



INSIGHTS

I *SPI1* something needed for B cells

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In this issue, Le Coz et al. (2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20201750>) describe a novel immunodeficiency syndrome caused by mutations in *SPI1*. Through a series of in-depth studies, the authors provide insights into how *SPI1* affects blood lineage specification, highlighting the important role of master transcription factors as cellular fate determinants.

Hematopoiesis, the process of blood cell production, consists of multiple highly orchestrated steps controlled by networks of transcription factors (TFs) that establish distinct cellular programs (Göttgens, 2015; Liggett and Sankaran, 2020). The activation of lineage-specific transcriptional programs is tightly governed by master hematopoietic TFs. Here, by using exome sequencing, Le Coz et al. (2021) examine a series of patients with agammaglobulinemia—a condition characterized by a paucity of B lymphocytes and therefore defective humoral immunity—and identify six unrelated patients with heterozygous germline mutations in the *SPI1* gene, which encodes the hematopoietic master TF PU.1.

Le Coz et al. (2021) define the clinical and molecular phenotype of this novel inherited PU.1 haploinsufficiency syndrome, which they term PU.1-mutated agammaglobulinemia (PU.MA). PU.1 is a key transcriptional regulator required in the development of multiple hematopoietic lineages (Scott et al., 1994). Somatic mutations in *SPI1* have been reported in the context of acute myeloid leukemia (AML; Pabst and Mueller, 2007), however germline variants impacting human *SPI1* have not been previously identified. Mouse models of *Sp1*-deficient hematopoiesis indicate a crucial role for PU.1 in early lineage commitment, differentiation of multiple myeloid lineages, as well as B cell development. However, the precise effect of *SPI1* loss on human hematopoiesis has remained unknown. PU.1 has

been shown to have multifaceted roles in hematopoiesis, with reduced PU.1 levels causing B cell developmental arrest, but other specific PU.1 perturbations can favor B cell over myeloid lineage development (DeKoter et al., 2007; Heinz et al., 2013; Houston et al., 2007). By providing comprehensive phenotypic data, Le Coz et al. (2021) help clarify some of the prior observations on the dosage-dependent role of PU.1 in hematopoietic lineage specification. The authors perform detailed immune phenotyping and transcriptional profiling of blood and bone marrow cells from PU.MA patients and observe a significant reduction of circulating B cells and dendritic cells, as well as a B cell maturation arrest.

To selectively examine the impact of PU.1 perturbation on human hematopoiesis, the investigators employed CRISPR/Cas9 editing of the *SPI1* locus in human cord blood-derived hematopoietic stem and progenitor cells, which were then differentiated toward T cells, B cells, and myeloid precursors. Using these *SPI1*-edited hematopoietic stem and progenitor cells as a model system for human hematopoiesis in the setting of PU.MA, the authors demonstrate that biallelic PU.1 expression is critical for B cell and myeloid cell development. Examining patient bone marrow cells by cellular indexing of transcriptomes and epitope sequencing, Le Coz et al. (2021) identify the B cell developmental defect in the pro- to pre-B cell transition. On a molecular level,



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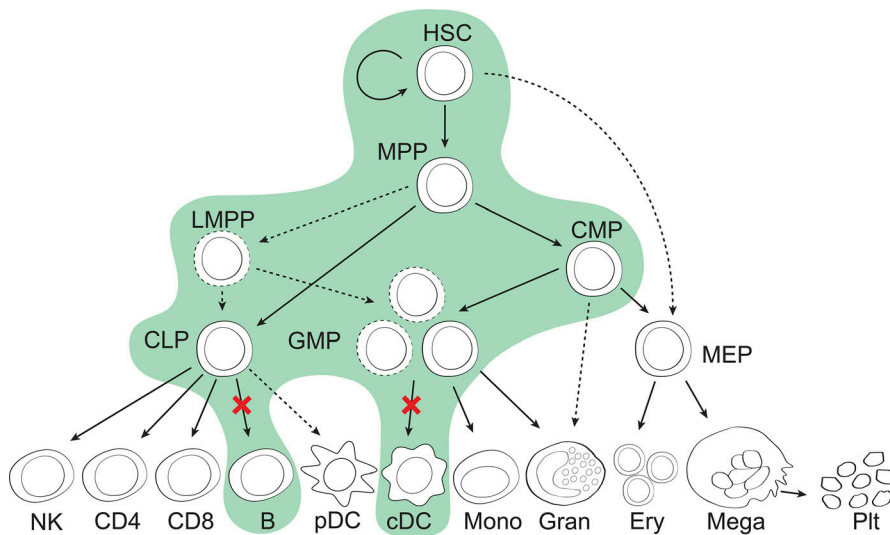
mutant heterozygous *SPI1* alleles were confirmed to result in destabilized, transcriptionally inert PU.1 proteins that do not appear to exert dominant negative effects. Finally, to examine the epigenetic and transcriptional effects of PU.1 haploinsufficiency, the authors employ the assay for transposase-accessible chromatin using sequencing and RNA-sequencing on edited human pro-B cell lines. Integrating these results, the authors demonstrate that the deleterious effects of PU.1 haploinsufficiency on early B cell development appear to be predominately mediated by constrained chromatin accessibility, resulting in transcriptional changes that disrupt critical pro-B cell gene expression patterns. Pioneer TFs are characterized by having the unique property of enabling the opening of previously closed chromatin sites that then enable activation of specific cellular programs (Mayran et al., 2019). The findings by Le Coz et al. (2021) further underscore the key role of PU.1 as a pioneer TF (Iwafuchi-Doi and Zaret, 2014; Minderjahn et al., 2020).

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Diverse roles for hematopoietic master TF PU.1 in humans and mice. Studies on the role of *Spi1*-deficient hematopoiesis implicate PU.1 at multiple stages along the hematopoietic hierarchy, as indicated by green shading. The principal phenotype of human *SPI1* haploinsufficiency seen in PU.MA patients is characterized by a paucity of circulating B and dendritic cells, as indicated with red crosses. HSC, hematopoietic stem cell; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; GMP, granulocyte–monocyte progenitor; MEP, megakaryocyte–erythrocyte progenitors; NK, natural killer cell; CD4, CD4⁺ helper T cell; CD8, CD8⁺ cytotoxic T cell; B, B cell; pDC, plasmacytoid dendritic cell; Mono, monocyte; cDC, conventional dendritic cell; Gran, granulocyte; Ery, erythrocyte; Mega, megakaryocyte; Plt, platelet.

The authors report a novel monogenic cause of a congenital blood disorder involving a master TF, thereby providing important phenotypic and molecular insight into the physiological roles of the *SPI1* gene in human hematopoiesis and extending the range of hematopoietic master TFs that are altered in disease. This report illustrates how deciphering rare congenital blood and immune disorders holds potential to gain fundamental insights into how cellular fate decisions in hematopoiesis are regulated. By adding to a growing list of hematopoietic master TFs that are mutated in inherited human diseases, including *BCL11A*, *BCL11B*, *ETV6*, *FLI1*, *GATA1*, *GATA2*, *GFI1*, *GFI1B*, *IRF4*, *IRF8*, *IZKF1*, *IFZK5*, *MECOM*, *PAX5*, *RUNX1*, and *TBX21*, many distinct and important phenotypes have been noted, including those that diverge from the observations made in knockout mice (Liggett and Sankaran, 2020). While some differences may be due to interspecies variation, disease-causing alleles can be distinct from complete knockout, particularly in the context of haploinsufficiency where unique requirements for specific master TFs may only become apparent after in depth study of human phenotypes. For example, while *BCL11A* is known to play a key role in B

lymphopoiesis, humans with haploinsufficiency of this factor have persistent fetal hemoglobin expression and neurodevelopmental phenotypes, but appear to have ostensibly normal humoral immunity (Basak et al., 2015).

This concept of pleiotropy and distinct phenotypes compared with mouse models is nicely illustrated for PU.MA syndrome (see figure). The current report defines B cell developmental arrest and absence of circulating dendritic cells as the principal pathological feature of the human phenotype of PU.1 haploinsufficiency. Compared with prior studies in model systems, some distinct differences are notable (DeKoter et al., 2007; Heinz et al., 2013; Houston et al., 2007; Ye et al., 2005). Studies in mouse models have suggested more global roles for PU.1 across different aspects of hematopoiesis. Further study of these and other patients that will likely be uncovered following this report may shed light on additional roles in earlier hematopoiesis that may not have been fully elucidated yet. The detailed insights from mouse models harboring PU.1 mutations are likely to provide key guidance on aspects of hematopoiesis that are important to examine in depth in these patients (see figure). In addition,

none of the PU.MA patients reported here have developed AML or other hematologic malignancies. In mouse models, even slight reductions in PU.1 expression can induce transformation to AML (Will et al., 2015). While the absence of malignancies in the reported six patients is intriguing, this finding requires cautious interpretation and close follow-up, given the low number of PU.MA patients reported and the unknown latency to develop malignancies.

This study highlights several key points that will be important to address in the future. First, from a broad perspective, it is clear that genome sequencing of patients impacted by blood and immune disorders will enable even more discoveries that provide further insights on the role of hematopoietic master TFs in regulating cellular fate decisions. As nicely illustrated here, some surprising observations are likely to be made that diverge from roles ascribed from studies in mice or other model systems. Second, the study also highlights the need for in-depth mechanistic studies that often may rely upon genome editing and other molecular tools that enable reductionist and well-controlled isogenic analyses. Third, the study also highlights a key area for future investigation: what underlies pleiotropy in such diseases. While some of the underlying variation may be due to genotype–phenotype relationships in mutant alleles, other factors such as background genetic variation are increasingly being appreciated to have a role in modulating presumed Mendelian phenotypes (Vuckovic et al., 2020). It is likely that additional patients with *SPI1* mutations may be uncovered following this report, and the range of phenotypes observed will be important to fully assess and integrate with our current knowledge. As nicely illustrated here, studies of rare human phenotypes are continuing to provide important new lessons on the seemingly well understood process of hematopoiesis.

References

- Basak, A., et al. 2015. *J. Clin. Invest.* <https://doi.org/10.1172/JCI81163>
- DeKoter, R.P., et al. 2007. *Blood Cells Mol. Dis.* <https://doi.org/10.1016/j.bcmd.2007.06.004>
- Göttgens, B. 2015. *Blood.* <https://doi.org/10.1182/blood-2014-08-570226>

- Heinz, S., et al. 2013. *Nature*. <https://doi.org/10.1038/nature12615>
- Houston, I.B., et al. 2007. *Exp. Hematol.* <https://doi.org/10.1016/j.exphem.2007.04.005>
- Iwafuchi-Doi, M., and K.S. Zaret. 2014. *Genes Dev.* <https://doi.org/10.1101/gad.253443.114>
- Le Coz, C., et al. 2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20201750>
- Liggett, L.A., and V.G. Sankaran. 2020. *Cell*. <https://doi.org/10.1016/j.cell.2020.08.030>
- Mayran, A., et al. 2019. *Nat. Commun.* <https://doi.org/10.1038/s41467-019-11791-9>
- Minderjahn, J., et al. 2020. *Nat. Commun.* <https://doi.org/10.1038/s41467-019-13960-2>
- Pabst, T., and B.U. Mueller. 2007. *Oncogene*. <https://doi.org/10.1038/sj.onc.1210765>
- Scott, E.W., et al. 1994. *Science*. <https://doi.org/10.1126/science.8079170>
- Vuckovic, D., et al. 2020. *Cell*. <https://doi.org/10.1016/j.cell.2020.08.008>
- Will, B., et al. 2015. *Nat. Med.* <https://doi.org/10.1038/nm.3936>
- Ye, M., et al. 2005. *J. Exp. Med.* <https://doi.org/10.1084/jem.20051089>