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HEMATOPOIESIS & STEM CELLS

Comment on Zhao et al, page 505

MYB and miR: a pair well matched

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In this issue of *Blood*, Zhao and colleagues describe a feed back loop between the transcription factor Myb and micro RNA 15a. Myb induces expression of *miR-15a* which, in turn, fine-tunes the abundance of Myb in human hematopoietic cells.

More than 30 years ago, Myb was first described as an oncogene carried by avian retroviruses (*v-Myb*). Shortly afterward, inappropriate activation of the cellular proto-oncogene counterpart *Myb* (= v-Myb myeloblastosis viral oncogene-homolog) by retroviral insertion was demonstrated as a cause for leukemia in mice and chickens.[1,2] Since then, it has become more and more evident that aberrant expression of Myb could be implicated in a multitude of human tumors, from colon carcinoma to breast cancer to leukemia.[3-5] Deregulation of *Myb* can result from multiple mechanisms, including genomic rearrangements, gene amplification, inappropriate or overexpression as well as structural changes.[1]

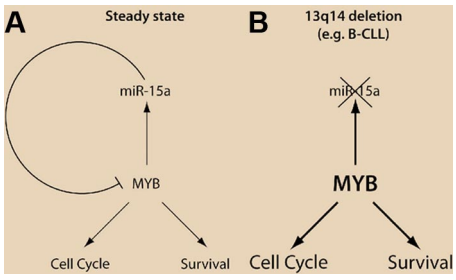
The physiologic functions of Myb in its genuine role as transcription factor have received a lot of attention. The protein is involved in multiple processes, such as stem cell self-renewal and lineage decisions, and needs to be down-regulated to permit cellular differentiation. Dozens of Myb target genes have been identified, many of which are involved in cell-cycle progression (*c-Myc, Cyclins A, B, E*) or survival (eg *Bcl2*). Obviously, Myb is an extremely well-studied protein. No more surprises to be expected? Even an old dog can learn new tricks!

In this issue of *Blood*, Zhao et al demonstrate that Myb is not only a regulator in its own right but is subject itself to a control mechanism receiving lots of attention these days: it is regulated by a micro RNA, specifically miR-15a. This finding is not unexpected; however, *Myb* and miR-15a are more closely, interconnected than other couples because they feed back on each other. Myb is the very transcription factor that enables expression of its own inhibitor, the *miR-15a* precursor gene, which, after maturation, interferes with *Myb* mRNA translation.

The authors identified 2 consensus Myb-binding sites (t/cAACt/gG), conserved between mouse and man, and demonstrated experimentally by chromatin immunoprecipitation that Myb actually binds to these sites in vivo. In an important control experiment, knockdown of Myb by RNA interference resulted in the expected reduction of miR-15a levels. Since these experiments were all done in K562 erythroleukemic cells, representing a rather artificial model system, Zhao et al extended their analysis to primary human cells. First, they determined Myb versus miR-15a levels in cord blood–derived CD34$^+$ hematopoietic progenitors undergoing gradual erythroid maturation in ex vivo cultures. There was a clear inverse correlation in the abundance of the 2 molecules at all time points. To substantiate this link in another experimental setting, either whole human bone marrow mononuclear cells or CD34$^+$ progenitors were transfected with a miR-15a mimic (partially double-stranded RNA). The ensuing colony assays for CFU-GM, BFU-E, and CFU-E suggested that indeed miR-15a interferes with lineage-
progression as well as proliferation-promoting functions of Myb.

This novel connection coupling of Myb and miR-15a reported by Zhao et al might have even more interesting pathophysiologic consequences because miR-15a has previously been reported to down-modulate expression of the antiapoptotic protein BCL2,[6] which is prominently expressed in many hematopoietic cell types. Myb upregulates BCL2, again arguing for a balancing act of the Myb/miR-15a couple, in this case affecting a down-stream target.

These considerations can be carried even further. In 68% of all B-cell chronic lymphocytic leukemia (B-CLL) cases, a 30kb chromosomal deletion can be detected (13q14), which encompasses the locus for miR-15a (and miR-16, another modulator of BCL2 expression).[7] Thus, even in the absence of any genetic alterations in the *Myb* gene, loss of the "tumor suppressor" miR-15a may be sufficient to induce leukemia by enabling increased Myb expression and, in turn, compromised lineage progression, enhanced survival as well as increased proliferation. It might become rewarding to assess these and other possibilities in the future.



(A) Steady state of the Myb/miR-15a regulatory loop, resulting in feedback inhibition of MYB, preventing excessive cell proliferation and survival. (B) Deletion of 13q14 (comprising the miR-15a gene locus) is frequent in B-CLL. This could result in an increased abundance of Myb, inducing increased cell proliferation and survival.

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Comment on Mullins et al, page 696

Bleeding hearts

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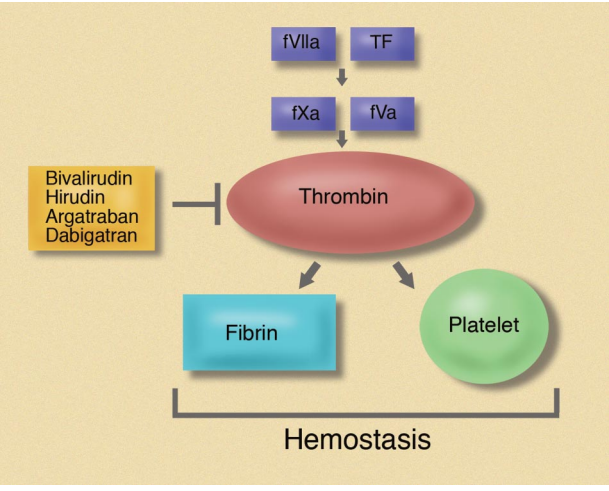
In this issue of *Blood*, Mullins and colleagues show that genetic elimination of the prothrombin (fII) gene in adult mice leads to death within 7 days. Unexpectedly, instead of a uniform hemorrhage in all tissues, the mice exhibited a tissue-specific pattern of hemorrhage particularly in the heart and brain.

Hemostasis, the arrest of bleeding from an injured blood vessel, is mediated by binding of platelets (primary hemostasis) and activation of the coagulation cascade (secondary hemostasis). Genetic studies in mice have demonstrated that "knock-out" of components of the extrinsic coagulation pathway (tissue factor and fVII) or the common coagulation pathway (fX, fV and fII) is not compatible with life due to defective vessel formation during embryonic development and hemostatic defects after birth. [1] However, mice can survive without fibrinogen, the end point of the coagulation cascade, or without protease activated receptor 4 (PAR-4), which mediates thrombin activation of platelets in mice.[2] Importantly, mice with a combined deficiency of both fibrinogen and PAR-4 die after birth due to uncontrolled hemorrhage.[3] These observations are consistent with the notion that thrombin is the central serine protease in the coagulation cascade and plays critical roles in both cleavage of fibrinogen and activation of platelets.



The extrinsic and common coagulation pathways are essential for hemostasis. Formation of the fVIIa:tissue factor (TF) complex after vessel injury leads to activation of fX to fXa, which, together with its cofactor fVa, cleaves prothrombin to thrombin. Thrombin activates fV, fVIII, fXI, and fXIII, cleaves fibrinogen to fibrin, and activates platelets by cleavage of PARs. Direct thrombin inhibitors include bivalirudin, which is used in invasive cardiology, hirudin and argatraban, which are used to treat patients with heparin-induced thrombocytopenia and dabigatran, an orally available drug. Professional illustration by Marie Dauenheimer.

The concept of hemostasis is easy to envisage in settings such as a knife cut where blood vessels are severed and the clotting system is activated by exposure to tissue factor. What is not considered by most people is that in daily life there are likely to be "spontaneous" breaks in blood vessels throughout the body. If one were to design a hemostatic system, it would be beneficial to provide a basic protection to all blood vessels, as well as additional protection to blood vessels in vital organs and organs in which blood vessels are more likely to be damaged by repetitive mechanical stress, such as the heart. Indeed, tissue factor is expressed by adventitial fibroblasts and vascular smooth muscle cells surrounding larger blood vessels and pericytes surrounding capillaries and thus provides basic hemostatic protection to all blood vessels. Tissue factor is also expressed in a tissue-specific manner, with high levels in the brain and heart. This pattern of expression is consistent with a design in which high levels of tissue factor provide additional hemostatic protection to these vital organs. Support for this idea was first provided by the observation that mice engineered to express very low levels of tissue factor (~1%) exhibited tissue-specific hemostatic defects, particularly in the heart.[4,5] Similar hemostatic defects were observed in mice expressing very low levels of fVII (~1%) and low levels of fX (approximately 5.5%).[4,6] In contrast, mice lacking either fVIII or fIX survive to adulthood and have no hemostatic defects in their hearts. However, patients with hemophilia A (fVIII deficiency) or hemophilia B (fIX deficiency) often experience spontaneous hemorrhages in tissues with low levels of tissue factor expression (ie, joints and skeletal muscle) and have excessive hemorrhage after injury. These observations indicate that the extrinsic and common coagulation pathways but not the intrinsic pathway are important for cardiac hemostasis.

In this issue of *Blood*, Mullins and colleagues use the poly I:C-inducible Mx1-Cre system to genetically eliminate *FII* in adult mice. This is an elegant approach to study the role of fII in hemostasis. As expected, loss of fII was associated with a profound effect on the prothrombin time and the activated partial prothrombin time. Administration of poly I:C led to a rapid reduction in circulating fII levels and uniform death of the mice within 7 days. If one believes that hemostasis is equal in all tissues, one would expect hemorrhage in the majority of organs in these mice. Rather, the exciting observation in this report is that mice lacking circulating fII exhibit tissue-specifichemorrhage. Postmortem analysis of 39 mice revealed that the majority had cardiac hemorrhage, and approximately 50% had intracranial hemorrhage. Mice expressing low levels of fII (10%-20%) also had a mild hemostatic defect in their hearts at 1 year of age. The phenotype of these acute and chronic fII-deficient mice

is remarkably similar to the phenotypes of mice with low levels of tissue factor, fVII and fX. Pharmacological inhibition of tissue factor in mice also leads to hemorrhage in the heart and brain.[7] These studies support the idea that hemostasis is not equal in all tissues.

Anticoagulant therapy is designed to reduce thrombosis without affecting hemostasis. However, not surprisingly, the major side effect of anticoagulant drugs is hemorrhage. New anticoagulants have been developed that selectively target thrombin or fXa.[8] The current study demonstrates that pharmacologic reduction in thrombin activity below a critical threshold could compromise cardiac and brain hemostasis.

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CLINICAL TRIALS

Comment on Federici et al, page 526

At least Type VWD2B is a discrete variant of VWD, isn't it?

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In this issue of *Blood*, Federici and colleagues demonstrate that some characteristics of VWD2B may be less than universal in identifying all patients diagnosed with the disease.

Type 2B von Willebrand Disease (VWD2B) is generally characterized as including patients with bleeding symptoms whose laboratory studies demonstrate (1) reduced von Willebrand factor (VWF), (2) loss of high- molecular-weight VWF multimers, (3) thrombocytopenia, and (4) platelet aggregation of patient platelet-rich plasma to low concentrations of ristocetin (usually $\leq$ 0.6 mg/mL).[1,2] The article by Federici et al is critical because it demonstrates the phenotypic diversity of laboratory tests in patients diagnosed with VWD2B. While clinical laboratory studies using ristocetin have recognized laboratory to laboratory differences, this collaborative group includes laboratories of excellence that are recognized for their precision in VWD diagnosis.

Looking at each of these key components separately, Federici et al made some interesting observations. There were 11 mutations identified as causing VWD2B. In 6 of the families with these mutations, some members had plasma von Willebrand ristocetin cofactor (VWF:RCo) assays greater than 50 U/dL. In a separate analysis of people with mutations and VWD2B, 6 of 16 had plasma VWF:RCo levels above 50 U/dL. Thus, not all patients with VWD2B will have recognized reduction of plasma VWF:RCo.

While most had abnormal VWF multimers, VWF multimers were normal in 5 families with 3 different VWD2B mutations. Only 30% of the subjects had platelet counts,[3] although many of those, but not all, with normal platelet counts exhibited thrombocytopenia following stress or DDAVP. They also demonstrated that those subjects with the combination of VWD2B and thrombocytopenia have significantly higher bleeding scores. Twenty-one of 67 had bleeding scores of less than 4. Thus, VWD2B patients do not all exhibit thrombocytopenia, loss of high-molecular-weight VWF multimers in their plasma, or even abnormal bleeding scores.

While all subjects exhibited heightened ristocetin-induced platelet aggregation, many of those identified in their study required more than 0.6 mg/mL of ristocetin to demonstrate this heightened interaction—a dose higher than many laboratories use to identify this interaction.

Thus, the clinical lab phenotypes that we have accepted for this disorder are not universally present, even in patients with defined type 2B VWF mutations. Assumptions that many of us have had for years about VWD2B and its diagnosis now need to be reexamined in light of this important study. While some might question whether the patients with normal VWF multimers, VWD New York[3] and VWD Malmo,[4] should be included in VWD2B, these investigators demonstrate much greater diversity in the laboratory studies, even in patients with abnormal multimers.

Since platelet VWF (and presumably endothelial) VWF multimers are normal sized in patients with VWD2B, more studies may be necessary to understand the reason in some subjects for the loss of high-molecular-weight multimers *without* concomitant thrombocytopenia. Some, but not all, of this might be explained by heightened cleavage of type 2B VWF by ADAMTS13.[5]

It seems that the more we learn about VWD, the more challenges we uncover to our accepted dogmas about it. Our work and learning continue!

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