

# blood

2008 111: 3301-3302  
doi:10.1182/blood-2008-01-131482

## Soluble protein C receptor: why?

Margarethe Geiger

---

Updated information and services can be found at:  
<http://bloodjournal.hematologylibrary.org/cgi/content/full/111/7/3301>

---

Information about reproducing this article in parts or in its entirety may be found online at:  
[http://bloodjournal.hematologylibrary.org/misc/rights.dtl#repub\\_requests](http://bloodjournal.hematologylibrary.org/misc/rights.dtl#repub_requests)

Information about ordering reprints may be found online at:  
<http://bloodjournal.hematologylibrary.org/misc/rights.dtl#reprints>

Information about subscriptions and ASH membership may be found online at:  
<http://bloodjournal.hematologylibrary.org/subscriptions/index.dtl>



# inside blood

1 APRIL 2008 | VOLUME 111, NUMBER 7

● ● ● HEMOSTASIS

Comment on Saposnik et al, page 3442

## Soluble protein C receptor: why?

Margarethe Geiger MEDICAL UNIVERSITY OF VIENNA

In this issue of *Blood*, Saposnik and colleagues show that soluble endothelial-cell protein C receptor (sEPCR) is derived not only from proteolytic ectodomain shedding, but also from alternative splicing of A3 haplotype mRNA transcripts and direct secretion of a truncated sEPCR form.

The serine protease activated protein C (APC) is a major natural anticoagulant, which proteolytically inactivates the clotting factors Va and VIIIa. In addition, APC has potent anti-inflammatory and cytoprotective capabilities.<sup>1</sup> PC circulates in plasma as a zymogen and is activated by thrombin bound to its endothelial receptor, thrombomodulin. Another protein important for the activation of PC is the endothelial protein C receptor (EPCR), which binds protein C and further enhances its activation by the thrombin/thrombomodulin complex.

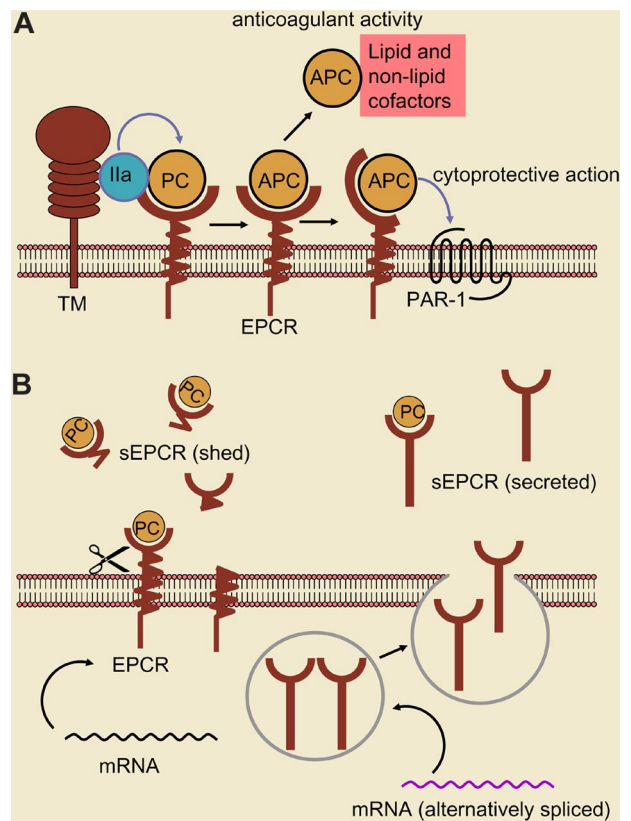
EPCR is a 46-kilodalton (kDa) type I transmembrane protein, which is expressed mainly on the luminal endothelial cell surface of large blood vessels and which is homologous to major histocompatibility complex class I/CD1 family proteins. In addition to the transmembrane EPCR, a soluble form of EPCR (sEPCR) has been described in plasma. sEPCR binds PC/APC with an affinity similar to that of membrane EPCR. Binding of APC to sEPCR interferes with binding of APC to phospholipids and inactivation of factor Va. Furthermore, binding of PC to sEPCR does not enhance APC generation, suggesting a procoagulant effect of sEPCR.<sup>2</sup>

Plasma levels of sEPCR show bimodal distribution: 80% of the healthy population have plasma levels between 75 ng/mL and 178 ng/mL, and 20% have levels between

200 ng/mL and 700 ng/mL.<sup>3</sup> There are polymorphisms in the human *EPCR* gene that define at least 3 different haplotypes. One of them (haplotype A3) is associated with increased plasma levels of soluble *EPCR*.<sup>3</sup> These increased plasma levels have been partially explained by increased shedding of this form.<sup>4</sup>

Saposnik and colleagues have now identified a truncated form of EPCR mRNA that is much more abundant in A3 haplotype EPCR. The deleted sequence (390 base pairs) corresponds to the entire coding region of exon 4 and to a large part of its noncoding region. The truncated mRNA therefore encodes a protein that lacks the transmembrane and intracellular domains, and has a theoretical new C-terminal stretch of 56 amino acids. By transfecting cells with a cDNA that encodes the recombinant

EPCR isoform, they also show that this form is not retained in the membrane, but rather is secreted. They also identify a sEPCR form in plasma samples from A3-carrying subjects, which is the same size as the protein arising from the alternatively spliced EPCR mRNA, suggesting that higher plasma levels of sEPCR in the A3-carrying population result not only from increased ectodomain shedding of membrane EPCR, but also from alternative mRNA splicing. Such a dual mechanism



(A) Binding of protein C (PC) to its membrane receptor, EPCR, enhances its activation by thrombin (IIa) bound to thrombomodulin (TM). Activated protein C (APC) can dissociate from its receptor and—in the presence of cofactors—act as anticoagulant. Membrane-receptor-bound APC exerts cytoprotective activities mediated via PAR-1. (B) sEPCR supports neither PC activation nor APC activity. sEPCR can be generated by ectodomain shedding (left) or, as shown by Saposnik and colleagues in this issue of *Blood*, by alternative mRNA splicing in haplotype-A3-carrying cells, resulting in a protein that is not retained in the membrane but rather secreted (right).

of soluble receptor generation has been shown already for the generation of soluble cytokine receptors.

Biological consequences of increased sEPCR (and/or the resulting decreased membrane EPCR density) may be related to increased procoagulant activity (eg, higher risk of thrombosis). There may also be an impact on the cytoprotective activity of APC, which depends on membrane EPCR and PAR-1. For both functions of PC/APC, the A3 haplotype would be unfavorable. However, there may also be beneficial effects of decreased EPCR receptor density on the cell surface (and thus of the A3 haplotype), since it also has been shown that APC mediates breast cancer cell migration through interactions with EPCR and PAR-1.<sup>5</sup>

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

#### REFERENCES

1. Mosnier LO, Zlokovic BV, Griffin JH. The cytoprotective protein C pathway. *Blood*. 2007;109:3161-3172.
2. Esmon CT. The endothelial protein C receptor. *Curr Opin Hematol*. 2006;13:382-385.
3. Saposnik B, Reny JL, Gaussem P, et al. A haplotype of the EPCR gene is associated with increased plasma levels of sEPCR and is a candidate risk factor for thrombosis. *Blood*. 2004;103:1311-1318.
4. Qu D, Wang Y, Song Y, Esmon NL, Esmon CT. The Ser219→Gly dimorphism of the endothelial protein C receptor contributes to the higher soluble protein levels observed in individuals with the A3 haplotype. *J Thromb Haemost*. 2006;4:229-235.
5. Beaulieu LM, Church FC. Activated protein C promotes breast cancer cell migration through interactions with EPCR and PAR-1. *Exp Cell Res*. 2007;313:677-687.

#### ● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Ghevaert et al, page 3407

## Sizing up platelet defects

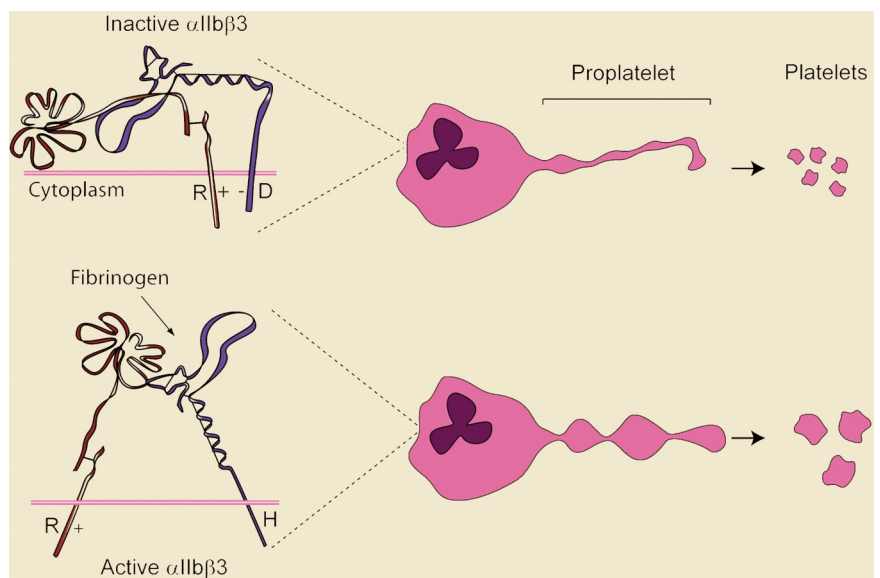
Paul F. Bray CARDEZA FOUNDATION FOR HEMATOLOGIC RESEARCH

In this issue of *Blood*, Ghevaert and colleagues show that a mutation in the cytoplasmic domain of integrin  $\beta_3$  is associated with dominantly inherited macrothrombocytopenia. This mutation is predicted to disrupt a conserved salt bridge with integrin  $\alpha_{IIb}$ , and constitutively activates the integrin.

Large platelets are often encountered in the evaluation of thrombocytopenias, but most often these are acquired conditions. A rather long list of inherited thrombocytopenias is now appreciated.<sup>1</sup> Among the macrothrombocytopenias, the most commonly responsible genetic variations are heterozygosity for mutations in platelet glycoprotein Iba and the Q43P polymorphism of  $\beta 1$  tubulin. The molecular regulation of platelet size is poorly understood, but circumstantial evidence supports a role for the platelet cytoskeleton and microtubular system. The lion's share of this evidence stems from the study of genetic defects in inherited disorders of platelet size. Although these disorders are quite uncommon, an understanding of the molecular basis of platelet size is important because subjects with abnormally high platelet volumes have enhanced platelet reactivity and are at risk for recurrent ischemic coronary syndromes.<sup>2</sup>

Ghevaert and colleagues performed a thorough series of studies characterizing a pedigree for macrothrombocytopenia, with the mean platelet volume of the proband measuring a whopping 17 fL. All affected individuals had heterozygous mutations: P53L in the gene encoding GPIIb $\alpha$  (*GP1BA*), and D723H in the gene encoding integrin  $\beta_3$  (*INTB3*). At least 4 syndromes (Bernard-Soulier, DiGeorge, benign Mediterranean macrothrombocytopenia, and platelet-type von Willebrand disease) are associated with macrothrombocytopenia and *GP1BA* mutations. However, convincing data were provided to suggest that the *GP1BA* P53L mutation was not responsible for the phenotype in this pedigree, and molecular modeling predicted that the *GP1BA* P53L substitution would not affect von Willebrand factor binding. Platelets from subjects with the *INTB3* D723H mutation showed spontaneous  $\alpha_{IIb}\beta_3$  activation, and CHO cells expressing  $\alpha_{IIb}\beta_3$ -D723H displayed enhanced binding to immobilized fibrinogen. Intriguingly, in vitro-differentiated megakaryocytes with the  $\beta_3$ -D723H mutation exhibited larger proplatelet buds than the wild type.

It was not long ago that molecular genetic studies were rather primitive compared with the thorough multidisciplinary approaches used by Ghevaert and colleagues to characterize this pedigree. Several novel findings are presented and questions raised. First,



Model summarizing how integrin activation state may regulate platelet size. The top panel indicates how the salt bridge between wild-type  $\alpha_{IIb}$ -R995<sup>+</sup> and  $\beta_3$ -D723<sup>-</sup> constrains the integrin in an inactive conformation. The  $\beta_3$ -D723H mutant disrupts this linkage and activates the receptor. Abnormally large platelets result via an unknown mechanism.