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Not All Heme is "Free" in Sickle Cell Disease

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Sickle cell disease (SCD) is an inherited hematologic disorder caused by a β-globin gene point mutation that results in the production of sickle hemoglobin (hemoglobin S [HbS)]. HbS tends to polymerize upon deoxygenation, causing the sickling of red blood cells (RBCs). RBC deformation initiates a sequence of events leading to multiple complications, including hemolytic anemia, vaso-occlusion, chronic inflammation, and tissue damage, which contribute to painful crises and aggravate disease severity.[1]

Extracellular vesicles (EVs) are a heterogeneous group of cell-derived membranous structures (0.1–1.0 μm) comprising exosomes and microvesicles, which originate from the endosomal system or are shed from the plasma membrane, respectively. EVs are released from cells under normal and activated conditions and can contain various cargos including proteins, lipids, and nucleic acids, which may reflect the state of activation of the cells from which they originate and can serve as vehicles for cellular communication. They are present in biological fluids and are involved in multiple physiological process as well as contribute to pathological processes.[1] The level of platelet-, leucocyte-, endothelial cell (EC)-, and RBC-derived EVs (REVs) is elevated in the bloodstream of patients with SCD at steady state and further increased upon vaso-occlusive crises. REVs are generated upon deformation of RBC involving membrane proteins such as band-3 and sphingomyelin, and are the most abundant in SCD. REVs in SCD (SCD REVs) are likely produced through accelerated ageing of RBCs during oxygenation/deoxygenation cycles, under the influence of stress factors, or during intravascular hemolytic events. SCD REVs have increased phosphatidylserine expression on their surface and carry heme, hemoglobin, and microRNAs.[1,2]

Being endothelial activation and sickle RBC adhesion central to the pathogenesis of SCD, a recent study assessed the impact of REVs generated from SCD RBCs on the microvasculature. Taking advantage of an endothelium-on-a-chip platform, An and co-authors analyzed RBC adhesion to REV-activated human pulmonary microvascular ECs under physiological flow conditions.[3] Interestingly, REVs were more abundantly generated from RBCs of SCD patients compared with RBCs from healthy individuals and contained higher levels of heme.[3] The exposure of ECs to REVs promoted EC activation as suggested by the elevation of Von Willebrand factor (VWF) expression.

Under microfluidic conditions, more RBCs (either from SCD or healthy individuals) adhered to ECs exposed to SCD REVs compared with control REVs, suggesting that REVs from SCD patients more potently activate the vascular endothelium and promote EC–RBC interaction.[3]

Intravascular hemolysis promotes endothelial activation and causes endothelial dysfunction through heme-mediated activation of TLR4 signaling pathway in ECs.[4–6] The observation that the incubation of REVs from SCD RBCs with the heme scavenger hemopexin reduced EC activation and RBC adhesion suggested that heme contained in REVs is the major mediator of EC activation. Importantly, this observation indicates that REV-derived heme represents an additional heme source other than the circulating one.

Heme is known to rapidly mobilize VWF from Weibel-Palade bodies and P-selectin onto the EC surface and cause vaso-occlusion in SCD.[4] VWF multimers released from ECs result in a significant increase in adhesion of SCD RBCs to endothelium, suggesting a key role of VWF in the pathophysiology of SCD-induced microvascular occlusion.[4] However, the relationship of this finding and SCD REVs has remained so far unexplored. The VWF-specific protease ADAMTS13 was observed to significantly reduce the adhesion of SCD RBCs to SCD REV-activated ECs, proving the dependence of REV-induced RBC adhesion on EC-released VWF. Finally, using a dorsal skin-fold chamber model to monitor vascular stasis in SCD mice, the authors demonstrated that infusion of hemin or SCD REVs induced microvascular stasis in SCD mice and this is inhibited by intravenous ADAMTS13 administration (Figure 1).

Interestingly, heme also retains the ability to activate immune cells, including macrophages and neutrophils, through TLR4 activation.[7,8] This process leads to inflammatory cytokine

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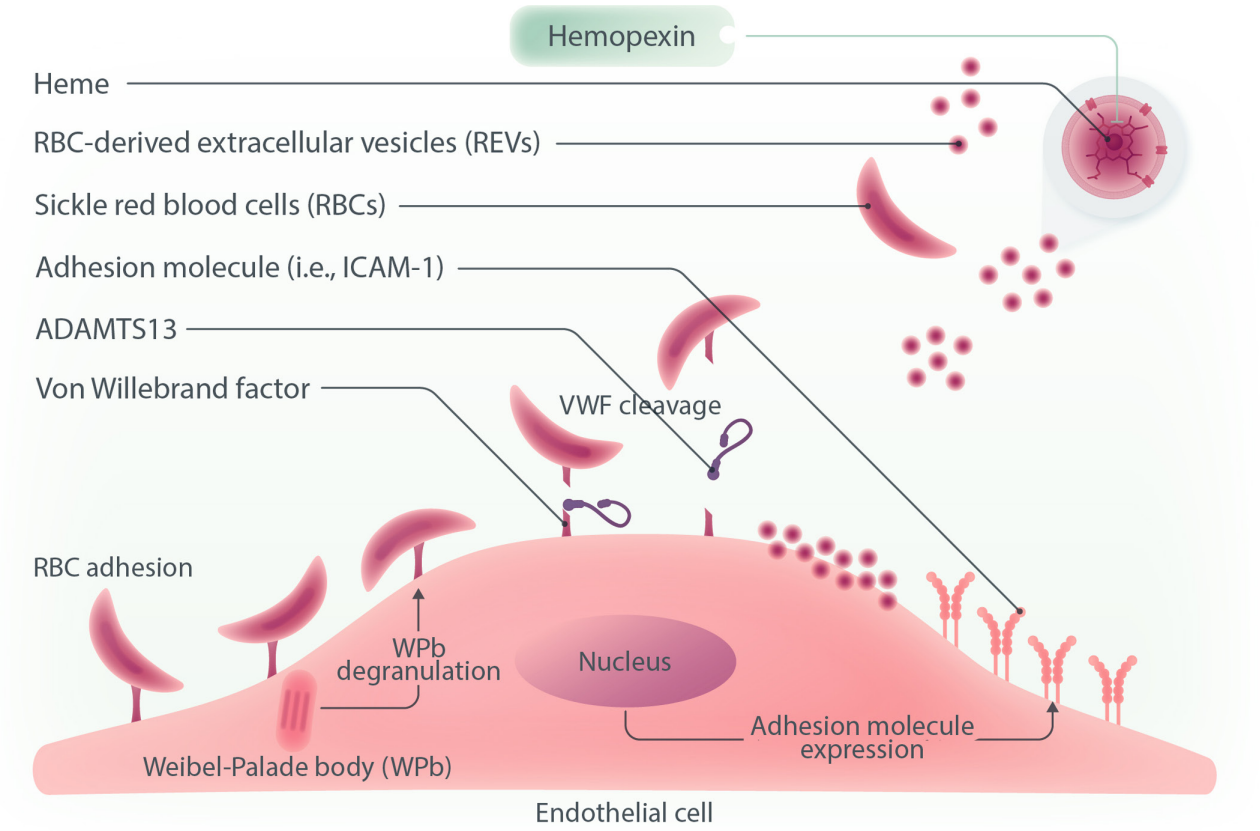

Figure 1. REVs mediate SCD microvasculature activation and promote RBC adhesion. SCD REVs promote VWF expression in ECs by releasing their heme content and stimulating Weibel–Palade body degranulation. Due to enhanced endothelial VWF, SCD RBCs adhere to REV-activated ECs. RBC adhesion is decreased by VWF cleaving and heme scavenging mediated by the protease ADAMTS13 and the heme-binding protein hemopexin, respectively. ECs = endothelial cell; RBC = red blood cells; REV = RBC-derived EVs; SCD = sickle cell disease; VWF = Von Willebrand factor.

production and underlies the chronic inflammatory landscape typically associated with SCD.[7,8] Heme-enriched REVs likely play a role in the activation not only of endothelial but also immune cells in SCD, contributing to the inflammatory state of SCD.

Lactate dehydrogenase (LDH) levels and absolute reticulocyte counts (ARCs) are relevant hemolysis biomarkers that have been linked to disease severity. An and coauthors found that SCD patients with higher LDH levels and ARCs had significantly greater RBC adhesion to REV-activated ECs compared with those with lower LDH and ARCs, indicating that severe hemolysis enhances RBC adhesive properties.[3] Similarly, patients with a documented history of deep vein thrombosis (DVT) had higher RBC adhesion to REV-activated ECs compared with those without DVT. The higher RBC adhesivity likely indicates more severe cell sickling, which promotes cell entrapment within venous clots and, thus, DVT.[3]

Overall, this recent study is a proof of how understanding the collective interplay between RBCs and REV-activated microvasculature helps characterizing the multicellular and multifactorial paradigm of adhesion triggering vaso-occlusive events in SCD. Especially, in the expanding field of EV biology, the contribution of EVs to pathologic mechanisms in diseases conditions is becoming evident, and these findings highlight the critical contribution of REVs to SCD pathophysiology through microvascular activation and abnormal RBC adhesion. Although much remains unknown regarding SCD EVs, this work indicates that REVs are a novel targetable pathological mechanism underlying vaso-occlusive crises and suggests that targeting REVs, their formation or content, may be an effective treatment to limit RBC–EC interaction in SCD.

AUTHOR CONTRIBUTIONS

FV conceived and wrote the article.

DISCLOSURES

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REFERENCES

1. Lamarre Y, Nader E, Connes P, et al. Extracellular vesicles in sickle cell disease: a promising tool. *Bioengineering*. 2022;9:439.
2. Camus SM, De Moraes JA, Bonnin P, et al. Circulating cell membrane microparticles transfer heme to endothelial cells and trigger vasoocclusions in sickle cell disease. *Blood*. 2015;125:3805–3814.
3. An R, Man Y, Cheng K, et al. Sickle red blood cell-derived extracellular vesicles activate endothelial cells and enhance sickle red cell adhesion mediated by von Willebrand factor. *Br J Haematol*. 2023;201:552–563.
4. Belcher JD, Chen C, Nguyen J, et al. Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. *Blood*. 2014;123:377–390.
5. Vinchi F, Tolosano E. Therapeutic approaches to limit hemolysis-driven endothelial dysfunction: scavenging free heme to preserve vasculature homeostasis. *Oxid Med Cell Longev*. 2013;2013:396527.
6. Vinchi F, Sparla R, Passos ST, et al. Vasculo-toxic and pro-inflammatory action of unbound haemoglobin, haem and iron in transfusion-dependent patients with haemolytic anaemias. *Br J Haematol*. 2021;193:637–658.
7. Dutra FF, Bozza MT. Heme on innate immunity and inflammation. *Front Pharmacol*. 2014;5:115.
8. Vinchi F, Costa da Silva M, Ingoglia G, et al. Hemopexin therapy reverts heme-induced proinflammatory phenotypic switching of macrophages in a mouse model of sickle cell disease. *Blood*. 2016;127:473–486.