HES6: an emerging player in human hematopoiesis

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In this issue of *Haematologica*, Tamara De Vos *et al.*[1] describe their study demonstrating that HES6, a member of the Hairy/Enhancer of Split (HES) family,[2] is required for human hematopoietic precursor cell repopulation and differentiation. Depletion of *HES6* in human hematopoietic stem and progenitor cells (HSPC) leads to a remarkable reduction in multilineage differentiation, compromises *in vitro* hematopoietic progenitor activity and impairs *in vivo* reconstitution capacity. Transcription factors involved in the Notch signaling pathway play pivotal roles in regulating normal hematopoiesis, and mutations of these factors are commonly associated with hematologic malignancies.[2-5] Notch activation induces expression of downstream target genes, including members of the HES family, which comprises seven members in humans (*HES1-7*).[2-5] Among these, *HES6* has been less explored. The study by De Vos *et al.* adds important information by unveiling a novel role for HES6 in regulating human HSPC function.

By analyzing published bulk RNA-sequencing and single-cell RNA-sequencing datasets, De Vos *et al.* found that *HES6* is expressed during erythroid/megakaryocyte and plasmacytoid dendritic cell (pDC) development. *HES6* is also expressed in multipotent precursors, as well as certain stages of T- and B-cell development following pre-B-cell receptor or pre-T-cell receptor engagement. Knockdown of *HES6* in cord blood-derived hematopoietic precursors inhibited the development of T-cell-specified precursors and $CD4^+CD8^+$ double-positive cells, limited the *in vitro* development of B cells beyond the stage that proliferating large pre-B cells receive pre-B-cell receptor signaling following heavy chain rearrangement, and reduced the frequency and absolute numbers of pDC ($CD45RA^+CD123^+$) in *ex vivo* differentiation assays, suggesting an important role for HES6 in regulating multilineage differentiation of human HSPC.

A colony-forming unit assay revealed that *HES6* knockdown decreased the numbers of myeloid and erythroid colonies. The authors further showed that both the frequencies and absolute cell numbers of megakaryocytes ($CD41^+$) and erythroblasts ($CD71^+CD235a^{high}$) differentiated from the *HES6* short hairpin (sh)RNA-transduced cells were significantly lower compared to those differentiated from the control cells in liquid culture, confirming that HES6 is essential for human erythrocyte and megakaryocyte development. In keeping with these *in vitro* findings, *HES6* shRNA-transduced HSPC exhibited diminished repopulating potential, leading to an *in vivo* reduction in the reconstitution of all lineages in the transplanted immunodeficient $IL2RG^{-/-}$ (NSG) recipient mice compared to reconstitution in the mice transplanted with control HSPC.

By using the sorted cells from *HES6* shRNA-transduced human HSPC after 4 days of induction in *ex vivo* liquid culture, the authors found that *HES6* knockdown significantly reduced erythroid-associated genes (*TFRC*, *HBB*, *EPCAM*, *E2F4*), and affected erythroblast-related gene sets. However, *HES6*-knockdown erythroid cells failed to downregulate *GATA2* and *ID2* expression, and had no impact on *GATA1* expression. *HES6* knockdown also decreased the expression of genes involved in megakaryocyte development (*CD36*, *DLK1*) and proliferation (*MKI67*, *HEMGN*). Furthermore, *HES6* shRNA-transduced cells demonstrated a remarkable reduction in pDC-associated genes (*IRF8*, *TCF4*), B-lineage genes (*BLNK*, *IGLL1*, *IGHM*), as well as hematopoietic precursor cell and pDC genes (*TCF3*, *RUNX2*).

Consistent with the reduced cellular output and downregulated genes associated with proliferation, RNA-sequencing illuminated changes in cell cycle-related gene sets in *HES6* knockdown cells. Specifically, cell proliferation-related genes, such as *TFRC*, *E2F4* and *E2F8*, were downregulated upon *HES6* knockdown in late erythroblasts. By employing an ethynyl-2´-deoxyuridine incorporation assay, the authors observed a marked decrease in the proportion of cells in the proliferative S phase, which was accompanied by a notable increase in cells in the quiescent G1 phase, in *HES6* knockdown cells compared to the control shRNA-transduced cells. These findings imply that HES6 is essen-

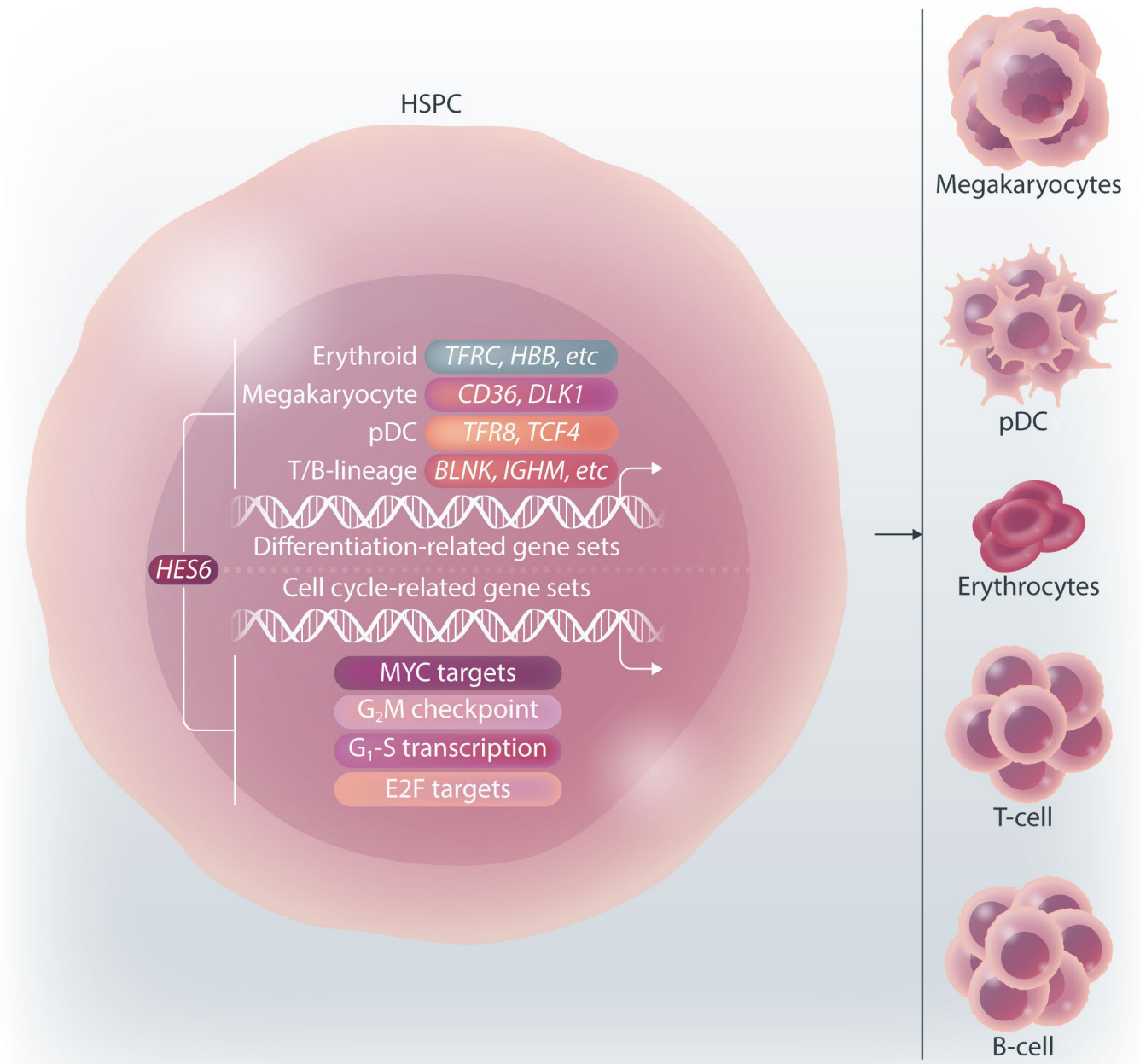

Figure 1. Schematic overview of HES6 regulation of hematopoiesis. HES6 modulates the expression of genes involved in lineage differentiation and cell cycle progression, thereby tightly controlling the maintenance and lineage commitment of human hematopoietic stem and progenitor cells. HSPC: hematopoietic stem and progenitor cells; pDC: plasmacytoid dendritic cells.

tial for human HSPC proliferation, potentially through the regulation of genes involved in cell cycle progression.

The study by De Vos *et al.* highlights the novel role of HES6 in orchestrating human HSPC multilineage development. The transcription factors GATA1 and GATA2 functionally overlap in primitive hematopoietic development.[6] HES6 has previously been shown to be a pivotal transcriptional cofactor of GATA1. Knockdown of *HES6* impaired human erythropoiesis by decreasing the expression of GATA1 as well as GATA1 downstream targets.[7] It is also known that GATA2 to GATA1 switch is critical for erythroid development.[8] De Vos *et al.* offer intriguing new insights into the intricate interplay between *HES6* and the *GATA* family. Notably, HES6 emerges as a crucial player in erythroid development, functioning independently of GATA2. This implies that HES6 might directly govern the expression of genes vital for HSPC differentiation.

This study also raises an important mechanistic question as to how HES6 regulates HSPC proliferation. The observations that HES6 facilitates HSPC maintenance through mediating G1-to-S transition, suggest that HES6 may be involved in the functional exhaustion of human HSPC. Previous studies have demonstrated a strong association between HES6 and the promyelocytic leukemia nuclear body, highlighting its role in modulating the cell cycle through upregulation of the expression of p21,[9] a negative regulator of the cell cycle.[10] The current RNA-sequencing and chromatin immunoprecipitation-sequencing data suggest direct regulatory roles for HES6 in positively regulating *TFRC* and *MKI67*, two important cell proliferation mediators, in a GATA1-independent manner. It will be important to determine the separated role of HES6 in regulating gene expression uncoupled from GATA transcription factor family members at different stages of HSPC development.

The current study by De Vos *et al.* provides new insight into the role of HES6 in HSPC maintenance (Figure 1). Their

findings add another layer of understanding of the HES family members in regulating hematopoiesis, shedding light on potential lineage- and stage-specific functions of this group of transcription factors in the intricate process of human hemostasis and lineage differentiation.

Disclosures

No conflicts of interest to disclose.

Contributions

JX and WD co-wrote the editorial.

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References

1. De Vos T, Oatman N, Boehme L, et al. HES6 knockdown in human hematopoietic precursor cells reduces their in vivo engraftment potential and their capacity to differentiate into erythroid, B, T and plasmacytoid dendritic cells. Haematologica. 2024:109(11):3578-3592.
2. Varnum-Finney B, Dallas MH, Kato K, Bernstein ID. Notch target Hes5 ensures appropriate Notch induced T- versus B-cell choices in the thymus. Blood. 2008;111(5):2615-2620.
3. Wendorff AA, Koch U, Wunderlich FT, et al. Hes1 is a critical but context-dependent mediator of canonical Notch signaling in lymphocyte development and transformation. Immunity. 2010;33(5):671-684.
4. De Decker M, Lavaert M, Roels J, et al. HES1 and HES4 have non-redundant roles downstream of Notch during early human T-cell development. Haematologica. 2020;106(1):130-141.
5. Ma Z, Xu J, Wang J, et al. Hes1 deficiency causes hematopoietic stem cell exhaustion. Stem Cells. 2020;38(6):756-768.
6. Fujiwara Y, Chang AN, Williams AM, Orkin SH. Functional overlap of GATA-1 and GATA-2 in primitive hematopoietic development. Blood. 2004;103(2):583-585.
7. Wang Z, Wang P, Zhang J, et al. The novel GATA1-interacting protein HES6 is an essential transcriptional cofactor for human erythropoiesis. Nucleic Acids Res. 2023;51(10):4774-4790.
8. Bresnick EH, Lee HY, Fujiwara T, Johnson KD, Keles S. GATA switches as developmental drivers. J Biol Chem. 2010;285(41):31087-31093.
9. Eun B, Lee Y, Hong S, et al. Hes6 controls cell proliferation via interaction with cAMP-response element-binding protein-binding protein in the promyelocytic leukemia nuclear body. J Biol Chem. 2008;283(9):5939-5949.
10. Ortiz-Alvarez G, Fortoul A, Srivastava A, et al. p53/p21 pathway activation contributes to the ependymal fate decision downstream of GemC1. Cell Rep. 2022;41(11):111810.