normal oxygen tension, the α-subunit of HIF is posttranslationally modified by proline hydroxylation so that it is rapidly degraded.[2] Only in hypoxic cells can HIF-α survive, allowing nuclear translocation, αβ dimer assembly, and induction (or repression) of gene expression.

Mechanisms underlying developmental and tissue-specific regulation of Epo expression are less clear.[3] In humans and other mammals, during fetal development, the liver is the primary site of Epo production, with a switch to the kidney at birth. A previous study by Dame et al[4] showed that the binding of the transcription factor GATA-4 to a cognate site on the *Epo* promoter contributes to expression in the fetal liver. In the paper published in this issue, Dame and colleagues demonstrate that the interaction of an isoform of Wt1 (Wt1(−KTS)) at a downstream site on the *Epo* promoter (see figure) is associated with transcriptional activation. They found that in mice deficient in Wt1, the expression of Epo mRNA in the fetal liver was about 60% of that in wild-type mice.

The determinants of tissue-specific expression of Epo appear to be complex.[3] The robust hypoxic induction of Epo in kidney and liver is due in part to cooperation in the 3′ enhancer between HIF and HNF-4, a nuclear receptor that is preferentially expressed in these organs. Both HIF and HNF-4 bind to the transcriptional adapter p300. In addition, as shown in the figure, there is a kidney-inducible element 9 to 14 kb upstream of the promoter. The paper of Dame et al suggests that lower-level production of Epo in the brain and testes is due to coexpression of Wt1 at these sites. These results help set the stage for understanding the biologic significance of low-level Epo production at sites beyond the kidney and liver. ■

REFERENCES

1. Semenza GL. HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol. 2001;13:167-171.
2. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol. 2004;5:343-354.
3. Fandrey J. Oxygen-dependent and tissue-specific regulation of erythropoietin gene expression. Am J Physiol Regul Integr Comp Physiol. 2004;286:R977-R988.
4. Dame C, Sola MC, Lim KC, et al. Hepatic erythropoietin gene regulation by GATA-4. J Biol Chem. 2004;279:2955-2961.

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Comment on Mangin et al, page 4346

GPVI: no magic bullet for thrombosis

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The platelet collagen receptor GPVI has been considered an attractive therapeutic target to treat human cardiovascular diseases. An article by Mangin and colleagues in this issue of *Blood* reveals that loss of GPVI does not produce the expected dramatic effect on arterial thrombosis in mice.

Platelet activation following plaque rupture is thought to initiate formation of the arterial thrombi that underlie stroke and myocardial infarction. Accordingly, antiplatelet therapies have become a mainstay of both chronic and acute treatment of these diseases. The ideal antiplatelet agent has been elusive, however, as agents that completely disable platelet aggregation (such as the αIIbβIII receptor antagonists) confer too great a bleeding risk to be safe chronic therapies, while milder agents that block secondary signaling pathways (such as ADP antagonists and aspirin) are better tolerated but not as effective. The identification of the platelet collagen receptor glycoprotein VI (GPVI) immediately raised hopes for the creation of an ideal antiplatelet therapy because of the receptor's narrowly defined thrombotic role,[1] as well as the observation that GPVI-deficient individuals manifest only a mild bleeding phenotype.[2]

Although earlier studies suggested that GPVI might indeed be the target for a platelet magic bullet,[3] Mangin and colleagues have popped this therapeutic bubble. Using 3 different models of arterial thrombosis in the mouse, they find that loss of GPVI alone has a minimal protective effect (significantly less than that of the ADP receptor antagonist clopidogrel) that can, however, be enhanced by simultaneous thrombin inhibition. Several important questions emerge from this study. First, can we gauge the potential therapeutic value of inhibiting GPVI or any other platelet receptor on the basis of arterial thrombosis models in the mouse? While this question will remain open, Mangin and colleagues directly compare established pharmacologic inhibitors of integrin αIIbβIII, the $P2Y_{12}$ ADP receptor, and thrombin to genetic loss of GPVI, and they observe relative inhibitory effects of these agents that correspond roughly to their efficacy in human trials. Thus, GPVI deficiency appears to have less effect than these agents on acute arterial thrombosis. However, GPVI blockade might be most clinically useful as chronic, prophylactic therapy, an end point not addressed by these studies. Second, do these findings suggest that the role of platelet collagen responses in arterial thrombosis has been overestimated? There is no "relative standard" by which to judge the predictive power of mouse models for pathogenic or physiologic platelet responses in humans, and so this question must remain open. In addition, platelets express 2 collagen receptors, GPVI and the integrin α2β1. α2β1 is essential for adhesion of platelets to collagen under flow but requires inside-out signals to engage collagen.[4] The authors do not directly test the role of α2β1, but suggest that it may be minimal because the loss of collagen adhesion in GPVI-deficient platelets cannot be compensated for by α2β1 ex vivo. However, in an ex vivo assay using anticoagulated blood, the only potential activating signal for integrin α2β1 is GPVI, while in vivo, α2β1 may be activated by other stimuli such as thrombin. Thus, there also remains the possibility of functional collagen receptor redundancy in vivo. Finally, the authors suggest that simultaneous targeting of both thrombin and GPVI signaling may provide synergistic blockade of arterial thrombosis. This is an intriguing finding that hints at the power of exploiting signaling cross talk therapeutically. ■

REFERENCES

1. Penz S, Reininger AJ, Brandl R, et al. Human atheromatous plaques stimulate thrombus formation by activating platelet glycoprotein VI. FASEB J. 2005;19:898-909.
2. Moroi M, Jung SM, Okuma M, Shinmyozu K. A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. J Clin Invest. 1989;84:1440-1445.
3. Massberg S, Gawaz M, Gruner S, et al. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. J Exp Med. 2003;197:41-49.
4. Sarratt KL, Chen H, Zutter MM, Santoro SA, Hammer DA, Kahn ML. GPVI and alpha2beta1 play independent critical roles during platelet adhesion and aggregate formation to collagen under flow. Blood. 2005;106:1268-1277.