenesis.

In 2013, findings on the cellular and molecular basis of VWD using patient-derived BOECs, were published in the same issue of *Blood*.[101,102] Two independent studies showed that it is feasible to isolate and culture BOECs from many patients with VWD, and that BOECs truly are endothelial cells. BOECs isolated from healthy, non-VWD individuals stored VWF

normally in elongated WPBs, while BOECs from type 1 and 2 VWD patients had mostly short and rounded WPBs.[101] These cellular defects in VWF storage in VWD BOECs also extended to molecular defects in VWF synthesis and processing.[102] The heterogenity of VWD was captured in these studies as BOECs derived from patients with similar plama VWF profiles but different mutations had unique cellular VWF phenotypes.[102]

In addition to uncovering that VWF was a negative regulator of angiogenesis, Starke and colleagues also recapitulated these findings in BOECs isolated from patients with type 1, 2A, and 2M VWD.[28] BOECs from VWD patients had reduced VWF levels in culture as well as an associated increase in the release of the pro-angiogenic molecule Ang-2.[28] Furthermore, VWD patient-derived cells also showed a significant increase in tube formation on Matrigel®, and increased VEGF-dependent proliferation and migration.[28] All of these cellular properties are characteristic of increased angiogenesis and mirror the angiogenic profile of siVWF-treated HUVECs. Interestingly, no differences in angiogenesis were found between the different VWD subtypes. However, this may have been because of the small sample size.

Groeneveld *et al.*, expanded on the aforementioned findings by culturing BOECs from type 1, 2A, 2B, and 3 VWD BOECs and determining their angiogenic characteristics.[103] This study highlighted the complexity in distinguishing the influence of different VWF mutations on angiogenesis, given that there was great variation across both control and VWD BOECs. A key observation was the loss of migration directionality in almost all VWD BOECs. While overall, VWD BOECs were not significantly more angiogenic than BOECs from controls, individual patient BOECs showed aberrantly angiogenic phenotypes when compared to controls.

Our research group is the first to successfully culture BOECs from patients of all VWD types and subtypes. Importantly, we have isolated BOECs from multiple type 3 VWD patients (Selvam *et al.*, submitted publication under review). In line with the above-mentioned studies, we have also determined the baseline angiogenic profiles of healthy non-VWD controls, and investigated the role of quantitative and qualitative VWD abnormalities on *in vitro* surrogates for the development of angiodysplasia. We found that overall there was great variability in the angiogenic properties of both control and VWD BOECs. Common features of BOECs from VWD patients included increased Ang-2 secretion, abnormal cell proliferation, and migration. In particular, type 1 and 3 VWD BOECs had reduced mean cell front migration velocity and increased directionality compared with control BOECs. We found that even within a single VWD type, BOECs from different patients presented with diverse angiogenic characteristics. This finding is consistent with results found by Dr. Eikenboom and Dr. Randi's groups and highlights the main difficulty in isolating the effects of VWF on angiogenesis in BOECs: inter-individual variability affects all expression profiles including those of many other angiogenic regulators.

Our lab is also interested in understanding the mechanism by which VWF HMWM modulate vessel formation. As previously mentioned, most currently available plasma-derived VWF concentrates lack VWF HMWM, while new recombinant VWF contains the full complement of multimers. BOECs present a unique opportunity to elucidate the

mechanisms through which these two different VWF replacement therapy influence angiodysplasia. To accomplish this, our current studies involve treating VWD patient-derived BOECs with plasma-derived VWF and recombinant VWF that has been digested to carry varying levels of multimers and subsequently, performing angiogenesis assays on these treated cells. These experiments have the potential to uncover the role of VWF HMWM in angiogenesis and identify differences in VWF replacement treatment efficacies.

CONCLUSIONS

While much research remains to be done to improve currently available treatments for patients suffering from GI bleeding, our understanding has come a long way since Dr. Erik von Willebrand first brought attention to this complex disease. VWF wears many hats in the vasculature, and its role in angiogenesis provides a potential avenue for treatment for a long fought clinical complication. These initial studies on VWD patient BOECs present a solid foundation from which future studies can be catapulted and holds promise for delineating the pathological mechanisms for different VWF mutations.

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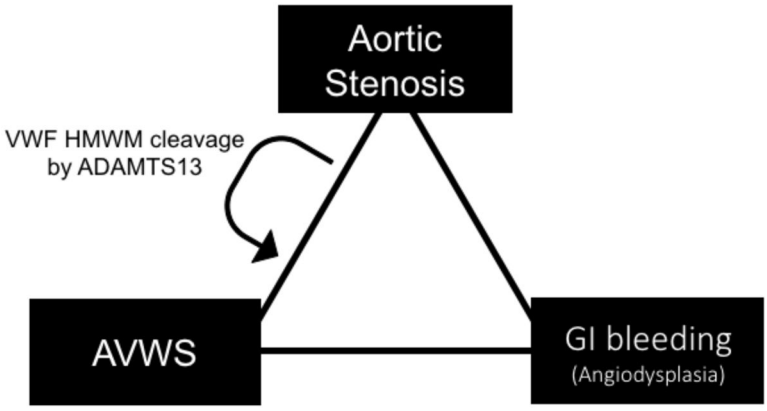

Figure 1. Heyde's syndrome refers to the triad of conditions including aortic stenosis, AVWS, and GI bleeding as a result of angiodysplasia

In individuals with aortic valve stenosis, the aortic valve can be become narrow causing blood jet. This high flow rate often makes VWF more susceptible to cleavage by ADAMTS13 resulting in the loss of HMWM of VWF and AVWS. Consequently, patients can develop angiodysplasia in the GI tract which can lead to severe and intractable bleeding.

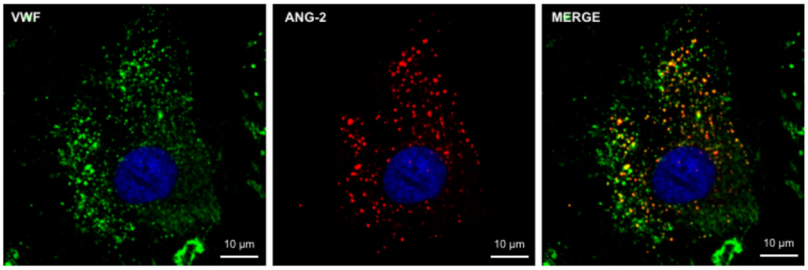

Figure 2. Ang-2 is synthesized by ECs and stored in WPBs alongside VWF

Confocal images of BOECs from healthy control non-VWD individuals with VWF (green), Ang-2 (red), and DAPI nuclear (blue) staining. Ang-2 co-localizes with VWF in yellow WPBs around the nucleus.

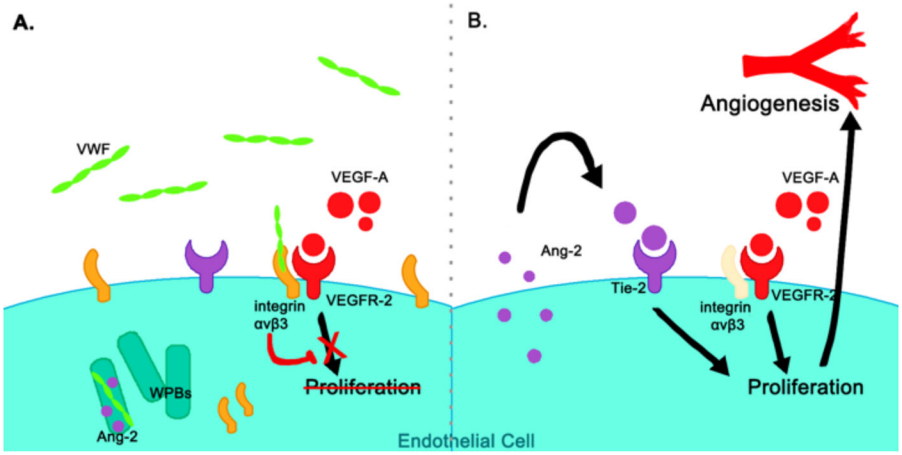

Figure 3. VWF is a negative regulator of angiogenesis[28]

(A) VEGF-mediated angiogenesis is regulated by extracellular VWF binding integrin αvβ3 to inhibit VEGFR-2 signaling and by intracellular VWF promoting the formation of WPBs to sequester the pro-angiogenic molecule Ang-2. (B) A lack of VWF allows Ang-2 to escapes the cell to promote EC proliferation, motility, and sprouting via signaling of the Tie-2 receptor.