careful study. In this study, the authors suggest that the PDGFRβ$^+$ hemangioprecursors may represent a transient, heterogeneous hemangioblastic cell population. PDGF-BB, functioning as a switch toward endothelial cell development, may induce endothelial cell differentiation directly, by directly stimulating PDGFRβ expressed on the hemangioprecursors, or indirectly, by stimulating the production of other growth factors (such as VEGF) by the committed perivascular cells. As a consequence, the differentiation of hemangioprecursors into the hematopoietic lineage was negatively suppressed. Additional proof for the authors' hypothesis might be provided by future research examining whether PDGFRβ inhibition, for example, via selective PDGFRβ kinase inhibitors (such as imatinib mesylate) or neutralizing antibodies to PDGFRβ or PDGF-BB, could reverse the trend of differentiation between the endothelial and hematopoietic lineages in the yolk sacs that express the kinase-activating PDGFRβ mutant or with ubiquitous PDGF-BB expression. However, it is worth noting that, in addition to hemorrhages and defects in kidney glomeruli because of a lack of mesangial cells, mice with PDGFRβ or PDGF-BB knockout also manifest severe hematologic abnormalities, including thrombocytopenia, erythroblastosis, and severe anemia.[1,2]

With the success of bevacizumab, an anti-VEGF antibody, the antiangiogenesis approach is gradually being accepted as an effective and practical means of anticancer therapy. A number of strategies are being explored to directly target the endothelial cells by inhibiting their growth/survival mechanisms (for example, with the use of antibodies to VEGF and its receptors[6]). Recently, pericytes have been heralded as potential targets for vascular destruction. Since pericyte recruitment to coat nascent vessels is essential for the stabilization and further establishment of the vascular network, vessels lacking adequate pericyte coverage are more vulnerable to VEGF inhibition.[7] Of the apparent approaches for negatively affecting pericyte coverage and function, perhaps the most logical and effective is to block the PDGFβ signaling pathway in these cells. However, it is rather surprising that only very limited antiangiogenic/antitumor benefits have been achieved so far when PDGFRβ antagonists have been tested as single agents. On the other hand, PDGFRβ antagonists have been shown to significantly enhance the antitumor activity of a number of chemotherapeutic agents when used in combination.[8] The enhanced antitumor effect usually correlates well with increase in tumor uptake of the cytotoxic agents, most likely as a result of reduction of interstitial fluid pressure in tumor after treatment with PDGFRβ antagonists.[8] A number of studies have demonstrated that PDGFRβ antagonists (antipericytes) could also generate additive/synergistic antitumor activity when combined with anti-VEGFR2 agents (antiendothelial cells), such as an anti-VEGFR2 antibody (DC101; J. Shen and Z. Z., unpublished observations, 2006) and small molecule inhibitors to VEGFR2 kinase.[9] Recently, Song and colleagues[10] reported the identification of a subset of PDGFRβ$^+$/sca-1$^+$ progenitor perivascular cells (PPCs) that could be recruited from bone marrow to perivascular sites in tumors. Through a paracrine mechanism involving growth factors produced by endothelial cells, including PDGF-BB, such precursors, like the previously described VEGFR2$^+$ embryonic stem cells,[4] could differentiate into mature pericytes and regulate vessel stability and survival in tumors. Specific inhibition of PDGFRβ signaling with a neutralizing antibody to the receptor eliminates PDGFRβ$^+$ PPCs and mature pericytes around tumor vessels, leading to increased endothelial cell apoptosis.[10] Taken together, these findings should lend support to the use of PDGFRβ antagonists in combination with other antitumor and/or antiangiogenic agents in the treatment of a broad range of human cancers. ■

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HEMOSTASIS

Comment on Zaitsev et al, page 1895

Change erythrocytes into thrombolytic agents

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In this issue of *Blood*, Zaitsev and colleagues describe an elegant technique of binding the tPA alteplase to the surface of erythrocytes via CR1, which is primarily expressed on erythrocytes. Anti-CR1 antibody tPA conjugates injected intravenously were bound to circulating erythrocytes, and effectively accelerated dissolution of lung emboli and prevented stable occlusive carotid arterial thrombi from forming without the consequence of bleeding.

For more than 10 years, recombinant tissue-type plasminogen activator (rtPA) has been used for thrombolytic therapy in acute ischemic stroke.[1] In this treatment, 0.9 mg/kg (in the United States and Europe) or 0.6 mg/kg (in Japan) of an rtPA such as alteplase is intravenously administered to patients within 3 hours of the onset of a stroke, successfully ameliorating the outcome of hyperacute embolic stroke. However, symptomatic

hemorrhagic transformation is the primary complication. Therefore, monitoring acute intracerebral hemorrhage with computed tomography (CT) and/or magnetic resonance imaging (MRI) is necessary to reduce risk of bleeding secondary to the thrombolytic therapy with rtPA. This has limited the application of rtPA to less than 5% of patients with stroke.

Many efforts have been made to reduce this problem, and several methods have been established, including the use of a combination of rtPA and a free-radical–trapping agent, NXY-059.[2] Another promising possibility would be the development of a novel thrombolytic agent, especially mutated or modified rtPA, that has higher affinity to fibrin clots, prolonged half-life in circulating blood, and lower neuronal toxicity.

A hint about how to develop such a modified rtPA is given in the elegant work by Zaitsev and colleagues in this issue of *Blood*. They prepared an anti–complement receptor type 1 (CR1)/tPA conjugate, namely tPA conjugated to a monoclonal antibody against CR1, that specifically bound to circulating erythrocytes via CR1, and demonstrated its potential use as a thromboprophylactic agent without significant bleeding both in a pulmonary emboli model and in an arterial occlusion model of thrombi.

On average, 2 tPA molecules were chemically cross-linked to one IgG molecule through NHS-ester reaction to sulfhydryl groups added on IgG; upon incubation with isolated human erythrocytes, about 1200 tPA molecules were bound to each erythrocyte via CR1, keeping its fibrinolytic activity. Zaitsev and colleagues next showed in vivo eligibility of the anti-CR1/tPA in a very smart way, using the transgenic mice expressing human CR1. About 40% of injected anti-CR1/tPA was found to remain within the circulation 3 hours after injection into transgenic mice, markedly contrasting with the 10% remaining after injection into wild-type mice (approximately 4% nonspecifically bound to erythrocytes, and approximately 6% in plasma). Compared with soluble tPA, anti-CR1/tPA dissolved 5-fold more microemboli in the lungs when injected 30 minutes before the injection of radiolabeled fibrin microemboli, whereas it dissolved only approximately 4-fold fewer microemboli when injected 10 minutes after the injection of microemboli. Zaitsev et al thought the latter was due to loss of relative enzymatic activity of tPA by conjugation to CR1 and/or binding to erythrocytes. Moreover, anti-CR1/tPA administered 30 minutes before artery injury with $FeCl_3$ did not affect the ratio of thrombus formation, but it did significantly accelerate thrombolysis within 30 minutes, as assessed by Doppler ultrasound. Finally, the authors showed that anti-CR1/tPA caused 20-fold less rebleeding in the transgenic mice than did soluble tPA.

This is an improvement of Zaitsev and colleagues' original method, in which tPA was directly coupled to the surface of erythrocytes using biotin-streptavidin as a cross-linker.[3] Nearly identical results were obtained in each measuring index between the previous and current methods. However, as phlebotomy from patients and reinfusion of the modified erythrocytes to patients are practically impossible in this context, the current method is superior to the previous one and much more promising for clinical use.

The authors predict clinical application of this method for prophylaxis against thrombosis. Their data suggest that erythrocytes bearing tPA would be unable to permeate preexisting hemostatic clots but would become entrapped within and dissolve nascent clots soon after formation, thus characterizing them as an ideal thromboprophylactic agents. That might be true. In addition, for the sake of prolonging half-life and markedly reduced extravascular toxicity, anti-CR1/tPA might be a useful agent for treatment of stroke,[1] as well as a novel thrombolytic agent for use prior to percutaneous coronary intervention (PCI) in the treatment of acute myocardial infarction (AMI),[4] even though the relative fibrinolytic activity is lower than tPA. This possibility should be tested in animal models to aim for application in patients in the near future.

Also, it would be interesting to use other genetically engineered mutant tPAs with longer half-lives and greater fibrin specificity, such as desmotoplase (recombinant desmodus salivary plasminogen activator α-1 [rDSPAα-1]); reteplase, a domain deletion mutant of tPA, comprising the kringle 2 and protease (K2P) domains; and tenecteplase, which has specific mutations at 3 sites in the alteplase molecule. ■

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IMMUNOBIOLOGY

Comment on Mansouri et al, page 1932

KSHV wages a 2-front war on PECAM/CD31

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In this issue of *Blood*, Mansouri and colleagues show that in addition to suppressing expression of histocompatibility complex class I molecules, the K5 gene product of KSHV also targets CD31. It does this by 2 separate mechanisms, one that targets newly synthesized CD31 and one that targets CD31 already expressed on the cell surface.

The interactions between viruses and their hosts are often quite complex. This is especially true with gamma-herpesviruses such as Kaposi sarcoma–associated herpesvirus (KSHV) and other viruses that persist in a latent state. Two KSHV-encoded genes, *K3* and *K5*, have previously been shown to target major histocompatibility complex class I (MHC I) and related molecules,[1-3] and thus serve to protect KSHV-infected cells against immune attack. Mansouri and colleagues now find that K5, but not K3, also targets CD31.