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Comment on Zhao et al, page 2383

ATF4, a new player in fetal HSC expansion

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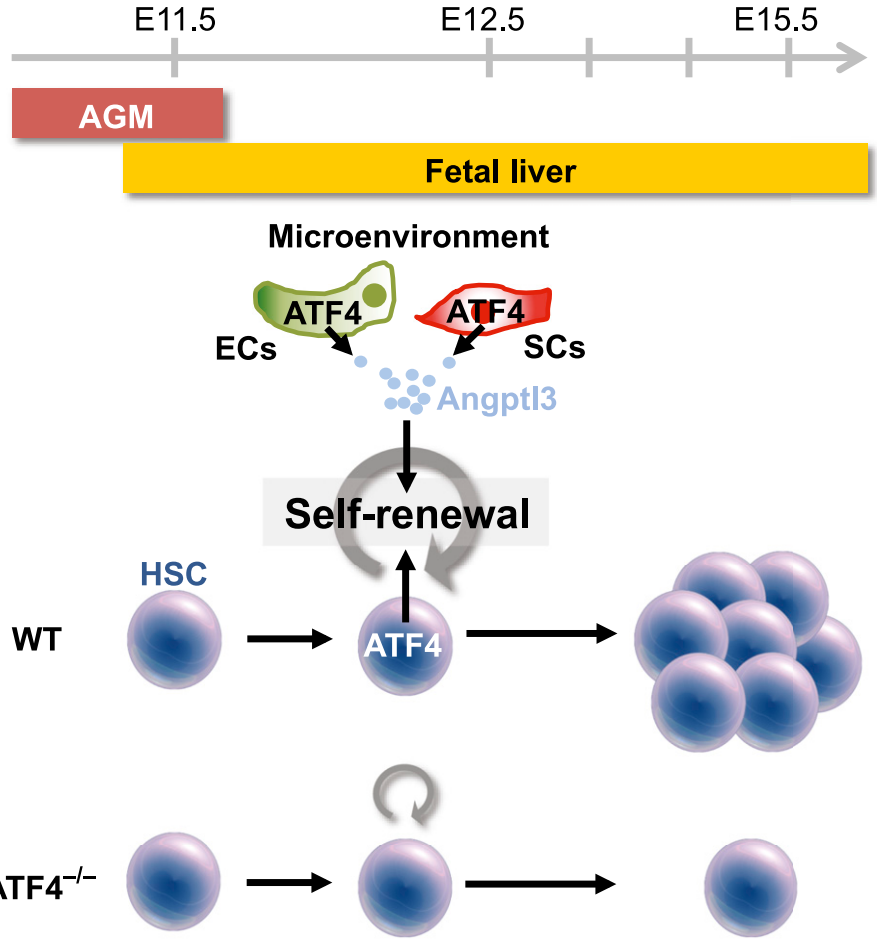
In this issue of *Blood*, Zhao et al have identified the basic region-leucine zipper transcription factor activating transcription factor 4 (ATF4) as a key molecule for the intrinsic and extrinsic regulation of the extensive expansion of fetal liver (FL) hematopoietic stem cells (HSCs) (see figure).[1]



The transcription factor ATF4 is essential for HSC self-renewal in the FL. Although the emergence and migration of definitive HSCs are largely independent of the presence of ATF4, the transcription factor is required for the intrinsic and extrinsic regulation of HSC self-renewal during their massive expansion phase between embryonic day 11.5 (E11.5) and E15.5 in the FL. ATF4 activates the expression and secretion of Angptl3 in the FL microenvironment, particularly in endothelial cells (ECs) and stroma cells (SCs), which further promotes the self-renewal of HSCs. AGM, aorta-gonad-mesonephros; WT, wild-type.

HSCs undertake an exciting journey during embryonic development from their first emergence to their final destination. The first definitive HSCs arise from hemogenic endothelial cells in the AGM region and in the placenta at E10.5 of mouse development.[2,3] They migrate to the FL and also to the spleen at E11.5 and start to massively renew themselves and thereby expand their numbers >100-fold within 4 to 5 days. This wave of HSC expansion provides the lifelong pool of stem cells for adulthood. From E15.5 until shortly after birth, HSCs settle in the bone marrow, where they largely reside in quiescence during adult hematopoiesis. In mice, there is a marked switch in their molecular program and in their functional behavior 3 to 4 weeks after birth. FL HSCs rapidly divide (1 division every 12-14 hours), while adult HSCs are deeply dormant and rarely divide (1 division every 145 days).[4]

The molecular profiling of FL long-term repopulating HSCs (LT-HSCs) during this massive expansion phase may provide the clues as to how we can manipulate adult HSCs to restart their self-renewal expansion for regenerative medicine. In adult HSCs, once they are "awoken" from their dormancy and enter cell cycle, they must rapidly return to their quiescent state to prevent exhaustion and long-term organ failure. Furthermore, extended replicative stress in adult HSCs holds the danger of DNA damage, which may occur once these cells are forced into cell cycle.[5] What makes fetal HSCs special enough to facilitate extensive self-renewal divisions without suffering from exhaustion, differentiation induction, or genomic instability? These are important questions that, once answered, may show us how to enforce HSC expansion for medical needs.

The study by Zhao et al introduces an important new player in the cell-intrinsic and -extrinsic regulation of FL HSC self-renewal and expansion. ATF4 is a basic region-leucine zipper transcription factor belonging to the ATF family which consists of 7 members in mice and humans. More than 10 years ago, the phenotype of the ATF4 homozygous knockout was reported in mice to result in perinatal lethality with a severe anemia and a low hematocrit.[6] The authors here revisited the consequences of ATF4 deletion on the biology of HSCs in great detail by investigating the intrinsically and extrinsically controlled cell fate decisions in the absence of ATF4 (see figure). They demonstrated that the FLs of

$ATF4^{-/-}$ mice between E12.5 and E15.5 have a severely reduced number of functional and phenotypic LT-HSCs. Importantly, there was no overt problem in the emergence of definite HSCs in the AGM region at E11.5 in the absence of ATF4 and almost the same number of LT-HSCs settled the FL at E11.5. These results suggest that FL HSCs in $ATF4^{-/-}$ mice have a reduced ability to self-renew and expand.

Zhao et al then went on to functionally quantify the amount of blood cell-repopulating LT-HSCs at different stages of development. The transplantation of whole FL equivalents and of limiting fetal cell dilutions (competitive-repopulating-unit assay) confirmed the strongly reduced number of functional LT-HSCs in FL. Only the serial transplantation of equal numbers of prospectively isolated LT-HSCs from $ATF4^{-/-}$ and $ATF4^{+/+}$ E14.5 embryos into WT recipients allowed the authors to manifest an intrinsic defect of the self-renewal capacity in $ATF4^{-/-}$ HSCs. When one deliberates on the term "stem cell self-renewal," many different cell fate decisions must come into place in HSCs: survival and division must be facilitated in the absence of differentiation. Zhao et al excluded increased cell death or changes in cell cycle progression in HSCs lacking ATF4 as alternative explanations for their diminished number.

On top of the intrinsic self-renewal defect caused by the absence of ATF4 in HSCs, the authors also elucidated an important contribution of the FL cell environment to this phenotype. They elegantly showed that ATF4 regulates the expression and secretion of angiopoietin-like 3 (Angptl3) in endothelial and stroma cells, which plays pivotal roles in supporting stemness in LT-HSCs.[7,8] LT-HSCs from WT donor mice rapidly lost their reconstitution ability in cocultures with stroma cells from $ATF4^{-/-}$ FLs, which could then be rescued by the addition of exogenous Angptl3.

Zhao et al present here a new molecule, ATF4, which is essential for sufficient LT-HSC expansion during development. However, its integration in the complex regulation with other known factors in FL hematopoiesis warrants future studies. Furthermore, dissection of the bivalent contribution of extrinsic and intrinsic functions governed by ATF4 during this important phase for the establishment of lifelong hematopoiesis requires investigation of the timed conditional knockout of ATF4 in distinct hematopoietic and nonhematopoietic lineages. A conditional system would also allow assessment of the involvement of ATF4 in adult steady-state and stress hematopoiesis.

Many individual molecules have been identified lately that are pivotal for FL HSC self-renewal,[9,10] some of them also being important in adult HSCs. However, the challenging goal is now to understand their exact interplay and to find molecular patterns and hubs that can be used for regenerative approaches. Nevertheless, we need to know many if not all components of the molecular network that governs HSC self-renewal, not only at the transcriptional level, but also functionally validated as successfully conducted in this study, to be able to mold the system toward our needs.

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

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Liang et al, page 2415

Factor V: an active player in inflammation

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In this issue of *Blood*, Liang et al identify a critical role of coagulation factor V in host inflammation response to infections that could guide our exploration for better therapy for sepsis.[1]

Severe sepsis is a common, life-threatening infection resulting from a number of pathogens. Understanding the complex host inflammation and coagulation response during sepsis is key to finding a solution to this severe disease. The uncontrolled inflammatory response by the host to the infection results in multiorgan failure and death in sepsis. Numerous studies have been performed to study the effects of anti-inflammatory and antithrombotic agents on sepsis outcomes. Only human recombinant activated protein C (aPC) demonstrated efficacy in clinical trials for improving the 28-day mortality rate of septic shock patients in 2001.[2] However, drotrecogin alfa (activated; recombinant human aPC) was withdrawn from the market in October 2011.[3] A multicenter investigator-led trial found no evidence for benefit of recombinant human aPC in adults with septic shock.[4] aPC cleaves activated factor Va and factor VIIIa and also demonstrates anti-inflammatory and cytoprotective effects.[5] Patients with severe sepsis who were heterozygote carriers of the factor V (fV) Leiden mutation, a prothrombotic mutation of fV resistant to aPC cleavage, demonstrated an improved survival rate.[6] These findings suggest that the roles of fV in aPC function are complicated and merit further exploration.