sickle cell disease in 87 consecutive patients who underwent transplantation between 1988 and 2004. Patients were initially conditioned with varying doses of busulfan and cyclophosphamide, but due to unstable mixed chimerism in several, busulfan dosing was adjusted to body area, and ATG was added. Rejection rates fell from 22.6% before the addition of ATG to 2.9% thereafter. Transplant-related mortality was 6.9% overall; however, no deaths occurred after the 40th patient. EFS at 5 years was 86.1% overall, similar to the previously reported studies. Importantly, multivariate analysis identified date of transplantation as the only variable that significantly affected EFS; the 5-year EFS was 95.3% for the 44 patients who underwent transplantation after January 2000. Although there has been recent enthusiasm for the development of nonmyeloablative regimens for transplantation in sickle cell anemia, an EFS of 95.3% will prove a high bar to overcome, especially given the high rejection rates observed in studies reported thus far,[3,4] and further supports the exploration of these regimens in older patients at high risk for conventional myeloablative transplantation due to comorbidities. These results represent a significant advance in the treatment of sickle cell anemia, and although the investigators argue that myeloablative stem-cell transplantation should be considered the standard of care for children at high risk for stroke, they might further argue that it should be considered standard of care for children with other disabling complications who have a suitable sibling-matched donor.

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

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● ● ● **HEMOSTASIS**

Comment on Lacroix et al, page 2432

It's not size, it's substance

Edward F. Plow and Elzbieta Pluskota CLEVELAND CLINIC

Lacroix and colleagues provide the first evidence that endothelium-derived microparticles provide an efficient surface for plasminogen activation in a urokinase-type plasminogen activator (uPA)– dependent and uPA receptor (uPAR)– dependent manner, and suggest that generated plasmin may influence angiogenesis.

Microparticles are usually defined as a heterogenous population of small (0.1-1 μm diameter) membrane-coated vesicles, which are released by all cell types upon activation or apoptosis. The first report of microparticles is ascribed to Wolf, who detected the presence of platelet-derived fragments in human plasma in 1967.[1] For many years, the existence of microparticles continued to be acknowledged, but they were simply regarded as "cell dust." However, over the past decade it has become clear that these "dwarf cells" are much more than inert debris. As examples with particular relevance to hemostasis, detection of microparticles in blood is now accepted as a diagnostic and prognostic marker for cardiovascular disease,[2] and both in vitro and in vivo studies implicate circulating microparticles in initiation and propagation of coagulation.[3]

Lacroix and colleagues now assign a new function to microparticles: they can express a profibrinolytic function, thereby complementing their procoagulant activity. These authors demonstrate that plasmin can be generated on the surface of microparticles derived from TNF-α–stimulated endothelial cells. Employing many approaches including electron microscopy and fluorescence-activated cell sorting (FACS), the authors provide convincing evidence that endogenous uPA and uPAR are present on the microparticle surface and, furthermore, that engagement of uPAR by exogenous uPA on the microparticles enhances plasminogen activation. Plasminogen interacts with the microparticles via its lysine binding sites, and α-enolase is identified as a pivotal receptor in mediating this interaction. Thus, the authors elegantly delineate the mechanism by which plasminogen is activated on the microparticle surface. This model does closely resembles the mechanism of plasminogen activation observed on cell surfaces, where plasminogen binding via a variety of its receptors is a prerequisite for its efficient activation,[4] but we now know that this mechanism is operative to microparticles.

Plasmin has a broad substrate repertoire and is capable of degrading fibrin and extracellular matrix proteins, activating various matrix metalloproteases, and participating in cytokine and growth hormone processing. Thus, plasmin not only contributes to fibrinolysis and maintenance of vascular patency, but also is a critical regulator of cell migration and has been implicated in inflammation and angiogenesis. Lacroix and colleagues take the initial steps to implicate microparticles and their regulation of plasmin generation in angiogenesis by analyzing their effect on tube formation by endothelial progenitor cells in an in vitro assay. At low concentrations, microparticles slightly enhance the response, and at higher concentrations they inhibit tube formation. How this model system and the concentration-dependent effects translate to angiogenesis in vivo is a question that remains to be resolved. There are more questions: would endothelium-derived microparticles bind to fibrin and mediate clot dissolution? Do microparticles derived from other cell types also bind and enhance activation of plasminogen? More globally, are microparticles involved in plasmin generation in vivo? These questions now become relevant based on the findings of Lacroix and colleagues. Answers to these and other questions are not likely to be determined by the size of the microparticles, but by the substances that they carry on their surfaces.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● ● **RED CELLS**

Comment on Paffett-Lugassy et al, page 2718

EPOR signaling: 450 million years' history

Ji Zhang and Paul A. Ney ST. JUDE CHILDREN'S RESEARCH HOSPITAL

Erythropoietin and the erythropoietin receptor have been cloned and studied in a lower vertebrate species. Key aspects of their structure and function have been evolutionarily conserved.

Erythropoiesis is a central feature of vertebrate development. Erythroid cells in vertebrates come from 2 lineages, primitive and definitive. Primitive erythropoiesis originates in the embryonic yolk sac or its equivalent and is transient in nature. Definitive erythropoiesis originates in the yolk sac or the aorta-gonad-mesonephros region, shifts to the kidney, spleen, liver, or bone marrow, and lasts for the lifespan of the organism. Erythropoietin receptor (EPOR) signaling is essential for definitive erythropoiesis in mice from the colony forming unit–erythroid progenitor stage onward,[1,2] but little is known about the role of EPOR signaling in lower vertebrates. In this issue of *Blood*, Paffett-Lugassy and colleagues take us back 450 million years, to the time when fish diverged from higher vertebrates in evolution, to show that the EPO–EPOR signal transduction axis is a highly conserved component of vertebrate erythroid development.

Paffett-Lugassy and colleagues have cloned the zebrafish homologs of EPO and the EPOR (see figure). Zebrafish *epo* is only 35% identical to human EPO; however, there is considerable homology between the proteins in their secondary and tertiary structure. Zebrafish *epo* possesses a subset of the N- and O-linked glycosylation sites that are important for mammalian EPO activity. Zebrafish *epor* is only 27% identical to the human EPOR, but also retains key structural features. In its extracellular domain, the zebrafish *epor* possesses 4 highly conserved cysteine residues and a "WSXWS" domain. In its cytoplasmic domain, zebrafish *epor* has a conserved hydrophobic patch, and box 1 and box 2 domains, which are essential for the binding and activation of janus kinase 2. Five out of 8 tyrosine

residues, which serve as docking sites for downstream signal transduction pathways, are also conserved.

Importantly, Paffett-Lugassy and colleagues demonstrate that the EPO-EPOR signal transduction axis is functional in zebrafish. Zebrafish *epo* expression is strongly induced in the hypochromic *weissherbst* mutant and, to a lesser extent, by hypoxia. Microinjection of zebrafish *epo* mRNA into 1-cell–stage embryos causes expansion of erythroid populations and polycythemia. Finally, morpholinos against the zebrafish *epor* cause a decrease in primitive erythropoiesis and a complete block of definitive erythropoiesis. Hu-

man EPO was not active in zebrafish, suggesting that the ligand-receptor pair has coevolved.

Zebrafish may provide a useful model for the investigation of signal transduction pathways downstream of the EPOR. In that regard, Paffett-Lugassy and colleagues identified a potential STAT5 docking site in the zebrafish *epor* and showed that morpholinos to zebrafish *stat5.1* cause decreased primitive erythropoiesis and a block of definitive erythropoiesis. However, STAT5 affects other hematopoietic compartments in mice,[3,4] and this experiment does not establish the developmental stage at which STAT5 is required. Furthermore, STAT5 may function as a transcriptional repressor to regulate primitive erythropoiesis in *Xenopus*, suggesting a different mechanism of action.[5] The role of STAT5 in EPOR signaling has been studied with receptor mutants in mice,[6] and this approach may also be informative in zebrafish. Additional biochemical and genetic studies in the zebrafish model should help define the essential aspects of EPOR signaling, as well as functionally significant changes that have occurred over the past 450 million years.

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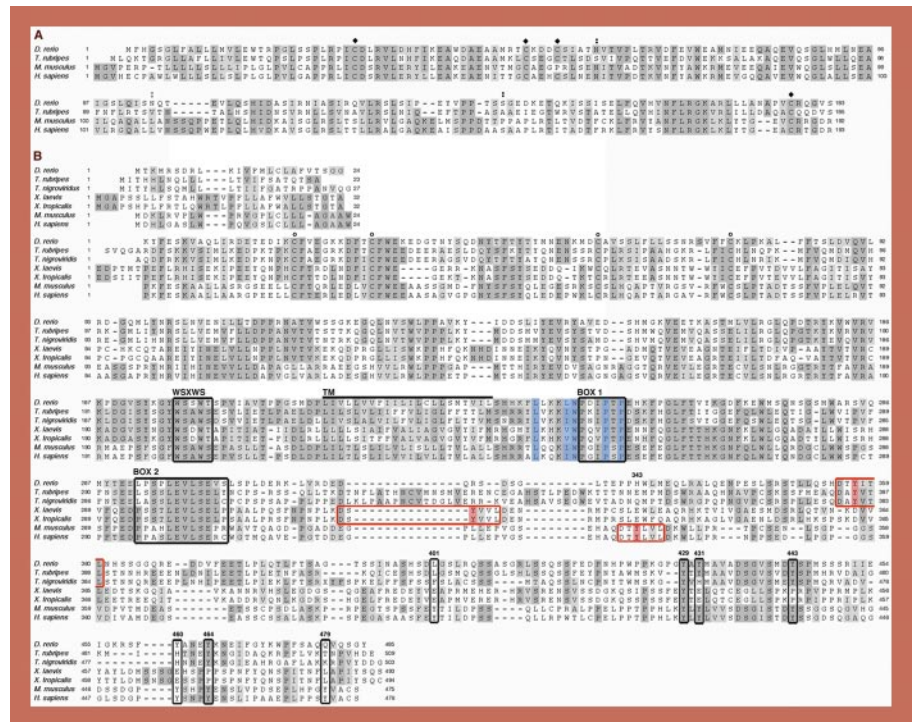
Alignment of vertebrate Epo and Epor sequences highlights conserved functional residues. See the complete figure in the article beginning on page 2718.