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Low von Willebrand factor phenotype: the enigma continues

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The cause of mildly reduced plasma levels of von Willebrand factor (VWF) has been elusive, but in this issue of *Blood*, Ng et al[1] describe results of a comprehensive series of studies that advance our understanding of this common condition.

The quantitative pathologies associated with abnormal plasma VWF levels range from a prothrombotic state with high VWF to bleeding with low levels of the protein. von Willebrand disease (VWD) is likely the most common inherited bleeding disorder,[2] and the most frequent subtype of the disease is type 1 VWD, manifested as mild to severe deficiencies of normally functional VWF.

Over the last 15 years, the molecular pathology of VWD has been extensively documented, and although pathogenic variants in the *VWF* gene are found in >90% of patients with types 2 and 3 VWD, causative genetic variants are only identified in ~65% of patients with type 1 VWD.[3-5] Furthermore, in patients with VWF plasma levels between 0.30 and 0.50 IU/mL pathogenic variants in *VWF* are even less evident. In accordance with the recent American Society of Hematology/International Society on Thrombosis and Haemostasis/National Hemophilia Foundation/World Federation of Hemophilia VWD guidelines, these patients are currently classified as either type 1 VWD, if they demonstrate a significant bleeding phenotype, or low VWF if they only have low VWF plasma levels.[6]

Although low levels of VWF could result from problems anywhere in the protein's life cycle, from VWF gene expression to protein clearance, Ng et al focus their efforts on circulating endothelial colony-forming cells (ECFCs) derived from patients with a low VWF phenotype. As previous examination has shown that accelerated VWF clearance seems to be only a minor contributor to low VWF levels,[7] the detailed analysis of biosynthesis, storage, and secretion from the endothelial cells of these patients is well justified.

There are 3 groups of findings in this study (see figure). The first is that there are abnormalities of VWF storage and secretion from endothelial Weibel-Palade bodies (WPBs). In the low VWF ECFCs, there are modest but statistically significant reductions in the numbers and size of WPBs, and the shape of these storage organelles is also abnormal. Furthermore, stimulated release of VWF was impaired from low VWF endothelial cells. This latter finding correlates with the study's demonstration in low VWF ECFCs of differential gene expression of several proteins involved in endothelial exocytosis.

The second group of observations derive from the study's single-cell mRNA sequencing (scRNA-seq) analysis. The results obtained in ECFCs show transcriptome similarities with human umbilical vein endothelium and indicate that the pattern of gene expression in these circulating cells most closely resembles venous and capillary endothelium.

Quantification of mRNA levels in the low VWF ECFCs showed an overall reduction of VWF transcripts, although ECFCs from 1 of the 5 patients with low VWF demonstrated normal levels of VWF transcript, suggesting that at least, in this subject, the pathogenic cause was located downstream of mRNA synthesis. Low VWF mRNA levels have been previously documented in the endothelial cells of patients with VWD.[8]

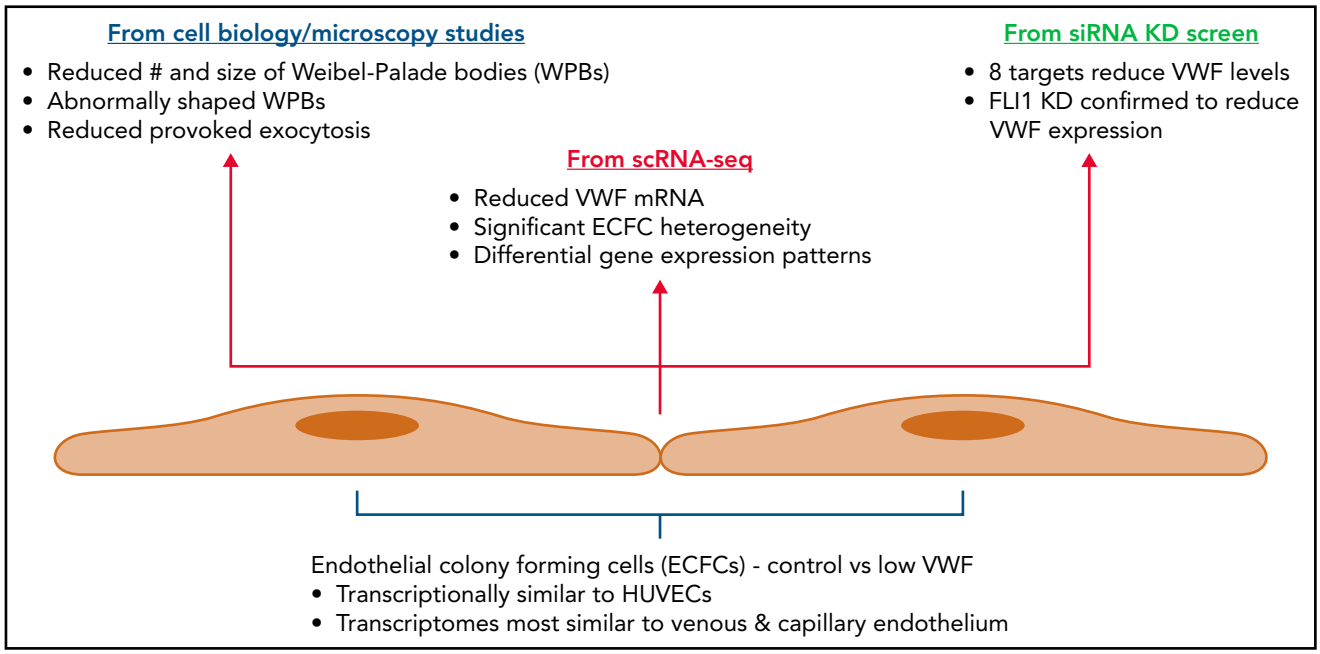
The other major finding from the study's scRNA-seq analysis was the profound heterogeneity of ECFC gene expression. Although, overall, the authors were able to conclude that gene expression patterns were different between low VWF ECFCs and control cells, they could not demonstrate a gene expression signature that consistently aligned with low VWF expression. These results are in agreement with prior recognition of the very significant vascular bed and even cell-to-cell variability in endothelial gene expression patterns.[9]

Finally, the authors used a combination of 3 differential gene expression analyses along with several endothelial biological rationales to develop a small inhibitory RNA screen to evaluate effects on VWF transcript production. These studies showed that *FLI1* was among the candidates for influencing VWF expression, and additional experiments focused on *FLI1* further confirmed this observation. The precise mechanisms responsible for how the eventual reduction of VWF expression occurs remain to be determined.

Overall, the findings of this comprehensive investigation of endothelial cell expression of VWF in patients with a low VWF phenotype have added important new information to this area of VWF pathobiology. However, there is more to be done. The study population here was small (5 cases with low VWF), and 1 of these patients did not demonstrate low VWF mRNA expression. In addition, several of the patients with low VWF also possessed both coding and noncoding *VWF* variants that remain of uncertain pathogenic significance. Last, the authors recognize that genomic variants (both frequent and rare) in the VWF regulatory region might influence VWF expression, as has previously been documented.[10]

This study has also shown evidence of VWF storage organelle abnormalities in low VWF ECFCs and has demonstrated a reduced response to provoked exocytosis. What has not been studied here is the potential pathogenic influence of mRNA processing or stability or the impact of altered VWF protein clearance.

In conclusion, the pathogenic basis of the common low VWF phenotype is almost certainly multifactorial and very likely involves variances in different stages in the VWF life cycle in different patients. This study confirms that one of



Studies of 5 isolated ECFCs from patients with the low VWF phenotype show several abnormalities of VWF expression, storage, and release. Of particular note, scRNA-seq demonstrated reduced VWF mRNA levels in 4 of 5 patients, and a different pattern of gene expression was seen in low VWF vs control ECFCs. HUVECs, Human umbilical vein endothelial cells; KD, knock down.

those mechanisms involves reduced VWF mRNA production in endothelial cells. Additional investigation is now required on larger study populations with complementary experimental approaches to achieve the next level of resolution of this enigmatic pathology.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood.2021013541