Challenges with this approach include a 10% manufacturing failure rate and a 21-day manufacturing period, resulting in 14% of patients not being able to receive the therapy, primarily due to disease progression. Future iterations of this approach will likely involve shorter culture durations or frozen off-the-shelf expanded products to extend this potentially lifesaving therapy to more patients.

Currently, the number of UCB units in the global UCB bank inventories exceeds 1.5 million, but many of these units are too small to support hematopoiesis, particularly for adults. UCB expansion technologies as reported here and by others[7] may provide a paradigm-changing opportunity to use smaller and better HLA-matched units for UCBT; they also set a benchmark for the expansion of other important UCB populations such as MSCs, NK cells,[8] or T cells.[9,10] Indeed, promising results with genetically engineered UCB NK cells targeting CD19+ cancers,[8] UCB-derived virus-specific T cells,[9] and T-regulatory cells[10] support the use of UCB units in the global inventory for the treatment of patients with cancer in the coming decades.

REFERENCES

1. Horwitz ME, Stiff PJ, Cutler C, et al. Omidubicel vs standard myeloablative umbilical cord blood transplantation: results of a phase 3 randomized study. *Blood.* 2021;138(16):1429-1440.

2. Brunstein CG, Gutman JA, Weisdorf DJ, et al. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood.* 2010;116(22):4693-4699.

3. Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med.* 2010;16(2):232-236.

4. de Lima M, McNiece I, Robinson SN, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *N Engl J Med.* 2012;367(24):2305-2315.

5. Wagner JE Jr, Brunstein CG, Boitano AE, et al. Phase I/II trial of StemRegenin-1 expanded umbilical cord blood hematopoietic stem cells supports testing as a stand-alone graft. *Cell Stem Cell.* 2016;18(1):144-155.

6. Peled T, Shoham H, Aschengrau D, et al. Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment. *Exp Hematol.* 2012;40(4):342-55.e1.

7. Cohen S, Roy J, Lachance S, et al. Hematopoietic stem cell transplantation using single UM171-expanded cord blood: a single-arm, phase 1-2 safety and feasibility study. *Lancet Haematol.* 2020;7(2):e134-e145.

8. Liu E, Marin D, Banerjee P, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N Engl J Med.* 2020;382(6):545-553.

9. Hanley PJ, Melenhorst JJ, Nikiforow S, et al. CMV-specific T cells generated from naïve T cells recognize atypical epitopes and may be protective in vivo [published correction appears in *Sci Transl Med.* 8(321):321er1]. *Sci Transl Med.* 2015;7(285):285ra63.

10. Brunstein CG, Miller JS, McKenna DH, et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: kinetics, toxicity profile, and clinical effect. *Blood.* 2016;127(8):1044-1051.

11. Horwitz ME, Wease S, Blackwell B, et al. Phase I/II study of stem-cell transplantation using a single cord blood unit expanded ex vivo with nicotinamide. 2018;37(5):367-374.

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**HEMATOPOIESIS AND STEM CELLS**

Comment on Thoms et al, page 1441

Transcriptional circuit dynamics in HSPCs

Goichi Tatsumi[1] and **Ulrich Steidl**[1,2] | [1]Montefiore Medical Centerr [2]Albert Einstein Cancer Center

In this issue of *Blood*, Thoms et al[1] report that chromatin accessibility at regulatory regions of a heptad of transcriptional factors (TFs; LYL1, TAL1/SCL, LMO2, FLI1, ERG, GATA2, and RUNX1) displays distinct patterns at major stages of hematopoiesis and can thereby predict cell identity.

Hematopoiesis is strictly regulated by transcriptional networks, consisting of TFs, regulatory regions, and complexes of multiple transcriptional regulators interacting with each other.[2] Regulatory regions are generally located in noncoding DNA sequences including intergenic and intragenic regions, and their epigenetic states such as DNA methylation and histone modifications dynamically control chromatin accessibility in a cell type–specific manner, and also contribute to cell fate decisions.[3] Aberrant expression of key hematopoietic TFs or alterations in their regulatory regions, caused via genetic or nongenetic dysregulation, can rewire transcriptional networks and eventually lead to leukemogenesis.[4] Although much work has been conducted to study the various effects of individual TFs or their regulatory regions on hematopoiesis and
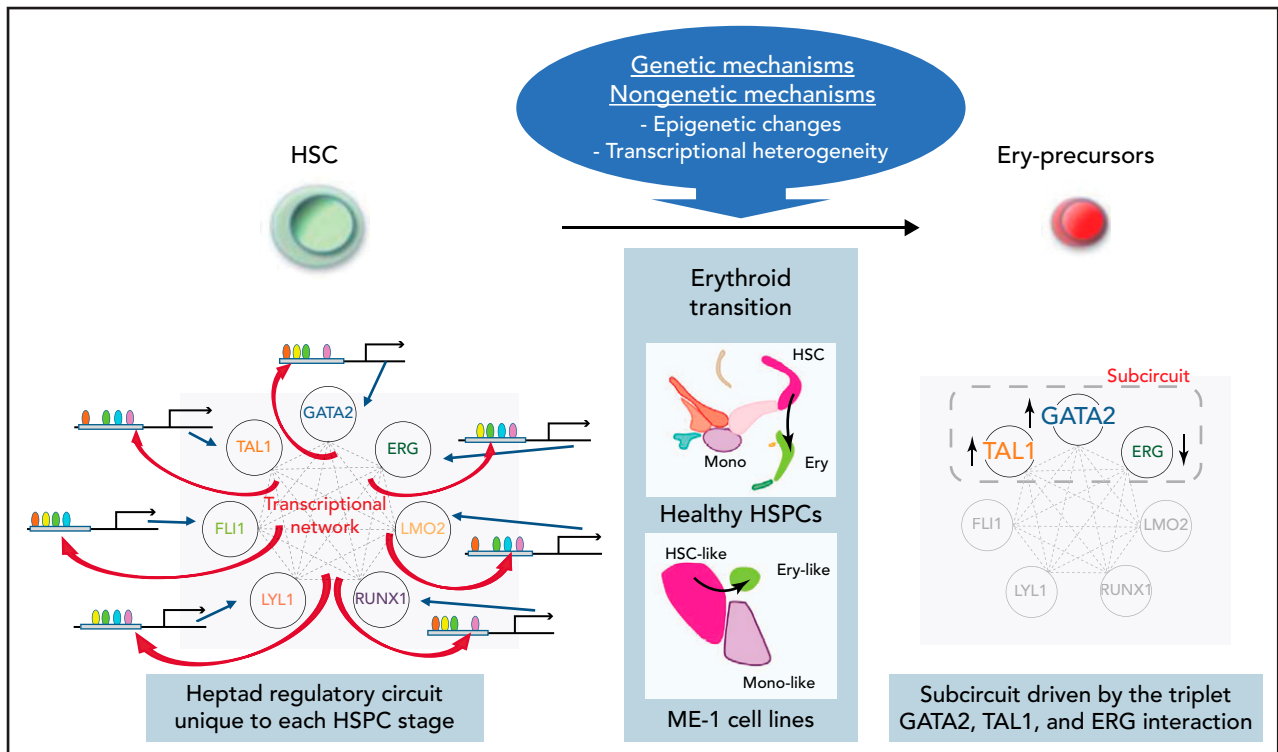
Schematic illustration of the transcriptional network consisting of heptad TFs and their regulatory regions and its dynamic change in erythroid transition.

leukemogenesis, the role and function of larger transcriptional networks and their dynamics are far less well understood.

Prior work by Thoms and colleagues and other groups have demonstrated an important role and impact of a heptad of key TFs both in normal hematopoiesis and acute myeloid leukemia (AML). These factors shape a robustly interconnected circuit by controlling themselves and each other in human CD34^{+} human hematopoietic stem and progenitor cells (HSPCs), and play an essential role in stem maintenance.[2,5] Furthermore, this heptad TF circuit was also reported to be active in AML, and heptad expression patterns were significantly associated with poor outcomes in patients.[6] These earlier results clearly indicated that this TF heptad plays a vital role in HSPCs and AML cells, but one of the questions that remained unanswered was whether this circuit also plays a role in dynamic situations such as cell fate decisions during hematopoiesis and leukemogenesis. To this end, the present study set out to study dynamic changes of the TF heptad circuit at different stages of hematopoietic differentiation and the functional effect of this circuit on cell identity.

First, to explore the heptad circuit dynamics during normal hematopoiesis, Thoms et al analyzed single-cell RNA sequencing data of human HSPCs as well as chromatin accessibility data of sorted bone marrow cells. They found that the expression of heptad TFs and chromatin accessibility of their regulatory regions showed unique and different patterns throughout hematopoietic development, with a tendency for the heptad connectivity to disappear at the terminal differentiation stage. They then asked whether the accessibility of heptad regulatory regions was altered in AML cells at different stages including preleukemic hematopoietic stem cells (HSCs), leukemic stem cells, and blasts. Remarkably, chromatin accessibility patterns at each AML stage were distinct from one another, but again closely related to their biologically closest normal counterpart (eg, preleukemic HSCs compared with normal HSCs/multipotent progenitor cells), suggesting that the TF heptad circuit state is a defining feature of cell identity in both normal HSPCs and AML cells.

Thoms et al next investigated whether the connectivity of heptad TFs and their regulatory regions had a functional impact on heptad circuit activity. Chromatin immune precipitation sequencing data from human CD34^{+} HSPCs and 2 AML cell lines revealed a robust landscape of heptad TF patterns bound to their regulatory regions both in healthy and leukemic states, which were again quite similar with only minor differences. Furthermore, DNA-binding motif mutagenesis studies showed that the physical interaction of heptad TFs with their regulatory regions via consensus motifs were indeed essential for transcriptional activation (see figure). And finally, Thoms et al asked whether these regulatory circuits change dynamically during the shift from HSC to a precursor state. To address this, they performed semisupervised clustering of single-cell RNA sequencing data in ME-1 cells and uncovered an erythroid precursor-like subpopulation, which was characterized by high expression of TAL1 and GATA2, as well as low expression of ERG. In addition, they found that experimental overexpression of GATA2 or knock-down of ERG in ME-1 cells was able to induce transcriptional programs similar to the ones found in erythroid precursors. Combined, these findings support a model in that the triplet of GATA2, TAL1, and ERG shape a transcriptional subcircuit that is functionally relevant in HSC–erythroid transition.

Overall, the work from Thoms et al suggests that transcriptional networks dynamically control cell fate decisions and shape unique subcircuits during phenotype transition in healthy blood and leukemia. The study also raises several interesting questions for future study. The elegant work of the authors shows that the chromatin accessibility state of only a handful of enhancers can classify essentially all major stages of early hematopoiesis. They also succeeded in the identification of a unique transcriptional subcircuit during erythroid differentiation. Based on this, it will be interesting to identify and study other types of subcircuits which may govern the transition to precursors of other lineages. Another intriguing question is what precise mechanisms are harnessed to dynamically regulate the transcriptional networks in HSPCs, and which ultimately drive the transition from HSCs to precursors (eg, recent work has monitored low-level cofluctuations of PU.1, Gata1, and Gata2 in murine HSPCs) and found that transcriptional stochasticity of these TFs played a vital role in the maintenance of transcriptional plasticity.[7] Future single-cell studies of changes of epigenetic states and transcriptional heterogeneity in the heptad circuit may lead to novel insights in that regard.

Another interesting question and future challenge are how the advances presented by Thoms et al could potentially be leveraged for therapeutic purposes. Although TFs are challenging drug targets in general, new approaches with small molecules or modified biologics are emerging.[8-10] If future studies were able to identify AML-specific subcircuits which are not critical in normal hematopoiesis, the therapeutic targeting of such key TFs may be a highly promising strategy to tackle the generation of leukemic cell fates at its root.

REFERENCES

1. Thoms JAI, Truong P, Subramanian S, et al. Disruption of a GATA2-TAL1-ERG regulatory circuit promotes erythroid transition in healthy and leukemic stem cells. *Blood.* 2021;138(16):1441-1455.

2. Wilson NK, Foster SD, Wang X, et al. Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. *Cell Stem Cell.* 2010;7(4):532-544.

3. Andersson R, Sandelin A. Determinants of enhancer and promoter activities of regulatory elements. *Nat Rev Genet.* 2020; 21(2):71-87.

4. Li S, Garrett-Bakelman FE, Chung SS, et al. Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. *Nat Med.* 2016; 22(7):792-799.

5. Beck D, Thoms JA, Perera D, et al. Genome-wide analysis of transcriptional regulators in human HSPCs reveals a densely interconnected network of coding and noncoding genes. *Blood.* 2013;122(14): e12-e22.

6. Diffner E, Beck D, Gudgin E, et al. Activity of a heptad of transcription factors is associated with stem cell programs and clinical outcome in acute myeloid leukemia [published correction appears in *Blood.* 2014;123(18):2901]. *Blood.* 2013;121(12): 2289-2300.

7. Wheat JC, Sella Y, Willcockson M, et al. Single-molecule imaging of transcription dynamics in somatic stem cells. *Nature.* 2020;583(7816): 431-436.

8. Illendula A, Pulikkan JA, Zong H, et al. Chemical biology. A small-molecule inhibitor of the aberrant transcription factor CBFβ-SMMHC delays leukemia in mice. *Science.* 2015;347(6223):779-784.

9. Antony-Debré I, Paul A, Leite J, et al. Pharmacological inhibition of the transcription factor PU.1 in leukemia. *J Clin Invest.* 2017;127(12):4297-4313.

10. Carvajal LA, Neriah DB, Senecal A, et al. Dual inhibition of MDMX and MDM2 as a therapeutic strategy in leukemia. *Sci Transl Med.* 2018;10(436):eaao3003.

LYMPHOID NEOPLASIA

Comment on Herrera et al, page 1456

Single-cell trajectories in Sézary syndrome

Audrey Gros[1,2] and Jean-Philippe Merlio[1,2] | [1]University of Bordeaux; [2]University Hospital of Bordeaux

In this issue of *Blood*, Herrera et al[1] identified a restricted phylogeny together with a proliferation and T-cell activation signature in the skin compartment of leukemic cutaneous T-cell lymphoma (CTCL) by utilizing a multimodal single-cell analysis of paired blood and skin samples.

In recent years, both phenotypic analysis and next-generation sequencing have revealed significant interpatient diversity of Sézary syndrome (SS).[2-4] Assessing blood tumor burden has always been a challenge in CTCL, requiring international efforts to define malignant cells because of their aberrant phenotype and/or T-cell receptor gene clonotype. Herrera et al employed a CRISPR-compatible cellular indexing of transcriptomes and epitopes by sequencing (ECCITE-seq), combining cell hashing plus phenotypic analysis by antibody-derived tags (ADT) with single-guide RNA capture of individual cells identified by molecule barcoding (see figure). This multimodal approach permits the detection of transcriptome, T-cell receptor α/β (TCRα/β) and TCRγ/δ clonotype, and surface proteins expression at the single-cell level.[5]

Thanks to integrated bioinformatics tools, Herrera et al established the transcriptional profile of individual skin and blood clonal T cells in 4 patients with SS and 1 patient with leukemic mycosis fungoides (MF), thus defining clusters in both compartments. Interestingly, malignant skin T cells mainly clustered together, whereas blood contained several clusters revealing a greater transcriptional heterogeneity. This approach also demonstrated inter- and intraindividual heterogeneity according to the transcriptional and phenotypic profile of the malignant T cells. Although the expression of transcription factors resulted in a relatively homogeneous T helper 2 cell (Th2) or Th17 signature, the study confirmed the heterogeneity of SS cells according to their naive, central, or effector memory phenotypes as previously shown using comparative phenotypic and molecular profiling of skin and blood