thrombosis because of less efficient activation of protein C. Recently, a mutation in the *EPCR* gene was described that consisted of a 23–base pair insertion that leads to an early stop codon.[4] Since such a mutation is likely to lead to loss of function due to less expression or lowered functionality, this opened a possibility to study the relevance of EPCR in vivo. The mutation was found in a few individuals with thrombophilia, but firm conclusions could not be reached due to its rarity (population prevalence estimated at less than 5 per 1000).[5]

In this issue, Saposnik and colleagues (page 1311) describe extensive screening of the *EPCR* gene, which led to the identification of a haplotype that is strongly related to sEPCR plasma levels, with over 6-fold increased levels in those homozygous for the haplotype. In addition, they report that the haplotype is associated with a 1.7-fold increased risk of venous thrombosis (95% confidence interval, 1.2-2.4) in a case control study including 338 patients and 338 controls. Even though this is a moderately increased risk, it may be highly relevant because of the high frequency of the haplotype in the population (18%), which implies that over 20% of all thrombotic events could be attributed to this variant (population attributable risk). However, since the same genotype was not associated with thrombosis in previous studies, the results on this association need confirmation.

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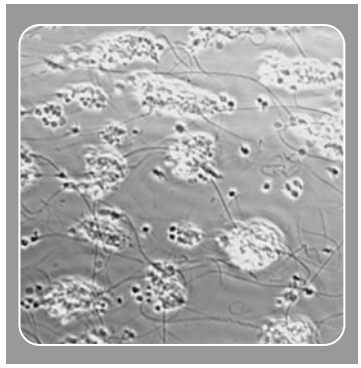
1. Fukudome K, Esmon CT. Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. J Biol Chem. 1994;269:26486-26491.
2. Stearns-Kurosawa DJ, Kurosawa S, Mollica JS, Ferrell GL, Esmon CT. The endothelial cell protein C receptor augments protein C activation by the thrombin-thrombomodulin complex. Proc Natl Acad Sci U S A. 1996;93:10212-10216.
3. Stearns-Kurosawa DJ, Burgin C, Parker D, Comp P, Kurosawa S. Bimodal distribution of soluble endothelial protein C receptor levels in healthy populations. J Thromb Haemost. 2003;1:855-856.
4. Biguzzi E, Merati G, Liaw PC, et al. A 23bp insertion in the endothelial protein C receptor (EPCR) gene impairs EPCR function. Thromb Haemost. 2001;86:945-948.
5. Poort SR, Vos HL, Rosendaal FR, Bertina RM. The endothelial protein C receptor (EPCR) 23 bp insert mutation and the risk of venous thrombosis. Thromb Haemost. 2002;88:160-162.

Platelet collagen receptors play molecular ping-pong

Following vessel injury, circulating platelets adhere to and form thrombi on exposed collagen. Traditionally, this response has been mechanistically compartmentalized by assigning adhesive and signaling roles to distinct collagen receptors. Such a neat division of labor fit nicely with the identification of 2 collagen receptors, the integrin $\alpha_2\beta_1$ and the immune receptor homologue glycoprotein VI (GPVI), from protein families with well-defined adhesive and signaling roles. Unfortunately, the experimental data have failed to draw clear lines between adhesive and signaling collagen receptors. The finding that platelet $\alpha_2\beta_1$, like integrin $\alpha_{IIb}\beta_3$, requires "inside-out" signals to bind collagen linked any adhesive role for this receptor to prior collagen signals.[1] Analysis of knockout mice has further eroded the notion of independent adhesive and signaling receptors: GPVI-deficient platelets lose both adhesion and signaling responses to collagen while $\alpha_2\beta_1$-deficient platelets exhibit near-normal signaling and, in some studies, normal adhesion to collagen under flow.[2] Recent work even has revealed that platelet integrins such as $\alpha_2\beta_1$ may couple to many of the same intracellular signaling molecules as GPVI.[3,4] It is now clear that the roles of GPVI and $\alpha_2\beta_1$ are not as easy to distinguish as once thought.

Two recent *Blood* papers address how human GPVI responds to collagen, both alone and with other platelet collagen receptors. Smethurst and colleagues[5] used differences in the binding of human and mouse GPVI extracellular domains to a collagen-related peptide and a blocking phage antibody to pinpoint the collagen-binding domain of human GPVI to the region surrounding K59. In this issue, Siljander and colleagues (page 1333) use the same blocking antibody to compare the role of GPVI with that of other collagen receptors during human platelet adhesion and signaling responses to collagen under flow. Remarkably, in both cases results obtained with human GPVI differ from those observed with mouse GPVI. The lysine at position 59 of human GPVI is replaced in mouse GPVI with glutamic acid, a nonconservative substitution. Human platelets



treated with blocking anti-GPVI antibody adhere normally to collagen under flow, whereas GPVI-deficient mouse platelets are unable to adhere to collagen under flow.

Do these results indicate that significant species differences will further complicate assigning molecular roles to platelet collagen receptors? Probably not. These discrepancies can be attributed to methodology and to collagen receptor interplay. The mouse studies are genetic: loss of GPVI function is absolute and loss of $\alpha_2\beta_1$ function follows due to loss of GPVI-generated inside-out activation signals. The human studies are pharmacologic: GPVI K59 may be more critical for collagen-related peptide (CRP) responses than for collagen responses, and loss of GPVI function due to blocking antibody is probably not absolute. Similar studies using antihuman GPVI antibody and antimouse GPVI antibody demonstrate that even minute amounts of GPVI signaling are sufficient to activate $\alpha_2\beta_1$ and permit that receptor to play a prominent signaling role.[2,6] These studies contribute to a growing body of evidence suggesting that platelet collagen responses are a game of molecular ping-pong between different collagen receptors in which GPVI is the first player to serve.

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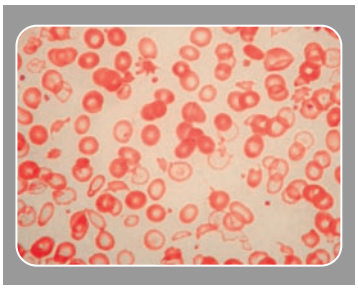
1. Jung SM, Moroi M. Platelets interact with soluble and insoluble collagens through characteristically different reactions. J Biol Chem. 1998;273:14827-14837.
2. Nieswandt B, Brakebusch C, Bergmeier W, et al. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. EMBO J. 2001;20:2120-2130.
3. Woodside DG, Obergfell A, Leng L, et al. Activation of Syk protein tyrosine kinase through interaction with integrin beta cytoplasmic domains. Curr Biol. 2001;11:1799-1804.
4. Inoue O, Suzuki-Inoue K, Dean WL, Frampton J, Watson SP. Integrin alpha2beta1 mediates outside-in regulation of platelet spreading on collagen through activation of Src kinases and PLCgamma2. J Cell Biol. 2003;160:769-780.
5. Smethurst PA, Joutsi-Korhonen L, O'Connor MN, et al. Identification of the primary collagen-binding surface on human glycoprotein VI by site-directed mutagenesis and by a blocking phage antibody. Blood. 2004;103:903-911.
6. Chen H, Kahn ML. Reciprocal signaling by integrin and nonintegrin receptors during collagen activation of platelets. Mol Cell Biol. 2003;23:4764-4777.

The myelodysplastic syndromes: heterogeneneity on many levels

The myelodysplastic syndromes (MDSs) are a heterogeneous group of disorders characterized by ineffective hematopoiesis and a variable risk of transformation to acute leukemia. This heterogeneity, both at the phenotypic and molecular level, is underscored by 2 papers in the current issue of *Blood.* In the first of these, Steensma and colleagues

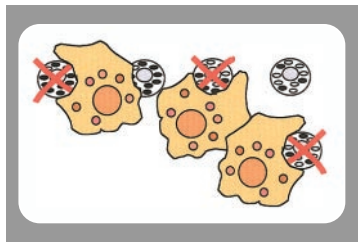


(page 1518) describe a patient with MDS associated with acquired α-thalassemia. This rare syndrome, referred to as the α-thalassemia myelodysplasia syndrome (ATMDS), is characterized by hypochromic microcytic anemia, anisopoikilocytosis, markedly reduced α-globin chain synthesis, and substantial amounts (> 10%) of hemoglobin H (beta globin tetramers). While inherited α-thalassemia is typically due to deletions or point mutations affecting the duplicated α-globin genes on chromosome 16, ATMDS has been shown to be due to mutations in a gene called *ATRX* (alpha thalassemia/mental retardation syndrome X-linked homolog).[1] This gene on the X chromosome encodes a chromatin-remodeling gene that regulates the expression of a number of genes, including the α-globin genes. Germ line mutations of *ATRX* have been described and cause a mild α-thalassemia syndrome associated with a mental retardation.[2]

In the current issue, Steensma and colleagues describe a man with mild anemia, marked microcytosis, hypochromia and anisopoikilocytosis, and bone marrow studies diagnostic of myelodysplastic syndrome (refractory cytopenia with multilineage dysplasia). Hemoglobin H inclusions were present at low levels (< 1%) in peripheral blood erythrocytes. Globin chain synthetic ratios were consistent with heterozygous α-thalassemia. Marrow chromosome studies revealed complex cytogenetic abnormalities. Fluorescence in situ hybridization (FISH) and Southern blot studies revealed a deletion larger than 1.9 Mb that removed the α-globin cluster from one chromosome 16. Thus, the α-thalassemia phenotype in this patient is due to the deletion of 2 of 4 α-globin genes (genotype αα/−−). Interestingly, the red cell changes are more marked than those typically seen in heterozygous α-thalassemia. In addition, the presence of hemoglobin H is also unusual for heterozygous α-thalassemia. It seems likely that this somewhat more severe thalassemic phenotype is due to an interaction between the α-thalassemia deletion and the ineffective erythropoiesis characteristic of a myelodysplastic bone marrow. This is the first report of ATMDS due to deletion of the α-globin gene cluster rather than a mutation in the *ATRX* gene.

In a second paper, Gattermann and colleagues (page 1499) examined the role of a mitochondrial DNA mutation in MDS. The potential role for mitochondrial DNA in acquired MDS was suggested by the constitutional disorder, Pearson syndrome, in which sideroblastic anemia is accompanied by pancreatic dysfunction. In this disorder a number of deletions in mitochondrial DNA have been described.[3] Studies of mitochondrial DNA in acquired MDS have suggested that mutations can be found in up to 50% of patients.[4] Other investigators have found that mitochondrial DNA mutations are less widespread in acquired MDS.[5] The significance of these mutations and their role in the pathogenesis of MDS remains a subject of ongoing investigation.

Gattermann and colleagues describe a patient with myelodysplastic syndrome



(refractory anemia with excess blasts [RAEB]) who was found to have a somatic mutation of mitochondrial transfer RNA (tRNA) in bone marrow cells. Approximately 40% of the mitochondrial DNA molecules in the marrow contained this mutation. The mutation was present at a higher level in marrow and peripheral blood $CD34^+$ cells than in unfractionated marrow. The mutation was not found in unfractionated peripheral blood leukocytes or in buccal mucosal cells. These findings suggested that marrow cells carrying the mitochondrial DNA mutation did not contribute to effective hematopoiesis. Thalidomide treatment resulted in improved red cell and platelet production and in a decrease in marrow blast percentage. Interestingly, there was a coincident decrease in the percentage of mutant mitochondrial DNA in the bone marrow. Hematopoietic colony assays were performed using $CD34^+$ cells derived from marrow and peripheral blood. In all cases, mature hematopoietic colonies were composed entirely of cells containing thewild-type mitochondrial DNA. These findings