Title

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Journal

Nature Cell Biology, 22(10)

Authors

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Publication Date

2020-10-01

DOI

10.1038/s41556-020-00585-7

Peer reviewed

HHS Public Access

Author manuscript

Nat Cell Biol. Author manuscript; available in PMC 2024 May 13.

Published in final edited form as:

Nat Cell Biol. 2020 October ; 22(10): 1155–1157. doi:10.1038/s41556-020-00585-7.

ERADicating stem cells from their niche

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Abstract

Protein homeostasis preserves stem cell function, but underlying mechanisms are largely unknown. A study reveals that protein quality control mediated by the endoplasmic reticulum-associated degradation pathway ensures proper expression of MPL, a key cell surface receptor that promotes haematopoietic stem cell function through niche interaction.

Protein homeostasis (proteostasis) is maintained by an integrated network of physiological mechanisms and stress response pathways that regulate proteome content and quality[1]. Despite being highly conserved, evidence suggests that proteostasis mechanisms can function in a cell-type-specific manner to support stem cell self-renewal[1–3]. However, our understanding of how proteostasis mechanisms uniquely function within stem cells is still an emerging topic. In this issue of *Nature Cell Biology*, Xu, Liu, Peng, Zhang et al. report that the endoplasmic reticulum-associated degradation (ERAD) pathway regulates haematopoietic stem cells (HSCs)[4]. The authors demonstrate that SEL1L, a conserved component of the ERAD pathway, is essential for HSCs by maintaining quality control and surface expression of MPL protein, which is required for HSCs to interact with their perivascular niche in the bone marrow.

The endoplasmic reticulum (ER) is the major site of protein folding for membrane and secreted proteins and requires organelle-specific mechanisms to maintain proteostasis. ERAD is one such mechanism, whereby misfolded proteins in the ER are identified, ubiquitylated, and retrotranslocated to the cytosol where they are ultimately degraded by the ubiquitin proteasome system[5]. However, a role for ERAD in HSCs had not yet been established.

After demonstrating that several components of the ERAD pathway are highly expressed by HSCs, the authors generated a conditional *Sel1l* knockout mouse, in which *Sel1l* is deleted from either the developing or adult haematopoietic system. *Sel1l* encodes a key adaptor protein for HRD1, an E3 ubiquitin ligase that acts as a retrotranslocon to transport misfolded

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Competing Interests

The authors declare no competing interests.

substrates from the ER to the cytosol[6]. The authors found that *Sel1l* deficiency severely disrupts HSC maintenance and function. Haematopoietic deletion of *Sel1l* diminished HSC quiescence and increased HSC proliferation, leading to a transient increase in bone marrow HSCs prior to a precipitous decline in their frequency and absolute number. This decline in HSCs was followed by a reduction of haematopoietic progenitors, the development of anaemia, and premature lethality. *Sel1l*-deficient HSCs also exhibited a loss of long-term multilineage reconstitution activity in transplantation experiments and increased sensitivity to serial 5-fluorouracil treatment. The authors therefore concluded that *Sel1l* is essential for HSC maintenance and function under both steady-state and stress conditions.

Surprisingly, Xu, Liu, Peng, Zhang et al. found that disrupting the ERAD pathway induces only mild ER stress and a modest activation of the ER unfolded protein response (UPR^{ER}) in HSCs, and that these pathways do not substantially contribute to the *Sel1l*-deficiency-induced HSC defect. Previous studies have demonstrated that the UPR^{ER} can have pleiotropic effects on HSC function, and moderate stress can promote HSC survival and reconstitution activity through either ATF4 or IRE1 activation[7–9]. Consistent with these previous findings, *Sel1l*-deficient HSCs preferentially, albeit modestly, activated the IRE1 branch of the UPR^{ER}, which partially protects HSCs from the deleterious effects of *Sel1l* deficiency. Deletion of either *Ire1α* or *Xbp1* exacerbated the *Sel1l*-deficiency HSC phenotype in vivo. In addition, the authors tested a multitude of genetic and pharmacological interventions that modulate UPR^{ER} activity, and found that they were unable to rescue the *Sel1l*-deficient HSC reconstitution defect. Furthermore, chemical induction of chronic ER stress did not recapitulate the *Sel1l* knockout HSC phenotype. For these reasons, disruption of the ERAD pathway impairs HSCs independently of either ER stress or activation of the UPR^{ER}.

To investigate alternative mechanisms underlying the severe HSC defect associated with ERAD dysfunction, the authors insightfully investigated whether *Sel1l* deficiency impairs HSC interactions with their niche. The rationale behind this decision was that the ER is a major site for the production and maturation of cell-surface and secreted proteins, some of which are essential for HSCs to engage with their perivascular niche in the bone marrow[10]. The authors found that *Sel1l*-deficient HSCs are located farther away from bone marrow vascular cells compared to control HSCs, suggesting that interactions between HSCs and their niche are impaired. This defect was further exemplified by efficient engraftment of wild-type HSCs in the bone marrow when transplanted into non-conditioned *Sel1l*-deficient mice. Together, these data demonstrate that *Sel1l*-deficient HSCs are mislocalized within the bone marrow.

Next, the authors considered candidate HSC surface proteins that could regulate both HSC quiescence and niche interactions, and focused their remaining studies on MPL and c-KIT, the cell surface receptors for thrombopoietin (TPO) and stem cell factor, respectively. They found reduced MPL expression on the surface of *Sel1l*-deficient HSCs, whereas c-KIT is unperturbed. The reduction in surface MPL expression was associated with reduced downstream signalling and accumulation of MPL in the ER. Xu, Liu, Peng, Zhang et al. further demonstrated that MPL interacts directly with SEL1L and HRD1, is polyubiquitylated by HRD1, and that SEL1L promotes MPL degradation, confirming

MPL as bona fide SEL1L-HRD1 client protein. Using a known disease-associated *MPL* mutation that disrupts normal protein folding, the authors found that misfolded MPL forms high molecular weight aggregates. Wild-type MPL also formed aggregates in *Sel1l*-deficient HSCs, suggesting that disrupted MPL regulation may contribute to *Sel1l*-deficient HSC defects. Similar to *Sel1l*-deficiency, genetic or antibody-mediated disruption of MPL or TPO induced HSC egress from their vascular niche, a phenotype that is not evident following chemical induction of ER stress. In summary, ERAD of misfolded MPL is necessary to prevent aggregation and to promote its normal surface expression in HSCs, which in turn maintains perivascular HSC localization (Fig. 1).

Finally, to confirm the contribution of dysregulated MPL to the *Sel1l*-knockout HSC phenotype, the authors used a MPL agonist that constitutively activates MPL signalling. The MPL agonist partially rescued steady-state HSC function as well as reconstitution in vivo. However, the potency of the rescue declined over time. The authors explained this diminished effect over time by demonstrating that MPL aggregates exert a dominant negative effect by recruiting properly folded MPL, thereby retaining it in the ER and preventing its surface expression. Consistent with these data, the surface expression of MPL continued to decline over time in *Sel1l*-deficient HSCs. However, it is likely that additional mechanisms contribute to HSC defects associated with *Sel1l* deficiency. In this regard, another study reported that *Sel1l* deficiency impairs HSCs in part by dysregulating the mTOR signaling pathway[11]. Overall, the study by Xu, Liu, Peng, Zhang et al. provides compelling evidence for a physiological role of ERAD in regulating MPL surface expression in HSCs, which is required for HSC-niche interactions, quiescence, and self-renewal[12].

In addition to regulating HSCs, MPL expression is also crucial for megakaryocyte lineage commitment[13]. Whether MPL surface expression in the megakaryocyte lineage is similarly regulated by SEL1L and the ERAD pathway remains to be explored. If MPL regulation in the megakaryocyte lineage is similar to HSCs, disruptions in proteostasis could contribute to the development of thrombocytopenia or other platelet disorders. Alternatively, if MPL surface expression is controlled differently in HSCs and megakaryocyte lineage cells, cell-type-specific differences in proteostasis regulation could potentially be leveraged to augment proteostasis maintenance and enhance stem cell fitness.

Precise cell surface protein expression is not only crucial for HSCs in mediating their interaction with the environment, but also helpful for identification and isolation. However, HSC surface phenotypes change throughout life[14], and in response to stressors[15] such as inflammation, chemotherapy, and cell culture, which can complicate the study of HSCs under certain conditions. An intriguing possibility is that these changes in cell surface protein expression are driven, at least in part, by disruptions in proteostasis and the ERAD pathway. If this is the case, changes in proteostasis may drive physiological changes in HSCs throughout life, and may also contribute to HSC dysfunction and haematological pathologies arising from acute or chronic stressors.

Overall, our understanding of proteostasis regulation in HSCs is still in its infancy, but it is clear that unique and highly-conserved proteostasis mechanisms are active in stem cells, but often function differently than in restricted progenitors and differentiated cells[1–3, 16]. The

study by Xu, Liu, Peng, Zhang et al. now establishes in detail how the ER protein quality control machinery acts in a cell-type-specific manner to regulate at least one key protein, MPL, that is required for HSC maintenance and function. Looking forward, as the sensitivity of proteomics and proteostasis monitoring technologies advances, the field will continue to unravel how the proteostasis network is uniquely wired to regulate proteome abundance, content, and quality to maintain long-lived stem cells at steady-state and during stress, with important implications for stem cell fitness and regenerative medicine.

References

1. Chua BA & Signer RAJ Hematopoietic stem cell regulation by the proteostasis network. Curr Opin Hematol 27, 254–263 (2020). [PubMed: 32452878]
2. Signer RA, Magee JA, Salic A & Morrison SJ Haematopoietic stem cells require a highly regulated protein synthesis rate. Nature 509, 49–54 (2014). [PubMed: 24670665]
3. Hidalgo San Jose L & Signer RAJ Cell-type-specific quantification of protein synthesis in vivo. Nat Protoc 14, 441–460 (2019). [PubMed: 30610239]
4. Xu L et al. Protein Quality Control Through Endoplasmic Reticulum-Associated Degradation Maintains Hematopoietic Stem Cell Identity and Interaction with Niche. Nature Cell Biology 22 (2020).
5. Brodsky JL Cleaning up: ER-associated degradation to the rescue. Cell 151, 1163–1167 (2012). [PubMed: 23217703]
6. Mueller B, Lilley BN & Ploegh HL SEL1L, the homologue of yeast Hrd3p, is involved in protein dislocation from the mammalian ER. J Cell Biol 175, 261–270 (2006). [PubMed: 17043138]
7. van Galen P et al. Integrated Stress Response Activity Marks Stem Cells in Normal Hematopoiesis and Leukemia. Cell Rep 25, 1109–1117 e1105 (2018). [PubMed: 30380403]
8. Chapple RH et al. ERalpha promotes murine hematopoietic regeneration through the Ire1alpha-mediated unfolded protein response. Elife 7 (2018).
9. Liu L et al. Adaptive endoplasmic reticulum stress signalling via IRE1alpha-XBP1 preserves self-renewal of haematopoietic and pre-leukaemic stem cells. Nat Cell Biol 21, 328–337 (2019). [PubMed: 30778220]
10. Crane GM, Jeffery E & Morrison SJ Adult haematopoietic stem cell niches. Nat Rev Immunol 17, 573–590 (2017). [PubMed: 28604734]
11. Liu L et al. Endoplasmic reticulum associated degradation preserves hematopoietic stem cell quiescence and self-renewal by restricting mTOR activity. bioRxiv 709964 (2019).
12. Yoshihara H et al. Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. Cell stem cell 1, 685–697 (2007). [PubMed: 18371409]
13. Methia N, Louache F, Vainchenker W & Wendling F Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit in vitro megakaryocytopoiesis. Blood 82, 1395–1401 (1993). [PubMed: 7689867]
14. Gekas C & Graf T CD41 expression marks myeloid-biased adult hematopoietic stem cells and increases with age. Blood 121, 4463–4472 (2013). [PubMed: 23564910]
15. Rabe JL et al. CD34 and EPCR coordinately enrich functional murine hematopoietic stem cells under normal and inflammatory conditions. Experimental hematology 81, 1–15 e16 (2020). [PubMed: 31863798]
16. van Galen P et al. The unfolded protein response governs integrity of the haematopoietic stem-cell pool during stress. Nature 510, 268–272 (2014). [PubMed: 24776803]

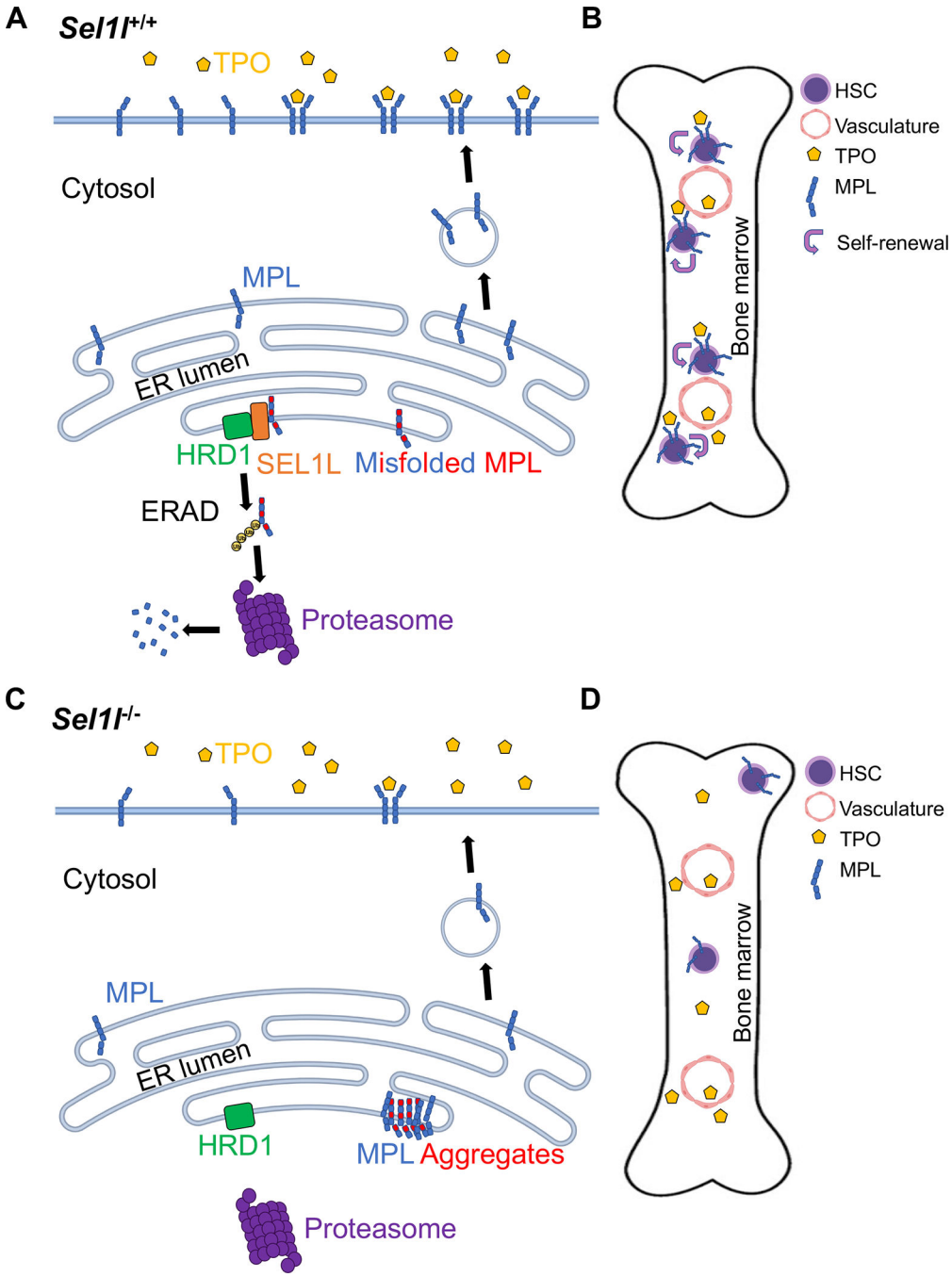

Figure 1. SEL1L promotes HSC maintenance, function and niche interactions by maintaining quality control and surface expression of MPL.

(A) In the endoplasmic reticulum (ER) of wild-type haematopoietic stem cells (HSCs), the SEL1L-HRD1 branch of the ER-associated degradation (ERAD) pathway recognizes, polyubiquitylates and retrotranslocates misfolded MPL to the cytosol where it is degraded by the proteasome. (B) MPL quality control promotes HSC surface expression of the thrombopoietin (TPO) receptor MPL, which enables HSCs to localize near perivascular cells in the bone marrow. TPO promotes HSC quiescence and self-renewal. (C) *Sel1l*-deficient HSCs fail to eliminate misfolded MPL, which subsequently forms high molecular

weight aggregates in the ER. MPL aggregates recruit and trap wild-type MPL and thus impair normal MPL surface expression. (D) *Sel1l*-deficient HSCs with reduced MPL surface expression are localized farther away from bone marrow perivascular cells, have reduced TPO signalling, and exhibit increased cell cycle entry, impaired self-renewal, and reconstitution.