Comment on Lemmerhirt et al, page 3061

Mouse VWF again teaches us diversity of mechanisms

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von Willebrand disease is a heterogeneous bleeding disorder in which the von Willebrand factor is abnormal in concentration, function, and/or structure. Each of these properties has a variable effect on the clinical and laboratory phenotype.

Human von Willebrand disease (VWD), particularly type 1 VWD, continues to be a *clinically* baffling disorder.[1] Two recent large VWD studies have identified families with low VWF that can be either genetically linked or nonlinked with the VWF gene.[2,3] While the spectrum of genetic mutations causing low VWF is wide[4,5] and may not be applicable across different species, studying broad mechanisms using inbred strains offers the opportunity to identify the spectrum of alterations that result in an increase or a decrease in VWF levels.

It is clear in the mouse that markedly different plasma levels have been selected through inbreeding and natural derivation of mouse strains. Since mouse strains have up to a 20-fold difference in levels of plasma VWF,[6] they offer a unique model to identify variable mechanisms for producing such marked variation. Some of these mechanisms are extragenic causes, such as the *Mvwf1* modifier previously reported by this group[7] that alters VWF clearance by means of altered glycosylation. In this issue of *Blood,* Lemmerhirt and colleagues report an intragenic variation within the VWF gene that results in a 20-fold greater level of VWF, and, notably, the alteration in the CASA/RkJ is a change that is present in human VWF but not in a variety of other inbred mouse strains. Whether this mutation results in increased biosynthesis and/or secretion, or decreased clearance, is not yet clear. Nevertheless, sequence differences can obviously account for differences in plasma levels that bracket the differences seen in humans—both in healthy individuals (50%-150% of normal) as well as those with VWD (10%-20% of normal). Whether this variability in murine VWF has been selected by chance during inbreeding or by natural biologic selection is not clear. This will undoubtedly be the subject of further study. The study also suggests that there is the biological possibility that human VWF gene alterations may potentially *increase* or *decrease* plasma VWF—although thus far only mutations that decrease VWF levels have been identified.[4,5]

The heterogeneity in our understanding of VWF and VWD continues. We will probably find VWF alterations that produce elevated VWF in humans, another variable to consider in our clinical diagnosis of VWD and the variability found within even a single family. Mice and creative scientists, including Lemmerhirt et al in this issue of *Blood,* continue to push our understanding of human biology. ■

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Comment on Poliseno et al, page 3068

MicroRNAs control angiogenesis

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The elegant report by Poliseno and colleagues indicates for the first time the functional role of microRNAs (miRNAs) in endothelial cells. Specifically, they identify miR-221 and miR-222 as key players in the control of angiogenesis, showing that they modulate the expression of kit receptor in human umbilical vein endothelial cells (HUVECs) and hence the angiogenic properties of the kit ligand, also called stem cell factor.

MicroRNAs (miRNAs) are a novel regulatory class of noncoding, single-stranded RNAs of approximately 22 nucleotides, that have been identified in plants and animals.[1] miRNAs repress protein expression at posttranscriptional level, mostly through base pairing to the 3′ untranslated region (UTR) of the target mRNA, thus leading to its degradation and/or reduced translation. miRNAs have been shown to control basic biologic functions, such as cell growth and differentiation. Despite these advances, few targets for the approximately 300 known mammalian miRNAs have been validated so far, hampering delineation of miRNA-based regulatory circuitries.

Little is known on the role of miRNAs in hematopoietic[2] and endothelial cells. Interestingly miR-221 and miR-222 control the growth of erythropoietic and erythroleukemic cells, as well as the hematopoietic stem cell activity, through regulation of kit receptor expression at translation level.[3] The report by Poliseno and colleagues identifies a

similar regulatory circuitry in endothelial cells.

A growing stream of research may unveil the functional role of miRNAs in endothelial and hematopoietic cells. In this regard, Polisena et al indicate that a series of miRNAs are expressed in endothelial cells, while at least 49 miRNAs are expressed in hematopoietic cells according to lineage- and/or stage-specific patterns (N. Felli and C.P., unpublished results, 2006). Future miRNA studies may have an extraordinary impact, not only at the basic research level but also in the biotechnology/therapy area; indeed, chemically modified anti-miRNAs (antagomirs) have been shown to effectively up-modulate the expression of diverse miRNA targets for extended time periods in vivo.[4]

Finally, the effects of miR-221 and miR-222 on both hematopoietic and endothelial cells through regulation of kit translation once again highlights the remarkable similarity of the expression/function of diverse growth factor receptors (Flt1, KDR, Tie2, and Kit) in hematopoietic and endothelial cells, particularly at the level of primitive cells deriving from the bipotent hemangioblast.[5] ■

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HEMOSTASIS

Comment on Hoffman et al, page 3053

Heavy metal FIX for Christmas wounds

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Hoffman and colleagues provide evidence for the link between the coagulation process, wound healing, and angiogenesis. Compared with wild-type mice, those with Christmas disease lacking coagulation factor IX (FIX) developed subcutaneous hematomas and exhibited delayed monocyte infiltration and impaired wound healing after 3-mm punch biopsies were placed in the skin. Surprisingly, the wounds of hemophilic mice contained twice as many blood vessels as those in wild-type animals

Wounds are a part of life, and wound healing affects everyone at some time. Nonhealing, chronic wounds impair the quality of life for millions of Americans, costing billions of health care dollars. In this issue of *Blood*, Hoffman and colleagues provide evidence for the link between the coagulation process, wound healing, and angiogenesis. Compared with wild-type mice, those with Christmas disease lacking coagulation factor IX (FIX) developed subcutaneous hematomas and exhibited delayed monocyte infiltration and impaired wound healing after 3-mm punch biopsies were placed in the skin. Surprisingly, the wounds of hemophilic mice contained twice as many blood vessels as those in wild-type animals.

Wound healing[1] is divided into 4 phases. The first includes triggering of the coagulation mechanism following injury to vessels with exposure of tissue factor and activation of a cascade of enzymes and cofactors that leads to thrombin generation, platelet activation, and fibrin deposition—the provisional wound matrix upon which platelets adhere and aggregate in the wound base. Growth factors, including basic fibroblast growth factor and platelet derived growth factor, are released from activated platelets and are the precursor to the inflammatory phase marked by the appearance of monocytes. Cytokines and other immunoeffector molecules result in rubor, calor, tumor, and dolor, and functio laesa—cardinal manifestations of wounds. The proliferation phase begins with the appearance of fibroblasts and is followed by the maturation phase in which collagen is deposited and then remodeled to enhance tensile strength. Vessel formation is critical to each phase of wound healing.[2] The mediators of this process are not known, but Hoffman and colleagues hypothesize that Fe may play a role. The formation of subcutaneous hematomas in hemophilic but not wild-type mice results in the deposition of Fe in the wound base that is released from erythrocyte heme by the action of heme-oxygenase, an enzyme from monocytes.

The multiple functions of Fe in cell metabolism were recently reviewed by Le and Richardson.[3] Fe is an obligate requirement for life and its depletion leads to G_1/S arrest and apoptosis. Fe deprivation inhibits the growth of tumors such as neuroblastoma, a highly vascular and aggressive malignancy, and reduces the activity of the R2 subunit of ribonucleotide reductase necessary for DNA synthesis, decreases the expression of cyclins A, B, and D, and results in hypophosphorylation of the retinoblastoma protein. The levels of p53 increase following Fe chelation via the ability of hypoxia-inducible factor to bind to and stabilize p53 and by phosphorylation at serine 15 and serine 37 that prevents the interaction of p53 with murine double minute-2 (mdm-2) leading to p53 degradation. Chelating Fe also increases the mRNA levels of the p53-inducible cyclin-dependent kinase (cdk) inhibitor, p21 (WAS1/CIP1). In contrast to the precious metals such as silver, the most abundant element on earth, iron (35% by mass), may be the key to healing wounds. ■

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