stem cell functions during steady-state conditions.

In the current study, Hu et al[1] find that mice lacking *miR-29a/b-1* bicistron have reduced HSC numbers correlating with enhanced HSC proliferation and cell death and reduced multilineage engraftment following competitive bone marrow reconstitution (see figure). Although this mouse strain is deficient in both *miR-29-a* and *miR-29-b1*, only expression of *miR-29a* was able to rescue this hematopoietic phenotype. Providing mechanistic insight, transcriptional profiling of *miR-29a/b-1*–deficient HSCs uncovered a battery of *miR-29* direct targets with relevance to stem cell biology, including *Dnmt3a*. Importantly, several *miR-29a/b1* phenotypes could be partially rescued by crossing *miR-29a/b-1*$^{-/-}$ mice with *Dnmt3a*$^{+/-}$ mice to specifically reduce Dnmt3a levels in HSCs lacking *miR-29a/b-1*. This provides genetic evidence that this particular DNA methyltransferase, a known regulator of HSCs,[7] is a functionally relevant target of *miR-29a* in this context.

In addition to expanding our understanding of how *miR-29a* has evolved to modulate HSC biology under physiological circumstances, the connection between *miR-29a* and *Dnmt3a* also provides important mechanistic insight into how *miR-29a* might epigenetically influence AML phenotypes. *Dnmt3a* acquires loss-of-function somatic mutations that result in reduced DNA methylation in a high percentage of AML patients with intermediate-risk cytogenetic profiles or FLT3 mutations, and is associated with a poor prognosis.[8] As *miR-29a* directly targets *Dnmt3a*,[1,9] this may prove to be an important step during the oncogenic process in certain types of AML.

With the identification of miRNAs that drive malignant phenotypes and recent progress in developing various methods to target specific miRNAs therapeutically, miRNAs such as *miR-29a* may one day be an effective target to treat AML. However, the decision to pursue *miR-29a* targeting as a means to mitigate AML might prove to be complicated, as a seemingly paradoxical role for exogenous *miR-29* family members in blocking AML cell line growth has also been reported.[10] This implies that the *miR-29* family may play varying roles in AML depending upon the cellular context and possibly the stage of disease where its expression becomes perturbed. Thus, further work is needed to unravel these complexities to both better understand its biology and enable intelligent targeting approaches in the clinic.

The discovery that endogenous *miR-29a* plays an indispensable role in HSCs through a mechanism involving repression of *Dnmt3* is an important step toward defining the molecular circuitry underlying HSC biology. It also justifies additional genetic studies to continue exploring other candidate miRNAs that may have imperative functions in this vital cellular compartment.

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

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Di Buduo et al, page 2254

Platelet biogenesis wears silkworm cocoons

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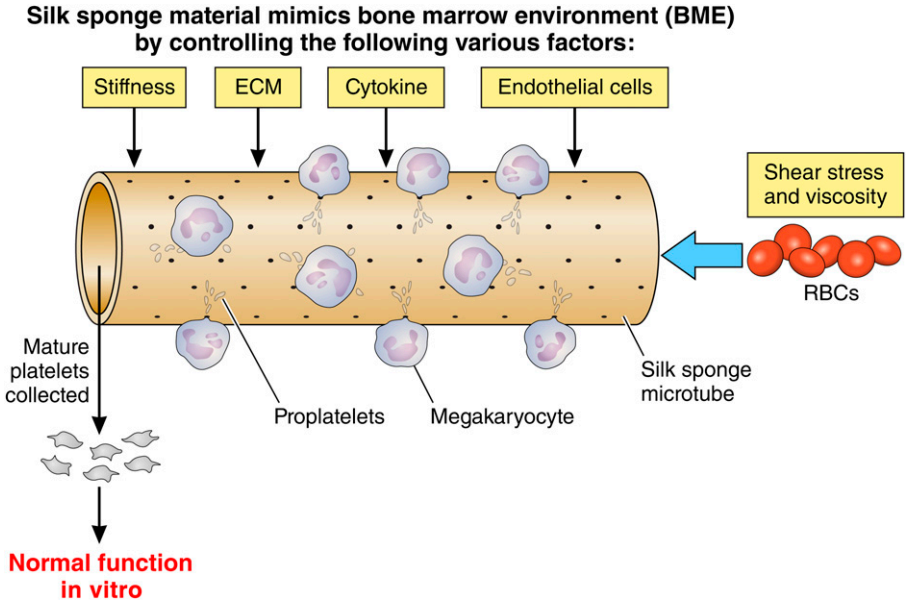
In this issue of *Blood*, Di Buduo et al report an artificial scaffold of the bone marrow niche whereby *Bombyx mori* silkworm cocoons appeared to coordinate several factors required for megakaryopoiesis and platelet biogenesis.[1]

A single bone marrow megakaryocyte (Mk) in the body produces >2000 platelets.[2] By recapitulating the chemical and physical signaling that promotes Mk differentiation, maturation, and the release of proplatelets and platelets into the blood stream in vivo, experimental trials ex vivo have tried but failed to achieve similar numbers.[3,4] Building on these ex vivo systems have been designs that incorporate switching the culture from 2-dimensional to 3-dimensional (3D) culture environments, for example, using scaffolds made of polyester fabric, hydrogel, or polydimethylsiloxane (PDMS) along with cytokines, extracellular matrix, or endothelial cells.[5-7] 3D environments likely make for more surface area, which could permit more proplatelets to interact with the endothelial wall, increasing the number of platelets acquired.

A series of bioreactor systems that better mimic the bone marrow environment by including extracellular matrix components, surface topography, stiffness, cytokines, and shear stress is also being investigated. Di Buduo et al,[1] to obtain higher production efficiency



Di Buduo et al demonstrated a combined model to mimic the bone marrow environment to study thrombopoiesis. They previously proposed that silk material-coated microtube structure is capable of recapitulating bone marrow environment, but it failed to obtain a higher rate of mature Mks.[8] The improved system included increasing stiffness, extracellular matrix, cytokines, endothelial cells, and modulating shear stress by red blood cells. In this article,[1] the authors developed the 3D bioreactor that enhances migration and maturation of Mks, proplatelet generation, and yield of functional platelets. Silk material may be suitable for integration of several factors required for thrombopoiesis ex vivo. Adapted from Figures 5, 6, and 7 in the article by Di Buduo et al beginning on page 2254.

of functional platelets from cultured Mks, prepared a bioreactor that uses a silk sponge, whereby a silk sponge-coated tube structure made by PDMS is also covered by endothelial cells or by vascular endothelial growth factor and vascular cell adhesion molecule-1 on the inside of the tube under the presence of shear stress. The idea of using the silk sponge was previously proposed by the same authors' group.[8] Di Buduo et al added additional factors necessary to fully recapitulate obtaining "functional platelets" through the artificial bone marrow environment (see figure). They previously designed a bioreactor that included both the artificial osteoblast and perivascular niches with the appropriate extracellular matrix proteins and growth factors. However, there were very few Mks displaying proplatelets and producing platelets with impaired function.[8] An advance by Di Buduo et al is the incorporation of various factors, ie, composed of stiffness for Mk adhesion, various extracellular matrixes, coated endothelial cells, and an intervention by red blood cells (RBCs) to modulate flow viscosity and shear stress. The subsequent flow by RBCs might influence the conditions of the silk sponge. The silk sponge should control the elements required for mimicking a true bone marrow environment. Thus, the authors concluded that silkworm cocoons contribute to making a comfortable and flexible environment for megakaryopoiesis and platelet biogenesis, which the authors referred to as a "programmable 3D silk bone marrow niche" (see figure).[1]

Because shear stress has been found to be an important factor in platelet number, Thon et al manufactured a microfluidic bioreactor that also incorporates bone marrow stiffness, the extracellular matrix composition, and other factors modulating shear stress.[7] They demonstrated that best rate of shear stress resulted in Mk maturation, as proplatelets were observed within seconds of trapping compared with the several hours seen in static conditions. The shear stress in their system was generated by parallel flows.[7] Nakagawa et al found, however, that a confluent system may be more effective, as flow intersecting at 60° achieves a 3.6-fold increase compared with a single-flow system.[6] Therefore, a silk-based sponge with PDMS may generate more complicated shear stress conditions by preincubating with RBCs. It has been reported that shear stress increases the expression of *RUNX1* in CD41a$^+$ cells,[9] also suggesting that shear stress per se facilitates megakaryopoiesis.

Ex vivo platelet production is still far from an industrial scale in transfusion medicine or a drug delivery system, but the bioreactor does offer an excellent model to study megakaryopoiesis from hematopoietic stem cells (HSCs), as demonstrated by the findings that HSCs and Mks are very close in proximity in the hematopoiesis hierarchy in the bone marrow.[10]

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